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Mapping the Neurotoxic Effects of Sub-Toxic Manganese Exposure: Behavioral Outcomes, Imaging Biomarkers, and Dopaminergic System Alterations

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Abstract : Manganese (Mn) is an essential trace element required for human health and is important in antioxidant defenses, as well as in the development and function of dopaminergic neurons. However, chronic low-level Mn exposure, such as through contaminated drinking water, poses risks that may contribute to neurodevelopmental and neurodegenerative conditions, including attention deficit hyperactivity disorder (ADHD). Pharmacological inhibition of the dopamine transporter (DAT) blocks reuptake, elevates synaptic dopamine, and alleviates ADHD symptoms. This study aimed to determine whether Mn exposure in juvenile mice modifies their response to DAT blockers, amphetamine, and methylphenidate and utilize neuroimaging methods to visualize and quantify Mn distribution across dopaminergic brain regions. Male and female heterozygous DAT^{T356M} and wild-type littermates were randomly assigned to receive control (2.5% Stevia) or high Manganese (2.5 mg/ml Mn + 2.5% Stevia) via water ad libitum from weaning (21-28 days) for 4-5 weeks. Mice underwent repeated testing in locomotor activity chambers for three consecutive days (60 mins.) to ensure that they were fully habituated to the environments. On the fourth day, a 3-hour activity session was conducted following treatment with amphetamine (3 mg/kg) or methylphenidate (5 mg/kg). The second drug was administered in a second 3-hour activity session following a 1-week washout period. Following the washout, the mice were given one last injection of amphetamine and euthanized one hour later. Using the ex-vivo brains, magnetic resonance relaxometry (MRR) was performed on a 7Telsa imaging system to map T1- and T2-weighted (T1W, T2W) relaxation times. Mn inherent paramagnetic properties shorten both T1W and T2W times, which enhances the signal intensity and contrast, enabling effective visualization of Mn accumulation in the entire brain. A subset of mice was treated with amphetamine 1 hour before euthanasia. SmartSPIM light sheet microscopy with cleared whole brains and cFos and tyrosine hydroxylase (TH) labeling enabled an unbiased automated counting and densitometric analysis of TH and cFos positive cells. Immunohistochemistry was conducted to measure synaptic protein markers and quantify changes in neurotransmitter regulation. Mn exposure elevated Mn brain levels and potentiated stimulant effects in males. The globus pallidus, substantia nigra, thalamus, and striatum exhibited more pronounced T1W shortening, indicating regional susceptibility to Mn accumulation ($p<0.0001$, 2-Way ANOVA). In the cleared whole brains, initial analyses suggest that TH and c-Fos co-staining mirrors behavioral data with decreased co-staining in DATT356M+/- mice. Ongoing studies will identify the molecular basis of the effect of Mn, including changes to DAergic metabolism and transport and post-translational modification to the DAT. These findings demonstrate that alterations in T1W relaxation times, as measured by MRR, may serve as an early biomarker for Mn neurotoxicity. This neuroimaging approach exhibits remarkable accuracy in identifying Mn-susceptible brain regions, with a spatial resolution and sensitivity that surpasses current conventional dissection and mass spectrometry approaches. The capability to label and map TH and cFos expression across the entire brain provides insights into whole-brain neuronal activation and its connections to functional neural circuits and behavior following amphetamine and methylphenidate administration.

Keywords : manganese, environmental toxicology, dopamine dysfunction, biomarkers, drinking water, light sheet microscopy, magnetic resonance relaxometry (MRR)
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