A Manganese-Enhanced Diet Alters Brain Metals and Transporters in the Developing Rat

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Manganese (Mn) neurotoxicity in adults can result in psychological and neurological disturbances similar to Parkinson’s disease, including extrapyramidal motor system defects and altered behaviors. However, virtually nothing is known regarding excess Mn accumulation during central nervous system development. Developing rats were exposed to a diet high in Mn via maternal milk during lactation (PN4-21). The high Mn diet resulted in changes in hematological parameters similar to those seen with iron (Fe) deficiency in dams (decreased plasma Fe; increased plasma transferrin [Tf]) and pups (decreased hemoglobin [Hb] and plasma Fe; increased plasma Tf and total iron binding capacity). Mn-exposed pups showed an increase in brain Mn, chromium, and zinc concurrent with a decrease in brain Fe. In conjunction with the altered transport and distribution of essential metals within the brain, there was enhanced protein expression of the divalent metal transporter-1 (DMT-1) and transferrin receptor (TfR) overall in the brain; there was a general increase in each region analyzed (cerebellum, cortex, hippocampus, midbrain, and striatum). Neurochemical changes were observed as an increase in γ-aminobutyric acid (GABA) and the ratio of GABA to glutamate, indicating enhanced inhibitory transmission in the brain. The results of this study demonstrate that developing rats undergo alterations in the transport and distribution of essential metals translating to neurochemical perturbations after maternal exposure to a diet supplemented with excess levels of Mn.

Key Words: manganese; iron deficiency; brain; development; rat.

Manganese (Mn) neurotoxicity in adults is associated with psychological and motor disturbances in a progressive disorder known as manganism, characterized by Parkinson’s-like symptoms, but distinct from Parkinson’s disease (Aschner, 2000; Erikson et al., 2002). Infants and children, Fe-deficient individuals, and patients with liver disorders are especially susceptible to Mn toxicity. Neonates accumulate more Mn than adults due to enhanced Mn absorption (70 vs. 3%, respectively); an incomplete blood-brain barrier; and little to no biliary excretion until weaning (Aschner, 2000; Dorman et al., 2000; Erikson et al., 2005). Indeed, brain Mn concentration is higher in neonates compared to adult rats (Aschner and Aschner, 1991; Takeda et al., 1999), suggesting an increased requirement for Mn during development. However, the higher levels of Mn in neonates may increase the risk of neurotoxicity. Despite the increased vulnerability of immature animals, developmental effects of Mn are not yet adequately studied. Additionally, there is potential for Mn toxicity in patients receiving total parenteral nutrition (TPN) (Spencer, 1999). Patients with liver damage and gastrointestinal disorders and preterm infants most commonly receive TPN, those already at risk for Mn accumulation due to inefficient heptobiliary elimination. Some children receiving TPN and exhibiting motor disturbances have abnormal MRI scans indicative of Mn accumulation (Barron et al., 1994; Devenyi et al., 1994; Fell et al., 1996; Komaki et al., 1999).

Chronic ingestion of well water containing elevated Mn levels was associated with elevated serum Mn concentrations and abnormal verbal and visual memory function in a 10-year-old boy (Woolf et al., 2002). Moreover, a cross-sectional investigation of intellectual function in 142 10-year-old children in Bangladesh demonstrated that consumption of well water with high Mn (793 μg Mn/l) was associated with dose-dependent reduced full-scale performance, and verbal raw scores from tests drawn from Wechsler Intelligence Scale for Children (Wasserman et al., 2006). However, cohorts of children in this study often had concurrent exposure to high arsenic levels in the water. In the United States, roughly 6% of domestic household wells have Mn concentrations that exceed 300 μg Mn/l, the current U.S. Environmental Protection Agency lifetime health advisory level (Wasserman et al., 2006). In all, these studies justify concern for potential Mn toxicity in children.

Fe and Mn are regulated within the central nervous system by influx into the brain via transferrin (Tf) and transferrin receptors (TfRs), as well as via the divalent metal transporter-1 (DMT-1) (Aschner, 2000; Connor et al., 2001; Malecki et al.,…
1999b), a nonspecific transporter implicated in the transport of Fe and Mn, and to a lesser extent zinc (Zn), copper (Cu), cobalt (Co), cadmium (Cd), and nickel (Garrick et al., 2003; Gunshin et al., 1999). Likewise, Tf binds several metals, potentially acting as a transporter for Fe, Mn, Zn, and chromium (Cr) as well as Cu, Co, Cd, vanadium (V), and aluminum (Al) (Aschner and Aschner, 1991). DMT-1 mRNA has been localized to the rat striatum, cortex, hippocampus, and cerebellum (Williams et al., 2000). Further, protein expression of DMT-1 and TfR is seen as early as PN5 and increases through PN15 in all regions examined (cortex, hippocampus, striatum) (Siddappa et al., 2002), verifying that DMT-1 and TfR are present in the developing brain. Because Mn and Fe transport is regulated by DMT-1 and TfR, there is an inverse relationship between Mn and Fe. Consequently, excess dietary Mn may lead to Fe deficiency and vice versa. Other essential metals sharing the transporters are also altered by excess Mn. Excess Mn exposure alters behavior, motor function, cognition, and neurotransmitter metabolism (Aschner et al., 2002; Dorman et al., 2000; Kontur and Fechter, 1985), most notably, dopamine (Montes et al., 2001; Tran et al., 2002a,b), glutamate (Takeda et al., 2002), and y-aminobutyric acid (GABA) (Erikson et al., 2002; Lai et al., 1984; Lipe et al., 1999). These neurotransmitters are vital to normal brain functioning and are key regulators of motor function. Glutamate and GABA are the predominant excitatory and inhibitory neurotransmitters in the brain, respectively. Although work traditionally has focused on the involvement of dopamine, Mn-induced neurodegeneration primarily involves GABAergic neurons within the globus pallidus, rather than dopaminergic neurons within the nigro-striatal pathway as in Parkinson’s disease (Olanow et al., 1996; Pal et al., 1999; Roth and Garrick, 2003; Verity, 1999).

The goal of this study is to assess the neurochemical alterations implicated in the etiology of manganism during development following a diet high in Mn by monitoring interactions with blood Fe, alterations of essential metals in the brain, mechanism of transport into the brain, and disturbances of neurotransmitters GABA and glutamate.

**MATERIALS AND METHODS**

**Animal treatment.** All animals were treated in accordance with IACUC protocols established by Wake Forest University Health Sciences. For a schematic view of the experimental design, refer to Figure 1. Timed pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN) were ordered to arrive on gestational day (GD) 7; GD0 was established by Harlan as the day a vaginal plug was observed after breeding. Upon arrival, groups of five to seven dams were fed ad libitum one of two pelleted, semi-purified AIN-93G certified diets from Bio-Serv (Frenchtown, NJ) formulated to vary in Mn content only: control (CN: 10 ppm Mn and 35 ppm Fe) or Mn supplemented (Mn: 100 ppm Mn and 35 ppm Fe). It is routine practice for the vendor to measure the concentrations of other metals in the diet and all were in the recommended range for rodent diet guidelines. Human average daily intakes are approximately 1–10 mg Mn/day, and the recommended Mn intake for laboratory rats is 10 mg Mn. On PN4, pups born to control dams were pooled and randomly cross-fostered to dams fed one of the two diets such that initial mean litter weights were approximately equivalent. Pups were exposed to each of these diets via maternal milk from PN4 to PN21 as well as via direct ingestion of solid chow when capable (near PN11). To assess maternal hemoglobin (Hb) during lactation, blood was collected from dams via tail prick on PN4, PN11, and PN21. On PN21, dams were euthanized using CO2. Pup brains were dissected into five regions (cerebellum, cortex, hippocampus, striatum, and midbrain—including thalamus), wet weights were recorded, and regions were immediately frozen on dry ice, and stored at −70°C until use. Trunk blood was collected in heparinized test tubes.

**Hematological parameters.** Blood collected from dams and pups was assayed for measures of Fe-deficiency anemia: Hb, plasma Fe, Tf, and total iron binding capacity (TIBC). Hb was measured colorimetrically by a standard cyanmethemoglobin method (procedure #525, Sigma, St Louis, MO). Whole blood was centrifuged 15 min at 2000 x g to separate cells from plasma. Plasma was frozen at −20°C until analysis in the Clinical Chemistry Laboratory at Wake Forest University (Dr Zak Shihabi). Plasma Fe was reacted with ferrozine as the color reagent and measured with the AVIDA 1650 (Bayer Corp., Tarrytown, NY). Plasma Tf was measured by turbidimetric immunoassay. TIBC was measured by a colorimetric diagnostic kit (procedure #565, Sigma).

**FIG. 1.** Experimental design. GD 7 timed pregnant rats were fed one of two diets that varied only in Mn content. Pups were exposed to each of these diets via maternal milk from PN4 to PN21. Maternal Hb was measured on PN4, PN11, and PN21. On PN21, blood was collected from dams and pups, and brains were collected from pups. Brain samples were analyzed for several metals, for DMT-1 and TfR expression, and for glutamate and GABA concentrations. See text for experimental details.
Metal levels via inductively coupled plasma mass spectrometry. To determine metal levels within the brain, regions (cerebellum, cortex, hippocampus, striatum, and midbrain) were lyophilized in glass tubes and sent to the Norwegian University of Science and Technology (Trondheim, Norway) for analysis as described previously (Erikson et al., 2004). Samples were analyzed for several essential trace metals: Cr, Co, Cu, Fe, Mn, molybdenum (Mo), V, and Zn; and nonessential metals: Al, Cd, and magnesium (Mg). Formalin (0.2 ml) was added to the samples 12 weeks before analysis. The samples were digested in sealed Teflon bombs in a microwave oven (Milestone MSL 1200) after 2 ml 65% HNO3 (Merck, Suprapur) was added. The microwave procedure was set for 3 min (gradient 100–600 W) and then for 4 min (600 W). After the microwave digestion, the samples were diluted directly in the Teflon bombs to 57.6 ml with ultrapure water (MilliQ, Millipore) to achieve a final acid concentration of 0.5 mol/l.

High resolution inductively coupled plasma mass spectrometry analysis was performed using a Thermo (Finnigan) model element instrument (Bremen, Germany). The RF power was 1150 W. The sample was introduced using a CETAC ASX 500 auto sampler (Omaha, NE) with a peristaltic pump (pump speed 1 ml/min). The instrument was calibrated using 0.5 mol/l HNO3 solutions of multielement standards at appropriate concentrations. Internal standards were not used. To check for possible drift in the instrument, a standard solution with known elemental concentrations was analyzed for every 10 samples. In addition, blank samples (0.5 mol/l HNO3, Suprapur) were analyzed for approximately every 10 samples. The samples were analyzed in random order, and the analyst did not know the identification of the samples. All elements were determined in the medium resolution mode (M/Δm = 4000).

DMT-1 and TfR expression via Western blot analysis. Brain tissues were homogenized in five volumes of cold buffer (10mM Tris, 1% SDS, 1mM sodium orthovanadate, pH 7.4) using a sonicator with microtip, boiled for 10 min, and centrifuged for 10 min. Protein concentrations were measured in the supernatants using the bicinchoninic acid method (Pierce Chemical, Rockford, IL). Prepared samples were run through an 8% SDS-PAGE gel and subsequently transferred to a nitrocellulose membrane (Schleicher & Schuell Biosciences, Keene, NH). Blots were washed and proteins blocked overnight at 4°C in phosphate buffered saline solution containing 5% dry milk and 0.05% Tween-20. Blots were washed and incubated with primary antibody (DMT-1 Natural resistance-associated macrophage protein gaζ polyclonal [Santa Cruz, CA], 1:400; TfR (CD-71) monoclonal [Dako Corporation, Carpentrya, CA], 1:200; or anti-β-actin monoclonal [Sigma], 1:8000) for 2 h, then with horseradish peroxidase (HRP)—conjugated secondary antibody for 1 h (rabbit anti-β-actin IgG-HRP [Santa Cruz], 1:1000–2000 for DMT-1; goat anti-mouse IgG [Kirkgaard & Perry Labs, Gaithersburg, MD], 1:1000 for TfR or 1:5000 for actin). Blots were developed with enhanced chemiluminescence reagents and exposed to film. To allow for subsequent reprobing with additional antibodies, blots were washed, stripped with Restore Western blot stripping buffer (Pierce Chemical), and proteins subsequently blocked as before.

Amino acid concentrations via high performance liquid chromatography. Glutamate and GABA concentrations were measured as previously in our lab (Erikson et al., 2002) with high performance liquid chromatography (HPLC) and fluorescence detection. Brain regions were pulverized in liquid nitrogen and stored at −80°C until extraction. Amino acids were extracted from 10–15 mg of frozen tissue powder with 0.4 ml 4°C methanol (MeOH). Homoserine was added to each sample as an internal standard. Homogenates (Gilson 121) (excitation 305–395 nm, emission 430–470 nm). The mobile phase was 0.1M sodium acetate, pH 6.2, containing 0.1mM EDTA and increasing concentrations of MeOH (15–50%); flow rate was 1.3 ml/min. Retention times for glutamate and GABA were 3.2 and 18.1 min, respectively. Proteins were measured in the pellets using the Lowry method (Lowry et al., 1951), and content (nmol/mg protein) was determined from the internal standards correcting for recovery for each sample.

Statistical analysis. Data were analyzed using NCSS software (Kaysville, UT). Data are presented as means ± SEM and considered significant at p < 0.05. The litter is the statistical unit. For body and brain weight data, and blood data, litter averages were analyzed; for all other measures, one male and one female from each litter were selected for each end point. For statistics where gender was collapsed due to no significant treatment × gender interaction, the sample size (n) equals two times the litter number to account for one male and one female from each litter. Outliers that were ± 2 SDs from the mean were omitted from statistical analyses. Technical errors were excluded prior to determining SDs. Treatment effects in the brain were analyzed by global ANOVA incorporating all factors: dietary treatment, gender (male, female), brain region (cerebellum, cortex, hippocampus, midbrain, and striatum), and measurement (Hb, plasma Fe, Tf, and TIBC; metal level; DMT-1 or TfR protein expression; and Glutamate or GABA concentration). Films containing DMT-1 and TfR blot were scanned into Photoshop, and band densities were measured using TINA program software. To normalize band densities across Western blots, bands from treated pups were analyzed as a percent of the control band on each blot. To verify consistent protein loading among gels, some blots were probed for actin and the ratio of DMT-1 or TfR:actin was compared to the analyzed values. There were no statistical differences between density percent control and their ratio with actin, so analyses are presented for percent control of each blot. Three-way ANOVA (treatment × region × gender) was tested for interactions between dietary treatments and measurement (metal level; transporter expression; and amino acid concentration) in each brain region with gender as a factor. If the global ANOVA indicated a significant interaction (treatment × region × gender, treatment × region, or treatment × gender), data were analyzed by one-(treatment) or two-way (treatment × region, or treatment × gender) ANOVA with Tukey-Kramer Multiple Comparison post hoc test used to evaluate differences in individual means within the larger comparison groups.

RESULTS

Animal Growth

Although initial pup body weights were equivalent at PN4, body weight during the treatment period was slightly lower in the pups exposed to the Mn diet such that PN21 body weights were statistically significantly (p < 0.02) lower compared to controls. Additionally, the total brain wet weight from Mn-treated pups was significantly (p < 0.005) less than controls. The ~10% decrease in both body and brain weights in treated pups resulted in equivalent brain: body weight ratios in control and treated groups. Thus, enhanced Mn in the diet during lactation resulted in slightly smaller pups by PN21.

Hematological Parameters

No differences were noted in Hb between control and Mn-fed dams; however, Mn-fed dams had decreased plasma Fe and increased plasma Tf on PN21, with a trend toward increased TIBC as well (p < 0.1) (Fig. 2a). Likewise, there were significant alterations in the hematological parameters of PN21 Mn-treated pups: decreased Hb and plasma Fe concurrent with increased plasma Tf and TIBC (Fig. 2b). Several values for
plasma Fe in the Mn-treated pups were reported as “<10” and were thus evaluated as “10”; therefore, the values are conservative in the experimental group. There were no gender differences for hematological measurements (no treatment × gender interaction).

**Brain Metal Levels**

Not surprisingly, Mn accumulated throughout the brains of pups raised by dams fed the Mn-supplemented diet; the cerebellum, midbrain, and striatum each achieved statistical significance (treatment × region interaction), while the cortex and hippocampus were not statistically significant compared with controls, although there was an increase of Mn in both regions in accordance with the overall accumulation within the brain (Fig. 3a). Conversely, Fe levels within the brains of Mn-exposed pups declined compared to control pups without a regional effect (no treatment × region interaction) (Fig. 3b). Additionally, Cr and Zn levels increased overall in the brain without a regional effect (no treatment × region interaction) (Figs. 3c and 3d). There was no effect on other metals analyzed, including Al (there was a trend toward increased Al in Mn-exposed pups, p < 0.06, data not shown), Cd, Co, Cu, Mo, or V. There were no gender differences for metal levels (no treatment × gender interaction), so analyses combined males and females.

**Brain DMT-1 and TfR Expression**

Two of the best characterized transporters of Mn and Fe thus far are TfR and DMT-1. Since neurotoxic outcomes depend upon the rate and extent of Mn accumulation in the brain, we investigated Mn and Fe regulation in the brain by measuring the expression of DMT-1 and TfR in each brain region at PN21 via Western blot analysis. DMT-1 and TfR expression increase by ~35 and 60%, respectively, in brains of pups nurtured by dams fed the high Mn diet, corresponding to Mn accumulation and Fe reduction within the brain (Fig. 4). There was no treatment × region interaction for DMT-1 expression; in other words, although expression increased by 20–50% across regions, a target region could not be identified statistically (Fig. 4a). On the other hand, there was a treatment × region interaction for TfR expression, with an increase in all regions, particularly the hippocampus. TfR expression increased ~35–45% in cerebellum, cortex, and striatum, 70% in midbrain, and 110% in hippocampus (Fig. 4b). There was no effect of gender on transporter expression (no treatment × gender interaction), so results were combined from males and females.

**Brain Amino Acid Concentrations**

In Mn-supplemented pups, GABA concentrations increased overall in the brain (p < 0.001) without a specific regional effect (no treatment × region interaction) (Fig. 5a). On the other hand, glutamate concentrations tended to decrease similarly across regions (Fig. 5b). For glutamate, a two-way
ANOVA indicated no treatment × region interaction, but there was a significant treatment effect; however, with a one-way ANOVA there was no significant treatment effect \((p < 0.09)\). Thus, although the statistical significance with regard to decreased glutamate is equivocal, the net ratio of GABA/glutamate was significantly increased \((p < 0.0001)\) (Fig. 5c). Because GABA is an inhibitory amino acid while glutamate is excitatory, the net result of an increase in GABA and/or a decrease in glutamate translates to more inhibitory action in the brains of Mn-exposed pups. There was no effect of gender on amino acid levels (no treatment × gender interaction), so results were combined from males and females.
Metals serve critical roles in the brain as essential cofactors, catalysts, second messengers, and modulators of gene, enzyme, and receptor activity. Calcium (Ca), Co, Cu, Fe, Mg, Mn, molybdenum (Mo), selenium (Se), and Zn are essential for normal brain development and function. Each must be present at specific levels to avoid deficiencies or toxic excess (Smith et al., 1997). Metal uptake into the brain is critically influenced by diet, and can cross-interact so that an imbalance in one metal can influence the uptake and concentration of other metals (Smith et al., 1997). The current results indicate that a high Mn diet leads to increased brain Mn, Zn, and Cr and decreased Fe levels. Mn accumulation was measured in both target (striatum and hippocampus) and nontarget (cortex, midbrain, and cerebellum) regions. Surprisingly, the striatum, midbrain, and cerebellum accumulated more Mn than the cortex and hippocampus, indicating that distribution differs between mature and immature animals. In support, increased Mn levels were observed in the striatum, hippocampus, hindbrain, and cortex of PN21 rats dosed with 25 or 50 mg/kg/day, but only in the striatum and cerebellum of adult rats dosed with 50 mg/kg/day (Dorman et al., 2000). Conversely, there were no significant treatment region interactions for Cr, Zn, or Fe; thus, no specific region was more or less susceptible to alterations of these metals.

Consistent with reduced brain Fe levels, plasma Fe levels also declined in Mn-exposed pups concurrent with decreased Hb and increased plasma Tf and TIBC, parameters that mimic Fe deficiency. A study of Fe-deficient patients showed elevated blood Mn; after Fe therapy, blood Mn decreased and Hb increased (Kim et al., 2005). Likewise, chronic Mn exposure in adult rats led to a 32% decrease in plasma Fe (Zheng et al., 1999). The current results corroborate the relationship between elevated Mn and hematological parameters of Fe deficiency, including reduced Hb and plasma Fe. Plasma Cr, Mn, and Zn were not measured following excess dietary Mn, so it is unknown whether alterations in the brain were mirrored in the body for these metals.

Zn is a component of multiple proteins and regulates gene expression, neurotransmitter homeostasis, and brain oxidation metabolism. In vitro studies have shown that Zn increases the overall accumulation of Mn (Aschner, 1999; Wedler and Ley, 1990); therefore, it is not surprising that both Mn and Zn are increased in the brain. Cr helps to maintain normal blood glucose levels. Physiological Cr, predominately in the trivalent state, binds to plasma Tf and is distributed throughout the body. Although relatively nontoxic, excess Cr(III) is associated with chronic renal failure (Barceloux, 1999). The functional implications of elevated brain Zn and Cr are not clear since there is no evidence yet that either is involved in neurodegenerative diseases in humans. Still, Zn may displace other metals such as Al, Fe, or Cu which may then be toxic to the system (Zatta et al., 2003).

Mn and Fe are transported by Tf-dependent and -independent pathways, both involving DMT-1 as the transport protein (Erikson et al., 2004). In vitro studies have shown that Zn increases the overall accumulation of Mn (Aschner, 1999; Wedler and Ley, 1990). Studies using a mouse mutant with < 1% of normal plasma Tf implicate the TfR system as the predominant mechanism for Fe delivery into the brain with less
importance for Mn transport (Aschner et al., 2005; Dickinson and Connor, 1994; Malecki et al., 1999a). Although both DMT-1- and TfR-mediated transport are important, other transport systems cannot be excluded.

DMT-1 and TfR protein expression was elevated in the brains from Mn-exposed neonates. While there was no significant treatment × region interaction for DMT-1 expression, there was a significant interaction for TfR; this may be due to more generalized distribution of DMT-1 in neonatal rat brains compared to adults (Barceloux, 1999), or to more variability in the DMT-1 Western blots. Unlike TfR and actin Western blots, the quality and reproducibility of band expression for DMT-1 blots was inconsistent, making DMT-1 analysis challenging. Nevertheless, overall both protein transporters appear elevated by 35–60% in the brains of pups maternally exposed to the Mn diet. Consistent with the increase in TfR protein in vivo, in vitro Mn incubation of cells derived from rat choroid plexus elevated TfR mRNA (Zheng et al., 1999). TfR

FIG. 5. GABA and glutamate concentrations (nmol/mg protein) in PN21 rat brain. Data represent means ± SEM ($n = 4–6$ per region per sex). Values for overall “brain” effects were determined by collapsing regional values together. (a) GABA levels increased overall in the brain, without a regional effect. (b) There was a nonsignificant trend for glutamate decrease overall. (c) The GABA/glutamate ratio was enhanced overall in treated pup brains.
mRNA is highest in cortex, hippocampus, and the white matter of the cerebellum. Thus, the marked increase in TfR expression in the hippocampus most likely mirrors the high levels of mRNA in this region (Malecki et al., 1999b). While an Fe-deficient diet has been linked to elevated DMT-1 (Erikson et al., 2004, 2005), it is novel that a diet high in Mn alone also elevates brain DMT-1.

Since Mn and Fe compete for both of these transporters, it is not surprising that an increase in brain Mn is linked to a decrease in Fe. Further, the increase in brain Cr and Zn may be explained by Tf and DMT-1 transport, since both transporters have been implicated in binding these metals. Evidently, DMT-1 and TfR are nonspecific for the transport of Fe or Mn, although both have a greater affinity to bind Fe. Accordingly, other transport proteins may be involved in the alterations of essential metals in the brain. Zn transporter 3 (ZnT3), for instance, is a more specific transporter of Zn (Valente and Auladell, 2002). The effect of dietary manipulation of Mn on ZnT3 is unknown; future studies should examine effects on ZnT3 as well of other potential metal transporters.

To study the transport and distribution of the metals, protein expression of DMT-1 and TfR were analyzed and found to be elevated in the brain. While there was no significant treatment × region interaction for DMT-1 expression, there was a significant interaction for TfR; this may be due to more generalized distribution of DMT-1 in neonatal rat brains compared to adults (Olanow et al., 1996; Pal et al., 1999; Verity, 1999). Our results showed brain Mn accumulation associated with an increase in GABA in Mn-exposed neonatal rats. In contrast, increased brain Mn was associated with a decrease in GABA in young adult rats (PN21-63); however, brain Mn accumulation resulted from Fe-deficient diets with and without Mn supplementation (Erikson et al., 2002). The same diets in neonates failed to alter GABA (Garcia et al.). Thus, GABA effects may be dependent upon development and/or diet. Specific target regions were not identified (no treatment × region interaction), in contrast to studies in adult rats in which the basal ganglia (e.g., globus pallidus) is targeted for Mn accumulation and toxicity (Erikson et al., 2002, 2004), suggesting developmental differences.

A trend ($p < 0.09$) combined with increased GABA resulted in an increase in the GABA/glutamate ratio, indicating enhanced inhibitory action. While elevated excitatory action can lead to disturbances in motor activity such as seizures, enhanced inhibition contributes to hypokinetia activity. Although we did not test for motor activity here, decreased locomotor activity occurred in Mn-exposed young adult rats (Normandin et al., 2004). Additionally, in a pre-Parkinsonism rat model generated by 6-hydroxydopamine-induced dopamine depletion, Mn treatment produced deficits in motor function concurrent with increases in striatal GABA (−20%), but did not affect dopamine (Gwiazda et al., 2002), suggesting that changes in GABAAergic neurotransmission in the globus pallidus precede those in striatal dopamine.

Although Mn exposures in rodent models produce inconsistent GABA alterations, a pattern may emerge. Lower (6 mg/kg/day), short-term exposures result in decreased levels of GABA, concurrent with increased brain Mn, indicating a decline in the inhibitory system in Mn toxicity (Chandra et al., 1982; Lai et al., 1984). Conversely, higher (20 mg/kg/day), long-term exposures result in elevated GABA (Gianutsos and Murray, 1982; Lipe et al., 1999; Shukla and Singhal, 1984). Accordingly, a relationship may exist between the severity of Mn exposure and GABA levels, likely dependent upon duration, dose, and/or timing of exposure. Mn affects motor function, and it appears that the GABAAergic system may be more sensitive than the dopaminergic system to increased Mn (Fitsanakis et al., 2005). Further studies are necessary to clarify the mechanisms involved.

In humans, nonhuman primates, and rodents, there is an association between high dietary Mn and cognitive deficits. Adverse neurological effects (learning or behavioral impairment; intellectual function) were observed in children (10–13 years) exposed to excess Mn in the drinking water and in food produced on land fertilized with sewage water (He et al., 1994; Wasserman et al., 2006; Zhang et al., 1995). However, although the groups were well matched, confounding issues (e.g., inadequate information regarding the duration and rate of Mn uptake and nutritional status including low Fe or Ca) preclude any definitive cause–effect relationship. Rhesus monkeys fed an Mn-supplemented diet during development had altered behavioral measures (less play behavior; more affiliative clinging in social interactions) and a blunted response to the dopamine agonist apomorphine (Golub et al., 2005); thus, Mn may influence brain development as reflected in behavioral measures. Further, neonatal rats supplemented orally with Mn from PN1-20 exhibited an increase of brain Mn, increased passive avoidance errors, and reduced striatal dopamine concentrations, suggesting deficits in neurodevelopment with high Mn exposure in infancy (Tran et al., 2002a,b). The current results demonstrate that developing rats undergo alterations in the transport and distribution of essential metals translating to neurochemical perturbations after maternal exposure to a diet supplemented with excess levels of Mn.

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