Manganese Synaptic Transfer Index (MSTI): A Novel Approach to Assess Neuronal Physiology in Mouse Models of Neurodegeneration

K. Smith[1], H. Zheng[2], R. Pautler[1]

[1]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, United States, [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States

Introduction

To date, there are few rapid and sensitive techniques that can detect changes in neurological phenotype in mouse models of neurodegeneration. Here, we extend the technique of Manganese-Enhanced MRI (MEMRI) to evaluate the *in vivo* efficiency of manganese movement across a synaptic junction as a sensitive biomarker for overall changes in synaptic physiology. We focus on the well-developed murine olfactory system because of its ease of access, and the vital role olfaction plays in the mouse's interaction with its environment. As proof of principle, we establish differences in two mutant mice with known neurological phenotypes. Hexosaminidase B (HexB) knockouts are a mouse model of the human Sandhoff's Disease, a severe lysosomal storage disease resulting in a gradual buildup of the ganglioside GM2 in the brain (1). The second mutant is a knockout of the Amyoid Precursor Protein (APP) protein, which is required for full synaptic function. These mice demonstrate mild deficits in synaptic function and learning from birth (2,3).

Materials and Methods

All MRI scans were conducted on a 9.4 T Bruker Avance Biospec spectrometer with a 21 cm horizontal bore using a 35 mm volume resonator (Bruker Biospin, Billerica, MA). HexB -/- mice were obtained from Jackson Labs (Bar Harbor, ME) and APP -/- mice were obtained in-house. Mice were anesthetized using a ketamine/xylazine cocktail (0.75/0.5 mg/ml, dose of 0.1 ml/10 g body weight) and 4 µl of 0.75 mg/ml $MnCl_2$ was administered nasally. Mice were allowed to recover and returned to their cages for 24 hours. At 24 hours, mice were scanned under isoflurane anesthesia using a multi-spin, multi-echo (MSME) sequence with the following parameters: TR/TE: 500/10.3 msec, FOV 30 mm, matrix 256 X 256, NEX=5. ROIs were selected using Bruker's Paravision software, and statistical analysis was performed utilizing Prism (Graphpad Software, San Diego, CA). Analysis was performed by taking the ratio of manually selected ROIs in the olfactory bulb (OB) and the primary olfactory cortex (OC), two anatomical regions connected by a single synaptic junction. Both ROIs were normalized to muscle (Fig. 1). This ratio reflects the efficiency of manganese transfer across the synapse, which we have termed the Manganese Synaptic Transfer Index (MSTI).

Results and Discussion

Figure 2 plots MSTI values vs. genotype for the APP -/- and HexB -/- mutant mice as a percentage of control, with age indicated in months. In each mouse model there is a significant and dramatic drop in MSTI values compared to age-matched, littermate controls. However, the pattern of decline is different. HexB -/- mice show a decline in MSTI values that is only apparent with advanced age, whereas the APP -/- show an age-independent drop. These changes parallel the pathology of these mice, as HexB -/- gradually lose neuronal function with GM2 buildup, but APP -/- mice show defects from birth. These results are comparable to behavioral studies we have conducted on these mice (data not shown). In conclusion, we present an *in vivo* extension of MEMRI as a sensitive biomarker for evaluation of neuronal function in novel mutant mice.

Figure 1

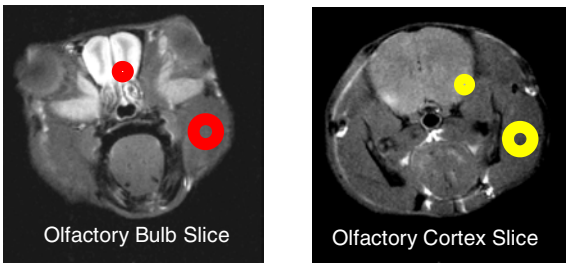

Fig. 1:Method of calculation of MSTI values

Figure 2

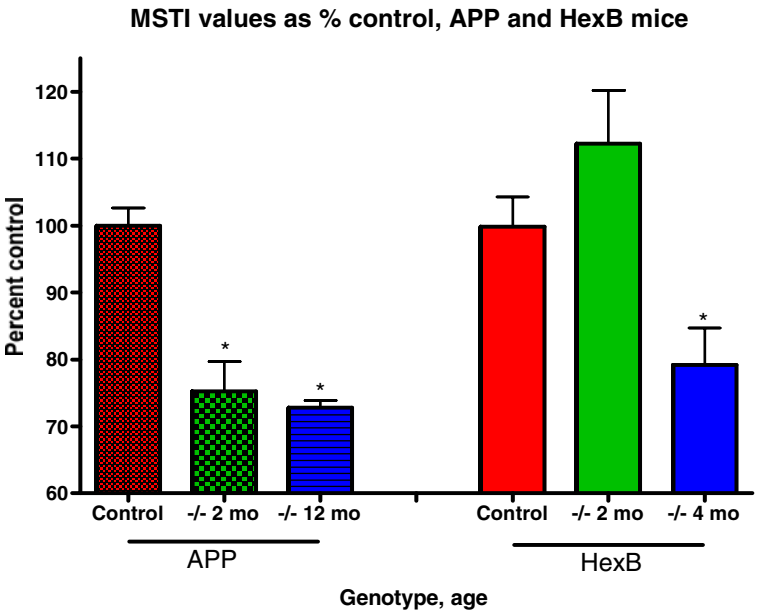

Fig. 2: APP (left) and HexB (right) mice show differences in MSTI values according to age; plotted as percent of control. For APP mice, n=2, for HexB mice, n=4. P values for indicated columns are less than 0.05 in all cases.

References

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