IN VIVO EVIDENCE OF AXONAL TRANSPORT PERTURBATION 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 phosphorylated tau protein. Recent research efforts in this field have focused on assessing the functional alterations of axonal transport that can occur in the early stages of the disease, i.e. prior to plaque and tangle formation. In vitro studies have shown that oligomers of Abeta have an adverse effect on axonal transport[1]. In vivo experiments using Manganese-Enhanced MRI (MEMRI), have reported an impairment of axonal transport in a plaque model of Alzheimer's disease before the plaque formation, thus suggesting an in-vivo toxic effect of the non-aggregated Abeta on axonal transport [2]. Additionally, it has been reported that tau filaments have an in-vitro inhibitory effect on axonal transport [3]. Thus, we hypothesize that tract-tracing MEMRI should detect an in-vivo axonal transport impairment associated with early tau pathology in a mouse model of tauopathy.

MATERIALS AND METHODS *Animals:* Five 6-month-old P301L transgenic mice [4] and four wild-type mice of the same background (WT) were used for this study. *Imaging:* A 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). The same in-house quadrature coil was used for all experiments. 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 [5]. Mice were imaged once 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 1, 4, 8, 12, 24, 36, 48 hours and 7 days post injection. *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 [6]), 5 regions of interest (ROI) were defined for the following structures using a mouse brain atlas: the glomerular layer, the mitral cell layer, the anterior part of the piriform cortex , the posterior part of the piriform cortex (Fig 1) and the pons which 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 [7] using an in-house Matlab fitting routine (The Mathworks 2009). The fitting process enabled the estimation in each ROI of the following parameters: timing T_{max} and intensity S_{max} of the bolus peak of Mn (Fig 2) such that $S(T_{max})=S_{max}$, drift velocity V, leakage rate λ, and diffusion coefficient D. For each mouse, 4 differential speeds of Mn transport were inferred by measuring the distance between 2 consecutive ROI over the difference of peak timings T_{max} in these ROI. For the first neuronal segment, we considered the distance between the injection site and the first ROI, with an initial $T_{max} = 0$. All parameters were processed and compared between WT and TG mice using a 2-tailed homoscedastic Student T-test.

RESULTS A decrease of S_{max} was observed in TG mice, which was statistically significant in the 2 proximal ROI (Fig 2A, C). An increase of T_{max} was also observed in TG mice, which was statistically significant in the mitral cell layer (Fig 2D). Apparent speed of Mn transport was decreased in TG mice in the 2 proximal ROI, although not statistically significant (Fig 2B); however this same parameter could not be estimated in the 2 distal ROI, due to the proximity of the 2 curves (Fig 2A). Velocity V and leakage rate λ were decreased in TG mice compared to WT (n.s., data not shown), whereas diffusion D was comparable between WT and TG (data not shown).

DISCUSSION This study provides the first *in vivo* evidence of axonal transport impairment assessed by MRI in a model of tauopathy. The choice of a long timeframe protocol, with 8 sequential examinations from H1 to D7, allowed us to detect statistically significant changes in peak value S_{max} and peak time T_{max}; these measurements would not have been feasible with a single examination protocol that is more commonly used for tract-tracing MEMRI [2]. According to the drift-diffusion model [7], an increase of T_{max} may be related to a decrease of velocity V, a decrease of leakage λ, or an increase of diffusion D. We did not observe any difference in D between WT and TG mice, but we observed a trend of decrease of V and λ in the TG mice. Surprisingly, statistically significant differences of S_{max} and T_{max} were observed in the more proximal part of the olfactory system, a region expected to have less expression of tauopathy than the distal part of the olfactory system [4]. We hypothesize that the leakage-induced decrease in signal intensity (Fig 2A) hinders the observation of the differences in S_{max} and T_{max}. Hence, the latter are more easily detected on the earlier curves (i.e. for the proximal part), as long as $\lambda T_{max}<1$. For the distal part, leakage dominates, $\lambda T_{max}>1$, suppressing the differences between the TG and WT.

CONCLUSIONS Our study shows that, as early as 6 month-old, P301L mice display significant differences in parameters of axonal transport. This suggests an early *in vivo* adverse effect of tauopathy on axonal transport, and gives new insights into the critical role played by tau pathology during Alzheimer's disease. This study also provides an exciting noninvasive tool for the evaluation of effects of emerging therapies that specifically aim at reducing tau pathology in Alzheimer's disease [8].

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Fig 1

Fig 2