MEMRI Reveals Novel Relationship Between Manganese and Calcium Channel Activity in Dopamine Neurons

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Introduction: Manganese is an essential trace element involved in many physiological processes. The clinical effects of manganese toxicity, many of which are Parkinson-like in nature, include a movement disorder characterized by tremor, rigidity, dystonia and/or ataxia and psychiatric disturbances including irritability, impulsiveness, agitation, obsessive-compulsive behavior, hallucinations and cognitive deficits such as memory impairment, reduced learning capacity, decreased mental flexibility and cognitive slowing. In the current study, we found a mechanistic link between manganese regulation of excitability of dopamine neurons, and manganese modulation of Ca channel and BK channels.

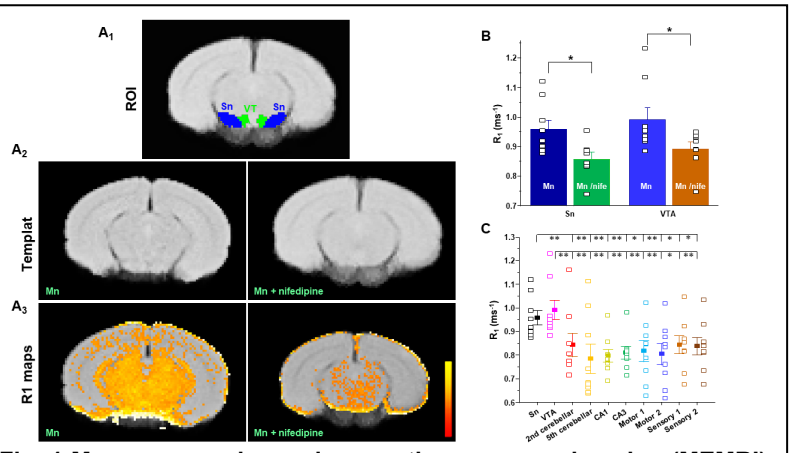

Fig. 1 Manganese enhanced magnetic resonance imaging (MEMRI) provides evidence of an *in vivo* calcium-channel dependent mechanism involved in midbrain Mn accumulation. *A_1*, Mouse brain atlas-based segmentation of the ventral tegmental area (VTA) and substantia nigra (Sn). ***A_2*,** Template brain for Mn alone or with nifepidine treatment that were aligned with mouse brain atlas. ***A_3*,** Parametric maps of T_1 relaxation rate (R_1 in $msec^{-1}$) show that calcium channel blockade with nifepidine treatment reduces R_1 in midbrain and surrounding areas. Scale bar indicates intensity of R_1. ***B*,** Nifepidine reduces T_1 relaxation rate (R_1) in Sn and VTA. *$p < 0.05$ Student's t-test. ***C*,** A greater Mn accumulation produces faster rates of T_1 relaxation (R_1) in Sn and VTA than in other cortical and subcortical nuclei. **p < 0.05 Student's paired t-test comparing to VTA or Sn.

Methods: The mice were either given an injection of nifedipine or saline 30 min before manganese (II) chloride tetrahydrate (x mg/kg) (Sigma-Aldrich Chemic Co, St. Louis, MO, USA). MR scanning was performed twenty-four after manganese exposure. Min write the injection protocol and your reference for it. On the scanning day, mice were induced using 3-4% isoflurane delivered in medical grade air (70% nitrogen, 30% oxygen; air flow rate 1.5ml/min). The anesthesia was maintained at 1.0-1.5% isoflurane during MRI scanning. Core body temperature and spontaneous respiratory rates were continuously recorded during MRI scanning (SA Instruments, Stony Brook, NY). Mice were maintained at normal body temperature levels (37–38 °C) using a warm water recirculation system. The MEMRI scans were collected in a 4.7T/33cm horizontal bore magnet (Magnex Scientific) at the Advanced Magnetic Resonance Imaging and Spectroscopy facility in the McKnight Brain Institute of the University of Florida. The MR scanner consisted of a 11.5cm diameter gradient insert (Resonance Research, Billerica, MA, USA) controlled by a VnmrJ 3.1 software console (Agilent, Palo Alto, CA, USA). A quadrature transmit/receive radiofrequency (RF) coil tuned to 200.6 MHz 1 H resonance was used for B1 field excitation and RF signal detection (airmri; LLC, Holden, MA). The MEMRI included a multiple TR sequence to calculate T_1 maps for each group using a fast spin echo sequence with multiple repetition times (TR's = 1.08, 2.33, 5.04 s, and TE = 6.02 ms) with the following geometric parameters: 16 x 16 mm^2 in plane, 14 slices at 0.8 mm thickness per slice, data matrix = 128 x 128 (125 µm in-plane resolution).

Results and Discussion: Our data suggest manganese dose-dependently increased the firing activity and reduced the action potential amplitude in dopamine neurons; whereas, blockade of Ca^{2+} channels prevented the manganese-stimulation of dopamine neurons and manganese accumulation in the cells expressing Cav1.2. The *in vivo* magnetic resonance imaging analysis (Fig. 1) further supported the single neuron analysis demonstrating nifepidine-blockade of Ca^{2+} channel reduced the manganese-enhanced magnetic resonance imaging in the midbrain. Finally, we found elevated intracellular manganese increased the plasma membrane levels of Ca^{2+}-activated potassium channels α subunits (BK- α subunits) and the amplitude of BK channels' currents, which we have previously shown to enhance excitability of dopamine neurons.

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