NON-INVASIVE EVALUATION OF TAU TARGETED IMMUNOTHERAPY: A TRACT-TRACING BOLUS MEMRI STUDY

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INTRODUCTION. Alzheimer's disease is defined by the presence of a progressive dementia, associated with 2 microscopic lesions: amyloid plaques, that are made of extracellular Abeta protein aggregates, and neurofibrillary tangles, that are made of intraneuronal aggregates of abnormally phosphorylated tau protein. The aggregation of this tau protein has been shown in previous studies to decrease the rate of axonal transport [1]. Immunotherapy targeting hyperphosphorylated tau is arising as a promising prospect to mitigate the neurodegenerative effects of such tauopathies [2] To assess the effectiveness of such immunotherapies often involves sacrifice of the animal. However, Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) permits the longitudinal study of neuronal function with minimal risk to the animal. We hypothesize that tract-tracing MEMRI in a mouse model of tau pathology should enable the non-invasive monitoring of various tau targeting therapies aimed at improving neuronal integrity.

MATERIAL AND METHODS. *Animals:* Twenty homozygous JNPL3 tangle transgenic mice [3] underwent MEMRI at 6 months of age. Twelve of the mice underwent tau immunotherapy(Treated) with Tau379-408[P-Ser396,404] in alum adjuvant from 3 months of age, and eight (Control) received an adjuvant alone. *Imaging:* Imaging studies were performed on a 7-T micro-MRI system, consisting of a Biospec Avance II console (Bruker, Ettlingen, Germany) interfaced to a 200-mm horizontal bore magnet (Magnex Scientific, Yarnton, 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=23.5-mm, AD=21.5mm Length=29mm) was used for all experiments. We used a 3D T1-SPGR sequence with the following parameters: FOV = 19.2 x 19.2 x 9.6 mm , Matrix= 128 x 128 x 64, resolution = $(150\mu)^3$, TR/TE = 15/4 ms, Averages = 6, acquisition time = 15 min. Flip Angle (18º) was chosen to provide the greatest T1-enhancement contrast [4]. Each mouse was imaged a first time with this sequence. One week later, each mouse was injected in one nostril (right or left alternatively), with 1.5 µL of a solution of 2.5M $MnCl_2$, under isoflurane anesthesia. Image sets were acquired subsequently at H=1, 4, 8, 12, 24, 36, 48 hours and at D= 7 days (Fig 2). During the MR image acquisition, mice were anesthetized with isoflurane and the body temperature was maintained at 35/37ºC using a heating water bed.. *Data processing:* All the MR dataset corresponding to the time course study for each individual mouse were comprised of 9 MRI sequences. The 3D datasets were processed using ImageJ software (NIH, Rockville, MD). After an automatic registration using the Rigid_Registration.jar plugin (J Schindelin & M Longair, [5]), Our regions of interest (ROI) were defined using a mouse brain atlas: the mitral cell layer and the pons that was used to normalize signal intensities. All the normalized measurements at the different time points for the mitral cell layer of each mouse were plotted and fitted to a previously described tract tracing bolus model [6] using an in-house MATLAB fitting routine (The Mathworks 2009). The fitting process enabled the estimation of the timing (Pt) and the intensity (Pv) of the bolus peak of Mn, and the maximal slope of uptake (Sv). All parameters were processed and compared between Control and Treated JNPL3 mice using a 1-tailed t-test.

RESULTS. A significant increase in maximal slope of manganese uptake, Sv, was observed (Fig 1) in the mitral cell layer in treated JNPL3 mice compared to identical Controls(39.0%, p=.01). Furthermore, in the Treated mice, there was a strong trend for an increase in the intensity of the bolus peak, Pv (4.6%, p=.12), and a decrease in the time to peak value, Pt (-13.0%p=.10), in the mitral cell layer, as compared to the Controls.

DISCUSSION : Our study, utilizing MEMRI's non-invasive, longitudinal analysis from H1 to D7, allowed us to detect changes in maximal uptake slope (Sv), time to peak signal (Pt), and peak value (Pv), which would not have been possible with a single examination protocol that is more commonly used for tract-tracing MEMRI [2]. We are currently analyzing tau pathology in olfactory brain sections from these mice to assess how these findings correlate with clearance of tau lesions, which we have shown previously to occur following tau immunotherapy as referenced above. While we did not obtain statistical significance in our determination of Pv and Pt, it is worthwhile to note that all three parameters did correlate as expected with respect to the treatment. Mice that have undergone immunotherapy should have less tau pathology, and showed in our experiment to have more rapid neuronal transport. We are currently performing these measurement in more animals which we expect will strengthen our results. Other ROI (Glomerular Layer, Posterior Pirifrom Cortex, and the Anterior Piriform Cortex) were also analyzed, but we are focusing on the mitral layer to compare with the immunohistochemistry semi-quantitative analysis.

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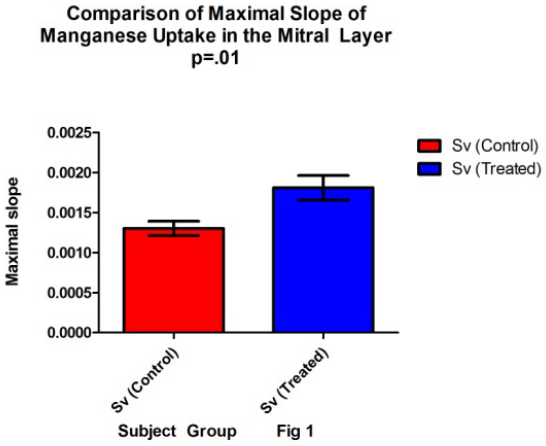

Fig 1

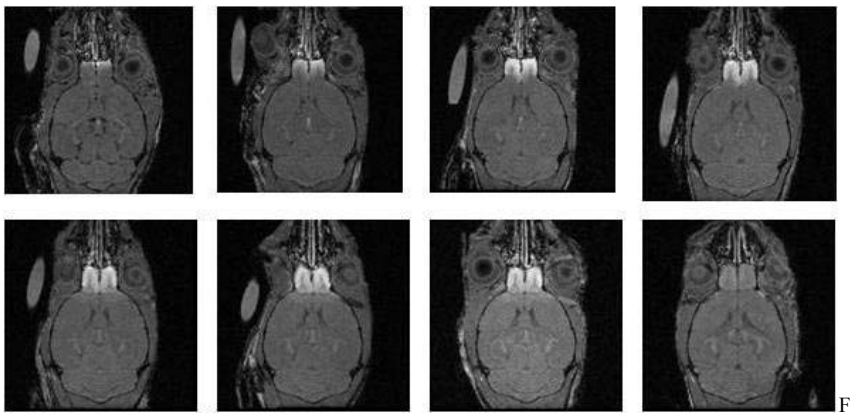

Fig 2 (Row 1: H1, H4, H8, H12 | Row 2: H24, H36, H48, D7)