Probing in vivo T2 relaxation time alterations in the corpus callosum of a mouse model of Alzheimer's disease

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

Purpose: In current years, a strong interest in white matter pathology, e.g. gliosis and demyelination, seen in Alzheimer's disease (AD) has developed. The corpus callosum (CC) is the largest white matter tract in the brain connecting cortical regions in the two cerebral hemispheres[1]. It plays an important role in interhemispheric transfer of cognitive information and has been shown to be susceptible to AD[2]. However little is known about tissue characteristics of biophysical alterations in the CC and how these abnormalities evolve during AD progression. The transverse relaxation time (T_2) measurements are sensitive to explore subtle microstructural changes in the CC. There is an emergent need for new in vivo non-invasive studies to monitor corpus callosum changes longitudinally in order to clarify how and when the integrity of CC alters in AD. The aim of this study was to probe *in vivo* T_2 changes longitudinally in the CC of the Tg2576 mice with age and to investigate potential biological mechanisms, such as demyelination, Aβ deposition and gliosis, contributing alterations in the biophysical environment of the CC.

Methods: Tg2576 (Tg) and age-matched wild-type (WT) littermates were used in this study[3]. Animals were studies between 4–18 months of age for histological (n= 12, Tg2576; n = 6 wild-type) and *in vivo* MRI measurements (n = 15, Tg2576; n = 14, wild-type) conducted on a 9.4T Bruker spectrometer, with a 1 Tm^{-1} imaging gradient insert (Bruker). *In vivo* T_2 values were collected from the CC, cortex (CX), hippocampus (HC) and thalamus (TH) regions (Fig. 1A) using MSME sequence[4] with following imaging parameters: number of echoes = 12 with echo spacing 8.5; TR= 1.5 s; in-plane resolution of 0.078 x 0.078 mm and a voxel resolution of 6.10 x 10^{-3} mm^3. To establish the test–retest reliability of T_2 measurements and to check systematic errors, the same C57BL/6J mice (n = 5) were scanned twice on two subsequent days. To study the dependence of T_2 on the refocusing interpulse interval (τ), the T_2 measurements were performed using the MSME sequence with 16 echo and 5 different refocusing interpulse intervals (i.e., 6.6, 8.5, 10.5, 15.5 and 18.5 ms). The T_2vtr fit function (y= A+C*exp (-t/T_2) was used for T_2 evaluation (A= Absolute bias, C= signal intensity). ROIs were manually defined. Statistical significance between Tg and WT groups was assigned for values of $^*P < 0.0031$ (Bonferroni corrected alfa value). The Hematoxylin and eosin stain (H&E) and Luxol fast blue staining (LFB) were used to compare white matter changes between WT and Tg mice. To detect gliosis (i.e., activated astrocytes) from brain slices, primary polyclonal anti-GFAP was used[5]. To detect Aβ, brain sections were subjected to immunohistochemistry using monoclonal anti Aβ40 (BC40) and polyclonal anti-Aβ 40–42 as described earlier[4]. ImageJ software (ImageJ, USA) was employed for quantitative image analysis of brain sections.

Results and Discussion:

In this study, T_2 relaxation measurements followed longitudinally to monitor *in vivo* changes in the integrity of the CC of same Tg2576 mice with age. Before the longitudinal T_2 study, the test-retest reliability of *in vivo* T_2 measurements and the dependence of T_2 on the τ was analysed (Fig. 1 B&C). The test-retest reliability was found to be excellent for almost all brain regions. No dependency of T_2 measurements on τ was observed suggesting T_2 values in individual brain structures depend on changes in tissue properties rather than magnetic field disturbances. The major finding of our longitudinal study was a significant prolongation of the T_2 in the CC, reflecting significant microstructural changes in Tg mice as compared to WT mice at and above 10 months of age ($P < 0.0031$) (Fig. 2A). In contrast, grey matter regions, namely the CX and HC, showed a significant T_2 decrease compared to WT mice at 18 months of age (data not shown). No statistically significant changes were observed at 4 months of age between Tg and WT groups. A quantitative histological assessment of activated glial cells and Aβ plaque load in the CC of Tg2576 mice with age is presented in Fig. 2B. A modest increase in activated glial cells in the CC was already present at 11 months of age in Tg mice compared to WT mice suggesting that there might be some diffuse changes in the normal appearing CC which activates the astrocytes, and these changes can be detected with quantitative T_2 measurements. Our visual assessment revealed that there is an increased demyelination and vacuole formation in Tg mice compared to WT mice (Fig. 2C&D). To our knowledge, this is the first longitudinal *in vivo* T_2 study assessing microstructural changes in the CC of the Tg2576 mice.

Conclusions: We have found promising results for the application of T_2 measurements to track changes in the CC of Tg2576 mice. Our results suggest that inflammatory pathology accompanied by demyelination may lead to prolonged relaxation times which can be mark as an early event during AD progression in this animal model. For future studies T_2 may serve as a viable biomarker for probing the onset of symptoms of AD in this animal model.

Acknowledgment: This work was partially supported by grants from Alzheimer Forschung Initiative e.V. (AFI, Grant Nr 13810), Centre for Medical Systems Biology (CMSB) and Stichting Alzheimer Onderzoek (SAO-FRA, Grant Nr 13026). Firat Kara is a Postdoctoral Fellow of the Research Foundation-Flanders (FWO) from University of Antwerp.

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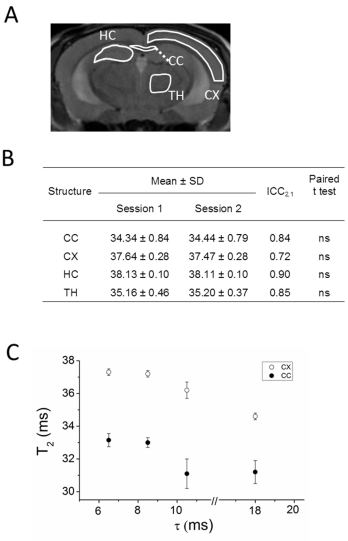

Fig. 1. (A) T_2-weighted image of a mouse brain. (B) Test-retest reliability of in vivo T_2 measurements in a variety of brain regions performed by intra-class correlation coefficient using a 2-way random-effects ANOVA (subject by session) and the absolute agreement definition. T_2 values are presented as means from five subjects ± SD; Paired t test results collected from two sessions show no significant (ns) difference in all cases. (C) Effect of τ on T_2 in the corpus collosum (CC) and cortex (CX) regions. T2 values were measured using 4 different τ values namely 6.6, 8.5, 10.5, and 18 ms. Values are expressed as mean T_2 in ms ± standard deviation (error bars); n = 3. Corpus callosum (CC), hippocampus (HC), cortex (CX) and thalamus (TH).

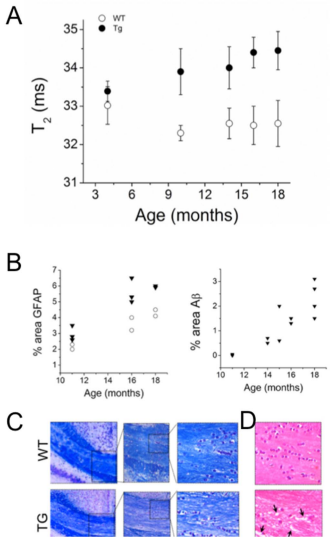

Fig. 2. (A) Age-dependent *in vivo* T_2 changes of the CC region of the wild-type (WT) and Tg2576 (TG) mice. Values are expressed as mean T_2 in ms ± SD (error bars) (95% C.I.). Two tailed student t- test show significant difference between wild type and Tg2576 mice at the age of 10 months ($P < 0.00313$), 14 months ($P < 0.00063$), 16 months ($P < 0.00063$) and 18 months ($P < 0.00063$). No significant difference was observed between WT and TG mice at the age of 4 months ($P > 0.05$). (B) Quantitaive analysis of GFAP stained area and Aβ load in the CC with age in Tg2576 (▼) and control mice (○). (C) Histological section of the brain of a WT and a Tg mouse, stained with the Klüver-Barrer method, which stains the myelin in blue, and standard H&E stain (D). Demyelination and vacuolation in the CC region of Tg mouse (C and D, lower layer) is more prominent compared to the WT mouse (C, D upper layer) as can be clearly seen (arrows) in the magnified subsampled areas. Scale bars: 500 μm.