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Olfactory tract/bulb metal concentration in Manganese-exposed mineworkers

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ABSTRACT

Background: Manganese (Mn) is an essential micronutrient as well as a well-established neurotoxicant. Occupational and environmental exposures may bypass homeostatic regulation and lead to increased systemic Mn levels. Translocation of ultrafine ambient airborne particles via nasal neuronal pathway to olfactory bulb and tract may be an important pathway by which Mn enters the central nervous system.
Objective: To measure olfactory tract/bulb tissue metal concentrations in Mn-exposed and non-exposed mineworkers.
Methods: Using inductively coupled plasma-mass spectrometry (ICP-MS), we measured and compared tissue metal concentrations in unilateral olfactory tracts/bulbs of 24 Mn-exposed and 17 non-exposed South African mineworkers. We used linear regression to investigate the association between cumulative Mn exposures and olfactory tract/bulb Mn concentration.
Results: The difference in mean olfactory tract/bulb Mn concentrations between Mn-exposed and non-Mn exposed mineworkers was 0.16 μg/g (95% CI −0.11, 0.42); but decreased to 0.09 μg/g (95% CI 0.004, 0.18) after exclusion of one influential observation. Olfactory tract/bulb metal concentration and cumulative Mn exposure suggested there may be a positive association; for each mg Mn/m^3-year there was a 0.05 μg/g (95% CI 0.01, 0.08) greater olfactory tract/bulb Mn concentration overall, but −0.003 (95% CI −0.02, 0.02) when excluding the three influential observations. Recency of Mn exposure was not associated with olfactory tract/bulb Mn concentration.
Conclusions: Our findings suggest that Mn-exposed mineworkers might have higher olfactory tract/bulb tissue Mn concentrations than non-Mn exposed mineworkers, and that concentrations might depend more on cumulative dose than recency of exposure.

1. Introduction

Manganese (Mn) is an environmentally ubiquitous heavy metal and a well-established neurotoxicant (Blanc, 2018; Bowman et al., 2011; Burton and Guilarte, 2009; Checkoway, 2010; Gonzalez-Cuyar et al., 2014; Guilarte, 2011, 2013; Perl and Olanow, 2007; Racette, 2014). Mn is also an essential micronutrient, important for neurotransmitter synthesis, bone and muscle development, protein metabolism, and a

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cofactor of essential antioxidant enzymes such as superoxide dismutase (Bowman et al., 2011; Erikson et al., 2005; Guilarte et al., 2008). Dietary Mn absorption and excretion are tightly regulated homeostatic processes, but non-gastrointestinal routes of absorption, including translocation of ultrafine ambient airborne particles via nasal neuronal pathway to olfactory bulb and tract, respiratory, and trans-integumentary, bypass this regulation, potentially leading to elevated blood Mn levels (Bowman et al., 2011; Milatovic et al., 2009; Moberly et al., 2012; Teeguarden et al., 2007b, 2007a). A study demonstrating higher olfactory tract/bulb metal concentrations in goats from heavily industrialized areas in Nigeria compared to those from unindustrialized locations supports the hypothesis that the olfactory tract/bulb may play an important role in uptake into the central nervous system from environmental and occupational exposures (Igado et al., 2008). Olfactory tract/bulb Mn deposition has also been identified in several controlled murine and primate Mn exposure models (Antonini et al., 2009; Fechter et al., 2002; Moberly et al., 2012; Normandin et al., 2002, 2004).

Occupational Mn exposures affect millions of workers worldwide, including those employed in dry battery and steel manufacturing, farming (fungicides), mining, welders, solderers, smelters, and boilermakers (Aschner et al., 2009; Criswell et al., 2011; Kieburtz and Kurlan, 2005; National Institute for Occupational Safety and Health NIOSH, 2011; Occupational Safety and Health Administration OSHA and Department of Labor, 2013; Rivera-Mancia et al., 2011; Santamaria et al., 2007). Notably, welding fume, which typically contains Mn, is classified by the International Agency for Research on Cancer as carcinogenic to humans (Guha et al., 2017; International Agency for Research on Cancer, 2018) and has been associated with Mn dose-dependent progression (worsening) of parkinsonian signs in welders (Racette et al., 2017).

Particle size is critical for uptake, delivery, and toxicity of inhaled particles, including Mn. Aerodynamic diameter determines deposition and penetration in the respiratory tract (Brown et al., 2013). Coarse particles (PM_{10}, diameter from 2.5 to 10 μm) tend to deposit in the upper respiratory tract. Respirable particles (diameter <4.0 μm) can reach the alveoli. Respirable particles include both fine particles ($PM_{2.5}$, diameter 0.1–2.5 μm) and ultrafine particles (UFPs, diameter <0.1 μm). The latter can translocate via the nasal neuronal pathway to olfactory bulb and tract (Oberdorster et al., 2004). Moreover, even though UFPs represent the smallest proportion of total dust by mass, they represent a large proportion of total dust by particle count (Hinds and Zhu, 2022) and the higher specific surface area of UFPs increases their ability to adsorb and exert toxicity (Schraufnagel, 2020).

Characterization of Mn particle size distribution (PSD) represents an important unmet need in the study of neurotoxicity from Mn mining exposure. While all mine workers would be exposed to a range of particle sizes, mine workers who are frequently undertaking hot work (e.g., welding, sintering, boilermaking, and blasting) would be more likely to be exposed to UFPs that result from combustion, whereas mineworkers conducting more mechanical processes (e.g., tramming, heavy equipment operator, and engineer) would likely be exposed to more coarse particles and fewer UFPs. Similarly, mines utilizing blasting processes likely generate more particles in the smallest size fractions relative to mines utilizing mechanical processes. Larger particles settle out of still air more quickly, whereas fine and ultrafine particles can stay suspended for days, weeks, or longer, and travel far from their source with moving air (Sioutas et al., 2005). Although, PSDs have not been quantified in Mn mines, PSDs from area aerosol samples collected at 13 locations in an underground coal mine indicated that 0.01–4.65% (by mass) and 20–87% (by particle count) of the aerosol was in the ultrafine size fraction (Skubacz et al., 2017). Fine particles made up 15.71–57.84% of the aerosol sample by mass, and 13–79% by particle count. It is also known that the content of Mn deposits varies even within a single mine field, but that the most abundant Mn oxides can consist of particles <10 μm (Preston, 2001). Taken together, bioaccessible fine and ultrafine Mn, as well as larger Mn particles, are likely present in mining operations, and worker exposure to each particle size varies by mine, location, prevailing atmospheric conditions, and job task.

Despite growing evidence of the critical impact of occupational Mn exposure on health, fewer than 20 decedents have undergone detailed neuropathological examinations (Criswell et al., 2015; Gonzalez-Cuyar et al., 2014; Guilarte, 2010; Perl and Olanow, 2007). Notably, most of these studies prior to 2014 presented predominately qualitative assessments, of presumably end-stage neuropathologic changes, in decedents with known high-level acute/sub-acute exposures estimated to be as high as 1,000,000 $\mu g/m^3$ (Gonzalez-Cuyar et al., 2014; Perl and Olanow, 2007). None of these reports provided characterization of the olfactory tract/bulb or insight into its potential role in Mn-induced neurotoxicity. To address this important question, we quantified tissue metal concentrations (including Mn) in olfactory tracts/bulbs from decedents that form part of our large occupational neuropathology study composed of South African Mn-exposed and non-exposed mineworkers. Workplace Mn exposures in these South African mines are reportedly below 5 mg Mn/m^3 (total dust) – the permissible exposure limit set by the United States Occupational Safety and Health Administration (Occupational Safety and Health Administration OSHA, 2018) and below the recommended exposure level set by the United States National Institute for Occupational Safety and Health (NIOSH) (1 mg/m^3, total dust). However, levels often exceed the current American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value for inhalable Mn exposure (TLV; 0.1 mg Mn/m^3) (American Conference of Governmental Industrial Hygienists ACGIH, 2013), a threshold based upon neurologic health effects (Korczynski, 2000; Susi et al., 2000).

2. Methods

2.1. Background study design, and decedents

The University of the Witwatersrand's Human Research Ethics Committee (Republic of South Africa; RSA) and the Washington University Human Research Protection Office (St. Louis, MO, USA) approved this study. As previously described, when working, all Mn-exposed decedents were employed at one or more of five Mn mines in the Northern Cape province of South Africa, and all non-Mn exposed decedents only worked in other types of mines in other regions of South Africa (Gonzalez-Cuyar et al., 2014; Nelson et al., 2012). All study subjects died during the years 2009–2018 either while still actively working at the mine or elsewhere, or after retiring. Each study decedent's next-of-kin provided informed consent for a restricted cardiopulmonary and brain autopsy, as well as consent for an occupational health nurse to review the decedent's relevant medical and employment records (Gonzalez-Cuyar et al., 2014; Nelson et al., 2012). For the study described in this paper, we also required: 1) a brain autopsy and no gross pathologic evidence of remote traumatic brain injury (i.e. chronic contusion) involving the olfactory bulbs and/or olfactory cortex (bilateral inferior frontal lobes); 2) intact olfactory tract/bulb tissue (e.g., olfactory bulbs and associated tracts not affected by artefactual loss of anatomical integrity such as may occur at the time of brain extraction) following prior *ex vivo* brain studies; and 3) adequate job history data to determine Mn exposure status and to approximate lifetime cumulative Mn (see below). We used the job history records to categorize each mineworker as having ever worked in Mn mine(s) (exposed) or only in non-Mn mine(s) (non-exposed) and excluded any mine worker with gaps in work history to minimize exposure misclassification. At the time we conducted the study, 43 intact olfactory bulbs from miners were available. We excluded one with parenchymal degeneration and focal hemorrhage extending into the olfactory bulb and one without sufficient exposure history to classify as Mn-exposed or non-exposed mineworkers. In total, we included 41 olfactory bulbs in 24 Mn-exposed and 17 non-Mn-exposed mineworkers. The latter group had worked in gold, asbestos, platinum, diamond, and/or iron mines. These mines are in

geological regions of South Africa distinct from the Mn mining region. While the asbestos mines are closest to the Mn mines, a geological study confirmed that samples taken from this region contain no Mn (von Plehwe-Leisen and Klemm, 1995). In addition, we divided decedents according to when we processed their brains (2011 or 2018–2019) because we handled olfactory tract/bulb tissue differently by time-period in accordance with established protocols (see below) at the time (24 in Cohort 1, 17 in Cohort 2).

2.2. Occupational Mn exposure quantification

To determine lifetime cumulative exposure to Mn for each decedent, we used both duration and intensity of exposure for each job to estimate mg Mn/m^3-years of exposure. Specifically, from each decedent's lifetime work history, we summed the product of the number of years of exposure for each Mn mining job and then estimated Mn exposure intensity for that job, in mg Mn/m^3. We estimated mg Mn/m^3 (total dust) for each job in the work history from an Mn mining-specific job exposure matrix (JEM) developed previously from total dust occupational hygiene personal sampling measurements taken in two of the five Mn mines in the present work (Myers et al., 2002, 2003). In that study, air Mn measurements were estimated from personal total dust samples collected over a four-year period from a variety of jobs performed above and/or below ground. These estimates were validated against those obtained using an IOM sampling head, optimal for measuring particles in the inhalable fraction (Myers et al., 2002). Then, air concentrations were averaged by job to obtain a mean time weighted average (TWA) of Mn in mg/m^3 by mine job. We used a full version of the JEM (Myers et al., 2002) to assign Mn exposure values for each job for each decedent in this study. In particular, we used both exact job title (which we translated to one of 24 JEM-specific job occupation codes relevant to workers in our study) and additional information about whether the person worked above ground, below ground, or both: mining (below ground), engineering (mixed), surface processing/sinter plants (above ground), or supervision/services (mixed). Finally, we calculated the number of years between last Mn exposure and death among the Mn-exposed mineworkers and subtracted the stop date of the last job from the date of death to calculate recency of exposure.

2.3. Olfactory tract/bulb sampling and tissue processing

At the time of autopsy, we suspended decedents' brains in 10% neutral buffered formalin (NBF) for at least three weeks and then shipped them to the University of Washington BioRepository and Integrated Neuropathology (BRaIN) laboratory (Seattle, WA, USA) for gross examination, tissue sampling, neurohistopathological evaluation, and metal quantification. A board-certified neuropathologist performed an external gross neuropathological examination of all study brains with particular attention to the olfactory tracts/bulbs and olfactory cortices. Olfactory tracts/bulbs without gross pathologic abnormalities were removed intact.

At the time of tissue sampling and neuropathological examination, we placed all olfactory tracts/bulbs in 10% NBF. For Cohort 1, we then processed the tissue for 24 hours in an automated tissue processor (ASP 6025; Leica, IL, USA), and embedded it in paraffin wax to produce formalin-fixed paraffin embedded (FFPE) tissue blocks. For Cohort 2, we did not embed the tissue in paraffin following formalin fixation. Once samples from Cohort 1 were prepared for metal quantification, FFPE tissue blocks corresponding to an intact unilateral olfactory tract/bulb were melted at 60℃ for less than 5 minutes in a tissue embedding station (Arcadia H; Leica, IL, USA). These samples were then retrieved from the wax and placed in deionized water. Unilateral samples from Cohort 2 were removed from formalin, rinsed, and placed in deionized water. Samples from both cohorts were simultaneously analyzed as described below. Investigators performing neuropathological examinations and metal quantification studies were blinded to Mn exposure status and cumulative Mn exposure.

2.4. Olfactory bulb metal quantification

Olfactory bulbs were removed from the original sample vials, blotted dry on MCE filters (mixed cellulose esters, SKC 225–5, PA, USA) to remove excess water, and transferred to pre-weighed 15-mL polypropylene tubes (Corning 430790, Sigma-Aldrich, MO, USA) provided by the analytical lab. The balance used was Mettler-Toledo model AG104 (OH, USA). Tubes with olfactory bulbs were then post-weighed to obtain sample masses that included residual moisture. Samples were hand-delivered to the analytical lab and refrigerated until analysis. The mean tissue masses were 58 mg (dry weight; range: 9.9–141 mg) and 92 mg (wet weight; range: 51–146 mg) for Cohorts 1 and 2, respectively.

Samples underwent microwave-assisted (MARS 5, CEM Matthews, NC, USA) digestion with 0.5 mL of nitric acid (trace metal grade, Fisher, WA, USA) prior to inductively coupled plasma–mass spectrometry (ICP-MS) analysis for Mn, Fe, Cu, Zn, Pb, Mg, P, and K with 50 ng terbium (Tb) added as recovery internal standard. Two rounds of a ramped microwave program were used (first round: 400 W 50% power, 10 min ramp to 40℃, hold 10 min at 40℃; 800 W 75%, 10 min ramp to 60℃, hold 10 min at 60℃; 800 W 100%, 10 min ramp to 90℃, hold 30 min at 90℃; second round: the last step was hold 60 min at 90℃), with inspection between rounds to ensure that tissue was submerged. Digestate was brought to 5 mL final volume with ultra-pure deionized water (≥18 MΩ).

We used an ICP-MS (Agilent 7900-CE; Santa Clara, CA, USA) with collision reaction cell in He mode (4.3 mL/min, OctP RF 200 V) to eliminate polyatomic interferences (U. S. EPA, 2014). The ICP-MS conditions were as follows: radiofrequency power - 1550 W; sampling depth - 8 mm; carrier gas - 1.03 L/min; no makeup gas; instrument internal standards - ^{193}Ir, ^{45}Sc. A 10-point calibration (0.01–5000 ng/mL in 10% nitric acid, R^2>0.999) was prepared from commercial stock (certified reference material, Aristar-BDH; VWR, Radnor, PA, USA) and confirmed with an independent check standard (Agilent IMS102, Santa Clara, CA, USA). The reporting limit was set at the lowest valid calibrant (relative standard error ≤10%) for Mn, Cu, and Pb (0.05 ng or 0.001 μg/g tissue assuming 50 mg sample size). Due to background, we used three times the procedural blank average (N=3) for Mg, P, K, Fe, and Zn. Mean spike recovery ranged from 93% (K) to 112% (Fe), and 8 separate aliquots of a NIST standard reference material (NIST SRM 1566b- homogenized oyster tissue) were analyzed alongside the tissue samples to verify the accuracy and precision of the method. Assay accuracies based on the SRM analyses were 81% (Fe), 91% (Cu), 93% (K, Mn), and 96% (Zn, Pb). Assay precision defined as the standard deviation of the SRM % recovery (N=8) was +/- 9.3% for Pb, and below +/- 4% for the other elements. All other quality control parameters were within historical ranges. Reporting limits (ng) were as follows: Mg- 5, P- 50, K- 10, Mn- 0.05, Cu- 0.1, Zn- 4, and Pb- 0.05. Of these metals assessed successfully in olfactory tract/bulb tissue, only Mn, Fe, and Pb were detectable in the mines in our study in an investigation of mineral content in these Mn mines (Costin et al., 2015).

2.5. Statistical analysis

All statistical analyses were performed using Stata MP 14.2 (StataCorp 2015, College Station, TX, USA). We performed linear regression to assess the association between exposure to Mn and other metal concentrations – with Mn exposure status (Mn-exposed vs non-exposed) as the primary independent variable and each metal concentration measured in the olfactory tract/bulb tissue as a continuous dependent (outcome) variable. We report estimates adjusted for age at death (continuous) and cohort. We adjusted for cohort due to the different specimen handling protocols established during processing as noted above and because preliminary analyses confirmed that even among the

non-exposed workers, olfactory bulb concentration of a non-target metal (K) differed by 34.2 (95% confidence interval [CI] 6.8–61.5) μg/g with age adjustment. This was equivalent to more than one standard deviation difference among non-Mn exposed mineworkers, an effect on K that we also confirmed among the Mn-exposed mineworkers (63.6, 95% CI 46.1–81.2) and to a lesser extent (approximately one standard deviation) for Cu and Pb. In the secondary analyses, we further examined the association between Mn exposure and olfactory bulb Mn as well as other metal concentrations, using cumulative Mn exposure and recency of Mn exposure as continuous exposure metrics, with each exposure modeled linearly in separate models. We used locally weighted scatterplot smoothing (LOWESS) to check for possible non-linear associations. In sensitivity analyses, we excluded influential observations, as assessed by Cook's distance (Cook's D) greater than four divided by the sample size in the respective model. Because of the small number of White and female decedents, we repeated our primary analysis restricted to Black/African male decedents and verified that the results were not materially different.

3. Results

3.1. Study decedent characteristics

Our study population (Table 1) was predominantly male (100.0% Mn-exposed, 76.5% non-exposed) and Black African (100.0% Mn-exposed, 88.2% non-exposed). Age at death was similar for the Mn-exposed and non-exposed decedents (median years, 55.2 vs 54.4), which is comparable to the life expectancy for men in that region (Department of Statistics South Africa, 2021). Clinical causes of death, as stated on the death certificates and/or the documents that are submitted to the National Institute for Occupational Health in South Africa, included pneumonia (n=2), diabetes (n=1), and "natural causes" (n=14) for controls, and pneumonia (n=4), mesothelioma (n=1), and "natural causes" (n=19) for Mn-exposed mineworkers. Among the Mn-exposed mineworkers, mean 8-hr TWA Mn ranged from 0.084 to 0.491 mg/m^3 (median 0.185 mg/m^3). For the Mn-exposed mineworkers, mean duration of exposure to Mn in a mining job was 15.7 years (SD=12.1) and the mean estimated cumulative Mn exposure was 3.5 mg Mn/m^3-years (SD=3.5). Decedents in Cohort 2 had greater Mn exposure than Cohort 1. Among Mn-exposed mineworkers the median elapsed time between last exposure and death was 1.2 years. Two (8.3%) were actively exposed at the time of death and in total nine (37.5%) appeared to be current employees of an Mn mine. Most (87.5%) Mn-exposed mine workers had at least some exposure after the Mn monitoring data utilized in the JEM was collected.

Table 1
Decedent characteristics, overall and by cohort, MINERS study, South Africa.

	All decedents (N=41)		Cohort 2 (N=17)		Cohort 1 (N=24)	
	Mn-exposed N=24 n (%)	Non-exposed N=17 n (%)	Mn-exposed N=13 n (%)	Non-exposed N=4 n (%)	Mn-exposed N=11 n (%)	Non-exposed N=13 n (%)
Sex assigned at birth						
Male	24 (100)	13 (76.5)	13 (100)	4 (100)	11 (100)	9 (69)
Female	0 (0)	4 (23.5)	0 (0)	0 (0)	0 (0)	4 (31)
Black/African	24 (100)	15 (88.2)	13 (100)	4 (100)	11 (100)	11 (85)
Mn-exposed at death	2 (8.3)	-	0 (0)	-	2 (18.1)	-
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age, years	55.6 (9.1)	58.4 (13.2)	54.2 (8.7)	51.0 (12.2)	57.3 (9.6)	60.6 (13.1)
Minimum	39.5	33.7	39.5	33.7	39.6	45.9
25th percentile	48.8	50.8	47.2	42.6	50.3	50.8
Median	54.4	55.2	53.0	54.2	57.5	55.2
75th percentile	61.8	63.6	58.6	59.3	67.4	74.5
Maximum	72.4	82.8	72.4	61.7	70.6	82.8
Duration of Mn exposure, years	15.7 (12.1)	0 (0)	18.7 (13.0)	0 (0)	12.1 (10.6)	0 (0)
Minimum	0.4	0	0.4	0	0.8	0
25th percentile	3.3	0	6.0	0	1.4	0
Median	15.4	0	22.2	0	13.8	0
75th percentile	26.3	0	27.1	0	22.2	0
Maximum	39.7	0	39.7	0	31.4	0
Time weighted average, mg Mn/m^3 (total dust)	0.243 (0.142)	0 (0)	0.222 (0.158)	0 (0)	0.269 (0.122)	0 (0)
Minimum	0.084	0	0.084	0	0.084	0
25th percentile	0.114	0	0.085	0	0.176	0
Median	0.185	0	0.159	0	0.318	0
75th percentile	0.354	0	0.368	0	0.339	0
Maximum	0.491	0	0.491	0	0.491	0
mg Mn/m^3-years[a] (total dust)	3.5 (3.5)	0 (0)	3.5 (3.5)	0 (0)	3.5 (3.7)	0 (0)
Minimum	0.2	0	0.2	0	0.2	0
25th percentile	0.6	0	0.8	0	0.4	0
Median	2.5	0	2.3	0	2.6	0
75th percentile	5.9	0	4.1	0	7.6	0
Maximum	11.5	0	11.5	0	10.3	0
Time elapsed between Mn exposure and death, years	9.4 (14.0)	-	7.6 (14.7)	-	11.4 (13.4)	-
Minimum	0	-	0.03	-	0.03	-
25th percentile	0.03	-	0.03	-	0.03	-
Median	1.2	-	0.3	-	4.6	-
75th percentile	18.9	-	5.0	-	20.3	-
Maximum	50.8	-	50.8	-	39.0	-

Abbreviations: Mn=manganese; SD=standard deviation

[a] From each decedent's work history, we summed the product between duration of exposure years for each Mn mining job and the estimated exposure intensity (8-hr time weighted average, TWA) for that job (mg Mn/m^3).

3.2. Occupational Mn exposure and olfactory tract/bulb metal concentration

With a few exceptions, Mn-exposed decedents had higher mean absolute metal concentration levels in the olfactory tract/bulb compared to non-exposed decedents within the cohorts, with the most notable differences for Mn (Cohort 1) and potassium (K; both cohorts) (Table 2). When adjusted for age and cohort, and after excluding influential observations, olfactory tract/bulb mean Mn and K concentrations were higher in Mn-exposed compared to non-exposed mineworkers [mean difference=0.09 (95% CI 0.004, 0.18) for Mn; 18.0 (95% CI 6.54, 29.6) for K] (Table 3). Further, there was some evidence of a positive association between cumulative Mn exposure and olfactory tract/bulb Mn concentration. For each mg Mn/m^3-year, there was a mean of 0.05 μg/g (95% CI 0.01, 0.08) higher concentration in the olfactory tract/bulb for Mn and 0.82 μg/g (95% CI 0.06, 1.59) for Zn, although both disappeared after excluding influential observations (Table 4). These results were unaffected by adjustment for medical comorbidities or when reassigning three control miners with iron mining exposure to the Mn mining category. There was no evidence of an association between recency of Mn exposure and olfactory tract/bulb metal concentration. Specifically, metal concentrations were not associated with the number of years that had elapsed since last Mn exposure, when we modeled the latter linearly (Table 5). LOWESS suggested that metal concentrations were higher among the nine workers who were Mn exposed at the time of death. However, in regression models comparing currently exposed to not currently exposed workers, only that for Mg was significant, and for Mn any positive association was modest and required exclusion of one influential observation.

Table 2
Olfactory tract/bulb metal concentrations, by cohort and Mn exposure status, MINERS study, South Africa (N=41).

	All decedents (N=41)		Cohort 2 (N=17)		Cohort 1 (N=24)	
Metal, μg/g	**Mn-exposed N=24 Mean (SD)**	**Non-exposed N=17 Mean (SD)**	**Mn-exposed N=13 Mean (SD)**	**Non-exposed N=4 Mean (SD)**	**Mn-exposed N=11 Mean (SD)**	**Non-exposed N=13 Mean (SD)**
Mn	0.3 (0.5)	0.2 (0.1)	0.4 (0.7)	0.2 (0.1)	0.3 (0.2)	0.2 (0.1)
Minimum	0.1	0.1	0.1	0.1	0.1	0.1
25th Percentile	0.1	0.1	0.1	0.1	0.2	0.1
Median	0.2	0.1	0.2	0.2	0.3	0.1
75th Percentile	0.3	0.2	0.3	0.3	0.4	0.2
Maximum[a]	2.5	0.3	2.5	0.3	0.8	0.3
Fe	55.9 (29.4)	55.3 (25.1)	54.3 (27.2)	47.7 (22.1)	57.8 (33.2)	57.6 (26.3)
Minimum	21.2	28	21.2	32.2	25.1	28
25th Percentile	36.0	33.8	38.6	33	28.3	37.4
Median	51.6	45.1	51.9	39.5	51.3	49.3
75th Percentile	65.4	74.3	60.9	62.5	72.5	74.3
Maximum	131.0	101	131	79.8	129	101
Mg	110.9 (58.2)	143.7 (79.1)	91.4 (23.6)	91.3 (11.9)	133.9 (77.8)	159.9 (84.4)
Minimum	59.5	38.6	59.5	82.1	70.6	38.6
25th Percentile	77.6	91.5	69.8	82.8	79.1	117
Median	96.2	118	87.6	87.5	100	154
75th Percentile	117	165	107	99.8	150	192
Maximum	284	389	141	108	284	389
P	875.7 (289.9)	775.4 (179.7)	785.8 (239.3)	758.3 (96.8)	982 (318.9)	780.7 (201.5)
Minimum	447	500	447	693	581	500
25th Percentile	696.5	658	672	702.5	714	627
Median	797	712	783	719	893	662
75th Percentile	962	963	907	814	1270	970
Maximum	1550	1040	1370	902	1550	1040
K	32.4 (38.2)	26.0 (24.2)	2.9 (0.9)	2.1 (0.4)	67.3 (29.6)	33.3 (23.1)
Minimum	1.7	1.7	1.7	1.7	32.1	4.6
25th Percentile	2.7	4.6	2.4	1.8	41.9	15.8
Median	4.7	17	2.7	2.1	69.3	28.3
75th Percentile	58.6	43.1	2.9	2.4	95	47.6
Maximum	120	87.1	5.2	2.5	120	87.1
Cu	13.9 (12.7)	16.5 (9.7)	6.6 (2.8)	8.4 (4.8)	22.6 (14.5)	18.9 (9.5)
Minimum	2.5	4.7	2.5	4.7	6.2	8.8
25th Percentile	5.8	8.8	5.1	5.3	11.8	11.7
Median	9.1	13.2	5.8	6.7	19.8	16.8
75th Percentile	19.6	21.5	6.9	11.5	27.4	23.1
Maximum	57.5	39.7	11.8	15.4	57.5	39.7
Zn	13.9 (9.5)	11.5 (3.8)	13.8 (11.6)	12.4 (2.0)	14.1 (6.7)	11.2 (4.2)
Minimum	6.3	4.6	6.3	10.1	7.6	4.6
25th Percentile	9.3	9.1	9.3	11	11.1	8.3
Median	11.2	11.8	9.6	12.3	12	11.5
75th Percentile	14.7	12.8	12.5	13.8	16.6	12.8
Maximum	50.8	21.5	50.8	14.8	31.6	21.5
Pb	0.3 (0.2)	0.2 (0.1)	0.2 (0.1)	0.1 (0.0)	0.4 (0.2)	0.3 (0.1)
Minimum	0	0.1	0	0.1	0.1	0.1
25th Percentile	0.1	0.1	0.1	0.1	0.3	0.2
Median	0.2	0.2	0.1	0.1	0.3	0.2
75th Percentile	0.3	0.4	0.1	0.1	0.4	0.4
Maximum	0.8	0.6	0.5	0.1	0.8	0.6

Abbreviations: Mn=manganese; Fe=iron; Mg=magnesium; P=phosphorus; K=potassium; Cu=copper; Zn=zinc; Pb=lead; SD=standard deviation.

[a] After excluding the individual with the highest level of Mn, the maximum Mn concentration was 0.8 μg/g overall and 0.5 μg/g in Cohort 2. With this exclusion the overall mean was 0.22 μg/g (SD 0.14 μg/g).

Table 3
Mn exposure status and olfactory tract/bulb metal concentrations, MINERS study, South Africa.

	Mean difference in metal concentration (μg/g) between Mn-exposed and non-exposed (95% CI)[a]	
	Age/cohort-adjusted	
Metal, μg/g	All decedents (N=41)	Excluding influential observations[b] (N≤40)[c,d,e,f,g,h]
Mn	0.16 (-0.11, 0.42)	**0.09 (0.004, 0.18)**[c]
Fe	2.70 (-16.3, 21.7)	-4.20 (-19.5, 11.1)[d]
Mg	-14.5 (-54.5, 25.5)	**-27.0 (-52.27, −1.68)**[e]
P	145 (-20.16, 311.9)	80.3 (-57.6, 218.2)[f]
K	**22.5 (7.92, 37.1)**	**18.0 (6.54, 29.6)**[g]
Cu	1.75 (-4.72, 8.23)	0.09 (-4.67, 4.84)[h]
Zn	2.44 (-2.87, 7.75)	0.10 (-2.50, 2.69)[d]
Pb	0.07 (-0.03, 0.18)	0.05 (-0.02, 0.12)[g]

Abbreviations: CI=confidence interval; Mn=manganese; Fe=iron; Mg=magnesium; P=phosphorus; K=potassium; Cu=copper; Zn=zinc; Pb=lead.
[a] Mean difference obtained as β-coefficient estimates from linear regression of the contrast between exposed and unexposed mineworkers. Models based on 24 Mn-exposed and 17 non-exposed decedents, unless specified otherwise. Age adjustment used age as a continuous variable; cohort was included as a binary variable.
[b] Decedents with Cook's distance >0.098 (4 divided by the sample size of 41).
[c] Excludes 1 Mn-exposed influential observation.
[d] Excludes 2 Mn-exposed influential observations.
[e] Excludes 4 influential observations (2 Mn-exposed and 2 non-exposed).
[f] Excludes 4 influential observations (3 Mn-exposed and 1 non-exposed).
[g] Excludes 3 influential observations (2 Mn-exposed and 1 non-exposed).
[h] Excludes 2 influential observations (1 Mn-exposed and 1 non-exposed).

Table 4
Cumulative Mn exposure and olfactory tract/bulb metal concentration, MINERS study, South Africa (N=41).

	Mean difference in metal concentration (μg/g) per mg Mn/m^3-year (95% CI)[a]	
	Age/cohort-adjusted	
Metal, μg/g	All decedents (N=41)	Excluding influential observations[b] (N≤40)[c,d,e,f,g]
Mn	**0.05 (0.01, 0.08)**	-0.003 (-0.02, 0.02)[c]
Fe	0.37 (-2.50, 3.24)	-0.86 (-3.80, 2.08)[c]
Mg	-0.74 (-6.82, 5.34)	-1.91 (-7.39, 3.57)[d]
P	7.43 (-18.6, 33.5)	-13.7 (-41.0, 13.6)[e]
K	2.27 (-0.09, 4.63)	1.63 (-0.81, 4.06)[f]
Cu	-0.28 (-1.26, 0.69)	0.18 (-0.57, 0.93)[d]
Zn	**0.82 (0.06, 1.59)**	0.16 (-0.35, 0.66)[e]
Pb	0.004 (-0.01, 0.02)	0.001 (-0.01, 0.02)[g]

Abbreviations: CI=confidence interval; Mn=manganese; Fe=iron; Mg=magnesium; P=phosphorus; K=potassium; Cu=copper; Zn=zinc; Pb=lead.
[a] Mean differences obtained as β-coefficient estimates from linear regression with metal exposure entered linearly as a continuous variable. Age adjustment used age as a continuous variable; cohort was included as a binary variable.
[b] Decedents with Cook's distance >0.098 (4 divided by the sample size of 41).
[c] Excludes 3 Mn-exposed influential observations.
[d] Excludes 3 influential observations (2 Mn-exposed and 1 non-exposed).
[e] Excludes 4 Mn-exposed influential observations.
[f] Excludes 2 Mn-exposed influential observations.
[g] Excludes 4 influential observations (3 Mn-exposed and 1 non-exposed).

4. Discussion

The role of Mn as a neurotoxic agent was first postulated in 1837 by Couper who described two Mn ore-exposed mineworkers with acute onset parkinsonism (Couper, 1837). Subsequently termed manganism, this multi-stage neurodegenerative disorder is characterized by postural instability, bradykinesia, dystonia, masked facies, and neuropsychiatric symptoms (Ashizawa, 1927; Bernheimer et al., 1973; Canavan et al., 1934; Casamajor, 1913; Cotzias, 1958; Cotzias et al., 1968; Couper, 1837; Kawamura et al., 1941; Parnitzke and Peiffer, 1954; Perl and Olanow, 2007; Rodier, 1955; Stadler, 1935; Trendtel, 1936; Yamada et al., 1986). These classic manifestations of manganism occurred at exposure levels as high as 1000 mg/m^3 (Flynn and Susi, 2009, 2010; Lucchini et al., 2009; Racette et al., 2012; Rodier, 1955; Santamaria et al., 2007). The clinical syndrome associated with current occupational and environmental Mn exposure levels is considerably different and consists primarily of parkinsonism and cognitive control dysfunction (Dlamini et al., 2020; Racette et al., 2012, 2021, 2022). As such, attention to chronic low-level occupational Mn exposures is critically important to understand exposure risks to miners' health and has regulatory policy implications (Antonini et al., 2009; Baker et al., 2014, 2015; Checkoway, 2010; Flynn and Susi, 2010; Lucchini et al., 2009; Sen et al., 2011).

Table 5
Olfactory tract/bulb metal concentration among Mn exposed workers in relation to years since last Mn exposure, MINERS study, South Africa (N=24).

	Mean difference in metal concentration (μg/g) per year since last Mn exposure (95% CI)[a]	
	Age/cohort-adjusted	
Metal, μg/g	All decedents (N=24)	Excluding influential observations[b] (N≤23)[c,d]
Mn	-0.005 (-0.03, 0.02)	0.001 (-0.01, 0.01)[c]
Fe	-0.58 (-1.91, 0.76)	-0.64 (-1.65, 0.38)[c]
Mg	-0.87 (-3.26, 1.53)	0.10 (-1.11, 1.32)[c]
P	-7.08 (-18.20, 4.03)	**-10.3 (-20.5, −0.12)**[c]
K	-0.69 (-1.54, 0.15)	-0.58 (-1.27, 0.12)[c]
Cu	0.07 (-0.38, 0.53)	0.10 (-0.22, 0.42)[c]
Zn	-0.26 (-0.66, 0.15)	-0.09 (-0.31, 0.14)[d]
Pb	-0.004 (-0.01, 0.004)	-0.0002 (-0.01, 0.005)[c]

Abbreviations: CI=confidence interval; Mn=manganese; Fe=iron; Mg=magnesium; P=phosphorus; K=potassium; Cu=copper; Zn=zinc; Pb=lead.
[a] Mean difference obtained as β-coefficient estimates from linear regression with metal exposure entered linearly as a continuous variable. Age adjustment used age as a continuous variable; cohort was included as a binary variable. Restricted to miners with any Mn exposure.
[b] Decedents with Cook's distance >0.1667 (4 divided by the sample size of 24).
[c] Excludes 2 Mn-exposed influential observations.
[d] Excludes 1 Mn-exposed influential observation.

Previous studies of animals exposed via inhalation routes of exposure have reported higher olfactory tract/bulb tissue Mn concentration in animals exposed to both soluble forms of Mn (Dorman et al., 2001, 2004) and insoluble forms of Mn (Elder et al., 2006; Fechter et al., 2002) as compared to non-exposed control animals. In rats exposed to MnO_2 aerosols of either 1.3 or 18 μm mass median aerodynamic diameter, only those exposed to the smaller particles had higher Mn in their olfactory bulb than control rats (Fechter et al., 2002). Similarly, rats exposed to Mn oxide UFPs had 3.5 greater concentrations of Mn in the olfactory bulb, suggesting that even insoluble forms of Mn, such as Mn oxides, could translocate via the neuronal nerve if the particle is ultrafine (Elder et al., 2006). In contrast, prior reports of Mn-exposed humans clinically diagnosed with manganism, utilizing older metal quantification methods to analyze Mn concentrations in different brain regions than the methods used in this analysis, did not demonstrate higher concentrations relative to non-exposed controls (Parnitzke and Peiffer, 1954; Stadler, 1935; Yamada et al., 1986). However, none of these studies reported quantitative values for Mn concentrations in olfactory tract/bulb tissue. A study characterizing the olfactory tracts/bulbs of seven asymptomatic welders and seven non-welder controls via high-resolution magnetic resonance imaging (MRI) found a higher T1 relaxation rate in the welders and suggested that this may be due to occupational Mn exposure (Sen et al., 2011). Our study builds on the MRI study by providing some evidence that Mn-exposed mineworkers have higher Mn olfactory tract/bulb tissue concentrations than

non-exposed mineworkers. We also found some evidence of a positive association between olfactory tract/bulb tissue Mn concentrations and cumulative Mn exposure, but not between olfactory tract/bulb tissue Mn concentrations and recency of exposure. Interestingly, these higher olfactory tract/bulb Mn concentrations occurred in a setting where Mn exposures were estimated to be substantially lower than the United States Occupational Safety and Health Administration (OSHA) 8-hr TWA occupational exposure limit of 5 mg/m^3 total dust but tended to be higher than the current ACGIH 8-hr TWA threshold limit value for inhalable Mn (0.1 mg/m^3) (Myers et al., 2003). However, total dust sampling and inhalable dust sampling, though both commonly used, are not directly comparable, as inhalable dust sampling collects particles with a diameter <100 μm, while total dust sampling collects particles with a diameter <30 μm. In addition, many decedents in our study continued to work many years after the sampling used to develop the JEM we utilized occurred, making accurate estimation of those later exposure levels less certain.

Divalent metal transporter 1 (DMT1), one of the primary Mn and Fe metal transporters, is typically expressed in enterocytes, astrocytes, endothelial cells, and sustentacular cells of the olfactory epithelium (Shawki et al., 2015; Tai et al., 2016; Thompson et al., 2007; Wolff et al., 2018; Zheng et al., 2012). Iron and Mn compete to cross the blood brain barrier and iron deficiency states may result in greater brain Mn accumulation (Fitsanakis et al., 2010). Moreover, there is also some evidence that lead may also be transported by DMT1 (Mani et al., 2021). Given that Fe and Pb are likely present in the Mn mines we studied (Costin et al., 2015), we cannot rule out the possibility that competition for DMT1 may have reduced tissue Mn concentrations.

Rodent and non-human primate studies, using occupational/environmental inhalation exposure models, have reported higher olfactory tract/bulb and striatal tissue Mn content in Mn-exposed animals than in control animals (Guilarte et al., 2008; Moberly et al., 2012; Normandin et al., 2002). The authors of these studies also hypothesized that higher olfactory tract/bulb Mn concentrations were linked to more central "downstream" effects, including dose-dependent central neurotransmitter release alterations, neuroinflammatory activity, release of proinflammatory cytokines, oxidative stress, and overexpression of striatal glial fibrillary acidic protein (GFAP) as a marker of induced astroglial response. Others have postulated similar downstream effects, including in a study in rats with short-term welding fume exposure which demonstrated significant elevations in DMT1 expression in the striatum and midbrain (Antonini et al., 2009). Our study finding of higher olfactory tract/bulb Mn concentrations in exposed vs. non-exposed humans provides evidence that inhaled Mn may enter the central nervous system through the olfactory bulb and may possibly contribute to neurotoxicity.

As with any human neuropathology study, there are limitations in our study. We cannot control who consents to have the brains of their relatives removed and examined, which creates the possibility of selection bias. We also have no information on clinical neurotoxicity in our study sample, but we have demonstrated that some workers from these same Mn mines have clinical parkinsonism and related disabilities that are comparable to our studies of Mn-exposed welders (Dlamini et al., 2020; Racette et al., 2017). A related concern is that neuropathology studies are, by nature, cross-sectional, and we cannot assess the impact of the relative importance of Mn influx and efflux in relation to recency of exposure. Nevertheless, our findings suggest that cumulative exposure may be more important in olfactory tract/bulb Mn concentration than recency of exposure. Methodologically, the differential sample processing (i.e., paraffin embedding for Cohort 1 and not for Cohort 2) could cause some differences between cohorts. Formalin fixing could leach metals or add metals from tissue specimens (Hasegawa et al., 2022). The metal concentrations that we observe are generally similar to those reported in a prior study of olfactory bulb sample (Gardner et al., 2017) in which the samples were snap frozen rather than being fixed in formalin. Snap freezing was not practical in our study, due to the remote location from which samples were collected. Additionally, paraffin embedding, followed by dewaxing could also have altered the moisture content of the tissue samples. We adjusted for cohort in addition to age to address potential for bias given evidence of systematic differences in concentrations of some metals among unexposed decedents. Moreover, in some of our sensitivity analyses, we had to exclude up to 10% of the combined cohorts because they were influential observations. Finally, our exposure estimates were informed by a JEM developed from occupational hygiene total dust sampling. While this sampling method would capture UFPs in the air, this method alone does not allow the quantification of the count or mass of UFP particles relative to the whole sample, and we do not have data on PSD, which would differ by job task and mine. Exposure to smaller particles is critical for both gas-exchange (respirable size fraction) and translocation to the olfactory bulb (ultrafine size fraction). Therefore, it is possible that the modest differences between Mn-exposed and non-exposed mineworkers are due to exposures in the Mn mines resulting in larger particle sizes that cannot access the brain through the olfactory bulbs. The weak dose-response association we observed could be due to differences in PSD (Hewett, 1991). Mn mineworkers undertaking hot processes in the mines would likely have more exposure to fine and ultrafine particles than Mn mineworkers undertaking more mechanical processes, a level of occupational detail that we were unable to capture. Despite the above potential limitations, this is a large occupational neuropathology study that provides some insight into a question that has been addressed previously only in animal model systems.

Future studies focusing on the topographical distribution of Mn within the olfactory tract/bulb and how this relates to the cumulative nature and timing of Mn exposure would expand on this work (Duncan et al., 2021; Hasegawa et al., 2020). Additionally, identification of the precise site of deposition at a cellular level (e.g. parenchymal, perivascular, and/or intracellular; since astrocytes, olfactory epithelium and endothelial cells express DMT1), and assessment of direct or indirect (via Fe homeostatic dysregulation) induction of metal driven oxidative stress cascade would help inform our understanding of tissue uptake, mechanisms of cytotoxicity, and potential preventive/therapeutic targets (Dobson et al., 2003; Gonzalez-Cuyar et al., 2008; Rivera-Mancia et al., 2011; Xia et al., 2011). Finally, a contemporary exposure assessment that quantifies exposure to the smallest particle sizes and investigates differences in PSD both between- and within- jobs would further aid in the understanding of this and related work, especially given the importance of particle size in relation to uptake and distribution of inhaled particles.

5. Conclusion

Findings from this relatively large occupational neuropathology study suggest that olfactory bulbs from workers employed in Mn mines have a higher Mn tissue concentration than non-exposed mineworkers, indicating that the olfactory route of exposure may be a potential route of entry across the blood-brain barrier.

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Formal analysis. **Luis Francisco Gonzalez-Cuyar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Shar Samy:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis. **Marissa G Baker:** Writing – review & editing. **Susan Searles Nielsen:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Brad A. Racette:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Funding acquisition, Data curation, Conceptualization. **Gill Nelson:** Writing – review & editing, Resources, Data curation, Conceptualization. **Lianne Sheppard:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Natalie Senini:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Susan R. Criswell:** Writing – review & editing, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2024.04.001.

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