Manganese Accumulation in the Olfactory Bulbs and Other Brain Regions of “Asymptomatic” Welders

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Received November 2, 2010; accepted January 31, 2011

Welding-generated metallic fumes contain a substantial amount of manganese (Mn), making welders susceptible to Mn toxicity. Although overt Mn toxicity manifests as a type of parkinsonism, the consequences of chronic, low-level Mn exposure are unknown. To explore region-specific Mn accumulation and its potential functional consequences at subclinical levels of Mn exposure, we studied seven welders without obvious neurological deficits and seven age- and gender-matched controls. Mn exposure for welders was estimated by an occupational questionnaire. High-resolution brain magnetic resonance imaging (MRI), Grooved Pegboard performance of both hands, Trail making, and olfactory function tests were obtained from all subjects. Compared with controls, the welders had a significantly higher T1 relaxation rate (R1) in the olfactory bulb (OB, p = 0.02), mean T1-weighted intensity at frontal white matter (FWM; p = 0.01), bilateral globus pallidus (GP; p = 0.03), and putamen (p = 0.03). The welders scored worse than the controls on the Grooved Pegboard test for both dominant (p = 0.06) and nondominant hand (p = 0.03). The dominant hand Grooved Pegboard scores correlated best with mean MRI intensity of FWM (R2 = 0.51, p = 0.004), GP (R2 = 0.51, p = 0.004), putamen (R2 = 0.49, p = 0.006), and frontal gray matter (R2 = 0.42, p = 0.01), whereas the nondominant hand scores correlated best with intensity of FWM (R2 = 0.37, p = 0.02) and GP (R2 = 0.28, p = 0.05). No statistical differences were observed in either the Trail-making test or the olfactory test between the two groups. This study suggests that Mn accumulates in OB and multiple other brain regions in “asymptomatic” welders and that MRI abnormalities correlate with fine motor but not cognitive deficits. Further investigations of subclinical Mn exposure are warranted.

Key Words: welding; manganese; MRI; olfactory bulb.

Welding fumes vary depending on the type of welding rod being used and the type of metal being welded, with iron (Fe) and manganese (Mn) being the two most common cations that are generated (Jenkins and Eager, 2005; Sowards et al., 2010). Of these metals, Mn has been well documented as a neurotoxicant for over a 100 years (Couper, 1837) and has been studied extensively in the past (Guilarte, 2010; Meyer-Baron et al., 2009). Mn toxicity is manifested by a neurological syndrome called managanism that initially consists of reduced response speed, irritability, intellectual deficits, mood changes, and compulsive behaviors. This may progress to more prominent, irreversible extrapyramidal dysfunction upon protracted exposure (Huang et al., 1993; Mergler and Baldwin, 1997; Olanow et al., 1996; Pal et al., 1999). Despite similarities between managanism/Mn-induced parkinsonism and Parkinson’s disease (PD), recent studies reveal clinically discernible differences (Chu et al., 1995; Guilarte, 2010; Ostiguy et al., 2006). Although any link to sporadic PD remains obscure, the potential neurobehavioral consequences of subclinical Mn exposure are of great public and occupational health importance.

The major health-relevant route of Mn exposure is inhalation (Environmental Protection Agency, 2010). Analyses of welding vapors indicate that Mn is predominantly in the divalent or trivalent states (Jenkins and Eager, 2005). In gas metal arc welding fumes, Mn is enriched in primary spherical particles of < 0.06μm (Sowards et al., 2010), sizes that can be easily inhaled. Once inhaled, these very small particles will deposit with relatively high efficiencies in the nasopharyngeal and alveolar regions due to diffusion. Welding fumes in the respiratory tract are either cleared by the mucociliary apparatus or absorbed systemically (Lam et al., 1978). Many recent studies using laboratory animals have shown distribution of Mn to the brain after tracheal instillation (Heilig et al., 2006), ip injection (Reaney et al., 2006), or aerosol exposure (Antonini et al., 2009; Dorman et al., 2001, 2006a,b; Elder...
et al. 2006). An alternate pathway for Mn transport to the brain is via the olfactory route, bypassing the blood-brain barrier (BBB). Rodent and other animal studies (Elder et al., 2006) suggest that metal particles deposited in the nose can be transported along the olfactory nerve directly into the olfactory bulb (OB) and further into the central nervous system, bypassing the protective BBB.

Mn is a paramagnetic metal that reduces the T1 relaxation time of magnetic resonance imaging (MRI), increasing the T1-weighted (T1W) intensity of brain structures (Newland et al., 1989) and the T1 relaxation rate (R1) (Dorman et al., 2006b). MRI has been extensively used to visualize Mn deposition in rodents (Fitsanakis et al., 2008; Silva and Bock, 2008), nonhuman primates (Bock et al., 2008; Dorman et al., 2006b; Newland et al., 1989), and in humans with manganism (Quaghebeur et al., 1996; Selikhova et al., 2008; Stephens et al., 2008). MRI has also been used previously to demonstrate Mn deposition in welders (Bowler et al., 2006b; Chang et al., 2009, 2010; Choi et al., 2007; da Silva et al., 2008). Nearly all MRI studies in humans, however, have focused on the pallidus with little attention to other regions. To our knowledge, there is no study that investigated the olfactory accumulation of Mn in humans despite the evidence from studies in laboratory animals that suggests an olfactory route of Mn to the brain. The present study was designed to investigate the Mn accumulation in different brain regions and to explore their functional correlations.

MATERIALS AND METHODS

Subjects. Seven welders (48 ± 13 years) and seven controls (50 ± 12 years) were recruited from the community for this study (see detailed demographic information in Table 1). All subjects were male and answered negatively for past diagnosis of PD and Parkinson’s symptoms (see Supplementary appendix A, Questions 17 and 18). As a part of the screening visit, a detailed clinical history was taken for all subjects that included age, education, history of smoking, and history of current and/or past major medical/neurological disorders. All subjects were also examined and ascertained to be free of any obvious neurological and movement deficits (scored zero on Unified Parkinson’s Disease Rating Scales, motor subscales) and any obvious cognitive deficits (Mini-Mental Status Examination [MMSE] above 28). All subjects had normal liver function test and blood calcium and magnesium levels. All welders also underwent an orbital radiograph to rule out any metal fragments around the orbit. Written informed consent was obtained from all subjects in accordance to the Internal Review Board/Human Subjects Protection Office guidelines of Penn State University, Hershey Medical Center.

Occupational Mn Exposure Assessment. Mn exposure was estimated by a detailed occupational questionnaire (Supplementary appendix A). Occupation-related questions included if the person was still employed as a welder and for how many years he had been welding. Other occupational questions included an estimate of what percentage of time on the job was related to welding (ranging from less than 25% to greater than 75%), where did the welding occur (indoors or outdoors), whether respiratory protection was worn, the ventilation at the workplace, the kind of welding primarily done (shielded metal arc, metal inert gas, heliarc, flux cored, other gas welding, or brazing), the kind of welding stick primarily used, the type of metal being welded, and how often welding occurred in confined spaces. Behavioral questions included whether individuals were primarily nose or mouth breathers, their smoking habits, and if they had worked in close contact with pesticides.

The cumulative lifetime exposures ($E_c$) to airborne Mn were determined for all subjects using the following formula:

$$E_c = (C_w T_w + \bar{C}_\lambda T_\lambda)$$

$$T_w = \frac{40}{168} Y_w = 0.24 Y_w$$

$$T_\lambda = Y_t - T_w = Y_t - 0.24 Y_w$$

Where $T_w$ is the time (in years), on the job working as a welder (assumes 40 h work week); $E_c$ is the cumulative lifetime exposure to Mn in milligrams per cubic meter—years; $Y_t$ is the welder’s age in years; $Y_w$ is the number of years employed as a welder; $C_w$ is the average concentration of Mn exposure during welding in milligrams per cubic meter; and $C_\lambda$ is the average concentration of Mn in ambient air in milligrams per cubic meter.

$$E_c = C_w (0.24 Y_w) + \bar{C}_\lambda (Y_t - 0.24 Y_w)$$

$$E_c = 0.24 W (C_w - \bar{C}_\lambda) + \bar{C}_\lambda Y_t$$

This analysis assumes that the exposures measured for the welder during his welding time included the exposure from ambient air as well. For all controls, $C_w$ was zero. An average ambient air exposure for Mn ($C_\lambda$) of 0.00003 mg/m³ is assigned to all subjects based on the Agency for Toxic Substances and Disease Registry (U.S. DHHS, 2008) (for further details of determining exposure levels from different types of welding, see Supplementary appendix B).

Functional Testing. Functional testing was performed on the same day as the screening visit. The Grooved Pegboard test was administered for both hands for all subjects as instructed by the accompanying manual (Lafayette Instrument Inc., Purdue, IN). Briefly, the subject is asked to pick the pegs one at a time using one hand and fill the board. For the right hand, the board is filled from left to right for each row beginning from the top. For the left hand, the board is filled from right to left for each row. The trial is done first with the dominant hand and then the nondominant hand. The total score is a sum of the time taken in seconds, total number of pegs dropped, and the total number of pegs correctly placed in the board.

The Trail making (parts A [TMT-A] and B [TMT-B]) (Reitan and Wolfson, 1975) also were administered to all subjects. Briefly, the TMT-A consists of numbers (1–25) within circles on a sheet of paper. The subject is asked to connect the circles sequentially and as quickly as possible without lifting the pen from the sheet. TMT-B consists of numbers (1–13) and letters (A–L) within circles. The subject is asked to connect the circles as before, but this time alternating between numbers and letters (e.g. 1-A-2-B-3-C, etc.). If the subject makes a mistake, he is asked to return to the last correct response and start again. The score is the total time taken to correctly complete the test.

The 40-item University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al., 1984) was mailed to all subjects for self-administration. Thirteen subjects (seven welders and six controls) returned the tests.

Magnetic Resonance Imaging. All subjects had their MRI scans done within 1 week of the screening visit. All images were acquired using a Siemens 3-T TrioTim MRI, with an 8-channel birdcage type In Vivo coil. First, a set of high-resolution T1W images (3D Multi Planar Reformating, Repetition Time (TR) = 1540 ms, Echo Time (TE) = 2.3 ms, voxel spacing 1 × 1 × 1, image resolution 256 × 256, 176 slices) was acquired for anatomical segmentation and mean intensity analysis. For estimating T1 relaxation time of the OB, coronal images were acquired by positioning the image acquisition grid perpendicular to the base of the frontal lobe, from anterior of the orbits to the anterior of the central sulcus, using multiple flip angles 10°, 20°, 30°, 45°, and 60° (3D gradient echo [GRE], TR = 35 ms, TE = 2 ms, voxel spacing 0.5 × 0.5 × 1.5, image resolution 128 × 128, 50 slices).
MRI Data Analysis. Defining regions of interest. Bilateral globus pallidus (GP) and frontal gray matter (FGM) (Fig. 1A) were defined using automatic segmentation software (AutoSeg) (Gouttard et al., 2007; Joshi et al., 2004) for each subject, whereas the frontal white matter (FWM) (Fig. 1B) was defined by a semi-automatic segmentation method in InsightSNAP (ITK-SNAP) (Yushkevich et al., 2006) on the T1W images. The OB, including both the OB neurons and surrounding epithelium (Fig. 1C), was defined by manually drawing a region of interest (ROI) on the coronal T1 image for all subjects by the same rater. Mean signal intensity and T1 values of all the voxels in the individual ROIs were calculated.

Estimation of T1 relaxation time in OB. To estimate the T1 relaxation time, the data from the 3D GRE MRI images acquired with multiple flip angles were fit into the following equation $\frac{\alpha(\theta)}{\alpha(0)} = \frac{E_1}{M_0} E_1 + M_0 \left[ 1 - E_1 \right]$ (Haacke et al., 1999), where $\alpha(\theta)$ is the MR signal acquired at the specific flip angle $\theta$, $M_0$ is the spin density, and $E_1 = e^{-TR/T1}$. After linear curve fitting, the computed slope reflects $E_1$, which in turn gives the estimate of $T1$. Computation was done using an in-house program written on MATLAB (Mathwork Inc.). The $T1$ relaxation rates ($R1 = 1/T1$) of the OB were computed from the mean $T1$ relaxation time.

Estimation of intensity in T1W images. To take into account variations in intensity of T1W images caused by imperfect magnetic fields, both within subjects and between subjects, a postacquisition image processing method was employed to correct the signal intensity variation. First, a bias field correction was used to reduce inhomogeneity within subjects for each scan using the standard, fully automatic FMRIB software library brain extraction tool with bias field and neck cleanup (Smith, 2002). Subsequently, histogram-based intensity standardization was used to correct inhomogeneities between subjects according to a recently published method (Ge et al., 2000; Nyul et al., 2000) that was implemented by a person blinded to welding exposure status. The mean intensities of all voxels in each ROI (i.e., GP, FWM, and FGM) for individual subjects were extracted using ITK-SNAP. Because the image processing procedure involves skull stripping (including the removal of nasal conchae, OB, and orbits) for internal bias removal, mean intensity of the OB could not be calculated. The pallidal index (PI) was derived from the ratio of GP intensity to FWM intensity (PI = GP/FWM) (Krieger et al., 1995).

Statistical Analysis. Comparison of the demographic information, specifically age, MMSE, blood chemistry results, and MRI data, between welder and control groups was conducted using Student’s t-test. Cumulative Mn exposures, duration of welding, and education between the welder and control groups were compared by t-tests assuming unequal variances. Smell test results were explored by comparing both raw scores, as well as the age-adjusted percentile scores, in a one-way ANOVA between the two groups. Analysis of covariance was used to test the group differences for the Grooved Pegboard test and the Trail-making test; age was the independent variable and welder or control was the group variable. Grooved Pegboard test scores for the dominant and nondominant hands were regressed on the mean signal intensity of FGM, GP, putamen, and FWM for all subjects. Correlations between variables were tested at a 95% confidence interval and $p$ values less than 0.05 were considered *Indicates significant differences ($p < 0.05$) between welders and controls.

### TABLE 1
Demographic Data

|                          | Welders ($n = 7$) | Controls ($n = 7$) | $p$ Value |
|--------------------------|-------------------|--------------------|-----------|
| Age (years)              | 48 (± 13)         | 50 (± 12)          | 0.79      |
| Education (years)        | 12                | 13.9 (± 1.9)       | 0.039*    |
| Current regular smoker   | 1/7               | 0                  | 1         |
| Durations of welding (years) | 24.1 (± 15.5) | 0                  | 0.006*    |
| Cumulative Mn exposure (mg/m$^3$—years) | 0.881 (± 0.567) | 0.002 (± 0.0003) | 0.006*    |
| MMSEx                    | 29.6 (± 0.8)      | 29.7 (± 0.5)       | 0.69      |
| Blood chemistry          |                   |                    |           |
| Aspartate aminotransferase (units/l) | 34 (± 4.6) | 36 (± 6)          | 0.49      |
| Alanine aminotransferase (units/l) | 26.9 (± 9.5) | 26.6 (± 7.9)       | 0.95      |
| Calcium (mmol/l)         | 9.8 (± 0.7)       | 9.4 (± 0.3)        | 0.20      |
| Magnesium (mmol/l)       | 2.0 (± 0.2)       | 2.0 (± 0.1)        | 0.72      |

Note. Means and SDs of demographic data for welders and controls. $p$ Value is derived after performing a Student’s $t$-test between the two groups; except education, duration of welding, and cumulative Mn exposure where $t$-test was performed assuming unequal variances.

*Indicates significant differences ($p < 0.05$) between welders and controls.
significant. All statistical analyses were performed on JMP 8.0.1 statistical software (SAS Institute Inc. Cary, NC).

RESULTS

There were no significant differences in age, MMSE, and blood chemistry between welders and controls. Welders had a lower education level and significantly higher cumulative Mn exposures compared with controls (Table 1). As seen in Table 2, welders scored significantly worse than controls on the Grooved Pegboard test for the nondominant hand (p = 0.03) and displayed a similar trend for the dominant hand (p = 0.06). There were, however, no significant differences in the Trail-making test (TMT-A [p = 0.58] and TMT-B [p = 0.29]) between welders and controls (Table 2). There was also no significant difference in either the UPSIT score (p = 0.66) or the age-adjusted UPSIT percentile scores (p = 0.74) between welders and controls (Table 2).

The volumes of all brain ROIs were not significantly different between welders and controls (data not shown). We also did not detect any hyperintense signal in the GP, putamen, and FGM in any of the welders by naked eye (see Supplementary appendix C, figure). Compared with controls, welders had significantly higher R1 values of OB (p = 0.02; Fig. 2A). In addition, the welders also had significantly higher T1W mean intensity of FWM (p = 0.01), GP (p = 0.03), and putamen (p = 0.03) (Fig. 2B) compared with controls. There was, however, no significant difference in the T1W mean intensity of the FGM (p = 0.22) and PI (p = 0.32; Fig. 2C) between controls and welders.

The Grooved Pegboard performance of the dominant hand (Fig. 3 upper panel) displayed a positive correlation with the mean intensity of FGM (R² = 0.42, p = 0.012), GP (R² = 0.51, p = 0.004), putamen (R² = 0.49, p = 0.006), and FWM (R² = 0.51, p = 0.004) in all subjects. The Grooved Pegboard performance of the nondominant hand had significant correlation (Fig. 3 lower panel) with FWM (R² = 0.37, p = 0.02) only and fell just short of significance with GP (R² = 0.28, p = 0.05). There were also no correlations between the Grooved Pegboard scores and PI for either the dominant hand (R² = 0.006, p = 0.8) or the nondominant hand (R² = 0.1, p = 0.26). There was also no significant correlation between the R1 values of OB and smell test performances (R² = 0.06, p = 0.41) in all subjects (data not shown).

DISCUSSION

To our knowledge, this is the first study to demonstrate higher T1 relaxation rates due to Mn accumulation in the OB of “asymptomatic” welders. This is consistent with the hypothesis that the olfactory nerve might be one of the routes of entry of Mn into the brain. This study also suggests that there is Mn accumulation in many other regions of the brain in asymptomatic welders and supports the importance of broadening the investigation of Mn exposure to regions of the brain other than just the pallidus. Lastly, our study provides preliminary evidence that there were potential brain region–specific neurobehavioral consequences of the subclinical Mn exposure that warrant further investigation.

The exact mechanisms by which Mn reaches different brain regions are not completely understood. Mn transport into the brain may occur via three pathways (Crossgrove and Zheng, 2004): (1) transport across the BBB by transporter-mediated kinetics, (2) transport across the blood-cerebrospinal fluid barrier of the choroid plexus, and (3) via the olfactory nerve bypassing the BBB. Of particular relevance are studies in laboratory animals showing that soluble metal particles (Chuang and Koretsky, 2006; Henriksson et al., 1999; Tjalve et al., 1996) as well as insoluble metal and nonmetal particles (Elder et al., 2006; Oberdorster et al., 2004; Wang et al., 2007, 2008) can be transported along the olfactory nerve directly into the OB and further into the central nervous system, bypassing the protective BBB. Furthermore, MRI studies of nonhuman primates exposed to aerosolized MnSO₄ demonstrated Mn accumulation in the OB (Dorman et al., 2006a,b). Although past reports have alluded to Mn distribution to the OB (Chu et al., 1995; Fitsanakis et al., 2006), we believe that this is the

| TABLE 2 | Mean and SD for Welders and Age- and Gender-Matched Controls on Neurobehavioral Tasks and Olfactory Tests |
|-----------------|-----------------|-----------------|
|                 | Welders (n = 7) | Controls (n = 7) | p Value |
| Grooved Pegboard scores (s) | Dominant | 76.1 (± 10) | 66.9 (± 11) | 0.06 |
|                 | Nondominant | 87 (± 11) | 73.7 (± 11) | 0.03* |
| Trails test scores (s) | TMT-A | 21.3 (± 7) | 19.4 (± 4) | 0.58 |
|                 | TMT-B | 56.7 (± 22) | 44.9 (± 17) | 0.29 |
| UPSIT test | UPSIT score | 34.7 (± 4.3) | 35.7 (± 3.1) | 0.66 |
|                 | UPSIT percentile score | 51.6 (± 30.5) | 57 (± 27) | 0.74 |

Note. Means and SDs of neurobehavioral and olfactory test scores of welders and controls. Olfactory test was self-administered by seven welders and six controls. p Value for Grooved Pegboard scores and Trails test scores were derived from analysis of covariance between the two groups after controlling for age. p Values for UPSIT scores were derived from one-way ANOVA between the two groups.
first MRI study in humans supporting the OB as a potential point of entry for Mn particles from welding fumes, as shown by high T1 relaxation rates in the OB.

It is conceivable that Mn may reach different brain regions through different mechanisms, thereby accumulating in varying rates and concentrations. After Mn is distributed to the brain tissue, intracranial Mn is known to have a very slow clearance rate (Newland et al., 1989), where it is primarily sequestered in tissue and intracellular compartments. The tissue half-life of Mn in brain has been reported to exceed 50 days (Newland et al., 1987; Takeda et al., 1995). MRI has been used to estimate Mn accumulation in the brain (Chang et al., 2009, 2010; Dorman et al., 2006b; Krieger et al., 1995) by calculating the PI, but a major deficiency of this approach is that it neglects brain tissues other than the GP (Dorman et al., 2006b; Maeda et al., 1997) and might be less sensitive to lower, subclinical levels of Mn exposure (Dorman et al., 2006a,b). Because none of our welders had any overt symptoms of Mn toxicity, it is not surprising that we neither detected any obvious hyperintense signals in the basal ganglia by naked eye (see Supplementary appendix C, figure) nor a significant higher PI in these asymptomatic welders. An obvious reason for the PI being less sensitive is that it is a ratio of GP intensity to FWM intensity, and because both these measures are significantly different between welders with subclinical Mn exposure and controls, the ratio of the two tissues may cancel out some of the Mn effects. The results of our study are consistent with this notion that Mn accumulates in regions outside of the pallidus in a region-specific manner; furthermore, some of these regions (e.g., OB and FWM) are more sensitive for detecting the differences between welders and controls than the PI.

Consistent with past studies (Bowler et al., 2006a; Chang et al., 2009; Ellingsen et al., 2008), we also found that welders performed worse than controls on the Grooved Pegboard test, but not on the Trail-making test. Few studies, however, had correlated the Grooved Pegboard tests with MRI findings. Chang et al. (2009, 2010) recently reported that the scores of the Grooved Pegboard test with the dominant hand correlated with blood Mn level and PI, the two most common exposure measurements. Although the number of subjects in that study was larger (43 welders) than in the current study, the correlations between functional and exposure correlations were weak ($R^2 < 0.20$). In our study, the correlations of Grooved Pegboard test scores were much stronger ($R^2 > 0.40$) with region-specific Mn accumulation. This supports that region-specific Mn accumulation may be a more clinically relevant marker for Mn exposure.

![FIG. 2. Student’s T-tests were conducted to compare between welders (black columns) and controls (gray columns). (A) The OB, T1 relaxation rate (R1); (B) the T1W intensity (T1WI) of the FGM, GP, and FWM; and (C) the PI. $p$ Values less than 0.05 at a 95% confidence interval were considered significant.](image)

![FIG. 3. Multiple regression analyses between T1W-MRI results and Grooved Pegboard scores from the nondominant and dominant hands of welders (solid circles) and controls (open circles). Correlation of FGM with scores of (A) dominant hand ($R^2 = 0.42$, $p = 0.01$) and (E) nondominant hand ($R^2 = 0.40$, $p = 0.006$) and (G) nondominant hand ($R^2 = 0.37$, $p = 0.02$). All analyses were performed with a 95% confidence interval.](image)
It is also worth noting that we observed a correlation of $T1$ intensity and Grooved Pegboard scores among controls as well. Many factors may contribute to the variations in either $T1$ intensity or motor skill testing in normal subjects. As we noted above, all subjects, even controls, are exposed to some concentration of Mn from ambient air. It is possible that some of the functional variations in controls are due to differences in Mn accumulation in the brain. This may result from several different exposure sources, including drinking water (Bouchard et al., 2011; Environmental Protection Agency, 2004), urban living (Willis et al., 2010), nearby ferromanganese alloy plants (Lucchini et al., 2007; Menezes-Filho et al., 2011), and the like. Previous reports have shown that aging also can affect both pegboard performance (Ruff and Parker, 1993) and MRI $T1$ intensity (Cho et al., 1997). In this context, it will be worthwhile investigating whether environmental Mn exposure affects $T1W$ imaging and contributes to aging-related cognitive decline in the general population.

Decreased olfactory function in welders has been previously reported (Antunes et al., 2007; Lucchini et al., 1997; Mergler et al., 1994). Our study of the seven welders and six controls failed to find a difference in the olfactory function between the two groups or any clear correlations between the smell test and $R1$ in OB. This may be due to our small sample size, relative to the study of Antunes et al. (2007) of 42 welders and 42 controls. Nevertheless, future studies with larger sample size are warranted to investigate the olfactory dysfunction and its relationship with Mn accumulation in OB.

There are other limitations to our study besides a small sample size. For example, welding fumes contain several other metals including Fe and copper that are known to be neurotoxic and chromium and nickel that are known to be more carcinogetic. Fe exposures tend to be well correlated with Mn exposures for many types of welding and have been determined to be about 10 times the Mn levels based on the analysis of several data sets (Flynn and Susi, 2011). Compared with Mn, both chromium and nickel are present in trace amounts in most welding wires but are not consistently detected in welding fumes unless stainless steels are involved (Jenkins and Eagar, 2005; Sowards et al., 2010). Even though Fe is the predominant component of welding wires and welding fumes, Fe itself has a very small $T1$ relaxivity (0.01 mM$^{-1}$s$^{-1}$) compared with Mn (7.1 mM$^{-1}$s$^{-1}$) (Yilmaz et al., 1999). Additionally, using a nonlinear competition model that accounts for Mn and Fe, Zhang et al. (2009) found that bound Mn, rather than Fe, is the most likely cause for the increase in $T1$ relaxation rates in different brain regions, particularly the striatum. Furthermore, a recent study that exposed rats to welding fumes via intratracheal instillation also showed that only Mn in welding fumes is distributed to the brain, whereas other metals (Cr, Fe, and Cu) are not (Antonini et al., 2010). Thus, based on existing data and literature, we can conclude that the changes in $T1W$ intensity and relaxation rates we observe in welders are primarily due to Mn. Nevertheless, further studies with larger sample size are required to properly assess the contribution of these other metals to neurobehavioral changes observed in welders.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

**FUNDING**

National Institute of Environmental Health Sciences (T32ES07018; University of North Carolina, Department of Biostatistics); National Institute of Neurological Disorders and Stroke (NS060722; Pennsylvania State University, Department of Neurology); General Clinical Research Center Grant from National Institutes of Health (M01RR10732), and General Clinical Research Center Construction Grant (CO6RR016499), Pennsylvania State University, College of Medicine.

**ACKNOWLEDGMENTS**

The authors would like to thank all those who participated in this study. The authors are indebted to Dr David Dorman for the suggestion of pursuing OB MRI studies in humans, to Jeff Vesek and the personnel at Penn State Hershey Center for NMR Research for use of their MRI facilities, and to Dr Richard Mailman and Dr Qing X. Yang for their comments and suggestions.

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