Nonoccupational Environmental Exposure to Manganese is Linked to Deficits in Peripheral and Central Olfactory Function

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Abstract

Manganese is of growing concern as a toxic air pollutant. It is readily transported from the olfactory epithelium to the olfactory bulb, and unlike other metals, it is transported transsynaptically to structures deep within the brain. However, little is known regarding the possible effect of nonoccupational exposure to manganese on olfactory function. Using the Sniffin’ Sticks test battery, we compared the olfactory performance of subjects from a manganese mining district living <1 km from a manganese processing plant, with nonexposed subjects living 50 km from the closest source of exposure (N = 30/group). Groups were matched for age, sex, and schooling, and none had ever worked in mining-related activities. Concentrations of manganese in hair were measured as a biomarker of exposure; exposed subjects had significantly higher concentrations than nonexposed subjects. They were also significantly outperformed by the nonexposed subjects on all olfactory measures (threshold, discrimination, and identification), indicating adverse effects of manganese exposure on a range of olfactory functions, including those involving higher order cognitive processes. This contrasts with previous findings showing adverse peripheral but not central effects on olfactory function of big city air pollution, which mostly consists of toxicants known to affect the olfactory epithelium but with lower transynaptic transport capacity compared with manganese. We conclude that nonoccupational exposure to airborne manganese is associated with decrements in both peripheral and central olfactory function.

Key words: age, cognitive function, manganese exposure, olfactory performance

Introduction

There is growing concern about the effect of environmental contaminants on human health, not only in the workplace but increasingly in the general public exposed to varying and often largely unknown levels of environmental pollution (Molina and Molina 2004; Chen and Kan 2008; Parrish and Zhu 2009). Metals such as lead, nickel, cadmium, mercury, and manganese are particularly worrying because of their neurotoxic effects resulting in part from their ability to bypass the blood–brain barrier and reach the central nervous system, including via inhalation and uptake by the olfactory receptor neurons originating in the nasal cavity (Tjälve and Henriksson 1999; Dorman et al. 2006; Thompson et al. 2011).

Manganese (Mn), although an essential trace element, is of particular concern as it is not only readily transported from the olfactory epithelium to the olfactory bulb, the first station in the central processing of olfactory information in vertebrates, but unlike a number of other metals such as cadmium and mercury, it is also transported transsynaptically to structures deep within the brain (Tjälve and Henriksson 1999; Aschner 2000; Dorman et al. 2006; Leavens et al. 2007). Consequently, exposure to excessive amounts of Mn
has been implicated in a number of psychiatric and motor disturbances (Hudnell 1999; Pal et al. 1999; Aschner et al. 2007; Bowler et al. 2007, 2012; Zoni et al. 2007), as well as in diminished olfactory function (Antunes et al. 2007; Bowler et al. 2007, 2011).

Although most human evidence for such pathology has come from studies of occupational groups exposed to high levels of Mn such as welders, miners, or workers in processing facilities (Mergler et al. 1994; Lucchini et al. 1995; Chu et al. 1996; Crump and Rousseau 1999; Antunes et al. 2007; Bowler et al. 2007, 2011; Zoni et al. 2007), there is growing evidence that lower level chronic exposure to airborne Mn in the general environment can also have lasting toxic effects on human health (Hudnell 1999; Mergler et al. 1999; Rodriguez-Agudelo et al. 2006; Zoni et al. 2007; Solís-Vivanco et al. 2009; Ríojas-Rodríguez et al. 2006; Solís-Vivanco et al. 2009; Riojas-Rodríguez et al. 2010). In particular, interest in the effect of low-level inhalation exposure to Mn has been stimulated by the use of methycyclopentadienyl manganese tricarbonyl as a gasoline octane booster in some countries (Davis 1998; Hudnell 1999; Pellizzari et al. 2001; Pfeifer et al. 2004).

Whereas previous studies have concentrated importantly on impaired motor and cognitive function or decreased quality of life (Catalán-Vázquez et al. 2010, 2012), no information is available regarding the possible effect of nonoccupational environmental exposure to Mn on olfactory function. And yet, apart from any negative effect on olfactory function itself, this could provide a sensitive, noninvasive indicator of the impact of Mn exposure on central nervous system processes more generally. To investigate this, we chose a region of rural Mexico where Mn has been extensively mined for the past 50 years (see Study site), where elevated levels of Mn in the soil, waterways, vegetation, and atmosphere have been recorded (Solís-Vivanco et al. 2009; Juárez-Santillán et al. 2010), and where significant deficits in motor and cognitive performance have been reported (Rodríguez-Agudelo et al. 2006; Solís-Vivanco et al. 2009; Ríojas-Rodríguez et al. 2010).

Previously, we found that otherwise healthy Mexico City residents, exposed to high levels of ambient air pollution, show a significant reduction in the ability to detect odorants presented at threshold concentrations, but little or no reduction in the ability to describe and to correctly name them when presented at above-threshold concentrations (Hudnell et al. 2006; Guarneros et al. 2009, 2011). This suggests that the negative effects of general big city air pollution on olfactory function are due primarily to damage at the periphery of the system, leaving centrally mediated cognitive processes largely intact (discussion in Hudson et al. 2006; Guarneros et al. 2009, 2011). Given the ability of Mn to reach and accumulate in central brain structures importantly involved in cognitive processes, and the vital role of learning and memory in olfactory function (Hudnell 1999; Wilson and Stevenson 2003; Herz 2009), we expected the Mn-exposed subjects of the present study to show impaired central and impaired peripheral olfactory function.

### Materials and methods

#### Study site

The study was conducted in the Molango district in the High Sierra of the central Mexican state of Hidalgo, where Mn has been extensively mined for the past 50 years (Rodríguez-Agudelo et al. 2006; Solís-Vivanco et al. 2009; Catalán-Vázquez et al. 2012). The mines and associated deposits of Mn are the second largest in Latin America and the fifth largest in the world (Rodríguez-Agudelo et al. 2006; Catalán-Vázquez et al. 2012). We chose as our focal site the adjacent villages of Tolago/Chiconcoac (T/C), population 1269 in 2010 (Instituto Nacional de Estadística y Geografía, Mexico), located approximately 20°80′N, 98°42′W; 1900 masl, and <1 km from the Mn processing plant. Our control site was the town of Calnali, population of about 15 000, located approximately 20°50′N, 98°36′W; 1800 masl, and 50 km from T/C and from any mining activity. The two sites have a similar temperate, humid upland climate, and the populations have a similar socioeconomic level and culture.

#### Subjects

We recruited 30 adults from each population with the help of staff of the local public health clinics; 25 women and 5 men matched as closely as possible for age and level of schooling (Table 1). According to the records of the clinics, all were in good general health. More women were recruited than men because most men in the T/C community either worked in the mines or had left to work abroad. All subjects were nonsmokers, all had spent all their lives in each of the respective

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<th>Table 1 General characteristics of the study groups</th>
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All subjects were nonsmokers.
communities, and none had ever been employed in mining-related activities (occupations given in Table 1).

Procedures conformed to the declaration of Helsinki for medical research involving human subjects and to the guidelines for the treatment of human subjects in research of the Instituto de Fisiología Celular and the Instituto de Investigaciones Biomédicas, UNAM, Mexico. The study was approved by the Subcomité del Campo de Conocimiento en Biología Experimental y Biomedicina, Posgrado en Ciencias Biológicas, UNAM, and we also obtained approval from the local health authorities (Desarrollo Integral de la Familia, Secretaria de Salud, Calnali, and Clinica de Salud, Secretaria de Salud, Chiconcoac). Testing was conducted in the presence of clinic staff and did not involve physical intervention or have treatment consequences. Subjects were fully informed about the purpose and procedures of the study, and anonymity was assured. All subjects gave their informed consent before being tested.

**Test procedures**

Subjects were tested at the local clinic of the public health service in their respective communities by the same experimenter (N.O.R.), who was familiar with the local culture and who had extensive experience with the Sniffin’ Sticks method (see below). After obtaining subjects’ informed consent, their age, sex, smoking history, work environment, level of schooling, and medical history were recorded (Table 1). Their olfactory performance was then evaluated using a standardized Sniffin’ Sticks test (see below), which we modified slightly to fit local conditions. Three odorants (licorice, green apple, and turpentine) were dropped from the identification test, because we have repeatedly found these to be unfamiliar to Mexicans and therefore largely unidentifiable. In addition, because of low levels of literacy, the experimenter read the list of alternatives by for each of theodorants to be identified (see below), and if requested by subjects, with repetitions. Each subject was tested in a single session lasting a maximum of 30 min. Tests were conducted in April and May 2011 so as to exclude possible seasonal effects between the two populations. In addition, samples of hair were taken from most subjects to confirm greater exposure to Mn in the focal group (see below).

**Olfactory performance**

As in a previous study of the effect of Mexico City air pollution on olfactory function (Guarneros et al. 2009), odorants were presented to blindfolded subjects following an established procedure (Hummel et al. 1997; Kobal et al. 2000). Odorants were presented in felt-tipped marker pens (Sniffin’ Sticks) filled with 4 mL of liquid odorant or odorant dissolved in propylene glycol. At the moment of testing, the cap was removed by the experimenter who held the tip of the pen approximately 2 cm in front of the subject’s nostrils for approximately 2–3 s. Subjects were instructed when to sniff, and except for the identification test, they could sample each stimulus only once.

The standard Sniffin’ Sticks procedure tests subjects’ ability to detect an odorant (threshold), to distinguish between odorants, and to identify (name) them using a verbal checklist. One advantage of combining these different measures of olfactory function is to help identify where in the chemoceptive pathway functional impairment occurs (Hudson et al. 2006; Guarneros et al. 2009, 2011). Another is that using more than one measure of olfactory function increases the likelihood of detecting olfactory loss (Dalton et al. 2006; Lötsch et al. 2008).

**Threshold**

Subjects’ ability to detect 2-phenyl ethanol (a rose-like odor) was determined using an ascending-staircase, 3-alternative forced-choice procedure. Sixteen dilutions were presented in a geometric series starting with a 4% solution (dilution ratio 1:2 in propylene glycol). In each trial, 3 pens were presented singly in randomized order, 2 containing only solvent and the third the odorant, and subjects were asked to identify the pen that smelled different (i.e., that contained 2-phenyl ethanol). The interval between presentation of pens within a triplet was approximately 3 s and between triplets approximately 20 s. The staircase was reversed when the target was correctly identified on 2 successive trials. Threshold was defined as the mean of the last 4 of 7 staircase reversals. Subjects’ scores could range from 0 (minimum sensitivity/anosmic for 2-phenyl ethanol) to 16 (maximum sensitivity) which, however, no subject achieved.

**Discrimination**

Again using a 3-alternative forced-choice paradigm, 16 triplets of pens containing concentrations of odorant well above threshold for normosmics were presented in randomized order, with 2 containing the same and 1 a different odorant. Subjects had to determine which one of the 3 pens smelled different. As for threshold determinations, the interval between presentation of pens within a triplet was approximately 3 s and between triplets approximately 20 s. Because 16 triplets were tested, subjects’ scores could range from 0 to 16.

**Identification**

Subjects were presented with 13 common odorants (see Test procedures above for elimination of 3 of the original 16 odorants) in the same order for all subjects and asked to choose the most appropriate (Spanish language) descriptor from 4 plausible alternatives (e.g., shoe leather as target, and glue, grass, and smoke). As mentioned above, because of low levels of literacy, subjects were read the list of alternatives by
the experimenter. The stimuli were well above threshold for normosmics, the interval between the presentation of each pen was approximately 10 s, and subjects’ scores could range from 0 to 13.

**Overall performance**

For each subject, results of the 3 subtests were summed to give a composite Threshold–Discrimination–Identification (TDI) score (maximum of $16 + 16 + 13 = 45$; cf. Hummel et al. 1997; Wolfensberger et al. 2000).

**Manganese hair content**

Mn accumulates in hair, and concentrations in hair have been suggested to provide a good general marker of Mn intoxication (Riojas-Rodríguez et al. 2010). We, therefore, took about 0.5 g of hair from the occipital region of subjects, close to the scalp: from 22 women and 3 men in the exposed group, and from 20 women and 3 men in the control group. The hair was stored under refrigeration in 5-mL metal-free polypropylene tubes until analysis. Samples from both groups were analyzed in the same session. Following a previously established procedure (Riojas-Rodríguez et al. 2010), samples were washed 3 times by vigorous agitation in a detergent solution of 2% Triton X-100 and rinsed with deionized water. The hair was then dried at 60 °C, cut into small pieces to facilitate acid digestion, and 300 mg placed for 30 min at 60 °C in metal-free polypropylene tubes with 250 μL of concentrated nitric acid (Suprapur; Merck, Naucalpan de Juárez). The resulting clear solution was analyzed using an atomic absorption spectrophotometer (Perkin-Elmer, AAAnylyst 600; Menezes-Filho et al. 2009; Riojas-Rodriguez et al. 2010). Quality control of the analysis was assured by measuring a biological matrix-based reference material (bovine liver 1577b; National Institute of Standards and Technology) together with the samples.

**Data treatment and analysis**

Because performance scores were not always normally distributed (Kolmogorov–Smirnov tests) and most were based on frequencies, data for the 2 groups are graphed as medians and interquartile ranges or as Spearman rank correlations, and compared using nonparametric Mann–Whitney $U$ tests. Two-tailed tests were performed throughout using the statistical program SYSTAT 12 and taking $P < 0.05$ as the level of significance. As the groups were matched for gender, scores for men and women have been combined.

**Results**

All subjects readily agreed to participate in the study, and there were no significant differences between the Mn-exposed and control groups in age, socioeconomic indicators such as employment or years of schooling, or in apparent general health (Table 1).

**Olfactory performance**

**Threshold**

The control subjects detected 2-phenyl ethanol in the ascending-staircase, 3-alternative forced-choice procedure at significantly lower concentrations (higher scores) than the Mn-exposed T/C subjects (Figure 1a; Mann–Whitney $U$ test: $U = 312.5_{30,30}, P = 0.042$). Based on the median scores, this represented a 2- to 4-fold difference between groups in the concentration needed to detect the presence of this stimulus.

**Discrimination**

The control subjects were better able to distinguish the target stimuli from the 2 alternatives in the 3-alternative forced-choice tests than the T/C subjects (Figure 1b). This difference was also significant ($U = 272_{30,30}, P = 0.008$).

![Figure 1](image)
Identification

Although the odorants in this test had been originally chosen for their familiarity for Europeans, after we eliminated the unfamiliar green apple, licorice, and turpentine stimuli (see above), the control subjects identified 8 of the 13 stimuli correctly in more than 90% of trials and 10 of 13 in more than 80% of trials. In contrast, the T/C subjects identified only 4 of 13 correctly (coffee, garlic, banana, fish) in more than 90% of trials, and 8 in less than 70% of trials. Again, the difference in performance between the 2 groups was significant (Figure 2c; $U = 265.5_{30,30}$, $P = 0.005$).

Overall performance

As a consequence of the above, the control subjects had significantly higher TDI scores than the T/C subjects, expressed as the sum of scores obtained in the threshold, discrimination, and identification tests (Figure 2d; $U = 213.0_{30,30}$, $P < 0.001$). Whereas 4 T/C subjects (13%) had scores below the lowest scoring control subject, 3 control subjects (10%) had scores above the highest scoring T/C subject.

Interaction with age

On all tests of olfactory ability, performance declined with subjects’ age (Figure 2). However, for threshold, identification, and overall performance (Figure 2a,c,d), the decline was greater for the T/C group than for the control group, even if only tendentially. The lack of a clear difference with age between the 2 groups in the test of olfactory discrimination (Figure 2b) was probably at least partly due to the younger Mn-exposed subjects already having scores so much lower than the age-matched control subjects that subjects in this group could not decline much further before approaching chance performance. Nevertheless, taken together the results suggest a negative interaction between age and Mn

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**Figure 2**  Spearman rank correlations between subjects’ age in years and the 3 tests of olfactory performance (a–c), as well as subjects’ TDI scores representing the sum of their scores on these tests (d) (cf. Figure 1). Crosses and solid lines give the values for the Mn-exposed subjects and open circles and broken lines for the control subjects; $N = 30$ per group; correlation coefficients: *$P < 0.05$, ns = not significant.
exposure, resulting in a greater decrement in olfactory performance with age in the T/C group (Figure 2d).

Manganese hair content

The T/C subjects had significantly higher concentrations of Mn in hair (MnH) than the control subjects (Figure 3; $U = 0.00, P < 0.001$). Based on the median scores of 9.73 μg/g versus 1.01 μg/g, this represented a 9-fold difference between the 2 groups. We also found a tendential negative correlation between MnH and the performance of subjects within each group on each of the olfactory tests of threshold, discrimination, identification, and TDI scores. Although this was clearer for the exposed than for the control subjects, none of the correlations reached significance for either group.

Discussion

The findings of the present study provide clear support for our prediction that subjects from the mining community of T/C, chronically exposed to elevated levels of Mn in the environment, would show impaired olfactory function, and despite having never worked in mining-related activities. Thus, compared with control subjects from the nearby town of Calnali, a comparable community but with no mining activity, the Mn-exposed subjects performed significantly worse on all 3 tests of olfactory function: detection, discrimination, and identification. In addition, the normal decline in olfactory function with age (Doty et al. 1984) was greater for the exposed than for the control group (see Davis 1998, p. 195, for relevant discussion of this issue). Particularly notable was the poorer performance of the exposed group on the odor identification task. This tests the ability of subjects to recognize and name odors and is thus dependent on cognitive processes associated with learning, memory, and language, important for a wide range of cognitive functions apart from odor recognition. That the T/C subjects understood the task, were able to manage the orally presented checklist, and were motivated to perform well was shown by the fact that on 4 particularly familiar, “easy” odorants they scored over 90% correct, doing almost as well as the control subjects.

For several reasons, it is likely that airborne Mn was an important contributor to the olfactory deficits of the T/C subjects. The T/C region has been reported to have atmospheric levels of Mn above the level of 0.05 μg/m$^3$ recommended by the USA Environmental Protection Agency (US EPA 1997; Solís-Vivanco et al. 2009), and air was found to be the most significant source of Mn exposure at T/C (Santos-Burgoa et al. 2001; Rodríguez-Agudelo et al. 2006). High levels of atmospheric Mn at T/C are also consistent with the significantly higher levels of Mn in the hair of T/C subjects compared with the control group. The finding of only a weak correlation between MnH and olfactory performance is perhaps not surprising given our rather small sample size and the considerable variance in MnH values, particularly among the Mn-exposed T/C subjects (Figure 3). Extraneous factors such as hair type, frequency of washing, and other forms of hair care can affect the accumulation of Mn considerably and contribute to such variance (Smolders et al. 2009). In addition, we do not know if Mn was the only metal contributing to the olfactory deficits reported here. However, we note that a study exploring possible contamination from lead reported levels in blood at T/C to be similar to other rural Mexican communities (Santos-Burgoa et al. 2001).

Also supporting an effect of atmospheric exposure to Mn, inhalation is reported to be a more efficient route than digestion for uptake of Mn by the body, and the concentration of Mn in hair is a good biomarker of chronic exposure (Andersen et al. 1999; Riojas-Rodríguez et al. 2010). Olfactory loss, particularly involving more central cognitive processes, due to airborne Mn, is also consistent with the animal studies reported in the Introduction showing ready uptake of Mn by the olfactory receptor neurons in the nasal cavity and transynaptic transport to central regions of the brain.

Given that none of the subjects had ever worked in Mn-related activities, our findings suggest that chronic exposure even to presumably low levels of Mn can affect health via inhalation, and even in younger and otherwise healthy persons. Furthermore, our findings might even be conservative given that we recruited subjects from local health clinics. Thus, they were possibly more health conscious and with a higher level of education than other members of the community, as indicated by most having mainly indoor, and several professional occupations (Table 1). Poorer residents of T/C

![Figure 3](image-url) Concentrations of Mn measured in the hair of Mn-exposed (N = 25) and control subjects (N = 23). Box plots: horizontal lines within boxes give medians, horizontal limits of boxes give the interquartile ranges, and vertical bars give the absolute ranges: ***$P < 0.001$ (Mann–Whitney U test).
working mainly outdoors in the fields, and possibly having a poorer diet and generally lower standard of living, might be even more vulnerable.

Our findings also demonstrate the suitability of olfactory testing for monitoring the effects of atmospheric pollution on health. Olfactory tests such as the Sniffin’ Sticks are non-invasive, quick, and easy to apply, and do not depend on subjects’ level of literacy as shown by the good performance of the exposed subjects on the “easy” odorants mentioned above. Furthermore, in our experience, olfactory testing has good subject acceptance. In the present study, all subjects readily agreed to participate and were eager to try the (familiar-looking) pens. Despite the fact that we had to exclude 3 odorants as unfamiliar to Mexicans, fresh pens can be readily filled with any suitable (nontoxic, soluble, nonperishable) odorant in order to match stimuli to particular test populations (e.g., Shu and Yuan 2008).

Particularly notable is the ability of the Sniffin’ Sticks standard test to detect and distinguish among deficits occurring at different levels in the olfactory system. Thus, using the same method in a previous study to test olfactory performance of Mexico City residents, we could show that big city air pollution has a detrimental effect on odor detection, less so on odor discrimination, and—in contrast to the present study—apparently no effect on odor identification (Guarneros et al. 2009, 2011; see also Hudson et al. 2006). As we have discussed previously (Hudson et al. 2006; Guarneros et al. 2009), this suggests that the air pollution of Mexico City, containing lower levels of toxic substances capable of reaching central brain structures via transynaptic transport compared with atmospheric levels of Mn at T/C (Tovalín et al. 2010), has a detrimental effect primarily on the periphery of the olfactory system (the receptor surface in the nasal cavity).

Taken together, the results of the present study suggest that chronic exposure to atmospheric Mn even outside the workplace can affect residents’ health. The results further suggest that standardized, noninvasive, inexpensive, and easily administered olfactory tests provide an efficient means of monitoring the effects on general health of exposure to potentially damaging levels of pollutants in the atmosphere such as Mn. In the case of Mn, this is relevant not only for occupationally exposed groups but also in relation to broader issues such as the use of Mn in gasoline or in pesticides, and to the debate over possible effects of this via atmospheric contamination on the health of communities in general (Boudia et al. 2006; Finkelstein and Jerrett 2007; Walsh 2007).

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