

# Maternal manganese exposure and subsequent infant cognition

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Neurodevelopmental disabilities such as learning, attention, and behavioral problems exact a significant toll on children. Smaller, subclinical decrements in brain function are more common and may diminish children's academic success and contribute to behavior problems. These conditions are associated with a growing list of potential neurotoxicants, including manganese. Manganese is found in groundwater across Minnesota and 20% of the population obtains drinking water from unregulated private wells. Our study examined neurodevelopmental effects of fetal/neonatal exposure to manganese, a potential neurotoxicant at excessive levels. We recruited 28 mother-infant pairs from two Minnesota cities with high levels of groundwater manganese, one of which filtered out manganese from its public water supply; the other did not. Infant neurocognitive function was assessed at 10 months of age in 22 infants by measuring changes in electrophysiologic response to a task dependent on intact frontal lobe function. Infants of mothers with increased concentrations of manganese in their hair and toenails showed smaller amplitudes in the electrophysiological response to distinct-far stimuli. Higher concentrations of manganese in tap water were also significantly and inversely associated with amplitude to distinct-far and distinct-close stimuli. Higher levels of manganese in maternal toenails were significantly and positively associated with latency difference score



for the far-distinct condition in their offspring, indicating slower latencies. Study findings provided evidence for perturbations in neurodevelopment in manganese-exposed infants. The association with maternal, not infant, biomarkers indicates these effects may be driven by fetal manganese exposure. Potential prevention measures include identifying women of reproductive years who get water from wells untreated for manganese, in order to educate them about manganese, and to encourage testing and treating wells for water quality.

Estimates of neurodevelopmental disabilities in the United States (U.S.) are that 4.9 million (8%) children are learning disabled and another 5.9 million (9.5%) have attention deficit disorder.<sup>1</sup> From the period 1998–2000 to 2007–2009, the prevalence of ADHD among children aged 5–17 years grew from 6.9% to 9.0%.<sup>2</sup> Smaller, subclinical decrements in brain function are more common than diagnosed disorders and such conditions may decrease children's academic success, disturb behavior, and diminish quality of life.<sup>3</sup> These conditions are associated with a growing list of potential neurotoxicants, including manganese.

Manganese is an essential micronutrient, low levels of which are required in a healthy diet. However, excessive exposure to manganese has adverse effects on brain function. Manganese overexposure in animal models is associated with cognitive and motor deficits and impaired dopamine release,<sup>4</sup> and induces chemical and structural changes in the frontal and parietal cortices.<sup>5</sup> Ingested manganese accumulates in tissue and bone; thus, cumulative exposure increases the risk of neurological issues.<sup>6</sup> In non-human primates, cumulative manganese exposure is associated with neuronal loss or dysfunction and white matter degeneration in the frontal cortex.<sup>5</sup>

An emerging body of literature suggests that childhood exposure to even low levels of environmental manganese may have adverse neurodevelopment effects and could contribute to neurobehavioral disorders.<sup>7</sup> Subtle decreases in memory, attention, and motor skills have been positively associated with manganese concentrations in drinking water. Most studies have analyzed the adverse effects of excessive manganese in school-age children, but in-utero and early childhood exposures are of greater concern because this is a critical time in brain development. While manganese levels are closely regulated by homeostatic mechanisms in adults, these mechanisms may be inefficient in newborns and infants resulting in increased absorption efficiency and reduced biliary excretion.<sup>8,9</sup> Infants are also at increased risk for toxicity because they consume a greater amount of fluids per body weight.<sup>10</sup> Drinking water, infant formula, and some baby foods may contain manganese. Combined with maternal exposures during pregnancy, a risk of cumulative exposure exists for the fetus and neonate, yet few studies have addressed infant outcomes.<sup>7</sup>

To address this concern, the Minnesota Department of Health (MDH) published a two-tiered, Risk Assessment Advice (RAA<sub>12</sub>). Tier 1 recommends limiting exposure to manganese in water to 100 µg/L

for infants less than 1 who drink untreated tap water or formula prepared with tap water. Tier II recommends limiting exposure to manganese in water to 300 µg/L for infants who never drink tap water or formula made with tap water, children 1 and older, and adults.<sup>11</sup> This guidance is important for Minnesotans, since manganese occurs naturally in groundwater across the state and concentrations vary from below reporting limits to more than 5,000 µg/L with a median of 101 µg/L.<sup>12</sup> (Figure 1) While most metropolitan public drinking water supplies are treated (e.g., filtered), some ex-urban and rural communities lack treatment for manganese. Approximately 1.35 million Minnesotans obtain drinking water from private wells that are not regulated for water quality. Individual well owners are responsible for testing and treating their water for contaminants, but it is unclear how many well owners test and treat their water for manganese. Additionally, 3% of Minnesotans who are on community public water systems receive drinking water with levels above 300 µg/L and 7% receive water with levels between 100-300 µg/L, although some households may treat drinking water at the tap.<sup>12</sup>

Our study examined the neurodevelopmental effects of fetal/neonatal exposure to manganese by enrolling mother-infant pairs from two U.S. cities that have naturally occurring, high levels of manganese in their ground water. Infant neurocognitive function was assessed using a test to assess infant frontal lobe integrity. We used an auditory oddball event-related potential (ERP) paradigm designed to elicit the P3a component. The P3a is elicited when improbable, distinct stimulus occurs against a background of a repeating stimulus. It represents the shift in attention to the distinct stimulus, and is dependent on frontal lobe integrity.<sup>13</sup> Attenuated P3a amplitudes have been found in populations with dopaminergic dysfunction<sup>13</sup> and frontal lobe lesions.<sup>14</sup> Based on previous studies of the P3a component and the established neurological implications of manganese exposure, we expected to find decreased amplitude and increased latency in the electrophysiological response to

distinct stimuli in manganese-exposed infants.

## Methods

Volunteer pregnant and postnatal women were recruited from two cities, both with high manganese concentrations in the drinking water and similar population demographics. One city filtered its water supply for manganese and had negligible levels in its water; the other city did not filter for manganese. Infants born full-term to pregnancies without risk factors to fetal neurologic health were enrolled in the study; in addition, mothers and infants must have resided in selected cities throughout the entire pregnancy and the infant's first 10 months of life. When an infant was 7 months old, we collected water samples from the family residence, as well as hair and toenail samples from mothers and infants. Of 28 infant-mother pairs who were originally surveyed, 22 were able to provide data for these analyses. Manganese levels in biomarkers were analyzed by inductively coupled plasma mass-spectrometry; the methodology and analysis of these biomarker data were reported in a separate publication.<sup>15</sup>

ERP data was collected when infants were 10 months old. Infants were fitted with a 64-channel Geodesic Sensor Net (EGI, Inc.) and presented with three tones (75 decibels sound pressure at the infant's head); the standard tone (440 Hertz) was presented for 75% of trials, while distinct-close (250 Hertz) and distinct-far (1000 Hertz) tones were equally presented for the remaining 25% of trials. (Figure 2) All infants heard a minimum of 250 tones (range 250-290). ERP data collection and processing parameters were consistent with previous literature.<sup>16</sup> The P3a is maximal over front-central leads<sup>13</sup> and occurs 250-500ms after stimulus presentation. For this analysis, one frontal midline lead, FCz, was chosen, and a P3a difference score was computed for the amplitude and

latency of each distinct condition by subtracting the value for the standard condition from the distinct conditions.

Paired data on ERP difference scores linked to corresponding biomarkers and tap water manganese were plotted, and Spearman's rank correlation (Rho) was used to quantify their association with minimal assumptions about their underlying distributions.<sup>17</sup> Levels of manganese measured in tap water that fell below the 10ug/L reporting limit were imputed as 9 micrograms/Liter (ug/L). All tests were run with a two-sided alternative hypothesis, and all statistics were computed in R.<sup>18</sup>

## Results

Differences in infants' response to distinct stimuli were associated with exposure to higher concentrations of maternal manganese. Infants of mothers with increased concentrations of hair-manganese (n=20 [71%], Rho = -0.47, p=0.040, 95%CI: -0.753,-0.30), and toenail-manganese (n=22 [79%], Rho = -0.42; p=0.052, 95%CI: -0.716, 0.000) showed smaller amplitudes in the electrophysiological response to distinct-far stimuli (Figure 3). Higher concentrations of tap water manganese were also significantly and inversely associated with amplitude to distinct-far (n=15 [54%], Rho = -0.61; p=0.015, 95%CI: -0.857, -0.148) and distinct-close (n=15 [54%], Rho = -0.54; p=0.038, 95%CI: -0.824, -0.039) stimuli. Infants' biomarkers of hair and toenails were not significantly associated with P3a amplitude. Analysis revealed the higher levels of manganese in maternal toenails were significantly and positively associated with latency difference score for the far-distinct condition in their offspring (n=22 [79%],

Rho = 0.50; p=0.018, 95%CI: 0.100, 0.761), indicating slower latencies. Latency difference scores were not significantly associated with water or other biomarkers.

## Discussion

The study's aim was to determine whether manganese exposure during the fetal and infancy periods was associated with changes in electrophysiologic response to auditory stimuli in a task dependent on intact frontal lobe function. Our results suggest that infants with increased gestational exposure to manganese may have frontal lobe impairments as indexed by decreased amplitude and increased latency of the P3a component to distinct stimuli. The processing deficits observed in manganese-exposed infants were primarily limited to the distinct-far stimulus, while processing of the distinct-close stimulus largely remained intact. Imaging evidence suggests a two-level approach to processing auditory deviance: small stimulus changes are processed by the superior temporal gyrus and inferior frontal gyrus; whereas large stimulus change processing requires the addition of the dorsolateral prefrontal cortex.<sup>19</sup> The associations between manganese exposure and reduced amplitude and increased latency of the distinct-far condition may be indicative of dysfunction in the prefrontal cortices of manganese-exposed infants, while processing dependent on ventrolateral regions remains intact.

The finding that maternal postnatal manganese levels were related to infant ERP response while infant biomarkers were not may indicate fetal exposure is driving these effects. Whole blood levels of manganese increase throughout pregnancy, and the fetus concentrates manganese, higher levels of which persist in the neonatal period.<sup>20</sup> While maternal and infant biomarkers may not reflect placental manganese concentrations,<sup>21</sup> the chronic maternal exposure implied by maternal nail and hair levels may have induced changes in the development of the fetal brain structure or circuitry.

## Study limitations

There are several limitations to this study:

- The sample size was small, precluding use of multivariate analyses to statistically adjust for confounders to the association of manganese exposure and infant neurocognitive function.
- Enrollment of study participants was voluntary, not random, creating the potential for selection bias.
- Participants were from two cities in the metropolitan Twin Cities, limiting the generalizability of study findings to other populations.
- Data collection did not include evaluation of manganese in infants' diet other than water and most infants began consuming baby food by the time they were 6 months old.

## Implications for research and practice

This study explored the relationship between manganese exposure and infant neurodevelopmental outcomes and provides evidence for perturbations in neurodevelopment in manganese-exposed infants. Studies with larger samples from various geographic locations are needed to facilitate more comprehensive study designs and robust analyses to learn if study findings generalize to other populations of pregnant women and fetuses. Longer follow-up periods would be necessary to understand whether the changes we observed in infant neurocognitive function are temporary or permanent, since similar perturbations in fetal and infantile iron deficient populations do carry forward into adulthood.<sup>22</sup>

The Minnesota Department of Health's two-tier Risk Assessment Advice (RAA<sub>12</sub>) for exposure to manganese in water provides important precautionary guidance for households including women of reproductive age and infants that obtain their drinking water from private wells or

public wells that lack treatment to reduce manganese levels. There is a critical need for physicians and public health practitioners to identify if women of reproductive years use well water and, if so, whether their drinking water is routinely tested for manganese and other contaminants by a certified laboratory. Providing these women with access to information on manganese in water, available on the Minnesota Department of Health's website, may be warranted; the website provides important information such as how to test well water quality and best protect their families. (<http://www.health.state.mn.us/divs/eh/water/contaminants/manganese.html#Protect>).

## Conclusion

This study explored the relationship between manganese exposure during the fetal and infant periods and infant electrophysiologic response in a task dependent on intact frontal lobe function, and provided evidence for perturbations in neurodevelopment in manganese-exposed infants. The association with maternal, rather than infant, biomarkers may indicate that these effects are driven by fetal manganese exposure. Given that subclinical decrements in children's brain function are more common than diagnosed disorders and such conditions may decrease children's academic success, disturb behavior, and diminish quality of life, families could benefit from learning about risk-mitigation strategies from medical and public health practitioners. Proactive measures, such as identifying women of reproductive years with wells untreated for manganese, to educate and encourage testing and treating wells for water quality in association with manganese and other contaminants is advised. **MM**

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