Higher Hippocampal Mean Diffusivity Values in Asymptomatic Welders

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ABSTRACT

Chronic high-level manganese (Mn)-induced neurotoxicity has been associated with Mn accumulation in the basal ganglia and higher risk for developing parkinsonism. Recent studies in Mn-exposed animals revealed Mn accumulation in the hippocampus, the presence of A β diffuse plaques, and deficits in associative learning, the latter being hallmarks of Alzheimer's disease (AD) or related disorders. This and recent evidence of hippocampal Mn accumulation in welders prompted us to test the hypothesis that welders with chronic Mn exposure would display changes in the hippocampus. Subjects with (welders; $n = 42$) or without (controls; $n = 31$) welding history were studied. Mn exposure was estimated by occupational questionnaires, whole blood Mn, and R1 imaging (estimate of short-term brain Mn accumulation). Hippocampal diffusion tensor imaging (DTI; estimate of microstructural brain changes) and volume were determined. Compared with controls, welders displayed no significant difference in hippocampal volume ($p = .165$). Welders, however, exhibited higher DTI hippocampal mean diffusivity (MD) values compared with controls ($p = .035$) that was evident particularly in older welders (>50 years, $p = .002$). Hippocampal MD was associated significantly with age in welders ($R = 0.59$; $p < .001$) but not in controls ($p = .16$). Moreover, higher hippocampal MD values (age adjusted) were associated with long-term cumulative Mn exposure ($R = 0.36$, $p = .021$). Welders with chronic exposure have higher MD values in the hippocampus that become greater with increasing age, a brain change that is similar to that observed in those at risk for AD. The current results suggest that Mn exposure, coupled with aging, may make welders more vulnerable to AD or AD-like changes.

Key words: welders; manganese; diffusion tensor imaging; mean diffusivity; hippocampus.

The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

Manganese (Mn) is an essential nutrient but can be neurotoxic to the central nervous system at high doses. Excessive exposure to Mn has been associated with neurobehavioral disorders such

as Mn-induced parkinsonism (Cersosimo and Koller, 2006; Colosimo and Guidi, 2009; Guilarte and Gonzales, 2015). Past studies largely have focused on Mn-related neurotoxicity in the

basal ganglia (BG) and its related functional consequences (Criswell et al., 2012; Dorman et al., 2006b) because Mn brain deposition reportedly is greatest in the globus pallidus (GP) of the BG (Criswell et al., 2012; Dorman et al., 2006b). Recently, Racette et al. (2012) reported that Mn-exposed workers had a higher frequency of parkinsonian features compared with unexposed workers, and parkinsonian symptoms increased with cumulative long-term Mn exposure (Racette et al., 2016).

Recent animal studies, however, demonstrated that Mn-exposed animals (rats and primates) had a higher Mn concentration in the hippocampus (Dorman et al., 2006a; Liang et al., 2015), as well as Alzheimer's disease (AD)-like pathology (eg, A β diffuse plaques) in the frontal cortex (Guilarte, 2010) and deficits in behavioral performance on associative learning tasks (Liang et al., 2015; Schneider et al., 2013), the latter being hallmark features of AD. In human studies, our team previously demonstrated Mn accumulation not only in the BG (the key location related to parkinsonism), but also in the hippocampus by showing a positive association between MRI R1 values (1/T1; an estimate for Mn brain accumulation) and recent welding hours (Lee et al., 2015). Compared with controls, asymptomatic welders also had performance declines in delayed verbal recall tasks that were greater than those for immediate memory (Chang et al., 2009, 2010), a feature of early AD. Together, these data suggest that, in addition to parkinsonism, welding-related neurotoxicity also may be related to neurodegenerative processes that occur in AD and/or AD-related disorders (ADRD).

As a common age-related neurodegenerative disease, AD is characterized by amyloid plaques, tau tangles, and loss of neuronal connections. The prominent neuronal damage initially was noted in the hippocampus (Costafreda et al., 2011; Sorensen et al., 2016; Wisse et al., 2014). The major behavioral deficits entail memory problems, particularly episodic memory (memory of personally experienced objects, people, and events) (Bäckman et al., 2001, 2005). There has been a broad interest in detecting AD-at-risk populations (eg, mild cognitive impairment [MCI]) or markers that predict AD. The MRI hallmark of AD is hippocampal atrophy. Recent studies, however, reported diffusion tensor imaging (DTI) differences in the hippocampus of subjects with AD and MCI that include higher mean diffusivity (MD) and lower fractional anisotropy (FA) (Douaud et al., 2013; Fellgiebel et al., 2006; Müller et al., 2007) that also were observed in several other brain regions (eg, fornix, cingulum, posterior cingulate, and precuneus) (Choo et al., 2010; Fellgiebel et al., 2005; Gordon et al., 2018; Mielke et al., 2012; Zhang et al., 2007). DTI measures the random translational motion of water molecules that are affected by tissue microstructural properties and, as such, often serves as an estimate of microstructural brain tissue changes (Basser and Pierpaoli, 1996; Le Bihan et al., 2001). These DTI changes may be more sensitive than hippocampal atrophy in predicting AD conversion (Douaud et al., 2013). Thus, the present study tested whether DTI can detect changes in the hippocampus of asymptomatic subjects who have chronic exposure to welding fumes, and also explored the association of DTI measures with aging and welding exposure levels.

MATERIALS AND METHODS

Study Subjects

Eighty subjects were recruited initially from labor unions in regions of central Pennsylvania (United States) and the local communities (Lee et al., 2015). Six subjects either failed to complete the DTI acquisition (two welders and one control) or had

poor image co-registration (two welders and one control). These subjects were excluded from the analysis resulting in 31 controls and 43 welders. Welders were defined as subjects who had welded at any point in their lifetime with a minimum of welding years ≥ 5 . Welders represented several different trade groups (eg, boilermakers, pipefitters, and a variety of different manufacturing jobs). Controls were volunteers from the same regional community with various occupations who did not have any lifetime history of welding. All subjects answered negatively for past Parkinson's disease (PD) diagnosis or other neurological disorders, and were free of any obvious neurological or movement deficits using the unified PD rating scale motor scores (UPDRS-III) with a threshold score of <15 (Lee et al., 2015). All subjects were male and had mini mental state exam (MMSE) scores >24 except for one welder. His data was excluded from the analysis. Montreal Cognitive Assessment (MoCA) was administered to assess global cognitive status. Demographic data, including age and education years, also were acquired. Written informed consent was obtained in accordance with guidelines approved by the Penn State Hershey Internal Review Board.

Exposure Assessment

Welding exposure was estimated using exposure questionnaires, whole blood Mn and iron (Fe) levels, and MRI R1 and R2* values (estimates of brain Mn and Fe accumulation, respectively) (Lee et al., 2015, 2016b). Our exposure questionnaire estimated recent exposure {hours welding, brazing, or soldering in the 90 days prior to the study visit [$\text{HrsW}_{90} = (\text{weeks worked}) * (\text{h/week}) * (\text{fraction of time worked related directly to welding})$] and E_{90} (an estimate of the cumulative exposure to Mn, past 90 days)} and lifetime exposure [welding years ($\text{YrsW} = \text{years spent welding during the subjects' life, lifetime}$) and ELT (an estimate of cumulative exposure to inhaled Mn over the individual's life)] (Lee et al., 2015). We also collected information on the type of welding processes and materials used, as well as other occupational exposures, that were integrated in calculating the E_{90} and ELT estimation (Lee et al., 2015). Whole blood Mn and Fe levels were obtained for all subjects from samples drawn the morning of the study visit.

Blood analysis. Whole blood Mn and Fe levels were measured by inductively coupled plasma mass spectrometry (ICP-MS) in batches from samples that had been collected the morning of the MRI acquisition and then stored at -80°C until analysis. Digestion was performed by microwave methods using the Discovery SPD digestion unit (CEM, Matthews, North Carolina). After digestion, the samples were analyzed for trace minerals using the Thermo (Bremen, Germany) Element 2 SF-ICP-MS equipped with a concentric glass nebulizer and Peltier-cooled glass cyclonic spray chamber. Bulk mineral concentrations were determined by ICP-OES (Optical Emission Spectrometry) analysis on the Thermo iCAP equipped with a polypropylene cyclonic spray chamber (Lee et al., 2015).

MRI Image Acquisition and Image Processing

All images were acquired using a Siemens 3T scanner (Magnetom Trio, Erlangen, Germany) with an 8-channel head coil. First, high-resolution T1-weighted (T1W) and T2-weighted (T2W) images were acquired for anatomical segmentation. For T1W images, MPRAGE sequences with repetition time (TR)/echo time (TE) = 1540/2.3 ms, FoV/matrix = $256 \times 256/256 \times 256$ mm, slice thickness = 1 mm, slice number = 176 (with no gap), and voxel spacing $1 \times 1 \times 1$ mm were used. T2W images were obtained using fast-spin-echo sequences with TR/TE = 2500/

Figure 1. Automatically segmented hippocampus region of interest on T1-weighted MPRAGE images for one representative subject.

316 ms and the same spatial resolution as the T1W images. For R1, TR/TE = 15/1.45 ms, flip angles = 4/25, FoV/matrix = 250 × 250/160 × 160 mm, slice thickness = 1 mm, slice number = 192, and voxel spacing = 1.56 × 1.56 × 1 mm were used. R2* images were acquired using five TEs ranging from 8 to 40 ms with an interval of 8 ms, TR = 51 ms, flip angle = 15°, FoV/matrix = 230 × 230/256 × 256 mm, slice thickness = 1.6 mm, and slice number = 88 were used. For DTI, TR/TE = 8300/82 ms, b value = 1000 s/mm², diffusion gradient directions = 42 and 7 b = 0 scans, FoV/matrix = 256 × 256/128 × 128 mm, slice thickness = 2 mm, and slice number = 65 were used.

Defining Hippocampal Regions of Interest

Bilateral hippocampi (Figure 1) were selected as regions of interest (ROI). The hippocampal ROIs were defined for each subject using automatic segmentation software (AutoSeg) (Gouttard et al., 2007; Joshi et al., 2004). The segmentation quality then was confirmed visually by a reviewer blinded to group assignment. For all MRI measures, the right and left hemisphere values were averaged.

Estimations of Brain MRI Measurements

DTI values. DTI quality control and tensor reconstruction were performed using DTIPrep (University of North Carolina, Chapel Hill, North Carolina) that first checks diffusion images for appropriate quality by calculating the inter-slice and inter-image intra-class correlation, and then corrects for the distortions induced by eddy currents and head motion (Liu et al., 2010b). DTI maps then were estimated via weighted least squares (Salvador et al., 2005). The segmented ROIs on T1W images were co-registered first onto T2W images using FSL flirt. DTI maps then were co-registered onto T2W images using ANTS, and the transformation matrix was inversely applied to bring ROIs on the T2W images to the DTI maps. Two DTI values (FA and MD) were calculated out of three diffusivity eigenvalues (Le Bihan et al., 2001). FA is a weighted average of pairwise differences of the three eigenvalues and may represent the degree of diffusion anisotropy. MD is an average of the three eigenvalues, providing the overall diffusion magnitude (Le Bihan et al., 2001).

R1 values. Longitudinal relaxation rate (R1; 1/T1) is an MRI estimate of short-term Mn brain accumulation (Lee et al., 2015). Mn has paramagnetic characteristics, and can shorten the MRI longitudinal relaxation time (T1) and increase T1-weighted intensity (T1WI). R1 signals, however, decay with time (Han et al., 2008) and show better associations with short-term rather than long-term cumulative exposure (Choi et al., 2007). To estimate R1 values, whole brain T1 time images were generated by the scanner using a published method (Venkatesan et al., 1998). ROIs were co-

registered onto the T1 maps using an affine registration implemented in 3D Slicer (www.slicer.org; Rueckert et al., 1999).

R2* values. The apparent transverse relaxation rate ($R2^* = 1/T2^*$) is an estimate for Fe brain accumulation because Fe has paramagnetic characteristics and shortens the apparent transverse relaxation time ($T2^*$; Haacke et al., 2005). To calculate R2* values, the magnitude images of multigradient echo images were used to generate R2* maps utilizing a voxel-wise linear least-squares fit to a mono-exponential function with free baseline using in-house Matlab (The MathWorks, Inc., Natick, Massachusetts) tools. The automatically segmented ROIs on the T1W images first were co-registered onto T2W images, and then the ROIs on T2W image space were co-registered again onto the R2* maps using an affine registration implemented in 3D Slicer (www.slicer.org; Rueckert et al., 1999). R2* values in each ROI were calculated as $1/T2^*$ in each voxel, and averaged over the entire ROI (Lee et al., 2016b).

The DTI, R1, and R2* measurements in the hippocampus were calculated using a trimmed mean (5%–95% percentile) to reduce possible segmentation error and imaging noise.

Volume calculation. Hippocampal volume was calculated by superimposing the automatically segmented ROI images on the individual T1W images and extracting volume values using Matlab R2016b.

Statistical Analysis

Group comparisons for both demographic and MRI data were conducted using one-way analysis of variance (ANOVA). For MRI measures, the ANOVA treated group (welders vs controls) as a between-subjects factor after adjusting for age and education effects. For the MRI R1 and R2* measures, the analyses additionally were adjusted by R2* or R1 values, and whole blood metal levels of Fe (for the R1 analysis) or Mn (for the R2* analysis) as covariates due to a potential interaction between Mn and Fe. Total intracranial volume (TIV) was used as a covariate when comparing hippocampal volumes. To test the associations between DTI markers and exposure metrics (HrsW₉₀, E₉₀, YrsW, ELT) in welders, Pearson correlation analyses were conducted with adjustment for age. For the association analyses of DTI with MRI exposure measures (R1 for Mn and R2* for Fe brain accumulation), the analyses also were adjusted by R2* and blood Fe (for the DTI-R1 association analysis) or R1 and blood Mn (for the DTI-R2* association analysis) values.

The age-dependent group differences in hippocampal DTI values were explored by means of a regression analysis with an age-by-group (welders and controls) interaction term in analyses adjusted for age and education factors, while including the

welding exposure group as the main effect. The age-dependent group difference was also examined by conducting a stratified analysis that divided subjects into two groups based on the welders' median age (51 years). This resulted in 20 welders and 21 controls for the younger subgroup, and 22 welders and 10 controls for the older subgroup. The rationale for the age-stratified analysis was based on a previous report demonstrating an exponential increase in hippocampal DTI values (especially MD) after 50 years of age among neurologically normal subjects (Carlesimo et al., 2010). To rule out the possibility that the age-stratified analysis results may be explained by the fact that welders in the older subgroup had higher chronic cumulative exposure than those in the younger subgroup, we further separated welders in each age-related subgroup into those with higher and lower exposure subgroups based on the median ELT value (0.987 mg-years/m³) and conducted ANOVA that treated group (controls vs welders with higher or lower exposure) as a between-subjects factor. SAS 9.4 was used for all statistical analyses. Statistical significance was defined by $\alpha = 0.05$.

RESULTS

Demographics and Exposure Types

Welders were older ($p = .048$) and had lower education years than controls ($p < .001$), but demonstrated comparable global cognitive performance assessed by the MoCA ($p = .797$). There was no significant difference in UPDRS-III scores between welders and controls ($p = .326$). Welders displayed higher short-term (HrsW₉₀ and E₉₀) and long-term (YrsW and ELT) exposure metrics, and had higher whole blood Mn and Fe levels ($ps < .004$; Table 1). Mean hippocampal R1 values were not significantly different between welders and controls ($p = .351$), but R1 values in welders increased significantly with increasing HrsW₉₀ ($R = 0.550$, $p = .034$) when the HrsW₉₀ exceeded welding equivalent to working half-time (eg, ~300 h). For MRI R2* measures, there was no significant group difference in the hippocampus ($p = .371$) and R2* values decreased in welders with increasing HrsW₉₀ ($R = -0.357$, $p = .028$).

In general, each welder performed multiple types of welding, but overall shield metal arc welding (SMAW), gas metal arc welding (GMAW), and gas tungsten arc welding (GTAW) accounted for most of the welding done by subjects in this study. The most frequent base metal reported was mild steel, followed by stainless steel. The major fume components were Fe and Mn and to a lesser degree chromium (Cr) and nickel (Ni). When asked to identify the most common SMAW electrode used, the E6010 (rutile) and E7018 (basic) electrodes were noted.

Group Comparisons of DTI and Volume Measures in the Hippocampus

Welders had significantly higher hippocampal MD values compared with controls after controlling for age and education ($p = .035$; Figure 2a), but no significant difference in FA values ($p = .129$; Figure 2b). Volume measures were similar in both groups after adjustment for age, education, and TIV ($p = .165$; Figure 2c).

Hippocampal DTI Associations With Welding-Related Exposure Measurements in Welders

Age-adjusted hippocampal MD values were significantly and positively correlated with questionnaire-based ELT (an estimate of cumulative Mn exposure, lifetime; $R = 0.36$, $p = .021$; Figure 3a), whereas there was no association between the age-adjusted hippocampal MD values and YrsW ($R = 0.16$, $p = 0.3$;

Figure 3b). In contrast, age-adjusted hippocampal FA values were significantly and negatively correlated with YrsW ($R = -0.31$, $p = .044$; Figure 3d), whereas no association was found for age-adjusted hippocampal FA and ELT ($R = -0.25$, $p = .104$; Figure 3c). Neither MD nor FA was significantly associated with short-term exposure measures (HrsW₉₀ and E₉₀; $ps > .104$). There were also no significant correlations of DTI measures (MD and FA) with R1 (MRI estimate for short-term Mn brain accumulation; $p = .679$ and $p = .527$, respectively) or R2* values (MRI estimate for Fe brain accumulation) in welders ($p = .710$ and $p = .103$, respectively). DTI (MD and FA) and whole blood Mn and Fe measures in welders were also not correlated ($ps > .100$).

The Influence of Age on Hippocampal DTI Measures

Association analysis. Hippocampal MD values were positively correlated with age in welders ($R = 0.59$, $p < .001$; Figure 4a), whereas the hippocampus MD-age association was not significant in controls ($R = 0.26$, $p = .161$). Hippocampal FA values were negatively correlated with age in welders ($R = -0.34$, $p = 0.029$; Figure 4b) but not in controls ($R = 0.24$, $p = .190$). The significant MD-age association in welders remained after controlling for long-term exposure using ELT ($R = 0.46$, $p = .002$).

Regression analysis. For hippocampal MD values, the regression analysis revealed a significant age by welding exposure group (welders vs controls) interaction effect ($t = 2.78$, $p = .007$) indicating that the welder-control group difference became greater as age increased. For hippocampal FA values, the regression analysis also revealed a significant age by welding exposure group (welders vs controls) interaction effect ($t = -2.38$, $p = .020$).

Age-stratified analysis. Demographics and exposure characteristics between welders and controls within each younger and older strata were similar to those of the whole group except for two things: (1) whereas the overall cohort showed a difference in age between welders and controls, this was not observed either in the younger or older strata, ($p = .221$ and $p = .661$, respectively), and (2) similar to the overall cohort, welders in the older strata had higher blood Mn levels than older controls ($p = .006$, details in Table 2), whereas there was no group difference in the younger strata ($p = .167$). DTI MD and FA values were similar between welders and controls in the younger strata after controlling for education ($p = .510$ and $p = .342$, respectively; Figure 5a). DTI MD values, however, were significantly higher in older welders compared with older controls after controlling for education ($p = .002$; Figure 5b), with no group difference in FA values ($p = .216$). When considering chronic cumulative exposure (ELT) in each age-stratified strata, welders with higher ELTs in the younger strata showed greater hippocampal MD values compared with younger controls (uncorrected $p = .041$) but this difference failed to be significant after correction for multiple comparisons ($n = 3$) using the stepdown Bonferroni method (Hochberg, 1988) (Supplementary Figure 1a). Welders in the older strata, however, still displayed higher hippocampal MD values compared with controls regardless of their ELT levels (uncorrected $p = .014$ for older welders with lower ELT and uncorrected $p = .001$ for older welders with higher ELT compared with controls; Supplementary Figure 1b).

DISCUSSION

High-level Mn exposure has been associated with a clinical syndrome called manganism that resembles PD in several

Table 1. Summary Statistics for Demographics (I) and Exposure Measures: Exposure, Whole Blood Metal, and MRI Metrics (II) in Welders and Controls

	Controls (N = 31)	Welders (N = 42)	Raw
	Mean $\pm$ SD	Mean $\pm$ SD	p-Values
I. Demographics			
Age (years)	43.6 $\pm$ 11.4	48.9 $\pm$ 10.7	0.048
Education (years)	16.2 $\pm$ 2.2	12.9 $\pm$ 1.6	<0.001
MoCA	26.2 $\pm$ 2.5	26.3 $\pm$ 2.1	0.797
Smokers (n, %)	1, 1.4%	6, 8.2%	0.113
UPDRS-III	1.5 $\pm$ 2.1	2.0 $\pm$ 2.5	0.326
II. Exposure measures: exposure, whole blood metal, and MRI metrics			
HrsW ₉₀ (h)	0 $\pm$ 0	241 $\pm$ 200	<0.001
E ₉₀ (mg-days/m ³)	0.003 $\pm$ 0	2.328 $\pm$ 2.019	<0.001
YrsW (years)	0 $\pm$ 0	26.2 $\pm$ 10.9	<0.001
ELT (mg-years/m ³)	0.001 $\pm$ 0.0003	1.177 $\pm$ 0.783	<0.001
Whole blood Mn (ng/ml)	8.9 $\pm$ 2.5	11.0 $\pm$ 3.2	0.004
Whole blood Fe (μ g/ml)	498 $\pm$ 76	556 $\pm$ 53	<0.001
MRI R1	0.51 $\pm$ 0.04	0.52 $\pm$ 0.04	0.351
MRI R2*	17.38 $\pm$ 2.1	18.4 $\pm$ 3.0	0.371

Data represent the mean $\pm$ SD for each measure. Groups were compared using one-way analysis of variance (ANOVA).

Abbreviations: y, years; MoCA, Montreal Cognitive Assessment; UPDRS, unified PD rating scale; HrsW₉₀, hours welding, 90 days; E₉₀, cumulative 90 day exposure to Mn; YrsW, years welding, lifetime; ELT, cumulative exposure to Mn, lifetime.

Figure 2. MRI measures in welders and controls. Hippocampal diffusion tensor imaging (DTI): a, MD (mean diffusivity), b, FA (fractional anisotropy), and c, volume. Values are raw means $\pm$ SEM.

ways. Accordingly, past studies largely focused on estimating risk of Mn exposure for conversion to parkinsonism. In the present study, for the first time, we used DTI measures to examine whether asymptomatic welders with chronic Mn exposure would display brain changes similar to an AD-at-risk population. The results demonstrated that welders had higher hippocampal MD values without differences in hippocampal volume or global cognitive status, a pattern similar to that observed in AD-at-risk groups. Moreover, higher hippocampal MD values became greater with age, and were associated with greater long-term cumulative Mn exposure. The current findings suggest that Mn exposure, coupled with

aging, may make welders more vulnerable to AD-related changes.

Exposure Characteristics

All Mn-related exposure measurements (YrsW, ELT, whole blood Mn, HrsW₉₀, E₉₀, and R1) indicated that our welders generally had chronic, but relatively lower, Mn exposure compared with previous studies: Whole blood Mn was considerably lower compared with other studies (eg, >14.2 ng/ml) (Chang et al., 2009; Criswell et al., 2012; Ellingsen et al., 2015); The average E₉₀ translates to ca. 0.08 mg/m³ for an 8-h time-weighted average. The average HrsW₉₀ was approximately equivalent to half-time welding and

Figure 3. Scatter plots show age-adjusted MD values in the hippocampus (y-axis) versus (a) ELT and (b) YrsW (x-axis); age-adjusted FA values in the hippocampus (y-axis) versus (c) ELT and (d) YrsW (x-axis) for welders and controls.

Figure 4. Scatter plots show (a) MD values in the hippocampus (y-axis) versus age (x-axis) for welders and controls. b, FA values in the hippocampus (y-axis) versus age (x-axis) for welders and controls.

the mean R1 was not significantly different from controls despite increasing with accumulating HrsW₉₀ (Lee et al., 2015).

Higher Hippocampal Mean Diffusivity in Welders

Our asymptomatic welders demonstrated higher hippocampal MD values compared with controls, evident particularly in older welders (>50 years). Mn-related microstructural changes previously were gauged using diffusion imaging, but these studies largely focused on changes in the BG (Criswell et al., 2012;

McKinney et al., 2004; Stepens et al., 2010) because Mn brain accumulation was noted prominently in the BG, principally in the GP (Dorman et al., 2006a; Lucchini et al., 2009). Recently, we found significantly lower FA values in the GP that were associated with chronic Mn exposure (Lee et al., 2016a).

Previous studies, including ours, reported welding-related Mn accumulation (estimated by R1 values) also occurred outside the BG (eg, frontal cortex, amygdala, and hippocampus) (Dorman et al., 2006a; Lee et al., 2015). In the present study, we

Figure 5. MRI diffusion tensor imaging (DTI) in the hippocampus: (a) MD (mean diffusivity), (b) FA (fractional anisotropy) in the younger and older subgroups of welders and controls. Values are raw means $\pm$ SEM.

Table 2. Summary Statistics for Demographics and Exposure Measures: Exposure, Whole Blood Metal, and MRI Metrics for Younger and Older Subgroups of Welders and Controls

	Younger Controls (N = 21)	Younger Welders (N = 20)	Rawp-Values
Age (years)	37.0 $\pm$ 6.9	40.2 $\pm$ 9.2	0.221
Education (years)	15.5 $\pm$ 2.0	12.8 $\pm$ 1.6	<0.001
MoCA	26.0 $\pm$ 2.9	26.5 $\pm$ 2.4	0.592
Smokers (n, %)	1, 2.4%	4, 9.8%	0.136
UPDRS-III	1.0 $\pm$ 1.8	1.6 $\pm$ 2.7	0.409
HrsW ₉₀ (h)	0 $\pm$ 0	262 $\pm$ 177	<0.001
E ₉₀ (mg-days/m ³)	0.003 $\pm$ 0	2.001 $\pm$ 1.883	<0.001
YrsW (years)	0 $\pm$ 0	19.10 $\pm$ 10.18	<0.001
ELT (mg-years/m ³)	0.001 $\pm$ 0.0002	0.848 $\pm$ 0.613	<0.001
Whole blood Mn (ng/ml)	9.4 $\pm$ 2.6	10.8 $\pm$ 3.4	0.144
Whole blood Fe (μ g/ml)	481 $\pm$ 78	545 $\pm$ 50	<0.001
MRI R1	0.51 $\pm$ 0.05	0.52 $\pm$ 0.02	0.861
MRI R2*	18.4 $\pm$ 1.8	18.3 $\pm$ 3.2	0.528
	Older Controls (N = 10)	Older Welders (N = 22)	
Age (years)	56.7 $\pm$ 3.4	57.4 $\pm$ 3.6	0.616
Education (years)	17.8 $\pm$ 1.5	12.9 $\pm$ 1.6	<0.001
MoCA	26.5 $\pm$ 1.6	26.2 $\pm$ 1.8	0.633
Smokers (n, %)	0, 0%	2, 6.25%	0.325
UPDRS-III	2.4 $\pm$ 2.5	2.4 $\pm$ 2.2	0.968
HrsW ₉₀ (h)	0 $\pm$ 0	222 $\pm$ 222	<0.001
E ₉₀ (mg-days/m ³)	0.003 $\pm$ 0	2.001 $\pm$ 1.883	<0.001
YrsW (years)	0 $\pm$ 0	32.7 $\pm$ 6.7	<0.001
ELT (mg-years/m ³)	0.002 $\pm$ 0.0001	1.475 $\pm$ 0.812	<0.001
Whole blood Mn (ng/ml)	7.9 $\pm$ 2.0	11.1 $\pm$ 3.2	0.006
Whole blood Fe (μ g/ml)	496 $\pm$ 70	545 $\pm$ 49	<0.001
MRI R1	0.52 $\pm$ 0.04	0.52 $\pm$ 0.04	0.139
MRI R2*	18.3 $\pm$ 2.5	18.5 $\pm$ 3.2	0.363

Data represent the mean $\pm$ SD for each measure. Groups were compared using one-way analysis of variance (ANOVA). Abbreviations: y, years; MoCA, Montreal Cognitive Assessment; UPDRS, unified PD rating scale; HrsW₉₀, hours welding, 90 days; E₉₀, cumulative 90 day exposure to Mn; YrsW, years welding, lifetime; ELT, cumulative exposure to Mn, lifetime.

found significantly higher MD values in the hippocampus, suggesting that welding-related microstructural changes may also occur in the hippocampus.

Welding-Related Increase in Hippocampal MD: A Marker for AD Risk?

The hippocampus is one of the regions where AD-related neurodegenerative processes are observed initially (Braak and Braak,

1991). Indeed, previous imaging studies have reported hippocampal atrophy in AD and prodromal (eg, MCI) AD populations (Killiany et al., 2002; Wisse et al., 2014). More recent studies, however, noted that hippocampal volume loss may not be sensitive enough to detect the earliest AD-related brain changes. Several neuroimaging markers (eg, shape of the hippocampus, DTI, or resting-state functional MRI) have been suggested to be more sensitive for predicting AD (Badhwar et al., 2017; Douaud et al.,

2013; Rathore et al., 2017). Among them, DTI methods (eg, higher MD and lower FA) have shown promise for assessing hippocampal changes in prodromal populations (Douaud et al., 2013; Fellgiebel et al., 2006; Kantarci et al., 2005; Müller et al., 2007). In asymptomatic elderly subjects, hippocampal MD values also better correlated with memory performance than did volume (Carlesimo et al., 2010; van Norden et al., 2012). These DTI differences may reflect gradual loss of barriers that restrict water diffusion in tissue compartments due to AD-related neuronal degeneration (eg, changes in water content, cytoarchitecture, and the demyelination process) (Beaulieu, 2002; Muller et al., 2005).

Although Mn-related neurotoxicity typically has been associated with parkinsonism rather than AD, there are several lines of evidence to suggest that Mn exposure may be a factor in AD and/or ADRD. First, Mn-exposed animals show increased Mn concentrations in the hippocampus (Dorman et al., 2006a; Liang et al., 2015), AD-like brain pathology (eg, A β diffuse plaques) (Guilarte, 2010), and deficits in associative learning (Liang et al., 2015; Schneider et al., 2013), the latter being hallmarks of AD. Second, Mn accumulates in human brains outside of the BG including in the hippocampus (Lee et al., 2015). Third, compared with controls, welders exhibited greater declines in delayed rather than immediate recall (Chang et al., 2010). Fourth, a higher prevalence of AD has been reported when an individual's birthplace had higher Mn levels (Emard et al., 1992). Fifth, AD-at-risk groups and dementia patients displayed dysregulated Mn metabolism and a dysfunctional Mn-superoxide dismutase (Mn-SOD) scavenger system (Du et al., 2017; González-Domínguez et al., 2014; Hare et al., 2016; Maeda et al., 1997). Lastly, a recent study reported higher serum blood Mn levels in dementia patients that were associated with severe cognitive decline and higher plasma A β peptide levels (Tong et al., 2014). Thus, our current results in combination with recent Mn-related neurotoxicity findings suggest that Mn-exposed welders may be at greater risk for AD or AD-related neurodegenerative processes.

Potential Mechanisms Leading to Higher Hippocampal MD

Hippocampal MD values were linearly and positively correlated with ELT, an estimate of long-term cumulative Mn exposure, but not with short-term exposure measurements (eg, HrsW₉₀, E₉₀, whole blood Mn, and R1). These results suggest that hippocampal microstructural changes may be associated with long-, rather than short-term exposure. Moreover, hippocampal MD values were not correlated with either whole blood Fe or R2* values (an estimate of Fe accumulation). Welding fumes contain several metals other than Mn (eg, Fe, Al, Cu, etc.) that can influence welding-related neurotoxicity and may contribute to AD-related brain changes (Banerjee et al., 2014; Liu et al., 2010a; Miu and Benga, 2006). Behaviorally, subjects exposed to Al and/or Fe (including welders) had deficits in memory performance (Hosovski et al., 1990). Several epidemiology findings, including a recent meta-analysis, reported positive relationships between chronic Al and/or Fe exposure and development of AD (Flaten, 2001; Kilburn, 1999; Killin et al., 2016; Shen et al., 2014; Wang et al., 2016). Consistent with these data, recent neuroimaging studies in AD patients confirmed higher Fe accumulation in brain areas that included the hippocampus (Acosta-Cabronero et al., 2013; Antharam et al., 2012; Raven et al., 2013). In addition, compared with nondemented controls, post-mortem analysis of AD patients revealed elevated Al or Fe concentrations in hippocampal neurons (Szabo et al., 2016; Walton, 2006), suggesting the involvement of Al and/or Fe in the pathogenesis of AD, although a causative role in AD is controversial (Tomljenovic, 2011).

Thus, although we cannot rule out the influence of other unmeasured co-exposures and other factors (eg, alcohol consumption) that may affect pathological changes in the hippocampus, the current hippocampal MD-ELT association, along with the lack of correlations with whole blood Fe and R2*, suggests that hippocampal MD variations in welders may be associated primarily with chronic Mn exposure. To our knowledge, this is the first study demonstrating elevated hippocampal MD values and their associations with Mn exposure measurements in welders.

Aging May Accelerate Welding-Related Hippocampal Changes: Implication for Occupational/Public Health

Welders in the current study demonstrated a significant linear relationship between hippocampal MD and age, whereas this association was not significant in controls. This was true even after controlling for chronic Mn exposure level (eg, ELT). When subjects were stratified by age, higher hippocampal MD effects were evident, especially in welders >50 years. Thus, the current results suggest that aging may play a role in making welders vulnerable to hippocampal changes. This finding is important because it implies that even chronic lower level Mn exposure may lead to hippocampal microstructural changes with advancing age. A previous study with healthy elderly subjects demonstrated higher hippocampal MD values, particularly after 50 years, in subjects ranging in age from 20 to 80 years; this was associated with poorer delayed memory performance (Carlesimo et al., 2010). The robust hippocampal MD-age association in our welders with ages ranging from 25 to 65, along with the lack of significance in controls from a similar age range, suggests that welding may have accelerated aging-related hippocampal degenerative processes. Thus, the current results indicate that Mn exposure, combined with the aging process, may play an important role in making welders more vulnerable to hippocampal changes and this may have implications for those exposed to metal toxicants being a potential risk factor for AD.

It is important to note that ascribing higher hippocampal MD values to higher risk for AD should be done with caution because several disorders other than AD (eg, multiple sclerosis and temporal lobe epilepsy) also display elevated hippocampal MD values (Assaf et al., 2003; Cercignani et al., 2001; Hakyemez et al., 2005; Rashid et al., 2004). We also found no group difference in MoCA scores, a measure of global cognitive status and a screening test for MCI, although this test may not be sensitive enough to detect subtle cognitive changes that may occur due to welding exposure, especially in subclinical populations. Future studies are warranted to elucidate the link between chronic welding and AD risk by including additional brain structures related to AD (eg, fornix, cingulum, entorhinal cortex, posterior cingulate, and precuneus) for the neuroimaging analysis and administering behavioral tasks highly sensitive in capturing AD-related early functional changes.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

SUPPLEMENTARY DATA

Supplementary data are available at Toxicological Sciences online.

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