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Associations between former exposure to manganese and olfaction in an elderly population: Results from the Heinz Nixdorf Recall Study

Swaantje Casjens[a,*], Beate Pesch[a], Sibylle Robens[a], Benjamin Kendzia[a], Thomas Behrens[a], Tobias Weiss[a], Nadin Ulrich[a], Marina Arendt[b], Lewin Eisele[b], Noreen Pundt[b], Anja Marr[b], Christoph van Thriel[c], Rainer Van Gelder[d], Michael Aschner[e], Susanne Moebus[b], Nico Dragano[f], Karl-Heinz Jöckel[b], Thomas Brüning[a]

[a] *Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bochum, Germany*
[b] *Institute for Medical Informatics, Biometry and Epidemiology (IMIBE), University of Duisburg-Essen, Essen, Germany*
[c] *Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany*
[d] *Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, Germany*
[e] *Department of Molecular Pharmacology, Albert Einstein College of Medicine, NY, USA*
[f] *Institute for Medical Sociology, Medical Faculty, University of Düsseldorf, Düsseldorf, Germany*

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ABSTRACT

Occupational exposure to manganese (Mn) has been associated with impairments in olfaction and motor functions, but it has yet to be determined if such effects persist upon cessation of exposure. The objective of this study was to evaluate the influence of former occupational Mn exposure on olfaction within the framework of a prospective cohort study among an elderly German population. Information on job tasks with recognized Mn exposure and data on odor identification assessed with Sniffin' sticks was collected during the second follow-up of the Heinz Nixdorf Recall Study. The study population consisted of 1385 men aged 55–86 years, 354 of whom ever worked in jobs with potential Mn exposure (median 58.3 $\mu g/m^3$ years, interquartile range 19.0–185 $\mu g/m^3$ years). Multiple exposure measures, including job tasks, cumulative Mn exposure, and Mn determined in blood samples (MnB) archived at baseline, were used to estimate effects of Mn on olfaction. Having ever worked as welder was associated with better olfaction compared to other blue-collar workers without Mn exposure. Blue-collar workers identified less odors in comparison to white-collar workers. Concentrations of previous Mn exposure >185 $\mu g/m^3$ years or MnB $\geq$15 $\mu g/L$ were not associated with impaired olfaction. In addition to a strong age effect, participants with lower occupational qualification identified less odors. We found no relevant association of former Mn exposure at relatively low levels with impaired olfaction. Possible neurotoxic Mn effects may not be persistent after cessation.

1. Introduction

Manganese (Mn) is an essential trace element naturally found in the environment. It is required for a variety of key physiological processes, including amongst others, antioxidant defense, energy metabolism, and immune function (Chen et al., 2015). Mn import, excretion and its concentration in blood (MnB) are tightly regulated. Control of Mn homeostasis involves a complex network of proteins, but none of these are specific for Mn. Particularly well studied is its interaction with iron (Fe), an important redox-active metal. Mn competes with Fe for protein binding, indicated by a negative association of MnB and serum ferritin (SF) (Aguirre and Culotta, 2012).

The tight homeostasis of Mn can be disturbed by occupational exposure, impaired hepatobiliary excretion and other circumstances (Perl and Olanow, 2007). Occupational settings with anticipated Mn exposure comprise the production and processing of steel, ferroalloys, and dry-cell batteries (ATSDR, 2008). A large

* Corresponding author at: Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789, Bochum, Germany.
E-mail address: casjens@ipa-dguv.de (S. Casjens).

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workforce of welders is exposed to inhalable Mn and Fe especially when welding steel with high-emission techniques (Pesch et al., 2012). Inhaled Mn can bypass the blood-brain barrier and reach structures deep within the brain by transport down the olfactory nerve to the olfactory bulb (Aschner and Dorman, 2006). Mn distributes non-uniformly in the brain accumulating in regions susceptible to oxidative injury, namely the globus pallidus, striatum, and substantia nigra (Bowman and Aschner, 2014).

Occupational exposure to Mn can result in various neurological dysfunctions. High exposures can cause neurotoxicity with the development of a movement disorder known as manganism which is distinct from idiopathic Parkinson's disease (Guilarte and Gonzales, 2015). There is clear evidence of neurologic effects from inhalation of high Mn concentrations, but results from studies at lower doses and on former exposure remain controversial. Several cross-sectional studies with small sample sizes in active workers or environmentally Mn-exposed persons found olfactory impairment (Antunes et al., 2007; Bowler et al., 2007; Guarneros et al., 2013; Lucchini et al., 2014). In large population-based studies focusing on occupations with Mn exposure olfaction is rarely considered. One such study examined the association of manufacturing occupation and olfaction and found no correlation (Schubert et al., 2011).

In addition to methodological shortcomings as possible reasons for inconsistent results, active and former workers with exposure to Mn may differ in the amount of accumulated Mn in the brain. Animal experiments showed that the Mn in the brain is eliminated after exposure (Aschner and Dorman, 2006). In humans the kinetics of Mn accumulation are less known. One study described no improvement in olfactory impairment in confined-space welders even after 3.5 years of exposure cessation (Bowler et al., 2011). Nevertheless, it is unclear whether Mn-induced neurotoxicity observed in active workers can persist as neurodegenerative effect after cessation of exposure.

Within the framework of the prospective Heinz Nixdorf Recall (Risk Factors, Evaluation of Coronary Calcium and Lifestyle) Study (HNRS) we investigated the association of exposure to Mn with olfactory dysfunction in elderly men. We evaluated multiple measures of exposure to Mn, including occupations with anticipated Mn exposure, cumulative inhalable exposure to Mn, and Mn in blood samples archived at baseline.

2. Materials and methods

2.1. Subjects

The study population of this analysis comprised 1474 men who underwent olfactory testing using Sniffin' sticks tests in the second follow-up survey of HNRS (2011–2014). HNRS is a prospective population-based cohort study in an industrial area in Germany. Its rationale, design, and conduct have been previously described (Schmermund et al., 2002). In brief, 4814 subjects (about 50% females) aged from 45 to 75 years were included. They were recruited from a random sample from the general population of the cities of Bochum, Essen, and Mülheim starting in December 2000. The overall recruitment efficacy proportion for the baseline recruitment (2000–2003) was 56% (Stang et al., 2005). This analysis was part of the project AeKo ("Arbeitsmedizinische Forschung in epidemiologischen Kohortenstudien"- Occupational medical research in epidemiological cohort studies), which aimed at investigating occupational risk factors within the HNRS. Approval for the study was obtained from the ethical commission of the Medical Faculty of the University Duisburg-Essen. All participants signed an informed consent.

From 1474 men enrolled for AeKo, we excluded 51 participants with missing olfactory testing, 26 participants with acute (e.g. a cold, a blocked nose or other sinonasal complaints) and six participants with sustained olfactory impairment (e.g. surgery), and six participants with conditions known to affect olfaction, such as Parkinson's disease. The dataset of the statistical analysis consisted of 1385 men with information on the occupational history and on job tasks with anticipated exposure to inhalable Mn as well as diverse blood parameters.

2.2. Odor identification test

Sniffin' sticks were applied to assess the identification of 12 odors (orange, leather, cinnamon, peppermint, banana, lemon, licorice, coffee, clove, pineapple, rose, and fish). Odors were presented in felt-tip pens. The individual pens were consecutively placed in front of both nostrils at a distance of approximately two cm. The participants could identify the odor as a multiple-choice task from a list of four potential answers resulting in a dichotomous variable of correct or false odor identification. The cumulative score of all 12 Sniffin' stick results yielded count data with values between 0 and 12. Subjects were classified as normosmic if ten or more odors were identified correctly, hyposmic if seven to nine odors were identified, and anosmic if less than seven odors were identified (Hummel et al., 2001).

2.3. Assessment of exposure to Mn

2.3.1. Job tasks with anticipated exposure to Mn

Exposure to Mn was initially assessed using a supplemental job-specific questionnaire for regular (N = 24), occasional welding (N = 187), and for other occupations with exposure to hot metal fumes (production of steel and alloys) or particulate matter that may contain Mn (battery production) (N = 99). In the main interview participants gave detailed information about their occupational history. Men who worked in their last job as blue-collar worker (N = 267) constituted the reference group. The remaining group comprised men with a white-collar job as last occupation (N = 618). Farmers were not among the participants from this industrial district.

2.3.2. Calculation of cumulative inhalable exposure to Mn

We established a job-exposure matrix (JEM) for occupations with Mn exposure using 4635 personal measurements of inhalable Mn compiled in the German exposure database MEGA from 1989 to 2015 (Gabriel et al., 2010). Mn was determined by inductively coupled plasma mass spectrometry (ICP-MS) in personal samples of inhalable particles. Multiple imputation was performed for 10% of all measurements that were below the limit of quantification (Lotz et al., 2013). Textual information about workplaces was assigned to the corresponding at-risk occupation assessed in the supplemental questionnaire of the HNRS. We classified welding measurements by major welding process and material. Mixed-effects models were applied to the natural log-transformed Mn concentrations with imputed non-detects to assess the geometric means of exposure to Mn in the various occupational settings according to a model developed in the WELDOX study (Pesch et al., 2012). In addition to the materials we implemented the median-centered year and sampling duration of measurement as covariate. Intensity scores were assigned for working as welder (=1) and for other occupations as welding frequently (=0.25) or less frequently (=0.1). We linked the exposure estimates with occupational histories of the HNRS participants and calculated inhalable Mn [$\mu g/m^3$ years] as the sum of the exposure in each job period with anticipated exposure using the product of the job-specific or welding-process average concentration [$\mu g/m^3$] and the corresponding duration [years], together with intensity scores for welding: inhalable Mn [μg/m^3 years] = Σ Shift concentration [μg/m^3] · Duration [years] · Intensity score.

2.4. Determination of Mn in archived whole blood samples

MnB was determined at the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance in 1 ml-aliquots stored at baseline by means of inductively coupled plasma-quadrupole mass spectrometry. A blank reagent was included in each analytical series. Plastic materials were used for sample preparation to prevent contamination. Prior to usage, the vessels were cleaned with 1 M nitric acid for 2 h, rinsed with ultrapure water and dried at room temperature. After thawing the frozen aliquots of whole blood, 400 μL was diluted 1:12.5 with a 0.5% solution of ammonium hydroxide and 100 μL of a 0.2% solution of Triton-X. Rhodium was used as internal standard. Analysis was carried out using a 7700 ICP-MS system from Agilent Technologies in He-mode (flow rate 5 mL/min) with a collision cell to avoid interferences. Skimmer and sampler cones were made of platinum. Calibration and calculation of the Mn concentration were carried out using standards prepared in sheep blood at eight different concentrations. LOQ was 2.0 μg/L. Materials from RECIPE (ClinChek Whole Blood Level, lyophil. for Trace Elements I and II, REF 8840, LOT 227) and SERONORM (Trace Elements Whole Blood Level I and II, LOT 1103129) served as internal control. Within-series and between-day imprecision were each lower than 8%. MnB above the German biological reference value for workplace substances (15 μg/L) was used as cutoff for an elevated concentration.

2.5. Determination of liver function and iron status

Analyses were performed in the central laboratory of the University Hospital Essen with standard methods at second follow-up as formerly described (Eisele et al., 2013). Gamma-glutamyl transferase (GGT) was measured with the ADVIA 1650 chemistry

Table 1
Characteristics of 1385 men of the Heinz Nixdorf Recall Study.

Variable	Total[a]	Welders	Occasional welding	Other occupations with Mn exposure	Blue-collar occupations without Mn exposure	White-collar occupations
n	1385	26	215	113	326	705
Age (years)						
Median [inter-quartile range]	69 [62; 74]	69 [67; 73]	68 [63; 73]	71 [62; 76]	69 [63; 74]	68 [62; 74]
Current employment						
Yes	376 (27.1%)	5 (19.2%)	46 (21.4%)	25 (22.1%)	69 (21.2%)	231 (32.8%)
Occupational qualification						
University	391 (28.2%)	0 (0%)	20 (9.3%)	19 (16.8%)	32 (9.8%)	320 (45.4%)
Foreman/technician	296 (21.4%)	8 (30.8%)	63 (29.3%)	25 (22.1%)	75 (23.0%)	125 (17.7%)
None/vocational	698 (50.4%)	18 (62.2%)	132 (61.4%)	69 (61.1%)	219 (67.2%)	260 (36.9%)
Smoking status						
Never	411 (29.7%)	13 (50.0%)	56 (26.0%)	24 (21.2%)	93 (28.5%)	225 (31.9%)
Former	783 (56.5%)	11 (42.3%)	124 (57.7%)	74 (65.5%)	189 (58.0%)	385 (54.6%)
Current	191 (13.8%)	2 (7.7%)	35 (16.3%)	15 (13.3%)	44 (13.5%)	95 (13.5%)
Gamma-glutamyl transferase [U/L]						
Median [inter-quartile range]	31 [22; 48]	25 [20; 42]	32 [23; 50]	30.5 [23.5; 49]	29 [20; 45]	31 [21; 50]
$\geq$ 81 (90th percentile)	141 (10.3%)	0 (0%)	30 (14.0%)	17 (15.2%)	21 (6.5%)	73 (10.4%)
Iron status						
Low (hemoglobin < 13 g/dL)	88 (6.4%)	3 (11.5%)	16 (7.4%)	6 (5.3%)	20 (6.1%)	43 (6.1%)
Normal	791 (57.1%)	16 (61.5%)	114 (53.0%)	68 (60.2%)	193 (59.2%)	400 (56.7%)
High (serum ferritin $\geq$ 400 μg/L)	92 (6.6%)	1 (3.8%)	18 (8.4%)	5 (4.4%)	27 (8.3%)	41 (5.8%)
Missing	414 (29.9%)	6 (23.1%)	67 (31.2%)	34 (30.1%)	86 (26.4%)	221 (31.3%)
Mn in blood [μg/L] at baseline						
Median [inter-quartile range]	8.1 [6.7; 9.7]	8.7 [7.2; 9.6]	8.2 [6.9; 10.3]	8.3 [6.9; 10.4]	8.3 [6.7; 9.7]	8 [6.7; 9.6]
$\geq$ 15	37 (2.8%)	2 (5.4%)	7 (18.9%)	1 (2.7%)	8 (21.6%)	19 (51.4%)
Cumulative inhalable exposure to Mn [μg/m^3 years]						
Median [inter-quartile range]	0 [0; 3.8]	1121 [465; 1766]	74.2 [27.2; 185]	25.1 [8.8; 78.8]	0 [0; 0]	0 [0; 0]

[a] Due to missing data, the total number of individuals for each variable may be less than 1385.

analyzer (Siemens Healthcare, Eschborn, Germany). GGT >90th percentile was considered as indicator of liver dysfunction. Hemoglobin (Hb) was measured with a STKR hematology analyzer (Beckman Coulter, Krefeld, Germany) and SF with the BN II nephelometer (Siemens Healthcare, Eschborn, Germany). Fe status was considered as low if Hb < 13 g/dL, and high if SF ≥400 μg/L.

2.6. Statistics

All calculations were done using SAS (version 9.4). Median and inter-quartile range (IQR) were used to describe the distribution of continuous variables. Study groups were compared using the Kruskal-Wallis test for continuous variables and the chi-square test for categorical data. The Jonckheere-Terpstra test was used to assess the trend of anosmia with age. Rank correlations between variables are presented by Spearman's correlation coefficient (r_S) with 95% confidence interval (95% CI). The strength of a relationship between two variables, while controlling the effect of other variables, is presented with the Spearman partial correlation coefficient (r_{Sp}) with 95% CI. Multiple Poisson regression models were applied to estimate the effects of at-risk occupations, inhalable Mn, MnB and other factors on the cumulative score of twelve Sniffin' stick odors using the GENMOD procedure with a log link function and the logarithm of the number of tested odors as offset. In this case, the offset variable serves to normalize the fitted cell means to the rate of correctly identified odors. As potential confounders we included age (log-transformed in decades), smoking status (never, former, current), and occupational qualification (university degree, foreman/technician, none or vocational). P-values < 0.05 were considered statistically significant.

3. Results

Table 1 depicts the characteristics of 1385 men of HNRS. The median age of all participants was 69 years (range 55–86). Overall, 50% of the participants worked as foreman or technician or had a university degree. Twenty-six men worked as welder with a median employment period of 22 years (IQR 15–40) and a median inhalable Mn of 1121 μg/m^3 years (IQR 465–1766). In other jobs 215 men welded occasionally and had a median inhalable Mn of 74.2 μg/m^3 years (IQR 27.2–185). Additional 113 men worked in other occupations with anticipated Mn exposure (median inhalable Mn 25.1 μg/m^3 years, IQR 8.8–78.8). Fewer occupational welders had ever smoked than other blue-collar workers (never smoked: 50.0% vs. 26.4%) and had lower GGT concentrations. In comparison to white-collar workers, blue-collar workers were more often born in countries other than Germany (3.7% vs. 7.1%, data not shown). MnB, anemia, and iron overload were indistinguishable between the occupational groups.

Overall, 173 men (12.5%) identified less than seven odors and were classified as anosmic (Table 2). The age of the participants had

Table 2
Characteristics of the study population according to Sniffin' sticks test results presented as identified odors, normosmia (12-10 odors), hyposmia (9-7 odors), and anosmia (6-0 odors).

	Age		Identified odors			Normosmia		Hyposmia		Anosmia	
	Median	n[a]	Median	IQR[b]		n	%	n	%	n	%
Total		1385	9	8	10	581	41.9	631	45.6	173	12.5
Age [years] (median, IQR[b])						67	61; 72	69	63; 74	74	69; 78
Occupation											
Welder	69	26	10	9	11	16	61.5	9	34.6	1	3.8
Occasional welding	68	215	9	8	10	79	36.7	113	52.6	23	10.7
Other occupations with Mn exposure	71	113	9	7	10	32	28.3	60	53.1	21	18.6
Blue-collar occupations without Mn exposure	69	326	9	7	10	122	37.4	153	46.9	51	15.6
White-collar	68	705	9	8	10	332	47.1	296	42.0	77	10.9
Occupational qualification											
University	68	391	9	8	10	191	48.8	159	40.7	41	10.5
Foreman/technician	69	296	9	8	10	115	38.9	145	49.0	36	12.2
None/vocational	69	698	9	8	10	275	39.4	327	46.8	96	13.8
Smoking status											
Never	69	411	9	8	10	177	43.1	187	45.5	47	11.4
Former	70	783	9	8	10	328	41.9	360	46.0	95	12.1
Current	64	191	9	8	10	76	39.8	84	44.0	31	16.2
Gamma-glutamyl transferase [U/L]											
<81 (90th percentile)	69	1233	9	8	10	521	42.3	562	45.6	150	12.2
≥ 81	68	141	9	8	10	59	41.8	60	42.6	22	15.6
Iron status											
Low (hemoglobin < 13 g/dL)	72	88	9	7	10	27	30.7	47	53.4	14	15.9
Normal	69	791	9	8	10	349	44.1	344	43.5	98	12.4
High (serum ferritin ≥ 400 μg/L)	68	92	9	8	10	39	42.4	47	51.1	6	6.5
Mn in blood [μg/L] at baseline											
<15	69	1293	9	8	10	539	41.7	589	45.6	165	12.8
≥ 15	66	37	10	8	11	20	54.1	15	40.5	2	5.4
Cumulative inhalable exposure to Mn [μg/m^3 years]											
=0	68	1031	9	8	10	454	44.0	449	43.5	128	12.4
>0–58 (median)	67	179	9	8	10	72	40.2	85	47.5	22	12.3
>58–185 (75th percentile)	70	87	9	8	10	25	28.7	50	57.5	12	13.8
>185	71	88	9	7	10	30	34.1	47	53.4	11	12.5

[a] Due to missing data, the total number of individuals for each variable may be less than 1385. [b]Inter-quartile ranges.

a strong effect on olfaction, with normosmic men being younger than hyposmic and anosmic men. Overall, there was a trend of increased anosmia with age in all men ($p < 0.001$), in Mn exposed blue-collar workers ($p < 0.001$), in blue-collar workers without Mn exposure ($p = 0.006$) and in white-collar workers ($p < 0.001$). In multiple regression models with different exposure measures we estimated a decrease of over 40% in the expected number of identified odors per 10 years of higher age in all men (Tables 3–6).

In addition, occupation and occupational qualification were associated with olfaction with 61.5% of welders being normosmic in comparison to only 36.7% of occasional welders and 28.3% of men working in other occupations with exposure to Mn (Table 2). On average, welders identified 10 odors correctly (IQR 9–11) and men in all other subgroups identified 9 odors. Likewise, participants with a university degree were presented with better odor identification results. Although current smokers were younger than never and former smokers (64 vs. 69 and 70 years), they were more often anosmic. Participants with low Hb (<13 g/dL), and normal MnB (<15 μg/L) concentrations were also more often anosmic. However, men without exposure to Mn were more often normosmic. Changes in olfaction were not associated with GGT concentration, alcohol intake or seasonality (data not shown).

Having ever worked as welder was associated with better olfaction determined as identified odors compared with other blue-collar workers without exposure to Mn (exp(β) 1.17, 95% CI 1.07–1.29). Working in a white-collar occupation was also associated with better olfaction (Table 3). Tables 4 and 5 depict MnB, inhalable Mn and other factors as potential predictors of odor identification. Both, MnB ≥15 μg/L and higher concentrations of inhalable Mn (>185 μg/m^3 years) were not found to influence olfaction. Seventeen men were exposed to very high concentrations of Mn (>1000 μg/m^3 years), which revealed a marginally improved odor identification. This very high exposure to Mn resulted in an increase of 1.12 (95% CI 1.00–1.26) in odor identification (Table 6). In addition, increased age, low occupational qualification, and current smoking were associated with impaired olfaction when using Poisson regression to estimate the effects on odor identification. GGT and iron status were not associated with olfaction (Supplemental Tables S1–S3). We found similar patterns of relative risks assessed with log-binomial regression for working in at-risk occupations as well as high concentrations of MnB and inhalable Mn with dysnosmic men correctly identifying less than ten odors (data not shown).

4. Discussion

In this analysis we did not observe impairment in olfaction in relation to multiple measures of former exposure to Mn among 1385 elderly men. Participants formerly working as regular welders correctly identified more odors than other participants.

Table 4
Manganese (Mn) in blood at baseline and other factors as potential predictors of identified odors with the Sniffin' stick test.

Variable	Value	n	Exp(β)	95% CI	
Intercept		1330	2.59	2.02	3.33
Mn in blood [μg/L]	<15	1293	1		
	≥ 15	37	1.05	0.97	1.14
Occupational qualification	University	372	1		
	Foreman/technician	283	0.97	0.93	1.01
	None/vocational	675	**0.96**	**0.93**	**0.99**
Age [log years in decades]		1330	**0.53**	**0.47**	**0.60**
Smoking status	Never	403	1		
	Former	751	0.99	0.96	1.02
	Current	176	**0.93**	**0.89**	**0.98**

The prospective design, the cohort's large size, and the comprehensive assessment of exposure to Mn are the major strengths of this analysis. To the best of our knowledge, this is one of the first approaches to quantitatively assess cumulative exposure to inhalable Mn based on measurements compiled in exposure databases. In AeKo hot metal fumes containing Mn together with Fe when welding and producing steel can be assumed to be the major source of Mn exposure. The production of batteries was a minor setting. The small number of regular welders in the population-based design, the lack of individual Mn exposure measurements, and the time gap between Mn exposure and olfactory testing are potential limitations.

Good or sufficient olfactory ability contributes to quality of life (Landis et al., 2004). Various factors are associated with the prevalence of olfactory impairment. These comprise age, gender, smoking status, alcohol abuse, nasal polyps, upper respiratory infection, other disorders, especially Parkinson's disease, medication, chemotherapy, and genetics (Casjens et al., 2013; Doty and Mishra, 2001; Riga et al., 2015). However, inhalation exposure to Mn may also lead to olfactory impairment as reported in different occupational settings and environmental health studies (Antunes et al., 2007; Bowler et al., 2007; Guarneros et al., 2013; Lucchini et al., 2014).

Three routes of Mn uptake into the central nervous system have been proposed including the direct uptake via the olfactory nerve. Studies in rats showed higher Mn concentrations in olfactory bulbs and other brain regions of exposed rats after inhalation of fine and ultrafine Mn-oxide aerosols in comparison to controls (Fechter et al., 2002). This olfactory neuronal translocation pathway was also shown in non–human primates (Dorman et al., 2006; Elder et al., 2006). These experimental findings offer a strong basis for a causal association between impairment in olfaction and inhalation exposure to Mn. However, we did not observe impairment in olfaction using multiple measures of former exposure to Mn. Seventeen participants of HNRS were exposed to Mn above

Table 3
Occupation and other factors as potential predictors of olfaction determined as identified odors with the Sniffin' stick test.

Variable	Value	n	Exp(β)	95% CI	
Intercept		1385	2.47	1.93	3.15
Occupation	Blue-collar jobs without Mn exposure	326	1		
	Welder	26	**1.17**	**1.07**	**1.29**
	Occasional welding	215	1.03	0.98	1.07
	Other jobs with Mn exposure	113	0.98	0.93	1.03
	White-collar	705	**1.05**	**1.02**	**1.09**
Age [log years in decades]		1385	**0.53**	**0.46**	**0.60**
Smoking status	Never	411	1		
	Former	783	0.99	0.96	1.02
	Current	191	**0.94**	**0.90**	**0.98**

Table 5
Cumulative exposure to inhalable manganese (Mn) and other factors as potential predictors of identified odors with the Sniffin' stick test.

		n	Exp(β)	95% CI	
Intercept		1385	2.67	2.09	3.42
Cumulative exposure to Mn [μg/m³ years]	0	1031	1		
	>0–58 (median)	179	0.99	0.95	1.03
	>58–185 (75th percentile)	87	0.99	0.94	1.05
	>185	88	1.02	0.97	1.08
Occupational qualification	University	391	1		
	Foreman/technician	296	0.97	0.93	1.01
	None/vocational	698	**0.96**	**0.93**	**0.99**
Age [log years in decades]		1385	**0.52**	**0.46**	**0.59**
Smoking status	Never	411	1		
	Former	783	0.99	0.96	1.02
	Current	191	**0.94**	**0.90**	**0.98**

Table 6
High concentrations of cumulative exposure to inhalable manganese (Mn) and other factors as potential predictors of identified odors with the Sniffin' stick test.

		n	Exp(β)	95% CI	
Intercept		1385	2.66	2.09	3.40
Cumulative exposure to Mn [μg/m³ years]	≤1000	1368	1		
	>1000	17	**1.12**	**1.00**	**1.26**
Occupational qualification	University	391	1		
	Foreman/technician	296	0.97	0.93	1.005
	None/vocational	698	**0.96**	**0.93**	**0.99**
Age [log years in decades]		1385	**0.52**	**0.46**	**0.59**
Smoking status	Never	411	1		
	Former	783	0.99	0.96	1.02
	Current	191	**0.94**	**0.90**	**0.98**

1000 μg/m³ years. This corresponds to five years working at 200 μg/m³, which is the occupational exposure limit for inhalable Mn in Germany, or to one year at 1000 μg/m³, which is the concentration that can induce manganism (Olanow, 2004). Even this very high cumulative exposure to Mn (> 1000 μg/m³ years) did not lead to an impairment in odor identification (Table 6). Furthermore, it has been shown that welding with materials and electrodes with enriched Mn content has a significant influence on MnB (Pesch et al., 2012). Here, 22 men welded with Mn alloys but did also not suffer from impaired olfaction (data not shown).

No improvement from the olfactory impairment was observed 3.5 years after cessation of Mn exposure (Bowler et al., 2011). However, it should be considered that we cannot confirm that former exposure to Mn is causal to the olfactory impairment in the elderly men included in this large population-based study. Here, the time since last Mn exposure was ten fold longer with a median cessation time of 33 years (IQR 16–44). In contrast to confined space welders (Bowler et al., 2007), we observed less welders with MnB >10 μg/L (15% vs. 43%). However, this study supports other findings that possible neurobehavioral Mn effects observed in active workers are not persistent (Ellingsen et al., 2015). Nevertheless, we confirmed findings of an increased olfactory impairment in blue-collar workers in comparison to white-collar workers (Murphy et al., 2002) (Supplemental Table S4).

As shown in several population-based studies age is strongly associated with olfactory impairment (Doty et al., 2011; Murphy et al., 2002; Schubert et al., 2011). In this study age had the strongest effect on odor identification. However, we did not find a stronger decline of olfaction with age in the Mn-exposed group in comparison to white-collar workers as found in an environmental study close to a Mn processing plant (Guarneros et al., 2013).

In current smokers we found some indication for higher risks of olfactory impairment, supporting results from previous large population-based studies (Murphy et al., 2002). In addition to the olfactory system, odor identification involves brain processing areas that include cognitive and language abilities. Hence, there is a risk that a decline in odor identification could be falsely attributed to olfactory dysfunction, when it, in fact, results from impaired cognitive or language abilities (Royet and Plailly, 2004). Therefore, we implemented stratified analysis by cognitive function according to the clock drawing test (good vs. impaired) (Shulman et al., 1986) and birthplace (Germany vs. other countries). All these analyses showed similar associations between Sniffin' stick scores, occupation, MnB, and inhalable Mn (data not shown). Similar results were also observed, when we restricted the analyses to blue-collar workers (Supplemental Table S5).

Alcohol consumption partially explained the association of Mn and neurobehavioral outcomes in a cross-sectional study of welders (Ellingsen et al., 2014). Liver cirrhosis may also lead to higher Mn concentrations in blood and brain (Krieger et al., 1995). Hence, we included elevated GGT concentrations levels > 90th percentile as indicator of liver dysfunction into the statistical models and found no significant association with odor identification (Supplemental Tables S1–S3). We also analyzed self-assessed alcohol consumption as potential confounder, but did not observe an association with odor identification (data not shown).

Hot metal fumes from steel production and processing contain, in addition to Mn, large amounts of Fe. Fe content in the brain changes over the life span and is involved in regulating motor and cognitive functions as well as neurodegeneration (Ward et al., 2014). A study examining the contribution of olfactory divalent metal transporter-1 (DMT1) in Mn uptake showed that the protein levels of olfactory DMT1 were elevated in iron-deficient rats, suggesting that olfactory uptake of Mn is facilitated by anemia (Thompson et al., 2007). Hence, the effect of Mn on olfactory tests has also to be controlled for iron status. Men with high iron stores (SF ≥400 μg/L) and anaemic men (Hb < 13 g/L) did not identify lesser number of odors in comparison to men with normal iron status (Supplemental Tables S1–S3). Nevertheless, low Hb may indicate a variety of disorders and chronic high SF may induce inflammation and liver dysfunction, and may contribute to tumor progression and poor clinical outcome in cancer patients (Wang et al., 2010). We found that welding with high-emission processes in confined spaces may lead to elevated MnB and SF (Casjens et al., 2014). However, such rare exposure settings are less likely to be captured in population-based studies.

5. Conclusions

With the exception of welders the average exposure levels on Mn were low in the investigated men. This is in agreement with the current occupational exposure limits to protect workers from neurotoxic or neurodegenerative effects of Mn. In this cohort, we failed to identify an association of former exposure to Mn at relatively low levels with impairment in odor identification. Welders performed even slightly better, when adjusting for covariates and in comparison to other blue-collar workers. Hence, possible neurotoxic Mn effects observed in active workers may not be persistent. In addition to a strong effect of age, working in a blue-collar job, lower occupational qualification, and current smoking were statistically significant predictors of impaired odor identification in men.

Conflict of interest

Authors from the Institute for Prevention and Occupational Medicine (IPA) and from the Institute for Occupational Safety and Health (IFA) work for the German Social Accident Insurance. The authors are independent from the German Social Accident Insurance in study design, access to the collected data, responsibility for data analysis and interpretation, and the right to publish. The views expressed in this paper are those of the authors and not necessarily those of the sponsor. All other authors have disclosed any potential conflicts of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neuro.2016.11.005.

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