Aging impacts significantly on neuronal transport in normal mice but not in an accelerated mouse model of Amyloid Beta pathology.

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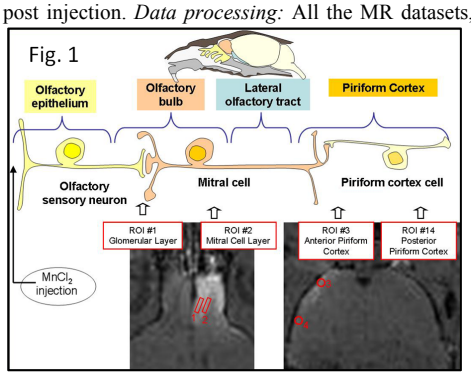
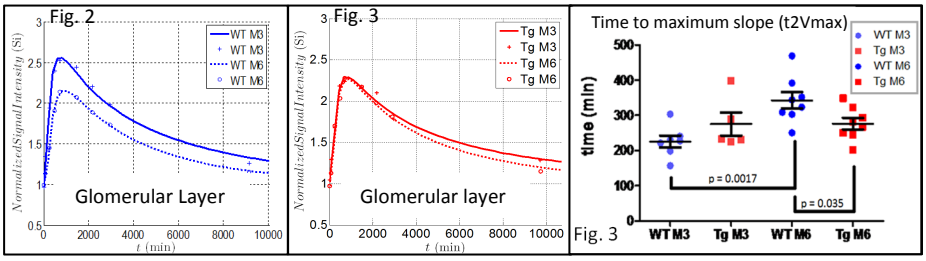
INTRODUCTION: Amyloid Beta (Aβ) and tau play an essential role in the Alzheimer's disease (AD) pathophysiology. There is *in vitro* evidence that Aβ oligomers can impair fast axonal transport [1]. Crucially lacking are in vivo non invasive techniques to evaluate neuronal function. Track-Tracing Manganese Enhanced MRI (TT-MEMRI) is currently the only non invasive 4-D volumetric imaging technique to demonstrate neuronal transport perturbations. Applying MEMRI in a transgenic model (Tg2576) of Aβ pathology by expressing human APP mutation confirmed the deleterious effect of Aβ on neuronal transport measured by a decrease in the rate of signal change [2]. We previously investigated a tau model (JNPL3) using a 7-day time-course period where we showed a significant decrease in neuronal transport function in Tg mice [3]. In the present study, we sought to examine with the same approach an accelerated Aβ mouse model (Tg6799 5xFAD) expressing both APP and PS1 human mutations [4]. Surprisingly, our results show significant decrease in neuronal conduction in the (C57/B6xSJL) WT mice with age contrasting with maintained transport function in Tg 5xFAD with age

MATERIALS AND METHODS *Animals:* Five 3-month-old and eight 6-month-old Tg 5XFAD transgenic model (Tg6799) [4] and seven 3-month-old and eight 6-month-old background matched wild type (WT) mice 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). An in-house quadrature coil (ID = 21.5mm, L= 29mm) was used for all experiments. A 3D T1-SPGR sequence was utilized with: FOV = 19.2 x 19.2 x 9.6 mm, matrix= 128 x 128 x 64, isotropic spatial resolution = 150μm^3, TR/TE = 15/4 ms, 6 averages and an acquisition time of 15 min. Flip angle 18º was chosen to provide the greatest T1-enhancement contrast [6]. Mice were imaged using a tract-tracing MEMRI (TT-MEMRI) protocol with 9 imaging time points, 1 pre and 8 post-intranasal injection of MnCl2 (1.5 μL of a solution of 5M), 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 [7]), four regions of interest (ROI) were defined on MR images (Fig.1), corresponding to 4 consecutive areas of the olfactory system (glomerular layer, mitral cell layer, anterior and posterior piriform cortex). A fifth ROI, Pons, was utilized 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 [5] using an in-house Matlab fitting routine (The Mathworks 2009). The fitting process enabled estimation in each ROI of the following parameters: maximal signal (Smax), time to Smax (t2Smax), maximal upslope of the curves (Vmax=ΔSi/t) and the time to Vmax (t2Vmax).



RESULTS: Time-curve plots obtained from wild type mice demonstrated visible difference over aging depicted in Fig.2. This unexpected decrease observed during normal aging was significant using both parameters Smax ($p = 0.0016$) and Vmax($p = 0.011$) in the glomerular layer. In the mitral cell layer, in addition to the decrease in Smax ($p = 0.0071$) and Vmax ($p = 0.0091$), an increase in t2Smax ($p = 0.0104$) and t2Vmax ($p = 0.0017$) were also observed. Surprisingly Tg 5xFAD mice failed to show an age associated decrease in transport as evidenced by the near superimposed time curves (Fig.3) suggesting maintained Mn transport function. Fig.4 summarizes the time-to-maximum-slope parameter in the mitral cell layer in both groups. These data taken together demonstrated a significant difference in t2Vmax in the mitral cell layer ($p = 0.035$) at 6-month between WT and Tg suggesting either decreased Mn intake or transport in WT. Expression of Aβ deposits was histologically confirmed both in the olfactory bulb and the piriform cortex in 6 month old Tg 5xFAD.

DISCUSSION: Smith et al. [2] showed a decrease in ΔSi/t in the olfactory bulb of Tg2576 mice with increased pathology within a single 30 min imaging session. Cross et al. [5] described an extended time study in rats measuring the same parameter but along the length of the olfactory tract also showing an age related conduction only in very advanced ages (24-25mth). Our design envisions a combined approach accessing transport rate with propagation parameters (Smax, t2Smax, Vmax and t2Vmax) in respective ROIs. Our C57/B6xSJL WT littermates show for the first time an altered Mn neuronal transport with age. We anticipated that the accelerated Aβ pathology in the Tg-5xFAD mouse model would lead to an even earlier neuronal impairment translating into a decrease in Smax, Vmax and an increase in t2Smax and t2Vmax. However, aging Tg 5xFAD appear to maintain their transport functions. These results are in contrast to our previous taupathy study [3] where the WT control demonstrated a stabilized Mn transport function while Tg mice showed a decrease. This suggests inherent differences in various mouse strains. Furthermore, maintained Mn transport parameters in 6 month-old Tg mice may reflect a preserved neuronal function, or may be due to other mechanisms distinct from axonal transport, such as excitotoxicity, which can also increase the neuronal uptake of Mn [8]. Nevertheless our imaging protocol appears to adequately assess the flow of the paramagnetic Mn through the olfactory system and as in case of C57/B6xSJL aging mice, it is sensitive enough to detect Mn transport perturbations.



CONCLUSIONS AND FUTURE DIRECTIONS: We show for the first time an early age associated decrease in Mn transport and/or intake in C57/B6xSJL WT mice not reported in other mouse strain that have been examined. Tg 5XFAD appear to maintain their Mn transport profiles. In vivo MEMRI studies will allow longer term follow up enabling opportunities of multiple same subject trials and evaluations of new therapies for diseases affecting neuronal functional integrity. On further improvements in our transport biomarkers we intend to develop a faster, efficient, reliable and most importantly a non-invasive yard stick to alleviate current dependence on subjective behavioral studies. For our present study we are looking to correlate our results with histological quantification. Particular microbiological features of the 5xFAD TG mice will also need to be studied for possible explanations for enhanced neuronal transport machinery. Our overall goal aims at better characterizing axonal transport impairment *in vivo* using a panel of different biomarkers over a long timeframe window [5].

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