Published in final edited form as:

Neurotoxicology. 2018 January ; 64: 43–49. doi:10.1016/j.neuro.2017.07.004.

[¹⁸F]FDOPA Positron Emission Tomography in Manganese-Exposed Workers

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Abstract

Occupational manganese (Mn) exposure is associated with the development of parkinsonism; however, the mechanism of neurotoxicity is unknown. Brain positron emission tomography (PET) imaging provides a non-invasive method of assessing dopaminergic neuronal function. 6-[¹⁸F]fluoro-L-DOPA (FDOPA) PET reflects in-vivo nigrostriatal function, but results in Mn exposure are conflicting. The objective of this study was to investigate the association between Mn exposure secondary to occupational welding, FDOPA striatal uptake, and clinical parkinsonism as

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COMPETING FINANCIAL INTERESTS DECLARATION:

The authors do not have any financial disclosures or conflicts of interest related to the research in the manuscript.

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measured by Unified Parkinson Disease Rating Scale motor subscore 3 (UPDRS3) scores. FDOPA PET scans were acquired on 72 subjects (27 Mn-exposed welders, 14 other Mn-exposed workers, and 31 non-exposed subjects). We estimated cumulative welding exposure from detailed work histories, and a movement disorders specialist examined all subjects. Striatal volumes of interest were identified on aligned magnetic resonance imaging (MRI) for each subject. Specific striatal FDOPA uptake was calculated with a graphical analysis method. We used linear regression while adjusting for age to assess the association between welding exposure and FDOPA uptake in the caudate, anterior putamen, and posterior putamen. Compared to the non-exposed subjects, mean caudate FDOPA uptake was 0.0014 min^{-1} (95% confidence interval [CI] 0.0008, 0.0020) lower in Mn-exposed welders and 0.0012 min^{-1} (95% CI 0.0005, 0.0019) lower in other Mn-exposed workers (both $p < 0.001$). There was no clear dose-response association between caudate FDOPA uptake and Mn exposure or UPDRS3 scores. Mn-exposed welders and workers demonstrated lower caudate FDOPA uptake, indicating pre-synaptic dopaminergic dysfunction in Mn-exposed subjects that was not associated with clinical parkinsonism.

Keywords

Manganese; [^{18}F]FDOPA PET

1. Introduction

Exposure to manganese (Mn) from occupational welding fume can cause neurologic dysfunction including clinical parkinsonism (Racette et al., 2012) and cognitive impairment (Bowler et al., 2007, Park et al., 2009, Rodier, 1955). Mn exposure is associated with Mn deposition within the brain (Dorman et al., 2006, Eriksson et al., 1987, Tapin et al., 2006), which can be detected with T1-weighted magnetic resonance imaging (MRI) (Kim et al., 1999a, Nelson et al., 1993), but its effects on brain physiology are not well characterized (Criswell et al., 2015, Gonzalez-Cuyar et al., 2014). Positron emission tomography (PET) is a non-invasive molecular imaging technique that can quantify biochemical and physiologic processes in the living brain. Striatal uptake in the brain of 6- ^{18}F fluoro-L-DOPA (FDOPA) primarily reflects neuronal aromatic L-aminoacid decarboxylase activity and provides a non-invasive method of assessing in-vivo dopaminergic pre-synaptic nerve terminal function within the nigrostriatal system (Yee et al., 2001). Some FDOPA PET studies in Mn-exposed subjects report normal dopamine function (Shinotoh et al., 1997, Wolters et al., 1989), whereas others found reduced striatal uptake of FDOPA suggesting pre-synaptic dopaminergic dysfunction (Criswell et al., 2011, Kim et al., 1999b, Racette et al., 2005).

In the present study, our objectives were to evaluate the association between Mn-containing welding fume exposure, clinical signs of parkinsonism, and striatal dopaminergic molecular imaging markers. We performed FDOPA PET imaging in an expanded cohort of welders and workers with a range of welding exposure and clinical signs of parkinsonism. We hypothesized FDOPA uptake in this expanded cohort would 1) validate our previous work (Criswell et al., 2011) demonstrating reduced FDOPA uptake in the caudate nucleus of asymptomatic welders compared to non-exposed subjects and 2) be inversely associated with welding fume exposure and clinical parkinsonism.

1. Methods

2.1 Protocol approvals and subject consents

This study was approved by the Washington University School of Medicine Human Research Protection Office and the Radioactive Drug Research Committee, and all subjects provided written informed consent prior to study conduct.

2.2 Subjects

Subjects (n = 72) were recruited from April 16, 2007 through January 25, 2016 from participants in a large cohort study (Racette et al., 2012, Racette et al., 2017) of 886 Mn-exposed workers at three Midwestern welding work sites: two shipyards and one heavy equipment fabrication company. All subjects had worked at the site within one year of the time of imaging. They were primarily engaged as welders or welder helpers (n = 27, hereafter “Mn-exposed welders” or “welders”). However, they also included workers otherwise exposed to welding fume at these work sites (n = 11). The latter included electricians, machinists, mechanics, and maintenance workers. Three additional workers with < 500 lifetime hours of welding fume exposure were available from a local carpentry union, providing a total of 14 subjects who had worked around welding fume (hereafter “Mn-exposed workers”). Eleven of the 27 Mn-exposed welders and 10 of the 14 Mn-exposed workers were newly added since our initial study (Criswell et al., 2011). In contrast to this initial study, these newly recruited workers could be clinically asymptomatic or symptomatic (defined as symptomatic complaints of parkinsonism). To allow for greater exposure contrasts, we also recruited reference subjects from the community or the same local carpentry union who had no occupational exposure to welding fume (n = 31). Eleven of the 31 non-exposed reference subjects were newly added since our initial study (Criswell et al., 2011). Exposed workers were excluded from the original cohort if they were < 18 years old or had a history of stroke, brain tumor, or other condition that could compromise the UPDRS3, as detailed previously (Racette et al., 2017). Therefore, we applied these same exclusion criteria to non-exposed reference subjects, and one individual was excluded on this basis.

For this imaging study in particular, we applied the following exclusion criteria for all exposed and non-exposed subjects according to a detailed assessment of medications/drugs that could affect imaging that is intended to assess dopamine uptake: any dopaminergic medications, any other anti-parkinsonian medications, any neuroleptics, or amphetamines. One Mn-exposed subject was excluded on this basis. In addition, we also ensured that the overall age range of Mn-exposed and non-exposed subjects was not markedly different as both were accrued. Specifically, we recruited men and women from the community who were similar in age to the Mn-exposed and non-exposed workers that we identified from the worksites. Accordingly, all exposed and non-exposed subjects in the present study were < 72 years of age.

2.3 Clinical assessments

A movement disorders specialist blinded to exposure levels and imaging results examined all subjects and rated them on the Unified Parkinson's Disease Rating Scale motor subsection 3 (UPDRS3) (Fahn et al., 1987).

2.4 Imaging

A high resolution 3D magnetization-prepared rapid gradient echo (MPRAGE) image ($1 \times 1 \times 1.25$ mm voxels) was acquired on each subject using a Siemens Magnetom Sonata 1.5 T scanner, or a Siemens Trio 3.0 T scanner (Erlangen, Germany). A reviewer blinded to the exposure and clinical status of the subject outlined volumes of interest (VOIs) in the caudate, anterior putamen, and posterior putamen on individual MPRAGE images as previously described (Criswell et al., 2011, Criswell et al., 2012a). A secondary reviewer performed a second reading of all VOIs to avoid observer bias. We further looked at the effect of primary rater with a multiple linear regression modeling VOI K_{occ} by age, exposure group, and rater. There was no statistical evidence for a rater effect on VOI K_{occ} (all $p > 0.43$). Striatal VOIs outlined the entire structure while the reference regions comprised a pair of semi-cylinders on either side of midline in occipital cortex with a mixture of grey matter (GM) and white matter (WM).

6- $[^{18}\text{F}]$ Fluoro-L-dopa (FDOPA) was synthesized via electrophilic destannylation using $[^{18}\text{F}]$ fluorine made with the Washington University RDS Eclipse cyclotron. The radiopharmaceutical was purified using preparative HPLC to give FDOPA with radiochemical purity $> 90\%$ and specific activity > 100 mCi/mmol (3.7 GBq/mmol). PET images were acquired using an ECAT EXACT HR, or EXACT HR+ scanner (Knoxville, TN). The PET scanners, EXACT HR and HR+, have similar 3D spatial resolutions of approximately 4.5 mm full width at half-maximum in all three dimensions for central regions of the field-of-view. All subjects were initially pretreated with carbidopa (Merck & Co., Inc) 200 mg orally one hour before the scan. FDOPA (3.18 – 7.20 mCi) was intravenously injected into an arm vein through a 20-gauge catheter. Attenuation was measured using ^{68}Ga - ^{68}Ge rotating rod sources. Dynamic emission scans were acquired in 3D mode following injection of FDOPA. Emission scans were corrected for attenuation, scatter, and randoms and reconstructed using filtered back projection with a ramp filter cutoff at the Nyquist frequency. Individual PET images were aligned to each other to correct movement between frames using Automated Image Registration (AIR) (Woods et al., 1993).

To correct for different partial volume effects of age and age-related atrophy the MPRAGE was segmented into GM, WM, and cerebral spinal fluid (CSF) using Statistical Parametric Mapping (SPM8, 2009). These individual tissue images were smoothed to the resolution of the PET using a 3D Gaussian filter prior to co-registration and reslicing to the PET images. We assumed that CSF has no FDOPA activity and that WM has uniform FDOPA uptake. While we corrected control regions to reflect GM activity, we did not correct striatal VOIs for any internal WM. Therefore, the computed values of K_{occ} represent FDOPA uptake for the entire structure relative to nonspecific GM uptake in occipital cortex. The partial-volume-corrected (PVC) GM FDOPA activity of each VOI was computed as follows: Corrected counts = (mean counts – WM fraction $\times$ mean WM counts)/GM fraction. The

transformation matrix generated by co-registering MPRAGE to PET was used to resample MR-defined VOIs. VOIs for PET comprised voxels in which at least 50% of each voxel was within the corresponding MR-defined VOI. Decay-corrected regional PET counts were extracted from dynamic PET images using the defined VOIs. Specific FDOPA uptake (K_{occ}) was computed using Patlak graphical analysis (Patlak et al., 1983) of the time-activity data from 24 to 94 minutes post-injection. Left and right VOIs for the caudate, anterior putamen, and posterior putamen were averaged. Using these values, we calculated the caudate/posterior putamen (C/P) ratio for each subject.

2.5 Exposure assessment

Workers completed or updated a validated, structured questionnaire in person at the time of imaging. Through the questionnaire we obtained a detailed work history (Hobson et al., 2009), which we used to estimate total years of welding fume exposure and weighted welding years as described previously (Racette et al., 2012). The weighted welding years metric takes into account total years of welding fume exposure and intensity of exposure, primarily by considering job title. For the third metric of cumulative Mn exposure, we refined this weighted welding years metric to also take into account the number of hours per week exposed, as well as the type of welding and degree of confinement, which affect Mn concentration (Hobson et al., 2011). Specifically, we first applied data on the type of welding and degree of confinement for each job to a model that predicts Mn concentration (mg Mn/m^3) (Hobson et al., 2011). We then applied this predicted Mn concentration to the respective portion of the weighted welding years. Finally, we summed these across all jobs in the work history, yielding $\text{mg Mn/m}^3\text{-years}$. We were able to calculate this most refined metric of cumulative Mn exposure for the Mn-exposed welder group, i.e. those subjects for whom we could estimate Mn concentration.

2.6 Statistical analysis

We used Intercooled Stata 11.0 (College Station, Texas) for all statistical analyses. We retained all outcome variables (FDOPA uptake, UPDRS3) and the exposure variables described above as continuous measures except where noted, and then used linear regression to assess the association between these outcome and exposure variables. We modeled all quantitative exposure variables linearly and verified the appropriateness of linearity by using locally weighted scatterplot smoothing (LOWESS) graphs (Cleveland, 1979).

We adjusted *a priori* for age (as a continuous variable) in all models, as previous papers have noted effects on FDOPA uptake (Gallagher et al., 2011, Nurmi et al., 2001). We have further described the effects of age on K_{occ} and volume in the Supplemental Material (Section 1). We also examined the effect of VOI volume before and after PVC on VOI PET K_{occ} to confirm there was no bias introduced by the segmentation procedure (Supplemental Material Section 2, Table 1). In models with UPDRS3 scores as the outcome we also adjusted for examiner. We repeated all analyses among the 32 subjects who were newly recruited since our prior investigation (Criswell et al., 2011). This permitted validation of our previous results in an independent sample. In addition, all but two of these subjects were assessed on the same PET scanner, allowing us to ensure that results were not influenced by scanner. Given the overall length of the study (eight years), we examined the effect of scan date

(time) on VOI FDOPA uptake. We did not find an association between scan date and any regional K_{occ} (all $p > 0.62$). We verified results in sensitivity analyses in which we restricted analysis to men to ensure that sex did not confound results, and restricted to subjects with no signs of parkinsonism (UPDRS3 < 6) (Racette et al., 2012) to ensure that any association between exposure and FDOPA uptake was not simply due to the lack of subjects with an abnormal UPDRS3 in the non-exposed group, including when further restricted to newly recruited subjects. Finally, given that rest tremor is less common in Mn toxicity than idiopathic Parkinson disease (PD), we repeated the sensitivity analysis excluding subjects with any rest tremor, and estimated the tremor effect within our primary models.

2. Results

3.1 Subject characteristics

The majority of subjects were Caucasian men. (Table 1) In the overall cohort, all subject groups were similar in age, but a greater proportion of the exposed workers were men. The newly recruited subjects were similar to the overall cohort with the exception that there were a higher percentage of males, and the Mn-exposed welders and workers had a slightly lower cumulative Mn exposure. The most common parkinsonian features in the Mn-exposed subjects included limb bradykinesia, axial signs, and limb rigidity. Action tremor was markedly more prevalent than rest tremor. (Table 1)

3.2 Mn exposure and FDOPA PET

Compared to the non-exposed subjects, the age-adjusted mean caudate FDOPA uptake was 0.0014 min^{-1} (95% confidence interval [CI] 0.0008, 0.0020) lower in Mn-exposed welders and 0.0012 min^{-1} (95% CI 0.0005, 0.0019) lower in other Mn-exposed workers (both $p < 0.001$). (Table 2) A difference between groups remained when we focused only on newly recruited subjects (both $p < 0.02$). (Table 2) FDOPA uptake was also 0.0006 min^{-1} (95% CI 0.0000, 0.0012) lower in the anterior putamen for Mn-exposed welders as compared to the non-exposed subjects ($p = 0.047$). Results were similar when we restricted analysis to the newly recruited subjects, albeit with larger 95% CIs. (Table 2) In this subset of newly recruited subjects, FDOPA uptake was also significantly lower in the Mn-exposed workers than the non-exposed subjects ($p = 0.049$). However, to the extent we could evaluate it, the association between exposure and FDOPA uptake in the anterior putamen, which was smaller in magnitude than for caudate FDOPA uptake, appeared to be largely due to its proximity and high correlation with FDOPA uptake in the caudate ($r = 0.70$). FDOPA uptake did not differ between groups for the posterior putamen (Table 2), which was more weakly correlated with FDOPA uptake in the caudate ($r = 0.31$). Accordingly, the C/P ratio of FDOPA uptake was lower in Mn-exposed welders (mean 0.83, SD 0.13) and Mn-exposed workers (mean 0.82, SD 0.09) (and the combined mean 0.83, SD 0.11) as compared to 0.95 (0.14) in the non-exposed subjects (both $p < 0.002$).

We confirmed the differences in caudate FDOPA uptake between exposure groups in all sensitivity analyses. Among men ($N = 55$), welders had 0.0016 min^{-1} (95% CI 0.0008, 0.0023) lower caudate FDOPA uptake than non-exposed subjects ($p < 0.001$), and Mn-exposed workers had 0.0012 min^{-1} (95% CI 0.0004, 0.0021) lower caudate FDOPA uptake

than non-exposed subjects ($p = 0.006$). When we restricted to subjects with a normal UPDRS3 (UPDRS3 < 6 , $N = 48$), caudate FDOPA uptake was again lower in both Mn-exposed welders (0.0017 min^{-1} , 95% CI 0.0008, 0.0027) and Mn-exposed workers (0.0012 , 95% CI 0.0003, 0.0020) as compared to non-exposed subjects (data not shown in tables). Although based on small numbers, a difference was still suggested when we further restricted to subjects who were newly acquired and had a UPDRS3 < 6 . (Table 2) Rest tremor was not significantly associated with VOI K_{occ} in any of our primary analysis when included in the model. Accordingly, caudate K_{occ} remained lower in Mn-exposed welders (0.0013 min^{-1} , 95% CI 0.00007, 0.0020) and Mn-exposed workers 0.0012 min^{-1} , 95% CI 0.0004, 0.0020) than non-exposed reference subjects when excluding all individuals with rest tremor ($n = 5$).

LOWESS suggested a U-shaped association between total years of exposure to welding fume and caudate FDOPA uptake (Figure 1), which we confirmed in linear regression using a squared term for this exposure variable in addition to the main effect. However, this U-shaped association was highly influenced by the 31 non-exposed subjects and welders/Mn-exposed workers with > 45 years of exposure ($n = 2$). Results were similar for weighted welding years. There was no association between caudate FDOPA uptake and mg Mn/m^3 -years among Mn-exposed welders.

3.3 FDOPA PET and UPDRS3

There also was no clear association between the FDOPA regional K_{occ} and UPDRS3 scores. In particular, UPDRS3 was not significantly associated with uptake in any of the three brain regions in the overall sample (all $p > 0.28$). Moreover, when we excluded non-exposed subjects, any suggested inverse association disappeared (0.07 [95% CI -1.8, 1.9] increase in UPDRS3 score for each 0.001 unit change in caudate FDOPA uptake).

3. Discussion

This cohort represents the largest cross-sectional molecular imaging study in Mn-exposed subjects. We found Mn-exposed welders and workers had clear evidence of pre-synaptic dopaminergic dysfunction as evidenced by reduced FDOPA uptake in the caudate nucleus compared to non-exposed reference subjects. The recruitment of additional subjects, including some with a broader range of clinical parkinsonism severity, validated our initial findings regarding reduced FDOPA uptake in the caudate nucleus of Mn-exposed welders, both overall and when we restricted to subjects with a UPDRS3 < 6 . However, these data did not support our hypothesis that striatal changes correlate with clinical parkinsonism since we did not find a significant reduction in the posterior putamen FDOPA uptake, and the reduction in caudate was not clearly associated with UPDRS3 scores. In contrast to previous early PET studies in Mn exposure (Racette et al., 2001, Racette et al., 2005, Wolters et al., 1989), our current expanded cohort represents a more than ten-fold increase in study size compared to the cumulative total of these initial reports. This emphasizes the importance of study size when the focus is to detect a relatively small, but significant 11 to 13% reduction in FDOPA caudate uptake in Mn-exposed welders and workers when compared to non-exposed subjects ($n = 72$).

We continue to see a distinct pattern of significantly reduced caudate FDOPA uptake in Mn-exposed subjects. This caudate reduction drives the difference in the C/P ratio between Mn-exposed and non-exposed subjects. Our expanded sample, which includes symptomatic Mn-exposed subjects, demonstrated a mean FDOPA uptake C/P ratio in Mn-exposed subjects of 0.83, SD 0.11. This extends, and is almost identical, to our previous report in asymptomatic Mn-exposed subjects (mean C/P ratio of 0.82, SD 0.13) and contrasts with the posterior putamen predominant pattern of PD (previously reported, mean C/P ratio 1.65, SD 0.41) (Brooks, 1998, Criswell et al., 2011, Criswell et al., 2012b). This indicates Mn-exposed welders and workers have caudate predominant effects and may explain some of the reported cognitive deficits associated with Mn toxicity including effects on attention and concentration, working memory, and cognitive flexibility (Al-Lozi et al., 2017, Bowler et al., 2007, Park et al., 2009, Rodier, 1955), as caudate lesions are associated with psychiatric and cognitive changes including disinhibition (Bhatia and Marsden, 1994). Similarly, neurodegenerative disorders of the caudate including PD and Huntington's disease are associated with deficits in tests of attention, planning, and working memory (Grahn et al., 2008). Alternatively, we previously hypothesized the caudate predominant pattern may reflect an early pattern of neurotoxicity that may progress with greater exposure to involve the posterior striatum. However, the similarity of the C/P ratio in the asymptomatic Mn-exposed subjects from our initial study (Criswell et al., 2011) and the current Mn-exposed cohort, which includes workers with clinically relevant parkinsonism (UPDRS3 range 0 – 22.5), would argue against this hypothesis. Unfortunately, minimal data exist on the progression of striatal FDOPA uptake in pre-symptomatic subjects at risk for the development of parkinsonism. Ultimately, the question of whether effects in the caudate precede those in the posterior putamen would best be addressed with a prospective longitudinal study in Mn-exposed subjects.

There was no clear inverse dose-response association between striatal FDOPA uptake and our primary exposure metrics. However, FDOPA uptake was reduced in Mn-exposed welders and workers. Therefore, exposure risk may extend to all employees with exposure to welding fume, not just welders. We also note that the lack of evidence of a dose-response relationship between Mn exposure and striatal FDOPA uptake may be related to possible up-regulation of dopa decarboxylase in the setting of pre-synaptic dopaminergic dysfunction (which is not addressed by our study), thereby attenuating the association between exposure and striatal FDOPA uptake (Doudet et al., 1998, Sossi et al., 2002). We also cannot exclude the possibility that the healthy worker survivor effect (Pearce et al., 2007) may have caused workers with lower FDOPA uptake or clinical parkinsonism to drop out of the work force and thus not be available to be included in the original cohort or the present study.

This study has several potential limitations. Welding fume contains a number of elements and gases (NIOSH, 1988) other than Mn; therefore, this study cannot fully exclude a contribution from other substances in welding fumes, particularly iron, which competes for the same metal transporter responsible for Mn uptake into the brain (Fitsanakis et al., 2010, Zhang et al., 2009). Some workers in our study were exposed to flux core arc welding (FCAW) and welding in confined spaces, suggesting that some of these workers may have been exposed to relatively high levels of airborne Mn in comparison to welders using other processes or open spaces. While our study included broad inclusion/exclusion criteria and

our workers were drawn from a well characterized, union-based cohort, we cannot exclude the possibility that our findings may not reflect the broader population of Mn-exposed workers in the U.S. Similarly, FDOPA PET is limited to the examination of the pre-synaptic dopaminergic system and therefore we cannot exclude the possibility that there may be significant pathologic effects in postsynaptic components of the dopaminergic systems or in other brain neurotransmitters. We also cannot exclude there may be some effect of sampling. In addition, we note that because almost all of the subjects were of working age (not elderly) and we excluded subjects who were taking dopamine (in effect excluding anyone who had been diagnosed with PD), the only subjects with a UPDRS3 > 6 were those who had been occupationally exposed to Mn. However, the consistency of our results in the validation sample and sensitivity analysis would argue against these being the cause of the difference in FDOPA uptake in exposed vs. non-exposed subjects. Ideally, imaging with a less regulated pre-synaptic marker of dopaminergic dysfunction such as (+)-¹¹C-dihydrotetrabenazine, could prove a superior marker of pre-synaptic dysfunction and eliminate the potential influence of dopa decarboxylase up-regulation. However, any study would have to be large enough to detect differences on the order of 10%. This study also does not include neurocognitive assessments in Mn-exposed workers, which will be an important area of future study given the effects on FDOPA uptake in the caudate nucleus.

4. Conclusion

In conclusion, we confirmed the presence of a modest but significant reduction in FDOPA uptake in the caudate nucleus of Mn-exposed welders and workers, indicating pre-synaptic dopaminergic dysfunction within the nigrostriatal system. Further study will be required to fully understand the relationship between Mn exposure, in-vivo dopaminergic neuronal function, and clinical symptomatology. Longitudinal follow-up of these subjects will be essential to determine if there is progression or reversibility of the dopaminergic dysfunction in these Mn-exposed welders and workers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FUNDING SOURCES:

This work was supported by the National Institutes of Health, Grant Numbers: K23ES021444-01, K24ES017765-075, R01ES019277, P42ES004696, R01ES021488, and ICTS UL1RR024992.

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Highlights

- The underlying mechanism of manganese neurotoxicity remains unknown.
- FDOPA PET is a non-invasive method assessing dopaminergic neuronal function.
- Mn-exposed welders and workers demonstrated lower caudate FDOPA uptake.
- Mn-exposure is associated with pre-synaptic dopaminergic dysfunction.

Figure 1. Scatterplot of the FDOPA Caudate K_{occ} (min^{-1}) and total years of exposure to welding fume (All Subjects).

LOWESS suggested a U-shaped association between the duration of welding fume exposure and caudate FDOPA uptake, which was largely influenced by non-exposed subjects and by exposed subjects who had > 45 years of exposure.

Table 1

Characteristics of participating subjects, all subjects and newly recruited subjects,^a by Mn exposure group

	All Subjects (N = 72)				Newly Recruited Subjects (N = 32)			
	Mn-Exposed Welders N = 27	Mn-Exposed Workers N = 14	Non-Exposed Reference Subjects N = 31		Mn-Exposed Welders N = 11	Mn-Exposed Workers N = 10	Non-Exposed Reference Subjects N = 11	
Male, n (%)	24 (89)	13 (93)	18 (58)		10 (91)	9 (90)	8 (73)	
Non-Hispanic Caucasian, n (%)	27 (100)	13 (93)	27 (87)		11 (100)	9 (90)	10 (91)	
Age, years								
Mean (SD)	44.3 (11.8)	46.9 (11.6)	48.8 (15.1)		41.0 (13.0)	48.8 (10.8)	46.5 (10.1)	
Median	46	49	49		37	50	43	
Range	26 – 63	23 – 64	19 – 71		26 – 63	23 – 64	32 – 64	
Duration welding job								
Mean (SD)	20.0 (12.8)	14.7 (12.5)	0 (0)		15.4 (13.3)	17.3 (13.8)	0 (0)	
Median	18.0	11.9	0		12.1	12.9	0	
Range	2.8 – 45.3	0.08 ^b – 46.4	0		2.8 – 45.3	0.08 ^b – 46.4	0	
Weighted welding years^c								
Mean (SD)	15.7 (12.5)	1.8 (1.6)	0 (0)		13.6 (13.7)	2.2 (1.7)	0 (0)	
Median	11.8	1.5	0		8.9	1.6	0	
Range	1.7 – 45.3	0.01 – 5.8	0		1.7 – 45.3	0.01 – 5.8	0	
Mg Mn/m³-years^d								
Mean (SD)	4.1 (5.2)	N/A	N/A		3.5 (6.0)	N/A	N/A	
Median	1.6	N/A	N/A		1.3	N/A	N/A	
Range	0.3 – 20.8	N/A	N/A		0.3 – 20.8	N/A	N/A	
UPDRS3^e								
Mean (SD)	9.1 (5.9)	5.4 (5.6)	1.3 (1.3)		10.3 (8.2)	5.3 (6.6)	1.5 (1.2)	
Median	8.5	3.5	1		9.5	1.75	1.5	
Range	0 – 22.5	0.5 – 19	0 – 4		0 – 22.5	0.5 – 19	0 – 3.5	
UPDRS3 < 6, n (%)	8 (30)	9 (64)	31 (100)		4 (36)	7 (70)	11 (100)	
Parkinsonism signs,^f n (%)								

	All Subjects (N = 72)				Newly Recruited Subjects (N = 32)		
	Mn-Exposed Welders N = 27	Mn-Exposed Workers N = 14	Non-Exposed Reference Subjects N = 31		Mn-Exposed Welders N = 11	Mn-Exposed Workers N = 10	Non-Exposed Reference Subjects N = 11
Limb bradykinesia	24 (89)	12 (86)	13 (42)		9 (82)	8 (80)	9 (82)
Limb rigidity	14 (52)	3 (21)	10 (32)		6 (55)	2 (20)	0 (0)
Action tremor	8 (30)	4 (29)	0 (0)		3 (27)	3 (30)	0 (0)
Rest tremor	3 (11)	2 (14)	0 (0)		1 (9)	1 (10)	0 (0)
Axial signs ^g	20 (74)	8 (57)	9 (29)		7 (64)	5 (50)	3 (27)

^aFor results in the original sample (N = 40) see Criswell et al., 2011.

^bMinimum was 0.5 years when restricted to workers recruited from a welding worksite.

^cCumulative weighted welding exposure as defined previously in Racette et al., 2012.

^dEstimated for welders and welder helpers according to welding type, degree of confinement, job title/classification, reported hours per week, and duration (years) in each job.

^eUnified Parkinson Disease Rating Scale motor subsection 3.

^fSubscore 0.5

^gAxial signs includes gait, posture, postural stability, arising from a chair, global bradykinesia, neck rigidity, expression, and speech.

Age-adjusted mean FDOPA K_{occ} (min^{-1}) by brain region and exposure group, overall and among newly recruited subjects and newly recruited subjects with UPDRS3 < 6

Table 2

	All Subjects		Newly Recruited Subjects		Newly Recruited Subjects with UPDRS3 < 6	
	N = 72	Difference from non-exposed (95% CI) ^{a,b}	Mean ^a	Difference from non-exposed(95% CI) ^{a,b}	Mean ^a	Difference from non-exposed(95% CI) ^{a,b}
Caudate						
Non-exposed reference subjects	0.0112	Reference	0.0112	Reference	0.0111	Reference
Mn-exposed workers	0.0100	-0.0012 (-0.0019, -0.0005) *	0.0099	-0.0013 (-0.0022, -0.0004) *	0.0100	-0.0011(-0.0022, -0.0000) *
Mn-exposed welders	0.0098	-0.0014 (-0.0020, -0.0008) *	0.0101	-0.0010 (-0.0019, -0.0002) *	0.0103	-0.0009 (-0.0022, 0.0005)
Anterior putamen						
Non-exposed reference subjects	0.0116	Reference	0.0120	Reference	0.0120	Reference
Mn-exposed workers	0.0111	-0.0004 (-0.0012, 0.0003)	0.0111	-0.0009 (-0.0018, -0.0000) *	0.0112	-0.0008 (-0.0019, 0.0003)
Mn-exposed welders	0.0110	-0.0006 (-0.0012, -0.0000) *	0.0114	-0.0006 (-0.0014, 0.0003)	0.0110	-0.0010 (-0.0024, 0.0003)
Posterior putamen						
Non-exposed reference subjects	0.0120	Reference	0.0123	Reference	0.0123	Reference
Mn-exposed workers	0.0123	0.0003 (-0.0008, 0.0014)	0.0123	-0.0000 (-0.0015, 0.0015)	0.0125	0.0002 (-0.0016, 0.0020)
Mn-exposed welders	0.0120	0.0000 (-0.0009, 0.0009)	0.0120	-0.0003 (-0.002, 0.0012)	0.0113	-0.0010 (-0.0032, 0.0012)

^a Adjusted for age as continuous variable

^b 95% confidence interval; difference may not match difference of presented means due to rounding

* p < 0.05