NeuroToxicology 47 (2015) 8–16

Contents lists available at ScienceDirect

NeuroToxicology

A follow-up study of neurobehavioral functions in welders exposed to manganese

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ARTICLE INFO

Article history:
Received 22 October 2014
Accepted 30 December 2014
Available online 8 January 2015

Keywords:
Welders
Follow-up
Manganese

ABSTRACT

Welders may be exposed to high amounts of manganese (Mn). In this study 63 welders and 65 referents were followed up with neurobehavioral tests approximately 6 years after the initial examination at baseline. The welders were exposed to the geometric mean (GM) Mn concentration of 116 $\mu g/m^3$ at baseline and 148 $\mu g/m^3$ at follow-up. Their mean duration of employments as welders was 19.5 years at follow-up. Being exposed as a welder was associated with a decline between baseline and follow-up in the performance on the Static Steadiness Test, Finger Tapping Test and Grooved Pegboard Test. However, the decline was also associated with having high concentrations of carbohydrate deficient transferrin in serum (sCDT), indicating high alcohol consumption. When subjects with sCDT above the upper reference limit of the laboratory ($\geq$1.7%) were excluded from the analyses, no difference in the decline in performance was observed between welders and referents for any of the applied neurobehavioral tests. Three welders had developed bradykinesia at follow-up, as assessed by a substantial decline in their Finger Tapping Test performance. They had also experienced a severe decline in Foot Tapping, Grooved Pegboard and Postural Sway Test scores (while blindfolded), while postural tremor as assessed with the CATSYS Tremor 7.0 was normal. Their neurobehavioral test performance at baseline 6 years previously had been normal.

1. Introduction

The severe central nervous system (CNS) disorder manganism, which is mainly characterized by movement disturbances, is caused by high, long-term occupational exposure to manganese (Mn) (Couper, 1837; McMillan, 1999). There is concern that the occupational exposure to Mn containing welding aerosols also may cause the disease, which traditionally has been diagnosed in workers employed in industries such as Mn alloy production, mining and crushing of Mn ore, and in steel and dry cell battery production (Rodier, 1955; Tanaka and Lieben, 1969; Emara et al., 1971; Cook et al., 1974; Huang et al., 1989).

Welders are by number the largest group of workers exposed to Mn. The predominant sizes of particles generated during welding are below 1 μm in aerodynamic diameter, thus the particles can easily penetrate into the alveolar region of the lung (Antonini et al., 2009a; Berlinger et al., 2011). The particles are typically agglomerated into chainlike structures formed by small primary particles with a complex chemical composition as well as compounds such as $KMnF_3$, $MnFe_2O_4$ or K_2MnO_4 (Voitkevich, 1995). Only a fraction of Mn in welding aerosol particles appears to be soluble in an artificial lung lining fluid and thus available for pulmonary uptake (Ellingsen et al., 2013).

Magnetic resonance imaging (MRI) has shown increased amounts of Mn in globus pallidus, midbrain, nucleus caudatus and putamen of welders (Kim et al., 1999; Criswell et al., 2012). PET-scan imaging suggested decreased striatal D2 receptor of non-human primates and dopamine D2 receptor density in the midbrain of Sprague-Dawley rats after Mn exposure (Guilarte, 2010; Sriram et al., 2010). Reduced striatal post-synaptic D2 receptor density was also reported to occur in chronic manganism (Aschner et al., 2009). However, the study of Sprague-Dawley rats pointed to additional neurotoxic manifestations of Mn exposure such as increased concentrations of cell markers of neuroinflammation (e.g. IL-1β and TNF-α) and astrogliosis (Antonini et al., 2009b).

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http://dx.doi.org/10.1016/j.neuro.2014.12.012
0161-813X/

Relatively few studies of Mn-exposed welders using neurobehavioral methods have been published. Cross-sectional studies have shown impaired motor functions (Siegl and Bergert, 1982; Sjögren et al., 1996; Bowler et al., 2003, 2006; Ellingsen et al., 2008; Chang et al., 2009; Laohaudomchok et al., 2011; Ellingsen et al., 2013) as well as impaired cognitive functions (Bowler et al., 2003, 2006; Wang et al., 2006; Yuan et al., 2006; Chang et al., 2009; Laohaudomchok et al., 2011). These studies may indicate that functional alterations of the CNS may occur as a result of exposure to welding aerosols containing Mn. However, several of the studies have been small and without appropriate exposure assessment. The results across studies appeared not to be consistent in a recent meta-analysis of neurobehavioral studies of occupationally Mn-exposed populations, and the authors pointed to various potential confounders not appropriately accounted for, e.g. alcohol consumption (Meyer-Baron et al., 2011). A recent review also pointed to lack of appropriate confounder control in several neurobehavioral studies (Santamaria and Sulsky, 2010).

There are few follow-up studies available of occupationally Mn-exposed populations. Such studies have methodological advantages compared to cross-sectional studies that are vulnerable to the effect of healthy workers staying in work while subjects with work-related disease may have left work. A prospective study of battery workers exposed to MnO_2 did not reveal a substantial decline in neurobehavioral performance during a 10 years follow-up period, while performance improved when exposure ceased (Roels, 1999). In a small 3.5 years follow-up of 26 subjects of whom 13 were welders and 13 were ex-welders, no significant differences in any of the neuropsychological or motor functions variables were shown between the two groups (Bowler et al., 2011). Only cognitive functions improved, while olfactory, extrapyramidal and mood disturbances remained constant or exacerbated.

In the present study, welders and referents who were examined with neurobehavioral methods at baseline in 2002/2003, were re-examined in 2008/2010 in order to assess a possible decline in performance over a 6 years period. The results from the examinations at baseline have been published (Ellingsen et al., 2008). This work is part of a larger study of welders' exposure and health in Russia, where manganism among welders for decades has been regarded as a serious occupational health issue.

2. Material and methods

2.1. Study design and subjects

During 2002/2003, 96 welders exposed to welding aerosols and 96 referents were examined with neurobehavioral methods (baseline). Details on the selection of participants and design of that study have been reported (Ellingsen et al., 2008). Briefly, at least 1 year of employment as a welder and currently employed were required for inclusion in that study. Referents were turners/fitters employed in the same plants as the welders, one plant is producing heavy machinery and one plant is a shipyard.

The purpose of the present follow-up study was to re-examine these subjects during 2008/2010 (follow-up). Out of the originally 96 examined welders, two had died and 18 had moved out of the region and were thus not available for the study. Five welders were on sick-leave and eight refused to participate. Fifteen referents had moved out of the region and were thus not available for participation at follow-up. Sixteen referents refused to participate. Thus, 63 welders and 65 referents were re-examined at follow-up.

Exclusion criteria were identical at baseline and at follow-up. Among the health-related exclusion criteria were known current or past diseases of the CNS that is probably unrelated to Mn exposure such as brain tumours or transitory ischaemic attacks. Known drug or alcohol abuse, diabetes mellitus, severe kidney or liver diseases and larger damage of the dominant hand also lead to exclusion. Subjects that had been on sick leave for more than 14 days at the examination day, were not considered for inclusion. Subjects occupationally exposed to organic solvents (>3 years) in jobs such as painters or spray-painters or occupationally exposed to neurotoxic metals (e.g. lead (Pb) or mercury (Hg)) for more than 1 year were also excluded. Subjects ever employed at plants producing solvents were not included.

Air samples were collected by personal sampling ideally on the 2 days preceding the neurobehavioral examinations. These examinations and a structured interview were carried out at the occupational health clinics of the respective facilities. A whole blood sample (from the cubital vein) was collected in the morning of the examination day. A first voided morning urine sample from the same day was also collected. The same sampling procedures were followed for the examinations at baseline (Ellingsen et al., 2008). Background characteristics for the studied subjects at follow-up are shown in Table 1.

Participation in the study was voluntary, and an informed written consent was obtained from each participant. The study was approved by the Norwegian Regional Ethical Committee for Medical Research (REK2), by the Ethics Committee of the Northwest Public Health Research Centre (NWPHRC) (St. Petersburg, Russia) and the Office of Research Protection, US Army Medical Research and Material Command (Fort Detrick, MD, USA).

2.2. Neurobehavioral examinations

The subjects were examined for around 1½ to 2 h. The same test sequence was used for all participants. Before being tested, the subjects were interviewed focusing on background variables,

Table 1
Background variables of welders and referents recorded at follow-up.

	Welders ($N=63$) AM[a] (range)	Referents ($N=65$) AM (range)	p-value
Age (years)	42.7 (26–70)	45.8 (22–70)	0.13
Weight (kg)	82.9 (53.7–120.1)	82.8 (55.7–117.8)	0.99
Education (years)	11.7 (7–17)	12.2 (8–19)	0.12
Alcohol consumption (g/year)	5260 (0–23920)	4610 (0–35360)	0.56
sCDT (%)[b]	0.8 (<DL-9.1)	0.7 (<DL-6.6)	0.23
Coffee consumption (cups/day)	1.0 (0–5)	1.2 (0–6)	0.51
Current smokers (in %)[c]	49.2	55.4	0.48
Shift workers (in %)[c]	31.7	29.2	0.76
Head injury (in %)[c]	11.1	10.8	0.95
Months of follow-up	70.8 (59–90)	70.7 (61–80)	0.92

[a] Arithmetic mean.
[b] Geometric mean.
[c] Prevalence.

medical and occupational history. Subjective neuropsychiatric symptoms were recorded by the self-administered questionnaire Q16, containing 16 questions (Lundberg et al., 1997). A comprehensive description of the neurobehavioral test battery has been given previously (Ellingsen et al., 2014).

2.2.1. *Tests for visuomotor processing speed and attention span*

The paper and pencil test digit symbol (WAIS) requires attention, psychomotor speed, perceptual organization and visual short-term memory (Lezak et al., 2012). The task is to recode symbols into digits. The number of correctly recoded symbols during 90 s was recorded. Digit span (WAIS) is a test of short-term memory and attentional capacity (Lezak et al., 2012). The maximum number of digits repeated after an oral presentation, either in the same (forwards) or in the reverse order (backwards) as presented, was recorded.

2.2.2. *Motor tests*

The Grooved Pegboard Test of motor speed and fine manipulative dexterity consists of a small board containing a 5 × 5 set of slotted holes angled in different directions. The task is to insert 25 pegs with a ridge into the holes as quickly as possible (Lafayette Instrument Company, Lafayette, IN, USA). Completion time (in second) was recorded. The Finger Tapping Test (Lafayette Instrument Company) requires the subject to press a tapping key with the index finger as fast as possible for 10 s (Reitan and Wolfson, 1985). The measure was the median number of presses based on three trials. The Foot Tapping Test requires the subject to press a lever with the foot as fast as possible for 10 s while standing (Matthews and Kløve, 1964). The measure was the mean number of presses from two trials. Grip strength was assessed with a hand dynamometer (Lafayette Psychological Instruments Model 78010). The subject is instructed to hold the arm down and away from the body and to squeeze the hand dynamometer as firm as possible (Reitan and Wolfson, 1985). The amount of pressure in kg was recorded. The subject is required to hold a stylus into nine successively smaller holes, each for 15 s, in the Kløve-Matthews Static Steadiness Test assessing hand steadiness (Bast-Pettersen and Ellingsen, 2005). Measures were the number and the duration (in second) of contacts between the stylus and the base plate.

2.2.3. *Computerized motor tests*

The computerized test system CATSYS 2000 was used for assessment of tremor, postural stability and coordination ability (Danish Product Development, 2000). The CATSYS Tremor Pen® (version 7.0) was used to assess postural tremor. The subject was required, while sitting in a chair, to hold a pen containing a biaxial micro-accelerometer like an ordinary pen in front without any support. The testing time was 16.4 s; 2 s to stabilize and 14.4 s for recording. Four measures were recorded; tremor intensity (TI), centre frequency (F50), dispersion of power (SF50) and harmonic index (HI). Further details have been published (Bast-Pettersen and Ellingsen, 2005; Ellingsen et al., 2008). The Sway Test consists of a platform with sensors recording the position of the centre force of a subject's position. The subjects were instructed to keep the balance while standing erect during two test sessions of 60 s each, one with eyes open and one when blindfolded. Recorded were mean, transversal and sagittal sway, sway area, sway intensity and sway velocity (Ellingsen et al., 2008).

Coordination ability was assessed with a hand pronation–supination test (maximum frequency test). The task is to lightly hit the surface of a touch-sensitive recording drum in an alternating hand pronation–supination as close as possible to the speed of a metronome beat increasing in frequency from 1.6 to 7.5 Hz for 12 s. The measure was the maximum frequency at which the sound could be followed (in Hz).

2.3. *Collection of biological samples*

Blood samples were collected from the cubital vein between 8.30 and 9.30 AM on the day of the clinical examinations at follow-up. First voided morning urine samples from the same morning were collected in 10 mL Sarstedt® polypropylene (PP) tubes (Sarstedt AG, Nümbrecht, Germany). One welder and seven referents refused to give a urine sample.

Whole blood for the determination of trace elements was collected in 4 mL Lithium-Heparine Vacuette® vacutainers (Greiner Labortechnik GmbH, Austria), and in 9 mL Vacuette® vacutainers without additives for the harvest of serum. The tubes were centrifuged for 10 min at 1500 × g after a wait of 30 min. Serum was stored in 1.5 mL Sarstedt® cryotubes. Eight referents declined blood sampling. The samples were kept frozen at −20 °C until analysis. The same sampling procedures were used at baseline (Ellingsen et al., 2006).

2.4. *Measurements of biological samples*

Whole blood samples were analyzed for Mn, Pb and Hg (B-Mn, B-Pb, B-Hg) and urine samples for Mn and Hg (U-Mn, U-Hg) with an inductively coupled plasma high-resolution magnetic sector-field mass spectrometer (ICP-SF-MS) at the National Institute of Occupational Health (Oslo, Norway). Details for the measurements of B-Mn, U-Mn and creatinine in urine have been described (Ellingsen et al., 2013). B-Pb, B-Hg and U-Hg were measured with the same procedure with ^{205}Tl as the internal spectrometric standard. The results obtained for all analytes were within the producer's recommended reference range for the control material used for quality assurance (Seronorm™ Quality Assurance Whole Blood Lot OK0337 and Urine Lot 0511545) (Sero Ltd., Asker, Norway). The methods' detection limits (DL) for B-Pb, B-Hg and U-Hg were 0.078, 0.055, 0.12 μg/L, respectively. The same analytical procedures were applied at baseline (Ellingsen et al., 2006).

The biomarker of alcohol consumption, carbohydrate deficient transferrin in serum (sCDT), was measured by capillary electrophoresis using Capillarys™ (Sebia Inc., Norcross, GA, USA) at Fürst Medical Laboratory (Oslo, Norway). Values below the method's DL of 0.4% were given the value ½ DL. Concentrations of sCDT $\geq$ 1.7% were considered above the upper limit of the laboratory. Serum was missing for 10 referents and one welder. A sCDT-value based on the association between self-reported alcohol consumption and sCDT among all participants in the study was assigned to these subjects. These assigned values were all well below 1.7%. SCDT was not measured at baseline.

2.4.1. *Welding aerosol exposure assessment*

Welding particulate matter in air was collected by full-shift personal sampling, ideally on the 2 days preceding the collection of biological samples and neurobehavioral examinations. Millipore (25 mm) closed-face aerosol plastic cassettes equipped with 5.0 μm pore-size polyvinyl chloride membrane filters (SKC Ltd., Dorset, UK) mounted in the breathing zone underneath the welding helmet were operated at an initial flow rate of 2.0 L/min with SKC Sidekick pumps (SKC Ltd., Dorset, UK). The welders used welding helmets, and two welders reported the use of respirators during work. Further details have been published (Ellingsen et al., 2013). The air sampling at baseline followed the same procedure (Ellingsen et al., 2006).

2.4.2. *Welding aerosol measurements*

The solubility of the elemental components in the particulate matter was assessed by leaching the air filters in the lung lining fluid simulant Hatch solution (Ellingsen et al., 2013). The filters were leached with 10 mL Hatch solution in 50 mL VectaSpin 20$^{\text{TM}}$ PP centrifuge tubes with 25 mL filter cup inserts equipped with 0.45 μm pore size nylon membranes (Whatman International Ltd., Maidstone, UK). The un-dissolved fraction (Hatch$_{\text{non-sol}}$) was acid digested and analyzed using a Perkin-Elmer Optima 7300 inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer, Waltham, MA, USA). The leached solutions (Hatch$_{\text{sol}}$) were analyzed using ICP-SF-MS. The measurements of air filters collected at baseline have been presented (Ellingsen et al., 2006).

2.5. Statistics

Continuous variables were considered to have a non-normal distribution when its skewness exceeded 2.0. These variables were log-transformed for the statistical calculations. The geometric mean (GM) is presented for these variables while the arithmetic mean (AM) is presented otherwise. Paired samples T-test was used to assess differences between baseline and follow-up in the same subjects. Students T-test for independent samples was applied for the comparisons between two different groups.

The differences in neurobehavioral test scores between baseline and follow-up were calculated and used as dependent variables in a multiple linear regression analysis. Multiple linear regression analysis (backward procedure) was used in order to assess the impact of exposure status (1/0), age (in years), years of education, current smoker (1/0), sCDT (lg), coffee consumption (cups/day), head injury with concussion (1/0) and shift work (1/0) on the differences in neurobehavioral test scores between baseline and follow-up.

The mean difference (X) and its standard deviation (SD) in performance between baseline and follow-up was calculated for the referents. A standard score (SS) was calculated for three welders that had received a diagnosis of manganism based on the formula $\text{SS} = (Z - X)/\text{SD}$, where Z is the difference in performance between baseline and follow-up in three cases of potential manganism.

A two-tailed p-value <0.05 was considered to be of statistical significance. The Statistical Package for Social Sciences, version 18.0 (IBM®, SPSS® Statistics, New York, USA) was used for the statistical calculations.

3. Results

There were only slight, non-significant differences in exposure indicators at baseline between welders participating or not participating at follow-up, the AM B-Mn being on average 8.6 μg/L (range 3.7–21.7) vs. 8.5 (range 4.6–23.5) ($p = 0.84$), the GM Air-Mn 116 μg/m^3 (range 6.5–1217) vs. 133 (range 9–2322) ($p = 0.61$), the GM U-Mn 0.15 μg/g cr. (range 0.03–4.5) vs. 0.22 (0.03–5.5)($p = 0.17$) and AM "Years of welding" 13.3 (range 1–39) vs. 13.8 (range 1–40) ($p = 0.81$). The non-participating welders had statistically significantly poorer test scores on all Static Steadiness Test measures at baseline than welders participating at follow-up. For the dominant hand the AM Timer was 9.4 s (range 0.8–37.7) vs. 5.4 (range 0.4–15.6) ($p = 0.01$) while the AM Number was 185 (17–656) vs. 120 (range 5–482) ($p = 0.03$). For the non-dominant hand the AM Timer was 11.1 s (range 1.2–41.8) vs. 7.8 (range 0.3–34.3) ($p = 0.04$) while the AM Number was 202 (27–624) vs. 134 (range 14–532) ($p = 0.03$). There were no statistically significant differences for any of the other neurobehavioral test scores at baseline between welders participating at follow-up and those not participating. The referents that participated at follow-up were substantially older than those lost to follow-up (mean 40 vs. 28 years at baseline). When adjusting for this age difference among the referents, no significant differences in neurobehavioral test scores at baseline were observed between referents participating or not participating at follow-up (not tabulated).

The welders were slightly younger than the referents at follow-up (Table 1). Other background variables, such as self-reported alcohol consumption and concentrations of sCDT, were similar in the two groups. The durations of follow-up were on average 70.8 and 70.7 months among the welders and referents, respectively. The welders had been exposed for nearly 20 years at follow-up (Table 2). All indicators of Mn exposure were higher at follow-up than at baseline, the GM concentration of Air-Mn being 116 and 148 μg/m^3, respectively. The welders' mean B-Mn concentration was almost 5 μg/L higher at follow-up.

There were few significant differences in neurobehavioral performance between the groups at baseline. Most noticeable was the welders' significantly better performance on all Static Steadiness Test measures (Table 3) and better postural sway performance (Table 4). The Static Steadiness Test performance was comparable in the two groups at follow-up, while the better postural sway performance at baseline remained at follow-up (Table 4). Most neurobehavioral test results shown in Table 3 are statistically significantly poorer at follow-up compared to baseline in the referents. However, the decline in performance appears to be larger in the welders, in particular for the Static Steadiness and the Grooved Pegboard Tests, but possibly also the Finger Tapping and Foot Tapping tests. The decline in the cognitive test scores, digit span and digit symbol, did not differ between the groups.

Table 2
Concentrations of selected trace elements in biological samples and workroom air collected at baseline and at follow-up.

	Welders ($N = 63$)		Referents ($N = 65$)	
	Baseline	Follow-up	Baseline	Follow-up
	GM[a] (range)	GM (range)	GM (range)	GM (range)
Years of welding[b]	13.3 (1–39)[***]	19.5 (7–45)	–	–
Air-Mn (μg/m^3)	116 (6.5–1217) [***]	148 (1–2040)[6]	–	–
Air-Fe (μg/m^3)	944 (127–4132) [***]	1201 (10–8430)[6]	–	–
B-Mn[b] (μg/L)	8.6 (3.7–21.7)[1,***]	13.6 (5.9–34.9)	6.8 (2.9–14.3)[*]	8.0 (4.2–12.3)[8]
B-Pb (μg/L)	48.5 (21–126)[1,***]	33.3 (11–91)	35.2 (13–208)[**]	25.6 (10–70)[8]
B-Hg (μg/L)	2.0 (0.2–25)[1,ns]	1.8 (0.3–7.6)	1.6 (0.2–8.1)[ns]	1.4 (0.1–6.4)[8]
U-Mn (μg/g cr.)	0.15 (0.03–4.5)[**]	0.26 (0.03–12.9)[1]	0.13 (0.02–10.2)[ns]	0.10 (0.02–1.5)[7]
U-Hg (μg/g cr.[c])	0.19 (0.03–1.8)[ns]	0.18 (0.03–1.1)[1]	0.13 (0.02–1.9)[ns]	0.16 (0.02–0.9)[7]

[a] Geometric mean.
[b] Arithmetic mean.
[c] Creatinine.
[1,6,7,8] Number of observations missing.
[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $p < 0.001$.
[ns] Not significant, between baseline and follow-up among the welders or referents, respectively.

Table 3
Neurobehavioral test results in welders and referents recorded at baseline and at follow-up 6 years later.

	Welders ($N = 63$)		Referents ($N = 65$)	
	Baseline AM[†] (range)	Follow-up AM (range)	Baseline AM (range)	Follow-up AM (range)
Grooved Pegboard (s)				
Dom. hand[‡]	62.8 (37–96)	72.2 (49–170)[***]	64.0 (45–91)[2]	68.3 (49–115)[**]
Non-dom. hand	66.3 (34–116)[1]	76.1 (45–145)[1,***]	68.6 (48–99)	74.2 (50–123)[**]
Finger tapping (no.)				
Dom. hand	46.9 (27–69)	42.1 (17–57)[***]	46.5 (27–63)	44.2 (23–57)[*]
Non-dom. hand	41.7 (26–72)	39.7 (12–57)	42.7 (26–59)	40.5 (28–54)[*]
Dynamometer (kg)				
Dom. hand	54.5 (37–71)	49.1 (20–72)[***]	54.2 (17–69)	49.6 (30–64)[***]
Non-dom. hand	51.8 (35–74)	47.5 (25–70)[***]	51.8 (16–70)	46.2 (25–60)[***]
Foot tapping (no.)				
Dom. foot	44.8 (33–58)	41.8 (17–59)[1,**]	42.8 (25–62)	41.2 (25–62)[1]
Non-dom. foot	43.3 (29–58)	40.6 (17–56)[1,*]	41.4 (25–63)	39.8 (25–55)
Max. freq. (Hz)				
Dom. hand	5.1 (1.0–7.5)[1]	7.0 (4.4–7.5)[***]	5.4 (1.0–7.5)	7.0 (4.9–7.5)[***]
Non-dom. hand	5.2 (1.0–7.5)[1]	6.9 (3.8–7.5)[***]	5.2 (1.0–7.5)[1]	7.0 (4.1–7.5)[***]
Static steadiness				
Dominant				
Timer (s)[a]	5.4 (0.4–15.6)	12.5 (2.3–39.9)[***]	11.9 (0.7–32.0)	14.0 (1.1–55.4)
Number[a]	120 (5–482)	281 (57–1067)[***]	191 (13–603)	273 (39–907)[***]
Non-dominant				
Timer (s)[a]	7.8 (0.3–34.3)	16.8 (2.7–42.6)[***]	13.7 (1.0–41.8)	19.7 (1.6–68.7)[**]
Number[a]	134 (14–532)	295 (80–865)[***]	199 (28–616)	299 (70–959)[***]
Digit span				
Forwards (no.)	5.9 (4–9)	5.9 (3–8)	5.8 (4–8)	6.2 (4–9)[*]
Backwards (no.)	3.8 (1–6)	4.2 (2–7)[*]	4.1 (3–7)	4.2 (3–7)
Digit symbol (no.)	47.0 (20–74)[1]	42.2 (15–74)[***]	48.6 (25–72)	43.4 (19–69)[**]
No. of symptoms[b]	3.7 (1–13)	4.6 (0–14)[1]	3.2 (0–14)	2.6 (0–9)[1]

[†] Arithmetic mean.
[‡] Geometric mean.
[1] One subject missing.
[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $p < 0.001$ between baseline and follow-up among welders or referents, respectively.
[a] $p < 0.05$ between the welders and referents at baseline.
[b] $p < 0.05$ between the welders and referents at follow-up.

Table 4
CATSYS Tremor 7.0 and Postural Sway Test results recorded at baseline and at follow-up.

	Welders ($N = 63$)		Referents ($N = 65$)	
	Baseline AM[†] (range)	Follow-up AM (range)	Baseline AM (range)	Follow-up AM (range)
CATSYS, Tremor 7.0				
Dominant				
Intensity (m/s^2)[‡]	0.14 (0.06–0.71)[1]	0.15 (0.07–0.74)	0.14 (0.07–0.46)	0.15 (0.08–0.63)
Central freq. (Hz)	7.9 (5.0–12.6)[1]	6.9 (3.4–11.6)[***]	7.4 (5.2–10.9)	6.5 (2.7–10.9)[***]
Dispersion (Hz)	3.2 (0.2–5.4)[1]	3.3 (1.3–4.8)	2.9 (0.2–5.4)	3.0 (1.4–5.1)
Harmonic index	0.85 (0.68–0.98)[1]	0.90 (0.80–0.96)[***]	0.87 (0.70–0.98)	0.91 (0.81–0.99)[***]
Non-dominant				
Intensity (m/s^2)[‡]	0.14 (0.06–0.54)[1]	0.15 (0.08–0.73)	0.15 (0.08–0.45)	0.15 (0.07–0.43)
Central freq. (Hz)	7.8 (4.5–12.6)[1]	6.9 (1.0–11.3)[**]	7.9 (5.0–12.3)	6.9 (2.9–11.3)[***]
Dispersion[a] (Hz)	3.5 (0.2–6.1)[1]	3.5 (0.2–5.0)	3.0 (0.2–4.9)	3.4 (1.3–4.9)[*]
Harmonic index	0.84 (0.64–0.98)[1]	0.89 (0.80–0.98)[***]	0.85 (0.70–0.97)	0.89 (0.78–0.96)[***]
CATSYS, Postural Sway				
Normal condition				
Transversal x (mm)	2.6 (1.1–5.4)[1]	2.7 (0.4–5.8)	2.7 (1.1–6.9)	3.0 (1.3–7.1)
Sagittal y (mm)[‡]	3.2 (1.4–14.3)[1]	3.4 (0.7–8.4)	3.6 (1.9–9.7)	4.0 (2.0–12.2)
Mean sway (mm)[‡,b]	4.6 (2.1–15.6)[1]	4.7 (0.9–9.8)	5.0 (2.7–11.2)	5.6 (2.8–12.8)[*]
Intensity (mm)[‡,b]	3.6 (1.9–7.2)[1]	3.4 (0.5–7.8)	3.9 (1.8–10.4)	4.4 (2.2–15.5)
Sway velo. (mm/s)[a,b]	8.5 (4.8–20.6)[1]	8.1 (1.1–20.7)	9.7 (4.9–23.2)	9.9 (5.1–18.8)
Sway area (mm^2)[‡,a,b]	173 (37–584)[1]	159 (6–957)[1]	222 (67–1328)	255 (77–1832)

[†] Arithmetic mean.
[‡] Geometric mean.
[1] One subject missing.
[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $p < 0.001$ between baseline and follow-up among welders or referents, respectively.
[a] $p < 0.05$ between the welders and referents at baseline.
[b] $p < 0.05$ between the welders and referents at follow-up.

The TI did not increase in any of the groups during the 6 years of follow-up (Table 4). The shift in central frequency towards lower frequency was similar in both groups and for both hands. The Postural Sway Test performed with eyes open and while blindfolded (results not shown) was almost identical at baseline and at follow-up in both groups.

Multiple linear regression analysis was carried out for all neurobehavioral tests with the difference in scores between baseline and follow-up as dependent variable (Table 5). Being exposed as a welder was significantly or nearly significantly associated with poorer test scores between baseline and follow-up for the Static Steadiness, Finger Tapping and Grooved Pegboard Tests. However, lifestyle habits at follow-up, in particular alcohol consumption as assessed by sCDT, had a substantial impact on the difference in test scores. Age and head injury with concussion were not associated with any of the differences (not tabulated). None of the other differences in neurobehavioral test results between baseline and follow-up were associated with exposure.

All subjects were stratified according to exposure status and having sCDT $\geq$ 1.7% with respect to the difference in neurobehavioral test performance between baseline and follow-up for the neurobehavioral tests presented in Table 5. Fig. 1A–D shows that the poorer development in the Static Steadiness Test scores was almost exclusively related to welders having sCDT $\geq$ 1.7% at follow-up. A similar pattern of decline in performance was observed for the Finger Tapping and Grooved Pegboard Test scores. However, the exposure-related decline in Finger Tapping was not of statistical significance in contrast to the Grooved Pegboard Test (results not shown). When excluding all subjects with sCDT $\geq$ 1.7% at follow-up, there were no statistically significant differences between the welders and the referents in the decline in any of the test results between baseline and follow-up.

Three welders experienced a substantial decline in the Finger Tapping Test performance from on average 40.3 to 18.0 for the dominant hand and from 37.3 to 15.0 for the non-dominant hand, indicating a development of bradykinesia during the study period. Their average time to completion (in second) on the Grooved Pegboard Test increased from 70.3 to 106.0 for the dominant hand and from 71.7 to 113.7 for the non-dominant hand. There was hardly any change in their performance on the cognitive tests, digit symbol and digit span. They were on average 59.3 years (range 47–70) old and had been welding for 27.3 years (range 20–32) at follow-up. Fig. 2 shows their decline in motor test scores between baseline and follow-up expressed as Standard Score (in SD) related to the age-related decline in performance calculated for the referents. The three welders' performance represented a severe bilateral decline with more than 2 SD on average reduced performance in Finger Tapping, Foot Tapping and Grooved Pegboard Tests. A moderate decline was observed for the Postural Sway Test while blindfolded, the Static Steadiness Test and Dynamometer. Postural Sway performed with eyes open and TI were hardly affected. A mean TI of 0.15 m/s^2 was measured on both hands at follow-up.

4. Discussion

This study shows that around 6 years of additional exposure to Mn in welders who had already been exposed for 13.3 years on average, had limited impact on the average decline in neurobehavioral performance compared to the decline of the referents, although the measured Air-Mn concentrations were higher than 100 $\mu g/m^3$ both at baseline and follow-up. However, the difference in the Static Steadiness Test results between baseline and follow-up was significantly higher in the welders, but the deterioration was limited to welders having sCDT $\geq$ 1.7%. Also the slightly larger declines in the welders as compared to the referents in Finger Tapping and Grooved Pegboard Test scores were mainly observed in welders having sCDT $\geq$ 1.7%. The decline in neurobehavioral performance did not differ significantly between welders and referents when only subjects with lower alcohol consumption (sCDT $<$ 1.7%) were compared. Three welders experienced a substantial decline in neurobehavioral performance between baseline and follow-up. These welders were given a thorough clinical diagnostics according to the Russian system for diagnosing

Table 5
Results from the multiple linear regression analysis of the difference in test scores between baseline and follow-up.

	β							
	α	Expo[1]	Edu[2]	Smoke[3]	sCDT[4]	Coffee[5]	Shift[6]	Mult. r
Grooved Pegboard (s)								
Dom. hand	4.4^{*}	-5.2^{*}	–	–	-12.0^{**}	–	-7.0^{**}	0.35^{***}
Non-dom hand	9.1^{****}	–	–	–	-12.9^{***}	1.8^{*}	-7.5^{**}	0.36^{***}
Finger tapping (no.)								
Dom. hand	-12.1^{**}	3.2^{**}	1.1^{***}	4.6^{***}	–	-1.1^{**}	–	0.40^{****}
Non-dom. hand	2.9^{****}	–	–	–	5.8^{**}	–	–	0.21^{**}
Static steadiness								
Dominant hand								
Timer (s)	-2.5^{*}	-4.0^{**}	–	–	-8.4^{***}	1.3^{**}	-8.7^{****}	0.57^{****}
Number	-42^{ns}	-69^{**}	–	-55^{*}	-160^{***}	–	-123^{****}	0.51^{****}
Non-dominant hand								
Timer (s)	-6.2^{***}	–	–	-3.5^{*}	-7.9^{**}	2.0^{***}	-8.7^{****}	0.50^{****}
Number	-61^{**}	-55^{**}	–	-104^{****}	-119^{***}	17^{*}	-71^{**}	0.52^{****}
Symptoms (no.)	0.7^{ns}	-1.6^{**}	–	–	–	–	–	0.22^{**}

[1] Exposure status.
[2] Years of education.
[3] Current smoker.
[4] sCDT (lg).
[5] Coffee consumption.
[6] Shift work.
^{ns} $p \geq 0.10$.
* $p < 0.10$.
** $p < 0.05$.
*** $p < 0.01$.
**** $p < 0.001$.

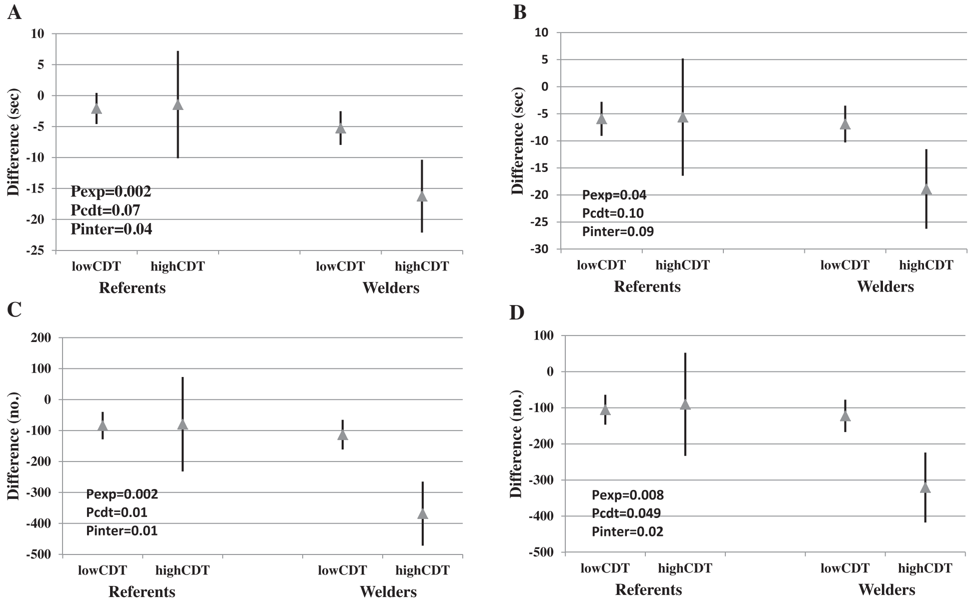

Fig. 1. Arithmetic mean (and 95% CI) difference in Static Steadiness Test scores between baseline and follow-up according in 63 welders and 65 referents to exposure status (Exp) and the concentration of sCDT (high: sCDT $\geq$ 1.7%; low: sCDT $<$ 1.7%) adjusted for age at follow-up. (A) Timer, dominant; (B) timer, non-dominant hand; (C) number, dominant hand; (D) number, non-dominant hand.

occupational diseases after closure of the study, and all three were diagnosed with chronic welding-related manganism.

The neurobehavioral test with the most pronounced difference in test scores between baseline and follow-up in the welders was the Static Steadiness Test. However, the deterioration in performance was almost exclusively related to welders with sCDT above the upper reference limit of the laboratory ($\geq$1.7%), indicating a daily alcohol consumption of at least 60–80 g of ethanol (Bortolotti et al., 2006). Since no significant deterioration was observed in the referents with sCDT $\geq$ 1.7%, these results could indicate an interaction between exposure to Mn and high alcohol consumption with respect to developing poorer neurobehavioral performance. We have previously reported from a cross-sectional study that exposure to Mn and high alcohol consumption may interact (Ellingsen et al., 2014). Decreased dopamine D2 receptor density in the midbrain of Sprague-Dawley rats and a significant decrease in striatal D2 receptor in non-human primates have been reported after exposure to Mn (Guilarte, 2010; Sriram et al., 2010). Reduced striatal post-synaptic D2 receptor density was also reported to occur in chronic manganism (Aschner et al., 2009). Further, PET and SPECT studies have shown reduction of dopamine transporter in striatum and reduced striatal D2 receptor density in alcohol dependency (Bühler and Mann, 2011; Urban and Martinez, 2012). Thus, it appears that both Mn exposure and alcohol dependency are associated with impaired dopamine metabolism in striatum. It is tempting to speculate that the statistical interaction observed between exposure as welder and sCDT $\geq$ 1.7% with respect to the Static Steadiness Test results may be due to impairment in the striatal dopamine metabolism related to Mn exposure and high alcohol consumption.

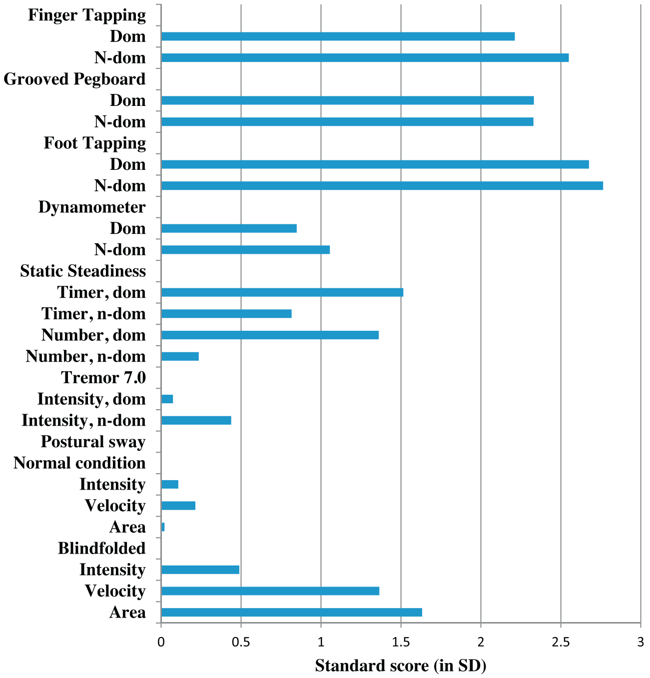

Fig. 2. Motor function decline between baseline and follow-up in three welders that have developed severe bradykinesia. The decline is expressed as the number of standard deviations above the background decline in the referents. Dom: dominant hand; N-dom: non-dominant hand.

We could in a previous cross-sectional study not show an interaction between Mn exposure and alcohol consumption for the Static Steadiness Test (Ellingsen et al., 2014). It is in this context of interest, however, that the only neurobehavioral test which the welders lost to follow-up scored significantly poorer than the participating welders at baseline, was the Static Steadiness Test. It is likely that the neurological functions assessed by this test of hand steadiness are important for the working operations performed by welders. Thus, the data suggest that welders performing poorer on this test tend to leave their job. If this is true, the consequence would be a tendency to underestimate effects related to hand steadiness in cross-sectional studies of welders. This interpretation is not necessarily valid for other Mn exposed populations than welders, where hand steadiness is less important for work performance.

The decline in neurobehavioral performance did not differ between the welders and the referents during the 6 years follow-up when all subjects with sCDT $\geq$ 1.7%, indicating high alcohol consumption, were excluded from the analysis. It might be suggested that subjects exposed to Mn, but not to high amounts of alcohol, do not experience a continuous decline in neurobehavioral performance during exposure. In a 3.5 years follow-up of 26 welders, of whom 13 were not working as welders at follow-up, several domains of cognitive functions improved substantially (Bowler et al., 2011). Also motor dexterity and graphomotor tremor improved significantly in that study, while psychomotor speed remained unchanged and dominant postural hand tremor and body sway worsened. Thus, the results from that study are quite different from our study. Roels (1999) studied Mn exposed battery workers during an 8 years follow-up. That study showed no decline in neurobehavioral performance during follow-up, which is in accordance with our observations that the welders did not have a continuous decline in neurobehavioral performance during exposure.

It is in this context of interest that three welders, who had been exposed for on average 27.3 years at follow-up, had a severe decline in neurobehavioral performance, most noticeable the Finger Tapping Test, but also the Grooved Pegboard and Foot Tapping Tests. In contrast, their performance on the CATSYS Tremor test was completely normal on both hands, and there was no decline in the cognitive test performance. Also their Postural Sway Test performance with eyes open was unaffected, while the performance was clearly impaired when blindfolded. Thus, these welders had developed a severe bilateral bradykinetic syndrome with impaired balance control and without postural tremor. No such observations were done in any of the referents. We have previously described a group of welders diagnosed with manganism in Russia. The neurobehavioral profile of the three welders in the present study was very similar to what was observed previously (Ellingsen et al., 2008), and thorough clinical examinations after completion of the present study detected manganism in all three cases. It should be emphasized that no tremor was observed, making the diagnosis of idiopathic Parkinson's disease less likely.

One should be cautious drawing too firm conclusions based on only three cases. On the other hand, manganism is a rather rare disease and the possible development of three cases is a serious outcome. If it is a reality that these welders have developed manganism, this can give information on the time development of the disease. At baseline, when they had been exposed for nearly 21 years on average, their neurobehavioral performances were normal. Around 6 years later they had developed serious neurological impairment as assessed by the applied neurobehavioral tests, which was later confirmed by more extensive clinical assessment. This may suggest that the development of manganism is not associated with a continuous decline in neurological function, but is a disease which manifests itself with a more sudden onset. It is in this context of interest that the development of the most common basal ganglia disease, idiopathic Parkinson's disease (PD), is associated with a substantial cell loss in substantia nigra pars compacta and striatal dopamine depletion before the appearance of motor symptoms (Mahlknecht and Poewe, 2013). It is known from autopsy studies that manganism is associated with nerve cell loss in pallidum and putamen (Guilarte, 2010). It is tempting to speculate that manganism is associated with cellular loss which does not cause clinical symptoms until the cell loss reaches a critical level, like in PD.

In summary, the different decline of the neurobehavioral test results in the groups was related to high alcohol consumption, suggestive of an interaction between high current alcohol consumption as assessed by sCDT and being occupationally exposed to Mn. The decline in performance during 6 years of follow-up did not differ between welders and referents with sCDT < 1.7%. Three welders had severely impaired neurobehavioral functions at follow-up, suggestive of a sudden onset of disease.

Conflict of interest

The authors declare no conflict of interests.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

The study was supported by Grant Number W81XWH-05-1-0239 from the United States Department of Defense, United States Army Medical Research and Material Command. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the United States Department of Defense, United States Army Medical Research and Material Command.

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