Brain Manganese Accumulation is Inversely Related to γ-Amino Butyric Acid Uptake in Male and Female Rats

By: Joel G. Anderson, Paula T. Cooney, and Keith M. Erikson

Anderson, J.G., Cooney, P.T., Erikson, K.M. (2007) Brain Manganese Accumulation is Inversely Related to GABA Uptake in Male and Female Rats. Toxicol. Sci.95(1): 188-195.

Abstract:

Iron (Fe) is an essential trace metal involved in numerous cellular processes. Iron deficiency (ID) is reported as the most prevalent nutritional problem worldwide. Increasing evidence suggests that ID is associated with altered neurotransmitter metabolism and a risk factor for manganese (Mn) neurotoxicity. Though recent studies have established differences in which the female brain responds to ID-related neurochemical alterations versus the male brain, little is known about the interactions of dietary ID, Mn exposure, and sex on γ-amino butyric acid (GABA). Male and female Sprague-Dawley rats were randomly divided into four dietary treatment groups: control (CN), control/ Mn supplemented, ID, and ID/Mn supplemented. After 6 weeks of treatment, both ID diets caused a highly significant decrease in Fe concentrations across all brain regions compared to CN in both sexes. Both ID and Mn supplementation led to significant accumulation of Mn across all brain regions in both sexes. There was no main effect of sex on Fe or Mn accumulation. Striatal synaptosomes were utilized to examine the effect of dietary intervention on ^{3}H-GABA uptake. At 4 weeks, there was a significant correlation between Fe concentration and ^{3}H-GABA uptake in male rats ($p < 0.05$). At 6 weeks, there was a significant inverse correlation between Mn concentration and ^{3}H-GABA uptake in male and female rats and a positive correlation between Fe concentration and ^{3}H-GABA uptake in female rats ($p < 0.05$). In conclusion, ID-associated Mn accumulation is similar in both sexes, with Mn levels affecting GABA uptake in both sexes in a comparable fashion.

Key Words: GABA; iron deficiency; manganese; rat; neurotoxicity.

Article:

Iron (Fe) is an essential trace metal involved in numerous and important processes in the body. Fe plays a crucial role in several cellular processes and as a component of various enzymes (Beard et al., 1993). Among the myriad of biological functions of Fe is the role it plays in the function and maintenance of the central nervous system, including neurotransmitter synthesis and metabolism, myelination, and energy metabolism (Beard et al., 1993, 2002). Iron deficiency (ID) is reported as the most prevalent nutritional problem in the world, affecting an estimated 600–700 million individuals worldwide (FAO/WHO, 1998). ID is associated with malformation of red blood cells, growth impairment, perturbations in thermoregulation, and deficits in cognitive function (see Beard et al., 1993).

The neurobiological sequelae of ID in humans include variations in behavior, cognition, and neurotransmitter metabolism (Beard, 2001). Over two decades ago, ID was linked to decreased dopamine D_2 receptors in striatum (Ashkenazi et al., 1982; Youdim et al., 1989), and presently it is well recognized that ID leads to increased extracellular dopamine (Beard et al., 1994; Chen et al., 1995; Nelson et al., 1997) and decreased dopamine transporter (DAT) and dopamine receptor functioning (Erikson et al., 2000, 2001). Knowing some of the behavioral alterations associated with ID, increasing evidence suggests that ID is associated with perturbations in additional neurotransmitter levels (e.g., γ-amino butyric acid [GABA], glutamate, and serotonin) (Burhans et al., 2005; Garcia et al., 2006; Li, 1998).

Recently, it has become clear that ID is a risk factor for manganese (Mn) accumulation (Davis et al., 1992; Erikson et al., 2002; Finley, 1999; Kwik-Uribe et al., 1999). High levels of Mn in the globus pallidus, caudate

putamen, and subthalamic nuclei may contribute to the susceptibility of the striatum to Mn toxicity (Montes et al., 2000; Verity, 1999). Transport of both Mn and Fe to extrahepatic tissues, including the brain, relies upon transferrin-mediated endocytosis (Crowe and Morgan, 1992; Malecki et al., 1999). Recently, divalent metal trans-porter (DMT-1) has been identified as an important protein in the proper cellular transport of Fe and Mn (Conrad et al., 2000; Roth and Garrick, 2003). To date, no study has looked at sex differences in this scenario.

Previous studies examining the sex differences on ID-related neurochemical alterations have targeted dopamine biology and established that there are clear differences in the manner that the female brain responds to ID compared to the male brain (Erikson et al., 2000, 2001). Specifically, ID male rats exhibited decreased DAT density in caudate putamen and nucleus accumbens, whereas female ID rats had decreased DAT density in nucleus accumbens only, compared to controls (CNs) (Erikson et al., 2000). Early ID in male mice had a greater impact on decreasing DAT density in caudate putamen compared to that in female mice (Morse et al., 1999). To date, very little is known about the interactions of dietary ID, exposure to Mn, and sex of the subject on GABA.

GABA is the most abundant inhibitory neurotransmitter in the adult brain (Beleboni et al., 2004; Olsen and DeLorey, 1999). Glutamate is converted to GABA by decarboxylation via glutamate decarboxylase (GAD) and is degraded via GABA transaminase. Cortical glutamate afferents project into the striatum where, in concert with GABA and dopamine, motor behaviors are controlled (Carlsson M. and Carlsson A., 1990). Altered GABA metabolism due to ID has been shown (Li, 1998), but absolute changes in GABA concentration were not detected. Further, Li's study demonstrated increased glutamate concentration due to ID, potentially caused by decreased GAD activity. Reports of GABA concentrations in the rat brain upon Mn exposure are inconsistent. For example, exposure to 6 mg Mn/kg/day led to a significant increase in brain Mn concentrations and significant decrease in GABA concentrations (Chandra et al., 1982). Another report showed that rats exposed to 20 mg Mn/kg/day had significantly increased brain Mn and GABA concentrations (Lipe et al., 1999). Gwiazda et al. (2002) found a significant 16% increase in striatal GABA concentrations resulting from cumulative low-dose Mn exposure in a pre-Parkinsonian rat model. Consequently, it appears that a relationship exists between the severity of Mn exposure and GABA concentrations, with lower Mn exposure leading to decreased GABA, and high Mn exposure leading to increased GABA concentrations. More recently, it has been shown that ID-associated Mn accumulation in the male striatum is negatively correlated with GABA concentrations (Erikson et al., 2002), but there are no data on the female striatum.

The goals of this study were to examine sex differences in (1) Mn accumulation due to environmental exposure (via the drinking water), (2) ID-associated Mn accumulation, and (3) the effect of the Mn accumulation on ^{3}H-GABA uptake.

MATERIALS AND METHODS

Animals. Male and female 21-day-old Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were randomly divided into four dietary treatment groups as in previous studies (Anderson et al., 2006; Erikson et al., 2002): CN (35 mg Fe/kg, 10 mg Mn/kg diet and drinking water), control Mn supplemented (CNMn; control diet and 1 g Mn [as $MnCl_2$]/l drinking water), iron deficient (ID; 4 mg Fe/kg, 10 mg Mn/kg diet and drinking water), and iron deficient/Mn supplemented (IDMn; 4 mg Fe/kg, 10 mg Mn/kg diet and 1 g Mn/l drinking water). Diets were obtained from Bio-Serv, (Frenchtown, NJ) and certified for metal content. Rats had free access to food and water 24 h/day, and the lights were turned off between 1800 and 600 h. Room temperature was maintained at 25 ± 1 °C. After 6 weeks of dietary treatment, the rats were sacrificed and brains removed for metal (n = 48) and synaptosomal (n = 48 in each of the three individual experiments) analyses. The University of North Carolina at Greensboro Animal Care and Use Committee approved all the animal procedures.

Hematological measurements. Hematocrit was measured weekly via blood samples acquired by tail prick, and determined by centrifugation of blood collected into heparinized microcapillary tubes. Blood samples were collected at the end of the experiment into heparinized tubes; aliquots were used for hematocrit and the remaining blood was cooled to 4 °C and centrifuged in a clinical centrifuge for 15 min to separate cells from

plasma. Plasma was frozen at -80 °C until analyzed for Mn. Plasma Mn was measured utilizing graphite furnace atomic absorption spectroscopy (Varian AA240, Varian, Inc. Palo Alto, CA).

Brain Fe and Mn. Brains were dissected into four regions: caudate putamen, globus pallidus, substantia nigra, and cerebellum. Regions were selected based on their varied concentrations of Fe and Mn and the known differential responses to alterations in dietary Fe and Mn levels (Anderson et al., 2006; Erikson et al., 2002). Tissue Mn and Fe concentrations were measured with graphite furnace atomic absorption spectroscopy (Varian AA240, Varian, Inc). Brain regions were digested in ultrapure nitric acid (1:10 wt/vol dilution) for 48–72 h in a sandbath (60 °C). An aliquot of 100 µl of digested tissue was brought to 1 ml total volume with 2% nitric acid and analyzed for Mn and Fe.

In vitro *studies.* We used striatal synaptosomes to indirectly assess the effect of ID and Mn supplementation on disturbances in GABA biology by measuring ^{3}H-GABA uptake. We followed a modified method of Cotman et al. (1981) (Erikson et al., 2000, 2001). Briefly, striata from two rats per treatment group of each sex were pooled for each individual experiment. The tissue was homogenized using a Teflon/glass homogenizer in 20 volumes of ice-cold 0.32M sucrose-HEPES, pH 7.4. The homogenate was then centrifuged at 2000 X g for 10 min at 4 °C. The supernatant was removed and centrifuged at 20,000 X g for 15 min at 4 °C. The pellet was resuspended in 10 ml fresh ice-cold Krebs-Ringer-HEPES (KRH) buffer (118.4mM NaCl, 1.18mM $MgSO_4$,4.7mM KCl, 1.2mM KH_2PO_4, 10.0mM HEPES, 5.6mM dextrose [pH 7.4]). An aliquot of 200 µl of the synaptosomes was placed in a tube with 50 µl KRH buffer. A 10-µl aliquot of ^{3}H-GABA (50nM at a specific activity of 94 Ci/mmol) (NEN, Boston, MA) was added to each tube, and tubes were incubated for 15 min at 37 °C. The reaction was stopped by adding 5 ml ice-cold KRH buffer to each tube followed by rapid filtration through a GF/F Whatman fiberglass filter on a Millipore sampling manifold. Filters were washed twice with ice-cold KRH, placed in scintillation vials with 5 ml scintillation cocktail, and counted on a Beckman LS scintillation counter (Beckman, Fullerton, CA). An additional set of tubes, one for each treatment group, was incubated on ice (approximately 4 °C) to determine nonspecific uptake. Protein analysis was performed utilizing the bicinchoninic acid method (Pierce, Rockford, IL).

To assess Mn and Fe concentration in the striatal synaptosomes, the un-used fraction of synaptosomes from each dietary treatment was centrifuged at 2000 X g for 10 min at 4 °C. The supernatant was removed and the pellet was digested in 500 µl ultrapure nitric acid for 48 h. Mn and Fe concentration was deter-mined by graphite furnace atomic absorption spectroscopy as described above.

Statistical analysis. The data were analyzed with the SPSS system v11.5 statistical analysis package. Data were examined for normality of distribution using a one-sample Kolmogorov-Smirnov test and presence of outliers by boxplot analysis. One-way ANOVA was used to test for effects of dietary treatment on clinical parameters. Repeated-measures ANOVA with repeated-measures factors (brain regions) and between-groups factors (sex and dietary treatment) was used to test for interactions between sex, dietary treatments, and brain regions for amino acid and metals data. For hematological measures, two-way ANOVA was performed with sex and diet as the main effects. Tukey's post hoc analysis was performed when the p value for the respective parameter was statistically significant ($p < 0.05$). Correlational analysis was performed to ex-amine the relationships between (1) Fe levels and Mn levels within brain regions, (2) Fe levels and ^{3}H-GABA uptake within synaptosomes, and (3) Mn supplementation and ^{3}H-GABA uptake within synaptosomes. Dunnett's procedure was used to evaluate mean differences from CN. The alpha level for the analyses was $p < 0.05$.

RESULTS

Body Weights, Tissue Weights and Hematology

Both ID diets (ID and IDMn) caused significant decreases in body weight (Table 1). Hematocrit was significantly lower in the ID and IDMn groups indicating severe anemia (Table 1). A significant difference ($p < 0.05$) was observed in plasma Mn in male rats, with those rats receiving the ID diet having higher Mn levels than CNs (Table 1). Plasma Mn was not significantly different between the dietary treatments in female rats

(Table 1). Mn supplementation did not affect body weight, hematocrit, or plasma Mn in the rats receiving the control diet.

TABLE 1
Body Weight, Hematocrit, and Plasma Mn Concentrations

	Diet	Body weight (g)	Hematocrit	Plasma Mn (nmol/l)
Female	ID ($n = 6$)	137 ± 15^{c}	0.22 ± 0.03^{b}	530 ± 53.5
	IDMn ($n = 6$)	104 ± 16^{d}	0.16 ± 0.03^{b}	560 ± 81.3
	CN ($n = 6$)	187 ± 20^{b}	0.48 ± 0.04^{a}	481 ± 42.0
	CNMn ($n = 6$)	191 ± 19^{b}	0.51 ± 0.04^{a}	613 ± 74.2
Male	ID ($n = 6$)	155 ± 27^{c}	0.20 ± 0.01^{b}	695 ± 69.9^{b}
	IDMn ($n = 6$)	108 ± 17^{d}	0.17 ± 0.03^{b}	702 ± 83.2^{b}
	CN ($n = 6$)	256 ± 34^{a}	0.53 ± 0.02^{a}	519 ± 13.0^{a}
	CNMn ($n = 6$)	$236 \pm 39^{a,b}$	0.54 ± 0.03^{a}	400 ± 45.6^{a}

Data are mean ± SD. Data were analyzed by two-way ANOVA with sex and diet as the main effects. Tukey's *post hoc* analysis was performed when the ANOVA for the respective parameter was statistically significant ($p < 0.05$). Means not sharing a superscript are significantly different. For Plasma Mn in the female rats, there was no significant difference between the four groups, thus there are no superscripts.

Brain Regional Fe and Mn Concentrations

Both iron-deficient diets (ID and IDMn) caused a significant decrease ($p < 0.001$) in Fe concentrations across all brain regions compared to CNs in both females (Fig. 1A) and males (Fig. 1B). Mn supplementation also significantly lowered Fe concentrations across most brain regions ($p = 0.001$) in both sexes. There were no significant interactions between dietary Fe, Mn, and sex across brain regions. Univariate analysis within brain regions revealed that there were significant differences in Fe levels in rats fed the experimental diets com-pared to CN rats ($p < 0.05$) (Figs. 1A and 1B).

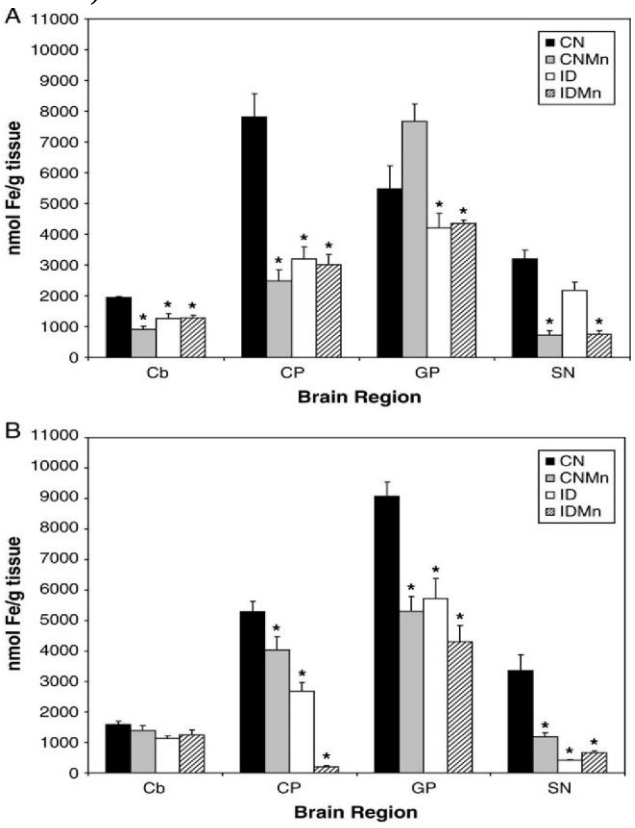

FIG. 1. Fe concentrations for four brain regions: cerebellum (Cb), caudate putamen (CP), globus pallidus (GP), and substantia nigra (SN) from female (A) and male (B) rats fed CN, CNMn, ID, and IDMn diets. Overall, both Fe and Mn levels had highly significant effects on decreasing brain Fe levels. Within brain regions, the low Fe diet and Mn supplementation caused a significant decrease in Fe concentrations in the CP, GP, and SN ($p < 0.05$) compared to CN as indicated by asterisk (*).

^{3}H-GABA Uptake

After 4 weeks of dietary treatment, there was a significant positive correlation between synaptosomal Fe concentration and ^{3}H-GABA uptake in male rats ($R = 0.342$; $p < 0.05$) (Fig. 3B). A similar relationship was not seen in female rats (Fig. 3D). There was no significant correlation between synaptosomal Mn concentration and ^{3}H-GABA uptake in those synaptosomes isolated from either male (Fig. 3A) or female (Fig. 3C) rats at 4 weeks. At 6 weeks of dietary intervention, there was a significant inverse correlation between synaptosomal Mn concentration and 3 H-GABA uptake in male rats ($R = -0.356$; $p < 0.05$) (Fig. 4A) and female rats ($R = -0.380$; $p < 0.05$) (Fig. 4C). Specifically, uptake of ^{3}H-GABA was significantly ($p < 0.05$) decreased in CNMn, ID, and IDMn in males (Fig. 4A inset) and in CNMn in female rats at 6 weeks (Fig. 4C inset). Additionally, there was a significant positive correlation between synaptosomal Fe concentration and ^{3}H-GABA uptake in female rats at 6 weeks ($R = 0.502$; $p < 0.01$) (Fig. 4D). There was no significant correlation between Fe concentration and ^{3}H-GABA uptake in male rats at 6 weeks (Fig. 4B).

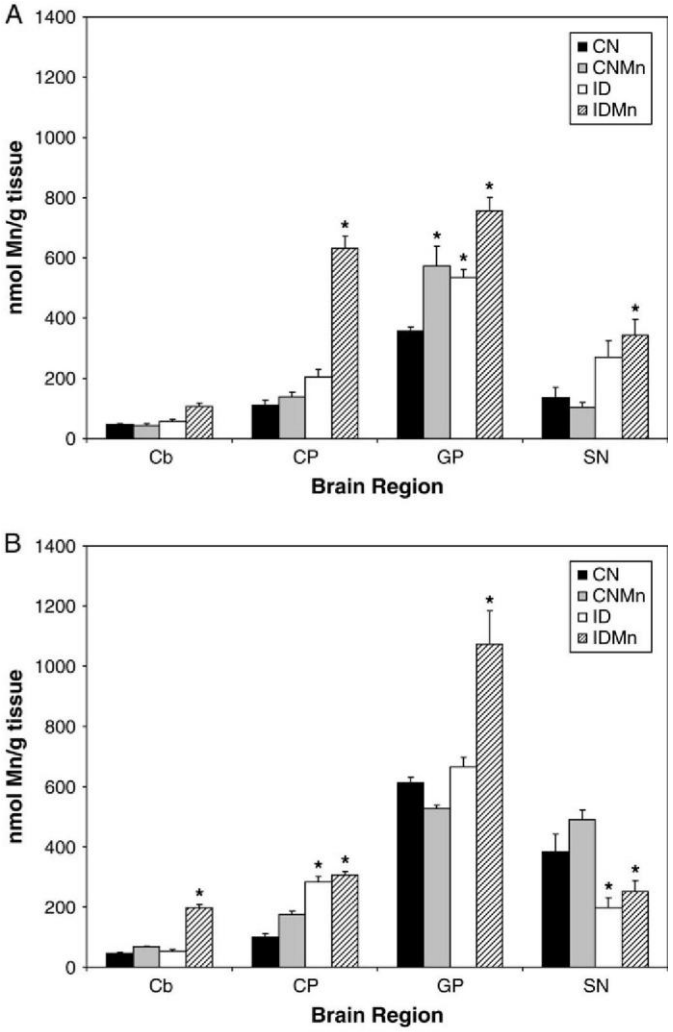

FIG. 2. Mn concentrations for four brain regions: cerebellum (Cb), caudate putamen (CP), globus pallidus (GP), and substantia nigra (SN) from female (A) and male (B) rats fed CN, CNMn, ID, and IDMn diets. Overall, both ID and Mn supplementation led to significant accumulation of Mn across all brain regions. There was a significant interaction between level of dietary Fe and Mn supplementation. Specifically, in both sexes, Mn supplementation caused a significant ($p < 0.05$) increase in Mn levels as indicated by asterisk (*).

DISCUSSION

Several novel findings emerged from the current study. First, our dietary protocol achieved elevated systemic Mn levels which were related to both dietary ID and Mn supplementation (Table 1). Second, Fe and Mn accumulated in a heterogeneous fashion similarly in both female (Figs. 1A and 2A) and male (Figs. 1B and 2B)

rats, with animals receiving the ID diet having lower Fe concentrations and Mn-supplemented animals having higher Mn concentrations than CN animals. There was significant accumulation of Mn in the striatum as a result of ID (Figs. 2A and 2B). Third, using striatal synaptosomes, a significant positive correlation was seen between synaptosomal Fe concentration and ^{3}H-GABA uptake in male rats only at 4 weeks of dietary intervention (Fig. 3B). At 6 weeks of dietary intervention, a significant negative correlation was seen between synaptosomal Mn concentration and ^{3}H-GABA uptake in both male and female rats (Figs. 4A and 4B). Additionally, a significant positive correlation between synaptosomal Fe concentration and ^{3}H-GABA uptake was observed in female rats at 6 weeks (Fig. 4D).

As in previous studies (Erikson et al., 2000, 2001, 2002), Fe accumulated in a heterogeneous fashion in the brain with those rats receiving the ID and IDMn diet accumulating significantly less Fe than CN rats in both females (Fig. 1A) and males (Fig. 1B) in most brain regions. Additionally, Mn supplementation significantly attenuated Fe accumulation in the striatum (Figs. 1A and 1B). Our data corroborate a previous study in which there was no statistical difference observed in brain regional Fe concentrations between male and female rats receiving an ID diet (Erikson et al., 2000).

The effect of ID-associated Mn accumulation in the female rat brain had yet to be examined. While ID is not a public health issue to the extent that it is in developing countries, the percentage of toddlers, adolescents, and particularly childbearing aged women (vulnerable subpopulations) affected by ID remain higher than the Healthy People 2010 Objectives set forth (Looker et al., 1997). Hence, it is imperative to include females in these studies that focus on ID-associated neurotoxicity.

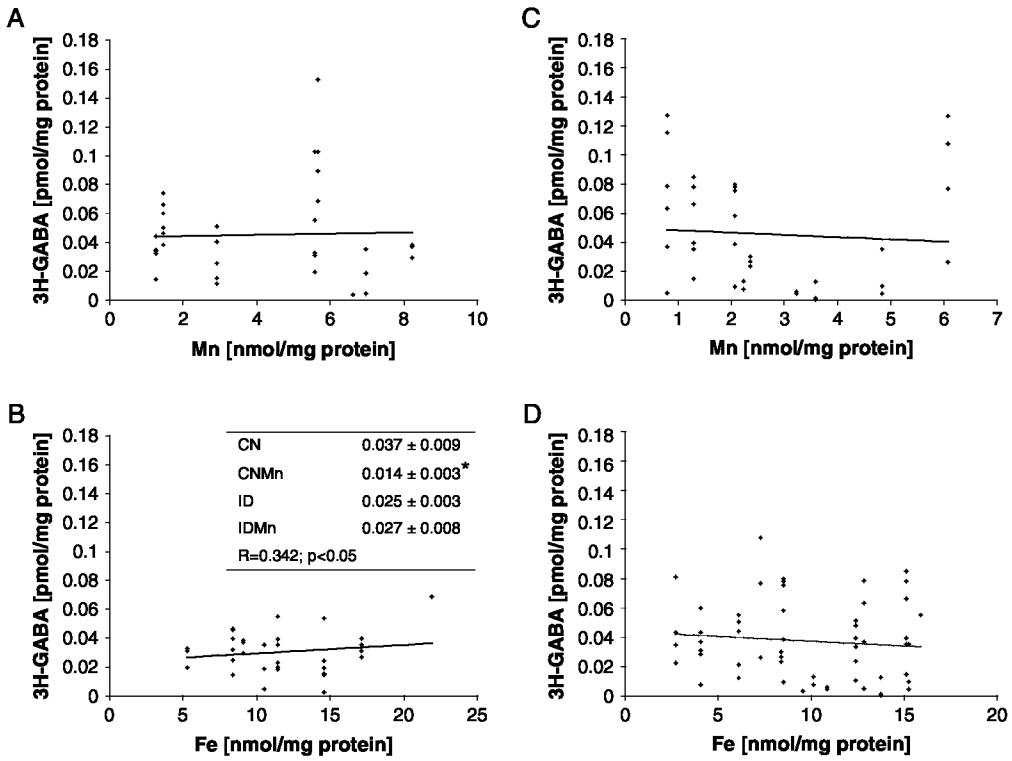

FIG. 3. Striatal synaptosome uptake of ^{3}H-GABA in male (A and B) and female (C and D) rats after 4 weeks of dietary intervention. (A) No significant correlation was observed between synaptosomal Mn concentration and ^{3}H-GABA uptake in male rats. (B) There was a significant positive correlation between synaptosomal Fe concentration and ^{3}H-GABA in male rats at 4 weeks ($R = 0.342$; $p < 0.05$) and the CNMn rats showed significantly decreased uptake as indicated by asterisk (*) ($p < 0.05$; see inset). No correlation between (C) synaptosomal Mn concentration and ^{3}H-GABA uptake (D) synaptosomal Fe concentration and ^{3}H-GABA uptake in female rats.

To our knowledge, this is the first study to examine this scenario. Mn accumulated in a heterogeneous fashion in the brain with supplemented animals accumulating significantly more Mn than CN animals in most brain regions (Figs. 2A and 2B). There was no main effect of sex on Mn accumulation, and the ID-associated Mn accumulation pattern was similar in the caudate putamen and globus pallidus. Specifically, the male and female

IDMn rats dramatically accumulated Mn in these two regions compared to CN rats. In a previous study, the effect of the dietary intervention on Mn accumulation in the male rat brain was examined and a similar accumulation was observed (Erikson et al., 2002). Although no statistically significant difference in Mn accumulation was observed between male and female rats, both genders had ID-associated Mn accumulation. How these changes in metal concentrations affect GABA uptake was our next question since decreased striatal GABA content (Erikson et al., 2002; Gwiazda et al., 2002) suggests altered reuptake GABA due to Mn exposure.

Previous studies suggest that decreased tissue levels of a neurotransmitter are due to decreased uptake of that neurochemical (reviewed in Gainetdinov et al., 1998). Knowing that Mn accumulation leads to decreased GABA levels in the striatum, we hypothesized that this effect is due to impaired uptake. Therefore, we set out to examine this relationship directly by measuring ^{3}H-GABA uptake in striatal synaptosomes. Synaptosomes were chosen due to their relative simplicity and their proven sensitivity to manipulation of neurotransmitter uptake. Dissociation from other interacting neuronal systems is advantageous in this case in order to provide direct interpretation of results. ID was associated with decreased ^{3}H-GABA uptake in male rats at 4 weeks (Fig. 3B). No significant correlation between Mn concentration and ^{3}H-GABA uptake was seen in either sex at 4 weeks (Figs. 3A and 3C). This finding was not surprising for we recently reported that 4 weeks of dietary treatment did not cause a significant accumulation of striatal Mn (Anderson et al., 2006). However, as reported here and previously (Erikson et al., 2002), 6 weeks of dietary treatment causes a significant accumulation of striatal Mn due to both ID and Mn supplementation (Figs. 2A and 2B). Thus, as expected Mn accumulation was associated with decreased ^{3}H-GABA uptake in both male and female rats after 6 weeks of dietary treatment (Figs. 4A and 4C).

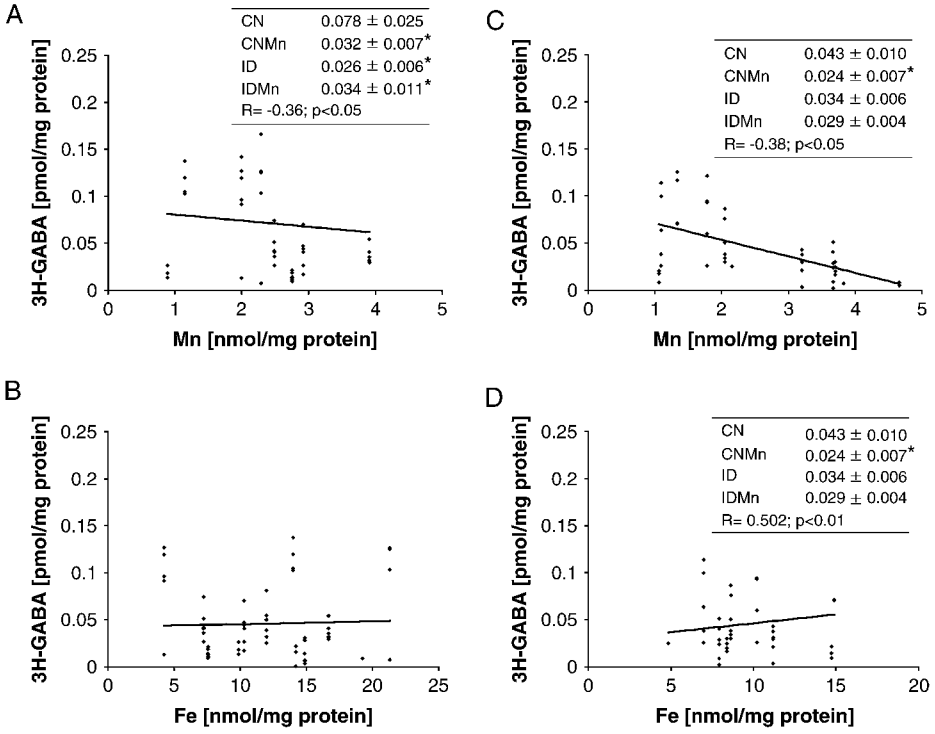

FIG. 4. Striatal synaptosome uptake of ^{3}H-GABA in male (A and B) and female (C and D) rats after 6 weeks of dietary intervention. (A) There was a significant negative correlation between synaptosomal Mn concentration and ^{3}H-GABA in male rats ($R = -0.356$; $p < 0.05$). ^{3}H-GABA uptake was significantly decreased ($p < 0.05$) in CNMn, ID, and IDMn versus CN as indicated by asterisk (*) (see inset). (B) No correlation between synaptosomal Fe concentration and ^{3}H-GABA uptake was observed in male rats. (C) There was a significant inverse correlation between synaptosomal Mn concentration and ^{3}H-GABA in female rats ($R = -0.380$; $p < 0.05$). ^{3}H-GABA uptake was significantly decreased ($p < 0.05$) in CNMn versus CN as indicated by asterisk (*) (see inset). (D) There was a significant positive correlation between synaptosomal Fe concentration and ^{3}H-GABA uptake in female rats ($R = 0.502$; $p < 0.01$).

Additionally, ID was associated with decreased ^{3}H-GABA up-take in female rats at 6 weeks (R = 0.502) ($p < 0.01$) (Fig. 4D). To our knowledge, this is the first report of impaired ^{3}H-GABA uptake in striatal synaptosomes derived from female rats fed an ID diet. Furthermore, the discovery that the male rats were impacted by ID in an opposite manner compared to females (i.e., 4 weeks of ID led to decreased uptake but 6 weeks had no effect)

clearly shows a sex difference in terms of the effect of ID on ^{3}H-GABA uptake. In this study, both male and female rats accumulated Mn due to IDMn treatment and this effect is likely causing the significant decrease in ^{3}H-GABA uptake ob-\served at 6 weeks in both sexes (Figs. 4A and 4C).

Decreased ^{3}H-GABA uptake could be a result of altered GABA transporter number and function. ID has been shown to affect DAT number and function (Erikson et al., 2000), and there may be a similar effect of ID on the GABA transporter. GABA transporter is a member of the SLC-6 transporter family that also contains DAT (Gether et al., 2006) and may have similar vulnerabilities to ID. In the current study, this question was probed pharmacologically with nipecotic acid, a GABA transporter inhibitor (data not shown). Treatment with the ED50 of nipecotic acid inhibited uptake of ^{3}H-GABA by at least 50% in all four treatment groups across both genders, indicating no change in drug efficacy or in transporter functioning. In other words, the treatment groups had decreased ^{3}H-GABA uptake compared to the CN group in both sexes before the addition of nipecotic acid to the synaptosomes, yet they responded to the drug in the same manner as CN, indirectly indicating decreased density of the GABA transporter. However, as this pharmacological approach is an indirect method of analyzing the functioning of the GABA transporter, future studies in our laboratory will explore this more directly using protein analysis (e.g., immunoblot) to assess density and radioligand-binding assays to assess functionality.

A decrease in GABA uptake would indicate increased extracellular GABA, which *in vivo* would lead to increased inhibitory activity leading to increased hypokinetic activity, a hallmark of Mn neurotoxicity. A study of Mn-exposed young adult rats observed a decrease in locomotor activity (Normandin et al., 2004). Motor deficits were also observed in a study utilizing a pre-Parkinsonian rat model and cumulative low-dose Mn exposure (Gwiazda et al., 2002). Furthermore, these changes did not alter dopamine, suggesting that changes in GABA precede changes in dopamine. Dystonia, a characteristic of Mn neurotoxicity (Cersosimo and Koller, 2006; Normandin et al., 2004), is associated with dysfunction of the globus pallidus (Calne et al., 1994), a GABAergic region of the brain and target of Mn accumulation during toxic exposure (Erikson et al., 2002; Fitsanakis and Aschner, 2005; Garcia et al., 2006).

In conclusion, ID affected ^{3}H-GABA uptake in male rats after 4 weeks of exposure and females after 6 weeks, demonstrating sex difference in the response to dietary ID in terms of a neurochemical outcome. ID-associated Mn accumulation was similar in the brains of both male and female rats, as was an inverse relationship between Mn and ^{3}H-GABA uptake observed in both sexes at 6 weeks supporting previous data illustrating disturbances in GABA levels as a result of Mn exposure. It is possible that alterations in dopamine metabolism observed in ID (Erikson et al., 2000, 2001) may be due to abnormal GABAergic firing as a result of Mn accumulation, supporting the hypothesis that disturbances in GABA may precede perturbations in dopamine metabolism as Mn accumulation is targeted to the globus pallidus (Figs. 2A and 2B) (Erikson et al., 2002), a GABA-rich brain region. Based on our findings in this study, we hypothesize that extracellular GABA (related to neurotransmission) levels are increased due to Mn accumulation compared to CN rats. Currently, there exists a lack of data evaluating the effect of ID and Mn on extracellular GABA concentrations *in vivo* which would reflect synaptic levels of GABA that modulate neurotransmission. Continuing studies in our laboratory will pharmacologically probe the effect of ID and Mn exposure on these extracellular GABA levels in order to fully understand the neurobiological consequences of Mn toxicity especially related to dietary ID.

REFERENCES

Anderson, J. G., Cooney, P. T., and Erikson, K. M. (2006). Inhibition of DAT function attenuates manganese accumulation in the globus pallidus. Environ. Toxicol. Pharmacol. Doi: 10.1016/j.etap.2006.08.006.
Ashkenazi, R., Ben-Shachar, D., and Youdim, M. B. H. (1982). Nutritional iron and dopamine binding sites in the rat brain. Pharmacol. Biochem. Behav. 17 (Suppl.), 43–47.
Beard, J. L. (2001). Iron biology in immune function, muscle metabolism, and neuronal functioning. J. Nutr. 131, 568s–579s.
Beard, J. L., Chen, Q., Connor, J. R., and Jones, B. C. (1994). Altered monoamine metabolism in caudate putamen of iron-deficient rats. Pharmacol. Biochem. Behav. 48, 621–624.

Beard, J. L, Connor, J. R., and Jones, B. C. (1993). Iron in the brain. Nutr. Rev. 51,157–170.
Beard, J. L., Erikson, K. M., and Jones, B. C. (2002). Neurobehavioral analysis of developmental iron deficiency in rats. Behav. Brain Res. 134, 517–524.
Beleboni, R. O., Carolino, R. O. G., Pizzo, A. B., Castellan-Baldam, L., Coutinho-Netto, J., dos Santos, W. F., and Coimbra, N. C. (2004). Pharmacological and biochemical aspects of GABAergic neurotransmission: Pathological and neuropsychobiological relationships. Cell. Mol. Neurobiol. 24,707–728.
Burhans, M. S., Dailey, C., Beard, Z., Wiesinger, J., Murray-Kolb, L., Jones, B. C., and Beard, J. L. (2005). Iron deficiency: Differential effects on monoamine transporters. Nutr. Neurosci. 8, 31–38.
Calne, D. B., Chu, N. S., Huang, C. C., Lu, C. S., and Olanow, W. (1994). Manganism and idiopathic parkinsonism: Similarities and difference. Neurology 44, 1583–1586.
Carlsson, M., and Carlsson, A. (1990). Interactions between glutamatergic and monoaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. Trends Neurosci. 13, 272–276.
Cersosimo, M. G., and Koller, W. C. (2006). The diagnosis of manganese-induced parkinsonism. Neurotoxicology 27, 340–346.
Chandra, S. V., Malhotra, K. M., and Shukla, G. S. (1982). GABAergic neurochemistry in manganese exposed rats. Acta Pharmacol. Toxicol. 51, 456–458.
Chen, Q., Beard, J. L., and Jones, B. C. (1995). Abnormal brain monoamine metabolism in iron deficiency anemia. J. Nutr. Biochem. 6, 486–493.
Conrad, M. E., Umbreit, J. N., Moore, E. G., Hainsworth, L. N., Porubcin, M., Simovich, M. J., Nakada, M. T., Dolan, K., and Garrick, M. D. (2000). Separate pathways for cellular uptake of ferric and ferrous iron. Am. J. Physiol. Gastrointest. Liver Physiol. 279, G767–G774.
Cotman, C. W., Foster, A. C., and Lanhorn, T. (1981). An overview of glutamate as a neurotransmitter. Adv. Biochem. Psychopharmacol. 27, 1–27.
Crowe, A., and Morgan, E. H. (1992). Iron and transferrin uptake in brain and cerebrospinal fluid in the rat. Brain Res. 592, 8–16.
Davis, C., Wolf, T., and Greger, J. (1992). Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats. J. Nutr. 122,1300–1308.
Erikson, K. M., Jones, B. C., and Beard, J. L. (2000). Iron deficiency alters dopamine transporter functioning in rat striatum. J. Nutr. 130, 2831–2837.
Erikson, K. M., Jones, B. C., Hess, E. J., Zhang, Q., and Beard, J. L. (2001). Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. Pharmacol. Biochem. Behav. 69,409–418.
Erikson, K. M., Pinero, D. J., Connor, J. R., and Beard, J. L. (1997). Regional brain iron, ferritin, and transferrin concentrations during iron deficiency and iron repletion in developing rats. J. Nutr. 127, 2030–2038.
Erikson, K. M., Shihabi, Z. K., Aschner, J. L., and Aschner, M. (2002). Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biol. Trace Elem. Res. 87, 143–156.
FAO/WHO. (2004). Vitamin and Mineral Requirements in Human Nutrition: A Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand, 21–30 September 1998. FAO/WHO, ROME/Geneva, p. 341.
Finley, J. W. (1999). Manganese absorption and retention by young women is associated with serum ferritin concentration. Am. J. Clin. Nutr. 70, 37–43.
Fitsanakis, V. A., and Aschner, M. (2005). The importance of glutamate, glycine, and y-aminobutyric acid transport and regulation in manganese, mercury, and lead neurotoxicity. Toxicol. Appl. Pharmacol. 204, 343–354.
Gainetdinov, R. R., Jones, S. R., Fumagalli, F., Wightman, R. M., and Caron, M. G. (1998). Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis. Brain Res. Brain Res. Rev. 26, 148–153.
Garcia, S. J., Gellein, K., Syversen, T., and Aschner, M. (2006). A manganese enhanced diet alters brain metals and transporters in the developing rat. Toxicol. Sci. 92, 516–525.
Gether, U., Andersen, P. H., Larsson, O. M., and Schousboe, A. (2006). Neurotransmitter transporters: Molecular function of important drug targets. Trends Pharmcol. Sci. 27, 375–383.
Gwiazda, R. H., Lee, D., Sheridan, J., and Smith, D. R. (2002). Low cumulative manganese exposure affects striatal GABA but not dopamine. Neuro-toxicology 23, 69–76.

Kwik-Uribe, C. L., Golub, M. S., and Keen, C. L. (1999). Chronic marginal iron intakes during early development in mice alter brain iron concentrations and behavior despite postnatal iron supplementation. J. Nutr. 130, 2040–2048.
Li, D. (1998). Effects of iron deficiency on iron distribution and y-aminobutyric acid (GABA) metabolism in young rat brain tissues. Hokkaido J. Med. Sci. 73, 215–225.
Lipe, G. W., Duhart, H., Newhart, G. D., Slikker, W., Jr, and Ali, S. F. (1999). Effect of manganese on the concentration of amino acids in different regions of the rat brain. J. Environ. Sci. Health B 34, 119–132.
Looker, A. C., Dallman, P. R., Carroll, M. D., Gunter, E. W., and Johnson, C. L. (1997). Prevalence of iron deficiency in the United States. JAMA 277, 973–976.
Malecki, E. A., Devenyi, A. G., Beard, J. L., and Connor, J. R. (1999). Existing and emerging mechanisms for transport of iron and manganese to the brain. J. Neurosci. Res. 56, 113–122.
Montes, S., Alcaraz-Zubeldia, M., Muriel, P., and Rios, C. (2000). Striatal manganese accumulation induces changes in dopamine metabolism in the cirrhotic rat. Brain Res. 891, 123–129.
Morse, A. C., Beard, J. L., Azar, M. R., and Jones, B. C. (1999). Sex and genetics are important cofactors in assessing the impact of iron deficiency on the developing mouse brain. Nutr. Neurosci. 2, 323–325.
Nelson, C., Erikson, K., Pinero, D. J., and Beard, J. L. (1997). In vivo dopamine metabolism is altered in iron deficient anemic rats. J. Nutr. 127, 2282–2288.
Normandin, L., Beaupre, L. A., Salehi, F., St Pierre, A., Kennedy, G., Mergler, D., Butterworth, R. F., Philippe, S., and Zayed, J. (2004). Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. Neuro-toxicology 25, 433–441.
Olsen, R. W., and DeLorey, T. M. (1999). Basic Neurochemistry, GABA and glycine, in basic neurochemistry. (G. J. Siegel, B. W. Agranoff, R. W. Albers, S. K. Fisher, and M. D. Uhler, Eds.), pp. 335–346. Lipincott Williams and Wilkins, Philadelphia, PA.
Pinero, D. J., Li, N. Q., Connor, J. R., and Beard, J. L. (2000). Variations in dietary iron alter brain iron metabolism in developing rats. J. Nutr. 130, 254–263.
Roth, J. A., and Garrick, M. D. (2003). Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. Biochem. Physiol. 66,1–13.
Takeda, A., Sotogaku, N., and Oku, N. (2002). Manganese influences the levels of neurotransmitters in synapse in rat brain. Neuroscience 114, 669–674.
Takeda, A., Sotogaku, N., and Oku, N. (2003). Influence of manganese on the release of neurotransmitters in rat striatum. Brain Res. 965, 279–282.
Verity, M. A. (1999). Manganese neurotoxicity: A mechanistic hypothesis. Neurotoxicology 20, 489–498.
Youdim, M. B. H., Ben-Shachar, D., and Yehuda, S. (1989). Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. Am. J. Clin. Nutr. 50, 607–617.