

## **Prenatal Manganese Levels Linked to Childhood Behavioral Disinhibition**

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## **ABSTRACT**

Although manganese (Mn) is an essential mineral, high concentrations of the metal can result in a neurotoxic syndrome affecting dopamine balance and behavior control. We report an exploratory study showing an association between Mn deposits in tooth enamel, dating to the 20<sup>th</sup> and 62-64<sup>th</sup> gestational weeks, and childhood behavioral outcomes. In a sample of 27 children, 20<sup>th</sup> week Mn level was significantly and positively correlated with measures of behavioral disinhibition, specifically, play with a forbidden toy (36 months), impulsive errors on a continuous performance and a children's Stroop test (54 months), parents' and teachers' ratings of externalizing and attention problems on the *Child Behavior Checklist* (1<sup>st</sup> and 3<sup>rd</sup> grades), and teacher ratings on the *Disruptive Behavior Disorders Scale* (3<sup>rd</sup> grade). By way of contrast, Mn level in tooth enamel formed at the 62-64<sup>th</sup> gestational week was correlated only with teachers' reports of externalizing behavior in 1<sup>st</sup> and 3<sup>rd</sup> grades. Although the source(s) of Mn exposure in this sample are unknown, one hypothesis, overabsorption of Mn secondary to gestational iron-deficiency anemia, is discussed.

Indexing terms: manganese exposure; tooth biomarker; developmental neurotoxicology; dopaminergic systems; behavioral disinhibition.

## 1. Introduction

Manganese (Mn), an essential nutrient, plays a part in bone mineralization, protein and energy metabolism, metabolic regulation, and cellular protection from damaging free radical species. It is a cofactor for enzymes such as Mn superoxide dismutase, arginase, and pyruvate carboxylase [34]. However, high concentrations of Mn can result in a neurotoxic syndrome affecting monoaminergic systems, in particular dopamine (DA) [19,20]. A number of mechanisms of Mn-induced neurotoxicity have been proposed, including DA receptor destruction mediated by free radicals [30], alteration of membrane processes via Mn-influenced neuronal oxygen reduction [20], increased iNOS mRNA and the release of neurotoxic nitric oxide [52], impaired glutamate uptake due to decreased glutamate/aspartate transporter (GLAST) mRNA [24], decreased metallothionein (MT-I) mRNA impairing the sequestration of oxidants by metallothionein [24], and enhanced expression of the transferrin receptor, inducing iron-induced oxidative stress in sensitive brain regions [71].

Exposure may occur via inhalation of Mn dust, bypassing the hepatic homeostatic process so that Mn remains available for tissue uptake over longer periods of time [4, 60]. This exposure route became a matter of particular concern with the introduction of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline additive in Canada, in 1977, and, since 1995, in the US (although its use is prohibited in some states,

notably California) [14]. It has been shown that increased tissue absorption of Mn, coupled with perturbations of brain chemistry and development, will occur in experimental animals exposed prenatally (via maternal inhalation) and/or neonatally to MnO<sub>2</sub> dust [13, 51]. There is also evidence of Mn-induced neurotoxicity from high concentrations of the metal in drinking water [36, 67].

However, food is the major source of absorbed Mn in the general population [60], and certain groups, such as neonates and infants, are more vulnerable than adults to Mn via intestinal absorption [40]. In the young rodent, intestinal absorption of Mn is on the order of 70%, compared to the 1-2% in the adult rat; further, Mn enters the neonatal brain at a much higher rate than in adult animals [21, 42]. With respect to prenatal exposure, Mn concentrations in umbilical cord blood have been found to be 33% to 50% higher than in maternal blood, suggesting not only an active transport system, but also a concentrating mechanism [37, 41].

Suckling animals exposed to high dietary levels of Mn show not only higher levels of Mn in the brain but also decreased striatal DA and increased behavioral deficits [57-59].

The pups of female rats exposed to high concentrations of Mn in their drinking water during pregnancy absorbed 35% to 150% more Mn into their brains than controls, accompanied by significantly increased locomotor activity [44]. This finding is of interest because perturbations of DAergic networks have been associated with behavioral disinhibition, as found in conditions such as attention deficit-hyperactivity disorder

(ADHD), which affects 3% to 5% of all children [54]. Furthermore, higher levels of Mn have been found in the head hair of children with hyperactivity [11, 17].

There are several methods for measuring Mn concentrations in humans, each with its unique advantages and drawbacks. Mn levels in whole blood, the most widely accepted measure of Mn status, provide a convenient record of recent Mn exposure (half life ca. 37 days) [40]. A more refined index of Mn exposure in industrial workers can be obtained by neutron activation analysis of liver, indicating recent exposure [6], and bone, showing body burden [7]. Mn exposure over a matter of months can also be determined by analysis of hair, a keratin protein that acts as a reporter for inorganic elements absorbed from the diet [53]. The analysis of tooth enamel provides a reliable measure of the accretion of Mn over a much longer time frame [22, 23]. Enamel crystals provide a longitudinal record of absorption, analogous to levels of pollutants recorded in tree rings. Thus, deciduous teeth provide a useful, accessible manner for assessing exposure to Mn at specific epochs in development. The cusp tip of the first molar, formed at 20 weeks gestation and shed at 10-11 years of age, provides a record of Mn exposure at approximately the 20<sup>th</sup> gestational week; the root tip of the molar provides a record of Mn exposure at approximately the 63<sup>rd</sup> gestational week (7 months postnatal) [64].

Here, we report relations between prenatal and early postnatal Mn, as reflected in Mn deposits in tooth enamel formed around the 20<sup>th</sup> and 63<sup>rd</sup> gestational weeks, and childhood behavioral outcomes. Because both iron (Fe) and lead (Pb) body burdens

have been demonstrated to correlate with Mn body burdens [25], and because excess Fe has been shown to be neurotoxic in infants [55], we also assessed the concentrations of these metals. Because Mn neurotoxicity preferentially affects dopaminergic networks, we expected to observe a correlation between Mn and measures of DA-mediated behavior, in particular behavioral disinhibition, which is considered the preeminent symptom of ADHD [9, 10].

## **2. METHODS**

### **2.1 Participants**

Participants were children from the NICHD Study of Early Child Care and Youth Development (SECCYD), a prospective longitudinal study of development that began in 1991 with 1364 normal newborns [43]. The institutional review boards of the participating university sites approved the original SECCYD study and permitted the use of unlinked archival behavioral data and discarded tissue (i.e., teeth) for the present analysis. From this national sample, shed molars were collected from 400 children who lost a tooth between 11 and 13 years of age. Those providing teeth were not significantly different from the SECCYD sample as a whole in terms of family income, ethnicity or child behavioral outcomes. However, parents in the former group had significantly higher education levels (14.7 years vs. 14.1 years,  $p < .05$ ).

For this exploratory study, 27 teeth were randomly selected, from children at seven of the ten study sites: Little Rock, AR, Lawrence, KS, Boston, MA, Pittsburgh, PA,

Philadelphia, PA, Morganton, NC, and Madison, WI. (Sample size was dictated by budgetary limitations; analysis of the full sample will be completed when additional funding becomes available.) The sample contained teeth from 11 boys and 16 girls. Mean maternal education was 14.2 years (s.d. = 2.31), average family income 2.3 times the poverty threshold (s.d. = 1.70), and 89% of the subjects were Caucasian. The representativeness of the selected sample was determined via a quasi Monte Carlo method, in which multiple random samples of  $n = 27$  were taken from the population of 400. No demographic characteristic of the study sample exceeded the 90% confidence limits derived from the multiple population samples.

## **2.2 Measures**

Included in the SECCYD database were measures of behavioral inhibition collected at ages ranging from 3 to 9 years (see Table 1 for the age of assessment and statistical properties of each measure). Three measures were based on direct behavioral assessment: The *Forbidden Toy Task* (FTT) was administered at 36 months to assess the child's ability to delay or inhibit play with an attractive toy when asked by the experimenter to do so. The measure of interest was the amount of time spent actively playing with the forbidden toy (inter-coder reliability = .98) [48, 61]. The *Mirsky Continuous Performance Test* (CPT) was administered at 54 months of age [47]. The CPT is a widely used assessment of sustained attention and impulse control with high construct validity, acceptable test-retest reliability ( $r$  s = .65 to .74) [31], and established predictive validity for clinical group membership [9, 15]. Computer-generated pictures of

ten familiar objects (e.g., butterfly, fish, flower) were presented on a 2-inch square screen. The child was asked to press the button “as fast as you can” each time a target stimulus (a chair) appeared on the screen. Errors of commission (impulsivity) were recorded when children pressed the button for a non-target. The *Children's Stroop Test* was administered at 54 months of age. Children were shown cards that were either black, with a yellow moon and stars, or white, with a bright sun, and instructed to say "day" when shown the night (black) card and “night” when shown the day (white) card. This instrument was designed to assess accuracy of performance in the context of a competing mental set, requiring behavioral inhibition. The measure of interest was impulsive errors, as defined by the proportion of trials in which the child answered incorrectly. Performance on this task has been correlated with other tests of frontal lobe development [27].

In Grades 1 and 3, mothers completed the *Child Behavior Checklist* (CBCL) and teachers, the *Teacher Report Form* (TRF) [1,2]. These questionnaires assess a broad range of behavioral and emotional problems; reliability and validity are well-established [1]. They yield a standard Total Problems score, two broad band factors (Externalizing, reflecting aggressive, defiant, disobedient, and destructive behavior, and Internalizing, reflecting anxious, fearful, depressed, and withdrawn behavior), and a narrow band factor for Attention Problems. Third grade teachers also completed the *Disruptive Behavior Disorders (DBD) Rating Scale*. This 26-item instrument was adapted from the Diagnostic and Statistical manual of Mental Disorders (DSM-IV) items for Attention-Deficit/Hyperactivity Disorder and Oppositional Defiant Disorder [4, 45]. The DBD yields

a Total Disruptive Behavior score, an ADHD score, and scores for Inattention, Hyperactivity/Impulsivity, and Oppositional/Defiant. Reliabilities of these scales (Cronbach's alphas) typically exceed .94 [47].

To provide a contrast with these measures of children's behavioral deficits, overall scores from the Woodcock Johnson Psycho-educational Battery [65, 66] at 54 months, and in grades 1 and 3 (alphas = .80, .83 and .90, respectively) were used as measures of children's intellectual ability. We did not expect to find that Mn was related to these measures.

[insert Table 1 about here]

### **2.3 Tooth analysis**

Tooth samples were cleaned, labeled, embedded in plastic and cross-sectioned by a diamond blade. One cross section was prepared for ion mass spectrometry (IMS) analysis. IMS analyses were performed using the CAMECA IMS 1270, a new generation high-resolution, high-sensitivity ion microprobe, located at the University of California Los Angeles. The instrument is optimized for the determination of heavy metal concentrations using high accuracy and precision in complex materials [32, 50]. Concentrations of Mn, Pb, and Fe were determined with a beam spot diameter of 10-50 Fm by sputtering the sample with a focused  $^{16}\text{O}$  beam and analyzing positively charged secondary ions at a mass resolving power of ~4000. The homogeneous Durango apatite standard, a widely known electron microprobe reference standard with known elemental concentrations, was

used to calculate the Mn, Pb, and Fe concentrations, reported as ppm, in the enamel samples. The accuracy of the method was better than 90% [70]. Concentrations of these metals in the molar cusp tip, an area near the dentine/enamel junction, formed at approximately the 20<sup>th</sup> gestational week, was used as an indication of prenatal mineral absorption; concentrations in the molar root tip, formed at approximately 62-64 weeks gestation, was used as an indication of postnatal absorption. These two points were selected for analysis because, being at the tips of the teeth, they were the most reliable points to measure and because they represent the earliest prenatal age and the latest postnatal age in the tooth enamel. Levels of prenatal and postnatal Mn were not correlated ( $r = 0.13$ , ns) nor were they related to concentrations of Pb (prenatal  $r = 0.19$ , ns; postnatal  $r = -0.08$ , ns). A significant correlation between Mn and Fe was found prenatally ( $r = 0.74$ ,  $p < .001$ ) but not postnatally ( $r = -0.06$ , ns).

### **3. RESULTS**

#### **3.1 Bivariate correlations**

[insert Table 2 about here]

Table 2 presents Pearson Product Moment partial correlations between ratings of child behaviors and tooth Mn levels, controlling for tooth Pb levels measured at the same time. Children with higher prenatal Mn levels received higher scores on all measures of behavioral disinhibition: they played more with the forbidden toy at 36 months, made

more impulsive errors on the CPT and Stroop test at 54 months, were rated by their mothers and teachers as having more externalizing and attention problems in first and third grades, and were rated by their teachers as having more disruptive behavior disorders (ADHD, hyperactivity/impulsivity, inattention) in third grade. Mothers, but not teachers, rated the children with higher levels of prenatal Mn as having more internalizing problems. Only five measures were not significantly correlated with prenatal Mn levels; general cognitive ability as measured by the Woodcock-Johnson tests and teacher ratings of internalizing problems.

Table 2 also presents the results of analyses correlating postnatal Mn levels (at 62-64 weeks gestation) with child outcomes with contemporaneous postnatal Pb levels partialled out. Only three correlations were significant: children with higher postnatal Mn were rated by their first and third grade teachers as having higher levels of externalizing behavior problems (also reflected in the teachers' rating of total behavior problems in third grade).

In addition, Table 2 presents analyses conducted to rule out the possibility that correlations with Mn were a consequence of the significant association between prenatal Mn and prenatal Fe. Controlling for prenatal Fe did reduce the significance of some of the correlations of child outcomes with prenatal Mn. However, there were still significant or near-significant ( $p < .10$ ) associations with forbidden toy play, impulsivity on the CPT, mother-rated internalizing and externalizing problems and mother- and teacher-rated attention problems in grade 1, and mother-rated internalizing and

externalizing problems in grade 3. In contrast, Fe, controlling for Mn levels, was related to *less* play with the forbidden toy, *fewer* mother-rated internalizing and externalizing problems in grade 1, and *fewer* mother-rated internalizing problems in Grade 3, and was unrelated to other measures of child outcomes. These analyses suggest that the significant correlations between child outcomes and prenatal Mn are not the result of a confounding of Mn concentrations with Fe levels.

Scatter plots were graphed and inspected to make sure that outliers were not responsible for significant correlations. To reduce the influence of outliers, the most extreme scores on scatter plots were pulled into the top of their respective distributions. As shown in Table 3, which shows the effect of trimming outliers on the resulting correlation coefficients for a number of critical behavioral measures, the reduction achieved by trimming outliers did not exceed .04, and significance levels were unaffected. In addition, inspection of the scatter plots revealed no curvilinear relationships.

[insert Table 3 about here]

### **3.2 Multiple regression analysis**

Four behavioral outcomes were selected to test the overall association between prenatal Mn and behavioral disinhibition in a multiple regression analysis: (a) CPT, (b) Stroop, (c) CBCL3-Internalizing, and (d) DBD3-Hyperactivity. These four variables were used because they were: 1) strongly correlated with Mn; 2) not highly intercorrelated

among themselves (mean  $r < .30$ ); and 3) collected using different instruments. The multiple correlation coefficient, predicting tooth Mn level from these behavioral outcomes was highly significant ( $R = .79$ ;  $R$  square = .62;  $F = 6.81$ ;  $p = .001$ ;  $df = 4, 26$ ). In a second step, multiple regression analysis was performed to determine the influence of three possible confounds on the prediction of prenatal Mn levels: (a) mother's education; (b) family income; and (c) child's ethnicity. Adding these variables to the multiple regression equation did not make a significant difference ( $F$  of change = .13,  $p = .97$ ).

#### **4. DISCUSSION**

These findings suggest that prenatal accretion of Mn, as reflected in tooth enamel deposits dating to the 20<sup>th</sup> gestational week, is significantly associated with childhood behavioral outcomes. Children with higher levels of prenatal manganese were more impulsive, inattentive, aggressive, defiant, disobedient, destructive, and hyperactive. Not unexpectedly, these children did not score lower on a standardized test of cognitive ability and achievement [49]. This finding is also consistent with experimental evidence from prenatally exposed rats, showing increased hyperactivity but no differences on tests of greater cognitive challenge, such as the radial arm maze or Morris water maze [44].

It is possible that the neurotoxic effects of Mn on DAergic systems may have played a part in the observed associations. Lesions to DAergic systems have long been

associated with inhibitory deficits [26, 38, 56], and, more recently, we have shown that Mn-induced depletion of striatal DA is associated with behavioral disinhibition in a rodent model [58, 59]. By the 20<sup>th</sup> gestational week, the neurons that form the substantia nigra are in place, and many have extended axons to striatal regions, including the caudate-putamen, as well as the cerebral cortex [28, 69]. Absorption of excess amounts of Mn before or during this time could result in reduced numbers and/or altered functioning of neurons, resulting in dysplasticity of neuronal networks that mediate behavioral inhibition [62, 63].

It has also been shown that maternal exposure to high levels of Mn can precipitate overabsorption of Mn by the fetal brain [12], which could in turn activate any one of a number of neurotoxic events, as mentioned in the introduction. However, we are unable to comment meaningfully on the source(s) of Mn deposits in tooth enamel in this sample, as the data collected from the SECCYD, although extensive, included no information about Mn exposure during pregnancy. One source of high Mn absorption to be ruled out would be that emanating from maternal iron (Fe) deficiency anemia during pregnancy, a condition that occurs worldwide in about 55% of all pregnancies, and in about 20% of pregnancies in industrialized countries. Fe-deficiency anemia is due to the added nutritional requirements of the developing fetus [3, 68]. During Fe deficiency, Fe absorption is dramatically up-regulated by homeostatic mechanisms in order to compensate for decreased tissue Fe. Since Mn and Fe share the same transport pathway, Mn absorption will increase substantially in situations of impaired Fe status [18, 39, 46]. It is possible, then, that our finding, dating to the 20<sup>th</sup> week of gestation, reflects

the presence of maternal Fe-deficiency anemia before the 20<sup>th</sup> gestational week. Support for this hypothesis may be found in a recent paper by Golub et al. [29], who showed significant reductions in inhibitory responses, as well as behavioral impersistence in young rhesus monkeys who experienced prenatal Fe deprivation. We are currently investigating this relationship prospectively in a cohort of 201 pregnant women, of whom 21% have been found to be anemic (hemoglobin <11 gm/dl) in the first trimester.

## **5. Conclusion**

The significance of this study is that it suggests a link between fetal Mn exposure and later behavioral disinhibition. The study introduces a novel perspective on risk, in that behavioral outcomes are associated with a measure of heavy metal exposure, occurring, in utero, that can be dated to a particular time in pregnancy. The fact that several statistically significant associations have been shown, all in a direction consistent with existing literature on behavioral effects of Mn exposure, supports the potential importance of this method and points to a need for prospective studies of larger populations.

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## **AUTHOR INFORMATION**

**Author Contributions:** Dr. Ericson conceptualized and designed the overall study, personally supervised the analysis of tooth enamel, and contributed to writing the manuscript.

Dr. Crinella was responsible for selecting relevant behavioral measures, assisting in statistical analysis of outcomes, interpreting behavioral outcomes in terms of known correlates of Mn neurotoxicity, and contributed to writing the manuscript.

Dr. Clarke-Stewart is one of the principal investigators in the NICHD Study of Early Child Care. She participated in conceptualization of the design, arranged for the initial logistics of data collection, supervised statistical analysis of outcomes, and contributed to writing the manuscript.

Dr. Allhusen was responsible for coordinating the acquisition of data, made day to day decisions regarding the logistics of data acquisition, managed the project database, served as the primary biostatistician for the project, and played a critical role in interpretation of outcomes.

Mr. Chan was responsible for preparing and analyzing tooth specimens by ion mass spectrometry (IMS) and assisted in statistical analysis of outcomes.

Dr. Robertson was responsible for integrating the outcome results with known findings in the neuroanatomical evolution of the dopaminergic system.

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**Table 1. Tests, Age at Administration, and Characteristics of Obtained Distributions**

Child outcome measure	Age	N	Min	Max	Mean	S.D.
Forbidden Toy Task (Total Time; FTT)	36 Mos.	27	0	150	34.7	54.2
Continuous Performance Test (Impulsive Errors; CPT)	54 Mos.	27	0	65	16.2	16.8
Stroop (Impulsive Error Score; STR)	54 Mos.	20	0	66.7	26.6	20.5
Child Behavior Checklist (Mother)						
Total Problems (CBCL1-TOT)	Grade 1	27	32	74	50.1	10.8
Internalizing (CBCL1-IN)	Grade 1	27	34	71	50.3	9.7
Externalizing (CBCL1-EX)	Grade 1	27	30	73	50.5	11.2
Attention Problems (CBCL1-AT)	Grade 1	27	50	75	54.4	7.3
Child Behavior Checklist (Teacher)						
Total Problems (TRF1-TOT)	Grade 1	26	37	64	47.8	7.6
Internalizing (TRF1-IN)	Grade 1	26	36	66	46.9	8.3
Externalizing (TRF1-EX)	Grade 1	26	39	71	49.7	7.8
Attention Problems (TRF1-AT)	Grade 1	26	50	70	52.2	5.03
Child Behavior Checklist (Mother)						
Total Problems (CBCL3-TOT)	Grade 3	27	26	74	48.6	12.0
Internalizing (CBCL3-IN)	Grade 3	27	34	72	50.3	9.5
Externalizing (CBCL3-EX)	Grade 3	27	32	73	48.6	11.2
Attention Problems (CBCL3-AT)	Grade 3	27	50	78	54.3	7.2
Child Behavior Checklist (Teacher)						
Total Problems (TRF3-TOT)	Grade 3	25	37	69	51.5	7.6
Internalizing (TRF3-IN)	Grade 3	25	37	64	52.5	6.3
Externalizing (TRF3-EX)	Grade 3	25	39	71	51.0	8.8

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Attention Problems (TRF3-AT)	Grade 3	25	50	74	53.8	6.6
Disruptive Behavior Disorders (Teacher)						
Total Score (DBD-TOT)	Grade 3	27	0	56	10.0	13.8
ADHD Score (ADHD)	Grade 3	27	0	47	7.9	12.1
Hyperactive-impulsive (HYP)	Grade 3	27	0	26	4.3	6.8
Inattentive (INATT)	Grade 3	27	0	21	3.6	5.8
Woodcock-Johnson Battery (WJP)	54 Mos.	27	78	121	98.5	12.2
Woodcock-Johnson Battery (WJ1)	Grade 1	27	90	131	105.8	11.0
Woodcock-Johnson Battery (WJ3)	Grade 3	27	95	1.29	110.4	10.2

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**Table 2. Correlations between Child Behavioral Measures and Metals in Tooth Enamel**

Child measures	Age	Prenatal concentration				Postnatal concentration			
		Manganese		Iron		Manganese		Iron	
		$r^{Pb}$	$r^{Fe}$	$r^{Pb}$	$r^{Mn}$	$r^{Pb}$	$r^{Fe}$	$r^{Pb}$	$r^{Mn}$
Forbidden Toy Task (Total Time; FTT)	36 Mos.	.48**	.52**	.21	-.42*	.07	.21	-.11	-.02
Continuous Performance Test (Impulsive Errors; CPT)	54 Mos.	.48**	.38 <sup>+</sup>	.24	.00	.28	.25	.25	.29
Stroop (Impulsive Error Score; STR)	54 Mos.	.38*	.27	.38 <sup>+</sup>	.10	.01	-.19	-.06	-.11
Total Problems (CBCL1-TOT)	Grade 1	.43*	.52**	.23	-.44*	.09	.09	.18	.20
Internalizing (CBCL1-IN)	Grade 1	.43*	.52**	.24	-.42*	-.16	-.16	.13	.11
Externalizing (CBCL1-EX)	Grade 1	.40*	.49**	.20	-.40*	.25	.28	.28	.33
Attention Problems (CBCL1-AT)	Grade 1	.40*	.37 <sup>+</sup>	.30	-.19	.11	.09	.00	.01
Total Problems (TRF1-TOT)	Grade 1	.38*	.19	.42*	.12	.21	.23	-.17	-.16
Internalizing (TRF1-IN)	Grade 1	-.23	-.14	-.15	.00	.04	.08	-.07	-.07
Externalizing (TRF1-EX)	Grade 1	.43*	.20	.43*	.18	.40*	.42*	-.25	-.21
Attention Problems (TRF1-AT)	Grade 1	.47**	.38 <sup>+</sup>	.47*	.05	.13	.13	-.03	-.03
Total Problems (CBCL3-TOT)	Grade 3	.44*	.43*	.34 <sup>+</sup>	-.29	.09	.17	.14	.15

Internalizing (CBCL3-IN)	Grade 3	.55**	.59**	.35 <sup>+</sup>	-.46*	.14	.31	.12	.12
Externalizing (CBCL3-EX)	Grade 3	.44*	.40*	.35 <sup>+</sup>	-.23	.03	.08	.29	.29
Attention Problems (CBCL3-AT)	Grade 3	.39*	.31	.38*	-.12	.02	-.04	-.08	-.10
Total Problems (TRF3-TOT)	Grade 3	.38*	.28	.36 <sup>+</sup>	-.05	.48**	.51**	.20	.23
Internalizing (TRF3-IN)	Grade 3	.08	.12	.14	-.10	.30	.32	.17	.20
Externalizing (TRF3-EX)	Grade 3	.38*	.16	.36 <sup>+</sup>	.12	.57**	.58**	.25	.30
Attention Problems (TRF3-AT)	Grade 3	.48**	.32	.47*	.04	.28	.25	.09	.08
Total Disruptive Disorder Score (DBD-TOT)	Grade 3	.44*	.23	.49*	.12	.38 <sup>+</sup>	.42*	.10	.11
ADHD Score (ADHD)	Grade 3	.48**	.29	.49*	.09	.31	.34	-.06	-.07
Hyperactive-impulsive (HYP)	Grade 3	.55**	.30	.58**	.17	.35 <sup>+</sup>	.40 <sup>+</sup>	-.07	-.07
Inattentive (INATT)	Grade 3	.38*	.25	.35 <sup>+</sup>	.00	.23	.21	-.05	-.06
Woodcock-Johnson Battery (WJP)	54 Mos.	-.23	-.30	-.22	.31	.20	.24	-.07	-.09
Woodcock-Johnson Battery (WJ1)	Grade 1	-.05	.04	-.23	-.05	.09	.10	-.11	-.11
Woodcock-Johnson Battery (WJ3)	Grade 3	-.00	.06	-.16	-.04	.21	.18	-.24	-.18

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+  $p < .10$ . \*  $p < .05$ . \*\*  $p < .01$  (2-tailed).

$r^{Pb}$  = partial correlation controlling for contemporaneous lead (Pb);  $r^{Fe}$  = partial correlation controlling for contemporaneous iron (Fe);  $r^{Mn}$  = partial correlation controlling for contemporaneous manganese (Mn).

**Table 3. Raw Correlations and Correlations with Outliers Trimmed between Selected Childhood Behavioral Measures and Manganese (Mn) in Prenatal Tooth Enamel, Controlling for Contemporaneous Lead (Pb)**

Child measures	Age	Manganese	
		$r^{\text{Pb}}$	$r^{\text{Pb}}$ with outliers trimmed
Attention Problems (CBCL1-AT)	Grade 1	.40*	.40*
Attention Problems (TRF1-AT)	Grade 1	.47**	.43*
Attention Problems (CBCL3-AT)	Grade 3	.39*	.39*
Attention Problems (TRF3-AT)	Grade 3	.48**	.46**
Total Disruptive Disorder Score (DBD-TOT)	Grade 3	.44*	.41*
ADHD Score (ADHD)	Grade 3	.48**	.47**
Hyperactive-impulsive (HYP)	Grade 3	.55**	.51**
Inattentive (INATT)	Grade 3	.38*	.39*

\*  $p < .05$ . \*\*  $p < .01$  (2-tailed).

$r^{\text{Pb}}$  = partial correlation controlling for contemporaneous lead (Pb)