The use of MEMRI to Measure *In Vivo* Axonal Transport Rates in a Murine Model of Alzheimer's Disease

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Introduction

Alzheimer's disease (AD) is a devastating neurological disorder characterized by extracellular amyloid-β plaques and intracellular neurofibrillary tangles. Current probable diagnosis of AD relies on psychological tests and can only be verified through brain biopsy or autopsy. The work of Braak and Braak (1) has indicated that there is a long progression of the disease before the probable diagnosis occurs. Recent molecular studies indicate that the proteins associated with AD have a detrimental effect on axonal transport prior to the accumulation of amyloid-β (Aβ) (2). Here we extend the use of MEMRI to detect axonal transport deficits *in vivo* using the murine olfactory system. We chose the olfactory system due to its ease of access in the mouse, but also because anosmia, loss of smell, is a prevalent symptom of AD in humans (3). We are looking specifically at a mouse model of AD known to overexpress mutant amyloid precursor protein (APP). This protein is indirectly associated with axonal transport, but, to our knowledge, no one has examined mutant APP mouse models for *in vivo* axonal transport rates. Detecting changes in the Mn^{2+} transport in the AD mouse model will not only be extremely useful as a research tool, but also open up the possibility of the use of MEMRI for diagnosis of AD.

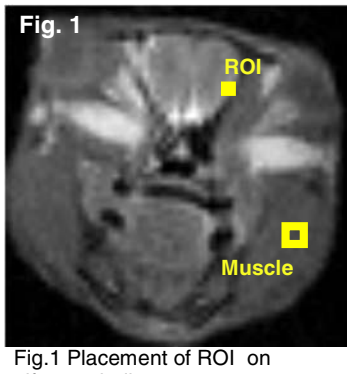

Fig.1 Placement of ROI on olfactory bulb

Materials & Methods

Homozygous mutant (K670N/M671L) tg2576 [tg (+)] (0) and age-matched littermate control [tg (-)] mice (50% C57B6, 50%SJL) were obtained from our home institution. Animals were anesthetized (0.1 ml/kg mouse) with ketamine/xylazine (0.75mg/ml)/(0.5 mg/ml) in phosphate buffered saline, 0.1 ml per 10g body weight, 10 min prior to lavage. A manganese lavage of 4 μl of 0.75 mg/ml $MnCl_2$ was administered 1 hr prior to imaging. Animals recovered prior to imaging and were then induced at 5% isoflurane and maintained with 2% isoflurane in 100% O_2. Images were acquired utilizing a 9.4T, Bruker Avance Biospec Spectrometer, 21 cm bore horizontal scanner with 35 mm volume resonator (Bruker BioSpin, Billerica, MA). The imaging parameters to acquire olfactory multi-spin/multi-echo MEMRI images were as follows: TR = 500 ms; TE = 10.2 ms; FOV = 3.0 cm; slice thickness = 1 mm; matrix = 128 x 128; NEX = 2; number of cycles = 15; each cycle ~2 min. Core temperature was maintained at 37°C during scanning. Data was acquired using Paravision (Bruker BioSpin) and then analyzed using linear regression and two-tailed t-tests with Prism (Graphpad Software, Inc). Region of interest (ROI) was placed on an axial slice 1.5 mm from the posterior of the olfactory bulb (OB). It measured 0.12 X 0.12 mm and was vertically centered on the dorsal olfactory neuronal layer. This ROI was copied for each cycle and each value normalized to the unaffected muscle of the same slice (Fig 1).

Results

Figure 1 illustrates the placement of the ROI for analysis of changes in Mn^{2+}. Figure 2 compares the tg2576 and control mice at different ages. The youngest age group, 3.5 months, shows no difference in transport rate as expected based on previous *ex vivo* axonal studies (4). The second time point, 7.5 months, demonstrates a significant decrease in the transport rate as compared to control of the same age group. It is important to note that this decline took place prior to the increased deposition of amyloid-β plaques (2).

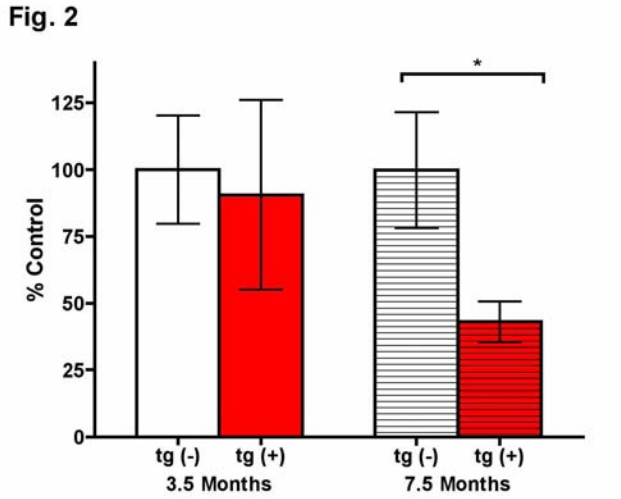

Fig. 2 3.5 month mice show no change p-value>0.05 $n_{tg(-)}=4$; $n_{tg(+)}=4$. 7 month tg (+) mice show decrease compared to tg (-) mice *p<0.05 $n_{tg(-)}=4$; $n_{tg(+)}=4$.

Discussion

For diagnosis of AD in humans, the only early indicator is histological change. However, our focus was on functional CNS changes in this model of AD, relative to the onset of histological changes. Here we demonstrate that MEMRI can detect a change in transport prior to the documented accumulation of amyloid plaques (2). To our knowledge this is the first *in vivo* study of axonal transport deficits in this APP overexpressing model. These results have clear implications for basic and clinical sciences in investigation of molecular mechanisms and early diagnosis of Alzheimer's Disease.

References

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