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Effect of environmental manganese exposure on verbal learning and memory in Mexican children

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ABSTRACT

Manganese (Mn) is an essential metal, but in excess it becomes neurotoxic. Children's developing nervous system may be especially vulnerable to the neurotoxic effects of overexposure to this metal. The aim of this study was to assess the effect of Mn exposure on verbal memory and learning in 7- to 11-year-old children. We tested 79 children living in the Molango Mn-mining district and 95 children from a non-exposed community in the same State of Mexico. The Children's Auditory Verbal Learning Test (CAVLT) was administered. Blood and hair samples were obtained to determine Mn concentrations using atomic absorption spectrophotometry. CAVLT performance was compared between the two groups and multilevel regression models were constructed to estimate the association between biomarkers of Mn exposure and the CAVLT scores. The exposed group presented higher hair and blood Mn ($p < 0.001$) than the non-exposed group (median 12.6 vs. 0.6 μg/g, 9.5 vs. 8.0 μg/L respectively), as well as lower scores ($p < 0.001$) for all the CAVLT subscales. Hair Mn was inversely associated with most CAVLT subscales, mainly those evaluating long-term memory and learning ($\beta = -0.47$, 95% CI -0.84, -0.09). Blood Mn levels showed a negative but non-significant association with the CAVLT scores. These results suggest that Mn exposure has a negative effect on children's memory and learning abilities.

1. Introduction

Manganese (Mn) is an essential metal (Aschner et al., 2007), but under certain conditions, exposure to high amounts can be neurotoxic (Keen et al., 2005). Mn overexposure in adults has been associated with cognitive and psychiatric impairment (Guilarte and Chen, 2007). In highly exposed workers, it can cause a neurological disabling disorder similar to Parkinson's disease, called "manganism" (Calne, 1997). The most important neurotoxic effect caused by Mn exposure is damage to the basal ganglia after chronic inhalation of dust containing Mn in both animals and humans (Aschner et al., 2007; Calne, 1997). Other studies with non-human primates have reported that Mn also affects the prefrontal cortex (Dorman et al., 2006), resulting in motor and executive function impairment (Guilarte et al., 2006; Schneider et al., 2009, 2006).

Results reported for adult populations show motor and cognitive impairment due to Mn exposure. To date, most of the research concerning Mn neurotoxicity has been carried out primarily with occupationally exposed populations (Bowler et al., 2006a; Bowler et al., 2006b, 1999; Mergler et al., 1994; Zoni et al., 2007; Lucchini et al., 1995); few studies of environmental exposure have been performed (Mergler, 1998; Mergler et al., 1994; Rodríguez-Agudelo et al., 2006; Solís-Vivanco et al., 2009). In the workplace, there are high peak-exposure periods, while environmental exposure is lower but continuous (Hudnell, 1999). The neuropsychological effects of

Abbreviations: As, Arsenic; ATSDR, Agency for Toxic Substances and Disease Registry; CAVLT, Children's Auditory Verbal Learning Test; Cd, Cadmium; CI, Confidence Interval; CONAPO, Consejo Nacional de Población (National Population Council); EDTA, ethylene diamine tetraacetic acid; ICC, Intraclass Correlation; INEGI, Instituto Nacional de Estadística, Geografía e Informática (National Institute of Statistics, Geography and Informatics); INNNMVS, Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez" (National Institute of Neurology and Neurosurgery); INSP, Instituto Nacional de Salud Pública (National Institute of Public Health); IQ, Intelligence Quotient; g/dL, Grams per deciliter; MeV, Mega Electron Volt; μg/dL, Micrograms per deciliter; μg/L, Micrograms per liter; μg/g, Micrograms per gram; μg/m^3, Micrograms per cubic meter; Mn, Manganese; NMDA, N-methyl-D-aspartate; Pb, Lead; $PM_{2.5}$, Particles less than 2.5 μm in diameter; ppb, Parts per billion; UNAM, Universidad Nacional Autónoma de México (National Autonomous University of Mexico); USEPA, United States Environmental Protection Agency

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chronic environmental exposure to Mn (Mergler et al., 1999; Rodríguez-Agudelo et al., 2006) may be on a "continuum of dysfunction" (Mergler et al., 1999).

Children are considered especially vulnerable to neurotoxic agents (Grandjean and Landrigan, 2006; Weiss, 2000), because damage to specific brain structures in early development can result in impaired cognitive functions (Weiss, 2000). Lead, for example, is a confirmed neurotoxicant, and its detrimental effect on IQ has been the outcome most often studied (Needleman, 2006; Weiss, 2000). To date, there are few reports assessing Mn neurotoxic effects in children, and most of these studies have analyzed intellectual functions (Bouchard et al., 2011; Menezes-Filho et al., 2011; Riojas-Rodríguez et al., 2010; Menezes-Filho et al., 2009; Wasserman et al., 2006a, 2011; Wright et al., 2006b).

Some studies have reported impairment of other cognitive functions, such as memory and learning, in children due to Mn exposure. A study carried out in the United States (Wright et al., 2006) with 11- to 13-year-old children living close to waste deposits with high arsenic (As), Mn, and cadmium (Cd) content reported a negative association between As and Mn levels and the Children's Auditory Verbal Learning Test (CAVLT) scores. He et al. (1994) found low scores in short-term memory tasks, hand skills, and perceptual speed in children exposed to Mn-containing drinking water. These studies suggest a probable effect of Mn exposure on children's memory and learning, though the reported routes of exposure to the metal have been different, and this could represent variable effects in terms of cognitive damage. To our knowledge, there are no reports assessing memory and learning in children exposed to Mn by inhalation.

Mexico has one of the main Mn mining deposits in the world, the Molango basin located in the North of Hidalgo State. Inhalation is the main route of exposure in this geographic area, due to high concentrations of Mn-laden particles suspended in the air (Rodríguez-Agudelo et al., 2006). In previous studies of adults from communities close to the Mn deposits and nearby processing facilities, motor and cognitive impairment has been reported (Rodríguez-Agudelo et al., 2006; Solís-Vivanco et al., 2009).

The aim of this study was to estimate the association between verbal memory and learning and biomarkers of Mn exposure, measured as Mn levels of the metal in blood and hair samples, from children whose age ranged from 7 to 11 years living in a mining district of Hidalgo, Mexico, compared to a non-exposed group of children.

2. Methods

2.1. Study design and population

This study was carried out in two communities (Chiconcoac and Tolago) of the Molango mining district in the northern area of Hidalgo State. These communities are close (less than 1 km away) to a Mn-processing facility named Otongo. A cross-sectional study was designed to sample 100 resident boys and girls from 7 to 11 years old, who attended the same elementary school and had lived in the community for a minimum of 5 years. Ninety-five children, also attending elementary school in four communities (Chichicaxtle, El Palizar, Los Cubes, and Plan Grande) within the municipality of Agua Blanca, located 80 km away from the mining district but in the same State, were tested as a comparison group. These communities had similar socio-economic to the exposed group, based on the National Population Council marginalization index (CONAPO, 2005). Children with a diagnosis of neurological or psychiatric illness and/or physical problems that would prevent them from responding to the test were excluded from the study.

The project was approved by the Bioethics Committee from the National Institute of Neurology and Neurosurgery "Manuel Velasco Suárez" (INNNMVS) and the National Institute of Public Health (INSP), both in Mexico. Resident children and parents of the communities were invited to participate voluntarily during school meetings where the study was described. Mothers, as well as participating children, signed an informed-consent letter in which the objective and procedure were explained. Children fulfilling the required criteria and whose parent had signed the informed consent were given an appointment in the community health center where hair and blood samples were obtained and the neuropsychological test battery was administered.

2.2. Cognitive Assessment

The Children's Auditory Verbal Learning Test (CAVLT) was used (Talley, 1997). The Semantic nature of the stimuli makes CAVLT a reliable test, and insensitive to cultural bias (Talley, 1997). The test was translated from English into Spanish and then back translated for this study, and applied in a pilot study to Mexican children to confirm comprehension of the instructions. CAVLT administration and scoring was carried out by a trained psychologist. CAVLT consists of three word lists: two of free recall, which the child must evoke as accurate as possible after the test administrator reads them, and one of recognition, in which the child must tell whether or not the word listened was in the first list. Its administration takes approximately 25–30 min.

CAVLT is composed of the following subscales:

a) *Learning curve*: progression of learning, measured as the number of words, from the original 16-word list, the subject has repeats over the course of five trials. Normally, the curve shows a consistent increase from one trial to the next.
b) *Immediate recall*: susceptibility of new information to be disrupted. Low scores suggest new learning is vulnerable to interference from exposure to other material (an interference word list).
c) *Delayed recall*: long-term memory functioning and retrieval ability. Low scores suggest retrieval deficits.
d) *Recognition accuracy*: reflects recognition memory ability. Low scores suggest difficulty in the initial coding of information into long-term memory.
e) *Immediate memory span*: reflects memory function when the information exceeds short-term storage capacity. Poor performance suggests a deficit in short-term memory function.
f) *Level of learning*: long-term memory coding abilities. Low scores suggest a reduced rate of learning.

2.3. Socio-demographic questionnaire

Mothers of participating children were asked to complete two questionnaires, the first to collect socio-demographic data and the second to inquire about the child's development including information about pregnancy and birth.

2.4. Blood metal measurements

Venous Blood (5 ml) was obtained from the cubital vein in metal-free Vacutainer tubes with EDTA as anticoagulant. Samples were refrigerated during transport and until analysis. Mn and Pb Analysis were performed using an atomic absorption spectrophotometer (Perkin Elmer 3110) equipped with a graphite furnace (HGA-600). Results for Mn are shown in micrograms per liter (μg/L) and for Pb in micrograms per deciliter (μg/dL; Montes et al., 2008). Quality control of the analytical procedure was assured by using the external standard Bovine liver from NIST (1577b); this biological-matrix based, reference material was digested and analyzed in the same session as samples, valid analytical sessions were considered only if metal measurements were 95–100% from those reported from the provider in the analysis certificate. Samples were analyzed in duplicates with less than 10% standard deviation. Quantification limits were 0.5 μg/L for Mn and 1 μg/dL for lead.

2.5. Hair metal measurements

Hair was obtained along with blood samples. Samples of approximately 1 g of hair were taken from the occipital region as close as possible to the scalp and stored in plastic bags for transportation. Then, they were cut into small pieces, washed thrice with non-ionic detergent (2% Triton X-100), rinsed with deionized water, and finally dried at 60 °C. Hair samples of 0.3 g were placed in metal-free polyethylene tubes with 250 μl of nitric acid (Suprapur, Merck, Mexico) and kept at 60 °C with continuous shaking. Results for Mn are given in micrograms per gram of hair (μg/g; Mortada et al., 2002). For hair Mn analysis quality control, we also used biological matrix NIST 1577 material, a 95% confidence interval for mean Mn in the reference material was considered along with the Mn hair determination. Limit of quantification of Mn in hair was 0.01 μg/g.

2.6. Air manganese sampling and measurement

Indoor and outdoor $PM_{2.5}$ air sampling was performed over 24-hour periods using MiniVol samplers (version 4.2; Airmetrics, Eugene, Oregon, USA). Samples

were collected using impactors, with a flow rate of 5 L/min through 47-mm Teflon filters (Gelman R2PJ047).

Sampling was performed during the dry season, for 1 week in April 2007 in the four Agua Blanca communities and for 1 week in May and 1 week in June 2007 in the two Molango communities. Mn content in the filters was measured at the Physics Institute, National Autonomous University of Mexico (UNAM) using PIXE (Proton Induced X-ray Emission), with a 2.2-MeV proton beam produced by a 9SDH-2 Pelletron accelerator (National Electrostatics Corporation, Middleton, WI, USA; Cortez-Lugo et al., 2008). The detection system response was measured using thin film MicroMatter standards (Deer Harbor, WA, USA). In order to estimate the accuracy of the PIXE analyses, a different set of MicroMatter standards was used (with an error no larger than 0.7% for the elements detected in this study).

2.7. Statistical analysis

Descriptive statistics were used to characterize socio-demographic data, exposure biomarkers, and CAVLT scores. Metal exposure biomarker distribution was skewed, so we used the median and range to describe the data and the Mann Whitney U test to compare the two groups. Standardized CAVLT scores were derived from the published manual (Talley, 1997). The CAVLT test outcomes and other continuous socio-demographic variables were normally distributed, so parametric statistics were used. Standardized CAVLT scores were considered as the dependent variables to estimate their association with Mn-exposure biomarkers for constructing linear multilevel models. Multilevel analyses allow the simultaneous examination of the effects of individual- and group-level variables on individual health outcomes. A common rule of thumb was applied by using the multilevel modeling when the intraclass correlation (ICC) was greater than 0.05 (Hox, 2002). A sex-stratified analysis was performed.

The information for potential covariates came from a maternal interview including biologically relevant variables, as reported previously in the literature. Models were adjusted for the same set of variables as in our previous report: children's sex, blood Pb (μg/dL), age (years), hemoglobin (g/dL), and maternal education (Riojas-Rodríguez et al., 2010). A significance level of 0.05 was specified. We examined residuals for normality and homoscedasticity to verify the linear regression assumptions. All of the analyses were performed with the statistical software Stata version 9.2 (Stata Corp., College Station, TX, USA).

3. Results

The final sample included 79 exposed and 95 non-exposed children with complete data (CAVLT scores and exposure biomarkers). Table 1 shows the description of the study population, including socio-demographic variables and exposure biomarkers for both groups. No significant differences in the proportion of girls and boys between the groups were observed, but a significant difference was observed for average age, with the exposed group was older than the non-exposed. There were no significant differences between groups for the children's education and maternal age. The maternal educational level of the exposed group was somewhat lower than the comparison group, but the differences did not reach statistical significance. On average, both outdoor and indoor area $PM_{2.5}$ Mn concentrations were higher where the exposed children lived compared to the comparison group, and the air Mn levels often surpassed the United States Environmental Protection Agency (USEPA) reference concentration for Mn (0.05 $\mu g/m^3$).

As shown in Table 1, a statistically significant difference was observed between the two groups in the median blood Mn levels of 8.0 and 9.5 μg/L for non-exposed and exposed children, respectively. Hair Mn levels in exposed children were, on average, 20 times higher (median 12.6 and mean 14.2 μg/g) than the non-exposed group (median 0.6 and mean 0.73 μg/g). Hair and blood Mn levels showed a low but significant correlation (Spearman rho = 0.22, $p < 0.01$). The comparison group presented significantly higher blood Pb levels than the exposed group.

Crude CAVLT scores showed significant differences between the two groups for the five-trial word list (Learning Curve), and for all the test subscales (Table 2). The exposed group average test result was in the low normal range, while the comparison group scored within the normal range (Talley, 1997). No significant differences were observed between girls and boys for the crude scores. Adjusted scores for relevant covariates are shown in Table 2. The adjusted values better reflect the difference that could be attributed to Mn exposure. The adjusted scores of the long-term memory test (trial 4, trial 5, immediate and delayed recall, level of learning) are, on average, lower for the exposed than for the non-exposed children,

Multiple regression results confirmed the association between Mn exposure and long-term memory test scores (Table 3). Hair Mn levels showed a strong, negative association, suggesting a deficit in long-term memory encoding abilities. A negative but non-significant association was observed between blood Mn levels and the CAVLT scores. When only the exposed group was examined, the results were in the same direction and of similar magnitude to those presented in Table 3 for the whole population, but non-significant (data not shown).

The negative association between hair Mn and long-term memory test scores (trial 4, trial 5, immediate recall, level of learning) was stronger for girls than boys, as can be observed in Fig. 1.

Table 1
Socio-demographic characteristics and exposure biomarkers by group.

Characteristics	Comparison group $N=95$		Exposed group $N=79$		*p*-Value
Children	**Mean (SD)**	%	**Mean (SD)**	%	
Girls		51.6		46.8	0.53
Age (years)	9.1 (1.5)		**9.8 (1.3)**		**0.002**
Education (years)	3.1 (1.4)		3.5 (1.3)		0.09
Hemoglobin (g/dL)	14.1 (0.9)		13.7 (0.8)		0.001
Families					
Maternal age (years)	34.7 (6.0)		35.0 (7.0)		0.77
Maternal education (years)	5.8 (3.2)		4.9 (3.7)		0.08
Mother married or living together		93.3		86.1	0.12
Housewife		89.0		92.3	0.45
Paternal education (years)	5.2 (3.4)		6.1 (3.8)		0.52
Exposure biomarkers	**Median (range)**		**Median (range)**		
Hair Mn (μg/g)	0.6 (0.06–3.6)		12.6 (4.2–48)		< **0.001**
Blood Mn (μg/L)	8.0 (5.0–14.0)		9.5 (5.5–18.0)		< **0.001**
Blood Pb (μg/dL)	8.0 (1.8–22.5)		3.3 (0.5–13.5)		< **0.001**
Outdoor air Mn ($\mu g/m^3$)	0.02 (0.003–0.09)		0.08 (0.02–0.24)		< **0.001**
Indoor air Mn ($\mu g/m^3$)	0.02 (0.005–0.03)		0.07 (0.01–0.31)		**0.008**

Table 2
Crude and adjusted[a] standard CAVLT scores by group, mean (standard deviation).

Trials and summary scales	Crude scores			Adjusted scores		
	Exposed group	Comparison group	*p*-Value	Exposed group	Comparison group	*p*-Value
Trial 1	85.4 (15.8)	94.9 (21.2)	< 0.001	92.1 (4.7)	90.1 (5.5)	0.010
Trial 2	88.5 (14.6)	100.1 (14.4)	< 0.001	94.6 (3.8)	95.0 (4.0)	0.441
Trial 3	88.1 (16.7)	100.1 (14.4)	< 0.001	94.2 (3.8)	95.1 (4.0)	0.124
Trial 4	88.6 (17.1)	104.7 (14.0)	< 0.001	96.4 (3.6)	98.2 (3.4)	0.001
Trial 5	90.5 (16.5)	105.7 (14.7)	< 0.001	97.7 (4.1)	98.8 (3.9)	0.073
Immediate memory span	82.9 (14.1)	93.2 (17.5)	< 0.001	89.7 (3.4)	87.5 (3.5)	< 0.001
Level of learning	87.9 (16.2)	103.8 (13.8)	< 0.001	95.7 (3.9)	97.1 (3.8)	0.019
Immediate recall	92.5 (16.4)	103.3 (15.0)	< 0.001	98.2 (2.3)	99.2 (2.4)	0.007
Delayed recall	92.9 (16.4)	102.0 (14.7)	< 0.001	97.4 (2.7)	99.0 (3.4)	0.001
Recognition accuracy[b]	27.9 (2.7)	29.2 (2.7)	0.002	28.9 (1.0)	28.3 (1.0)	< 0.001

[a] Adjusted for blood Pb (μg/dL), age (years), sex, hemoglobin (g/dL), and maternal education.
[b] For the recognition accuracy scale, the raw score is used.

Table 3
Association between Mn-exposure biomarkers and standard CAVLT scores, adjusted linear multilevel models.

Standard scores[a]	Hair Mn (μg/L)				Blood Mn (μg/g)			
Trials	Coeff	*p*-Value	95% CI	ICC	Coeff	*p*-Value	95% CI	ICC
Trial 1	0.14	0.581	−0.35, 0.62	0.19	−0.01	0.988	−1.23, 1.21	0.15
Trial 2	−0.21	0.260	−0.58, 0.16	0.13	−0.44	0.359	−1.39, 0.50	0.18
Trial 3	−0.34	0.087	−0.72, 0.05	0.07	−0.37	0.475	−1.39, 0.65	0.15
Trial 4	−0.48	0.019	−0.88, −0.08	0.11	−0.62	0.236	−1.66, 0.41	0.24
Trial 5	−0.50	0.010	−0.88, −0.12	0.08	−0.05	0.930	−1.06, 0.97	0.24
Summary scales								
Immediate memory span	−0.06	0.777	−0.48, 0.36	0.20	−0.14	0.788	−1.20, 0.91	0.21
Level of learning	−0.47	0.016	−0.84, −0.09	0.11	−0.37	0.464	−1.35, 0.62	0.25
Immediate recall	−0.41	0.033	−0.78, −0.03	0.02	−0.17	0.755	−1.22, 0.89	0.12
Delayed recall	−0.25	0.196	−0.62, 0.13	0.03	−0.44	0.401	−1.48, 0.59	0.06
Recognition accuracy[b]	−0.03	0.379	−0.09, 0.04	0.13	−0.01	0.860	−0.18, 0.15	0.18

Coeff: coefficient; 95% CI: 95% confidence interval; ICC: intraclass correlation

[a] Adjusted for blood Pb (μg/dL), age (years), sex, hemoglobin (g/dL), and maternal education.
[b] For the recognition accuracy scale, the raw score is used.

4. Discussion

The main finding of this study was the negative association between hair Mn levels and long-term memory and learning abilities, evaluated with the CAVLT test in environmentally exposed children.

Previous results by He et al. (1994), Zhang et al. (1995), and Wasserman et al. (2006b) reported similar results, but in children exposed to Mn-containing water. In the present study, air-borne Mn is the primary source; and water Mn concentrations in the region has been shown to be low (Rodríguez-Agudelo et al., 2006). In our study, the difference between hair Mn from exposed versus non-exposed children was much larger than the corresponding difference in blood Mn levels. Hair Mn levels found in exposed children in this study were higher compared to other studies. He et al. (1994) and Bouchard et al. (2007) found mean concentrations of 1.25 μg/g and 6.2 μg/g, respectively, in water Mn-exposed children, whereas Wright et al. (2006) reported 0.47 μg/g concentrations in hair from children living close to an industrial waste site. Only one study in Brazil has reported higher hair Mn levels (Menezes-Filho et al., 2009) than those found in the present study. It is noteworthy that children in that sample were also exposed to Mn by inhalation. These results suggest that Mn hair levels are on average higher in air-borne Mn exposure studies than those reported for water-borne Mn exposure. Blood Mn levels showed a low but statistically significant difference between groups. Mean blood Mn in the exposed group was 10.04 μg/L, near the guideline level (10 μg/L) established by the ATSDR (2000). Other studies have found similar blood Mn levels for children exposed to environmental Mn. Wasserman et al. (2007) and Röllin et al. (2005) reported mean blood Mn levels of 12.8 μg/L, and 9.8 μg/L, respectively. In our study, blood Mn levels were significantly correlated with hair Mn levels. It has been proposed that hair Mn concentration may constitute a reliable marker for chronic Mn exposure (Rodrigues and Batista, 2008), whereas blood Mn levels may provide a better indication of recent or severe exposure (Aschner et al., 2007; Montes et al., 2008).

Wright et al. (2006) reported that low scores in CAVLT significantly correlated with hair Mn levels in children. He et al. (1994) reported an inverse association between hair Mn and cognitive functions, such as short-term memory in 11- to 13-year-old children exposed to Mn in drinking water. In a single case study, Woolf et al. (2002) reported impairment in verbal and visual memory of a child exposed to water containing high Mn levels. Likewise, Pihl and Parkes (1977), and Collip et al. (1983) reported high hair Mn concentrations in children with learning difficulties. Mergler et al. (1999) found that blood Mn was associated with defective learning and memory in environmentally exposed adults.

Previous results suggest that Mn exposure affects specific cognitive functions in children additional to memory and learning, such as intellectual (Riojas-Rodríguez et al., 2010; Wasserman et al., 2006b, Wasserman et al., 2011) and behavioral areas (Bouchard et al., 2007). Negative effects on working memory have also been observed in non-human primates exposed to

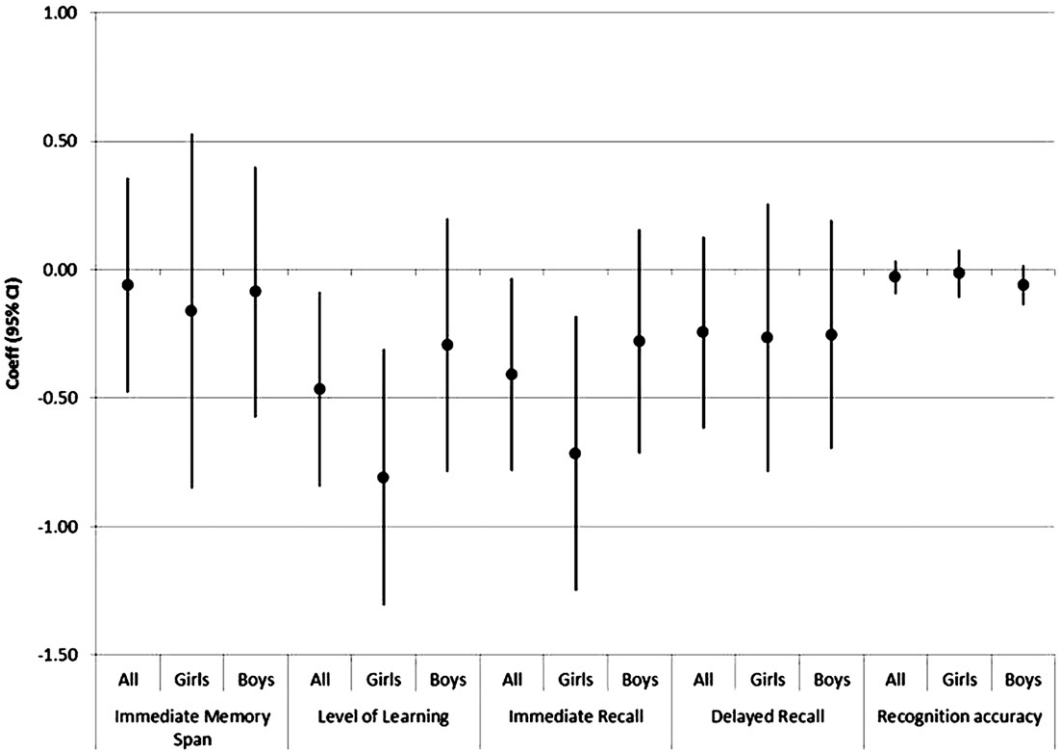

Fig. 1. Adjusted coefficients of the association between hair Mn and CAVLT scores for girls and boys.

manganese sulfate: Schneider et al. (2009) showed a deficit in a working-memory task after Mn exposure.

Although the neurobiological mechanisms by which Mn affects these functions are not fully understood, Guilarte and Chen (2007) have proposed an inhibitory effect of Mn on the N-methyl-D-aspartate (NMDA) receptors, needed to consolidate learning, as a possible explanation. In addition, dopamine, the most-studied neurotransmitter affected by Mn (Fitsanakis et al., 2006), is capable of modulating synaptic plasticity related to NMDA receptors, specifically long-term potentiation in regions such as the hippocampus, striatum, and prefrontal cortex, consequently affecting learning and memory (Jay, 2003). However, mechanistic studies are needed to explain the neurobiological basis of the cognitive effects associated with Mn exposure.

The population studied here is a representative of rural populations with low educational and socioeconomic status (SES) in Mexico (INEGI, 2004). This is an important consideration because the distribution of SES is lower overall compared to the normative groups reported for the neuropsychological tests (Ardila and Ostrosky-Solís, 1991; Herrans and Rodríguez, 1992; Sattler, 2001). It is likely that SES presents an additional factor of vulnerability to Mn exposure. Populations living in poor settings are frequently exposed to high environmental pollution (Evans, 2004) and to an unfavorable economic and social environment, which may interact or exacerbate the effect of toxic environmental agents (Koger et al., 2005), especially during childhood (Weiss, 2000). For example, it is known that low-iron diets favor high Mn levels in the organism, especially in brain (Chandra and Shukla, 1976; Finley and Davis, 1999), increasing children's susceptibility to the toxic effects of Mn (Ali et al., 1983).

Our cross-sectional study design does not allow establishing the temporal relationship between Mn exposure and cognitive function at school age. We cannot establish if the associations presented here resulted from specific exposure windows during pregnancy or childhood. To answer this question, it would be necessary to follow a birth cohort measuring prenatal and postnatal Mn exposure. Such a follow-up has been performed for lead exposed children and the age of greatest susceptibility to childhood lead exposure has been discussed (Hornung et al., 2009).

The strengths of this study are the inclusion of a comparison group with similar socio-economic and demographic conditions but not exposed to Mn, the adjustment of the models for potentially confounding variables (Mink et al., 2004), and the comparison of both blood and hair Mn levels with cognitive function.

The cognitive effects described show the need for risk management programs as well as Mn emission regulations in order to prevent or reduce the health impact on poor residents living close to sources of Mn emission.

In conclusion, results from the present study suggest a Mn effect on memory and learning for environmentally exposed school children. This study provides further evidence that Mn exerts toxic effects on children's cognitive development.

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