Changes in Dietary Iron Exacerbate Regional Brain Manganese Accumulation as Determined by Magnetic Resonance Imaging (MRI).

By: Vanessa A. Fitsanakis, Na Zhang, Malcolm J. Aviso, Keith M. Erikson, John C. Gore and Michael Aschner

Fitsanakis, V.A., Zhang, N., Avison, M.J., Erikson, K.M., Gore, J.C., Aschner, M. (2011) Changes in Dietary Iron Exacerbate Regional Brain Manganese Accumulation as Determined by Magnetic Resonance Imaging (MRI). *Toxicological Sciences* 120(1): 146-153.

Abstract:

Manganese (Mn) is an essential metal required for normal homeostasis. Humans chronically exposed to high Mn levels, however, may exhibit psychomotor signs secondary to increased brain Mn. As Mn and iron (Fe) share several cellular membrane transporters, decreased Fe levels resulting from Fe deficiency or anemia lead to increased brain Mn deposition. Conversely, decreased Mn levels are associated with abnormal brain Fe accumulation. To reduce potential Mn toxicity resulting from brain Mn accumulation, we proposed that increased dietary Fe would attenuate brain Mn deposition. To test this hypothesis, three groups of Sprague-Dawley rats were injected weekly (14 weeks) with Mn (3 mg/kg) and fed normal Fe (TX), Fe-supplemented (FeS), or Fe-deficient (FeD) chow. Control (CN) rats received normal dietary Fe and saline injections. Using magnetic resonance imaging, rats were imaged biweekly for 14 weeks to qualitatively monitor brain Mn and Fe accumulation. Both FeS and FeD had greater brain Mn deposition than TX rats. By week 3, R1 values, which correlate with Mn deposition, were statistically significantly increased ($p < 0.05$) in brain stem, cerebellum, cortex, midbrain, and striatum compared with CN or TX animals. By week 14, R1 values for all brain regions in FeS and FeD animals were statistically significantly increased ($p < 0.05$). By the end of the study, similar results were obtained for R2 values, a marker of Fe accumulation. These data suggest that Fe supplementation does not effectively protect and may even exacerbate brain Mn accumulation in mammals subchronically exposed to Mn.

Keywords: manganese | dietary iron | magnetic resonance imaging | rats | neuroscience | nutrition | biology

Article:

Although manganese (Mn) is an essential metal required for proper growth and homeostasis (Wedler, 1994), exposure to high levels of this micronutrient can lead to a neurological disorder, termed manganism, which shares multiple clinical similarities with Parkinson's disease (Calne et

al., 1994; Pal et al., 1999). Early symptoms of manganism include psychiatric changes, such as increased anxiety and impairments in learning and memory. As the disease progresses, the basal ganglia become affected, resulting in motor involvement manifesting as tremors and rigidity. Once motor functions deteriorate, the disease is often irreversible, even if the patient is removed from the source of Mn exposure (Pal et al., 1999). Typically, humans occupationally exposed to Mn from welding (Josephs et al., 2005; Racette et al., 2005), Mn mining (Myers et al., 2003a), or smelting (Myers et al., 2003b) are at the greatest risk for the disease when Mn exposure approaches 5 mg Mn/m3 (National Institute for Occupational Safety and Health, 2005). Additionally, neonates and older populations administered total parenteral nutrition (Fitzgerald et al., 1999) or patients suffering from liver disease (Klos et al., 2005; Park et al., 2003) may accumulate abnormally high concentrations of brain Mn.

Rodents, nonhuman primates, and humans suffering from iron (Fe) deficiency or anemia have greater propensity to accumulate Mn in several tissues, including the brain (Erikson et al., 2004; Park et al., 2007b). This is particularly alarming as approximately 2 billion people worldwide suffer from Fe deficiency or Fe-deficient (FeD) anemia (de Benoist et al., 2008). Additionally, numerous countries are either considering the addition of methylcyclopentadienyl Mn tricarbonyl to gasoline as an antiknock agent replacement for lead or they have already implemented this practice (Boudia et al., 2006; Rollin et al., 2005). Such usage could lead to a scenario whereby human populations are chronically exposed from birth to high levels of atmospheric Mn (Boudia et al., 2006; Rollin et al., 2005).

Although numerous studies have examined brain Mn accumulation at various and discrete time points in numerous tissues (Erikson et al., 2007; Park et al., 2007a), few have simultaneously followed brain Mn and/or Fe deposition during a subchronic exposure paradigm. Furthermore, only a small number of studies have examined the effects of dietary Fe on brain Mn deposition (Chua and Morgan, 1997). Accordingly, the present study was designed to test the following hypothesis: Rodents subchronically exposed to Mn and fed an FeD diet would have greater brain Mn deposition compared with animals fed a diet with normal Fe concentrations. Additionally, an Fe-supplemented (FeS) diet would result in brain Mn levels more consistent with those of animals receiving saline rather than Mn injections. The kinetic profile of brain Mn accumulation over the course of the 14-week experimental period was conducted by means of magnetic resonance imaging (MRI). A final objective of this study was to determine whether changes in Fe status result in differential accumulation of brain Mn.

MATERIALS AND METHODS

Animals.

All animal protocols were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (240–250 g) were ordered from Harlan (Indianapolis, IN). Rats had free access to food and water during the 14-week experimental protocol. Control (CN; n = 5) and Mn-treated (TX; n = 6) rats received normal rodent chow containing 30 mg Fe/kg and 10 mg Mn/kg chow. Similar to other studies from our laboratory (Erikson et al. 1997, 2002, 2004; Fitsanakis et al. 2008), two additional groups received diets with modified Fe levels: the FeD (FeD; n = 6) group was fed a specially formulated diet with reduced levels of Fe (3 mg Fe/kg chow) and the FeS (n = 4) group received a diet with 300 mg Fe/kg chow. Both Fe-modified diets were formulated and assayed for Fe content by BioServ (Frenchtown, NJ) and were designed to provide 10× less (FeD) or 10× more (FeS) Fe than the normal daily allowances for rodents (National Research Council, 1995), whereas Mn levels in the chow remained unchanged. In addition to dietary modifications, animals from the three treatment groups (TX, FeD, and FeS) received weekly intravenous (iv) tail injections of a sterile, isotonic Mn solution (3 mg Mn/kg) of $MnCl_2$ for a total of 14 weeks. CN rats received injections of similar volumes of sterile, isotonic saline. Although humans are generally exposed to Mn via inhalation, this results in rapid entry into the blood stream. As such, iv exposure was chosen for this subchronic study. Rats were weighed weekly as a gross measure of general health.

Magnetic resonance imaging.

Upon arrival at Vanderbilt, rats were allowed to adjust to their new environment for 4–6 days prior to any handling or testing. Following this acclimation period, all animals were imaged as a baseline prior to initiation of their respective treatments. Rats were also imaged every 2 weeks, starting at week 1 of the treatment (weeks 1, 3, 5, 7, 9, 11, and 13). Finally, they were imaged at week 14, the last week of the study. In order to maintain consistency during the study, animals were always imaged 24 h after the respective week's injection of $MnCl_2$ or saline.

During the imaging, animals were anesthetized with 2% isoflurane and placed in a stereotaxic support cradle with their heads secured by surgical adhesive tape. The cradle was put in the volume coil to ensure that the rat's head was located in the coil′s center. Isoflurane was then lowered to 1.5–1.75%, and this ventilation mixture was maintained for the duration of the scanning protocol, usually 1.5 h, to ensure an appropriate level of unconsciousness sufficient to prevent duress in the animals. During the scan, body temperature was maintained at 37°C with warm air controlled by a rectal temperature probe (SA Instruments). Respiration was monitored throughout and maintained at 50–70 breaths per minute.

All experiments were acquired using a 4.7-T, 31-cm bore Varian INOVA magnet with actively shielded gradients (40 G/cm, rise time full amplitude of 130 μs) and 63 mm transmit/receive quadrature imaging volume coil of Litz design with high B1 homogeneity over the rat brain. Rat brains were scanned from both axial (field of view [FOV] = 40 × 40 mm, 30 slices) and coronal directions (FOV = 40 × 50 mm, 20 slices) with 0.75 mm slice thickness. T1 was measured by progressive saturation (multiple flip angles) using a standard Varian 2-D multislice Fast Low Angle Shot sequence with radio frequency (RF) and gradient spoiling: repetition time/echo time = 489/6.59 ms; flip angles = 10°, 30°, 55°, or 70°; 2 acquisitions; and image matrix = 256 × 256. T2 was measured using a multislice multi-cecho fast spin echo sequence with parameters as follows: TR = 5100 ms; echo train length = 8; k-space center = 4; echo spacing = 5, 6.7, 10, 13, and 15 ms (effective TE = 20, 26.8, 40, 52, and 60 ms); 2 acquisitions; and image matrix = 128 × 128.

Brain dissection.

At the conclusion of the study (week 14), rats were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg). When animals no longer responded to deep toe pinch, they were rapidly decapitated. Brains were removed and dissected into the following regions: cerebellum, brain stem (medulla and pons), midbrain, hippocampus, striatum, and cortex. The regions, which corresponded to those selected for imaging analysis, were quick frozen in dry ice and stored at −80°C until metal analysis by atomic absorption spectroscopy (AAS).

Brain metal analysis by AAS.

Tissue Mn and Fe concentrations were measured with graphite furnace AAS (Varian AA240, Varian, Inc.). Brain regions were digested in ultrapure nitric acid (1:10 wt/vol dilution) for 48–72 h in a sand bath (60°C). One hundred microliters of digested tissue was brought to 1 ml total volume with 2% nitric acid and analyzed for Mn and Fe. Bovine liver (10 μg Mn/l) was digested in ultrapure nitric acid and used as an internal standard for analysis.

Image analysis.

T1 and T2 maps were calculated by fitting the series of T1- and T2-dependent images to the appropriate theoretical expressions using two-parameter least squares fits. The parametric maps were then co-registered to a high-resolution rat template and resliced using statistical parametric mapping (http://www.fil.ion.ucl.ac.uk/spm). Based on the rat brain template, multislice regions

of interest (ROIs) were chosen for seven brain regions: cerebellum, brain stem, midbrain, striatum, hippocampus, cortex, and olfactory bulbs. Averaged values for the longitudinal relaxation rates, R1 (1/T1), and transverse relaxation rates, R2 (1/T2), within each of the ROIs were calculated for each rat and used in further statistical analyses.

Statistical analyses.

One-way ANOVA, with Bonferonni′s posttest, was used to compare brain metal data or total body mass gain for all four groups. All analyses were completed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA; http://www.graphpad.com). Either Tukey's honestly significant difference technique or Dunnet's post hoc analyses were used for multiple comparisons of the magnetic resonance (MR) data by treatment groups or weeks. All data were accepted as statistically significant if $p < 0.05$.

RESULTS

Changes in Body Mass

Rats were dosed as outlined in “Materials and Methods” section. The Mn dose was chosen to approximate blood Mn levels observed in populations demonstrating Mn toxicity (Hauser et al., 1996; Kim et al., 2005) without causing acute and overt toxicity in the rats. Beginning at week 3 and continuing throughout the duration of the study, animals receiving the diet with normal Fe levels along with Mn injections (TX), those animals with an FeS diet receiving Mn injections (FeS), and those on an FeD diet that received Mn injections (FeD) all gained significantly less body mass compared with CN animals ($p < 0.001$; Fig. 1). Rats that received altered Fe levels (FeD and FeS) also gained significantly more than TX animals ($p < 0.001$; Fig. 1). Furthermore, although animals did not undergo a battery of behavioral tests, they were routinely handled during weighing and MR scans. In conjunction with these interactions, animals were carefully observed for increased tendency to bite handlers or alterations in walking pattern. None of the animals from any treatment groups (TX, FeS, or FeD) demonstrated overt changes in behavior (increased aggressiveness, abnormal posturing, and gait disturbances) sometimes associated with end-stage Mn toxicity.

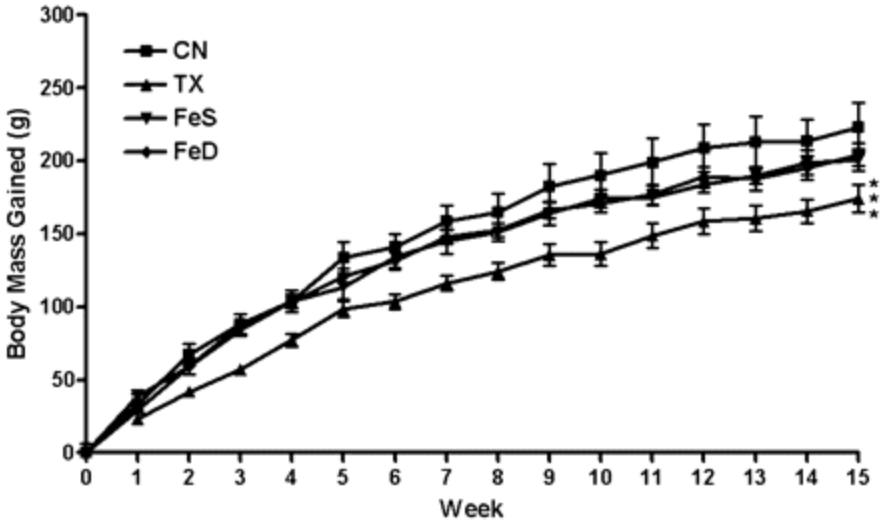


FIG. 1.

Total body mass gain—Rats in each group were weighed weekly as a general assessment of overall health. Only the group receiving normal dietary levels of Fe and injected with Mn (TX) gained statistically significantly less body mass compared with CN rats (***$p < 0.001$) or TX rats (^^^$p < 0.001$). Data are normalized to average body mass at week 0 and presented as mean body mass gained ± SEM.

Regional Changes in Longitudinal (R1) and Transverse (R2) Relaxation Rates

We previously showed that both R1 and R2 depend in a complex way on tissue Mn and Fe levels and on the numbers of available binding sites for which Mn and Fe compete (Zhang et al., 2009). Nonetheless, these studies demonstrated that, under most conditions, regional increases in R1 generally correlated with brain Mn deposition. It must be noted, however, that a quantitative determination of changes in tissue Mn from changes in R1 and/or R2 is complicated by the influence of concomitant changes in tissue Fe, as well as regional variations in the molar relaxivities of both species and in the concentration of available binding sites. We therefore monitored changes in R1 as a qualitative measure of brain changes in Mn deposition, inferring only that significant changes in R1 in a given region reflected significant changes in local Mn concentration. Following the first Mn injection at week 1, there were statistically significant increases ($p < 0.05$) in R1 values in all brain regions (Figs. 2A–F), except the striatum (Fig. 2G), in rats from all treated groups (TX, FeS, and FeD) compared with CN. By week 3, R1 values from all brain regions, except hippocampus (Fig. 2D) and olfactory bulb (Fig. 2F), in the FeS and FeD groups were statistically significantly higher ($p < 0.05$) compared with the TX group. The significantly increased R1 values persisted throughout the 14-week study period for all groups compared with the initial scan at week 0. Notably, the longitudinal relaxation rates from the discrete brain regions monitored in treated animals generally increased from the initial measured

values at week 0. This is in contrast to R1 values for CN animals, which remained unchanged during the course of the study.

Figure 2 has been omitted from this formatted document.

Whereas it is well known that high tissue Fe concentrations lead to increased R2 and associated hypointensity on T2 and -weighted MRIs (McNeill et al., 2008), increases in tissue Mn can also significantly increase R2, and these effects may dominate (Zhang et al., 2009). By week 7, R2 values for the brain stem, cerebellum, cortex, and hippocampus of animals in the FeS and FeD groups were statistically significantly increased ($p < 0.05$) compared with CN (Figs. 3A–D); no increases were observed in TX animals. In the midbrain and striatum, statistically significant increases in R2 values were apparent by week 5 (Figs. 3E and 3G), whereas increases in the olfactory bulbs of FeS and FeD animals were statistically significantly increased ($p < 0.01$) at week 3 (Fig. 3F) compared with CN. These increases in R2 values typically persisted throughout the 14-week study period. Compared with week 0, the mean differences in transverse relaxation rates for each brain region and each group did not reach the level of statistical significance until week 13.

Figure 3 has been omitted from this formatted document.

Regional Brain Metal Levels

At the conclusion of the study, regional brain Mn (Fig. 4) and Fe (Fig. 5) levels were measured by AAS. Mn accumulation in the brain stem, striatum, and cortex from FeS and FeD animals was statistically significantly higher than both CN and TX animals ($p < 0.05$; Fig. 4). This was not the case for the hippocampus, however, where Mn accumulation was significantly lower compared with TX ($p < 0.001$; Fig. 4). Additionally, compared with CN and TX animals, rats in both the FeS and FeD groups had statistically significantly lower Fe accumulation in the cerebellum and cortex ($p < 0.05$; Fig. 5).

FIG. 4.

Brain Mn content—AAS was used to determine the amount of Mn in discrete brain regions at the conclusion of the study. The following symbols are used to denote statistical significance: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with CN; ^$p < 0.05$, ^^$p < 0.01$, and ^^^$p < 0.001$ compared with TX.

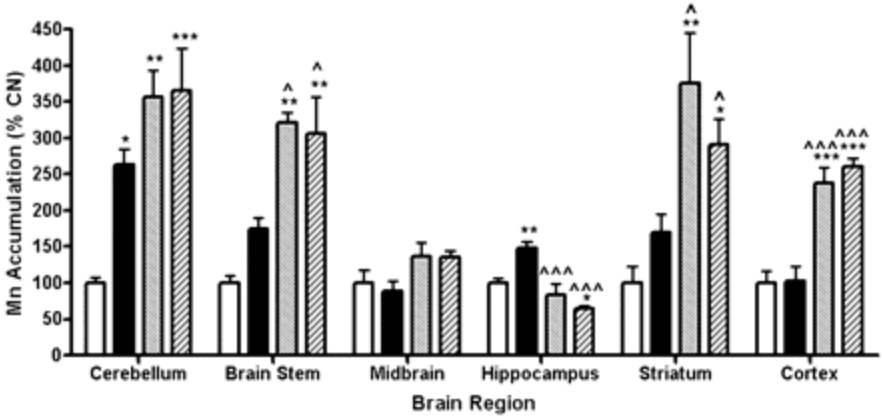
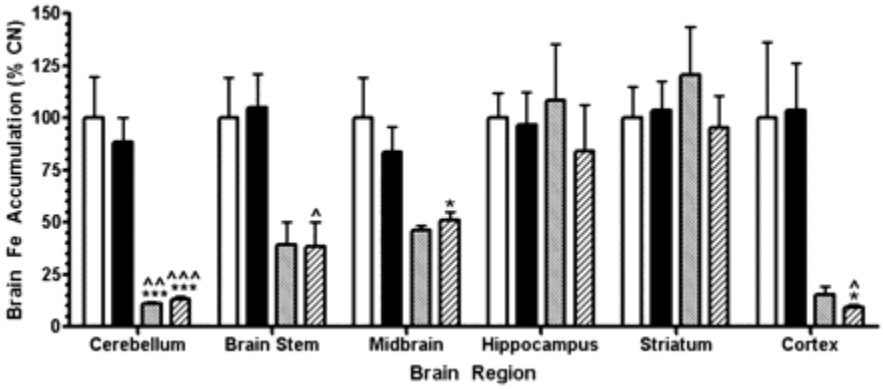


FIG. 5.

Brain Fe content—AAS was used to determine the amount of Fe in discrete brain regions at the conclusion of the study. The regions listed correspond to those analyzed by MR throughout the time course of the study. The following symbols are used to denote statistical significance: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with CN; ^$p < 0.05$, ^^$p < 0.01$, and ^^^$p < 0.001$ compared with TX.



DISCUSSION

Although rats have dietary metal requirements that exceed those of humans (National Research Council, 1995), rats have been used extensively as a model organism for both Fe and Mn regulation in nutritional and toxicological studies (Anderson et al., 2007; Carpenter, 2001; Chua and Morgan, 1996, 1997; Davis et al., 1990). Additionally, it is well established in both rats and humans that Mn and Fe share common transport proteins (Forbes and Gros, 2003; Wang et al., 2006) and that Mn homeostasis is affected by Fe levels (Chua and Morgan, 1996; Garcia et al., 2006). Similarly, when Mn levels are disturbed, Fe homeostasis is also disrupted (Chen et al., 2006; Garcia et al., 2006). In light of this inverse relationship, our original hypothesis was as follows: Because Fe deficiency, even in the absence of Mn exposure (Erikson et al., 2002, 2004), leads to increased brain Mn deposition, dietary Fe supplementation will decrease brain Mn

accumulation. Interestingly, a literature review indicated a gap in our knowledge related to how Fe supplementation might affect the regulation of other trace metals in the brain.

To test this hypothesis, we used noninvasive MRI over the course of a 14-week, subchronic study to determine regional R1 and R2 values for rats in four distinct groups (CN, TX, FeS, and FeD). These relaxation rates have been used in clinical and research settings to follow brain Mn (R1) (Kim, 2004; Zhang et al., 2009) and brain Fe (R2) deposition (McNeill et al., 2008; Zhang et al., 2009). The quantitative interpretation, however, of R1 and R2 changes in terms of changes in regional Mn and Fe is complex and beyond the scope of this study. Nonetheless, a unique strength of MRI R1 and R2 mapping for assessing qualitative changes in heavy metal deposition lies in the fact that each animal can be used as its own CN, thus requiring fewer total animals and reducing the effects of intersubject variance in longitudinal studies.

The present study offers novel information on the temporal accumulation of brain Mn and its relationship to dietary Fe levels. Indication of statistically significant increases in Mn accumulation (vs. CN) began at week 1 in all brains regions, except the striatum (Fig. 2G), as suggested by increased longitudinal relaxation rates (R1; Figs. 2A–F). Interestingly, motor signs in latter stages of manganism rely on the involvement of the basal ganglia, including the striatum (Pal et al., 1999). Furthermore, increased Mn deposition, as suggested by R1 values, persisted throughout the study (week 14). Because the tissue Fe concentrations measured in brain regions collected from all three Mn treatment groups immediately following the last MRI measurements were the same as (hippocampus and striatum), or lower than (cerebellum, brain stem, midbrain, and cortex), but never higher than, in CN (Fig. 5), we interpreted the persistent increases in regional R1 for all three treatment groups as a reflection of regional increases in regional Mn levels. Consistent with this interpretation, Mn levels were increased or unchanged in all but the hippocampus (Fig. 4). The R1 changes were most pronounced in animals fed Fe-modified diets, either FeS or FeD. Indeed, TX animals (normal dietary Fe) typically did not demonstrate statistically significant increases in R1 values until either week 3 (brain stem; Fig. 2A) or week 5 (cortex; Fig. 2C).

Fe deficiency, both in humans and rodents, leads to an increase in brain Mn deposition (Erikson et al., 2004; Park et al., 2007b). Thus, it was not unexpected that brain Mn levels were increased in FeD rats (Fig. 2). Others have reported that Fe deficiency not only leads to the upregulation of divalent metal transporter-1 (DMT-1) and transferrin (Tf; Siddappa et al., 2003) but also stabilizes messenger RNA (mRNA) of various Fe regulating proteins (IRPs) with Fe regulating elements (IRE-1 or IRE-2). These nucleic acid (mRNA) and protein modifications are thought to regulate overall Fe homeostasis. Many of the IRPs, including DMT-1 and Tf, are also known to

transport Mn (Au et al., 2008) and are upregulated in the brain following conditions of Fe deficiency (Erikson and Aschner, 2006; Garcia et al., 2007). Thus, when Fe concentrations are low, more Mn is likely transported into the brain because of decreased competition from blood or plasma Fe and increased expression of DMT-1 and Tf (Garcia et al., 2007). Our data extend these observations by demonstrating that, as early as week 3, combining an FeD diet with low doses of Mn is sufficient to significantly increase R1 values above those observed in animals on a normal diet (Fig. 2). This phenomenon persists throughout the exposure period, such that by week 14 the R1 values for all brain regions are significantly increased in FeD compared with TX animals ($p < 0.01$; Fig. 2). These increases were corroborated by means of AAS, showing elevated Mn levels in the cortex, striatum, brain stem, and cerebellum in the FeD and TX groups (Fig. 4).

Although it has been suggested that changes in brain Fe levels can be tracked using T2-weighted or R2 and mapping MR protocols (Kruit et al., 2008), our recent work (Zhang et al., 2009) has demonstrated that a complex multisite model is required to describe the quantitative dependence of regional tissue R1 and R2 on tissue Fe and Mn. Consistent with this, it is noteworthy that in both FeS and FeD groups, R2 values increased or remained unchanged in regions showing significant decreases in tissue Fe, suggesting that R2 lacks specificity for Fe changes, and indeed can be dominated by concomitant increases in tissue Mn. This study did, however, confirm that R1 changes provide a better qualitative method for tracking changing Mn levels noninvasively in vivo, albeit with questions remaining regarding the specificity of the measurement. As demonstrated in Figure 3, there was generally no statistically significant difference between R2 values from CN and TX animals throughout the course of the study. The exceptions occur in the midbrain, olfactory bulbs, and striatum (Figs. 3E–G), where TX groups reach a statistically significant increase in R2 values ($p < 0.05$) by week 13, again consistent with an increase in tissue Mn.

As mentioned previously, there is an interdependent relationship between Mn and Fe transport. As a result, it was anticipated that animals injected with Mn would show increased brain Mn and decreased Fe accumulation. Consistent with the postmortem findings that Mn levels were increased or unchanged, R1 and R2 values increased in FeD (and FeS) animals (Figs. 2 and 3), whereas tissue Fe levels measured at the end of the study were unchanged or decreased. Whereas our data do not provide direct evidence for a mechanism of action, recent in vitro data suggest that decreased Fe leads to increases in functional ferroportin (Yin et al., 2009), which then promotes the extrusion of Fe from internal stores (De Domenico et al., 2007).

It is more difficult to explain the brain Mn accumulation in animals receiving an Fe-fortified diet, although this has been reported previously by others (Chua and Morgan, 1996). Fe supplementation, however, results in the degradation of ferroportin. Thus, when dietary Fe levels are high, there is decreased cellular Fe efflux, ultimately resulting in decreased Fe availability for Tf/Tf receptor (TfR) transport (Rossi, 2005). As Tf/TfR is known to transport Mn (Li et al., 2006; Takeda et al., 2000), decreased extracellular Fe (from ferroportin degradation) may result in increased Mn accumulation via Tf/TfR (Roth, 2006). Thus, as extracellular Fe concentrations decrease, it is possible that brain Mn transport increases. Although this hypothesis is certainly testable, it was beyond the scope of the work presented here.

In summary, our data indicate that both subchronic Fe supplementation and deficiency coupled with a low dose of Mn lead increased brain Mn deposition. Whereas Fe deficiency, in the absence of Mn exposure, can result in increased brain Mn deposition, Fe supplementation does not ameliorate this accumulation. Future research should continue to focus on mechanisms related to Mn dysregulation in the presence of changes in dietary Fe.

FUNDING

U.S. Department of Defense (W81XWH-05-1-0239 to M.A.); National Institutes of Health (1R15NS061309-01 to K.M.E., R01EB000461 to J.C.G.).

References

Anderson JG, Cooney PT, Erikson KM. Brain manganese accumulation is inversely related to gamma-amino butyric acid uptake in male and female rats. Toxicol. Sci. 2007;95:188-195.

Au C, Benedetto A, Aschner M. Manganese transport in eukaryotes: the role of DMT1. Neurotoxicology 2008;29:569-576.

Boudia N, Halley R, Kennedy G, Lambert J, Gareau L, Zayed J. Manganese concentrations in the air of the Montreal (Canada) subway in relation to surface automobile traffic density. Sci. Total Environ. 2006;366:143-147.

Calne DB, Chu NS, Huang CC, Lu CS, Olanow W. Manganism and idiopathic parkinsonism: similarities and differences. Neurology 1994;44:1583-1586.

Carpenter DO. Effects of metals on the nervous system of humans and animals. Int. J. Occup. Med. Environ. Health 2001;14:209-218.

Chen MT, Cheng GW, Lin CC, Chen BH, Huang YL. Effects of acute manganese chloride exposure on lipid peroxidation and alteration of trace metals in rat brain. Biol. Trace Elem. Res. 2006;110:163-178.

Chua A, Morgan E. Effects of iron deficiency and iron overload on manganese uptake and deposition in the brain and other organs of the rat. Biol. Trace Elem. Res. 1996;55:39-54.

Chua A, Morgan E. Manganese metabolism is impaired in the Belgrade laboratory rat. J. Comp. Physiol. B 1997;167:361-369.

Davis CD, Ney DM, Greger JL. Manganese, iron and lipid interactions in rats. J. Nutr. 1990;120:507-513.

de Benoist B, McLean E, Egll I, Cogswell M, editors. Worldwide Prevalence of Anaemia 1993-2005. New York, NY: World Health Organization; 2008.

De Domenico I, Ward DM, Langelier C, Vaughn MB, Nemeth E, Sundquist WI, Ganz T, Musci G, Kaplan J. The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol. Biol. Cell. 2007;18:2569-2578.

Erikson K, Shihabi Z, Aschner J, Aschner M. Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biol. Trace Elem. Res. 2002;87:143-156.

Erikson KM, Aschner M. Increased manganese uptake by primary astrocyte cultures with altered iron status is mediated primarily by divalent metal transporter. Neurotoxicology 2006;27:125-130.

Erikson KM, Dorman DC, Lash LH, Aschner M. Manganese inhalation by rhesus monkeys is associated with brain regional changes in biomarkers of neurotoxicity. Toxicol. Sci. 2007;97:459-466.

Erikson KM, Pinero DJ, Connor JR, Beard JL. Regional brain iron, ferritin and transferrin concentrations during iron deficiency and iron repletion in developing rats. J. Nutr. 1997;127:2030-2038.

Erikson KM, Syversen T, Steinnes E, Aschner M. Globus pallidus: a target brain region for divalent metal accumulation associated with dietary iron deficiency. J. Nutr. Biochem. 2004;15:335-341.

Fitsanakis VA, Zhang N, Anderson JG, Erikson KM, Avison MJ, Gore JC, Aschner M. Measuring brain manganese and iron accumulation in rats following 14 weeks of low-dose manganese treatment using atomic absorption spectroscopy and magnetic resonance imaging. Toxicol. Sci. 2008;103:116-124.

Fitzgerald K, Mikalunas V, Rubin H, McCarthey R, Vanagunas A, Craig RM. Hypermanganesemia in patients receiving total parenteral nutrition. JPEN J. Parenter. Enteral. Nutr. 1999;23:333-336.

Forbes J, Gros P. Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2 (Slc11a2) expressed at the plasma membrane. Blood 2003;102:1884-1892.

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

Garcia SJ, Gellein K, Syversen T, Aschner M. Iron deficient and manganese supplemented diets alter metals and transporters in the developing rat brain. Toxicol. Sci. 2007;95:205-214.

Hauser RA, Zesiewicz TA, Martinez C, Rosemurgy AS, Olanow CW. Blood manganese correlates with brain magnetic resonance imaging changes in patients with liver disease. Can. J. Neurol. Sci. 1996;23:95-98.

Josephs KA, Ahlskog JE, Klos KJ, Kumar N, Fealey RD, Trenerry MR, Cowl CT. Neurologic manifestations in welders with pallidal MRI T1 hyperintensity. Neurology 2005;64:2033-2039.

Kim Y. High signal intensities on T1-weighted MRI as a biomarker of exposure to manganese. Ind. Health 2004;42:111-115.

Kim Y, Park JK, Choi Y, Yoo CI, Lee CR, Lee H, Lee JH, Kim SR, Jeong TH, Yoon CS, et al. Blood manganese concentration is elevated in iron deficiency anemia patients, whereas globus pallidus signal intensity is minimally affected. Neurotoxicology 2005;26:107-111.

Klos KJ, Ahlskog JE, Josephs KA, Fealey RD, Cowl CT, Kumar N. Neurologic spectrum of chronic liver failure and basal ganglia T1 hyperintensity on magnetic resonance imaging: probable manganese neurotoxicity. Arch. Neurol. 2005;62:1385-1390.

Kruit MC, Launer LJ, Overbosch J, van Buchem MA, Ferrari MD. Iron accumulation in deep brain nuclei in migraine: a population-based magnetic resonance imaging study. Cephalalgia 2008;29:283-285.

Li GJ, Choi BS, Wang X, Liu J, Waalkes MP, Zheng W. Molecular mechanism of distorted iron regulation in the blood-CSF barrier and regional blood-brain barrier following in vivo subchronic manganese exposure. Neurotoxicology 2006;27:737-744.

McNeill A, Birchall D, Hayflick SJ, Gregory A, Schenk JF, Zimmerman EA, Shang H, Miyajima H, Chinnery PF. T2* and FSE MRI distinguishes four subtypes of neurodegeneration with brain iron accumulation. Neurology 2008;70:1614-1619.

Myers JE, teWaterNaude J, Fourie M, Zogoe HB, Naik I, Theodorou P, Tassel H, Daya A, Thompson ML. Nervous system effects of occupational manganese exposure on South African manganese mineworkers. Neurotoxicology 2003a;24:649-656.

Myers JE, Thompson ML, Ramushu S, Young T, Jeebhay MF, London L, Esswein E, Renton K, Spies A, Boulle A, et al. The nervous system effects of occupational exposure on workers in a South African manganese smelter. Neurotoxicology 2003b;24:885-894.

National Institute for Occupational Safety and Health. NIOSH Pocket Guide to Chemical Hazards. Atlanta, GA: Department of Health and Human Services (Ed.). Centers for Disease Control and Prevention; 2005.

National Research Council. Nutrient Requirements of Laboratory Animals. 1995. Nutrient requirements of the laboratory rat. (Committee on Animal Nutrition, Subcommittee on Laboratory Animal Nutrition, Board of Agriculture, Eds.). pp. 11–79. National Academy Press, Washington, DC.

Pal P, Samii A, Calne D. Manganese neurotoxicity: a review of clinical features, imaging and pathology. Neurotoxicology 1999;20:227-238.

Park JD, Chung YH, Kim CY, Ha CS, Yang SO, Khang HS, Yu IK, Cheong HK, Lee JS, Song CW, et al. Comparison of high MRI T1 signals with manganese concentration in brains of cynomolgus monkeys after 8 months of stainless steel welding-fume exposure. Inhal. Toxicol. 2007a;19:965-971.

Park JD, Kim KY, Kim DW, Choi SJ, Choi BS, Chung YH, Han JH, Sung JH, Kwon IH, Mun JH, et al. Tissue distribution of manganese in iron-sufficient or iron-deficient rats after stainless steel welding-fume exposure. Inhal. Toxicol. 2007b;19:563-572.

Park NH, Park JK, Choi Y, Yoo CI, Lee CR, Lee H, Kim HK, Kim SR, Jeong TH, Park J, et al. Whole blood manganese correlates with high signal intensities on T1-weighted MRI in patients with liver cirrhosis. Neurotoxicology 2003;24:909-915.

Racette BA, Tabbal SD, Jennings D, Good L, Perlmutter JS, Evanoff B. Prevalence of parkinsonism and relationship to exposure in a large sample of Alabama welders. Neurology 2005;64:230-235.

Rollin H, Mathee A, Levin J, Theodorou P, Wewers F. Blood manganese concentrations among first-grade schoolchildren in two South African cities. Environ. Res. 2005;97:93-99.

Rossi E. Hepcidin—the iron regulatory hormone. Clin. Biochem. Rev. 2005;26:47-49.

Roth JA. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. Biol. Res. 2006;39:45-57.

Siddappa AJ, Rao RB, Wobken JD, Casperson K, Leibold EA, Connor JR, Georgieff MK. Iron deficiency alters iron regulatory protein and iron transport protein expression in the perinatal rat brain. Pediatr. Res. 2003;53:800-807.

Takeda A, Ishiwatari S, Okada S. Influence of transferrin on manganese uptake in rat brain. J. Neurosci. Res. 2000;59:542-552.

Wang X, Li GJ, Zheng W. Upregulation of DMT1 expression in choroidal epithelia of the blood-CSF barrier following manganese exposure in vitro. Brain Res. 2006;1097:1-10.

Wedler F. Biochemical and nutritional role of manganese: an overview. In: Klimis-Tavantzis D, editor. Manganese in Health and Disease. 1994. Chapter 1, pp. 1--38. CRC Press, Boca Raton, FL.

Yin Z, Jiang H, Lee ES, Ni M, Erikson KM, Milatovic D, Bowman AB, Aschner M. Ferroportin is a manganese-responsive protein that decreases manganese cytotoxicity and accumulation. J. Neurochem. 2009;112:1190-1198.

Zhang N, Fitsanakis VA, Erikson KM, Aschner M, Avison MJ, Gore JC. A model for the analysis of competitive relaxation effects of manganese and iron in vivo. NMR Biomed. 2009;22:391-404.