Detection of altered axonal transport a mouse model of neurofibromatosis using manganese enhanced MRI

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Introduction: The goal of this work is to determine whether fast axonal transport rates in the CNS of mice heterozygous for the neurofibromin 1 (NF1) gene differ from transport rates in WT controls. Neurofibromatosis 1 (NF1) is a cogenital neurodegenerative disease caused by mutation of the NF1 gene, which encodes neurofibromin. Neurofibromin is a tumor suppressor that modulates the RAS signaling pathway (1,2). Although tumor growth (peripheral nerve tumors and CNS tumors) has received the greatest attention, the most common clinical problem in children with NF1 is cognitive impairment (2). Defects in visuospatial learning and memory are reproduced in the haploinsufficient NF1-/+ mouse model (1). The cognitive deficits in the NF1-/+ mouse can be corrected by genetic or pharmacological treatments that restore RAS activity to normal levels (3). The implication is that NF1 mutation, via RAS activation, disrupts one or more cellular processes in neurons that directly affects synaptic plasticity. Four key observations drive this study: 1) Neurofibromin interacts with microtubules via its GAP-related domain 2) Neurofibromin interacts with kinesin-1. 3) Neurofibromin interacts with amyloid precursor protein. 4) Altered vesicular trafficking is a potential mechanism for cognitive impairment. Based on these observations, we hypothesize that neurofibromin mutation affects fast axonal transport, and vesicular trafficking. In order to determine whether fast axonal transport is altered with development of a neurofibromin mutation, we used Manganese Enhanced MRI (MEMRI) to detect axonal transport in olfactory sensory neurons *in vivo*. MEMRI has been used to measure axonal transport in healthy animals (4), and axonal transport rates through changes in the MRI signal associated with transport of paramagnetic Mn^{2+} *in vivo* (5), making it a potential technique to detect altered axonal transport in NF-/+ mice. We also studied whether lovastatin, a pharmacological inhibitor of RAS, would restore any reduced fast axonal transport in NF1-/+ mice. This is a first step toward the use of MEMRI to enable discovery of drugs to restore fast axonal trasnsport in patients with NF.

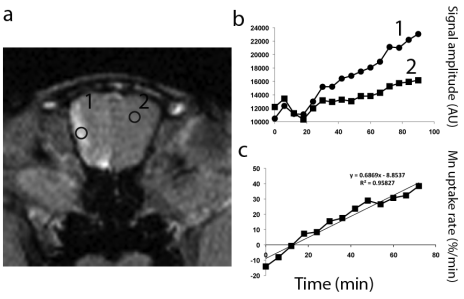

Figure 1. (a) Typical MRI image mouse olfactory bulb (OB) 40 mnutes after intranasal administration of MnCl2. Note enhancement in the ipsilateral OB, and ROIs 1 and 2 in the ipsilateral and contralateral OB, respectively. (b) The signal amplitude (arbitrary units) in the ipsilateral OB (ROI 1) increases rapidly compared to the contralateral OB, but baseline drift is visible in ROI 2. (c) To estimate axonal transport rate, we plot a normalized [SI(1) – SI(2)]/SI(2) percent as a function of time. The slope of the best straight line fit to this data is taken as a measure of Mn uptake rate.

Methods: Animals: Our NF1-/+ colony was established using breeders obtained from Jackson Labs (Stock No.002646), and maintained in the C57/Bl6 strain. Animals were genotyped using DNA from tail snips, by PCR. This resulted in a 2 kbp band in NF1-/+ heterozygotes, and no product in WT animals. A total 22 Male mice, 2 months old, were used in this study. Animals were anesthetized and 10 ml of a 1 M solution of $MnCl_2$ was instilled into the left nostril. The total volume (10 ml) was given in three boluses of 3.3 ml. Animals were then imaged in a Bruker 7T MRI scanner. Temperature was maintained at 37°C. T1-weighted multi-slice gradient-echo images were acquired with a 30° flip angle and TE/TR = 5/100 ms, with 100 X 100 X 500 um resolution. The most an terior slice was placed over the tip of the olfactory bulb, localized by a scout image. Images were acquired every 6 minutes for a total over 2 hours. Typically contrast enhancement was visible in the outer layers of the ipsilateral olfactory bulb within 20-30 minutes of $MnCl_2$ injection. Image analysis: Image reconstruction was done in Paravision (Bruker), and MR images were analyzed ImageJ (NIH). A 9-voxel region of interest was placed over the enhancing region of the ipsilateral olfactory bulb (ROI-1) and in the center of the contralateral olfactory bulb (ROI-2) [see Fig 1-a]. For each time point, the normalized signal amplitude is calculated as a percent change over contralateral olfactory bulb. We thus calculated a Mn^{2+} uptake rate as the slope for each animal. Lovastatin was purchased in inactive lactone ring form (mevinolin, Sigma) and converted into active form. 40 mg of mevinolin was dissolved in 1 ml of ethanol. 1.5 ml of 0.1 M Na OH is added, the mixture was heated for 2 hours at 50°C. After cooling, the pH was adjusted to 7.2 with 0.1 M HCl. The solution was diluted with PBS to a concentration of 0.25 mg/100 ul, aliquoted and kept frozen until ready for use. Lovastatin was administered to both WT and Nf1-/+ animals by subcutaneous injection of 10 mg/kg body weight daily for 4 consecutive days. Animals were imaged by MEMRI on the 5th day.

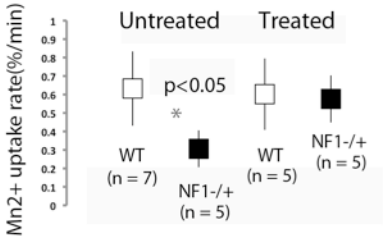

Figure 2. Mn uptake rate was measured in 7 WT and 5 NF1-/+ animals. Data analysis for each mouse was conducted without prior knowledge of mouse genotype. Average Mn uptake rate in WT animals was about 0.8%/min, but in NF1-/+ animals, the uptake rate was about 0.35%/minute ($p < 0.01$, Wiclcoxon rank sum test). Lovastatin treatment restored transport to normal in NF-/+ mice.

Results: We used MEMRI to measure axonal transport rates in healthy and NF-/+ mice (Fig 1). Transport rates in the axons of olfactory sensory neurons are significantly lower ($p < 0.05$) in NF1-/+ animals than in WT littermates – rates in Nf1 mice are approximately ½ that in WT mice (Fig 2). Further, treatment with lovastatin restored axonal transport in the Nf1-/+ mice to normal levels.

Conclusions: This work demonstrates the use of MEMRI to measure alterations in axonal transport in a mouse model of NF1. Treatment with lovastatin rescued the axonal transport defect in Nf1-/+ mice. This suggests that RAS activation may play an important role in axonal transport. MEMRI may be useful for the discovery of drugs to restore fast axonal transport in humans with neurofibromatosis.

References: 1.Gottfried et al. Neurosurg Focus 18: E8. (2010). 2.Costa et al. Nature 415: 526-530 (2002). 3.Li et al. Current Biology 15: 1961-1967. (2005). 4. Pautler et al. MRM 40: 740-748 (1998). 5.Smith et al. NeuroImage 35: 1401-1408 (2007).