Longitudinal monitoring of transverse relaxation time changes in the corpus callosum of a mouse model of Alzheimer's disease

Firat Kara[1,2], Reinhard Schliebs[3], Stephan Roßner[3], Annemie van der Linden[1], Huub J.M. Groot[2], and A. Alia[2,4]

[1]*Bio-imaging Lab, Biomedical Sciences, University of Antwerp, Antwerp, Belgium,* [2]*Leiden Institute of Chemistry, Gorlaeus Laboratoria, Leiden University, Leiden, Netherlands,* [3]*Paul Flechsig Institute for Brain Research, Leipzig University, Leipzig, Germany,* [4]*Institute of Medical Physics and Biophysics, Leipzig University, Leipzig, Germany*

Target audience: Those who are studying white matter pathology in Alzheimer's disease using relaxometry will benefit from this study.

Purpose: The corpus callosum (CC) is the largest commissural fiber connecting left and right hemisphere of the brain[1]. Emerging evidence suggests that a variety of abnormalities, detected in the microstructure of this white matter fiber tract, can be an early event in Alzheimer's disease (AD) pathology[2]. However, little is known about tissue characteristics of these abnormalities and how these abnormalities evolve during AD progression. In this study, we measured *in vivo* magnetic resonance transverse relaxation times (T_2) to longitudinally monitor changes in tissue integrity and abnormalities related to myelination and demyelination processes in the CC of an AD mouse models.

Methods: Tg2576 and age-matched wild-type littermates were used in this study[3]. The number of animals per age is as follows: control mice: age 10 months (n = 8), 14 months (n = 8), 16 months (n = 6) and 18 months (n = 6); Tg2576: age 10 months (n = 8), 14 months (n = 9), 16 months (n = 8) and 18 months (n = 7). All measurements were conducted on a vertical wide bore 9.4T Bruker spectrometer, with a 1 Tm^{-1} actively shielded imaging gradient insert (Bruker). Radio frequency transmission and reception was performed with a volume coil (20 mm). Bruker ParaVision 5.0 was used for scan control and image acquisition. *In vivo* T_2 values were collected from same animals at age 10, 14, 16, and 18 months from CC, cortex (CX), hippocampus (HC) and thalamus (TH) regions (Fig. 1) using MSME sequence. MSME experiments were performed as described previously[4] with following imaging parameters: number of echoes = 12 with echo spacing 8.5; TR= 1.5 s; voxel resolution = 7.8 x 7.8 μm^2; Slice number=10; Slice thickness = 1 mm. To calculate relaxation times following fit functions were used: T_2vtr fit function (y= A+C*exp (-t/T_2) for T_2 evaluation (A= Absolute bias, C= signal intensity). ROIs were manually defined. Statistical significance was assigned for values of $P < 0.05$. The Hematoxylin and eosin stain (H&E) and Luxol fast blue staining (LFB) were used to compare white matter changes between wild-type and Tg2576 mice. To detect gliosis from brain slices, primary polyclonal anti-GFAP and anti-IBA-1 were used[5]. To detect Aβ, brain sections were subjected to immunohistochemistry using monoclonal anti-Aβ42 (BC42), anti Aβ40 (BC40) and polyclonal anti-Aβ 40–42 as described earlier[4].

Results and Discussion: In this study, *in vivo* T_2 changes were followed longitudinally in the CC of Tg2576 mice between 10 and 18 months of age (Fig. 2). The major finding of this study was a significant prolongation ($P<0.05$) of the T_2 in the CC, reflecting significant microstructural changes in Tg2576 mice as compared to wild-type mice. Interestingly, the T_2 of CC was already significantly longer for 10 months old Tg2576 mice, compared to age-matched wild-type mice, at the onset of Aβ deposition. In contrast, grey matter regions surrounding the CC, such as the CX and HC, showed a significant T_2 decrease compared to wild-type mice (Fig. 3). No change in T_2 values in TH region was observed at any age, which was associated with very low Aβ deposition in this region. Histological analyses clearly revealed demyelination (Fig. 4), gliosis and amyloid-plaque deposition in the CC. Our results suggest that demyelinating and inflammatory pathology may lead to prolonged relaxation times and can mark an early event during AD progression. To our knowledge, this is the first longitudinal *in vivo* T_2 study assessing microstructural changes in the CC of the Tg2576 mice.

Acknowledgment: This work was partially supported by grants from Alzheimer Forschung Initiative and Centre for Medical Systems Biology.

References: [1] Hinkley B.N.L., Marco E.J., Findlay A.M., et al., The role of corpus callosum development in functional connectivity and cognitive processing. PloS One 2012; 7:e39804; [2] Di Paola M., Iulio Di F., Cheruni A., et al., When, where, and how the corpus callosum changes in MCI and AD: a multimodal MRI study. Journal of Alzheimer's Dis. 2010; 20:67-95; [3] Hsiao K., Chapman P., Nilsen S., et al., Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996; 274:99-103; [4] Braakman and Matysik et al., Longitudinal assessment of Alzheimer's β-amyloid plaque development in transgenic mice monitored by in vivo magnetic resonance microimaging. J Magn Reson Imaging 2006; 24: 530-36; [5] van Duijn S., Nabuurs R., van Duinen S.G., et al., Longitudinal monitoring of sex-related in vivo metabolic changes in the brain of Alzheimer's disease transgenic mouse using magnetic resonance spectroscopy. J Alzheimers Dis. 2013 DOI 10.3233/JAD-122188.

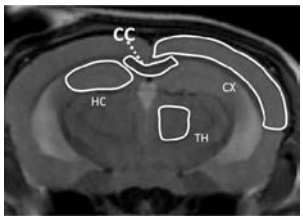

Fig. 1. Anatomical T_2-weighted MR coronal slices of a mouse brain, showing corpus callosum (CC), hippocampus (HC), cortex (CX) and thalamus (TH) acquired with RARE sequence (TR= 6000, TE= 17ms) at 9.4 T.

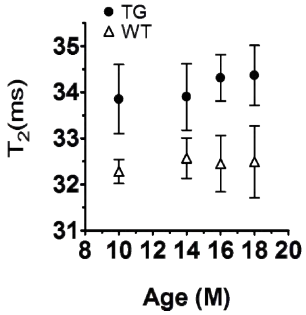

Fig. 2. Age-dependent *in vivo* T_2 changes of the CC region of the wild-type (WT) and Tg2576 (TG) mice. Values are expressed as mean T_2 in ms ± SD (error bars) (95% C.I.). Two tailed student t-test, $^{**}P < 0.01$, $^{***}P < 0.001$ significant from T_2 of WT mice. The number of animals per age was presented in Fig. 2.

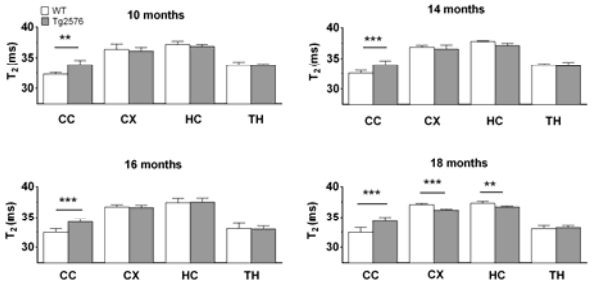

Fig. 3. *In vivo* T_2 (ms ± SD) changes in the corpus callosum (CC), cortex (CX), hippocampus (HC) and thalamus (TH) regions of the wild-type (WT) and Tg2576 mice at the age of 10, 14, 16 and 18 months. Two tailed student t- test, $^{**}P < 0.01$, $^{***}P < 0.001$ significant from T_2 of control mice.

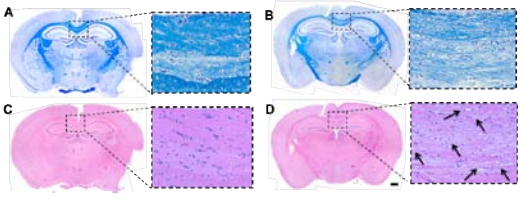

Fig. 4: Histological section of the brain of a wild-type (A,C) and a Tg2576 (B,D) mouse, stained with the LFT method (A,B), which stains the myelin in blue, and standard H&E stain (C,D). Demyelination and vacuolation in the CC region of transgenic mouse (B and D) is more prominent compared to the wild-type mouse (A, C) as can be clearly seen (arrows) in the magnified subsampled areas. Scale bars: 500 μm.