IN VIVO NEURONAL TRANSPORT IMPAIRMENT REFLECTS THE LEVEL OF ABNORMAL TAU IN A MOUSE MODEL OF TAUOPATHY : A TRACK-TRACING MEMRI STUDY

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INTRODUCTION Alzheimer's disease is defined by a progressive dementia associated with 2 microscopic lesions: amyloid plaques, that are made of extracellular Abeta protein, and neurofibrillary tangles, that are made of intraneuronal abnormally phosphorylated tau protein. There is evidence from cellular studies that tau hyperphosphorylation can induce microtubule breakdown and subsequently impair axonal transport [1]; however, very few studies of neuronal transport have been conducted in animal models of tauopathy, and their results are often contradictory [2,3]. Our aim was to study neuronal transport in vivo and non invasively, in a mouse model of tauopathy, using Manganese-Enhanced MRI (MEMRI) [4]. In a pilot study of 9 animals, we previously demonstrated that in vivo neuronal transport is affected in a transgenic mouse model of tauopathy [5]. Here, we extend these results on a larger group of animals, including repeated follow-up examinations, and we show that neuronal transport parameters measured by MEMRI correlate with the levels of pathological tau immunostaining on histological sections.

MATERIALS AND METHODS *Animals:* 19 transgenic mice (Tg) of the JNPL3 line [6] and 17 wild-type mice (WT) from the same strain were used for this study. For the follow-up study, a subgroup of 9 Tg and 8WT mice were examined both at 3 and 6 months of age; other mice were imaged either at 3, 6 or 9 months of age. *Imaging:* 7-T micro-MRI system was used, consisting of a 200-mm horizontal bore magnet (Magnex Scientific, UK) with an actively shielded gradient coil (Bruker BGA-9S; ID 90 mm, 750 mT/m gradient strength, 100 µs rise time), and an in-house quadrature RF coil. A 3D T1-SPGR sequence with the following parameters was used: FOV = 19.2 x 19.2 x 9.6 mm, matrix= 128 x 128 x 64, resolution = $(150\mu m)^3$, TR/TE = 15/4 ms, 6 averages, acquisition time 15 min. Flip angle 18º was chosen to provide the greatest T1-enhancement contrast [7]. Initially, each mouse was imaged with this sequence. One week later, mice were injected in one nostril (right/left alternatively) with 1.5 µL of a solution of 5M $MnCl_2$, under isoflurane anesthesia, and image sets were acquired subsequently at H=1, 4, 8, 12, 24, 36, 48 hours and at D=7 days. *Data processing:* All the MR datasets, corresponding to the time course study for each individual mouse (9 MRI sequences), were processed using ImageJ software (NIH, Rockville, MD). After an automatic registration with the Rigid_Registration.jar plugin (J Schindelin, M Longair [8]), 3 regions of interest (ROI) were defined on the following structures using a mouse brain atlas: the glomerular layer, the mitral cell layer, and the pons that was used to normalize signal intensities. All the normalized measurements at the different time points for each ROI of each mouse were plotted and fitted to a previously described tract-tracing bolus model [9] using an in-house Matlab fitting routine (The Mathworks 2009). The fitting process enabled the estimation of the following parameters in each ROI: timing T_{max}, value S_{max} of the bolus peak of Mn such that $S(T_{max})=S_{max}$, maximal slope V_{max} of the ascending part of the curve. *Histology*: After the last MR examination, mice were anesthetized and perfused transaortically. The brain was extracted, fixed and cryo-preserved before coronal brain sections (40µm) were cut for histology. Immunohistochemistry was performed on brain sections of each animal using the following antibodies: MC1 (abnormal tau conformation), PHF1 (abnormally phosphorylated tau), GFAP (astrogliosis). Degree of staining was assessed quantitatively using a manual threshold. A subset of sections was also stained with cresyl violet for unbiased neuronal counting in the mitral cell layer of the olfactory bulb. Tau antibodies MC1 and PHF1 were generously provided by Dr. Peter Davies, AECoM, Bronx, NY.

RESULTS At 6 months of age, Tg mice displayed a decreased peak value ($p<0.05$, Wilcoxon signed rank test, fig.A), a decreased maximal slope ($p<0.001$, paired T-test, fig.B), and a delayed time-to-peak ($p<0.05$, paired T-test, fig.C). The peak value correlated significantly with the degree of tauopathy, as quantified both in the soma and in the dendrites of neurons, either whith MC1 antibody (Spearman correlation coefficient, r= 0.46 and 0.5, fig. D&E) or with PHF1 antibody (data not shown). Quantitative evaluation of gliosis (GFAP staining) and unbiased cell counting of neurons (Cresyl Violet) ruled out any significant gliosis or neuronal loss in the Tg mice, which could have affected our results.

CONCLUSIONS Our study shows that the progressive neuronal transport impairment observed in the JNPL3 mice is correlated with their level of pathological tau immunostaining. This highlights the significance of axonal transport perturbations as a key event during the course of Alzheimer's disease. As an indicator of tau functional changes, MEMRI provides a useful biomarker for the pre-clinical evaluation of potential drugs against Alzheimer's disease and related tauopathies, such as immunotherapies [10].

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