Aging deficits in axonal transport are exacerbated by Abeta plaques: An MEMRI study

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Introduction

Vesicular transport within the axon between the neuronal cell body and nerve terminals delivers materials for maintenance and elaboration of synapses, and carries signals from the synapse back to the cell body. Because transport is critical for neuronal function, and because protein components of the cellular transport machinery, amyloid precursor protein (APP) and tau, are involved in the pathogenesis of Alzheimer's disease (AD), defects in transport are hypothesized to be contributing factors. We use time-lapse high-field, high resolution, manganese-enhanced MRI (MEMRI) in mouse models of AD to investigate this hypothesis. Manganese gives a hyper-intense signal in T1-weighted MRI, enters neurons, is carried within axons by the transport system and preferentially transmits across active synapses [1, 2]. We recently reported deficits in transport of the visual system and the important memory circuit from hippocampus to basal forebrain in mice lacking APP [3]. Here we report results from aged transgenic mice over-expressing a mutant form of APP from two different types of familial AD, $APP^{swe/ind}$, under control of the Tet-Off promoters, TTA [4].

Materials and Methods $MnCl_2$ (600 mM, 3-5 nL) was injected into the right hippocampus (coordinates x −3.2 mm (midline), y −4.1 mm (Bregma), z 3.4 mm (down)) of 8 mice (13-15 mo old, with the following genotypes: WT littermate (1 animal), $APP^{swe/ind}$ (2), TTA (2), and $APP^{swe/ind}$/TTA double transgenics (3)). Since expression of the transgene is under control of TTA, only the double transgenic expressed the mutant APP. Immediately after injection, mice were anesthetized with 0.8% isofluorane and MR images acquired at 11.7T (Bruker BioSpin Inc.) using a 35mm linear birdcage RF coil with a 3D RARE imaging sequence, a RARE factor of 4 and 4 averages, TR/TE_{eff} = 300 ms/10.18 ms; matrix size of 256×160×128; FOV 23.04 mm × 14.4 mm × 11.52 mm; image reconstruction involved zero-filling of y from 160 to 256 yielding voxel sizes of 56.25 × 89.84 × 89.84 microns with a 46 minute scan time. Full brain images were begun 30 minutes and repeated at 6 hr and 25 hr post injection. After MRI, mice were sacrificed, perfusion fixed, embedded in gelatin and sectioned in parallel for microscopy correlation. Sections were stained for Abeta plaques with Campbell-Switzer silver stain. Analysis of the MR images involved skull-stripping using BrainSuite9, then all 3D MR images from 3 time points for each mouse were align-warped to a group-specific minimal deformation atlas. Statistical parametric mapping (SPM) analysis was completed in a similar fashion to previous MEMRI studies published by our group. Comparisons between time-points of WT (5 mice) and APP overexpressors (3 mice) were performed in SPM8 using paired Student's *t*-test and significance was considered reached at an False Discovery Rate (FDR) adjusted p value of less than 0.05. Analysis was performed on a region of interest in the contralateral hippocampus using 2-way ANOVA and post-hoc Bonferroni tests.

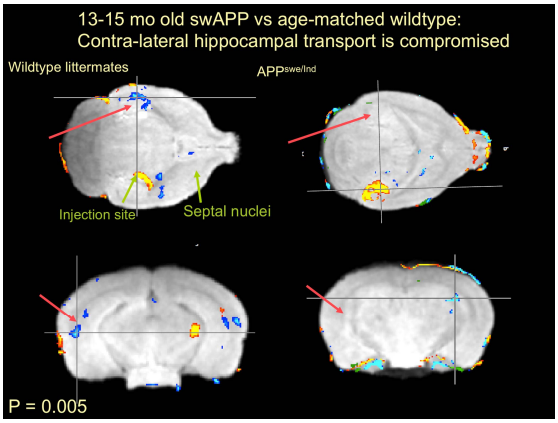

Figure 1: SPM maps of 6hr>30 min (yellow) and 25hr>30 min (blue) images projected onto a WT gray scale image from either the WT (n=5) dataset or the mutant $APP^{swe/ind}$ over-expressors (n=3). Note the difference in the contralateral hippocampus of WT compared to $APP^{swe/ind}$ (red arrows).

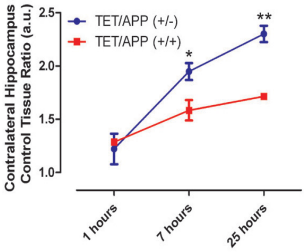

Figure 2: ROI analysis of intensity increases in contra-lateral hippocampus after Mn^{2+} injection

Results and Discussion Histologic studies demonstrated that the $APP^{swe/ind}$/TTA double mutants had high levels of Abeta throughout the brain whereas no plaques were found in the WT animals. We thus grouped the three WT genotypes (WT, $APP^{swe/ind}$ and TTA single transgenes) together for statistical analysis of the MR images. We compared within group differences between the two datasets (WT group and $APP^{swe/ind}$/TTA set) between time-points. Mn^{2+} enhanced intensity persisted in the injection site over 25 hr after injection in both groups, but appears to occupy a larger volume in the mutants. Transport to the medial septal nucleus at 25 hr post-injection, normally robust in young mice, was barely detectible in the aged WT group, and not found in the mutant group (Figure 1). Analysis across all 8 mice demonstrated that injections were consistent with respect to location in CA3 of the hippocampus, within 0.5mm, with similar intensity and dimension of the halo. ROI analysis of the intensity change in the contralateral hippocampus was consistent with the statistical mapping, with little change in the $APP^{swe/ind}$ and highly significant increase of intensity in the WT group. Statistical analysis of the increases observed at the contralateral hippocampus identified both time ($p < 0.0001$; $F(2, 31.33)$; 2-way ANOVA) and genotype ($p < 0.001$; $F(1, 17.57)$; 2-way ANOVA) as significant factors in explaining these increases.

Conclusion Our data show that transport within the brain between CA3 of the hippocampus to its distal projection, the medial septal nucleus and the contralateral hippocampus, declines in aging mice. This decline is further exacerbated in mice over-expressing human mutant $APP^{swe/ind}$. We are currently in investigating transport in young $APP^{swe/ind}$/TTA. Supported by NINDS NS062184.

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