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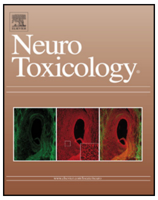

Neuro Toxicology

Elevated airborne manganese and low executive function in school-aged children in Brazil

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ABSTRACT

Exposure to airborne manganese (Mn) has been associated with neurotoxic effects, including motor and cognitive deficits. The main deficits related to excessive exposure to Mn are predominantly the dysfunction of fronto-striatal and dopaminergic circuits observed in animal experimental studies, which are involved in attention, working memory and motor function. The present study aims to assess the association between elevated Mn exposure and performance on executive function and attention neuropsychological tests in children living in two communities near a ferro-manganese alloy plant. Seventy children aged between 7 and 12 years with no history of neurologic disease and an estimated IQ >68 (Vocabulary and Block Design subtests) that had lived near the iron-Mn production alloy plant for at least 1.5 years were included. Participants were assessed for cognitive functioning with neuropsychological measures for sustained attention (Test of Visual Attention – TAVIS-3R), cognitive flexibility (WCST), and verbal and visual working memory (WISC-III Digit Span subtest and Corsi Block). Manganese hair (MnH) levels were used as a biomarker of exposure. Mean scores among study participants were lower than general population norms/averages for block design, digit span, reaction time and commission errors. The median MnH level was 11.48 (range 0.52–55.74) μg/g, and no difference between sexes was observed. Spearman's correlation analysis showed a significant inverse correlation between MnH levels and estimated IQ (rho = −0.448, $p = 0.0001$), Vocabulary (rho = −0.272, $p = 0.02$), Block Design (rho = −0.485, $p = 0.00002$) and Digit Span (rho = −0.410, $p = 0.0004$). Multiple regression analyses detected inverse associations between log MnH and scores on estimated IQ ($\beta = -9.67$; 95%CI = −16.97 to −2.37), Block Design ($\beta = -2.50$; 95%CI = −3.91 to −1.10) and Digit Span Total ($\beta = -2.59$; 95%CI = −4.13 to −1.05) standardized scores and the number of correct answers in forward and backward Digit Span methods, after adjusting for covariates ($\beta = -1.32$ = 95%CI = −2.23 to −0.40; $\beta = -1.09$ 95%CI = −2.02 to −0.16, respectively). The results suggest that airborne Mn exposure may be associated with lower IQ and neuropsychological performance in tests of executive function of inhibition responses, strategic visual formation and verbal working memory. Executive function is dependent on the fronto-striatal circuit, which may be disrupted by Mn accumulation in the brain.

1. Introduction

Manganese (Mn) is an essential metal for humans. High concentrations of manganese can cause damage to cognitive functions. High Mn levels in the brain are associated with manganism, which has clinical symptoms similar to those of Parkinson's disease (Dobson et al., 2004). Most studies on Mn toxicity have primarily been performed in occupational settings with adults, in which exposure occurs mainly through the inhalation of airborne particles (ATSDR, 2008). There are two main routes of exposure: the gastrointestinal route, which is the most common in the general population, and inhalation. The inhaled route of exposure is considered the fastest and exhibits the greatest potential to transfer Mn to the brain, especially in cases of occupational (welders and miners) and environmental exposure (people that live near of Mn mining and/or transformation). Another source of environmental exposure is the ingestion of Mn in drinking water.

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The process of Mn transport in the brain has not yet been clearly established. Some experimental animal studies suggest that after inhalation exposure, Mn can reach the brain via axonal transport from nerve endings in the nasal mucosa (Brenneman et al., 2000; Dorman et al., 2002, 2006). This may explain, at least in part, the Mn accumulation in certain brain regions (Brenneman et al., 2000; Dorman et al., 2006). The basal ganglia have been identified as the brain region with a higher accumulation of Mn (Dobson et al., 2004), which is related to the fact that Mn has tropism for brain regions rich in dopaminergic neurons (Rivera-Mancía et al., 2011). Beyond the region of the basal ganglia, other studies in rats found an increase of Mn in regions of the hippocampus, frontal cortex and brainstem (Dorman et al., 2001; Guilarte et al., 2006; Burton and Guilarte, 2009; Schneider et al., 2009). There is also evidence that chronic exposure to Mn produces a cellular stress response and neurodegeneration in the frontal cortex of non-human primates (Guilarte et al., 2008).

The accumulation of Mn causes alterations in neurotransmitter systems, especially the dopaminergic system in brain areas responsible for attention, motor coordination and cognition (Dobson et al., 2004; Kern et al., 2010). The neurobiological basis of chronic exposure to Mn has not yet been explained. Animal studies have found associations between Mn exposure and cognitive and motor functions (Burton and Guilarte, 2009). Research with animal models has provided more concrete data on the action of Mn on the dopaminergic system (Tran et al., 2002; Aschner et al., 2007; Burton and Guilarte, 2009), which is usually responsible for the modulation of neuronal activity and controls responses appropriate to the context (Kern et al., 2010).

Few studies have investigated environmental exposure to airborne Mn in children. In Mexico, the cross-sectional investigation of children between 7 and 11 years of age exposed to airborne Mn found a negative association between Mn and intelligence, memory and motor function (Riojas-Rodríguez et al., 2010; Hernández-Bonilla et al., 2011; Torres-Agustín et al., 2012). In our previous study, carried out in one of the communities of this study in Simões-Filho, Bahia, Brazil, the median level of airborne Mn was 0.11 μg Mn/m^3 (Menezes-Filho et al., 2011), close the value reported in a study in Mexico, which was 0.13 μg Mn/m^3 (Riojas-Rodríguez et al., 2010). Intelligence has been studied in Mn-exposed children, and the results showed a negative association between IQ (Intellectual Coefficient) and different indicators of Mn exposure in the hair of the scalp and water consumed (Wasserman et al., 2006, 2011; Riojas-Rodríguez et al., 2010; Bouchard et al., 2011; Menezes-Filho et al., 2011). The highest levels of Mn in hair (MnH) in children were registered in studies of environmental exposure from airborne Mn in Brazil (Menezes-Filho et al., 2011) and Mexico (Riojas-Rodríguez et al., 2010).

The ability to generate new responses adapted to the context and the voluntary control of actions have been identified in the domain of executive functions (EF) and have been related to the fronto-striatal circuit. EFs integrate skills such as self-monitoring, self-regulation, inhibition, flexibility and abandon or replace ineffective strategies in favor of others that are more effective to solve problems (Shallice, 1988; Leon-Carrion et al., 2004; Stuss, 2011). Impairments in EFs have been frequently related to injuries in the frontal lobes, as well as in other brain regions such as the basal ganglia and thalamus (Stuss, 2011). It is known that the development of the brain's frontal areas starts in the first months of life, reaching full maturity at the end of adolescence.

Attention is the cognitive domain responsible for processing information properly and filtering relevant information instead of distractors (Posner, 1994). Sustained attention is the ability to maintain attention for extended periods of time (Oken et al., 2006). Generally, sustained attention is assessed by tasks that require efficient performance over time with instruments such as Continuous Performance Tests (CPT). Literature has indicated that sustained attention is significantly impaired in a number of disorders and is a component affected mainly in children with Attention Deficit Hyperactivity Disorder (ADHD) (Aguiar et al., 2010; Miranda et al., 2012). Brazilian researchers have developed the Visual Attention Test (TAVIS-III), which was validated and standardized for Brazilian children and adolescents between 6 and 17 years old (Duchesne and Mattos, 1997). Coutinho et al. (2009) showed that children with ADHD presented significantly more commission errors than those without the disorder in the sustained attention task from TAVIS-III.

Ericson et al. (2007) evaluated components of executive functions in preschool children. The researchers found that high Mn levels in pre-natal life was significantly correlated with impulsive errors on CPT and a children's Stroop test at four years old (Ericson et al., 2007). Some studies have shown a correlation between Mn and externalizing behaviors (Ericson et al., 2007; Khan et al., 2011) and hyperactivity (Bouchard et al., 2007).

This research is part of a study that has evaluated children exposed to airborne Mn in a manganese alloy plant in Simões-Filho, Bahia, Brazil. The results of previous studies conducted in Cotegipe Village revealed high levels of Mn in children's hair (mean 11.5 $\mu g/g$) and showed a negative association with intelligence (Menezes-Filho et al., 2009, 2011). The results of this study are the application of a neuropsychological battery in the Cotegipe Village and another nearby community, Santa Luzia. This study is part of the project "Effects of environmental Mn exposure on the health of residents of two communities." The present study aims to assess the association between elevated Mn exposure and performance on executive function and attention neuropsychological tests in children living in two communities near an iron-manganese alloy plant.

2. Materials and methods

2.1. Study design and population

This is a cross-sectional study that was conducted in the Simões-Filho district, Bahia, Brazil. Residents are vulnerable to Mn exposure by air emissions arising from an iron-manganese alloy plant. Cotegipe Village is located within a radius of 1.5 km from the metallurgical plant, while the community of Santa Luzia is located at a distance between 2 and 3.5 km from the plant. All children between the ages of 7 and 12 of both communities were invited to participate in the study. Inclusion criteria were: living in the community for at least a year and a half, registration and school attendance in their communities, and an IQ greater than or equal to 67 (which is the low limit for borderline intelligence IQ 70 ± 3.2) (Fiigueiredo, 2002). Three children were excluded: one for having an IQ less than 67, a child with Down syndrome, and a deaf child. The study population consisted of 70 children, representing 87% of the potential group of sample children in these communities. Parents provided informed consent to participate in the research. This project was approved by the Committee on Ethics in Research from the Climério de Oliveira Maternity, Federal University of Bahia, with registration approval 027/2011.

The first step was to carry out visits to check the number of children and families in the communities. Each community has a public school, in which the majority of children attended. The schools were contacted and provided the researchers with a list of names and ages of the attending children. In addition to this information, visits were made in the homes of the two communities and meetings with parents and teachers were scheduled to explain the purpose of the research and to invite dyads (mother-child) to participate.

2.2. Data collection

Five psychologists were trained and monitored for the application of neuropsychological tests. To ensure the quality of the neuropsychological evaluation, the supervisor trained the psychologists and observed their performance before starting the assessment. Besides that, during the evaluations, the supervisor made sure the psychologists followed the tests instructions demonstrating reliability during children's evaluation. Psychologists were blinded to exposure status but not to study area.

2.3. Socio-demographic questionnaire

Psychology students were trained to administer the caregivers' interview questionnaires on socio-economic characteristics. The questionnaire included information about maternal education, parents' occupations, home characteristics, maternal age, and characteristics of pregnancy and child health.

2.4. Maternal estimated IQ

To evaluate the intelligence of mothers or caregivers, the Vocabulary and Block Design subtests from the Wechsler Adult Intelligence Scale – 3a̲ version, standardized to Brazil (Nascimento, 2005), were used. The abbreviated version of the scale with only two tests generates an estimated intelligence quotient (IQ) score, a reliable and quick method of intelligence assessment (Ringe et al., 2002). We assessed mother's intellectual status because it is a known determinant of a child's performance (Breslau et al., 2001).

2.5. Neuropsychological assessment

Estimated IQ: The estimated Intelligence Coefficient (estimated IQ) was generated from the sum of the standardized scores of the Vocabulary and Block Design subtests from the Wechsler Intelligence Scale for Children and converted into an IQ score (Mello et al., 2011).

Digit Span WISC-III (Wechsler, 2002) and the Corsi Block-Tapping Task (Kessels et al., 2000) are two classical tests that are well-known in the literature to assess short-term memory and working memory. Both tasks have the same logic that consists of recall sequences of numbers (digits) or taps onto cubes (Corsi Blocks) in forward and backward methods, representing immediate memory and working memory, respectively. The task of Digit Span evaluates the verbal component and Corsi Blocks assesses the visual component.

A sustained attention task were selected from the Visual Attention Test (TAVIS-III) that has three attention tasks: selective, shifting and sustained attention (Duchesne and Mattos, 1997). In the Sustained Attention Task, the child is requested to press a control button as quickly as possible as soon as the stimulus appears on a black screen for a few minutes. For children aged 7–11 years, the stimulus is a clock and lasts for 6 min, and for children aged 12–16 years, the stimulus is a red dot and lasts for 10 min. The scores are given by the reaction time, commission errors and omission errors. Greater numbers of commission errors indicate difficulty in inhibiting responses, while a greater number of omission errors is considered to represent an attention deficit.

The Wisconsin Card Sorting Test-64 Card Computer Version (Kongs et al., 2008) – WCST evaluates cognitive flexibility, perseveration and strategy maintenance to solve problems. The goal is to find different possibilities for classification (color, shape and number) through feedback offered for each card that changes every 10 correct answers.

2.6. Anthropometric measurements

The children's height, weight and head circumference were registered. Height and weight, along with age, were used to obtain the following anthropometric indices: weight for age, height for age, and body mass index (BMI) for age. These indices were converted in terms of z-scores from the WHO AnthroPlus software, version 1.0.4, which provides references from the World Health Organization (2009) for children between the ages of 5 and 19 years.

2.7. Hair manganese measurements

A tuft of hair approximately 0.5 cm in diameter was cut off as close as possible to the scalp in the occipital region with a surgical stainless steel scissor and stored in plastic bag until processing. Detailed information on hair sampling, washing procedure and Mn determination by GFAAS is reported elsewhere (Menezes-Filho et al., 2009).

2.8. Statistical analysis

Descriptive statistics were used to examine the distribution of socio-demographic information and neuropsychological test scores. Differences between groups were evaluated with Student's t-test and Mann–Whitney U tests for continuous or discrete variables and the chi-square test or Fisher's exact test for categorical variables. Standardized WISC-III scores were converted from the published Brazilian manual (Fiigueiredo, 2002) and the TAVIS-III percentile classification were obtained from Brazilian norms (Duchesne and Mattos, 1997). The standardized WISC-III scores and estimated IQ were normally distributed, so parametric statistics were used. Spearman's correlation tests were applied to evaluate bivariate correlations between co-variables and exposure bioindicators and possible confounding variables. Standardized neuropsychological tests and raw scores were considered as the dependent variables to estimate their association with Mn in hair (exposure biomarker) for constructing multiple linear regression models. Variables were selected to enter if $p < 0.05$ and $r > 0.100$ (age, maternal education, family income, Height for age z-score, weight for age z-score, body mass index for age, weight in the birth, place of residence during pregnancy). Because the distributions of hair Mn levels were skewed, data were log 10 transformed in order to make distributions more symmetrical for further analysis. All of the analyses were performed with the statistical software IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.).

3. Results

Table 1 contains a description of the population, including the sociodemographic characteristics of children and their families. Participants were 70 mother/child dyads. The demographic questionnaires were completed by all responsible parties. Approximately 53% of children lived with both parents and 39% lived with only their mother. Mothers had an average of seven years of study. In relation to pregnancy, 85% of mothers experienced their pregnancy in the delineated communities, indicating that the children residing in the communities were exposed to Mn since pregnancy. The families' residences contained an average 5.1 ± 1.5 people living in each house and 4.2 ± 0.8 rooms per residence. Some families were receiving grants from Brazilian social programs.

Table 2 presents the distribution of MnH in the children: the mean was 14.6 μg/g (±11.8), and the values ranged from 0.52 to 55.74 μg/g. No significant differences in the levels of MnH and gender were observed. Children from the Santa Luzia community presented higher levels of MnH than children from Cotegipe Village

Table 1
Characteristics of the study population.

Child characteristics	*N* (%)	Mean (SD)	Range
Age (years)	70	9.43 (1.64)	(7–12)
Gender – Female	36 (51%)		
Anthropometric measurements – *z*-score			
Height for age		−0.15 (1.12)	(−3.18 to 2.6)
Weight for age		−0.04 (0.73)	(−1.58 to 1.97)
Body mass index (BMI) for age		−0.38 (1.23)	(−3.07 to 3.44)
Village			
Santa Luzia	34 (49%)		
Cotegipe	36 (51%)		
Family characteristics			
Live with:			
Both parents	36 (53%)		
Only with mother or others	32 (47%)		
Gestation in the community	58 (85%)		
Complications in childbirth	9 (14%)		
Mother's education (years)		6.96 (3.47)	(0–14)
Mother's estimated IQ		85.78 (10.16)	(74–117)
Mother's age (years)		35.06 (9.11)	(17–62)
Main caregiver			
Mothers	61(88%)		
Others	8 (12%)		
Mother's occupation			
Housewife	30 (42%)		
Housekeeper or diarist	21 (29.4%)		
Others	13 (18.2%)		
Father's occupation			
Farmer	14 (19.6)		
Construction worker	22 (30.8%)		
Welder	3 (4.2%)		
Others	13 (18.2%)		

Table 3
Neuropsychological test scores.

	Mean (SD)	Median	Range
Standardized Scores			
WISC-III			
IQ (estimated)	91.55 (13.28)	91	6–123
Vocabulary	9.57 (2.57)	9	4–15
Block Design	6.56 (2.43)	7	3–12
Digit Span	8.67 (2.78)	8	3–15
Raw Scores			
Immediate and Working Memory			
Digits forward (Span)	4.46 (0.86)	4	3–7
Digits backward (Span)	2.73 (1.25)	3	0–6
Corsi Blocks forward (Span)	4.22 (1.17)	4	2–8
Corsi Blocks backward (Span)	3.54 (1.5)	3	0–6
WCST			
Total errors	27.76 (8.95)	29	12–44
Perseverative responses	17.45 (8.28)	17	1–43
Nonperseverative errors	12.85 (7.03)	12	2–34
Perseverative errors	14.91 (6.37)	14	1–35
Trials to complete first category	–	15.5	10–65
Categories completed	1.91 (1.15)	2	0–4
TAVIS-III (Sustained Attention)			
Reaction time (ms)	–	559	383–1477
Omission errors	–	0	0–6
Commission errors	–	1	0–37
	%		
Reaction time percentile < 9	55.2%		
Omission errors ≥ 1 (χ^2)	16.2%		
Commission errors ≥ 5 (χ^2)	25.4%		

WCST – Wisconsin Card Sorting Test; TAVIS – Visual Attention Test.

($M = 16.7 \pm 12.3 > M = 12.6 \pm 11.2$ μg/g MnH, respectively), but the differences did not reach statistical significance (Mann–Whitney *U*; $p = 0.072$).

3.1. Neuropsychological performance and Mn

Table 3 shows the children's average performance in neuropsychological tests. The children's reaction time on TAVIS-III was, on average, 566 ms, ranging between 383 and 953 ms. Compared with Brazilian national standards, children in this study performed below average on reaction time (TAVIS-III). The percentile classification on reaction time was: 34.3% extremely low (percentile <2); 21.4% borderline (percentile 2 to <9); 15.7% low average (9 to <25); and 28.6% average or normal (percentile 25 to <75). We did not find significant differences between the percentile classification and levels of MnH. Moreover, some children exhibited scores below the expected average: 18.8% on IQ, 15.9% in Vocabulary, 38.6% in Block Design and 17.1% in Digit Span tests. In Corsi Blocks, 17.1% performed below average in a forward way and 31.4% in a backward way.

To further describe the dose-response relationships, we categorized MnH into tertiles and analyzed the neuropsychological performance by tertiles (T): lowest-T1 (0.5–8 μg/g), average-T2 (8–16 μg/g) and highest-T3 (>16 μg/g) (Table 2). Compared with the lowest tertile, the highest had significantly lower standardized scores on Estimated IQ, Vocabulary, Block Design, Digit Span (Fig. 1a). Children in the highest tertile exhibited significantly lower raw scores (correct answers) than the lowest tertile in backward Digits (Median T1 = 4 and T3 = 2; $p = 0.026$) and forward Corsi Blocks (Median T1 = 5.5 and T3 = 5; $p = 0.048$) (Fig. 1b). In TAVIS-III, commission errors ≥ 5 refer to a percentile ≤ 2 (this percentile represents a clearly impaired performance relative to standard references). The number of children who committed commission errors ≥ 5 was significantly higher in tertile 2 (31.8%) and tertile 3 (39.8%) compared to the lowest tertile (4.3%) (Fig. 2).

Table 4 shows the Spearman's correlation matrix considering the main variables in this study. Inverse correlations were found between MnH and estimated child IQ (rho = −0.448) and standardized scores on Vocabulary (rho = −0.272; $p < 0.05$), Block Design (rho = −0.485; $p < 0.01$), and Digit Span (rho = −0.410; $p < 0.01$). The number of correct sequences in backward Digit span was negatively correlated with MnH (rho = −0.296; $p = 0.01$). No

Table 2
Distribution of children's manganese hair levels.

Village	*N*	Mean (SD)	Percentiles – MnH μg/g						
			5%	10%	25%	50%	75%	90%	95%
Cotegipe	36	12.6 (11.2)	1.2	1.8	5.2	10.1	16.2	24.8	41.1
Santa Luzia	34	16.7 (12.3)	1.3	3.2	7.6	14.5	22.9	33.2	49.3
Total	70	14.6 (11.8)	1.4	2.9	6.6	11.5	19.1	28.8	42.9

Tertiles (T) – MnH	*N*	MnH μg/g
T1 Lower	24	0.5–8
T2 Middle	23	8–16
T3 Highest	23	>16

MnH: manganese in hair.

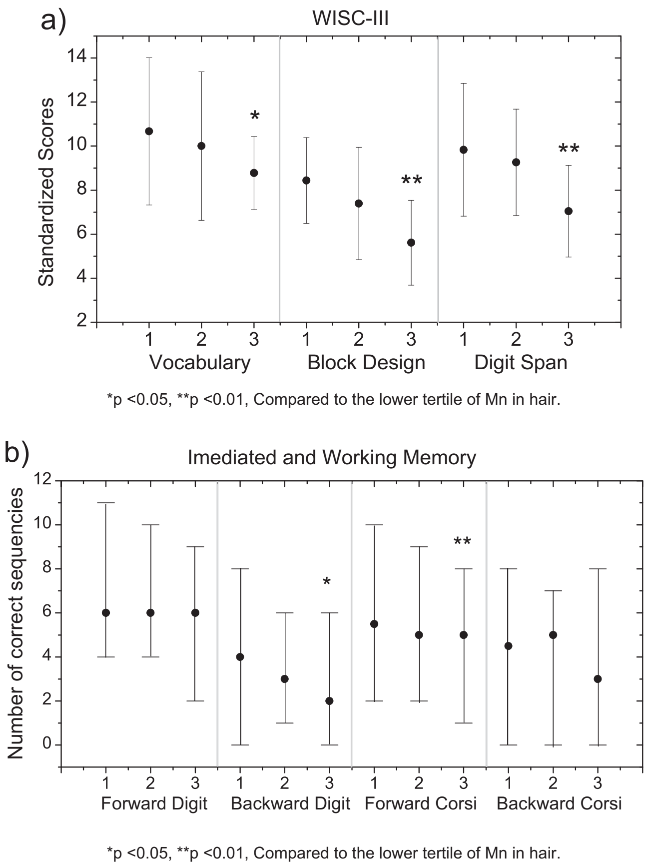

Fig. 1. Comparison of test scores between children with different Mn levels. Children were grouped by tertile levels of Mn in hair: lowest-1 (0.5–8 μg/g), average-2 (8–16 μg/g) and highest-3 (>16 μg/g). (a) WISC-III standardized scores (analyzed by Student's *t*-test; mean and SD); (b) forward and backward correct answers from the Digit Span and Corsi Block Tapping Tasks (analyzed by the Mann–Whitney *U* Test; median and range). $^{*}p < 0.05$, $^{**}p < 0.01$, Compared to the lower tertile of Mn in hair

significant correlations were found between MnH and other neuropsychological outcomes. Correlated inversely and significantly with child MnH were the maternal IQ and maternal education. No significant sex differences for concentrations of MnH were observed. The sex of the child was significantly correlated with omission and commission errors in the sustained attention task TAVIS-III, and we found no significant sex differences for other neuropsychological scores.

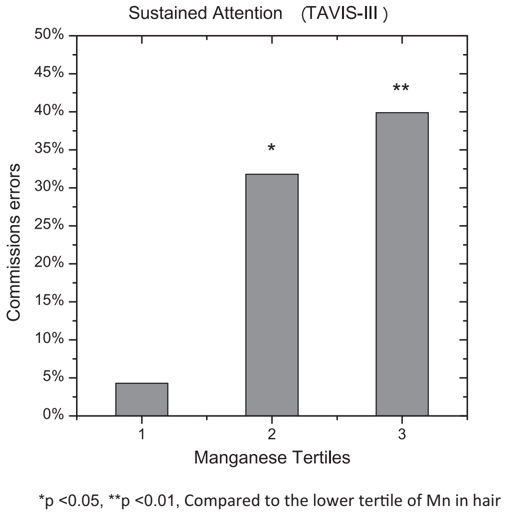

Fig. 2. Frequency comparison between children with different Mn levels that had commission errors ≥ 5. Children were grouped by tertile of Mn levels in hair: lowest-1 (0.5–8 μg/g), average-2 (8–16 μg/g) and highest-3 (>16 μg/g). Commission errors ≥ 5 refer to a percentile ≤ 2 (this percentile is regarded as a clearly impaired performance relative to standard references).

Significant negative correlations were found between age and estimated IQ and WISC-III standardized subtests scores accordingly general average population, which indicate that older children performed worse than younger children (Table 4). The analysis divided by age groups revealed that children 11–12 years old ($N = 21$) exhibited a moderate and positive correlation between MnH and omission errors (rho = −0.51; $p = 0.02$) in sustained attention task (data not shown). For children aged 7–8 years and 9–10 years, there were not significant correlations between MnH and the scores on the sustained attention task and WCST.

The results of the multiple regression analysis are shown in Table 5. We observed a significant negative association between Log transformed MnH and estimated IQ ($\beta = -9.67$; 95%CI = −16.97 to −2.37) and the standardized scores on Block Design and Digit Span ($\beta = -2.50$, 95%CI = −3.91 to −1.10; $\beta = -2.59$, 95%CI = −4.13 to −1.05, respectively). This means that independent of maternal education, for each 1 μg/g increase of Mn in hair, there could be a decrease of approximately 1.0 point in IQ and a decrease of 0.25 in the standardized scores of Block Design and Digit Span. There was a negative association between Log MnH and scores in the number of correct sequences in forward and backward Digit Span.

Table 4
Spearman's correlation matrix.

	1	2	3	4	5	6	7	8	9	10	11	12
1 Age (years)	1.00	0.19	−0.27*	−0.31*	−0.13	−0.29*	0.09	0.25*	−0.31**	−0.30*	−0.14	−0.26*
2 MnH		1.00	−0.45**	−0.27*	−0.49**	−0.41**	−0.30*	−0.12	−0.04	−0.11	−0.42**	−0.35**
3 Child IQ[a]			1.00	0.85**	0.79**	0.45**	0.33**	0.35**	−0.04	−0.10	0.49**	0.41**
4 Vocabulary[a]				1.00	0.38**	0.30*	0.20	0.27*	0.12	0.05	0.35**	0.31**
5 Block Design[a]					1.00	0.48**	0.40**	0.32**	−0.16	−0.23	0.45**	0.37**
6 Digit Span[a]						1.00	0.65**	0.39**	0.04	−0.23	0.43**	0.32**
7 Digit backward[b]							1.00	0.61**	−0.18	−0.23	0.46**	0.34**
8 Corsi backward[b]								1.00	−0.37**	−0.18	0.40**	0.25*
9 Time of reaction[b]									1.00	0.09	−0.11	−0.08
10 Perseverative errors (WCST) [b]										1.00	0.09	0.09
11 Maternal IQ											1.00	0.58**
12 Maternal education												1.00

WCST – Wisconsin Card Sorting Test.

[a] Standardized WISC-III Scores.

[b] Raw tests scores.

* $p < 0.05$.

** $p < 0.01$.

Table 5
Multiple regression coefficients for the associations between neuropsychology test scores and manganese hair levels ($N = 70$).

	Log MnH (μg/g)				
	β	SE	Beta	95% IC	
Standardized Scores					
WISC-III					
IQ (Estimated)[a]	−9.67[*]	3.66	−0.30	−16.97	−2.37
Vocabulary[a]	−0.74	0.90	−0.10	−2.53	1.05
Block Design[a]	−2.50[**]	0.71	−0.40	−3.91	−1.10
Digit Span[a]	−2.59[**]	0.77	−0.39	−4.13	−1.05
Raw Scores					
Immediate and Working Memory (correct answers)					
Digit Span forward[b]	−1.32[**]	0.46	−0.34	−2.23	−0.40
Digit Span backward[b]	−1.09[*]	0.47	−0.27	−2.02	−0.16
Corsi Blocks forward[b]	−0.31	0.52	−0.07	−1.34	0.72
Corsi Blocks backward[b]	−0.09	0.62	−0.02	−1.32	1.15
TAVIS-III (Sustained Attention) ($N = 68$)					
Reaction time[b]	0.00	0.07	0.00	−0.13	0.13
WCST ($N = 66$)					
Trials to complete first category[b]	0.27	5.83	0.01	−11.38	11.92
Perseverative errors[b]	−1.31	1.95	−0.09	−5.20	2.58

Note: values for Mn in hair have been log-transformed, MnH – manganese on hair.

[a] Adjustment for maternal education.

[b] Adjustment for maternal education and age.

[*] $p < 0.05$.

[**] $p < 0.01$.

4. Discussion

We found a statistically significant negative association between concentrations of MnH and intelligence, visuospatial organization and Digit Span scores. Previous studies have found negative associations between IQ and Mn in children who consumed water with high concentrations of Mn (Wasserman et al., 2006, 2011; Bouchard et al., 2011) and airborne exposure derived from industrial activity and mining (Riojas-Rodríguez et al., 2010; Menezes-Filho et al., 2011). Combining the results of 617 children from studies in Mexico, Brazil and Canada, researchers found a decrease in total IQ of 2.62 points for each increase of 10 units MnH μg/g (Roels et al., 2012). We use the estimated IQ based on only two WISC-III subtests, which confirmed previous results of studies that showed decreased total IQ related to Mn exposure through different exposure indicators (Wasserman et al., 2006, 2011; Riojas-Rodríguez et al., 2010; Bouchard et al., 2011; Menezes-Filho et al., 2011).

The verbal immediate and working memory scores showed a negative association with MnH levels. This negative association in Digit Span showed that the increase of 10 μg/g of Mn in children's hair may decrease 2.5 points in the standardized digit score and decrease in one correct sequence in forward and backward ways, after adjusting for covariables. Age and mother's formal education level were the main confounding variables. These results are in concordance with evidences that show that mother's education is associated with neuropsychological performance related to manganese exposure. They are also consistent with previous studies that showed impairments associated with Mn exposure in immediate memory (Torres-Agustín et al., 2012), working memory (Wasserman et al., 2011) and memory for histories and a word learning list (Wright et al., 2006).

The Digit Span test of the Wechsler scales has been classically used to identify alterations in immediate and working memory, as related to auditory attention and working memory, respectively (Lezak et al., 2004). Two studies, one in China (He et al., 1994) and another in Mexico (Riojas-Rodríguez et al., 2010), used the Digit Span subtest and found significantly lower performance in the Mn-exposed group when compared to a reference group. Despite the fact that these two studies used the Digit Span test, the decomposed forward and backward scores were not analyzed as in the present study. The study conducted in Mexico (Riojas-Rodríguez et al., 2010) used the WISC-R and the results showed that children in the Mn exposed-group obtained significantly lower scores on Digit Span subtest, but not in the Block Design and Vocabulary subtests.

We found an inverse association between concentrations of MnH and the performance on the Digit Span and Block Design subtests. In our study, the impairment in visuospatial organization may have been influenced by the children's skills in executive functions related to low self-monitoring and ineffective strategy choices for solving the task. In Bangladesh, a recent study with 299 children aged 8–11 years reported similar results, and there was negative association between levels of Mn in blood and the performance on the working memory subscale that was derived from the Digit Span and Letter-Number Sequencing subtests (Wasserman et al., 2011).

A study with non-human primates reported the significant effect on tasks of spatial and non-spatial working memory that resulted from chronic Mn exposure (Schneider et al., 2009). The authors noted that changes in performance were negatively correlated with the concentration of Mn in brain regions such as the frontal white matter, caudate, putamen and globus pallidus (Schneider et al., 2009). The results of this study, together with those of previous studies, suggest that exposure to Mn interferes with immediate recall that is dependent on auditory attention and alters the maintenance of online information and its effective use in mental operations. This latter process is known as working memory, which involves the ability to retain and manipulate information for a brief period (Alloway et al., 2006).

In the sustained task, we observed that the number of children who performed below the 2nd percentile increased in tertiles 2 and 3 of MnH levels compared to the lower tertile. We also identified a high frequency of children that performed below average in reaction time, suggesting that the speed of the response was slower in those children with chronic exposure to Mn, although we did not find an association between reaction time and levels of MnH. A high number of commission errors has been related in the literature to impulsivity and difficulty to inhibit predominant responses which can characterize an impairment in inhibitory control. It is probable that deficits in inhibitory control are exacerbated by environmental exposure to toxic agents. Like our study, the prospective study of Ericson et al. (2007) was the only one that used a continuous performance task (CPT) and aimed to correlate high levels of pre- and postnatal Mn absorption with behavioral disinhibition in a sample of 27 children. In this research, children with higher levels of Mn prenatally exhibited more impulsive errors in CPT at 4.5 years.

Low performance on EF tests has been associated in the literature to impulsivity and ADHD and the impairment of the fronto-striatal circuit (Semrud-Clikeman and Ellison, 2009; Aguiar et al., 2010). Slower reaction time in continuous performance tasks and a larger number of commission errors have been found in children with ADHD (Lezak et al., 2004; de Zeeuw et al., 2008; Coutinho et al., 2009; Miranda et al., 2012). Failures in inhibition may indicate an impairment in working memory (Semrud-Clikeman and Ellison, 2009). Performance on working memory and commission errors were related to MnH, and these results indicate that executive functions may be affected by chronic exposure to Mn. In addition, the storage capacity is related to processing speed, which means that the faster the system operates, the more it can process at once (Lezak et al., 2004). The slowed response required to detect the stimulus was determined in our study and we also observed that Digit Span decreases significantly in children with high levels of MnH.

We found no associations between WCST raw scores and the concentrations of MnH, even after adjusting for covariates. In this study, we used only the raw scores of the computerized version of the WCST with 64 cards, as normative data are not available for the Brazilian population. Performance data of Brazilian children were published for the computerized version of the WCST with 128 cards (Coelho et al., 2012), which did not allow us to compare studies. The WCST was reported by Woolf et al. (2002) in a case study of a 10-year-old boy that was exposed to Mn by drinking contaminated water, obtaining a performance within the average. The WCST evaluates the "Cold" component of EF and has been related to rational ability and abstraction (Malloy-Diniz et al., 2010), which is apparently not associated with Mn exposure.

The results of this study included children from the Santa Luzia and Cotegipe communities, in which mean MnH level was 14.6 μg/g, close to the levels observed previously (15.2 μg/g) in children's hair of the Cotegipe community (Menezes-Filho et al., 2011) The MnH reference level in the regular Brazilian population is 0.25–1.15 μg/g (Miekeley et al., 1998). The cross-sectional study of Mexican children had lower mean of MnH (12.6 μg/g), which was reported in three publications (Riojas-Rodríguez et al., 2010; Hernández-Bonilla et al., 2011; Torres-Agustín et al., 2012). The studies were conducted in the Mexican district of Molango, which is regarded as one of the largest deposits of manganese mining in the world, and the population's exposure is primarily airborne. Our study found levels of MnH in school age children that were consistent with previous studies whose exposure to Mn occurred by inhalation and in an environmental setting (Riojas-Rodríguez et al., 2010; Menezes-Filho et al., 2011).

Despite important advances that this study represents, we observed some limitations. The most important is the small number of children, due to the size of the communities and the number of eligible children, beside the fact that a control group could not be used. These facts limited our statistical analysis, reducing its power. Furthermore, not all neuropsychological tests used had developed standards for Brazilian children. In this case, it limited some analysis and the generation of standardized scores by age because children of varying ages were evaluated.

5. Conclusions

The results suggest that airborne Mn exposure may be associated with lower IQ and neuropsychological performance in tests of executive function of verbal working memory, inhibition responses and strategic visual formation. Executive function is dependent on the fronto-striatal circuit that may be disrupted by Mn accumulation in the brain.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neuro.2013.11.006.

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