Published in final edited form as:
Toxicol Rep. 2014 ; 1: 1046–1061. doi:10.1016/j.toxrep.2014.10.004.

Effects of developmental manganese, stress, and the combination of both on monoamines, growth, and corticosterone

Charles V. Vorhees[a], **Devon L. Graham**[a,1], **Robyn M. Amos-Kroohs**[a], **Amanda A. Braun**[a], **Curtis E. Grace**[a,2], **Tori L. Schaefer**[a,3], **Matthew R. Skelton**[a], **Keith M. Erikson**[b], **Michael Aschner**[c], and **Michael T. Williams**[a]

Devon L. Graham: devon.graham@vanderbilt.edu; Robyn M. Amos-Kroohs: robyn.amos-kroohs@cchmc.org; Amanda A. Braun: amanda.braun@cchmc.org; Curtis E. Grace: curtis.grace@covance.com; Tori L. Schaefer: tori.schaefer@cchmc.org; Matthew R. Skelton: matthew.skelton@cchmc.org; Keith M. Erikson: kmerikso@uncg.edu

[a]Department of Pediatrics, Division of Neurology, Cincinnati Children's Research Foundation and University of Cincinnati College of Medicine, Cincinnati, OH, USA

[b]Department of Nutrition, University of North Carolina, Greensboro, NC, USA

[c]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA

Abstract

Developmental exposure to manganese (Mn) or stress can each be detrimental to brain development. Here, Sprague-Dawley rats were exposed to two housing conditions and Mn from postnatal day (P)4–28. Within each litter two males and 2 females were assigned to the following groups: 0 (vehicle), 50, or 100 mg/kg Mn by oral gavage every other day. Half the litters were reared in cages with standard bedding and half with no bedding. One pair/group in each litter had an acute shallow water stressor before tissue collection (i.e., standing in shallow water). Separate litters were assessed at P11, 19, or 29. Mn-treated rats raised in standard cages showed no change in baseline corticosterone but following acute stress increased more than controls on P19; no Mn effects were seen on P11 or P29. Mn increased neostriatal dopamine in females at P19 and norepinephrine at P11 and P29. Mn increased hippocampal dopamine at P11 and P29 and 5-HT at P29 regardless of housing or sex. Mn had no effect on hypothalamic dopamine, but increased norepinephrine in males at P29 and 5-HT in males at all ages irrespective of rearing condition.

Correspondence: Charles V. Vorhees, Ph.D. or Michael T. Williams, Ph.D., Division of Neurology, MLC 7044, Cincinnati Children's Research Foundation, 3333 Burnet Ave., Cincinnati, OH 45229-3039, Tel: (513) 636-8624 (MTW); (513) 636-8622 (CVV), Fax: (513) 636-3912, charles.vorhees@cchmc.org; Michael.williams@cchmc.org.
[1]Current address: Department of Pharmacology, Vanderbilt University School of Medicine, 23rd Ave. South; 476 RRB, Nashville, TN 37232-6600 (DLG)
[2]Current address: Covance Laboratories, Inc., Discovery and Translational Service, 671 South Meridian Rd., Greenfield, IN 46140 (CEG).
[3]Current address: Div. of Psychiatry, Cincinnati Children's Research Foundation, Cincinnati, OH 45229

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Barren reared rats showed no or opposite effects of Mn, i.e., barren rearing + Mn attenuated corticosterone increases to acute stress. Barren rearing also altered the Mn-induced changes in dopamine and norepinephrine in the neostriatum, but not in the hippocampus. Barren rearing caused a Mn-associated increase in hypothalamic dopamine at P19 and P29 not seen in standard reared Mn-treated groups. Developmental Mn alters monoamines and corticosterone as a function of age, stress (acute and chronic), and sex.

Keywords

Manganese; barren cage rearing; corticosterone; dopamine; norepinephrine; serotonin; shallow water stress

Introduction

Manganese (Mn) is an essential nutrient for maintaining homeostasis (Aschner and Aschner, 2005). However, Mn over exposure (MnOE), most commonly seen in adults from occupational exposure, can produce symptoms similar to Parkinson's disease (manganism), especially motor deficits (Aschner and Aschner, 2005;Barceloux, 1999;Roth, 2009). Cognitive and other behavioral deficits also occur (Farina et al., 2013;Racette et al., 2012). This phenotype is seen in rodent models of MnOE as well. For instance, MnOE results in spatial working memory deficits and increases in compulsive behaviors in non-human primates (Schneider et al., 2006) and in spatial memory deficits in rodents in the Morris water maze (Blecharz-Klin et al., 2012). MnOE also has effects when it occurs during development (Erikson et al., 2007) that include deficits in executive function and passive avoidance (Tran et al., 2002b). Neonatal rats accumulate Mn more than similarly exposed adults because of lower excretion shortly after birth (postnatal day (P) 8–10) compared with later time points (P18–19); however, even P18–19 rats excrete Mn at lower rates than adults (Ballatori et al., 1987;Dorman et al., 2000;Garcia et al., 2006); this developmental pattern is mediated in part by reduced biliary excretion of Mn during the preweaning period (Kontur and Fechter, 1988;Fechter, 1999;Yoon et al., 2011). $^{54}MnCl_2$ tracer analysis in rats found that Mn uptake was highest in brain (with regional specificity), followed by liver and blood. Developmentally, the highest uptake is at P5 compared with other ages from 5 weeks to almost 2 years of age (Takeda et al., 1999). Physiologically-based pharmacokinetic modeling in rats verifies the above findings and the higher Mn uptake in the neonatal period is likely because of higher Mn requirements during rapid growth as seen during the preweaning period (Yoon et al., 2009). This leaves open the question of whether the same developmental mechanisms that permit greater Mn uptake for nutritional requirements may act to increase exposure when Mn levels are increased beyond what is nutritionally needed. Ingestion of excess Mn in children occurs for a number of reasons, including, but not limited to infant baby formulas or polluted air, soil, or well water. MnOE children show cognitive deficits, behavioral disinhibition, decreased IQ, and decreased performance on school-related tasks (Bouchard et al., 2011;Khan et al., 2011;Khan et al., 2012;Lucchini et al., 2012;Zhang et al., 1995).

Soy-based baby formula (Tran et al., 2002a;Tran et al., 2002b) can contain 5, 10, or more times the levels of Mn found in cow-based formulas and 100 times or more than found in human breast milk (Lonnerdal, 1994;Aschner and Aschner, 2005;Collipp et al., 1983). Unfortunately, one of the factors that makes soy-based formulas attractive is that they are often less expensive. Thus, children in lower socioeconomic status (SES) families are more likely to be fed soy-based formulas, and this is in addition to having a greater risk for exposure to stress because of the impoverished environments associated with lower SES. The combination of MnOE and stress during development may interact to create greater risk than either factor alone.

Chronic stress is a known risk factor to the developing nervous system. Children in low SES families have higher cortisol levels suggesting a heightened level of stress compared with children of higher SES strata (Cohen et al., 2006;Lupien et al., 2000;Lupien et al., 2001). During late gestation and early infancy humans go through a period of stress-induced adrenal quiescence. In rodents a similar period occurs, known as the stress hyporesponsive period (SHRP), that takes place from P4 to P14 in rats. During the SHRP, the adrenal response of the hypothalamic-pituitary-adrenal (HPA) axis is down-regulated resulting in lower circulating glucocorticoids to stressors. This period is hypothesized to be protective to defend the developing brain from excitotoxicity produced by stress-induced elevations in corticosteroids (De Kloet et al., 1988;Sapolsky and Meaney, 1986). Hence, the SHRP, while buffering the effects of stress has a finite capacity; chronic stress during this period may exceed this buffering capacity and result in adverse effects (Anisman et al., 1998;Carrion et al., 2007;Gos et al., 2008;Gruss et al., 2008;Lupien et al., 2011).

The purpose of this study was to test the hypothesis that chronic stress alters the effects of MnOE during neonatal development in rodents. Accordingly, we combined MnOE with a model of developmental stress already shown to result in long-term effects, i.e., the barren cage model that uses cages without normal bedding (Gilles et al., 1996). This cage condition was used to mimic aspects found in impoverished low SES human environments (Baum et al., 1999;Ivy et al., 2008;Rice et al., 2008). We previously used this model to assess developmental lead exposure in combination with barren cage rearing (Graham et al., 2011). Because the effects of chronic stressors are not reliably reflected in basal corticosterone levels, we also used an acute stressor (shallow water stress) to induce an acute stress response to test for differences in stress reactivity. The Mn-stress interaction exposure reported here is intended to be a model for future experiments on Mn in combination with other factors.

Materials and methods

Animals

All protocols were approved by the Institutional Animal Care and Use Committee. Animals were maintained in a AAALAC-accredited vivarium with regulated light cycles (14:10 h light:dark cycle, lights on at 600 h) and controlled temperature (19 ± 1°C) and humidity (50 ± 10%). Rats had access to NIH-07 rodent chow and reverse osmosis filtered, UV sterilized water provided *ad libitum*. The NIH-07 diet contains consistent levels of metals, minerals, and other nutrients, thus providing a consistent background nutritional formulation. Male

and nulliparous female Sprague-Dawley CD (IGS) rats (strain 001, Charles River Laboratories, Raleigh, NC) were bred following acclimation to the facility for a minimum of 1 week. The morning a sperm plug was found was designated embryonic day 0 (E0). On E1 females were transferred to polycarbonate cages (46 cm × 24 cm × 20 cm) with woodchip bedding containing a stainless steel semicircular enclosure as partial environmental enrichment (Vorhees et al., 2008). Birth was counted as P0. On P1, litters were culled to 12 pups (6 males and 6 females). If a litter had 10 or 11 pups, 1 or 2 pups from another litter with the same date of birth were fostered into the litter short of pups to achieve uniform litter sizes. The study design by litter was for 2 housing conditions x 3 assessment ages x 20 litters/cell = 120 litters. Litters were enrolled in the study on a rolling basis. Since litter outcomes could not be predicted at the time of enrollment, the number of litters actually enrolled was 124. Two litters were removed because the dams did not nurse their pups, hence 122 litters were used for data collection (Table 1). Mn treatment and acute stress (0 or 30 min in shallow water: Shallow Water Stress (SWS)) were within-litter factors (see below). Pups were individually identified by ear punch on P4.

Manganese over exposure and rearing

MnOE and differential rearing conditions were begun on P4. Rearing conditions (standard vs. barren) were adapted from (Gilles *et al.*, 1996) as described (Graham *et al.*, 2011). Briefly, the woodchip bedding was removed from cages in the barren condition and replaced with a paper towel, and the cages changed daily. Standard cages with bedding and enclosures were also changed daily to control for cage changing frequency. For MnOE, equal numbers of males and females per litter were randomly assigned (using a random number table) to vehicle or one of two Mn dose groups. Mn was given as Mn chloride tetrahydrate dissolved in distilled water (Sigma-Aldrich, St. Louis, MO). Within each litter, 4 pups (2 males and 2 females) were orally gavaged with 0, 50, or 100 mg/kg Mn in a volume of 3 ml/kg distilled water (VEH) using a 24-gauge gavage needle with ball tip. Doses were expressed as the free metal. Pups were gavaged to avoid Mn exposure to the dams and therefore prevent effects on maternal behavior. We showed (Graham et al., 2011) that gavaging by experienced personnel does not significantly alter plasma corticosterone levels when compared with non-gavaged pups. Mn was administered every other day from P4–28. Offspring were sacrificed at three different ages: P11, 19, and 29. For the group that continued to P29, weaning occurred on P28. On P28 pups were placed in standard cages in same sex pairs until sacrifice 24 h later. One male and one female pair per Mn group were euthanized by decapitation at each assessment age.

Acute Stress and Corticosterone Assessment

For the acute stressor, rats were placed in shallow water for 30 min (SWS) as described (Graham et al., 2011;Mineur et al., 2006;Mineur et al., 2003). SWS consisted of placing rats in a standard rat cage with room-temperature water filled to a depth of 2 cm on P11; 3 cm on P19, and 4 cm on P29. Some rats were euthanized immediately after removal from the water (time-0), while the remaining animals were placed back in their home cages and euthanized 30 or 60 min later. Litters not exposed to the SWS were used for baseline plasma corticosterone and Mn determinations, and brains were dissected for monoamine neurotransmitter assay. Blood was collected in 12 × 75 mm polyethylene tubes containing

0.05 ml of 2% EDTA, while an additional blood sample was taken from animals on P29 for Mn analysis. Corticosterone levels were determined from plasma using a commercially available EIA kit (Immunodiagnostic Systems Inc., Fountain Hills, AZ) as described (Graham et al., 2011).

Monoamines

Rats not exposed to SWS were used for neurotransmitter determinations via high performance liquid chromatography with electrochemical detection (HPLC-ECD) and neostriatal Mn concentrations. The neostriatum (caudate and putamen), hypothalamus, and hippocampus were dissected over ice using a 1 mm brain block (Grace et al., 2010) and rapidly frozen until analysis of monoamines was performed as described (Graham et al., 2011). Body weights were obtained from the same animals. Dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and norepinephrine (NE) were obtained from single chromatograms for each region per animal. Frozen tissues were weighed, thawed, and sonicated in appropriate volumes of 0.1 N perchloric acid (Fisher Scientific, Pittsburgh, PA). Samples were centrifuged for 14 min at 13,000 RCF at 4°C. The supernatant sample was transferred to a new vial for injection onto a Supelco Supelcosil™ LC-18 column (150 × 4.6 mm, 3 μm; Sigma-Aldrich Co., St. Louis, MO). The HPLC system consisted of a Waters 717plus autosampler (Waters Corp., Milford, MA), ESA 584 pump (ESA, Inc., Chelmsford, MA), and ESA Coulochem III electrochemical detector. The potential settings were −150 mV for E1 and +250 mV for E2, with a guard cell potential of +350 mV. MD-TM mobile phase (ESA, Inc.) was used and consisted of 75 mM sodium dihydrogen phosphate (monohydrate), 1.7 mM 1-octanesulfonic acid sodium salt, 100 μl/l triethylamine, 25 μM EDTA, and 10% acetonitrile, with a final pH of 3.0. The pump flow rate was set at 0.7 ml/min, and the samples were run at 28°C. Standards for DA, DOPAC, HVA, NE, 5-HT, and 5-HIAA (all obtained from Sigma-Aldrich Co., St. Louis, MO) were prepared in 0.1 N perchloric acid.

Mn concentrations

Rats from the P29 age group were used for serum and neostriatal Mn determination as described (Cowan et al., 2009). Neostriatal Mn concentrations were measured with graphite furnace atomic absorption spectrometry (GFFAAS, Varian AA240, Varian, Inc., Palo Alto, CA). Neostriata were digested in ultrapure nitric acid (1:10 wt/vol dilution) for 48 72 h in a sand bath (60ºC); 100 μl of digested tissue was brought to 1 ml of total volume with 2% nitric acid and analyzed for Mn. For serum, a 400-μl aliquot was vortexed with 100 μL of 0.5% Triton-X for 30 s and brought up to 1 ml of total volume with 2% nitric acid for analysis. The mixture was then centrifuged and the clear supernatant was used for analysis (100-μl aliquot brought up to a 1-ml volume with 2% nitric acid). A bovine liver (NBS Standard Reference Material, USDC, Washington, DC) (10 μg Mn/g) was digested in ultrapure nitric acid and used as an internal standard for analysis (final concentration 5 μg Mn/L).

Statistical analyses

All data, except weekly body weights and mortality, were analyzed using mixed linear factorial analysis of variance (ANOVA; Proc Mixed, SAS v9.2, SAS Institute, Cary, NC). Factors were Mn (0, 50, or 100 mg/kg), sex, rearing condition (Standard or Barren), age (P11, P19, or P29), and time following SWS (baseline, 0, 30, or 60 min). Litter was a randomized block factor in a completely randomized block design to account for litter effects. Significant interactions were followed-up using slice-effect ANOVAs. Body weights in the group euthanized on P29 were analyzed by general linear model ANOVA on even numbered days (Proc GLM, SAS). Where significant interactions occurred on body weight, they were further analyzed by slice-effect ANOVA and pairwise group comparisons using the False Discovery Rate (FDR) method to control for multiple comparisons. Mn exposure, day, and sex were within-subject factors in GLM analyses, while rearing condition was a between-subject factor. Mortality data were analyzed by Fisher's tests for uncorrelated proportions. Significance was set at $p \leq 0.05$. GLM data are presented as mean ± SEM, and Mixed data are presented as least square (LS) mean ± LS SEM.

Results

Mortality—Mortality data are shown in Table 1. Manganese at the high dose (Mn100) caused a significant increase in offspring mortality irrespective of rearing condition, i.e., both the Mn100 Standard and Mn100 Barren cage reared groups showed increased mortality (10.1 and 12.9%, respectively). The apparent 3% increase in mortality in the Barren Mn100 group was not significantly different from that in the Standard Mn100 group. There was an apparent difference in mortality as a function of rearing condition in the Mn50 groups inasmuch as the Standard cage reared Mn50 group had less mortality than the Barren Mn50 group (i.e., 5.6 vs. 9.6%) but the difference was not significant ($X^2(1) = 2.84$, $p>0.05$.

Body Weights—Because treatment was from P4–28, body weight data were analyzed during this period separately from body weights after MnOE. A Mn x sex x rearing condition x age ANOVA with age as a repeated measure, showed effects of Mn ($F(2,362) = 82.7$, $p<0.0001$), sex ($p<0.005$), day ($p<0.0001$), Mn x day ($F(12,2378) = 41.6$, $p<0.0001$), sex x day ($p<0.0001$), and rearing condition x day ($p<0.0001$). The Mn x day interaction was followed up with slice-effect ANOVAs on each day. In these analyses, the effect of Mn was significant on P8–28 (p's<0.001) but not on P4. Pairwise comparisons by FDR tests are summarized in Table 1. At P8 only the Mn100 group differed from control, whereas from P12–28 both Mn groups differed from VEH in both standard and barren cage reared rats. For all biochemical determinations, group sizes are summarized in figure captions.

Mn Concentrations—Rats treated with Mn (100 mg/kg) had significantly elevated levels of Mn in the neostriatum relative to VEH-treated rats ($F(1,23) = 230.3$, $p<0.0001$), i.e., VEH = 0.39 ± 0.12 μg/g vs. Mn100 = 2.39 ± 0.12 μg/g tissue. Serum Mn levels were somewhat elevated ($F(3,31) = 1.58$, $p<0.10$), i.e., VEH = 11.67 ± 4.75 μg/L vs. Mn100 = 16.62 ± 4.75 μg/L (note: SEMs are the same because they are LS SEMs).

Corticosterone—In the overall ANOVA, only one significant Mn-related effect was found, which was the Mn x rearing condition x age interaction ($F(4,1004) = 2.78$, $p < 0.05$). In order to sort this, ANOVAs were conducted for Mn for each age and rearing condition. At P11 there were no significant effects of Mn, rearing condition, or SWS (Fig. 1, top). At P19 for Standard housing, there were both Mn ($F(2,198) = 3.11$, $p < 0.05$) and Mn x SWS effects ($F(6,198) = 3.87$, $p < 0.01$). The Mn main effect was the result of increased corticosterone in the Mn-exposed groups (Fig. 1, middle, left inset). Slice ANOVAs on the interaction showed a Mn effect immediately upon removal from the SWS (time-0) in which the Mn-exposed groups showed increased corticosterone compared with VEH (Fig. 2, middle-left). At P19 for Barren housing, there was a significant main effect of Mn ($F(2,231) = 3.38$, $p < 0.05$), but no interactions. The main effect was attributable to reduced corticosterone in the barren Mn100 group, but note that this was against a higher pre-SWS in the Barren housing groups than in the Standard housing groups (Fig. 1, middle, right inset, and ***cf.* middle, left inset**). There was, at this age, a significant SWS main effect ($F(3,231) = 15.47$, $p < 0.0001$). At P29 for both Standard and Barren housing, there were no significant main effects or interactions with Mn. There were main effects of SWS (Standard $F(3,198) = 51.44$, $p < 0.0001$; Barren $F(3,176) = 50.58$, $p < 0.0001$) (Fig. 1, bottom).

Monoamines

Neostriatum—There were no significant effects of Mn or rearing condition on 5-HT in the neostriatum at any age (not shown). For DA, there was a significant Mn × rearing condition × age × sex interaction; ($F(4,220) = 2.45$, $p < 0.05$, Fig. 2A–D). Slice and FDR analyses revealed the effect to be in the female Standard housed Mn100 group (Fig. 2C) at P19 in which the Mn100 group showed increased DA compared with Standard housed VEH females at this age. No significant treatment effects were found on DOPAC. For neostriatal NE, there were significant Mn ($F(2,186) = 9.11$, $p < 0.001$) and Mn × sex × age effects ($F(4,186) = 5.3$, $p < 0.001$). Slice ANOVAs and pairwise comparisons showed the pattern illustrated in Fig. 3 in which there were increases in NE in female Mn50 and Mn100 groups at different ages irrespective of housing condition compared with VEH females. Similarly, there was a Mn main effect on HVA in the neostriatum ($F(2,209) = 5.59$, $p < 0.01$: Fig. 4) and a Mn × age × sex interaction ($F(4,209) = 3.28$, $p < 0.05$). When the latter effect was further analyzed, the effects occurred in the male Mn50 and Mn100 groups at P19 and in females in the Mn100 group at P29, both irrespective of rearing condition (Fig. 4, panels E and F in which rearing conditions were combined).

Hippocampus—Monoamine levels in the hippocampus were also affected by Mn and rearing condition (Fig. 5). A significant main effect of Mn ($F(2,129) = 3.43$, $p<0.05$) was evident for DA, such that Mn groups had increased DA compared with VEH (Fig. 5E); the main effect of rearing condition showed a trend toward increased DA across all Barren reared groups ($F(1,129) = 3.59$, $p\leq 0.06$). For hippocampal NE, there were significant Mn x age ($F(4,168) = 3.53$, $p < 0.01$: Fig. 6) and Mn × sex × age interactions ($F(4,168) = 2.46$, $p < 0.05$). Further analyses showed these to be predominantly expressed in males irrespective of rearing condition and occurred in the Mn50 group at P11 and in the Mn100 group at P29 (both were increases; Fig. 6E). Hippocampal 5-HT showed a Mn main effect ($F(2,171) = 11.33$, $p < 0.0001$: Fig. 7) and a Mn x age interaction ($F(4,171)=2.42$, $p < 0.05$). Further

analysis showed that the main effect was attributable to increased 5-HT in the Mn groups, whereas the Mn x age interaction showed the effect to be predominately on P29 (Fig. 7E). For 5-HIAA, the only effect was a Mn x age interaction which when further analyzed was attributable to reduced 5-HIAA in the Mn groups at P19 irrespective of sex or rearing condition (Fig. 7F).

Hypothalamus—Monoamines in the hypothalamus were altered (Fig. 8 and 9). For DA there was a 4-way interaction of Mn × sex × rearing condition × age ($F(4,206) = 2.4$, $p < 0.05$). When further analyzed, this interaction was attributable to DA increases in the barren-housed female Mn100 group at P19 and both Mn groups at P29 compared with VEH animals at those ages (Fig. 8D). There were no significant treatment effects found on DOPAC. For hypothalamic NE, there was also a 4-way interaction of Mn × sex × rearing condition × age ($F(4,216) = 3.03$, $p < 0.05$). In this case, further analysis showed increases in NE in standard-housed males at P29 in the Mn100 group and a trend in the Mn50 group (Fig. 9A) and a similar trend in the barren Mn100 females at this age (Fig. 9D). For HVA, there was a significant Mn × sex interaction ($F(2,123) = 3.33$, $p < 0.05$; Fig. 10) which when further analyzed was attributable to increased HVA in the Mn100 males compared with VEH males (Fig 10E). There were no significant Mn or rearing effects on hypothalamic 5-HT (Fig. 11A–E). A main effect of Mn was found for 5-HIAA ($F(2,213) = 3.75$, $p < 0.05$) in which the Mn groups had lower 5-HIAA levels than VEH animals irrespective of sex or housing condition (Fig. 11F).

As noted in Methods, litters 1 or 2 pups short of the 12 needed per litter had 1 or 2 pups in-fostered from litters born within 24 h of the litter that had too few born. Out of the 116 litters used for corticosterone and monoamine determinations, a total of 36 pups out of 1392 pups were in-fostered or 2.6%. Within the Standard housing condition a total of 22 pups were in-fostered out of 696 pups or 3.2%. Within the Barren housing condition a total of 14 pups were in-fostered out of 696 pups or 2.0%, making it unlikely that this proportionately small amount of in-fostering would significantly impact either the corticosterone or monoamine responses of the treatment groups.

Discussion

This experiment tested whether two dose levels of Mn during postnatal development under standard or barren cage rearing conditions altered corticosterone and brain monoamines at different developmental ages. This was based on the observation that excess Mn exposure during development is a significant problem in some areas in the U.S. and many other countries where industrial Mn pollution or well water naturally high in Mn result in MnOE-induced neurotoxicity. We included an environmental deprivation rearing condition (barren housing) to model chronic developmental stress because MnOE more often occurs in regions of lower SES, stress, and physical and social hardship. In order to test these conditions on the HPA axis we measured corticosterone before and after an acute stressor (standing in shallow water for 30 min).

Reduced body weight was found during treatment in both Mn-treated groups regardless of housing condition. The literature on developmental Mn exposure and body weight effects is

mixed. One study that exposed rats to Mn throughout gestation and lactation and, similar to the present experiment, found significant body weight reductions during treatment (Molina et al., 2011) as did a study giving Mn in drinking water to rats from P1–80 in which reduced body weight occurred in the high dose group but not in the mid or lower dose groups (Leung et al., 1982). In another study in rats treated with Mn by gavage, as we did, from P1–21, MnOE rats had reduced body weights at the two doses tested (25 and 50 mg/kg/day); their high dose being our low dose (Kern et al., 2010). There is also a report of prenatal Mn exposure causing reduced fetal weight (Sanchez et al., 1993). In contrast to these reports, there is one report of gestational and lactational Mn exposure in rats finding increased body weight in females during and three weeks after the end of treatment but no changes in males (Betharia and Maher, 2012). Several studies report no change in body weight resulting from preweaning Mn exposure: one found no change in body weight on P8 or P29 in rats from Mn exposure from P8–27, however, this study used doses lower than ours (Cordova et al., 2013). Another study found no change in body weight in rats at P21 after Mn exposure from P1–20, again at doses lower than ours (Tran et al., 2002a); and another study found no differences in body weight after P1–21 Mn exposure in rats long after exposure when the animals were adults, but report no data on body weight during treatment (Beaudin et al., 2013). There is also a study in rats using Mn that found no body weight differences in the offspring at P21 after prenatal-only exposure, also at doses below ours (5 mg/kg/day vs. our 50 mg/kg/2 days) (Chandra et al., 1983); and a study in rats using later Mn exposure starting at P21 that found no body weight differences (Chandra and Shukla, 1978). Somewhat surprisingly, there are also a number of developmental Mn studies that are silent concerning body weight. Four preweaning exposure studies in rats (Fitsanakis et al., 2009;Cordova et al., 2012;Kern and Smith, 2011;Tran et al., 2002b) and five using exposures that started on P21 (Anderson et al., 2008;Anderson et al., 2009;Fordahl et al., 2010;Ali et al., 1983b;Ali et al., 1983a) make no mention of body weight. It is difficult to draw conclusions from the above with so many studies not mentioning body weight. Several studies gave 25 mg/kg every day, essentially comparable to our 50 mg/kg every other day dose, and found body weight reductions during treatment and one study found increased body weight after gestational and lactational exposure, a finding difficult to reconcile with the overall pattern of reduced or no change in body weight in the majority of studies. In in a follow-up experiment using 100 mg/kg Mn/2 day we have replicated the body weight reduction seen here (unpublished observations), indicating that the present body weight changes are not a false positive result.

We found a modest increase in prenatal mortality associated with MnOE at the 100 mg/kg/2 day dose reared under standard housing conditions (10.1%) but not at 50 mg/kg/2 day. In barren cages, both doses increased mortality (9.6% and 12.9% in the 50 and 100 mg/kg/2 day doses, respectively). The latter is presumably the result of an interaction of Mn and stress on survival. Of all the studies reviewed above, most make no statement of morality, i.e., they fail to state that there was or was not any change. There is one report of increased mortality in rats associated with P21–81 Mn exposure (Chandra and Shukla, 1978), and one report of increased resorptions from prenatal Mn exposure (Sanchez et al., 1993). Interestingly, there is one human epidemiological study showing a significant association between infant mortality and Mn ground water concentrations across the state of North

Carolina (Spangler and Spangler, 2009). It is difficult to interpret the present mortality data in light of the silence of other reports on this point.

Neither Mn nor barren cage rearing altered baseline corticosterone at the ages tested, but immediately after the acute SWS stressor exposure, standard housed Mn groups showed an exaggerated increase in corticosterone on P19. This response was absent in Mn exposed groups raised in barren housing, suggesting that chronic stress attenuates the normal acute stress response at this age. In terms of rearing condition alone, housing produced only a trend main effect ($F(1,1004) = 2.87$, $p \leq 0.09$) but it modified the corticosterone response to acute stress. This influence appeared on P19 also in which Barren housed rats showed increased corticosterone after acute stress compared with Standard housed controls. This change was different when Mn effects were overlaid on this pattern. Barren housing suppressed the corticosterone increase caused by Mn at P19. At P29, where no Mn effects on corticosterone were observed, there was a large effect of housing in which Barren housed animals showed a larger response to acute stress at time-0 as reflected in a 3-way interaction of housing x age x time ($F(6,1004) = 4.16$, $p < 0.001$).

Housing had no main effects on neostriatal, hippocampal, or hypothalamic monoamines or their principal metabolites, although it was an interacting factor with Mn at some ages. These interactions with Mn were complex as they were age-specific and in some cases both age and sex-specific. However, some common threads may be discerned. The most apparent of the effects of Mn on neurotransmitters was that Mn, in nearly every case where it had significant effects, increased NE, DA, and 5-HT. In no case, did Mn exposure decrease monoamine neurotransmitters. Moreover, where changes in the metabolites HVA or 5-HIAA were seen, they were decreased in one or both of the Mn exposed groups.

What is more difficult to ascertain was the age by region effects of Mn exposure. Overall, P11 showed the fewest effects; only one P11 effect was seen (P11 Mn50 increase in hippocampal NE). It seems likely that since Mn exposure began on P4, and if the effects of Mn are cumulative, P11 may have been too soon after exposure to show many effects. In terms of number of effects, P19 appeared to be the most sensitive; most of the effects found were at this age. P29 showed an intermediate number of effects, the most striking of which were Mn-induced increases in hippocampal NE and hypothalamic NE and DA. While these effects were not always dose-dependent, in general the Mn100 groups showed more effects than the Mn50 groups. Table 2 shows a summary of the pattern of monoamine changes.

Two other studies from the same laboratory measured striatal DA concentrations in rats after developmental Mn exposure. Rats were exposed to Mn by gavage from P1–21 and reduced striatal DA was found, on P40, 19 days after the end of treatment (Tran et al., 2002a) and on P65, 41 days after the end of treatment (Tran et al., 2002b). While these findings contrast with ours, Tran et al.'s findings were long after treatment whereas ours were during treatment. Two studies, from the same group, measured DA D1 and D2 receptors and DA transporter (DAT) in frontal cortex, striatum, and nucleus accumbens (Kern et al., 2010;Kern and Smith, 2011). Data for rats at two different ages are reported, P24 and P107 after P1–21 Mn exposure to 25 or 50 mg/kg/day. The P24 data are the same in both reports. Effects were varied by brain region, age and outcome. D1 levels were unchanged in frontal

cortex, decreased in striatum at P24 but not at P107, and decreased in nucleus accumbens at 50 mg/kg at P24 but only in the 25 mg/kg group at P107. D2 levels were increased in frontal cortex in the 50 mg/kg group at both ages, whereas D2 was unchanged at both ages and at both doses in the striatum and nucleus accumbens. DAT levels were unchanged in frontal cortex, and reduced in the 50 mg/kg group at P24 but not at P107. These DA-related markers suggest a complex pattern of decreases in D1 and DAT in basal ganglia with opposite increases in D2 in frontal cortex. Another study in rats looked at postsynaptic markers of monoaminergic signaling, (Cordova et al., 2012), including striatal ERK1/2, AKT, and DARPP32 phosphorylation, after short-term, P8–12, exposure to Mn by i.p. injection and these parameters were measured 48 h later on P14. They used three doses of Mn (5, 10, and 20 mg/kg) and found increased pERK1/2 at 20 mg/kg Mn, increased pAKT at 10 and 20 mg/kg, and increased pDARPP32 at 5 and 10 mg/kg. While not specifically DA-related, these striatal markers all overlap with dopaminergic signaling, suggesting again that developmental Mn exposure affects striatal DA pathways. But DA is not the only neurotransmitter reported to be affected by developmental Mn exposure. Two studies report changes related to GABA (Anderson et al., 2008;Fordahl et al., 2010) when Mn exposure started on P21. One found that Mn reduced GABA release in striatum following nipecotic acid-induced release. The other found that Mn exposure reduced hippocampal glutamic acid transaminase (GAT-1), increased $GABA_A$ protein, and reduced $GABA_B$ mRNA expression. We also found an increase in hippocampal 5-HT which no one else has examined. While most of the reported effects of developmental Mn suggest decreased DA-related markers, these findings are mostly found long after Mn exposure whereas we measured during exposure. Taken together with data from these other studies, Mn can induce neurotransmitter changes, but these changes are likely specific to the timing of the exposure and when the neurotransmitters are assessed. Takeda et al. (1999) found that the deposition of Mn in the brain was dependent on the age of exposure which suggests that the effects of Mn during different exposure periods may differ. It will be necessary to determine if increases we observed change after exposure has ended.

The results support the general notion that developmental Mn exposure causes brain monoamine changes. How long these changes persist is unknown, as are whether they result in functional changes to neurobehavior. It may be that neuroplastic compensatory processes occur such that after a recovery period neurotransmitters return to control levels or even decrease. Alternatively, these early changes may result in enduring functional changes as others have found with developmental MnOE (Schneider et al., 2006;Blecharz-Klin et al., 2012;Tran et al., 2002b), i.e., that while the level of a neurotransmitter may change following treatment there may be downstream, enduring changes to receptors, second messengers, or modulators that result in neurobehavioral changes (see above). Functional changes to behavior and cognitive development may be present either during or after Mn exposure and will require further experiments to determine if this is the case.

We found few interactions between the chronic stress of barren housing and Mn exposure except on corticosterone. For monoamines, barren housing caused no effects in and of itself. However, chronic developmental stress has been shown to affect brain and behavior in other studies (Anisman et al., 1998;Gruss et al., 2008;Lupien et al., 2011;Carrion et al., 2007;Gos et al., 2008). There is a critical period for neonatal stress that results in altered behavior,

reduced LTP, and lower spine density in cortical layer 5 and anterior cingulate compared with non-stressed animals (Anisman et al., 1998;Gruss et al., 2008;Gos et al., 2008) whereas in humans increased amygdala size is reported after chronic early stress (Lupien et al., 2011).

Limitations of the present experiment include that only two doses of Mn were assessed and only one period of developmental exposure was used (P4–28). It may be that other doses or other developmental exposure periods, perhaps including prenatal exposure, would produce different effects. Only monoamine neurotransmitters were assessed and in only three brain regions. Mn may affect other neurotransmitter, neurotrophins, receptors, transporters, or morphology that were not examined here. As noted above, this experiment did not include assessments of the permanence of the changes observed or test for their effects on cognitive or other behavioral functions. We fostered 1–2 pups into litters short 1 or 2 pups; across the study this amounted to 2.6% of pups in-fostered, a proportion unlikely to impact the findings (see (Maccari et al., 1995)). It is also worth mentioning that rats were weaned on P28 and the last samples were taken on P29, only 24 h post-weaning which could conceivably be an added stressor. A comparison of the P19 baseline levels of corticosterone and the P29 baseline levels in controls shows that corticosterone levels were lower on P29 than on P19, suggesting that weaning was not a stressor. Despite limitations, the data demonstrate that developmental Mn alters brain neurotransmitters in several brain regions important for behavior and the effects were age- and sex-dependent. The data suggest that developmental Mn exposure should be investigated further for possible long-term effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
DA	dopamine
NE	norepinephrine
Mn	manganese
DOPAC	dihydroxyphenylacetic acid
HVA	homovanillic acid
5-HIAA	5-hydroxyindolacetic acid
SWS	shallow water stress

References

Ali MM, Murthy RC, Saxena DK, Chandra SV. Effect of low protein diet on manganese neurotoxicity: II. Brain GABA and seizure susceptibility. Neurobehav Toxicol Teratol. 1983a; 5:385–389. [PubMed: 6410294]

Ali MM, Murthy RC, Saxena DK, Srivastava RS, Chandra SV. Effect of low protein diet on manganese neurotoxicity: I. Developmental and biochemical changes. Neurobehav Toxicol Teratol. 1983b; 5:377–383. [PubMed: 6410293]

Anderson JG, Fordahl SC, Cooney PT, Weaver TL, Colyer CL, Erikson KM. Manganese exposure alters extracellular GABA, GABA receptor and transporter protein and mRNA levels in the developing rat brain. Neurotoxicology. 2008; 29:1044–1053. [PubMed: 18771689]

Anderson JG, Fordahl SC, Cooney PT, Weaver TL, Colyer CL, Erikson KM. Extracellular norepinephrine, norepinephrine receptor and transporter protein and mRNA levels are differentially altered in the developing rat brain due to dietary iron deficiency and manganese exposure. Brain Res. 2009; 1281:1–14. [PubMed: 19481535]

Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci. 1998; 16:149–164. [PubMed: 9785112]

Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. Mol Aspects Med. 2005; 26:353–362. [PubMed: 16099026]

Ballatori N, Miles E, Clarkson TW. Homeostatic control of manganese excretion in the neonatal rat. Am J Physiol. 1987; 252:R842–R847. [PubMed: 3578551]

Barceloux DG. Manganese. J Toxicol Clin Toxicol. 1999; 37:293–307. [PubMed: 10382563]

Baum A, Garofalo JP, Yali AM. Socioeconomic status and chronic stress. Does stress account for SES effects on health? Ann N Y Acad Sci. 1999; 896:131–144. [PubMed: 10681894]

Beaudin SA, Nisam S, Smith DR. Early life versus lifelong oral manganese exposure differently impairs skilled forelimb performance in adult rats. Neurotoxicol Teratol. 2013; 38:36–45. [PubMed: 23623961]

Betharia S, Maher TJ. Neurobehavioral effects of lead and manganese individually and in combination in developmentally exposed rats. Neurotoxicology. 2012; 33:1117–1127. [PubMed: 22732189]

Blecharz-Klin K, Piechal A, Joniec-Maciejak I, Pyrzanowska J, Widy-Tyszkiewicz E. Effect of intranasal manganese administration on neurotransmission and spatial learning in rats. Toxicol Appl Pharmacol. 2012; 265:1–9. [PubMed: 23022103]

Bouchard MF, Sauve S, Barbeau B, Legrand M, Brodeur ME, Bouffard T, Limoges E, Bellinger DC, Mergler D. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ Health Perspect. 2011; 119:138–143. [PubMed: 20855239]

Carrion VG, Weems CF, Reiss AL. Stress predicts brain changes in children: a pilot longitudinal study on youth stress, posttraumatic stress disorder, and the hippocampus. Pediatrics. 2007; 119:509–516. [PubMed: 17332204]

Chandra SV, Murthy RC, Saxena DK, Lal B. Effects of pre- and postnatal combined exposure to Pb and Mn on brain development in rats. Ind Health. 1983; 21:273–279. [PubMed: 6197396]

Chandra SV, Shukla GS. Manganese encephalopathy in growing rats. Environ Res. 1978; 15:28–37. [PubMed: 620661]

Cohen S, Doyle WJ, Baum A. Socioeconomic status is associated with stress hormones. Psychosom Med. 2006; 68:414–420. [PubMed: 16738073]

Collipp PJ, Chen SY, Maitinsky S. Manganese in infant formulas and learning disability. Ann Nutr Metab. 1983; 27:488–494. [PubMed: 6651226]

Cordova FM, Aguiar AS Jr, Peres TV, Lopes MW, Goncalves FM, Pedro DZ, Lopes SC, Pilati C, Prediger RD, Farina M, Erikson KM, Aschner M, Leal RB. Manganese-exposed developing rats display motor deficits and striatal oxidative stress that are reversed by Trolox. Arch Toxicol. 2013; 87:1231–1244. [PubMed: 23385959]

Cordova FM, Aguiar AS Jr, Peres TV, Lopes MW, Goncalves FM, Remor AP, Lopes SC, Pilati C, Latini AS, Prediger RD, Erikson KM, Aschner M, Leal RB. In vivo manganese exposure modulates Erk, Akt and Darpp-32 in the striatum of developing rats, and impairs their motor function. PLoS ONE. 2012; 7:e33057. [PubMed: 22427945]

Cowan DM, Fan Q, Zou Y, Shi X, Chen J, Aschner M, Rosenthal FS, Zheng W. Manganese exposure among smelting workers: blood manganese-iron ratio as a novel tool for manganese exposure assessment. Biomarkers. 2009; 14:3–16. [PubMed: 19283519]

De Kloet ER, Rosenfeld P, Van Eekelen JA, Sutanto W, Levine S. Stress, glucocorticoids and development. Prog Brain Res. 1988; 73:101–120. [PubMed: 3047791]

Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J, Miller R. Neurotoxicity of manganese chloride in neonatal and adult CD rats following subchronic (21-day) high-dose oral exposure. J Appl Toxicol. 2000; 20:179–187. [PubMed: 10797470]

Erikson KM, Thompson K, Aschner J, Aschner M. Manganese neurotoxicity: a focus on the neonate. Pharmacol Ther. 2007; 113:369–377. [PubMed: 17084903]

Farina M, Avila DS, da Rocha JB, Aschner M. Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury. Neurochem Int. 2013; 62:575–594. [PubMed: 23266600]

Fechter LD. Distribution of manganese in development. Neurotoxicology. 1999; 20:197–201. [PubMed: 10385883]

Fitsanakis VA, Thompson KN, Deery SE, Milatovic D, Shihabi ZK, Erikson KM, Brown RW, Aschner M. A chronic iron-deficient/high-manganese diet in rodents results in increased brain oxidative stress and behavioral deficits in the morris water maze. Neurotox Res. 2009; 15:167–178. [PubMed: 19384579]

Fordahl SC, Anderson JG, Cooney PT, Weaver TL, Colyer CL, Erikson KM. Manganese exposure inhibits the clearance of extracellular GABA and influences taurine homeostasis in the striatum of developing rats. Neurotoxicology. 2010; 31:639–646. [PubMed: 20832424]

Garcia SJ, Gellein K, Syversen T, Aschner M. A manganese-enhanced diet alters brain metals and transporters in the developing rat. Toxicol Sci. 2006; 92:516–525. [PubMed: 16705042]

Gilles EE, Schultz L, Baram TZ. Abnormal corticosterone regulation in an immature rat model of continuous chronic stress. Pediatr Neurol. 1996; 15:114–119. [PubMed: 8888044]

Gos T, Bock J, Poeggel G, Braun K. Stress-induced synaptic changes in the rat anterior cingulate cortex are dependent on endocrine developmental time windows. Synapse. 2008; 62:229–232. [PubMed: 18088062]

Grace CE, Schaefer TL, Graham DL, Skelton MR, Williams MT, Vorhees CV. Effects of inhibiting neonatal methamphetamine-induced corticosterone release in rats by adrenal autotransplantation on later learning, memory, and plasma corticosterone levels. Int J Dev Neurosci. 2010

Graham DL, Grace CE, Braun AA, Schaefer TL, Skelton MR, Tang PH, Vorhees CV, Williams MT. Effects of developmental stress and lead (Pb) on corticosterone after chronic and acute stress, brain monoamines, and blood Pb levels in rats. Int J Dev Neurosci. 2011; 29:45–55. [PubMed: 20920575]

Gruss M, Braun K, Frey JU, Korz V. Maternal separation during a specific postnatal time window prevents reinforcement of hippocampal long-term potentiation in adolescent rats. Neuroscience. 2008; 152:1–7. [PubMed: 18255235]

Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. Neuroscience. 2008; 154:1132–1142. [PubMed: 18501521]

Kern CH, Smith DR. Preweaning Mn exposure leads to prolonged astrocyte activation and lasting effects on the dopaminergic system in adult male rats. Synapse. 2011; 65:532–544. [PubMed: 20963817]

Kern CH, Stanwood GD, Smith DR. Preweaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels. Synapse. 2010; 64:363–378. [PubMed: 20029834]

Khan K, Factor-Litvak P, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, Levy D, Mey J, van GA, Graziano JH. Manganese exposure from drinking water and children's classroom behavior in Bangladesh. Environ Health Perspect. 2011; 119:1501–1506. [PubMed: 21493178]

Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, Levy D, Mey J, van GA, Graziano JH, Factor-Litvak P. Manganese exposure from drinking water and children's academic achievement. Neurotoxicology. 2012; 33:91–97. [PubMed: 22182530]

Kontur PJ, Fechter LD. Brain regional manganese levels and monoamine metabolism in manganese-treated neonatal rats. Neurotoxicol Teratol. 1988; 10:295–303. [PubMed: 3226372]

Leung TK, Lai JC, Lim L. The effects of chronic manganese feeding on the activity of monoamine oxidase in various organs of the developing rat. Comp Biochem Physiol C. 1982; 71:223–228. [PubMed: 6122537]

Lonnerdal B. Nutritional aspects of soy formula. Acta Paediatr Suppl. 1994; 402:105–108. [PubMed: 7841612]

Lucchini RG, Guazzetti S, Zoni S, Donna F, Peter S, Zacco A, Salmistraro M, Bontempi E, Zimmerman NJ, Smith DR. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. Neurotoxicology. 2012; 33:687–696. [PubMed: 22322213]

Lupien SJ, King S, Meaney MJ, McEwen BS. Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. Biol Psychiatry. 2000; 48:976–980. [PubMed: 11082471]

Lupien SJ, King S, Meaney MJ, McEwen BS. Can poverty get under your skin? Basal cortisol levels and cognitive function in children from low and high socioeconomic status. Dev Psychopathol. 2001; 13:653–676. [PubMed: 11523853]

Lupien SJ, Parent S, Evans AC, Tremblay RE, Zelazo PD, Corbo V, Pruessner JC, Seguin JR. Larger amygdala but no change in hippocampal volume in 10-year-old children exposed to maternal depressive symptomatology since birth. Proc Natl Acad Sci U S A. 2011; 108:14324–14329. [PubMed: 21844357]

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le MM. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. J Neurosci. 1995; 15:110–116. [PubMed: 7823121]

Mineur YS, Belzung C, Crusio WE. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav Brain Res. 2006; 175:43–50. [PubMed: 17023061]

Mineur YS, Prasol DJ, Belzung C, Crusio WE. Agonistic behavior and unpredictable chronic mild stress in mice. Behav Genet. 2003; 33:513–519. [PubMed: 14574128]

Molina RM, Phattanarudee S, Kim J, Thompson K, Wessling-Resnick M, Maher TJ, Brain JD. Ingestion of Mn and Pb by rats during and after pregnancy alters iron metabolism and behavior in offspring. Neurotoxicology. 2011; 32:413–422. [PubMed: 21458486]

Racette BA, Aschner M, Guilarte TR, Dydak U, Criswell SR, Zheng W. Pathophysiology of manganese-associated neurotoxicity. Neurotoxicology. 2012; 33:881–886. [PubMed: 22202748]

Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology. 2008; 149:4892–4900. [PubMed: 18566122]

Roth JA. Are there common biochemical and molecular mechanisms controlling manganism and parkisonism. Neuromolecular Med. 2009; 11:281–296. [PubMed: 19757210]

Sanchez DJ, Domingo JL, Llobet JM, Keen CL. Maternal and developmental toxicity of manganese in the mouse. Toxicol Lett. 1993; 69:45–52. [PubMed: 8356567]

Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: Neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Research Reviews. 1986; 11:65–76.

Schneider JS, Decamp E, Koser AJ, Fritz S, Gonczi H, Syversen T, Guilarte TR. Effects of chronic manganese exposure on cognitive and motor functioning in non-human primates. Brain Res. 2006; 1118:222–231. [PubMed: 16978592]

Spangler AH, Spangler JG. Groundwater manganese and infant mortality rate by county in North Carolina: an ecological analysis. Ecohealth. 2009; 6:596–600. [PubMed: 20232227]

Takeda A, Ishiwatari S, Okada S. Manganese uptake into rat brain during development and aging. J Neurosci Res. 1999; 56:93–98. [PubMed: 10213480]

Tran TT, Chowanadisai W, Crinella FM, Chicz-Demet A, Lonnerdal B. Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels, and neurodevelopmental status. Neurotoxicology. 2002a; 23:635–643. [PubMed: 12428735]

Tran TT, Chowanadisai W, Lonnerdal B, Le L, Parker M, Chicz-Demet A, Crinella FM. Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. Neurotoxicology. 2002b; 23:645–651. [PubMed: 12428736]

Vorhees CV, Herring NR, Schaefer TL, Grace CE, Skelton MR, Johnson HL, Williams MT. Effects of neonatal (+)-methamphetamine on path integration and spatial learning in rats: effects of dose and rearing conditions. Int J Dev Neurosci. 2008; 26:599–610. [PubMed: 18502078]

Yoon M, Nong A, Clewell HJ III, Taylor MD, Dorman DC, Andersen ME. Lactational transfer of manganese in rats: predicting manganese tissue concentration in the dam and pups from inhalation exposure with a pharmacokinetic model. Toxicol Sci. 2009; 112:23–43. [PubMed: 19726576]

Yoon M, Schroeter JD, Nong A, Taylor MD, Dorman DC, Andersen ME, Clewell HJ III. Physiologically based pharmacokinetic modeling of fetal and neonatal manganese exposure in humans: describing manganese homeostasis during development. Toxicol Sci. 2011; 122:297–316. [PubMed: 21622944]

Zhang G, Liu D, He P. Effects of manganese on learning abilities in school children. Zhonghua Yu Fang Yi Xue Za Zhi. 1995; 29:156–158. [PubMed: 7648952]

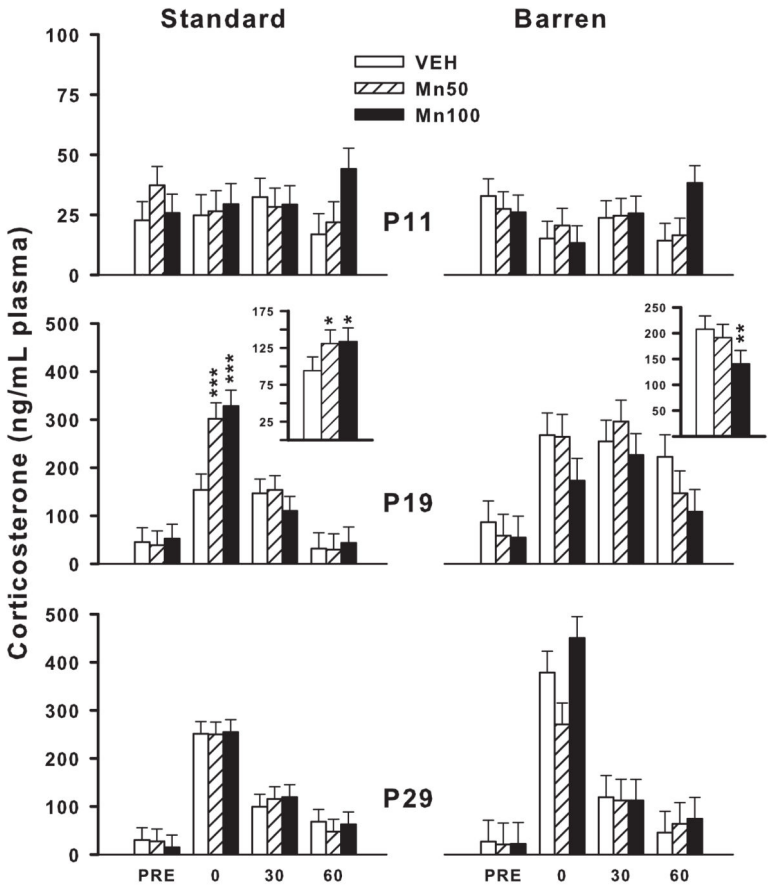

Fig. 1. Plasma corticosterone

Plasma corticosterone prior to, immediately after (time-0), or 30 or 60 min after removal from acute stressor (SWS) as a function of rearing condition and Mn exposure; see text for details. Top, corticosterone on P11; Middle, corticosterone on P19; Bottom, corticosterone on P29. In each panel, corticosterone for rats reared in standard bedding is shown on the left and for those reared in barren cages is shown on the right. Insets on P19 represent main effects averaged across time of sacrifice. Group sizes were: number of litters (number of pups): Std. P11 = 24 (264), Std. P19 = 17 (192), Std. P29 = 24 (288), Barren P11 = 21 (237), Barren P19 = 20 (212), Barren P29 = 16 (192). For each Housing x Treatment x Age x Acute Stress N = 5–12 per cell. Values are mean ± SEM. *P < 0.05; **P < 0.01.

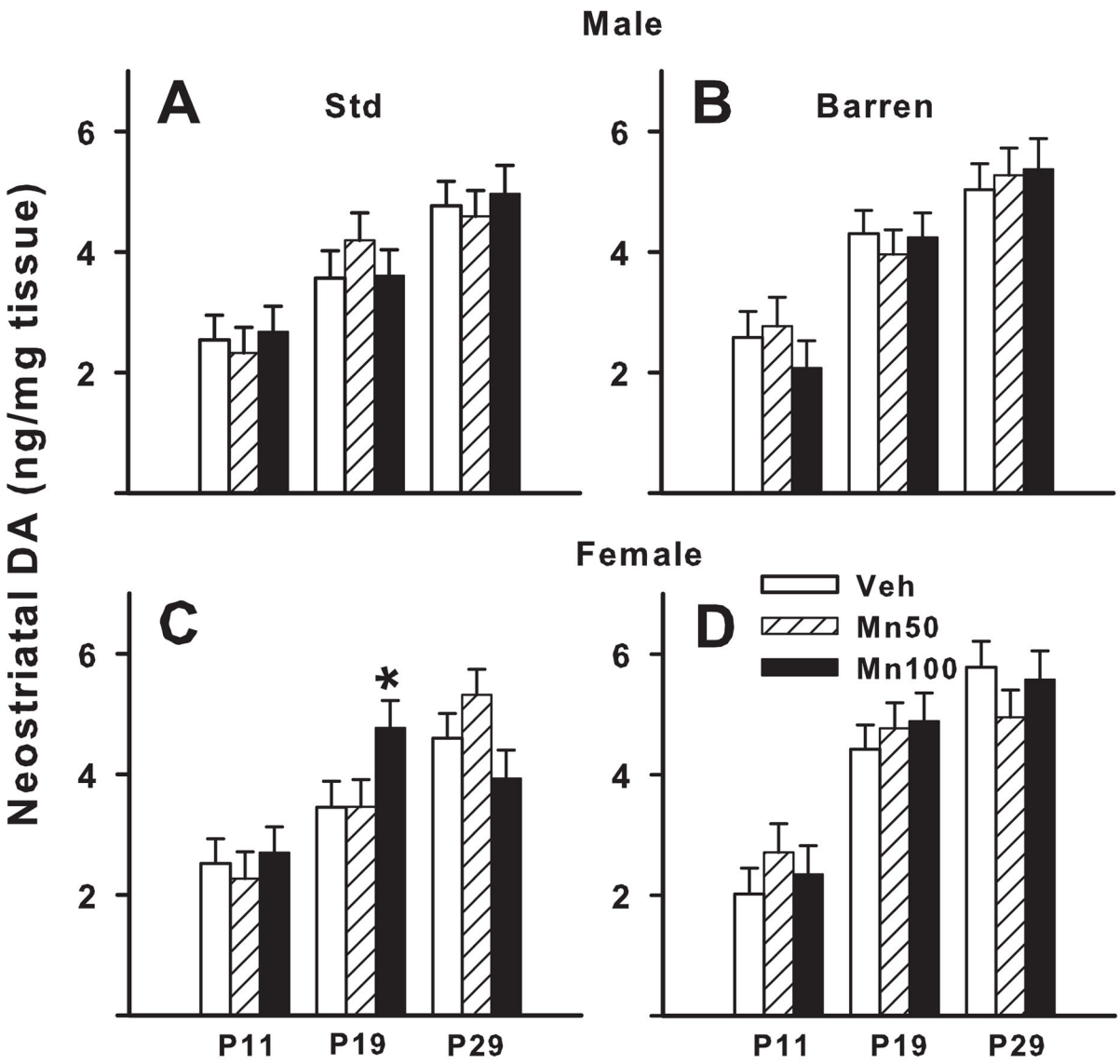

Fig. 2. Neostriatal dopamine (DA)

DA concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. Group sizes for this and all remaining figures are as follows: P11, Std. housing (M/F): Saline = 5–10/4–10; Mn50 = 5–9/4–9; Mn100 = 5–9/6–9; Barren housing (M/F): Saline = 6–10/5–9; Mn50 = 3–8/4–8; Mn100 = 4–9/4–8; P19, Std. housing (M/F): Saline = 5–9/6–9; Mn50 = 4–8/6–8; Mn100 = 5–9/6–8; Barren housing: Saline = 6–11/5–10; Mn50 = 7–10/6–9; Mn100 = 4–10/4–7; P29, Std. housing: Saline = 4–10/2–10; Mn50 = 3–9/5–9; Mn100 = 2–7/4–7; Barren housing: Saline = 4–9/3–9; Mn50 = 5–9/4–8; Mn100 = 4–6/3–8. Values are mean ± SEM. *P < 0.05.

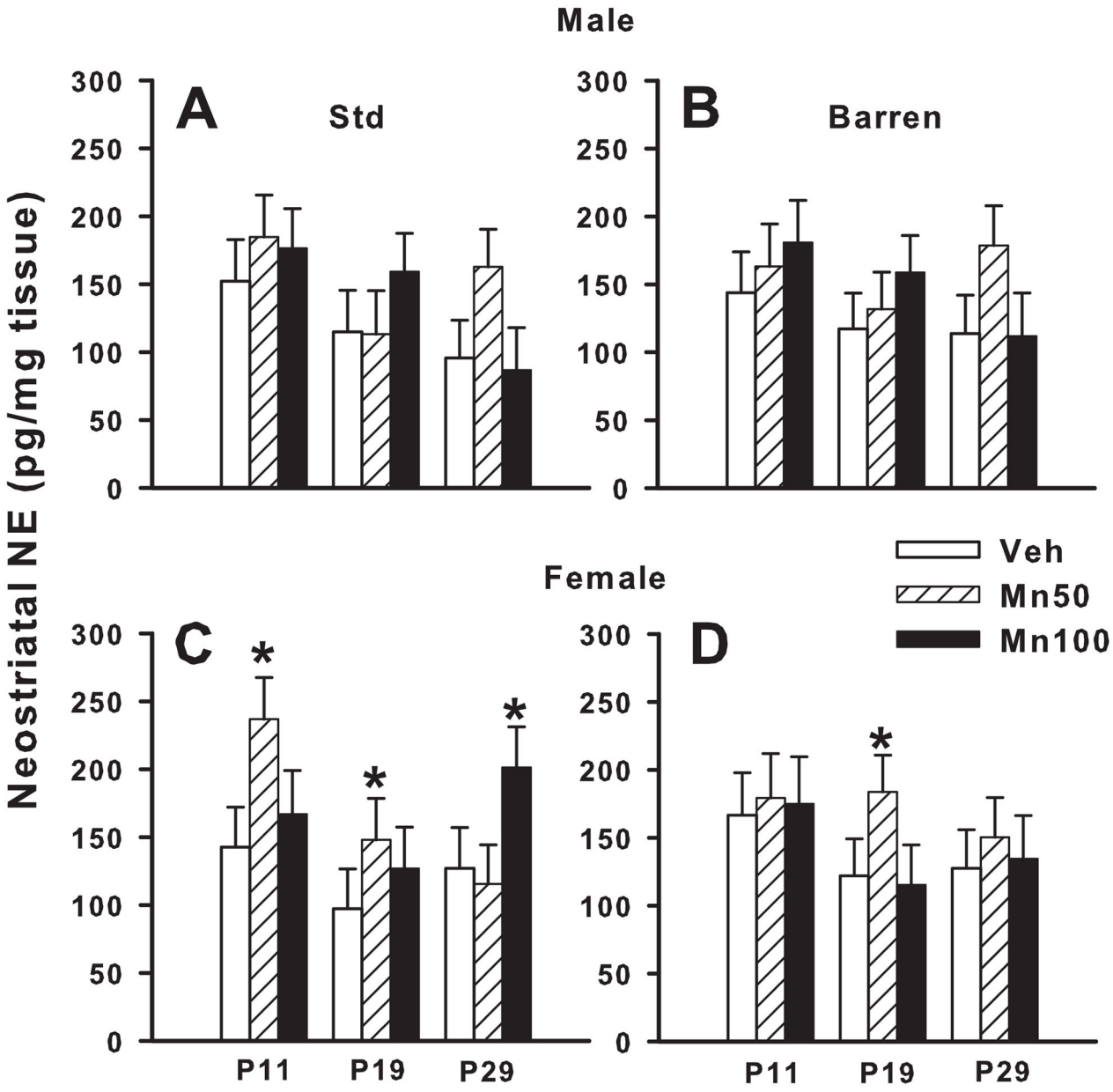

Fig. 3. Neostriatal norepinephrine (NE)

NE concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05. Note: the asterisk on the Mn50 P19 females is for the mean effect across housing condition.

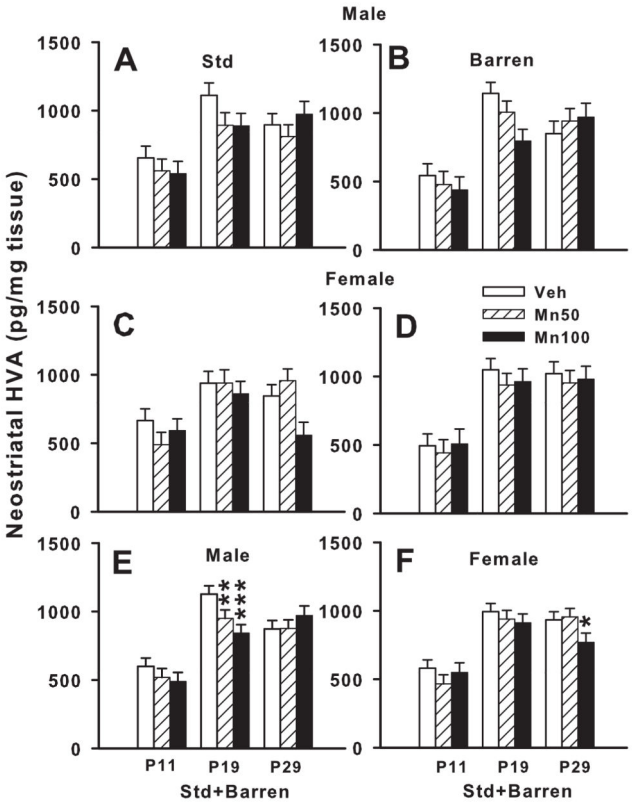

Fig. 4. Neostriatal homovanillic acid (HVA)

HVA concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. ***E***, effects in males averaged across housing condition; ***F***, effects in females averaged across housing condition. Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

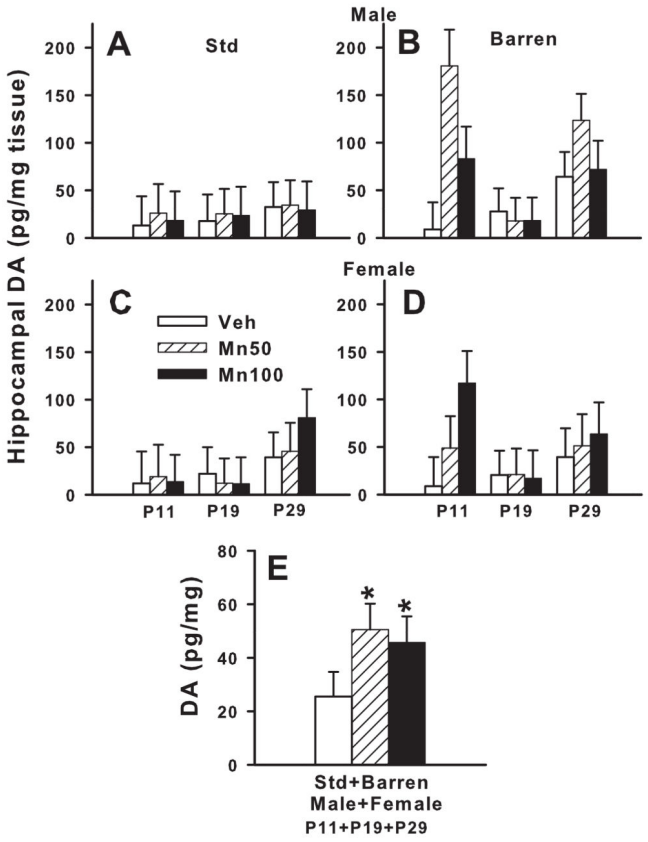

Fig. 5. Hippocampal dopamine (DA) and main effect norepinephrine (NE)

DA concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. ***E***, NE averaged across sex, housing condition, and age to show main effect of Mn (see Fig. 7 for hippocampal NE in greater detail). Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05.

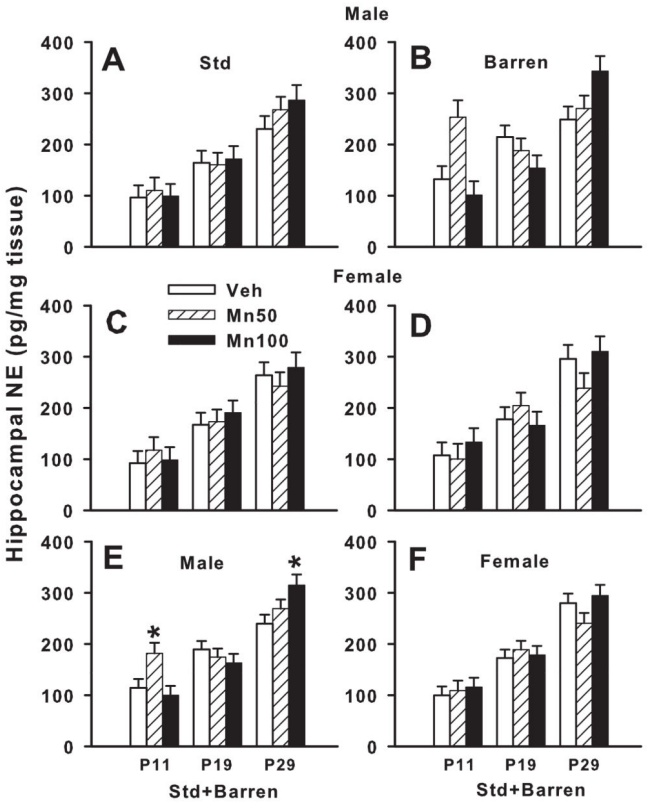

Fig. 6. Hippocampal norepinephrine (NE)

NE concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. ***E,F***, effects averaged across housing to show Mn effects on males (E) and females (F). Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05.

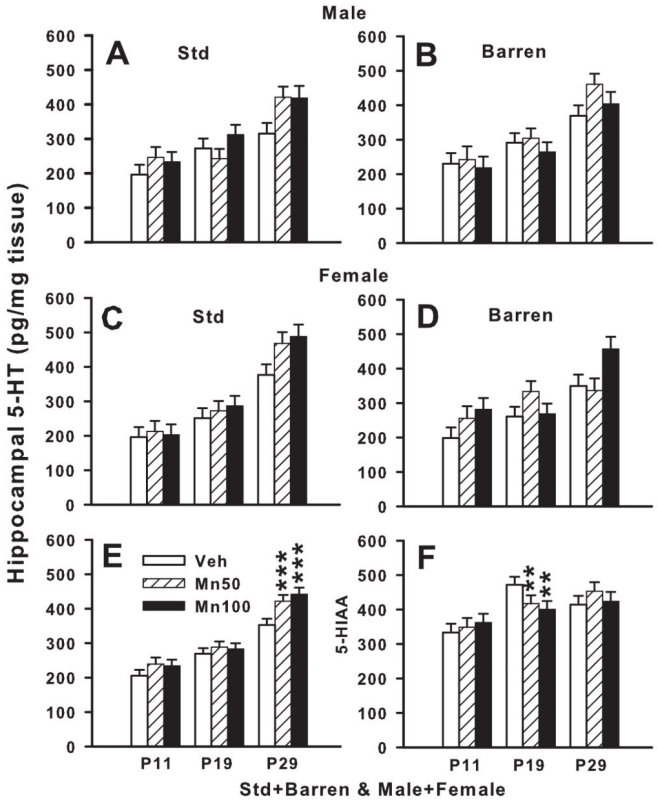

Fig. 7. Hippocampal serotonin (5HT)

5HT concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. ***E, F***, Main effect of Mn on 5-HT (***E***) and 5-HIAA (***F***) across housing condition and sex. Range of group sizes shown in Table 3. Values are mean ± SEM. **P < 0.01; ***P < 0.001.

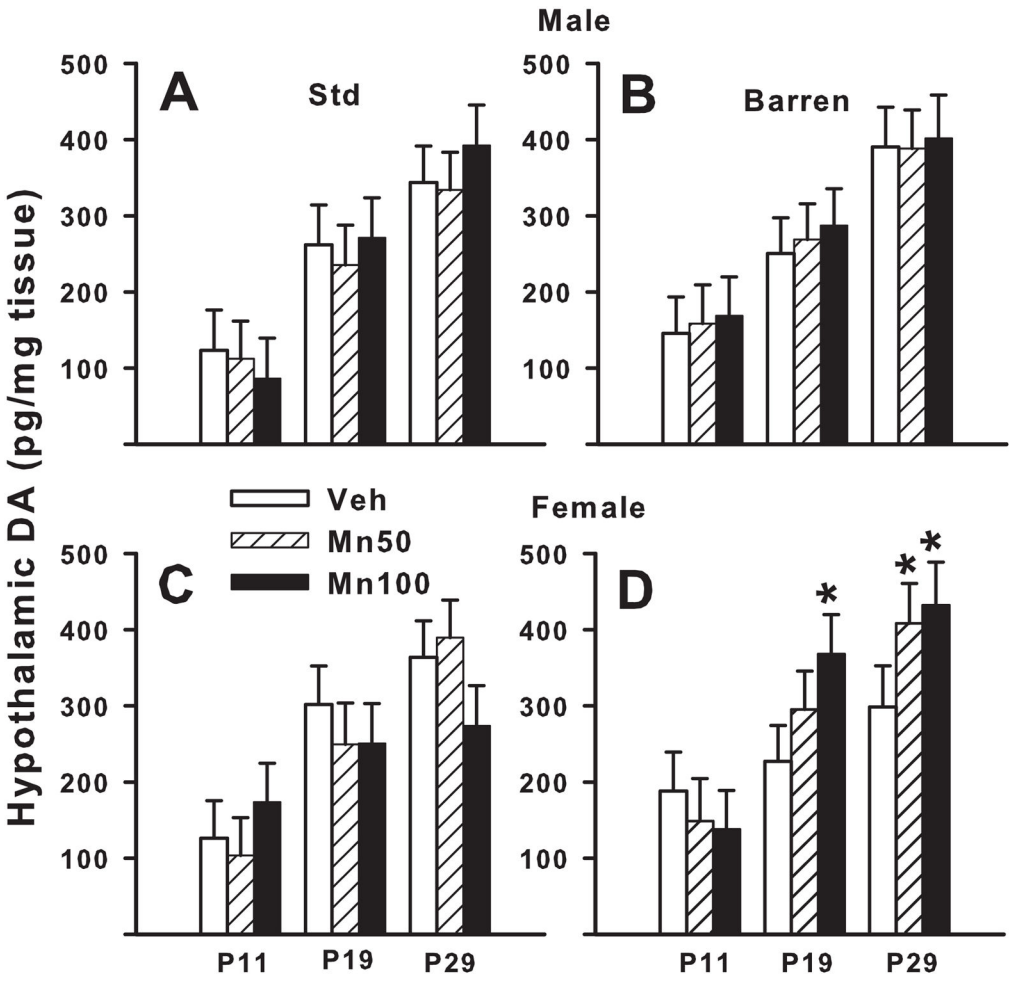

Fig. 8. Hypothalamic dopamine (DA)

DA concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05.

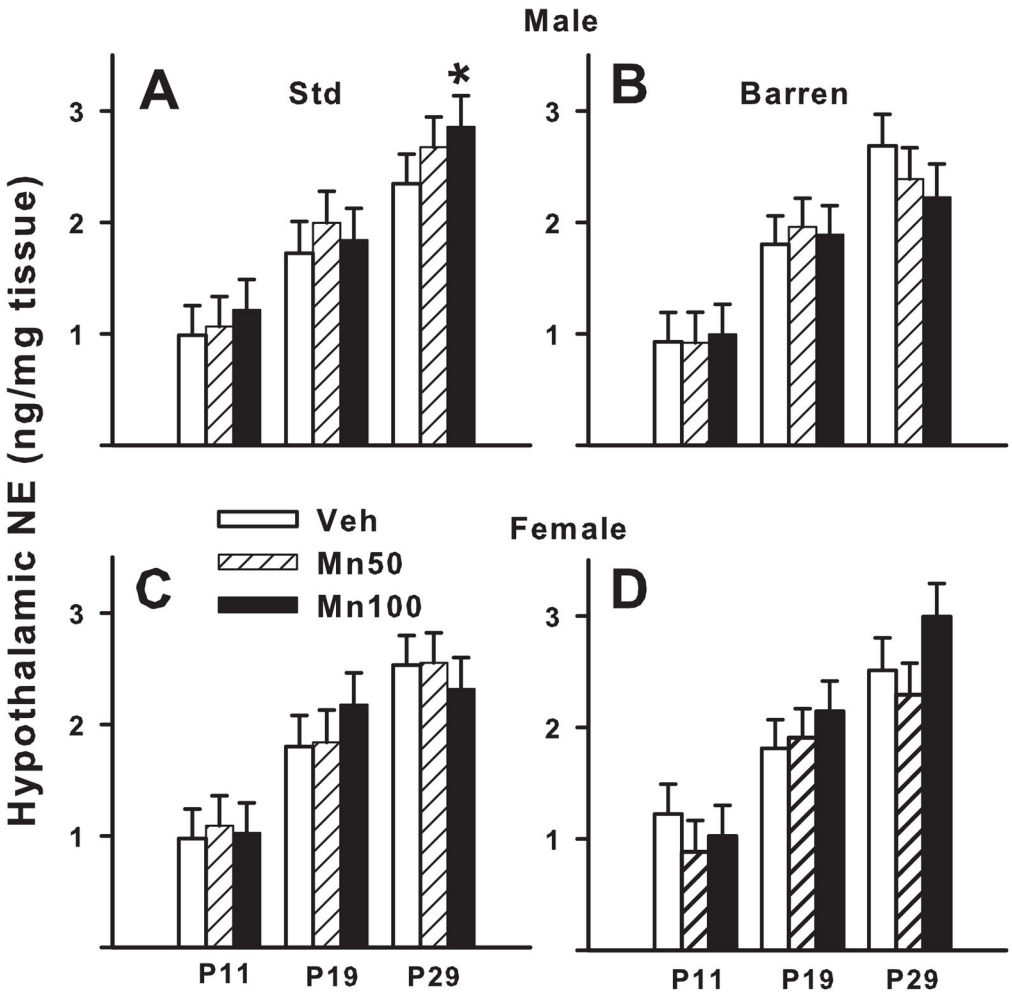

Fig. 9. Hypothalamic norepinephrine (NE)

NE concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05.

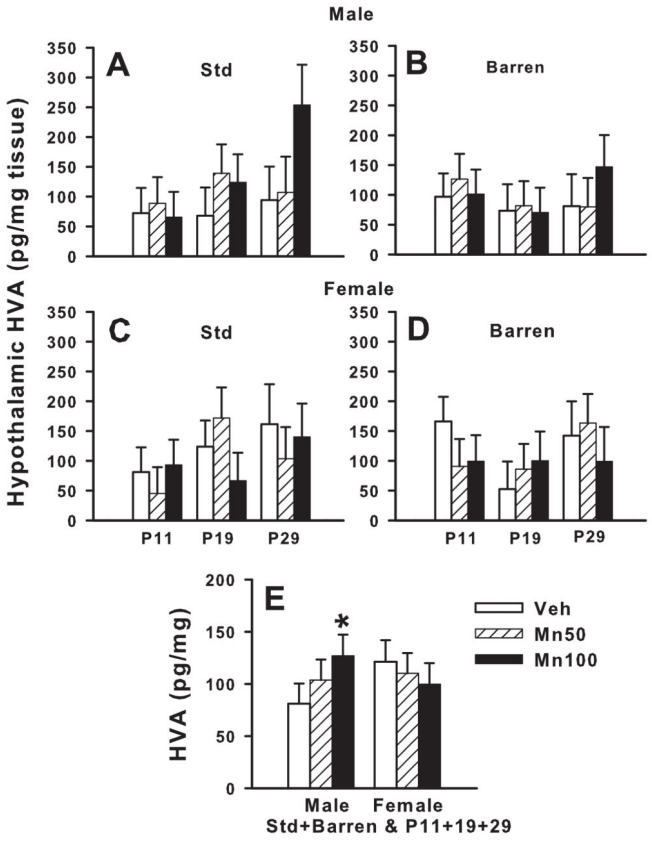

Fig. 10. Hypothalamic homovanillic acid (HVA)

HVA concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. ***E***, effects averaged across sex, housing, and age to show main effects of Mn. Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05.

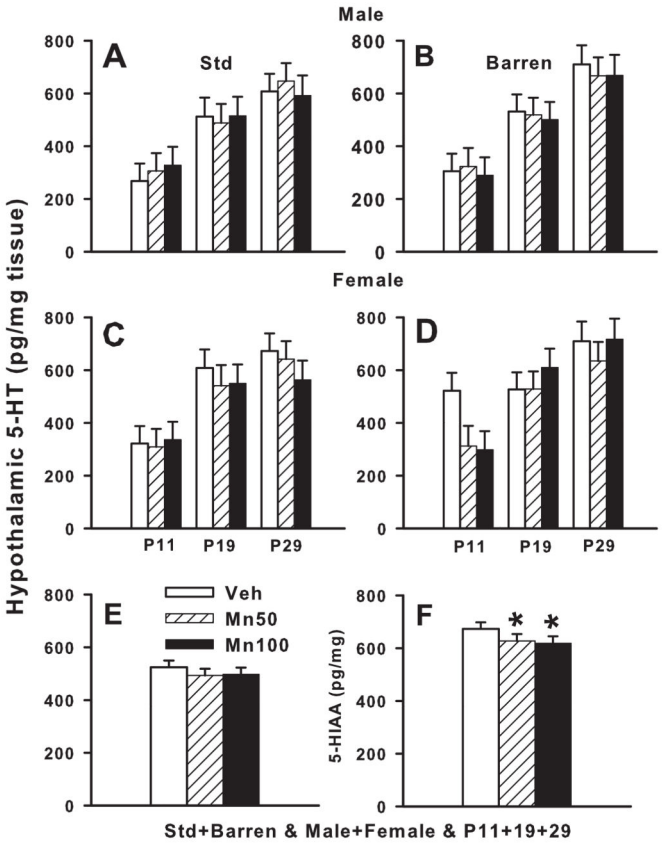

Fig. 11. Hypothalamic serotonin (5HT)

5HT concentrations in neostriatum as a function of age, rearing condition, Mn exposure, and sex. ***A,B***, males; ***C, D***, females; ***A,C***, standard cage condition; ***B, D***, barren cage condition. ***E,F***, Main effect of Mn on hypothalamic 5-HT (***E***) and 5-HIAA (***F***) across housing condition, sex, and age. Range of group sizes shown in Table 3. Values are mean ± SEM. *P < 0.05.

Table 1

Offspring Mortality and Body Weight (g)

Preweaning Housing												
Standard (62 litters)							Barren (60 litters)					
Control			Mn50		Mn100		Control		Mn50		Mn100	
	M	F	M	F	M	F	M	F	M	F	M	F
Mortality												
	6/248 (2.4%)		14/248 (5.6%)		25/248 (10.1%)**		6/239 (2.5%)		23/240 (9.6%)**		31/241 (12.9%)**	
	3/124	3/124	8/124	6/124	14/125	11/123	3/119	3/120	10/120	13/120	11/120	20/121
	2.4%	2.4%	6.4%	4.8%	11.2%	8.9%	2.5%	2.5%	8.3%	10.8%	9.2%	16.5%
P	Offspring Body Weight (g)											
4	10.7±1.3	10.2±1.3	10.5±1.4	10.5±1.4	11.0±1.4	10.2±1.4	11.2±1.4	10.9±1.4	11.4±1.5	10.3±1.5	11.6±1.5	10.9±1.4
8	19.0±1.3	18.4±1.3	16.3±1.4*	16.9±1.4*	16.2±1.4**	15.1±1.4**	19.1±1.4	18.1±1.4	16.8±1.5*	15.9±1.5*	16.3±1.5**	15.7±1.4**
12	28.6±1.3	27.9±1.3	25.0±1.4**	24.8±1.4**	23.1±1.4**	22.4±1.4**	27.7±1.4	26.6±1.4	24.9±1.5**	23.6±1.5**	23.6±1.5**	22.3±1.4**
16	37.1±1.3	36.1±1.3	31.9±1.4**	31.2±1.4**	29.5±1.4**	28.7±1.4**	35.3±1.4	34.2±1.4	31.0±1.5**	30.0±1.5**	29.4±1.5**	27.9±1.4**
20	49.3±1.3	48.4±1.3	42.1±1.4**	42.0±1.4**	38.7±1.4**	37.6±1.4**	45.8±1.4	44.3±1.4	38.2±1.5**	37.8±1.5**	35.8±1.5**	34.0±1.4**
24	69.2±1.3	66.9±1.3	59.9±1.4**	59.1±1.4**	56.2±1.4**	54.0±1.4**	65.4±1.4	62.3±1.4	54.8±1.5**	54.0±1.5**	50.4±1.5**	47.9±1.4**
28	93.9±1.3	88.4±1.3	82.6±1.4**	79.6±1.4**	77.5±1.4**	73.4±1.4**	89.0±1.4	82.4±1.4	75.7±1.5**	73.0±1.5**	70.3±1.5**	66.2±1.4**

Standard housing mortality Chi-square = 12.9, df = 2, P < 0.001; Barren housing mortality Chi-square = 17.6, df = 2, P < 0.001

** P < 0.01 compared with Control within housing (but not across) conditions by Fisher's test for uncorrelated proportions

Body weight:

* p < 0.01;

** P < 0.001 compared with Control within housing condition. Values represent least square mean ± SEM

Table 2

		Standard						Barren					
		Male			Female			Male			Female		
Region		11	19	29	11	19	29	11	19	29	11	19	29
Neostr.	DA	-	-	-	-	↑H	-	-	-	-	-	-	-
	NE	-	-	-	↑L	↑L	↑H	-	-	-	-	↑L	-
	5HT												
Hipp.	DA	↑H&L main effect											
	NE	-	-	-	-	-	-	↑L	-	↑H	-	-	-
	5HT	-	-	↑H&L	-	-	↑H&L			↑H&L			↑H&L
Hypo.	DA	-	-	-	-	-	-	-	-	-	-	↑H	↑H&L
	NE	-	-	↑H	-	-	-	-	-	-	-	-	-
	5HT	-	-	-	-	-	-	-	-	-	-	-	-

L = Low dose (Mn50)

H = High dose (Mn100)