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Prenatal manganese exposure and intrinsic functional connectivity of emotional brain areas in children

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ABSTRACT

Manganese (Mn) is an essential trace metal that is neurotoxic at high levels of exposure. Disruption of brain maturation processes during the prenatal period may have lasting consequences. During this critical period, the developing human brain is uniquely vulnerable to exposure to environmental toxicants such as Mn, and prenatal Mn exposure has been associated with changes in brain areas involved in emotion processing and regulation. The goal of the present pilot study was to examine whether prenatal Mn exposure is associated with changes in the intrinsic functional connectivity (iFC) of the brain in childhood, focusing on changes in emotional brain areas. We selected 15 subjects (age 6–7 years) from an ongoing longitudinal birth cohort study to participate in a resting state functional magnetic resonance imaging (fMRI) study. Prenatal Mn exposure was determined from maternal blood collected during the 2nd and 3rd trimesters of pregnancy. We used seed-based correlation analyses and independent component analyses to examine whether prenatal Mn exposure was associated with the iFC of the brain in children. We found that the right globus pallidus showed reduced iFC with the dorsal anterior cingulate cortex and lateral prefrontal cortex in children who were exposed to higher prenatal Mn levels, after controlling for sociodemographic confounders (SES, maternal education, child sex, home environment support) and environmental confounders (prenatal lead exposure and air pollution). These findings suggest that prenatal Mn exposure is associated with reduced iFC of brain areas involved in emotion processing and regulation in children. Future studies should investigate whether this reduced iFC mediates the association between prenatal Mn exposure and emotional dysfunction in childhood.

1. Introduction

Manganese (Mn) is one of the most prevalent metals on earth (Roth, 2006). Widely used in industrial settings resulting in occupational exposure in adults, the general population is exposed to Mn through inhalation, dietary intake and drinking of contaminated water (Wright and Baccarelli, 2007). As an essential trace metal, homeostatic levels of Mn are required for a variety of enzymatic and cellular processes within the human body, but levels outside of the homeostatic range can be neurotoxic (Roth, 2006). Neurotoxic effects of excess Mn include oxidative damage to neuronal cells, dopaminergic dysfunction and changes in the function of astrocytes (Normandin and Hazell, 2002). Extensive literature demonstrates the neurotoxic effects of excess Mn exposure in adults, particularly among exposed occupational workers (Mergler and Baldwin, 1997; Registry AfTSaD, 2017). In adults, excess Mn characteristically accumulates in the basal ganglia and exposure is most commonly associated with clinical signs and symptoms resembling Parkinson's disease (termed manganism) (Bowler et al., 2006). While non-occupational exposure to Mn is often lower than occupational exposure, a rapidly growing body of literature reveals the complexity of associations between early life Mn exposure and adverse neurodevelopmental outcomes (Zoni and Lucchini, 2013).

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During prenatal and early postnatal periods, the developing human brain is uniquely vulnerable to exposure to environmental toxicants (Rice and Barone, 2000; Rodier, 1995). Human brain development is a protracted process beginning early in pregnancy that relies on the temporal and regional emergence of critical developmental processes (i.e., proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis) (Rice and Barone, 2000; Rodier, 1995; Osmond and Barker, 2000). The complexity and extent of human brain development throughout early life (Faustman et al., 2000) result in a unique susceptibility to environmental chemicals, including metals such as Mn, which can override a normal growth trajectory towards a maladaptive phenotype (Grandjean and Landrigan, 2006, 2014). Pregnancy is a period of rapid growth and cell differentiation for both the mother and fetus and is associated with increased demand of many micronutrients including Mn (Al-Jameil et al., 2014). In humans, blood Mn levels increase markedly during pregnancy, peaking in the 3rd trimester (Al-Jameil et al., 2014). Mn is actively transported across the placental barrier (Adinolfi, 1985), and accumulates in fetal and neonatal tissue (Fechter, 1999). Infants and children absorb and retain a larger fraction of Mn than adults (Fechter, 1999; Aschner and Aschner, 2005; Yoon et al., 2011) and the fetal blood-brain barrier provides only partial protection against Mn (Aschner and Aschner, 1991; Aschner, 2000; Ljung and Vahter, 2007). Brain areas implicated in emotion processing and regulation, including the prefrontal cortex (PFC), anterior cingulate cortex (ACC), insula, basal ganglia, and parietal cortex (Ochsner et al., 2012), are particularly vulnerable to prenatal and early-life exposure to Mn (Aschner et al., 2015). Indeed, prenatal exposure to Mn has been linked to deficits in emotion processing and regulation in childhood, such as increased internalizing (e.g., anxiety and depression) and externalizing (e.g., aggression) symptoms (Khan et al., 2011; Mora et al., 2015), behavioral disinhibition (Ericson et al., 2007) and hyperactivity (Mora et al., 2015).

While a growing number of researchers have examined associations between prenatal Mn exposure and behavioral outcomes including emotion regulation (Khan et al., 2011; Mora et al., 2015; Ericson et al., 2007), the effects of prenatal Mn exposure on the functioning of brain areas subserving emotion processing and regulation in children remain poorly understood. Studying these neural mechanisms would improve our mechanistic insights into the effects of prenatal Mn exposure on emotional dysfunction in childhood, which is a growing public health problem (Belfer, 2008). One recent functional magnetic resonance imaging (fMRI) pilot study explored the effects of Mn exposure on brain function in teens (Iannilli et al., 2016). Iannilli et al. (2016) found that teens raised in an Mn contaminated area showed reduced activity of brain areas involved in emotion processing and regulation during olfactory stimulation, compared to teens who were not exposed to Mn. Specifically, reduced activation of the dorsolateral PFC (DLPFC), parietal cortex and insula was observed in Mn-exposed teens.

The goal of the present study was to investigate whether prenatal Mn exposure is associated with intrinsic functional connectivity (iFC) of the brain (Biswal et al., 1995; Fox and Raichle, 2007) in children aged 6–7 years. These children were selected from the ongoing longitudinal birth cohort study PROGRESS (Braun et al., 2014; Burris et al., 2013) in Mexico City. We focused on prenatal Mn exposure specifically, because: 1) Mn is an essential nutrient and ubiquitous in the environment leading to widespread exposure (Roth, 2006); 2) while literature demonstrates associations between early life Mn exposure and adverse neurodevelopmental sequelae, the neural mechanisms underlying Mn neurotoxicity are poorly understood (Sanders, 2015); 3) it is highly relevant to study the effects of prenatal Mn exposure, since Mn concentrations increase threefold during pregnancy and it is actively transported across the placenta (Adinolfi, 1985; Zota et al., 2009); 4) Mn exposure in Mexico is higher than in the US and Canada (Santos-Burgoa et al., 2001), which makes the PROGRESS cohort uniquely poised to examine the effects of prenatal Mn exposure on iFC. We used resting state fMRI (rs-fMRI) to measure associations between spontaneous fluctuations in blood oxygen level dependent (BOLD) activity at rest, in the absence of a cognitive task (Kelly and Castellanos, 2014; Smyser et al., 2011). An rs-fMRI scan is used to measure correlations between distinct areas of the brain at rest, allowing one to focus on the iFC of distributed networks, instead of only focusing on activity of isolated brain areas (Kelly and Castellanos, 2014; Smyser et al., 2011). Moreover, rs-fMRI is a promising method to use in young children, as a scan lasting only a couple of minutes is administered, and participants are not required to perform a specific task (Smyser et al., 2011). For the present study, we used both theory-driven (i.e., seed-based correlation analyses) and data-driven (i.e., independent component analysis) methods to assess iFC of the brain in children aged 6–7 years old. Given that Mn is both an essential nutrient (at low levels of exposure) and a neurotoxicant (at higher levels of exposure) (Roth, 2006), we examined both linear and quadratic associations between prenatal Mn exposure and iFC of the brain in childhood, since quadratic associations between prenatal and early postnatal Mn exposure and neurodevelopmental outcomes have been reported (Chung et al., 2015; Claus Henn et al., 2010).

2. Materials and methods

2.1. Participants

A sample of 20 children were selected from the ongoing longitudinal birth cohort study PROGRESS (Programming Research in Obesity, Growth, Environment and Social Stressors) to participate in this pilot neuroimaging study. Original enrollment into the PROGRESS cohort is described at length elsewhere (Braun et al., 2014; Burris et al., 2013). Briefly, between July 2007 and February 2011, women attending a prenatal consult in 4 clinics belonging to the Mexican Social Security System (IMSS) in Mexico City were approached for enrollment. If women were in their first trimester, they completed a screening questionnaire and, if eligible, were invited to participate in the study. Inclusion criteria considered: being $<$20 weeks pregnant, $\geq$18 years old (Mexican legal voting age), being heart or kidney disease free, having access to a telephone, planning to reside in Mexico City for the next 3 years, no use of steroids (including glucocorticoids) or anti-epilepsy drugs, and not consuming alcohol on a daily basis (Braun et al., 2014). At each visit the study protocol was explained to women, who provided informed consent before any procedure was carried out. From the 760 mother-infant pairs actively enrolled in PROGRESS we selected 20 subjects with the following criteria; 1) a 2nd and/or 3rd trimester maternal blood sample analyzed for metals and 2) a neurodevelopmental assessment completed at 5-years of age.

Socioeconomic status (SES) was calculated based on an index created by the Mexican Association of Market and Public opinion Research Agencies (Spanish acronym AMAI) using 13 variables derived from questionnaire results (education of the head of household, number of rooms, number of bathrooms with showers, type of floor, number of light bulbs, ownership of car/hot water/automatic washing machine/videocassette recorder/toaster/vacuum cleaner/microwave oven/personal computer) (Carrasco, 2002). Participants were classified as coming from a family of either low, middle or high SES (Rodosthenous et al., 2016; Stroustrup et al., 2016). Maternal education was defined as low ($<$high school), medium (high school) or high (>high school). The Infant/Toddler version of the Home Observation for Measurement of the

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Environment (HOME) Inventory (Bradley et al., 2003) was administered when subjects were 2 years old, to measure the quality and quantity of stimulation and support available to the child in the home environment.

All procedures of the pilot neuroimaging study were approved by the institutional review boards of the Icahn School of Medicine at Mount Sinai, Harvard T. H. Chan School of Public Health, the National Institute of Public Health Mexico, the Mexican Social Security System, and the National Institute of Perinatology, Mexico.

2.2. Blood manganese measurements

Venous whole blood samples were collected from the mothers of participants in trace metal-free tubes during the 2nd (between the 16th and 20th gestational weeks) and 3rd (between the 30th and 34th pregnancy weeks) trimesters of pregnancy (Tamayo et al., 2016). Manganese was measured with a dynamic reaction cell/inductively-coupled plasma mass spectrometer (ICP-MS) (Elan 6100; PerkinElmer, Norwalk, CT) using previously described methods and quality control measures (Zota et al., 2009).

For subjects with both 2nd and 3rd trimester blood Mn concentrations ($n = 18$), we averaged the two measures and used the mean level in our analyses. For subjects with only 2nd trimester blood manganese ($n = 2$), we used the available sample. 2nd and 3rd trimester blood Mn concentrations were highly correlated ($rho = 0.55$, $p = 0.017$). Blood Mn concentrations were skewed (Shapiro-Wilk statistic = 0.83, $p = 0.012$) and therefore log-transformed for analyses.

2.3. Environmental confounders

Lead (Pb) exposure is positively correlated with Mn exposure and Mn-Pb interactive effects on children's neurodevelopment have been reported (Claus Henn et al., 2012). We therefore assessed maternal blood Pb concentrations during the 2nd and 3rd trimesters of pregnancy. Data collection methods and quality control measures are described in detail elsewhere (Braun et al., 2014; Stroustrup et al., 2016; Tamayo et al., 2016). For subjects with both 2nd and 3rd trimester blood Pb concentrations ($n = 18$), we averaged the two measures. For subjects with only 2nd trimester blood Pb ($n = 2$), we used the available sample. Blood Pb concentrations were skewed (Shapiro-Wilk statistic = 0.72, $p < 0.001$) and therefore log-transformed for analyses.

Air pollution is another important potential environmental confounder, as Mn is often a component of air pollution which could lead to inhalation exposure (Wright and Baccarelli, 2007). We estimated ambient fine particulate matter ($PM_{2.5}$ concentrations ($\mu g/m^3$)) based on subjects' residential address during enrollment (2nd trimester of pregnancy), using a hybrid satellite-land use regression model (Just et al., 2015). Estimated daily $PM_{2.5}$ concentrations were averaged for the 2nd and 3rd trimesters of pregnancy.

2.4. MRI data collection

MRI data were collected using a Philips Achieva 3T scanner equipped with an 8-channel head coil (Sense Head 8) at the Centro Nacional de Investigación en Imagenología e Instrumentación Médica (Ci3M) in Mexico City.

For registration purposes, an anatomical T1-weighted scan was collected using an MPRage sequence (301 vols, TR = 7.45 ms, TE = 3.44 ms, FOV = 25 cm, Matrix = 256 × 256, slice thickness = 1.2 mm, slice gap = 0.6 mm). Participants watched an age-appropriate cartoon video during the T1 acquisition.

In order to measure instrinsic functional connectivity, a 10 min rs-fMRI scan was collected using a Field Echo-EPI gradient pulse (300 vols, TR = 2000 ms, TE = 27 ms, slice thickness = 3 mm, 37 slices, ascending slice acquisition, FOV = 22 cm, Matrix = 80 × 80).

2.5. Resting state fMRI data analyses

2.5.1. Exclusion of participants

From 20 children invited to participate in the pilot imaging study, data from 15 was included in analyses. Two participants were excluded from the rs-fMRI analyses because the lateral PFC and superior parietal cortex were not covered in their collected images. Further, we excluded three additional participants because of excessive head motion during the rs-fMRI scan. Even small head movements have been shown to influence functional connectivity measures. Specifically, motion leads to overestimation of short-distance connections and underestimation of long-distance connections (Power et al., 2012; Van Dijk et al., 2012). Therefore, we used the fsl_motion_outliers tool (implemented in FSL version 6.00) to determine volumes that were corrupted by excessive motion based on the stringent threshold described in Power at al. (Power et al., 2013): relative framewise displacement (FD) > 0.2 mm. Participants were excluded from the analyses if removal of these motion corrupted volumes resulted in having less than 4 min (120 vols) of useable data (Satterthwaite et al., 2013).

Thus, 15 participants (7 girls) were included in all analyses described below.

2.5.2. Seed-based correlation analyses

Preprocessing was carried out using FEAT Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The first two volumes were discarded to allow for T1-equilibration effects. We performed motion correction using MCFLIRT (Jenkinson et al., 2002), slice-timing correction using Fourier-space time-series phase-shifting, non-brain removal using BET (Smith, 2002), spatial smoothing using a Gaussian kernel of 6 mm FWHM, and highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 100.0 s). Registration of functional images to participants' high resolution structural images was carried out using FLIRT (Jenkinson et al., 2002; Jenkinson and Smith, 2001). Given participants' young age, we created a study-specific template by averaging participants' high resolution structural images and registered this study-specific template to standard space (MNI-152) using FNIRT nonlinear registration (Andersson et al., 2007a, 2007b). Next, we registered participants' functional and high resolution structural images to this standard space, study-specific template using FNIRT.

We used FSL's Featquery to extract the mean timeseries of each seed region in participant's native space focusing on 6 brain regions shown to be affected by prenatal and early-life Mn exposure in prior research (Aschner et al., 2015; Iannilli et al., 2016; Dion et al., 2016). Specifically, we selected four seeds from the probabilistic Harvard-Oxford Cortical Structural Atlas (Kennedy et al., 1998): bilateral ACC, bilateral insula, bilateral middle frontal gyrus, and bilateral superior parietal lobule. We further selected two seeds from the probabilistic Harvard-Oxford Subcortical Structural Atlas (Lieberman and Cunningham, 2009): right and left globus pallidus. Using FEAT, the extracted timeseries of each seed was included as a predictor in a lower-level multiple regression analysis for each participant and seed separately, which produced Z-value correlation maps of all voxels that positively and/or negatively correlated with the seed timeseries. This analysis was carried out using FILM with local autocorrelation correction (Woolrich et al., 2001). In order to control for the confounding effects of head motion, we included 24 motion parameters (Friston et al., 1996) as nuisance regressors, and also regressed out volumes

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that were corrupted by excessive motion (relative FD > 0.2 mm; i.e., motion scrubbing).

Group-level analyses were carried out using a mixed-effects model implemented in FSL FLAME (stage 1). The general linear model included the mean-centered linear and quadratic effects of Mn exposure as predictors. Statistic images were thresholded using clusters determined by Z > 2.3 and cluster-corrected (using Gaussian Random Field theory) threshold of $p < 0.05$.

2.5.3. Independent component analysis (ICA)

We used ICA-AROMA (Pruim et al., 2015) to first denoise the data by regressing out motion components. Denoised data were preprocessed for the ICA with FSL MELODIC ICA version 3.14, using the same preprocessing steps that were used for the seed-based correlation analyses. Multi-session temporal concatenation was performed to obtain group-level average spatial maps. We limited the output of this analysis to 20 components. Visual inspection of these components revealed 12 components that were core resting state networks: a default mode network, a fronto-temporal-occipital network, a cognitive control network (i.e., lateral PFC, insula), two subcortical reward networks (i.e., ventral striatum, thalamus), two motor networks (left and right pre- and postcentral gyrus), two visual networks (occipital cortex), two emotion/memory networks (OFC, inferior frontal gyrus, temporal cortex), and a posterior ACC network (dACC, PCC, postcentral gyrus) (Damoiseaux et al., 2006; Jolles et al., 2011; van Duijvenvoorde et al., 2016). The remaining 8 components represented physiological noise (e.g., heart rate and respiration) or white matter.

The set of 12 spatial maps that were identified as core resting state networks from the group-average analysis was used to generate subject-specific versions of the spatial maps, and associated timeseries, using *dual regression* (Filippini et al., 2009). First, for each subject, we regressed the group-average set of spatial maps (as spatial regressors in a multiple regression) into the subject's 4D space-time dataset. This results in a set of subject-specific timeseries, one per group-level spatial map. Next, those timeseries are regressed (as temporal regressors, again in a multiple regression) into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per group-level spatial map. We then tested for the linear and quadratic effects of prenatal Mn exposure (using mean-centered scores) with FSL's *randomise* permutation-testing tool (5000 permutations) (Winkler et al., 2014). Statistical maps were family-wise error (FWE) corrected with a threshold of $p < 0.05$, based on the threshold-free cluster enhancement (TFCE) statistical image (Smith and Nichols, 2009).

3. Results

3.1. Participant demographics and Mn exposure estimates

Demographic characteristics of the 15 subjects included in this rs-fMRI pilot study are presented in Table 1. The average age of the subjects was 6.8 years (SD: 0.4 years, range: 6.3-7.6 years). Participants were born full term (mean gestational age: 38 weeks, SD: 0.9 weeks) and birth weight ranged between 2000 and 4000 g (mean: 2970 g, SD: 314). Based on our indicator of SES, the majority of subjects were born to families of low- to middle SES.

Maternal blood Mn concentrations were detectable in all maternal blood samples and ranged from 2.7 to 41.1 μg/L (mean μg/L: 17, SD: 6). Mn concentrations were not related to socio-demographic variables, and the participants of the rs-fMRI pilot study did not differ significantly from the participants enrolled in the overall study ($n = 948$) or those still participating in the most recent follow-up ($n = 760$) in terms of sociodemographic variables.

Maternal blood Mn concentrations were significantly correlated with maternal blood Pb concentrations ($r = 0.59$, $p = 0.021$). $PM_{2.5}$ concentrations during the 2nd and 3rd trimesters of pregnancy were not correlated with maternal blood Mn concentrations (p's > 0.76).

3.2. Seed-based correlation analyses

3.2.1. Bilateral ACC seed

We found negative associations between prenatal Mn and functional connectivity between the ACC and orbitofrontal cortex (OFC), inferior frontal gyrus, insula and amygdala (see Table 2 and Fig. 1A). Thus, children who were exposed to higher levels of Mn during pregnancy showed reduced functional connectivity between the ACC and these prefrontal and limbic regions.

3.2.2. Bilateral insula seed

We found negative associations between prenatal Mn and functional connectivity between the insula and occipital cortex, middle temporal gyrus and angular gyrus (see Table 3 and Fig. 1B). Thus, children who were exposed to higher levels of Mn during pregnancy showed reduced functional connectivity between the insula and these occipito-temporal regions.

3.2.3. Right globus pallidus seed

We found a negative correlation between Mn exposure and functional connectivity between the right globus pallidus and dorsal ACC (dACC; see Table 4 and Fig. 1C). Thus, children with higher maternal blood Mn concentrations during pregnancy, showed reduced functional connectivity between the right globus pallidus and dACC. Additionally, we found a quadratic association

Table 1
Demographic and Mn exposure characteristics of the PROGRESS cohort and rs-fMRI pilot subjects.

Characteristic	PROGRESS ($n = 760$) Mean or% (SD)	rs-fMRI pilot ($n = 15$) Mean or% (SD)
Child age (years)	6.8 (0.4)	6.9 (0.4)
Child sex (% female)	50.1	46.7
Birth weight (grams)	3100 (400)	2970 (314)
Gestational age (weeks)	38.3 (1.8)	38.0 (0.9)
Maternal SES (%)		
Low	53	53
Middle	37	47
High	10	0
Maternal blood Mn at 2nd and/or 3rd trimester (ug/L)[a]	17 (6)	17 (6)

Note. The demographic and exposure characteristics of the 5 subjects excluded from these analyses were not different from the 15 included in this study, except for child age (the 5 excluded subjects were younger than the 15 included subjects).

[a] Mn blood manganese represented the average of 2nd and 3rd trimesters or of the 2nd or 3rd trimester, depending on sample availability.

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Table 2
Negative correlations between Mn (log) measured in maternal blood and functional connectivity with the bilateral ACC seed of 6–7-year-old children (corrected $p < 0.05$).

Brain regions	MNI coordinates			Z value
	X	Y	Z	
Inferior frontal gyrus	51	17	−1	4.00
Orbital frontal cortex	38	26	−11	3.72
Orbital frontal cortex/Subgenual ACC/Amygdala	27	21	−15	3.64
Orbital frontal cortex	14	21	−15	3.56
Insula	40	17	−9	3.38
Orbital frontal cortex	40	20	−9	3.35

Note. ACC = anterior cingulate cortex. All regions are part of a single, extended cluster (11263 voxels). MNI coordinates refer to the peak voxels, i.e., the locations of maximum activation.

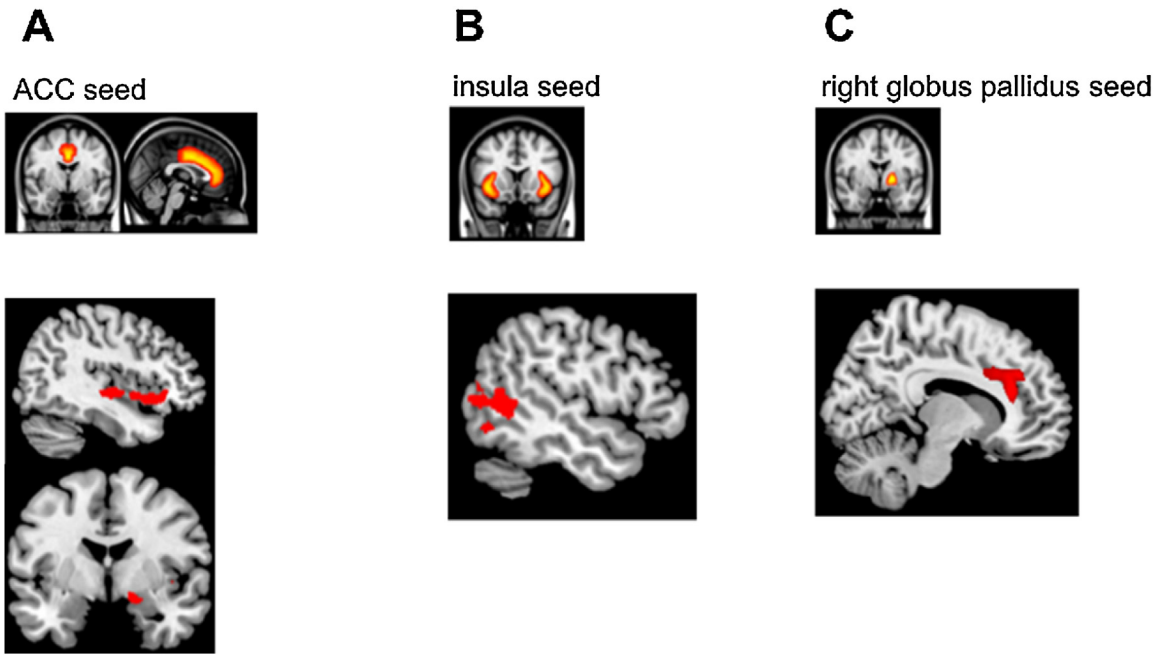

Fig. 1. Negative correlations between maternal blood Mn (log) and functional connectivity with the (A) bilateral ACC; (B) bilateral insula; (C) right globus pallidus of 6–7-year-old children.
Note: Seed-based correlation analyses were performed. Seed regions are displayed in the top row, while brain areas showing reduced functional connectivity with these seed regions (depicted in red) are displayed in the bottom row.

between prenatal Mn and connectivity between the right globus pallidus and inferior frontal gyrus (see Table 4 and Fig. 2).

There were no significant associations between prenatal Mn concentrations and functional connectivity with the middle frontal gyrus, superior parietal lobule and left globus pallidus seeds.

3.2.4. Controlling for multiple comparisons, sociodemographic variables and environmental confounders

In order to test whether these seed-based correlation analyses survived correction for the number of seeds ($n = 6$) that were analyzed, we repeated these analyses with a Z-score >2.3 and a corrected $p < 0.0083$ ($p = 0.05/6$). The findings for the bilateral ACC seed and right globus pallidus seed remained significant, even with this more conservative statistical threshold. The findings for the insula seed did not survive this more stringent threshold.

Moreover, adding either child sex, parental SES, maternal education or HOME environment as covariates in the seed-based correlation analyses did not change the findings for the bilateral ACC seed and right globus pallidus seed. However, the findings for the bilateral insula seed were no longer significant after controlling for either child sex or maternal education. Further, the findings for the right globus pallidus seed remained significant after controlling for prenatal Pb exposure and air pollution, separately, but the findings for the bilateral ACC seed were no longer significant when Pb exposure was included as a covariate.

3.3. Independent component analysis

There were no resting state networks derived from the independent component analysis that significantly correlated with prenatal Mn concentrations at a FWE- corrected threshold of significance.

However, when we used a more liberal, uncorrected threshold (uncorrected $p < 0.005$, with an extent threshold of 20 contiguous voxels (Lieberman and Cunningham, 2009)), we observed several correlations between prenatal Mn concentrations and a fronto-temporal-occipital resting state network that mirrored the findings for the seed-based correlation analyses (see Table 5). Specifically, children who were exposed to higher prenatal Mn levels showed reduced functional connectivity between different parts of the fronto-temporal-occipital network, including the insula, lateral occipital cortex and medial and lateral PFC.

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Table 3
Negative correlations between Mn (log) measured in maternal blood and functional connectivity with the bilateral insula seed of 6–7-year-old children (corrected $p < 0.05$).

Brain regions	MNI coordinates			Z value
	X	Y	Z	
Occipital pole	−38	−95	2	4.01
Angular gyrus/ Middle temporal gyrus	−61	−56	13	3.86
Occipital pole	−26	−98	7	3.86
Middle temporal gyrus/ Lateral occipital cortex	−48	−54	7	3.57
Occipital pole	−29	−100	1	3.56
Middle temporal gyrus	−56	−57	1	3.50

Note. All regions are part of a single, extended cluster (8537 voxels). MNI coordinates refer to the peak voxels, i.e., the locations of maximum activation. MNI coordinates refer to the peak voxels, i.e., the locations of maximum activation.

4. Discussion

The goal of this pilot study was to examine whether prenatal Mn exposure is associated with the intrinsic functional connectivity of the brain in childhood. Fifteen subjects aged 6–7 years from the ongoing longitudinal birth cohort study PROGRESS participated in a resting state fMRI study, in order to measure the intrinsic functional connectivity of their brains. Among these 15 subjects, higher levels of maternal blood Mn were associated with reduced functional connectivity of brain areas implicated in emotion processing and regulation in children. Specifically, three brain regions of interest (i.e., seeds) showed reduced functional connectivity with other brain areas in children who were exposed to higher prenatal Mn: the globus pallidus, ACC, and insula. Other studies have demonstrated similar associations between early life Mn exposure and the changes in the globus pallidus and insula in childhood, suggesting these areas may be particularly sensitive to Mn exposure during brain development (Aschner et al., 2015; Iannilli et al., 2016; Dion et al., 2016).

Our three main findings are as follows; First, the right globus pallidus showed reduced connectivity with the dACC and lateral PFC in children who were exposed to higher prenatal Mn levels. This finding remained significant after controlling for sociodemographic confounders (SES, maternal education, child sex, home environment support) and environmental confounders (prenatal Pb exposure and air pollution). The globus pallidus is part of the basal ganglia, and is involved in reward anticipation and processing (O'Doherty, 2004), while the dorsal ACC plays a key role in performance monitoring, and may help track the extent to which rewarding behaviors are performed (Botvinick et al., 2004). The lateral PFC is involved in regulating emotions (Aron et al., 2004; Rolls, 2004).

Second, the ACC showed reduced functional connectivity with the OFC, inferior frontal gyrus, amygdala and insula in children who were exposed to higher prenatal Mn. Third, the insula showed reduced functional connectivity with the occipital cortex and middle temporal gyrus in children who were exposed to higher prenatal Mn levels. However, the ACC and insula findings were no longer significant after controlling for prenatal lead exposure, suggesting that there may be complex interactive effects of manganese and lead exposure on brain development (Claus Henn et al., 2012), which should be explored in more detail in future research.

Reduced connectivity between these brain areas, particularly between the globus pallidus and medial and lateral PFC, may partially explain why prenatal Mn exposure is linked to emotional dysfunction in childhood in other studies, such as such as increased internalizing (e.g., anxiety and depression) and externalizing (e.g., aggression) symptoms (Khan et al., 2011; Mora et al., 2015), behavioral disinhibition (Ericson et al., 2007) and hyperactivity (Mora et al., 2015). In the future, we aim to extend the findings of this pilot study by collecting data on internalizing and externalizing symptoms and intrinsic functional connectivity in a larger sample of children. This would enable us to investigate whether the reduced functional connectivity between emotion processing and regulation areas we observed in this study mediates the association between prenatal Mn exposure and emotional dysfunction in childhood.

There are several potential mechanisms that might underpin the association between Mn exposure during the 2nd and 3rd trimesters of pregnancy and reduced functional connectivity of the brain in childhood. Structural connections underlie the brain's functional connectivity, although the relationship between structure and function is not perfect (Damoiseaux and Greicius, 2009). The structural foundation critical to the development of functional connectivity is established early in gestation (Tau and Peterson, 2010). The structural framework and functional capacities of the major neurotransmitter systems, including dopamine, are established early in gestation, and exposure to environmental toxicants may significantly affect the development of neural circuits and neurotransmitter systems (Tau and Peterson, 2010). Indeed, exposure to Mn is associated with dopaminergic dysfunction (Normandin and Hazell, 2002). Further, Mn has been shown to impair the function of astrocytes (Normandin and Hazell, 2002). Astrocytes and other glia cells play an important role in white matter development and myelination, which increases five-fold during the 3rd trimester of pregnancy (Huppi et al., 1998). Disruption of myelination by environmental toxicants, such as Mn, during this time could predispose to poor neurodevelopmental outcomes (Tau and Peterson, 2010). Moreover, given that optimal brain development requires extraordinarily complex processes to occur at the right time and in the right sequence, disruption of these processes during prenatal life might have lasting consequences that become apparent only later in life, such as in childhood (Grandjean and Landrigan, 2006, 2014).

In this study, maternal blood Mn concentrations during the 2nd and 3rd trimesters of pregnancy ranged from 2.7–41.1 μg/L, with a mean of 17 μg/L. While the *range* of blood Mn concentrations in our sample was comparable to prior studies that measured blood Mn concentrations in pregnant women in the US and Canada (Oulhote

Table 4
Correlations between Mn (log) measured in maternal blood and functional connectivity with the right globus pallidus seed of 6–7-year-old children (corrected $p < 0.05$).

Brain regions	MNI coordinates			Z value
	X	Y	Z	
Negative linear correlation				
Dorsal ACC	8	31	21	4.02
Dorsal ACC	6	35	32	3.48
Dorsal ACC	7	29	31	3.35
Rostral ACC	−11	30	20	3.34
Dorsal ACC	−6	15	34	3.19
Paracingulate gyrus	1	29	33	3.10
Quadratic correlation				
Frontal pole	53	40	8	4.40
Middle frontal gyrus	41	53	8	4.18
Inferior frontal gyrus	52	10	16	4.14
Inferior frontal gyrus	54	37	10	4.05
Inferior frontal gyrus	57	34	9	4.01
Middle frontal gyrus	40	56	9	3.89

Note. All regions showing a negative linear correlation with prenatal Mn are part of a single, extended cluster (9774 voxels). All regions showing a quadratic correlation with prenatal Mn are part of a single, extended cluster (17242 voxels). ACC = anterior cingulate cortex. MNI coordinates refer to the peak voxels, i.e., the locations of maximum activation.

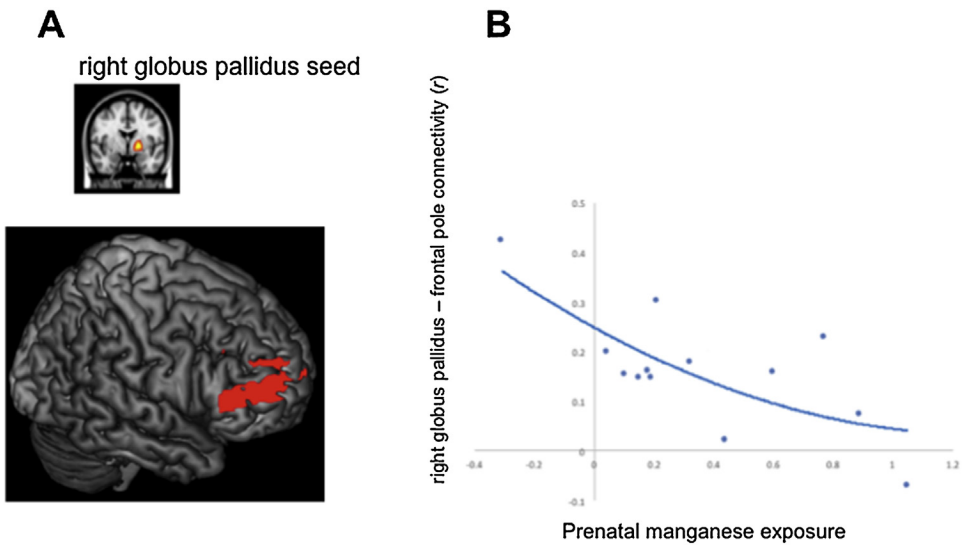

Fig. 2. Quadratic correlation between prenatal Mn (log) and functional connectivity with the right globus pallidus of 6–7-year-old children.
Note: A seed-based correlation analysis was performed. (A) the right globus pallidus seed region is displayed in the top row, while the brain areas that showed reduced functional connectivity with this seed region (i.e., the frontal pole, inferior frontal gyrus and middle frontal gyrus; depicted in red) are displayed in the bottom row. (B) Quadratic association between prenatal manganese exposure (x-axis) and functional connectivity (r) of the right globus pallidus seed with the frontal pole (y-axis). In order to compute the functional connectivity with the seed for each subject, we masked the significant activation (displayed in red in Fig. 2A) with the corresponding region from the probablistic Harvard-Oxford cortical atlas (i.e., frontal pole). We calculated the mean r value for this region using matlab and python scripts.

et al., 2014; Takser et al., 2004), *mean* blood Mn concentrations were higher in our sample than in the studies of Oulhote and colleagues (Oulhote et al., 2014) and Takser et al., (Takser et al., 2004), who both reported a mean of 13.1 μg/L. Several unique demographic and geographic aspects of Mexico, and Mexico City in particular, might explain the higher prenatal Mn concentrations in the present study. First, Mn concentrations in soil and water and consumption of Mn in foods are higher in Mexico than in the US and Canada (Santos-Burgoa et al., 2001). Second, Mexico City has among the highest levels of air pollution in the world, partly because the city sits in an elevated basin and is surrounded on three sides by mountain ridges (Just et al., 2015). Importantly, however, our most robust finding of reduced functional connectivity between the right globus pallidus and dACC and lateral PFC in children with relatively high prenatal Mn exposure remained significant after controlling for air pollution.

Inconsistent with previous studies (Chung et al., 2015; Claus Henn et al., 2010), we did not observe inverted u-shaped

Table 5
Negative correlations between Mn (log) measured in maternal blood and functional connectivity within the fronto-temporal-occipital network of 6–7-year-old children (uncorrected $p < 0.005$, $k = 20$ voxels).

Fronto-temporal-occipital network	MNI coordinates			*t* value
	X	Y	Z	
Inferior temporal gyrus	−50	−58	−20	5.81
Lateral occipital cortex	18	−86	32	8.57
Temporal pole	58	18	−12	7.22
Lateral occipital cortex	−30	−78	24	6.00
Precentral gyrus	10	−26	52	5.62
Lateral occipital cortex	−18	−70	52	5.64
Middle temporal gyrus	46	2	−32	5.08
Orbitofrontal cortex	34	58	−4	4.89
Fusiform cortex	−26	−46	−20	5.07
Precentral gyrus	42	−14	48	7.26
Brain areas showing reduced functional connectivity with the fronto-temporal-occipital network in children with higher prenatal Mn exposure				
Insula	−34	−6	12	6.63
Superior temporal gyrus	−58	−2	−8	8.44
Lateral occipital cortex	38	−74	36	5.32
Frontal pole	10	54	44	6.79
Inferior temporal gyrus	−54	−18	−28	5.31
Occipital pole	2	−98	−4	5.62
Supramarginal gyrus	58	−30	56	7.61
Lateral occipital cortex	−22	−82	32	4.94
Middle temporal gyrus	58	−42	0	5.67
Lateral occipital cortex	46	−82	−8	6.05
Fusiform cortex	30	−42	−12	4.32

Note. An independent component analysis was performed. Brain areas that are consistent with the seed-based correlation analyses are printed in bold. MNI coordinates refer to the peak voxels, i.e., the locations of maximum activation.

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associations between Mn exposure and functional connectivity in the present study. In prior research, inverted u-shaped associations were observed between prenatal and early postnatal Mn exposure and infant neurodevelopment (Chung et al., 2015; Claus Henn et al., 2010). Specifically, infants with lower levels (<20 μg/L) and higher levels (>30 μg/L) of Mn exposure had lower neurodevelopmental scores than infants with moderate exposure (20–30 μg/L). These discrepant findings between the current study and prior studies might be explained by differences across studies in the distribution of Mn exposure (i.e., only 2 subjects in the present study had Mn levels >30 μg/L), the age of participants (6–12 months in prior studies vs. 6–7 years in this study), and the neurodevelopmental outcome measure that was used (mental and psychomotor development as assessed by the Bayley Scales of Infant Development in prior studies vs. resting state functional connectivity in the present study).

To our knowledge, this study is the first to explore the association between prenatal Mn exposure and functional brain connectivity in children. The use of neuroimaging tools in studies of environmental exposure in children is an emerging science, with the potential to provide mechanistic insights into the effects of environmental toxicants on neurodevelopment, cognition and behavior (Horton et al., 2014). However, several limitations of the current study need to be mentioned as well. The modest sample size may have precluded us from finding additional associations between Mn exposure and intrinsic functional connectivity. Nevertheless, we did observe robust findings that were consistent with prior studies, and the observed correlations between Mn exposure and functional connectivity survived a stringent Bonferroni correction for the number of brain areas (i.e., seeds) that we focused on. In addition, the right globus pallidus findings remained significant after controlling for potential sociodemographic confounders, (i.e., child sex, SES, home environment support and maternal education) and environmental confounders (i.e., prenatal lead exposure and air pollution). We aim to replicate and extend these promising pilot findings, by adding measures of emotional dysfunction in a larger sample, in order to examine whether reduced functional connectivity mediates the association between Mn exposure and emotional dysfunction in children and adolescents.

We did not collect information on the exact sources of Mn exposure in our subjects, which is a limitation. Mn exposure might have occurred through inhalation of polluted air, dietary intake and drinking water (Wright and Baccarelli, 2007; Dion et al., 2016).

We measured Mn exposure in maternal blood at two time points during pregnancy (i.e., 2nd and 3rd trimester), and concentrations were highly correlated. While blood Mn is considered an appropriate indicator of environmental exposure (Draft Toxicological Profile for Manganese, 2008), measuring Mn exposure in blood at two timepoints does not allow one to determine windows of susceptibility, during which exposure may be particularly detrimental. A newly developed biomarker of metal exposure in deciduous teeth does allow for the identification of potential windows of susceptibility during prenatal life and early childhood (Arora and Austin, 2013; Arora et al., 2014, 2012). Future studies should determine temporally resolved Mn exposure from teeth in order to examine whether associations between Mn exposure and functional connectivity differ as a function of the timing of the exposure. Future researchers may also include children and adolescents from a wide age range, to test whether the reduced functional connectivity in Mn exposed children simply indicates a developmental delay, or that it remains stable or even increases with age. Further, measures of structural connectivity (e.g., Diffusion Tensor Imaging; DTI) could be included in future investigations, to provide more insight into the potential underlying mechanisms of reduced functional connectivity in Mn exposed children. Finally, we focused specifically on prenatal Mn exposure, since Mn concentrations increase during pregnancy (Al-Jameil et al., 2014); Mn is an understudied metal (Sanders, 2015); and environmental exposure to Mn is relatively high in Mexico, where this study was performed. However, several other environmental toxicants and environmental factors may influence intrinsic functional connectivity of the developing brain. We included several of these variables as covariates, such as prenatal lead exposure, air pollution, and home environment support (HOME). However, the effects of prenatal exposure to other environmental toxicants (e.g., other metals, phtalates, PBDEs, pesticides) on the developing brain deserve to be explored in future investigations.

To conclude, we found that the right globus pallidus showed reduced intrinsic functional connectivity with other brain areas involved in emotion processing and regulation in children who were exposed to higher prenatal Mn levels, even when controlling for sociodemographic and environmental confounders. Future studies need to examine whether this reduced functional connectivity underpins the association between prenatal Mn exposure and emotional dysfunction (e.g., internalizing and externalizing problems) in childhood.

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