Neuroinflammation and white matter alterations in occupational manganese exposure assessed by diffusion basis spectrum imaging

Susan R Criswell  
Washington University School of Medicine in St. Louis

Susan Searles Nielsen  
Washington University School of Medicine in St. Louis

Irene M Faust  
Washington University School of Medicine in St. Louis

Joshua S Shimony  
Washington University School of Medicine in St. Louis

Robert L White 3rd  
Washington University School of Medicine in St. Louis

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/oa_4

Part of the Medicine and Health Sciences Commons

Please let us know how this document benefits you.

Recommended Citation
Criswell, Susan R; Nielsen, Susan Searles; Faust, Irene M; Shimony, Joshua S; White, Robert L 3rd; Lenox-Krug, Jason; and Racette, Brad A, "Neuroinflammation and white matter alterations in occupational manganese exposure assessed by diffusion basis spectrum imaging." NeuroToxicology. 97, 25 - 33. (2023).  
https://digitalcommons.wustl.edu/oa_4/2976

This Open Access Publication is brought to you for free and open access by the Open Access Publications at Digital Commons@Becker. It has been accepted for inclusion in 2020-Current year OA Pubs by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Authors
Susan R Criswell, Susan Searles Nielsen, Irene M Faust, Joshua S Shimony, Robert L White 3rd, Jason Lenox-Krug, and Brad A Racette

This open access publication is available at Digital Commons@Becker: https://digitalcommons.wustl.edu/oa_4/2976
Neuroinflammation and white matter alterations in occupational manganese exposure assessed by diffusion basis spectrum imaging

Susan R. Criswell a,b,*, Susan Searles Nielsen b, Irene M. Faust b, Joshua S. Shimony b,c, Robert L. White 3rd b,d, Jason Lenox-Krug b, Brad A. Racette a,b

a Department of Neurology, Barrow Neurological Institute, 2910 N. 3rd Ave, Phoenix, AZ 85013, USA
b Department of Neurology, Washington University School of Medicine, 660 S. Euclid Ave, St. Louis, MO 63110, USA
c John Cochran Division, St. Louis VA Medical Center, Neurology Section, 915 N. Grand Blvd, St. Louis, MO 63106, USA
d School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, 27 Andrews Rd, Parktown 2193, South Africa

Objective: To evaluate in-vivo neuroinflammation and white matter (WM) microstructural integrity in occupational manganese (Mn) exposure.

Methods: We assessed brain inflammation using Diffusion Basis Spectrum Imaging (DBSI) in 26 Mn-exposed welders, 17 Mn-exposed workers, and 26 non-exposed participants. Cumulative Mn exposure was estimated from work histories and the Unified Parkinson’s Disease Rating Scale motor subsection 3 (UPDRS3) scores were completed by a movement specialist. Tract-based Spatial Statistics allowed for whole-brain voxel-wise WM analyses to compare WM DBSI-derived measures between the Mn-exposed and non-exposed groups. Exploratory grey matter region of interest (ROI) analyses examined the presence of similar alterations in the basal ganglia. We used voxelwise general linear modeling and linear regression to evaluate the association between cumulative Mn exposure, WM or basal ganglia DBSI metrics, and UPDRS3 scores, while adjusting for age.

Results: Mn-exposed welders had higher DBSI-derived restricted fraction (DBSI-RF), higher DBSI-derived nonrestricted fraction (DBSI-NRF), and lower DBSI-derived fiber fraction (DBSI-FF) in multiple WM tracts (all p < 0.05) in comparison to less-exposed workers and non-exposed participants. Basal ganglia ROI analyses revealed higher DBSI-NRF in Mn-exposed welders relative to non-exposed participants (p < 0.05). DBSI-NRF was also associated with greater cumulative Mn exposure and higher UPDRS3 scores.

Conclusions: Mn-exposed welders demonstrate greater DBSI-derived indicators of neuroinflammation-related cellularity (DBSI-RF), greater extracellular edema (DBSI-NRF), and lower apparent axonal density (DBSI-FF) in multiple WM tracts suggesting a neuroinflammatory component in the pathophysiology of Mn neurotoxicity. Caudate DBSI-NRF was positively associated with both cumulative Mn exposure and clinical parkinsonism, indicating a possible dose-dependent effect on extracellular edema with associated motor effects.

1. Introduction

Manganese (Mn) is a neurotoxicant that, in excess, produces parkinsonism, however the underlying mechanism has yet to be fully elucidated (Rodier, 1955). Multiple studies suggest neuroinflammation may play a key role in this pathologic process. Glial activation is a robust indicator of neuroinflammation and is a prominent pathologic feature in both humans and non-human primates exposed to Mn (Bauer et al., 1994; Cordova et al., 2013; Erikson and Aschner, 2006; Gonzalez-Cuyar et al., 2014; Harischandra et al., 2019; Huang et al., 2007; McGeer et al., 1988b; McGeer et al., 1988a; Perl and Olanow, 2007; Veneti et al., 2006; Wiley et al., 1986). Glial activation plays a key role in the central nervous system’s inflammatory response to environmental stressors and toxins by removing cellular debris and releasing proinflammatory cytokines (Harischandra et al., 2019; Tansey et al., 2008). Inducible nitric oxide synthase (iNOS), released by microglia in response to inflammatory mediators, produces large quantities of nitric oxide (NO). We previously demonstrated strong epigenetic evidence that Mn exposure also

* Corresponding author at: Department of Neurology, Barrow Neurological Institute, 2910 N. 3rd Ave, Phoenix, AZ 85013, USA.
E-mail address: susan.criswell@barrowneuro.org (S.R. Criswell).

https://doi.org/10.1016/j.neuro.2023.04.013
Received 13 August 2022; Received in revised form 4 April 2023; Accepted 28 April 2023
Available online 29 April 2023
0161-813X/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
increases production of NO through increased iNOS, further suggesting the presence of a pro-inflammatory state in Mn-exposed welders (Searles Nielsen et al., 2015). However, deeper investigations of in-vivo inflammation in environmental and occupationally exposed humans have been challenging, given that current imaging and pathology options are frequently restricted by cost, accessibility, and technical limitations.

Diffusion Basis Spectrum Imaging (DBSI) is a novel magnetic resonance imaging (MRI) approach designed to detect neuroinflammation and white matter (WM) microstructural alterations. In comparison to conventional diffusion tensor imaging (DTI) modeling, DBSI modeling differentiates and quantifies multiple intravoxel pathological processes by assigning specific diffusion components to different pathologic mechanisms (Wang et al., 2011; Wang et al., 2014). DBSI accomplished this by simultaneously modeling the diffusion signal with both anisotropic and isotropic components. The DBSI anisotropic tensor components evaluate voxel-level water diffusion, including water diffusion parallel to the axon (DBSI-axial diffusivity [DBSI-AD]) and perpendicular to the axon (DBSI-radial diffusivity [DBSI-RD]). DBSI also models fiber-tract specific diffusion anisotropy (DBSI-fractional anisotropy [DBSI-FA]) reflecting the integrity of axon bundles and DBSI-derived fiber fraction (DBSI-FF), an indicator of axonal density. Simultaneously, DBSI models isotropic diffusion which can be further separated into DBSI-restricted fraction (DBSI-RF), an indicator of neuroinflammation-related cellularity, and non-restricted isotropic diffusion (DBSI-NRF) or DBSI-hindered fraction, an indicator of extracellular tissue edema (Samara et al., 2019). These latter two DBSI measures are sensitive to the increased cellularity and extracellular tissue edema present in neuroinflammatory conditions (Cross and Song, 2017; Frohman et al., 2006; Stamatovic et al., 2006) (Supplemental Table 1). As such, DBSI has indicated the presence of neuroinflammation in a wide range of neurologic conditions including multiple sclerosis (Chiang et al., 2014), Alzheimer’s disease (Wang et al., 2019), traumatic spinal cord injury (Sun et al., 2017), and HIV (Strain et al., 2017).

Importantly, DBSI can provide current, in-vivo information about ongoing neuroinflammatory processes (Chiang et al., 2014; Cross and Song, 2017; Lin et al., 2017; Samara et al., 2019; Strain et al., 2017; Sun et al., 2017; Wang et al., 2011; Wang et al., 2014; Wang et al., 2015).

The goal of this study was to apply DBSI in Mn-exposed humans to evaluate the presence of neuroinflammation and investigate WM microstructural integrity. We hypothesized that Mn-exposed welders and Mn-exposed workers (non-welders exposed to welding fume while working at the same site) would have greater DBSI-RF (an indicator of neuroinflammation-related cellularity), greater DBSI-NRF (an indicator of extracellular edema), and lower DBSI-FF (an indicator of lower apparent axonal density) within the WM compared to non-exposed participants. We tested these hypotheses in participants recruited from a well characterized cohort of Mn-exposed and non-exposed workers. We tested these hypotheses in participants recruited from a well characterized cohort of Mn-exposed and non-exposed workers (Racette et al., 2017). Further, since Mn exposure is associated with parkinsonism and possible neuroinflammation within the basal ganglia, we selected the caudate, anterior/posterior putamen, and globus pallidus (GP) to perform regions of interest (ROI) analyses, and explored the presence of similar variations in these gray matter regions and their relation to motor dysfunction as measured by the Unified Parkinson’s Disease Rating Scale motor subsection 3 (UPDRS3) (Fahn et al., 1987).

2. Materials and methods

2.1. Protocol approvals and participant consents

This study was approved by the Washington University Human Research Protection Office and was carried out in accordance with the principles expressed in the Declaration of Helsinki. All participants gave written, informed consent prior to participation.

2.2. Participants

All participants (N = 69) were from the U.S. Midwest and underwent brain MRI from 2007 to 2015. To obtain a range of Mn exposures, we recruited from two participant sources: 1) 38 participants were International Brotherhood of Boilermakers (IBB) union members who participated in a previous cohort study (Racette et al., 2017) of Mn-exposed welders and workers and five carpentry construction workers who were exposed to welding fume as part of their same site occupation; and 2) 26 non-IBB union participants with no history of occupational welding or Mn exposure from the community or the local carpentry union who were recruited through IBB contacts. Thus, in total, 43 workers conducted welding as part of their occupation (26 Mn-exposed welders) or worked around welding fume as part of their occupation (17 Mn-exposed workers), and 26 participants who were not occupationally exposed to Mn (26 non-exposed). All participants from the greater IBB cohort (Racette et al., 2017) were offered participation in this study. The socioeconomic status of the non-exposed participants (both from the community and carpentry union) was similar to the Mn-exposed welders and workers and included carpenters, general laborers, machinists, and construction workers. Exclusion criteria were history of liver disease, stroke, brain tumor, or other condition that could compromise the neurologic examination; use of anti-parkinsonian medications, neuroleptics, or amphetamines; and age <18 years, all of which were uncommon.

2.3. Clinical assessments

A movement disorders specialist blinded to Mn exposure levels examined all participants and rated them using the UPDRS3. Prior to rating participants for this study, the two primary examiners in this study each rated ten Parkinson disease (PD) patient videos and the intraclass correlation coefficient for UPDRS3 ratings was > 90%. This was repeated annually throughout this study. We utilized the UPDRS3 rating closest to the MRI scan date; > 81% of participants had a UPDRS3 rating on the same day as their MRI scan. To account for potential differences by examiner, we adjusted UPDRS3 scores for examiner as previously described (Racette et al., 2017). One participant did not have examiner-adjusted UPDRS3 data because their exam was completed by a third examiner.

2.4. Exposure assessment

All participants completed or updated a validated, structured questionnaire in person at the time of imaging, which included a detailed work history (Hobson et al., 2009). Using this information, we calculated cumulative Mn exposure in mg Mn/m$^3$-years which takes into account both duration (years) and intensity (mg Mn/m$^3$) of exposure as previously described (Racette et al., 2017). This variable was available for all participants.

2.5. Magnetic Resonance Imaging (MRI) acquisitions

All magnetic resonance imaging scanning was performed on a Siemens Trio 3 T scanner (Erlangen, Germany) with a 12-channel head coil. We acquired high resolution three-dimensional (3-D) magnetization-prepared rapid gradient echo (MPRAGE) images on each participant (repetition time [TR]=2400 ms, inversion time [TI]=1000 ms, echo time [TE]=3.14 ms, flip angle=8°, 0.9 × 0.9 × 0.9 mm voxels). A minimum of two sequential diffusion-weighted scans (acquisition time 4 min 25 s) were acquired in 25 directions, at multiple b values (Supplemental Table 2), using an echo planar imaging sequence (TR=9200 ms, TE=90 ms, 2.0 mm isotropic voxels, b values ranging from 0 to 1400 s/mm$^2$, and one non-diffusion weighted image).
2.6. Image preprocessing and DTI processing

All volumes were manually inspected to exclude the presence of large artifacts. We used FMRIB Software Library (FSL) (Smith et al., 2004) for all preprocessing steps and to fit the standard DTI diffusion tensor model at each imaging voxel. Non-brain tissue was removed using FSL brain extraction tool (BET) (Smith, 2002), followed by motion and eddy-current distortions correction. For DTI analyses, FSL DTIFIT tool was used to compute diffusivities from fitting the diffusion tensor model and to generate DTI-fractional anisotropy (DTI-FA), DTI-mean diffusivity (DTI-MD), DTI-radial diffusivity (DTI-RD), and DTI-axial diffusivity (DTI-AD) volumes for each participant.

2.7. DBSI processing

We calculated DBSI measures using in-house software scripted in MATLAB and Statistics Toolbox Release (2012) (Wang et al., 2011). By solving the DBSI model, we obtained the following anisotropic and isotropic metrics: DBSI-FA, DBSI-AD, DBSI-RD, DBSI-FF, DBSI-RF, and DBSI-NRF for each participant.

2.8. Tract-based spatial statistics

We completed post-processing of DTI- and DBSI-derived volumes with Tract-based Spatial Statistics (TBSS) (Smith et al., 2006). We used DTI-FA images to create an average WM skeleton as previously described (Samara et al., 2019). To achieve this, all DTI-FA were slightly eroded to remove potential outliers from diffusion tensor fitting. Subsequently, all images were non-linearly registered to a FMRIB58-FA standard-space image as a target image. Aligned FA images were then averaged to create a mean FA image and fed into the skeletonization step to create a WM skeleton using a threshold of FA > 0.3. Using the same transformation process, all DTI- and DBSI-derived images, for each participant, were projected onto the mean FA skeleton for voxel-wise and region of interest (ROI)-based analyses.

2.9. WM tracts and ROI identification

We used the JHU-ICBM-DTI-81 WM labels atlas to create masks to label anatomic areas within the WM (Mori et al., 2008). Exploratory grey matter DBSI-derived metrics (both anisotropic and isotropic) in the right and left caudate, globus pallidus, and anterior/posterior putamen were extracted using hand drawn ROIs in standard atlas space, as previously published (Criswell et al., 2011). The ROIs were then projected onto the atlas aligned images of all participants; hence, ROI volumes were identical for all participants. Voxel values were averaged within each grey matter ROI and left and right brain ROI DBSI-metrics were averaged for comparison between Mn-exposed welders and workers, and non-exposed participants.

2.10. Statistical analyses

We used Stata MP Version 14.2 and 17.0 for all analyses unless otherwise specified. We assessed differences in race, sex, and age between Mn exposure groups using multiple linear regression with the non-exposed participants as the reference group. We assessed differences in motor performance between the exposure groups with multiple linear regression controlling for age using Harrell's age (continuous in five linear terms) as a covariate. We verified linearity for each ROI DBSI metric against cumulative Mn exposure and UPDRS3 scores using locally weighted scatterplot smoothing (LOWESS). Additionally, we used linear regression controlling for age using Harrell’s method to assess the relationships between the following: 1) DBSI metrics in the basal ganglia ROIs with cumulative Mn exposure (with basal ganglia DBSI metrics as the dependent variable) and 2) DBSI metrics and UPDRS3 score (with UPDRS3 score as the dependent variable). We did not correct for multiple comparisons for the exploratory grey matter ROI-based analyses. To ensure this modeling did not result in overfitting, we performed a sensitivity analysis by examining the same comparisons using a linear age adjustment. We also explored the effects of alcohol use by adjusting for alcoholism as liver dysfunction can cause Mn toxicity (acquired hepatocerebral degeneration) with the characteristic basal ganglia T1-weighted signal intensities secondary to poor hepatic clearance (Meissner and Tison, 2011). Similarly, we explored the effects of the time interval between the first participant imaged to subsequent participants and the time interval between imaging and clinical examination for each participant by adjusting for the specified time interval.

3. Results

3.1. Characteristics of participants

Most workers were non-Hispanic white men (Table 1). As compared to the non-exposed participants, the two Mn-exposed groups were similar in terms of sex (Mn-exposed welders p = 0.64, Mn-exposed workers p = 0.54) and age (Mn-exposed welders p = 0.67, Mn-exposed workers p = 0.55), with means across all three groups ranging from 43.8 to 46.2 years. The Mn-exposed welders and workers had a relatively wide range of duration of welding exposure (0.01–44.8 years), with substantially greater duration among the former (p < 0.01). The Mn-exposed workers had a similar age and sex distribution as compared to the larger cohort (Racette et al., 2017) from which they were recruited (not shown). Mn-exposed welders and Mn-exposed workers each demonstrated higher examiner-adjusted UPDRS3 scores (age-adjusted Mn-exposed welders p < 0.01, Mn-exposed workers p = 0.03) than the non-exposed participants, with the Mn-exposed workers having intermediate values on average (8.3) as compared to the welders (12.5) and the non-exposed participants (5.9) (Table 1).

3.2. Voxel-wise comparison of white matter DTI and DBSI metrics

In TBSS voxel-wise analyses that adjusted for age, we observed significantly lower DTI-FA and DTI-AD in the WM of Mn-exposed
welders when compared to either Mn-exposed workers (data not shown) or non-exposed participants (Fig. 1A-B). WM DTI-MD and DTI-RD were not significantly different between the Mn exposure groups (data not shown). There was also no difference in WM DBSI-FA, or DBSI-RD between the Mn-exposed welders or workers when compared to non-exposed participants (Fig. 2A-B). WM DBSI-AD and DBSI-RF in Mn-exposed welders were higher in Mn-exposed welders compared to non-exposed participants (Fig. 4). There were no consistent age-adjusted associations between cumulative Mn exposure, WM volume, time from first participant imaging, the time interval between imaging and clinical examination, or UPDRS3 scores and voxel-based WM DBSI metrics.

3.3. Basal Ganglia DBSI metrics and Mn exposure

When we compared DBSI-derived metrics (DBSI-AD, DBSI-FA, DBSI-FF, DBSI-NRF, DBSI-AD, DBSI-RF) between Mn-exposed welders, Mn-exposed workers, and non-exposed participants, caudate DBSI-NRF and DBSI-RD were significantly higher in Mn-exposed welders compared to non-exposed participants, while there was a trend toward higher caudate DBSI-AD in Mn-exposed welders (Table 2). Caudate DBSI-NRF was also positively associated with cumulative Mn exposure measured in mg Mn/m$^3$-years ($p = 0.04$), adjusting for age (Table 3). GP DBSI-AD was higher in Mn-exposed welders compared to non-exposed participants (Table 2). Anterior and posterior putamen DBSI metrics did not significantly differ by exposure group. Adjusting for the time interval from first participant imaging or the time interval between imaging and clinical examination did not result in any significant change in the associations between DBSI metrics and Mn exposure. Utilizing an alternative model with a linear age adjustment did not result in meaningful changes in these associations.

3.4. Clinical associations with Basal Ganglia DBSI metrics

We observed positive associations between caudate DBSI-NRF and the examiner-adjusted UPDRS3 score ($p = 0.03$) and caudate DBSI-AD ($p = 0.05$) as well as between the GP DBSI-AD and the UPDRS3 score ($p = 0.07$) (Table 4). All other associations between ROI DBSI and measures and examiner-adjusted UPDRS3 scores were not significant or of borderline significance (all $p > 0.10$). Adjusting for alcoholism, time interval from first participant imaging, or

---

**Table 1**

<table>
<thead>
<tr>
<th>Characteristics of Participants, by Manganese Exposure Category.</th>
<th>No welding exposure</th>
<th>Mn-exposed workers</th>
<th>Mn-exposed welder</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 26</td>
<td>N = 17</td>
<td>N = 26</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>23 (88.5)</td>
<td>16 (94.1)</td>
<td>24 (92.3)</td>
</tr>
<tr>
<td>Non-Hispanic white, n (%)</td>
<td>23 (88.5)</td>
<td>16 (94.1)</td>
<td>26 (100)</td>
</tr>
<tr>
<td>Age, years</td>
<td>43.8 (13.5)</td>
<td>46.2 (11.3)</td>
<td>45.3 (12.6)</td>
</tr>
<tr>
<td>Median</td>
<td>48.0</td>
<td>47.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22-69</td>
<td>23-64</td>
<td>23-63</td>
</tr>
<tr>
<td>Welding fume exposure, years</td>
<td>0.0</td>
<td>0.5 - 5.8</td>
<td>0.5 - 44.8</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.8 (1.6)</td>
<td>17.1 (14.0)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.5 - 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welding fume exposure, Mn/m$^3$-years</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.3 (0.2)</td>
<td>2.4 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.001 - 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPDRS3 score$^b$</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8.3 (5.0)</td>
<td>12.5 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.0-9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welding fume exposure, Mn/m$^3$-years</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.0-19.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.0-25.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ANOVA = analysis of variance; Mn = manganese; SD = standard deviation; UPDRS3 = Unified Parkinson’s Disease Rating Scale motor subsection

$^a$-values for male sex, race, age, and UPDRS3 scores were compared using multinomial logistic regression with non-exposed workers as the reference group. UPDRS3 score $p$-values are adjusted for age using Harrell’s placement method (continuously in five linear terms). Welding fume and manganese exposure $p$-values are from ANOVA.

$^b$Examiner-adjusted. UPDRS3 score missing from one participant without welding exposure.

---

Fig. 1. Voxel-wise comparison of DTI derived fractional anisotropy (DTI-FA) and axial diffusivity (DTI-AD) in the white matter (WM) of Mn-exposed welders and non-exposed participants (N = 52). Mn-exposed welders had lower age-adjusted (A) WM DTI-FA and (B) WM DTI-AD than non-exposed participants. Green, WM skeleton; red ($p < 0.05$).
the time interval between imaging and clinical examination did not result in any meaningful change in these associations. Similarly, applying the alternative model with a linear age adjustment did not result in any significant changes in these associations.

4. Discussion

The findings of this DBSI MRI study indicate the presence of multifocal neuroinflammation (higher DBSI-RF), extracellular tissue edema (higher DBSI-NRF), and greater water diffusion parallel to the axon (higher DBSI-AD) in Mn-exposed welders when compared to Mn-exposed workers and non-exposed participants in multiple WM tracts.
Mn-exposed welders also demonstrated multiple WM areas of lower apparent axonal density (DBSI-FF) in comparison to non-exposed reference participants; however, there were no differences in WM DBSI-FA and DBSI-RD between exposure groups. We observed differences in WM DBSI measures between Mn-exposed welders, workers, and non-exposed participants within the left > right internal capsule, genu of the corpus callosum, cingulate, right > left corticospinal tracts, and anterior corona radiate as defined by the JHU-ICBM-DTI-81 atlas (Fig. 2-4). Exploratory grey matter ROI analyses revealed age-adjusted dose-response associations between cumulative Mn exposure and caudate DBSI-NRF, suggesting greater extracellular edema with increasing Mn exposure. In addition, caudate DBSI-NRF was also positively associated with UPRDS3 score indicating a relationship with DBSI measures in the caudate and both Mn exposure and clinical severity of parkinsonism. These results suggest Mn exposure may be associated with ongoing, multi-focal white and grey matter neuroinflammation with associated clinical neurotoxicity.

Previously, DTI studies in Mn-exposed welders identified lower DTI-FA in the corpus callosum and frontal WM with marked increases in DTI-RD when compared to non-exposed participants suggesting microstructural abnormalities in these areas (Kim et al., 2011). DTI-FA measured in the cerebellar peduncle and the superior longitudinal fasciculus also differentiates between welders using respirators and those who do not (Rechtmann et al., 2020). We similarly identified multiple areas of lower WM DTI-FA (and additionally, lower DTI-AD) in Mn-exposed welders in comparison to non-exposed participants in the corpus callosum, bilateral internal capsule, corticospinal tracts, frontal WM (anterior corona radiata) and the superior longitudinal fasciculus; however, there were no differences in DTI-RD identified between the exposure groups. In traditional DTI interpretation, the combination of these findings would suggest impairment in WM integrity and axonal injury in Mn-exposed welders. However, because DTI models the diffusion of water both within and outside the axon, increased cellularity associated with neuroinflammation may lead to symmetric decrease in diffusion in all directions, resulting in an observed decrease in DTI-AD and DTI-FA, even in the absence of axonal injury. After the isotropic diffusion is accounted for using the DBSI modeling, there was no difference in WM DTI-FA between groups, while DBSI-AD was higher in the Mn-exposed welders. This may indicate the presence of greater water diffusion parallel to the axons in the extracellular compartment secondary to tissue edema, as suggested by the higher WM DBSI-NRF in Mn-exposed welders when compared to the less exposed workers and non-exposed participants. These findings are consistent with previous DBSI MRI studies (Samara et al., 2019; Wang et al., 2014; Winklewski et al., 2018; Zhan et al., 2018), suggesting that neuroinflammation-related processes (cellular infiltration and tissue edema) may confound DTI-derived metrics. Alternatively, the patterns of differences across DBSI and DTI metrics might represent different stages of neuroinflammation and WM structural reorganization including remyelination and axon sprouting in the presence of a chronic neuroinflammatory exposure.

In the Mn-exposed welders, higher DBSI-RF and DBSI-NRF in WM tracts would indicate an infiltration of central nervous system inflammatory cells and resulting extracellular tissue edema in response to Mn exposure. While information related to WM pathology in Mn neurotoxicity is limited, multiple MRI studies suggest Mn deposition can occur throughout the brain (Guiarté et al., 2006; Sen et al., 2011). This observation combined with the previous DTI-based studies demonstrating WM abnormalities in Mn-exposed individuals indicates that Mn neurotoxicity likely has direct effects on WM structures. The current study suggests some of the differences in DBSI metrics are associated with an underlying neuroinflammatory process, but pathologic confirmation will be needed.

We also identified higher caudate DBSI-AD, DBSI-NRF, and DBSI-RD...
4.1. Limitations

This study has several potential limitations. Welding fume contains a number of elements and gases (NIOSH, 1988); therefore, we cannot fully exclude a contribution from other neurotoxicants present in welding fume. Our study relied on Mn exposure estimates based on work histories, rather than on-site measurements of concentrations of Mn in the air. Our findings were also limited to group differences in DBSI WM metrics from a cross-sectional study; as a result, we had very limited ability to assess dose-response relationships between Mn exposure and WM changes. Nevertheless, our observation that Mn-exposed welders had higher DBSI-RF, DBSI-NRF, and DBSI-AD in WM tracts than the less exposed worker group is consistent with a causal role for Mn, as this does suggest a dose-response relationship with exposure. Similarly, while we identified a dose-response relationship between a continuous measure of cumulative Mn exposure and caudate DBSI-NRF, the use of DBSI in grey matter structures remains exploratory. Of note, Mn deposition in the brain is clearly associated with changes in T1 measures in occupational exposure studies (Jensen et al., 2022). However, regardless of any Mn related effects on susceptibility, the diffusion measurement is a relative change in the signal intensity between the b= 0 state (reference image effectively cancelling baseline signal intensity and a b= ~0 image effectively cancelling out any metal related effects). Further, without histopathological validation, the relationship between DBSI measures and neuroinflammation in Mn exposure remains limited to a highly plausible hypothesis and may be related to non-Mn causes of neuroinflammation. Future longitudinal studies including plasma, cerebrospinal fluid inflammatory markers, and neuropsychiatric testing may be helpful in clarifying the temporal relationships between neuroinflammation, DBSI metrics, and the full cognitive and motor phenotype associated with Mn neurotoxicity. As such, further histopathologic confirmation of DBSI as a marker of neuroinflammation in Mn neurotoxicity will be required to determine if these differences are secondary to glial activation. Similarly, DBSI-NRF is an indicator of non-restricted water diffusion in the extracellular space, reflecting tissue edema, which has been identified in multiple acute neuroinflammatory conditions (Wang et al., 2011; Wang et al., 2014; Zhan et al., 2018). Fully understanding differences in DBSI-NRF in the setting of chronic neuroinflammation, like long-term Mn exposure, will require further study.
In summary, we demonstrated differences in DBSI-derived indicators of neuroinflammation within the WM and the basal ganglia of Mn-exposed welders when compared to non-exposed participants. These include indicators of increased neuroinflammation-related cellularity (DBSI-RF) and extracellular edema (DBSI-NRF) and may suggest a neuroinflammatory process in the pathophysiology of Mn neurotoxicity, however this will require further confirmatory studies. Caudate DBSI-HF may be of particular interest as it demonstrated a dose-response relationship with both cumulative Mn exposure and severity of parkinsonism.

**Funding**

This work was supported by the following National Institute of Health, USA Grant Numbers: R01ES021488, R01ES029524, K23ES021444, K24ES017765, R01ES028295, R01ES013743, R01ES021488-02S1, P42ES004696, and ICTS UL1RR024992, the American Parkinson Disease Association (APDA), USA, the Advanced Research Center at Washington University, USA, and the Greater St. Louis Chapter of the APDA, USA. These sponsors had no involvement in the study design; collection, analysis or interpretation of the data; writing of the report; or decision to submit for publication.

**CRediT authorship contribution statement**

Susan R. Criswell: Conceptualization, Visualization, Investigation, Writing – original draft preparation. Susan Searles Nielsen: Methodology, Formal analysis, Writing – review & editing. Irene M. Faust: Methodology, Formal analysis, Writing – review & editing. Joshua S. Shimony: Methodology, Validation, Writing – review & editing. Robert L. White: Methodology, Validation, Writing – review & editing. Jason Lenox-Krug: Data curation, Software, Formal analysis, Writing – review & editing. Brad A. Racette: Conceptualization, Funding acquisition, Writing – review & editing.

**Declaration of Competing Interest**

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests Dr. Criswell reports grant funding from the NIH including K23ES021444, R01ES029524, and R01ES013743. Dr. Searles Nielsen reports grant funding from the NIH including R01ES028295 and R01ES021488, other NIH grants unrelated to the work, and grant funding from the U.S. Department of Defense, Cure Alzheimer’s Fund, The Michael J. Fox Foundation, and American Parkinson Disease Association unrelated to the submitted work. Dr. Shimony reports grant funding by the P50 HD103525 to the Intellectual and Developmental Disabilities Research Center at Washington University. Dr. White reports grant funding from the VA and Michael J. Fox Foundation. Dr. Racette reports grant funding from NIH including K24ES017765, R01ES021488, R01ES013743, P42ES004696, and UL1RR024992, grant funding from American Parkinson Disease Association and grant funding from The Michael J. Fox Foundation, U.S. Department of Defense, and Cure Alzheimer’s Fund unrelated to the submitted work. Mr. Lenox-Krug, and Ms. Faust have nothing to disclose.

**Data availability**

Data will be made available on request.

**Appendix A. Supporting information**

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2023.04.013.


