Manganese Accumulates in Iron-Deficient Rat Brain Regions in a Heterogeneous Fashion and Is Associated with Neurochemical Alterations

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Abstract:
Previous studies have shown that iron deficiency (ID) increases brain manganese (Mn), but specific regional changes have not been addressed. Weanling rats were fed one of three semipurified diets: control (CN), iron deficient (ID), or iron deficient/manganese fortified (IDMn+). Seven brain regions were analyzed for Mn concentration and amino acid (glutamate, glutamine, taurine, γ-aminobutyric acid) concentrations. Both ID and IDMn+ diets caused significant (p<0.05) increases in Mn concentration across brain regions compared to CN. The hippocampus was the only brain region in which the IDMn+ group accumulated significantly more Mn than both the CN and ID groups. ID significantly decreased GABA concentration in hippocampus, caudate putamen, and globus pallidus compared to CN rats. Taurine was significantly increased in the substantia nigra of the IDMn+ group compared to both ID and CN. ID also altered glutamate and glutamine concentrations in cortex, caudate putamen, and thalamus compared to CN. In the substantia nigra, Mn concentration positively correlated with increased taurine concentration, whereas in caudate putamen, Mn concentration negatively correlated with decreased GABA. These data show that ID is a significant risk factor for central nervous system Mn accumulation and that some of the neurochemical alterations associated with ID are specifically attributable to Mn accumulation.
Index Entries: Rat; brain; iron deficiency; manganese; GABA; glutamate.

Article:
INTRODUCTION
Iron deficiency (ID) is one of the most prevalent nutritional problems in the world, affecting approximately 2 billion people *(1).* ID is also a known risk factor for metal toxicity (e.g., cadmium, aluminum, lead) *(2–4),* with an inverse relationship existing between dietary iron (Fe) content and the gastrointestinal absorption of a host of other metals. Fe also shares similar absorption mechanisms with essential divalent metals, particularly manganese (Mn) *(5),* and a deficiency of one of these minerals in the diet can lead to excess absorption of the other mineral. For example, high Fe intakes decrease gastrointestinal Mn absorption, whereas ID leads to increased Mn absorption, *(6,7).* In addition, rats exposed to high doses of Mn exhibit altered brain Fe metabolism *(8).* Furthermore, ID is associated with increased Mn accumulation in the brain *(9,10);* however, brain regional changes are unknown.

Both Mn and Fe transport to extrahepatic tissues, including the brain, is dependent on transferrin-mediated endocytosis *(11,12).* Because ID causes increased brain regional transferrin (Tf) and transferrin receptor (TfR) concentrations in a heterogeneous fashion *(13–15),* it is likely that ID-associated brain Mn accumulation occurs in a heterogeneous manner. High levels of manganese in globus pallidus, caudate putamen, and sub-thalamic nuclei have contributed to the vulnerability of the striatum to manganese toxicity *(16,17).* Accordingly, a goal of this study was to investigate if ID is associated with increased accumulation of Mn, especially in brain regions that inherently contain high Mn levels (e.g., striatum).

The neurobiological sequelae of ID include alterations in behavior, cognition, and neurotransmitter metabolism *(18).* Over three decades ago, ID was linked to decreased dopamine D2 receptors in striatum *(19,20),* and, presently, it is well established that ID leads to increased extracellular dopamine *(21–23)* and decreased dopamine transporter and dopamine receptor functioning *(24,25).* There is growing evidence that ID is associated with perturbation in additional neurotransmitter levels [e.g., γ-aminobutyric acid (GABA), glutamate, and serotonin] *(26).* However, a direct effect of ID on these neurotransmitters has yet to be determined. Accordingly, a secondary goal of this study was to examine the effect of ID and/or Mn accumulation on brain regional concentrations of GABA, glutamate, and aspartate.

Glutamate is the most prevalent excitatory neurotransmitter in the brain *(27),* whereas GABA is the most abundant inhibitory neurotransmitter in the brain *(28).* Cortical glutamate afferents project into the striatum where, in concert with GABA and dopamine, motor behaviors are controlled *(29).* Glutamate is converted to GABA by decarboxylation via glutamate decarboxylase (GAD) and is degraded via GABA-transaminase. Altered GABA metabolism as a result of ID has been shown *(26),* but absolute changes in GABA concentration were not detected. Further-more, Li's study demonstrated increased glutamate concentration as a result of ID, caused by decreased GAD activity. Reports of GABA concentrations in the rat brain upon manganese exposure are inconsistent. For example, exposure to 6 mg Mn/kg/d led to a significant increase in brain manganese concentrations and significant decrease in GABA concentrations *(30).* Another report showed that rats exposed to 20 mg Mn/kg/d had significantly increased brain manganese and GABA concentrations *(31).* Accordingly, 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.

Taurine is present in high concentrations in the mammalian central nervous system (CNS), particularly in the striatum. It has been implicated as a neuroprotectant *(32)* and a possible neurotransmitter. Past studies have focused on its relationship with GABA in striatum and substantia nigra *(33).* Briefly, taurine injected into rat substantia nigra induces inhibition of dopamine neurons *(34)* and GABA-like behaviors *(35).* Furthermore, taurine has been shown to interact with $GABA_A$ receptors *(36),* which are abundant in the substantia nigra. Mn exposure has been shown to increase taurine transporter expression in astrocytes *(37)* and increase taurine concentration in the cerebellum *(31),* potentially representing adaptive responses to Mn-induced oxidative damage *(38,39).*

The first goal of this study was to examine the effect of ID on Mn accumulation in seven brain regions: cortex, cerebellum, hippocampus, caudate putamen, globus pallidus, substantia nigra, and thalamus. These brain regions include those known to be vulnerable to Mn toxicity (e.g., globus pallidus, caudate putamen, thalamus), as well as regions less sensitive to Mn (e.g., cortex). We also included an ID diet fortified with 100 mg Mn/kg diet (10 mg Mn/kg diet is the recommended level) to examine the interaction between dietary Mn and brain Mn concentration in response to ID. The working hypothesis was that ID will lead to Mn accumulation in the brain and that an ID diet that is fortified with Mn will not increase brain Mn accumulation beyond levels that are associated with ID *per se.* Furthermore, we hypothesized that ID will increase Mn accumulation in Mn-sensitive "target" regions (i.e., thalamus, caudate putamen, and globus pallidus) to a greater extent than Mn-insensitive "nontarget" (i.e., cortex) brain regions. The final goal of this study was to measure neurochemical changes in these seven brain regions as a result of ID and to determine if Mn accumulation correlates with these changes.

MATERIALS AND METHODS

Animals

Male 21-d-old Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were randomly divided into three dietary treatment groups: control (CN; 35 mg Fe/kg, 10 mg Mn/kg diet), iron deficient (ID; 4 mg Fe/kg, 10 mg Mn/kg diet), and iron deficient/manganese supplemented (IDMn+; 4 mg Fe/kg, 100 mg Mn/kg diet). Diets were obtained from Bio-Serv (Frenchtown, NJ) and certified for mineral content. Rats had free access to food and water 24 h/d, and the lights were turned off between 1800 and 0600 h. Room temperature was maintained at 25 ± 1°C. After 6 wk of dietary treatment, the rats were killed and their brains were removed for

mineral and neurochemical analyses. The Wake Forest University School of Medicine Animal Care and Use Committee approved all of the animal procedures.

Hematological Measurements

Hematocrit was measured weekly via blood samples acquired by tail prick. It was 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 hemoglobin (procedure no. 525; Sigma Chemical, St. Louis, MO) measurements. 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 Fe and transferrin (Tf). Plasma Fe was measured with the ADVIA 1650^{TM} (Bayer Corp., Tarrytown, NY) and Tf by a turbidimetric immunoassay *(40).*

Brain Mn

Brains were dissected into seven regions: caudate putamen, globus pallidus, thalamus, hippocampus, substantia nigra, cerebellum, and cortex. Neutron activation analysis (NAA) was used to measure Mn and Fe in the brain regions. Unfortunately, NAA was not sufficiently sensitive enough to detect Fe in all brain regions. Therefore, only Mn concentrations are reported. Samples were irradiated for 60 s each in PULSTAR pneumatic terminus at 900 kW_{th}. Samples were decayed for approximately 60 min and counted for 10 min each on a gamma spectroscopy system analyzing for Mn.

Amino Acid Analysis

Amino acid standards were purchased from Sigma Chemicals (Sigma Aldrich, Natick, MA). Brain amino acid concentrations were measured according to a published method *(41).* Briefly, tissues were homogenized in 4°C absolute methanol (#A 454-4; Fisher Scientific, Fairlawn, NJ). Homoserine was added as an internal standard and several concentrations of the amino acids were added to brain extracts and processed in parallel for quantifying unknowns. Homogenates were centrifuged at 1000 g for 10 min and the supernatants were dried at 37°C under a stream of dry nitro-gen. The dried samples were stored at –20°C until analyzed. Samples were reconstituted with methanol (A 454-4) and reacted with OPT reagent (100 mg *O*-pthaldialdehyde in 0.5 mL methanol, 100 µL of 2-mercaptoethanol in 1.9 mL of 0.4 *M* borate, pH 9.5) for 2 min and injected into a gradient high-performance liquid chromatography (HPLC) system (Gilson Model 201) using an autoinjector (Gilson 401 and 231). A C_{18} reversed-phase column (0.46 × 15 cm, 5 µm) was used with fluorometric detection (Gilson 121) (excitation 305–395 nm, 430–470 nm). The mobile phase was 0.1 *M* sodium acetate, pH 6.2, containing 0.1 µ*M* EDTA and increasing concentrations of methanol (15–50%) with a 1.3-mL/p min flow rate. Retention times in minutes were as follows: aspartate (Asp), 2.35; glutamate (glu), 3.47; glutamine (gln), 7.9; homoserine (Hser), 9.9; glycine (Gly), 11.16; taurine (Taur), 17.0; γ-amino butyric acid (GABA), 20.2.

Statistical Analysis

The data were analyzed with the SAS system for Windows v6.12 statistical analysis package (SAS, Cary, NC). Data were examined for normality of distribution and presence of outliers. Data two standard deviations above or below the mean were considered outliers. One-way analysis of variance was used to test for the effects of dietary treatment on clinical parameters. Simple repeated-measures analysis of variance with repeated-measures factors (brain regions) and between-groups factors (dietary treatment) was used to test for interactions between dietary treatments and brain regions for amino acid and mineral data. Tukey's HSD post hoc test was used to evaluate mean differences. The α 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 and hemoglobin were significantly lower in the ID and IDMn+ groups, indicating severe anemia (Table 1). Furthermore, plasma transferrin (higher than controls) and iron (lower than controls) concentrations were characteristic of anemia in the ID and IDMn+ groups (Table 1) and consistent with earlier reports of ID *(13–15,42).*

Table 1
Body Weight and Hematological Parameters

Diet		Body Wt	Hb	Hct	Plasma Fe	Plasma Tf
		(grams)	(g/L)		(μg/L)	(mg/L)
CN	**Mean**	**277. 1**	**160.3**	**0.45**	**44.8**	**121.3**
	SEM	5.5	8.5	0.02	5.1	3.4
ID*	**Mean**	**201.8**	**45.2**	**0.17**	**12.4**	**301.6**
	SEM	4.1	1.3	0.01	2.2	8.1
IDMn+*	**Mean**	**205.7**	**46**	**0.16**	**12.9**	**295.3**
	SEM	4.2	3.4	0.01	1.4	14.6

Note: Data are mean ± SEM. CN = control, ID = iron deficient, IDMn+ = iron deficient/high manganese. Asterisk * denotes significantly different, $p < 0.05$, from control.

Brain Regional Mn Concentrations

Both iron-deficient diets (ID and IDMn+) caused a significant increase ($p<0.05$) in Mn concentration in all brain regions compared to controls (CN), except substantia nigra, where the trend was similar but the p-value was not statistically significant ($p=0.091$) (Table 2). The IDMn+ diet did not significantly increase Mn concentration in any brain region compared to the ID diet, except in the hippocampus (CN<ID<IDMn+).

Table 2
Brain Regional Mn Concentration

Diet		HC	Cx	Cb	Th	CP	GP	SN
		μg Mn/g tissue	μg Mn/g tissue	μg Mn/g tissue	μg Mn/g tissue	μg Mn/g tissue	μg Mn/g tissue	μg Mn/g tissue
CN	**Mean**	**0.308^{a}**	**0.374^{a}**	**0.359^{a}**	**0.444^{a}**	**0.375^{a}**	**0.261^{a}**	**0.443**
	SEM	0.014	0.022	0.029	0.011	0.016	0.084	0.046
ID	**Mean**	**0.407^{b}**	**0.403^{a,b}**	**0.471^{b}**	**0.644^{b}**	**0.500^{b}**	**0.468^{b}**	**0.559**
	SEM	0.019	0.027	0.012	0.041	0.043	0.022	0.069
IDMn+	**Mean**	**0.479^{c}**	**0.491^{b}**	**0.442^{b}**	**0.663^{b}**	**0.449^{b}**	**0.500^{b}**	**0.667**
	SEM	0.022	0.024	0.009	0.051	0.024	0.036	0.092

Note: Data are mean ± SEM. CN = control, ID = iron deficient, IDMn+ = iron deficient/high manganese. Brain regions are hippocampus (HC), cortex (Cx), cerebellum (Cb), thalamus (Th), caudate putamen (CP), globus pallidus (GP), and substantia nigra (SN). Superscript letters signify mean differences within regions as determined by Tukey's HSD post hoc analysis, $p < 0.05$.

Brain Regional Amino Acid Concentrations

Amino acid concentrations in the seven brain regions are summarized in Table 3. The cortex, glutamate, and glutamine were significantly decreased ($p<0.05$) in both ID and IDMn+ groups compared to CN. However, glutamate concentration was significantly higher ($p<0.05$) in the caudate putamen of IDMn+ rats compared to ID and CN. Glutamine and GABA concentrations were significantly reduced ($p<0.05$) in the caudate putamen of ID and IDMn+ rats compared to CN. GABA concentration was also decreased in the hippocampus (ID and IDMn+) and the globus pallidus (IDMn+) compared to CN rats ($p<0.05$). Taurine concentration in the substantia nigra of IDMn+ rats was significantly higher ($p<0.05$) than in CN and ID rats.

Correlational Analysis

Only two significant relationships emerged when Mn concentration was correlated with each of the amino acids in all of the brain regions. Mn concentration significantly correlated with increased taurine concentration in the substantia nigra ($r = 0.65$; $p < 0.05$) (Fig. 1), and in the caudate putamen, increased Mn negatively correlated with GABA concentration ($r = –0.54$; $p < 0.05$) (Fig. 2).

Table 3
Brain Regional Amino Acids

Amino Acid	Diet	HC	Cx	Cb	Th	CP	GP	SN
Glutamate (nmol/g tissue)	**CN**	1733±38	1647±57[a]	1600±44	1188±31	1185±79[b]	1065±99	1055±145
	ID	1792±168	1421±15[b]	1631±121	1152±93	1163±31[b]	1293±175	699±95
	IDMn+	1948±97	1465±45[b]	1696±52	1041±29	1611±67[a]	1112±136	875±121
Glutamine (nmol/g tissue)	**CN**	993±23	862±35[a]	1063±33	723±11[a]	1137±46[a]	685±51	747±92
	ID	878±84	620±27[b]	949±31	594±44[b]	814±48[c]	550±124	553±51
	IDMn+	798±10	617±34[b]	748±130	567±18[b]	964±63[b]	567±64	677±66
Taurine (nmol/g tissue)	**CN**	981±42	792±59	1014±13	405±46	1306±46	1295±274	449±28[b]
	ID	1140±132	841±35	876±49	469±35	1320±27	868±181	424±15[b]
	IDMn+	1151±68	846±50	922±27	415±26	1500±108	1048±126	550±21[a]
GABA (nmol/g tissue)	**CN**	748±55[a]	399±14	403±5	546±54	715±21[a]	360±48[a]	996±105
	ID	515±27[b]	360±17	345±8	702±69	538±8[c]	432±16[a]	918±63
	IDMn+	556±6[b]	333±19	343±43	586±89	594±20[b]	257±29[b]	1175±26

Note: Data are mean ± SEM. CN = control, ID = iron deficient, IDMn+ = iron deficient/high manganese. Brain regions are hippocampus (HC), cortex (Cx), cerebellum (Cb), thalamus (Th), caudate putamen (CP), globus pallidus (GP), and substantia nigra (SN). Superscript letters signify mean differences within regions as determined by Tukey's HSD post hoc analysis, $p < 0.05$.

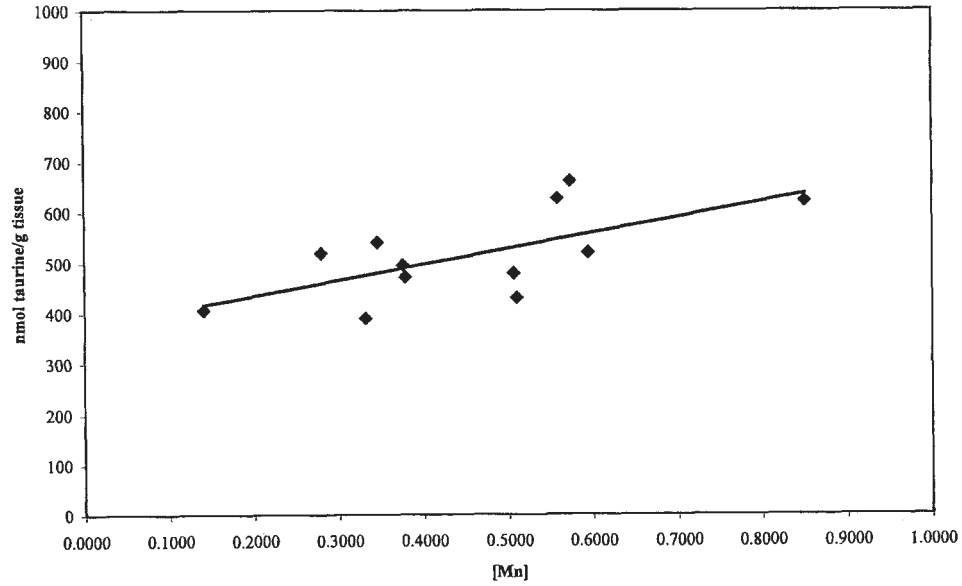

Fig. 1. Analysis of manganese concentration with taurine concentration in the substantia nigra reveals a significant positive correlation ($r = 0.65$, $p < 0.05$).

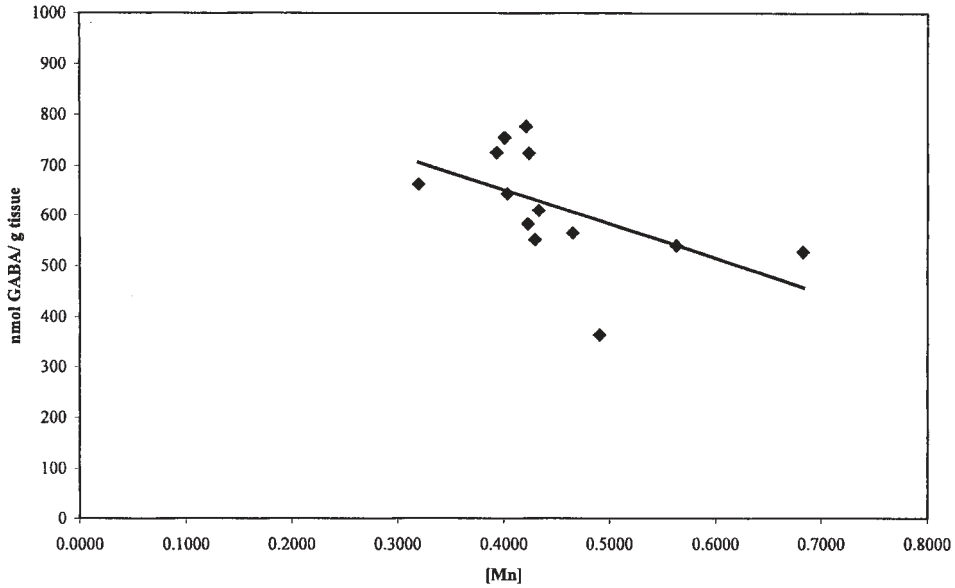

Fig. 2. Analysis of Mn concentration with γ-amino butyric acid (GABA) concentration in the caudate putamen reveals a significant negative correlation ($r = 0.54$, $p < 0.05$).

DISCUSSION

Several novel findings emerged from this study. First, although ID caused a significant increase in brain Mn concentration, increased dietary Mn (IDMn+ diet) did not increase brain Mn concentration beyond the level associated with ID, except in the hippocampus. Second, ID led to brain-region-specific alterations in amino acid concentrations (e.g., GABA concentration was significantly decreased in hippocampus, caudate putamen, and globus pallidus, but not other regions). Finally, increased taurine in the substantia nigra was highly correlated with Mn concentration.

Although it was previously shown that ID increases Mn concentration in rat brain *(9,10)*, this is the first study to assess ID-associated region-specific changes in brain Mn levels. ID led to a significant increase ($p<0.05$) in the Mn concentration of all brain regions, except substantia nigra ($p=0.091$) (Table 2).

Brain regional Mn concentrations were measured with NAA, which offers highly sensitive detection for Mn. Unfortunately, NAA has a very low sensitivity for Fe detection. Nevertheless, Fe measurements were detected in cerebellum, hippocampus, cortex, and thalamus and were similar to levels reported in the literature *(14,15,24).* ID caused a 20–40% decrease in Fe concentration in these brain regions, with hippocampus showing the greatest reduction in iron concentration.

Iron deficiency leads to a significant increase in transferrin (Tf), transferrin receptor (TfR), and a decrease in Fe concentrations in various rat brain regions *(13–15).* Therefore, because ID causes increased Tf and TfR heterogeneously in the brain, it was logical to expect increased Mn in the brain in a heterogeneous manner, given earlier findings that link Mn trans-port to TfR endocytosis *(43).* Although we did not measure brain Tf levels in these rats, it is interesting to note that ID increased hippocampal and cortical Tf more robustly than other regions *(14)* and that the IDMn+ diet caused a significant increase in Mn compared to the ID diet exclusively in these two regions (Table 2). Hence, ID-induced Mn accumulation occurred in a heterogeneous fashion in the brain, with regions that have been shown to respond profoundly to ID with increased Tf and TfR, accumulating more Mn.

An additional novel finding of these studies suggests that even at nor-mal dietary Mn concentration, ID is sufficient to induce brain regional Mn accumulation (i.e., increased dietary manganese [IDMn+ diet] was not needed to raise regional Mn concentrations). The data also corroborate other studies that have shown that even under extreme conditions of Mn exposure, brain Mn concentration were not increased beyond twofold to threefold *(44).* The public health ramification of this observation is pro-found, for segments of the population in which ID is most prevalent (pregnant women, adolescents, toddlers) are at risk for increased brain Mn accumulation, even in the absence of environmental Mn exposure.

Numerous reports show that ID is associated with disturbances in neurotransmitter metabolism. For example, ID is linked to decreased dopamine D_2 and D_1 receptor densities *(19,20,25),* increased extracellular dopamine *(21–23),* and decreased dopamine uptake and dopamine transporter density *(24).* Similarly, manganese neurotoxicity is associated with altered neurotransmitter metabolism. Manganese is linked to alterations of dopamine, glutamate, and GABA metabolism in rats *(16,31,45,46).*

In addition to the known disturbances in dopamine metabolism, ID affects GABA metabolism in rat brain tissues *(26).* Whereas the absolute GABA concentration was not different from control levels, GAD activity was significantly lower in ID rats *(26).* In contrast, we found that both ID diets (ID and IDMn+) caused a significant decrease in GABA concentration in the hippocampus and caudate putamen, whereas only the IDMn+ diet decreased GABA concentration in the globus pallidus (Table 3). The level of ID was more severe in our rats than in Li's (e.g., Hb 46 g/L vs 84 g/L; Hct 16% vs 31%, respectively). Therefore, because our study employed a more severe ID condition, we observed a significant ID-dependent decrease in GABA concentration and Li *(26)* did not, even though the overall amino acid concentrations in each brain region tested were similar in both studies. Consistent with the caudate putamen's known sensitivity to Mn-induced alterations in neurochemistry *(16,45),* GABA concentration in this brain region negatively correlated with Mn concentration (Fig. 2). This finding is in accordance with a previous study in which Mn accumulation in rat brains led to decreased GABA concentrations *(30).* It is also important to note that the increased dopamine levels in caudate putamen observed in ID rats *(21–23)* may be associated with decreased GABA concentration. It is well established that striatonigral GABAergic neurons are inhibitory neurons, which project onto the substantia nigra. Thus, it is probable that decreased striatal GABA concentration is indicative of attenuated inhibitory signals to the nigrostriatal dopaminergic pathway (i.e., substantia nigra), thereby contributing to the observed elevation of dopamine levels in caudate putamen.

Glutamate (Glu) is increased in striatum of ID rats *(26).* This ID-associated increase in striatal Glu was the result of decreased GAD (rate-limiting enzyme in GABA formation) activity, according to Li *(26).* Our study showed that in caudate putamen, the IDMn+ diet caused a significant increase in Glu, but both ID diets caused a decrease in cortical Glu levels. Because glutamatergic afferents projecting into striatum control motor behavior,

decreased cortical Glu could exacerbate and/or precipitate motor behavioral changes associated with ID *(20,25,47,48).* A plausible explanation for this significant decrease in cortical Glu concentration is that ID-induced Mn accumulation caused attenuation of Glu recycling. It has been suggested that Glu uptake by astrocytes, its conversion to glutamine (Gln) via glutamine synthetase, and deamination to Glu is the primary Glu recycling pathway *(49–51).* Indeed, Mn exposure is associated with decreased Glu uptake in astrocytes *(37,52).* Furthermore, the decreased Gln observed in caudate putamen, cortex, and thalamus associated with ID might be caused by Mn-induced attenuation of astrocytic Glu uptake. The implications of the potential neurotoxic effects of this increased Glu *(53)* secondary to ID are profound and extend our knowledge of neurobiological sequelae of ID from primarily neurochemical to neurotoxic.

Taurine concentration was significantly increased in substantia nigra resulting from the IDMn+ diet compared with CN or ID diets. There is no known link between ID and taurine levels in the brain. However, Mn is linked with increased taurine transporter gene expression in astrocytes *(37).* Taurine is one of the most abundant amino acids in the brain, particularly in the striatum. It is thought to be a neuroprotectant, as well as a putative neurotransmitter *(33).* Its role as a neurotransmitter is putatively mediated via interactions with GABAA receptors *(36).* A potential explanation for the significant correlation between Mn and taurine concentration in substantia nigra is that Mn accumulation leads to an adaptive response (i.e., increased taurine) *(39),* especially in the substantia nigra, an area that is vulnerable to oxidative processes (e.g., Parkinson's disease).

In conclusion, the present study establishes that ID leads to brain Mn accumulation in a heterogeneous fashion. Further, the IDMn+ diet does not enhance brain Mn accumulation beyond the ID diet *per se*, except in the hippocampus, a region shown to respond vigorously to ID with an increase in Tf and TfR *(14,15).* Our data unequivocally support a role for Fe in the metabolism of certain amino acid neurotransmitters, namely glutamate and GABA. Both amino acids are critical for proper functioning of the striatum, which is known to be perturbed in ID. Thus, this study broadens the understanding of neurochemical alterations in ID from being solely associated with dopamine to identifying changes in glutamate and GABA metabolism as a consequence of ID. Additionally, the significant correlation between caudate putamen concentration of GABA and Mn warrant further investigation, particularly because this relationship is brain region-specific (i.e., ID-associated Mn accumulation may account for some of the neurochemical alterations formerly attributed to brain Fe loss). Finally, taurine concentration was significantly correlated with Mn concentration in the substantia nigra, potentially representing a neurochemical change that is specific to combined ID and Mn fortification (i.e., not observed with ID alone). The ID diets utilized in this study were sufficient to ascertain changes in brain Mn and amino acid transmitters associated with the lack of Fe. A less severe ID diet (10–15 mg Fe/kg diet) will be tested in future studies to examine these issues under less physiologically extreme conditions.

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