NeuroToxicology 57 (2016) 230–240

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Effects of manganese exposure on visuoperception and visual memory in schoolchildren

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ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form 8 October 2016
Accepted 8 October 2016
Available online 11 October 2016

Keywords:
Manganese
Schoolchildren
Neurotoxicity
Visuoperception
Visual memory

ABSTRACT

Background: Manganese (Mn) is an essential metal involved in multiple physiological functions. Environmental exposure to airborne Mn is associated with neurocognitive deficits in humans. Children, whose nervous system is in development, are particularly susceptible to Mn neurotoxicity.
Objective: The objective of this study was to assess the association between Mn environmental exposure, and effects on visuoperception and visual memory in schoolchildren.
Methods: We assessed schoolchildren between 7 and 11 years old, with similar socioeconomic status, from the mining district of Molango (n = 148) and Agua Blanca (n = 119, non-mining area) in Hidalgo state, Mexico. The Rey-Osterrieth Complex Figure (ROCF) test was used to assess visuoperception and short-term visual memory. Hair manganese (MnH) concentrations were determined. Linear regression models were constructed to estimate the associations between MnH and ROCF scores, adjusted for potential confounders.
Results: The geometric mean MnH was nine times higher in schoolchildren from the Mn mining area (5.25 μg/g) than in schoolchildren from the non-mining area (0.55 μg/g). For the ROCF Copy trial, MnH was significantly associated with an increase in distortion errors (tangency, closure), angle errors, overtracing (partial overtracing). In the Immediate Recall trial, MnH was significantly associated with increased overtracing (partial overtracing) and omissions, and negatively associated with the number of perceptual drawn units, total score and percentage immediate recall.
Conclusions: MnH is associated with alterations in visuoperception and short-term visual memory in schoolchildren exposed to airborne Mn.

1. Introduction

Manganese (Mn) is an essential trace element, involved in enzymatic reactions necessary for cellular function (Gwiazda et al., 2007), however, exposure to dust with high Mn content is associated with altered Central Nervous System (CNS) functioning (Butterworth, 2010; Zhang et al., 2010; Schroeter et al., 2012; Guerra et al., 2013). The most important route of Mn exposure from

Abbreviations: CNS, central nervous system; μg/dL, micrograms per deciliter; μg/g, micrograms per gram; μg/m^3, micrograms per cubic meter; μL, microliter; μg, micrograms; GM, geometric mean; IC, confidence interval; Hb, hemoglobin; Mn, manganese; MnH, hair manganese; LogMnH, natural logarithm hair manganese; Pb, lead; PbB, blood lead; LogPbB, natural logarithm blood lead; PM, particulate matter; ROCF, Rey-Osterrieth Complex Figure; ROCF-C, Rey-Osterrieth Complex Figure – copy; ROCF-IR, Rey-Osterrieth Complex Figure – immediate recall; FTT, finger tapping test.

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http://dx.doi.org/10.1016/j.neuro.2016.10.006
0161-813X/

the point of view of availability and access to the CNS is via inhalation (Tjalve and Henriksson, 1999; Aschner et al., 2007). Mn enters the body through the respiratory tract and through olfactory nerve uptake, avoiding some of the homeostatic mechanisms that eliminate excess Mn from the body (Dorman et al., 2006). Since children's respiration rate and air intake are proportionally higher than adults, they are considered to be at higher risk than adults for inhaled Mn toxicity (Weiss, 2000; Dorman et al., 2006; Grandjean and Landrigan, 2014).

Excess Mn can disrupt CNS function at cortical and subcortical areas (Tiffany-Castiglion and Qian, 2001; Jiang et al., 2007; Guilarte, 2013). Basal ganglia and frontal cortex accumulate significant amounts of Mn, which disturbs their maturation and functioning (Reaney et al., 2006; Jiang et al., 2007). Children's increased susceptibility to Mn neurotoxicity may stem from its effects on the myelination process in the frontal cortex and subcortical connections, which starts, on average, at 6–8 months of age, and concludes in early adulthood. This neurological maturation process promotes appropriate cognitive development (Deoni et al., 2011; Dubois et al., 2014; Croteau-Chonka et al., 2015).

There is growing epidemiologic evidence of the deleterious effects of Mn on cognition and behavior in environmentally exposed schoolchildren. Cognitive deficits include attention problems (Oulhote et al., 2014; Shin et al., 2015), motor function impairment (Hernández-Bonilla et al., 2011; Oulhote et al., 2014; Mora et al., 2015), decreased verbal memory and learning (Torres-Agustín et al., 2013; Oulhote et al., 2014), compromised language development (Khan et al., 2011; Rink et al., 2014), executive function, working memory deficit, (Carvalho et al., 2013), and poor intellectual performance (Wasserman et al., 2006; Menezes-Filho et al., 2010; Riojas-Rodríguez et al., 2010; Bouchard et al., 2011; Khan et al., 2012; Haynes et al., 2015). Behavioral disinhibition, hyperactivity and behavior problems have also been reported (Bouchard et al., 2007; Ericson et al., 2007; Menezes-Filho et al., 2014).

Mexico has the eighth highest Mn deposit in the world and the second in Latin America (INEGI, 2014). It is located in Hidalgo state, in the east of the country, comprising an area of 125 km^2, with a reserve of 32 million tons of this metal (INEGI, 2014). Several studies have been conducted in the area since 1998 to document neurotoxic effects of Mn in the inhabitants of the surrounding communities. Initial studies carried out with adult residents of this mining district reported associations between high blood Mn levels ($\geq$15 μg/L) and the risk of a poor performance on cognitive tests (Santos-Burgoa et al., 2001); these authors likewise observed a negative association between exposure to high concentrations of air-borne Mn and effects on motor function (Rodríguez-Agudelo et al., 2006) and attention function (Solís-Vivanco et al., 2009). Following these results for the adult population, the cognitive function of schoolchildren residing in this mining area was assessed and compared with a similar group residing in a non-mining region in the same state in 2006. Mn exposure was negatively associated with the intellectual function (Riojas-Rodríguez et al., 2010), motor function (Hernández-Bonilla et al., 2011), and verbal memory and learning (Torres-Agustín et al., 2013), adjusting for the following variables: blood lead level, hemoglobin, child's age and gender, mother's schooling years and intellectual quotient. A new population of schoolchildren of the same areas were assessed again in 2013.

To our knowledge, there is no literature describing the effects of Mn exposure on visuoperception and short-term visual memory. In the current study, these functions were assessed and compared in schoolchildren living in the mining and in a non-mining region, both located in Hidalgo state, Mexico in the years 2006 and 2013.

2. Materials and methods

2.1. Study design and population

Cross-sectional studies were carried out in 2006 and 2013. At both time points, children from the same elementary school in each of two municipalities were tested.

The environmentally Mn exposed schoolchildren resided in the rural communities Chiconcuac and Tolago, within the municipality of Lolotla in the mining district of Molango, Hidalgo. The non-mining area schoolchildren lived in the rural municipality of Agua Blanca, Hidalgo, with no known Mn exposure (Fig. 1). The non-mining area group was selected on the basis of similar socio-economic conditions to the exposed communities, according to the *Marginalization Index* of the *National Population Council* (CONAPO, 2005).

Particulate Matter (PM) Mn concentrations were measured for both groups in 2006 and 2013. Mean 24 h Mn concentration in PM_{10} in 2006 for the mining area was 0.47 ± 0.64 $\mu g/m^3$ and in the non-mining area 0.02 ± 0.01 $\mu g/m^3$. The mean Mn concentration in $PM_{2.5}$ in the mining area was 0.08 ± 0.06 $\mu g/m^3$ and in the non-mining area 0.03 ± 0.03 $\mu g/m^3$ (Cortez-Lugo et al., 2015). Mean concentration of manganese in PM_{10} in 2013 for the mining area was 0.24 ± 0.35 $\mu g/m^3$ and in the non-mining area 0.02 ± 0.02 $\mu g/m^3$. Mean Mn concentration in $PM_{2.5}$ for the mining area was 0.01 ± 0.03 $\mu g/m^3$ and in the non-mining area 0.03 ± 0.02 $\mu g/m^3$ (unpublished data).

Both studies were approved by the Bioethics and Research Committees of the National Institute of Neurology and Neurosurgery "Manuel Velasco Suarez" and the National Institute of Public Health in Mexico.

In all communities, children and their parents were invited to participate voluntarily through information sessions held in the communities. Participant children were selected on the basis of age (between 7 and 11 years old), attending elementary school, and with a minimum residence of 5 years in their community. Children with any psychiatric or neurological problems, or some disability that would interfere with the execution of the neuropsychological tests, were excluded from the study. A total of 267 children (136 girls and 131 boys) were assessed: 159 children in 2006 and 108 children in 2013. Their parents signed informed consent forms.

2.2. Socio-demographic and child development variables

We collected child's socio-demographic and developmental information with an interview to the children's mothers (Lussier and Flessas, 2001). Raven's Progressive Matrices test was applied to all mothers to assess *intellectual function* (Raven, 1960).

2.3. Children's neurocognitive assessment

2.3.1. Visuoperception and short-term visual memory

To assess visuoperception and short-term visual memory, we used the Rey-Osterrieth Complex Figure test (ROCF). This test has been used in clinical and epidemiological studies to assess development of visuoperception and visual memory in children with Central Nervous System alterations or learning disabilities (Rey, 1999; Bellinger et al., 2003; Senese et al., 2015). The ROCF has been standardized for the Mexican child population (Cortés et al., 1996; Salvador and Cortés, 1996), with a normalized procedure for scoring (Galindo y Vila et al., 1996).

The ROCF was designed for use with children ages 7 and above. It consists of a complex geometric design made up of 18 perceptual units, which the child is required to copy and reproduce as accurately as possible (Fig. 2). The test took approximately 15 min, depending on the abilities of each child. It was administered and

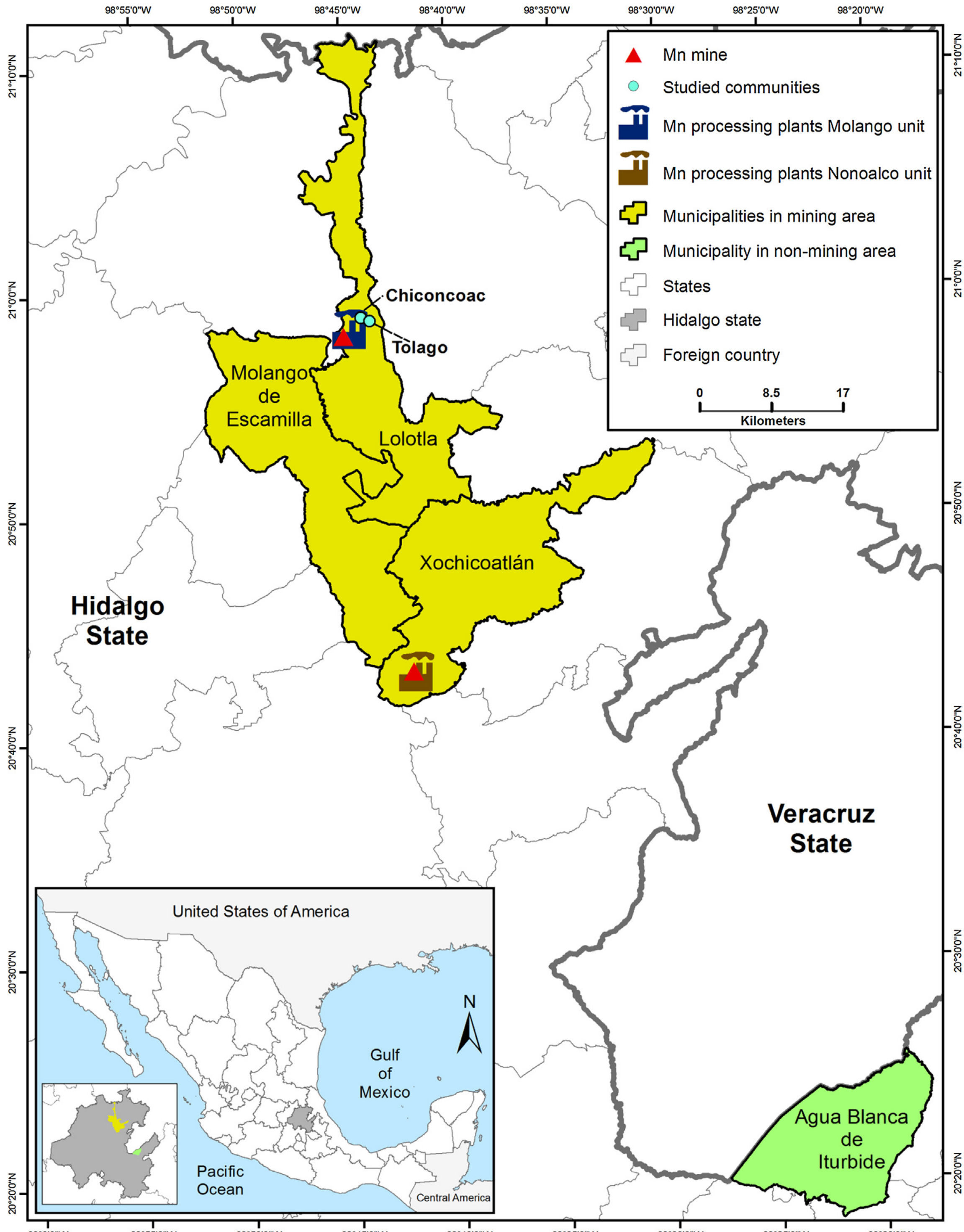
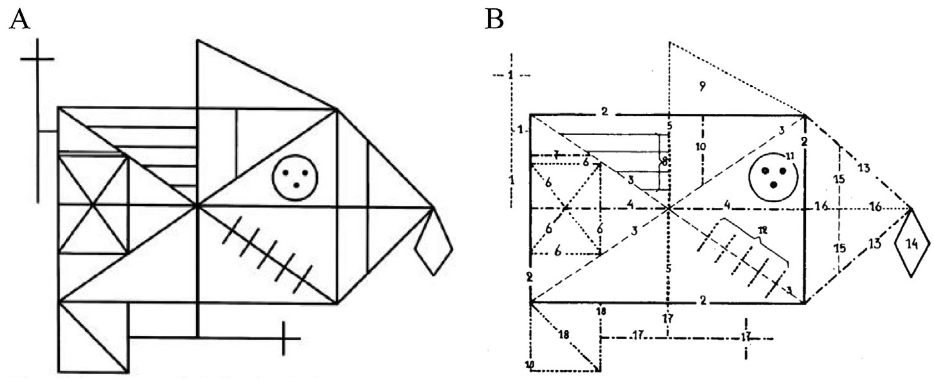


Fig. 1. Mining district of Molango, Hidalgo, Mexico.

scored by a pediatric neuropsychologist (DHB). The neuropsychologist has a master's degree, was trained in pediatric neuropsychology and has 10 years of clinical experience. At each school, testing was carried out in a classroom, with adequate lighting and minimum external noise (Lezak et al., 2012).

In the initial trial, children a were asked to copy the complex geometric figure as accurately as possible; then, after 5 min they were asked to remember it and draw it again. Two series of measurements were obtained from this test, one for Copy (ROCF-C), which reflects the degree of accuracy of visuoperceptual function and the other for Immediate Recall (ROCF-IR) (Kaplan, 2003).

The child's execution of both drawings was assessed by evaluating the overall construction of the figure and each one of the perceptual units. To evaluate the total execution of ROCF-C, several items were taken into account, including rotation errors,



A. Original stimulus (Rey, 1999)
B. Perceptual units (Galindo y Villa et al., 1996)

Fig. 2. Rey-Osterrieth Complex Figure.

size, disintegration and replacement. Each of the 18 perceptual units drawn were assessed using the following nine criteria: *rotation errors* (45°, 90°, 180°), *location errors* (type a, b, c, d), *repetition errors* (full, partial), *distortion errors* (uncoordinated drawing, tangency, closure, incompletely drawn, change length to width ratio), *angle errors*, *overtracing* (partial/full), *size errors* (macrographia, micrographia), *omission errors*, *added details*. For the total score, each perceptual unit was scored according to the quality of the execution, 2 points corresponded to no recordable error; 1 point to any type of error(s) in the horizontal line of the reproduction –not combined with *location* or *rotation* errors; 0.5 points when the *rotation* or *location* error, or both are scored, or either of these two errors are scored relative to another type of errors, and 0 points when the perceptual unit is omitted or unrecognizable. The scores from each of the 18 perceptual units were summed; a score of 36 points indicated perfect execution. The ROCF-IR score was calculated using the same method and evaluation criteria of ROCF-C. The ROCF standardization offered average scores by age group (Cortés S et al., 1996; Galindo y Vila et al., 1996). These scores in ROCF-C in children 7 years were 16.1 points, in the group 8–9 years is 17.6 points, and children of 10–11 years is 21.4 points. The scores for ROCF-IR in the children of 7 years were 9 points, in the group of 8–9 years is 10.6 points and in the group of 10–11 years is the 12 points. Finally, a Percentage Immediate Recall was calculated by dividing the number of units drawn in ROCF-IR by the total number of units drawn in the ROCF-C multiplied by 100 (Karapetsas and Kantas, 1991; Kaplan, 2003).

2.3.2. Motor dexterity

The Finger Tapping Test (FTT) has been used as a test of simple motor speed being part of the Halstead-Reitan Neuropsychological Test Battery. The FTT has been generally used to assess the manual dexterity and motor speed (Lezak et al., 2012).

The FTT was designed for use with children ages 6 and above. The Finger Tapper apparatus consists of a tapping key attached to a counter that records the number of taps completed. Examinees were asked to tap as fast as they can with their index finger. The test was administered in a series of five 10-s trials for each hand, with brief rest periods in between trials. The dominant and non-dominant hand trials were registered. The score for each trial was recorded separately in the proper sequence of occurrence. Five consecutive trials with scores greater than five-taps were used to calculate the mean number of taps. A higher number indicated better performance (Reitan and Wolfson, 1985; Lafayette Instrument, 2002; Hernández-Bonilla et al., 2011; Lezak et al., 2012).

2.4. Exposure assessment

For MnH determination, hair samples of approximately 0.5 g were obtained from the occipital region closest as possible to the scalp and stored in plastic bags until analysis. MnH determination was carried out as described previously by Menezes-Filho et al. (2009) and Riojas-Rodríguez et al. (2010). Hair samples were washed three times by vigorous agitation with 2% Triton X-100 detergent solution and rinsed twice with deionized water. Samples were dried at 60 °C and cut from the nearest side to the scalp for their acidic digestion. A hair sample (200 mg) was then placed in a polyethylene tube (metal-traces free) with 500 μL concentrated nitric acid (Suprapur, Merck, Mexico). The samples were digested for 30 min at 60 °C. The resulting solution was analyzed in the graphite-furnace atomic absorption spectrophotometer Analyst 600, (Perkin Elmer). Quality control for Mn determination was ensured by including a biological reference standard (bovine liver 1577b, National Institute of Standards and Technology, Gaithersburg, MD, USA); this biological-matrix based reference material was digested and analyzed in the same session as samples. Quantification limits for MnH were 0.5 μg/g.

Due to the possible influence of lead (Pb) on the cognitive development and its relationship with Mn (Sanders et al., 2015; Claus Henn et al., 2014), blood lead (PbB) levels were determined in the above described atomic absorption spectrophotometer with a specific light source for Pb. A sample of blood was diluted with matrix modifier consisting in 0.05% Triton X-100. Quality control was ensured through the analysis of blood with known amounts of Pb from the Wisconsin State Lab's Hygiene Program. Results are expressed as micrograms of Pb per deciliter of blood (Montes et al., 2008). Analytical sessions were considered valid only if metal measurements were 95–100% of the values provided in the analysis certificate. Samples were analyzed in duplicates, with less than 10% standard deviation, the quantification limits for PbB were 1 μg/dL.

Hemoglobin (Hb) levels were determined in blood by using the routine procedure of the Clinical Laboratory facilities of the National Institute of Neurology and Neurosurgery. The Hb normal reference value considered for children was 13.5 g/dL, a cut-off for iron deficient anemia (Valenzuela et al., 2010).

2.5. Statistical analysis

Statistical analyses included data from 148 schoolchildren from the mining area and 119 schoolchildren from the non-mining area with complete data (ROCF scores and biomarkers). The sociodemographic characteristics, exposure biomarkers and ROCF scores were compared between the two groups using Mann-Whitney test or Chi-Square test, according to the type of variable.

The distribution of MnH and PbB exposure biomarkers were reported as geometric mean and 95% confidence interval. The median with the 25 and 75 percentiles or mean and standard deviation were used to describe the errors and total scores for ROCF-C and ROCF-IR.

To explore the association of MnH and the ROCF's errors and total scores, linear regression models were constructed. For these analyses, ROCF errors and scores were considered as the outcome variables and exposure biomarkers concentrations (MnH and PbB) were transformed into natural logarithmic scale, to normalize the distribution of residuals.

The models included potential confounders selected according to *prior* knowledge: logPbB (μg/L), Hb (g/dL), child's age (months), sex, motor dexterity assessed as the average in 5 trials for the dominant hand in the FTT, and the raw score of the mother's Raven test.

The interaction terms between exposure biomarkers (logMnH and logPbB), Mn exposure and children's age, and gender and FTT results were tested considering a p-value ≤ 0.15.

We assessed the goodness of fit and conducted residual diagnosis and influential observations for each model. Analyses were conducted using the statistical package STATA (Version 14; Stata Corp, College Station, TX, USA.).

3. Results

3.1. Characteristics of the study population

Table 1 shows the sociodemographic characteristics of the study populations. The mean age of the children was 9 ± 1.3 years. Fifty-one percent (51%) were girls and there was no difference in the proportion of girls and boys between groups. There was a statistical significant difference in children's years of education; the children in the mining area had more schooling years. The Hb levels were in the normal range in both groups, although, the mean Hb level in the children from non-mining area was higher than the level in the children from the mining area. A statistical difference was observed in the FTT scores, the children from non-mining area had higher scores than schoolchildren from mining area.

Mothers from the non-mining area were older compared with those from the mining area. Mothers' mean education was six years of schooling. Mothers from the non-mining area showed higher scores on the Raven test than the mothers from the mining area group.

3.2. Exposure biomarkers

There was a significant difference in hair Mn concentration between the two groups, the geometric mean for MnH was 9 times higher for the schoolchildren from the mining area (5.25 μg/g, CI 95% 4.38–6.29) than that from schoolchildren non-mining area (0.55 μg/g, CI 95% 0.49–0.62). In contrast, the PbB geometric mean was significantly higher in the schoolchildren from the non-mining area (6.21 μg/dL, CI 95% 5.53–6.97) than those from the schoolchildren in the mining area (2.59 μg/dL, CI 95% 2.31–2.90). Table 1 shows the distribution of the biomarker levels of schoolchildren from the two groups.

3.3. Children's neurocognitive assessment

3.3.1. Visuoperception and short-term visual memory

The schoolchildren from the non-mining area presented more *location* (type c and d), *distortion* (closure and change in the ratio of length to width), and *size errors* (micrographia and macrographia) in the ROCF-C compared to children from the mining area (Table 2), while the schoolchildren from mining area had more *overtracing errors* (partial and total).

For the ROCF-IR, the children from the non-mining area showed more *location* (type b and c), *repetition* (partial), *distortion* (tangency and closure), *angle*, *overtracing* and *size* errors (micrographia), while for the children from the mining area, there were a greater number of *distortion* (incompletely drawn) and *omission errors*.

No significant differences between groups for the total scores of ROCF-C and ROCF-IR were found nor in the *percentage immediate recall*; on average, the children in both groups recalled up to 67

Table 1
Sociodemographic characteristics and exposure biomarker of the study population.

Characteristics	Mining area n = 148	Non-mining area n = 119	p-Value
Schoolchildren			
Age (years) P50(P25–P75)	9 (8–10)	9 (8–10)	0.21
Girls%	74 (50)	62 (52)	0.73
Education (years) P50 (P25–P75)	4 (4–6)	4 (3 k 6)	0.01
Hb (g/dL) $\bar{x} \pm$ SD	13.67 ± 0.71	14.09 ± 0.90	<0.01
Motor dexterity			
Finger Tapping Test $\bar{x} \pm$ SD	17.70 ± 7.00	21.28 ± 7.24	< 0.01
Exposure biomarkers			
MnH (μg/g) GM (CI 95%)	5.25 (4.38–6.29)	0.55 (0.49–0.62)	<0.01
PbB (μg/dL) GM (CI 95%)	2.59 (2.31–2.90)	6.21 (5.53–6.97)	<0.01
Mothers			
Age (years) P50 (P25–P75)	33 (29–37.5)	35 (30–40)	0.05
Education (years) P50(P25–P75)	6 (3–9)	6 (5–9)	0.80
Raven raw score P50 (P25–P75)	17 (13–22)	22 (16–27)	<0.01

Abbreviations: GM, geometric mean; CI, confidence interval.
p-value Mann-Whitney or Chi-Square test.

Table 2
ROCF errors and total score for schoolchildren from the mining and non-mining areas.

Errors and total score	ROCF-C			ROCF-IR		
	Mining area n = 148 P50 (P25–P75)	Non-mining area n = 119 P50 (P25–P75)	p-Value	Mining area n = 148 P50 (P25–P75)	Non-mining area n = 119 P50 (P25–P75)	p-Value
Rotation errors	1 (1–2.5)	1 (1–2)	0.56	1 (1–2)	1 (1–2)	0.65
45°	1 (0–2)	1 (0–2)	0.56	1 (0–1)	1 (0–1)	0.35
90°	0 (0–1)	0 (0–1)	0.57	0 (0–1)	0 (0–1)	0.50
180°	0 (0–0)	0 (0–0)	1.00	0 (0–0)	0 (0–0)	0.87
Location errors	5 (4–8)	7 (5–9)	<0.01	4 (3–6)	5 (4–7)	0.01
a	0 (0–0)	0 (0–0)	0.16	0 (0–0)	0 (0–0)	0.45
b	5 (3–7)	6 (3–8)	0.08	3 (1–5)	3 (3–5)	0.03
c	0 (0–0.5)	0 (0–1)	0.03	0 (0–1)	1 (0–2)	<0.01
d	0 (0–1)	0 (0–1)	0.03	0 (0–1)	0 (0–1)	0.57
Repetition errors	1 (0–1.5)	1 (0–2)	0.94	1 (0 − 1)	1 (0–2)	0.29
Full	0 (0–0)	0 (0–0)	0.20	0 (0 − 1)	0 (0–0)	0.34
Partial	1 (0–1)	1 (0–1)	0.80	0.5 (0 − 1)	1 (0–1)	0.05
Distortion errors	15 (12–16)	15 (13–16)	0.41	9 (7–11.5)	10 (8–12)	0.04
Uncoordinated drawing	7 (3.5–9)	7 (4–10)	0.48	4 (2–6)	4 (3–7)	0.07
Tangency	11 (8–13)	11 (9–13)	0.14	6 (4–8)	7 (6–9)	<0.01
Closure	1 (1–2)	2 (1–2)	0.03	1 (0–1)	1 (0–2)	0.05
Incompletely drawn	0 (0–1)	0 (0–1)	0.16	2 (1–3)	1 (0–2)	0.03
Change length to width ratio	1 (1–2)	1 (1–2)	0.03	1 (1–2)	1 (1–2)	0.24
Angle errors	7 (5–8.5)	8 (7–8)	0.01	5 (3–6)	6 (5–7)	<0.01
Overtracing	3 (1–5.5)	2 (1–3)	<0.01	1 (0–2)	1 (0–2)	0.03
Partial Overtracing	2 (1–4)	1 (0–3)	<0.01	1 (0–2)	1 (0–2)	0.06
Full Overtracing	0 (0–1)	0 (0–0)	<0.01	0 (0–0)	0 (0–0)	0.22
Size errors	2 (1–3)	4 (2–5)	<0.01	2 (1–3)	2 (2–4)	<0.01
Macrographia	0 (0–1)	1 (0–2)	<0.01	0 (0–1)	0 (0–1)	0.05
Micrographia	1 (1–2.5)	2 (1–4)	<0.01	1 (0–2)	2 (1–3)	<0.01
Omission errors	1 (0–2)	1 (0–2)	0.77	8 (5–10)	7 (5–9)	0.05
Added details	0 (0–1)	0 (0–0)	0.31	0 (0–1)	0 (0–1)	0.93
Total units drawn	17 (16–18)	17 (16–18)	0.77	10 (8–13)	11 (9–13)	0.05
	x ± SD	**x ± SD**	**p-Value**	**x ± SD**	**x ± SD**	**p-Value**
Total score	13.3 ± 3.2	13.3 ± 3.2	0.38	7.8 ± 3.3	8.3 ± 2.8	0.20
Percentage Immediate Recall				62.5 ± 21.2	66.3 ± 14.6	0.29
Total score by age[a]						
7	10.6 ± 3.6	11.6 ± 2.9	0.74	5.8 ± 2.9	7.2 ± 2.6	0.12
8–9	12.7 ± 3.2	12.7 ± 2.8	0.87	6.9 ± 3.0	8.1 ± 2.9	0.04
10–11	14.9 ± 2.2	15.0 ± 3.0	0.88	9.4 ± 3.1	9.3 ± 2.4	0.62

p-value Mann-Whitney or Chi-Square test.
[a] Reference values corresponding to mean by age for total score (ROCF-C/ROCF-IR): 7 years (16.1/9.0); 8–9 years (17.6/10.6); 10–11 years (21.4/12.0) (Cortés et al., 1996).

percent of the figure drawn in the copy task. Children from 8 to 9 years old from the non-mining area showed higher *total scores* in ROCF-IR (Table 2).

3.3.2. Motor dexterity

A statistical difference was observed in the FTT scores, the children from non-mining area had higher scores than schoolchildren from mining area (Table 1).

3.4. Association between MnH and ROCF-C – ROCF-IR

Table 3 shows the results of the linear regression models, each change in MnH (0.14 μg/g) was associated with different types of errors in ROCF-C and ROCF-IR (β/100). The main exposure variable was logMnH and all models were adjusted for logPbB, Hb (g/dL), child's age (months) and sex, motor dexterity measured as the dominant hand average in 5 trials in the FTT, and the mother's Raven test score. The interaction terms were tested but not included in the statistical models, because, none of them showed statistical significance, except for a sex interaction for total scores in ROCF-C (Fig. 3).

For ROCF-C, MnH was associated with an increased probability of *distortion* (tangency and closure), *angle* and *overtracing* (partial overtracing) errors, as well as a marginally decrease in *distortion* (uncoordinated drawing) error and *total score*. We observed that the negative association of MnH with the total score in the copy trial was significantly modified by sex; girls, but not boys, showed a significant decrease in total score as shown in Fig. 3.

For ROCF-IR, MnH concentrations were associated with an increase in *overtracing* (partial overtracing) and *omission* errors, as well as a decreased number of *perceptual units drawn*, *total score* and *percentage immediate recall* (Table 3).

No statistically significant association with MnH was found for *rotation* (45°, 90°, 180°), *location* (type a, b, c and d), *repetition* (full and partial), *distortion* (incompletely drawn and modification of the long-width ratio), *overtracing* (full overtracing), *size errors* (micrographia and macrographia) and *added details* in ROCF-C or ROCF-IR (data not shown).

Table 3
Results of linear regression for ROCF errors and scores with respect to MnH and PbB for the total population.

Errors and total score	ROCF				
	logMnH β (95% CI)	p-Value	logPbB β (95% CI)	p-Value	R^2 adjusted
		ROCF-C			
Distortion	0.30 (0.05–0.56)	0.01	0.84 (0.40–1.28)	<0.01	0.07
Uncoordinated drawing	0.31 (−0.02–0.65)	0.06	0.94 (0.35–1.53)	<0.01	0.10
Tangency	0.41 (0.14–0.67)	<0.01	1.14 (0.68–1.6)	<0.01	0.10
Closure	0.15 (0.06–0.24)	<0.01	0.35 (0.19–0.51)	<0.01	0.09
Angle	0.17 (0.01–0.33)	0.03	0.71 (0.43–0.99)	<0.01	0.09
Overtracing	0.90 (0.64–1.15)	<0.01	0.39 (−0.04–0.84)	0.08	0.22
Partial Overtracing	0.53 (0.35–0.71)	<0.01	0.24 (−0.07–0.55)	0.13	0.15
Size	0.05 (−0.12–0.23)	0.53	0.95 (0.64–1.27)	<0.01	0.17
Micrographia	0.10 (−0.04–0.26)	0.16	0.70 (0.43–0.96)	<0.01	0.15
Omissions	0.11 (−0.05–0.29)	0.19	−0.08 (−0.38–0.21)	0.58	0.15
Total units drawn	−0.11 (−0.29–0.05)	0.19	0.08 (−0.21–0.38)	0.58	0.15
Total score	−0.21 (−0.46–0.03)	0.09	−0.54 (−0.97–−0.10)	0.01	0.30
		ROCF-IR			
Distortion	−0.21 (−0.5–0.06)	0.13	0.42 (−0.07–0.92)	0.09	0.09
Uncoordinated drawing	−0.04 (−0.29–0.21)	0.74	0.55 (0.10–1.00)	0.01	0.04
Tangency	−0.09 (−0.35–0.16)	0.47	0.61 (0.15–1.06)	<0.01	0.06
Closure	0.02 (−0.05–0.11)	0.49	0.18 (0.04–0.33)	0.01	0.02
Angle	−0.08 (−0.26–0.1)	0.38	0.44 (0.11–0.76)	<0.01	0.03
Overtracing	0.26 (0.09–0.43)	<0.01	0.09 (−0.19–0.39)	0.49	0.07
Partial Overtracing	0.16 (0.02–0.29)	0.01	0.02 (−0.20–0.25)	0.81	0.06
Omissions	0.55 (0.26–0.84)	<0.01	0.22 (−0.27–0.72)	0.37	0.17
Total units drawn	−0.55 (−0.84–−0.26)	<0.01	−0.22 (−0.72–0.27)	0.37	0.17
Total score	−0.42 (−0.68–−0.16)	<0.01	−0.55 (−1.00–−0.09)	0.01	0.22
% Immediate Recall	−2.88 (−4.58–−1.18)	<0.01	−2.21 (−5.18–0.75)	0.14	0.09

Exposure variable logMnH: natural logarithm transformation. One percent change of MnH (0.14 μg/g) represent an increase of ROCF errors (β/100).
Exposure variable logPbB: natural logarithm transformation. One percent change of MnH (0.53 μg/dL) represent an increase of ROCF errors (β/100).
Linear regression models (n = 267): all models were adjusted for logPbB (μg/dL), HbB (g/dL), child's age (months), sex, Finger Tapping test dominant hand average in 5 trials, and the raw score of the mother's Raven test.

We also observed a statistically significant association with an increase of PbB (0.53 μg/dL) and some of ROCF errors (β/100). For ROCF-C, PbB was associated with an increase in *distortion* (uncoordinated drawing, tangency and closure), *angle* and *size* (micrographia) errors. PbB also was negatively associated with the *total score*. For ROCF-IR, PbB concentrations were associated with an increase in *distortion* (uncoordinated drawing, tangency and closure) and *angle errors*, as well as a decrease in *total score* (Table 3).

According to previous knowledge about the influence of the motor dexterity on the visuoperception and visual memory (Mayor-Dubois et al., 2010; Bo and Lee, 2013), we included the

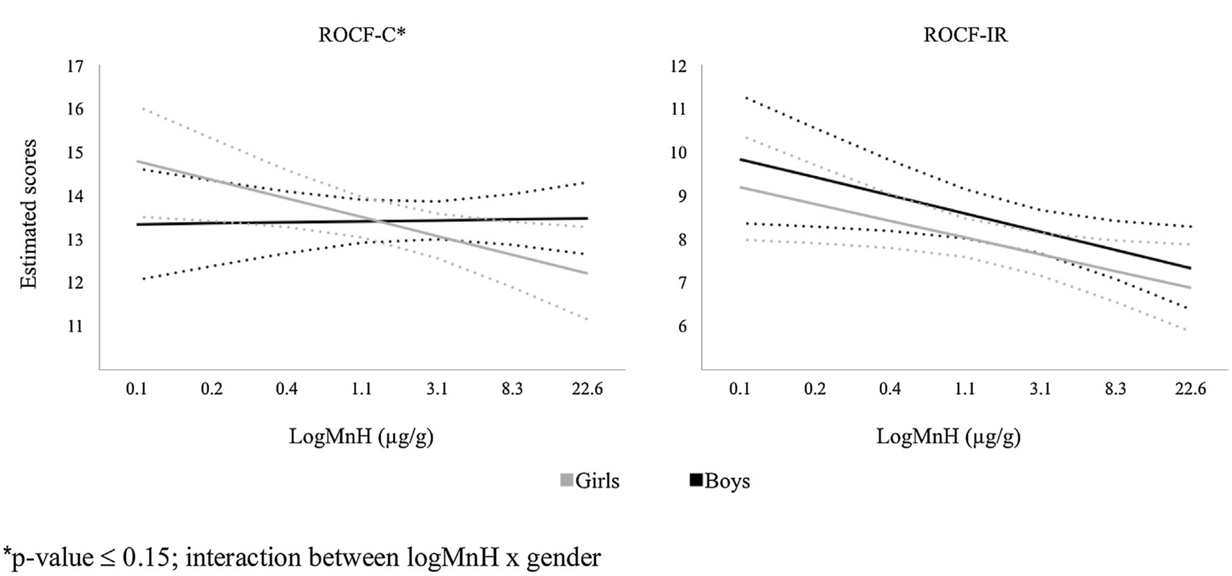


Fig. 3. Estimated ROCF total score for different MnH concentrations, stratified by gender.

FTT in the regression models, although no significant associations were found except for a significant decrease in *overtracing* (*partial overtracing*) errors in the ROCF-C and ROCF-IR.

It is known that the maternal IQ influences the child cognitive development (Lussier and Flessas, 2001; Mazeau, 2005), so we included the raw Raven Test scores in the regression models, because the obtained scores were low not allowing the estimation of standard scores. The maternal IQ tended to decrease the *distortion* (uncoordinated drawing, tangency), *angle* and *size* (micrographia) errors in ROCF-C. In ROCF-IR, we observed the same trend to decrease *distortion* (uncoordinated drawing) and *size* (micrographia) errors. Also, the maternal IQ significantly increased the total *units drawn*, *total score* and *percentage immediate recall*.

4. Discussion

In this study, the ROCF test was used to assess the potential neurotoxic effects on visuoperceptual and visual memory function of schoolchildren. Mn exposure levels were associated with increased *distortion*, *angle*, *overtracing* and *size errors* as well as omission of *perceptual units* in visuoperceptual assessment. Mn exposure was also associated with an increased number of *overtracings* and *omissions errors*, and a decreased number of *perceptual units* drawn, *total score* and *percentage immediate recall* in the short-term visual memory assessment.

Previous studies have demonstrated the usefulness of MnH as a biomarker of environmental exposure in assessing associations with cognitive deficits in children. For several of these studies Mn exposure was from drinking water (Bouchard et al., 2011; Oulhote et al., 2014; do Nascimento et al., 2015; Molina-Villalba et al., 2015; Vibol et al., 2015), while for others the source of exposure was airborne Mn (Menezes-Filho et al., 2009; Rugless et al., 2014; Riojas-Rodríguez et al., 2010; Lucas et al., 2015; Haynes et al., 2015). MnH levels in the present study were higher than those reported in these studies.

In the present study, MnH parallels the airborne Mn PM_{10} and $PM_{2.5}$ concentrations measured in 2006 and 2013 in the mining and non-mining areas. At both times, Mn concentrations in PM_{10} for the mining area surpass the US Environmental Protection Agency reference concentration of 0.05 μg/m3 (US EPA, 2015).

To our knowledge, this is the first study to use ROCF to assess the effects on visuoperceptual abilities and visual memory associated with Mn exposure in schoolchildren. Previous studies have shown associations with motor reaction times (Vibol et al., 2015), non-verbal intelligence (do Nascimento et al., 2015) and IQ (Wasserman et al., 2006; Menezes-Filho et al., 2010; Riojas-Rodríguez et al., 2010; Bouchard et al., 2011), visuospatial organization (Carvalho et al., 2013), verbal memory-learning (Torres-Agustín et al., 2013), motor function (Hernández-Bonilla et al., 2011) and behavioral problems and inattention (Menezes-Filho et al., 2014).

The ROCF has been typically used in the clinical context to determine alterations of visuoperception and visual memory (Kaplan, 2003; Lezak et al., 2012; King et al., 2015). Although its use is more frequent in adult populations (Kaplan, 2003; Baerresen et al., 2015), it has also been used to describe and evaluate development of perception and visual memory as a consequence of development disorders and/or neurological alterations (Bellinger et al., 2003; Kaplan, 2003). In occupational studies the ROCF has been used to evaluate the association between Mn exposure and neurocognitive effects. Park et al. (2006) demonstrated a significant decrease of total ROCF-C and ROCF-IR scores in welders with chronic exposure to Mn.

Compared to the Mexican standardization sample (Cortés et al., 1996; Salvador et al., 1996), the number of errors made by the children from both the mining and the non-mining area in the execution of the ROCF-C and ROC-IR was higher and the total scores were lower than expected, probably reflecting the socioeconomic, educational and exposure conditions of these children. Moreover, on some subtests, the schoolchildren from the non-mining area performed more poorly than schoolchildren from mining area when MnH concentrations was not taken into account.

Alterations in the development of visuoperception and visual memory are known to have an impact on the acquisition of educational skills, mainly on those that are necessary for the development of writing and reading (Lussier and Flessas, 2001; Ardila et al., 2005). Poor performance on ROFC has been associated with special educational needs (Khan et al., 2012; Bellinger et al., 2003).

Sex stratification revealed differences between boys and girls in ROCF total score. Previous studies of schoolchildren have reported gender differences: Mn-exposed girls present a more marked decrease in IQ compared to boys (Roels et al., 2012; Bouchard et al., 2011; Riojas-Rodríguez et al., 2010) as well as more pronounced externalizing behavioral problems and inattention (Menezes-Filho et al., 2014). However, no gender differences were observed on the California Verbal Learning Test or on motor tests (Oulhote et al., 2014). Animal studies have observed long-lasting changes in neuronal morphology in female manganese-exposed adult mice, but not in males despite similar concentrations of manganese accumulation in the brain (Madison et al., 2011). While our data do not allow us to speculate on the possible underlying mechanisms or whether these are gender- or sex-related, it does provide further evidence of the importance of stratifying for sex when examining manganese toxicity (Mergler, 2012; Roels et al., 2012).

In this study, the use of ROCF offers details of the different components of visuoperception and visual memory, providing a way to better identify the specific areas which may be affected by toxic exposures, in this case, to Mn and Pb. Errors that appear during visuoperception are primarily related to visuospatial alterations and poor visuomotor coordination, as well as to a deficient planning for the reproduction of figures (Karapetsas and Kantas, 1991; Bartolomé et al., 1998; Lezak et al., 2012). These three components are anatomically related to the functioning of the frontal lobe, some thalamic nuclei and basal ganglia (Kandel et al., 2001; Afifi and Bergman, 2005), which are involved in motor response necessary for proper visuoperception. In the present study, we examined whether motor dexterity, assessed with the FTT, influenced the results on the ROFC. This was not the case, suggesting that the deficits were not related with motor function.

The errors on short-term visual memory reflect alterations in analysis, recording, and recall of visual information required for the visual memory, which depends directly on the functioning of the temporo-occipital cortex and the hippocampus (Vannetzel et al., 2011). Several studies conducted in animal models and human provide evidence for the affinity and effect of Mn on the frontal cortex (Takeda, 2003; Calderón-Garcidueñas et al., 2013), as well as on the basal ganglia and hippocampus (Bekiesinska-Figatowska et al., 2013; Neal and Guilarte, 2013; Dion et al., 2016).

In this study we found highest PbB levels in schoolchildren from non-mining area (GM 6.21 μg/dL, CI 95% 5.53–6.97) in comparison to the children from the mining area (GM 2.59 μg/dL, CI 95% 2.31–2.90), showing mean levels above internationally recommended levels for this neurotoxic (Betts, 2012; OMS, 2015). This high levels in the comparison group was an unexpected finding and we do not know which is the source of Pb exposure in schoolchildren from the non-mining area. Pb exposure levels were associated with an increase *distortion*, *angle* and *size* errors, also was negatively associated with the *total score* in visuoperceptual assessment. Pb exposure was also associated with an increased number and *angle errors*, as well as a decreased in *total score* in the in the short-term visual memory assessment.

It has been shown that the Pb induce damage in some brain areas as prefrontal cortex, hippocampus, and cerebellum (Lezak et al., 2012). Several studies have demonstrated the Pb impact on children's cognitive function: attention, memory and learning, visual and verbal abilities, processing speed, and motor and coordination functions (Lidsky and Schneider, 2006; White and Janulewicz, 2009). Another study in Mexico, in schoolchildren between 6 and 11 years old exposed to different mixtures of neurotoxic, found an inverse association between ROCF-IR total score and PbB (Rocha-Amador et al., 2009).

The main limitation of this study is the epidemiological design since cross-sectional studies do not address temporality and causality to identify early neurological and cognitive susceptibility windows. A birth cohort study would be the most appropriate methodology for those purposes. Additionally, it is necessary to perform further functional studies to validate and better understand the present findings and their consequences for these children. Because of the nature of the study design, it was impossible to blind the pediatric neuropsychologist to potential exposure in the selected groups. This may have led to information bias, but, in order to reduce this possibility, the neuropsychologist followed the same standardized procedure to evaluate ROCF in all of the participating children.

The findings in the present study suggest that Mn induced deficits are widespread and cover several domains. Other forms of cognitive deficits have been reported for these same children (Torres-Agustín et al., 2013; Hernández-Bonilla et al., 2011; Riojas-Rodríguez et al., 2010). The efforts to decrease Mn exposure in this region should be continued through an environmental intervention plan to reduce emission and Mn exposure in the area.

Conflict of interest

The authors declare no conflict of interest.

Funding sources

This project, received funding from the International Development Research Centre (IDRC), project number 100662 and from the Consejo Nacional de Ciencia y Tecnología (CONACYT) project number 141385.

Bioethics and Research Committees

This study was approved by the Bioethics and Research Committees of the National Institute of Neurology and Neurosurgery "Manuel Velasco Suarez" and the National Institute of Public Health in Mexico.

Acknowledgments

We appreciate the support and participation in this study of the psychologists V. Martinez-García and R. Ramos-Clatempa. We thank the assistance of SJ. Velázquez-Juárez and JL. Texcalac with the map of the study area. Our special thanks to all families who participated in this study and the teachers of the elementary schools "Gral. Ignacio Manuel Altamirano" and "Belisario Dominguez" for all the assistance offered.

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