

# T2 RELAXATION PARALLELS PROGRESSIVE CLINICAL SYMPTOMS OF DEMYELINATION IN A NOVEL TRANSGENIC MOUSE MODEL

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## INTRODUCTION

Loss of myelinating cells is a common hallmark of demyelinating diseases such as Multiple Sclerosis (MS), and its impact on axonal damage is still poorly understood. A novel mouse model of demyelination has been generated using intrinsic, temporally and spatially controlled triggering of oligodendrocyte cell death. This model will enable further insights into damage mechanisms triggered by demyelination. Axonal damage seems to be the major disability correlate in these diseases - converse to primary affection of myelinating glial cells. Sensitive, specific, non-invasive imaging marker for monitoring de- and remyelination in vivo would be of paramount importance. To date, there is no established MRI marker for assessing demyelination, although quantitative magnetization transfer ratio (qMTR) MRI [1] and Relaxivity-weighted MRI [2] has shown high sensitivity in detecting myelin pathology. Similarly the use of iron-oxide nanoparticles (USPIOs) to visualize macrophage infiltration has been proposed as biomarker for MS and animal models thereof [3], primarily reflecting inflammatory events. To evaluate the sensitivity of various MR readouts for monitoring demyelination pathology we quantitatively assessed T2, MTR and infiltration of macrophages using USPIOs in control and diseased mice over the time-course of five weeks.

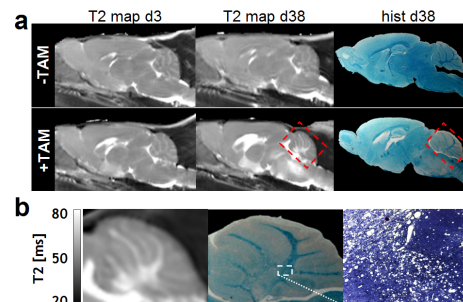
**METHODS** In vivo experiments were carried out in strict adherence with the Swiss law for animal protection. **Mouse model:** Generated by crossing a tamoxifen (TAM) inducible, oligodendrocyte specific Cre expressing mouse line [4] with a mouse line expressing diphtheria toxin fragment A (DT-A) [5] allowing spatially and temporal controlled cell ablation. Induction of DT-A expression was achieved by i.p. injection of tamoxifen (TAM) for 5 consecutive days (start at d=1). The study design comprised 2 experimental groups: group1=recording of MTR and T2 maps; group2=recording of T2 maps prior- and 24h post USPIO (Sinerem®, Guerbet, France) injections (i.v. 340µM Fe/kg). Each experimental group consisted of 2 control [no TAM injection: -TAM] and 4 mice which received i.p. injection of TAM (i.p. dosemg/kg; +TAM) Longitudinal MRI measurements of group 1 were performed at days 3, 10, 17, 24, 31, 38 and 42. Animals of group 2 received USPIOs immediately after the first T2 acquisition (preUSPIO) and were followed up 24 h later (postUSPIO). All MRI experiments were carried out on a Bruker Pharmascan 47/16 (Bruker BioSpin MRI, Ettlingen, Germany) small animal system operating at 200 MHz. MRI measurements were performed using a birdcage transmit-receive resonator. Mice were anesthetized using 1.8% isoflurane applied via a face-mask in an oxygen/air (20% /80%) mixture and placed on water-heated cradle. **MRI:** Magnetic transfer imaging (MTI) was performed using a 3D-GE sequence (FOV: 15x15x12mm; acquisition matrix: 60x60x48; isotropic voxel dimension of 250µm; TE/TR: 2.0/30.0ms; flip angle 30°). A gauss pulse with B1 amplitude of 80µT and an offset frequency of 5 kHz was used as MT saturation pulse. The reference sequence was obtained with the same parameters but without application of the saturation pulse. Accordingly the MTR was calculated as:  $MTR [\%] = (M_s/M_0) \times 100$ , where  $M_0$  is the magnitude of signal without MT saturation and  $M_s$  is the steady-state magnitude of signal during MT saturation. T2 values were obtained using a multi-spin-echo sequence with the following parameters: FOV: 19x19mm; acquisition matrix: 132x132; in plane resolution of (144µm)<sup>2</sup>; TE/TR: 10.0/2000ms; echo spacing 10.0 ms; N° echos: 14; NA: 6 and SLTH: 0.8mm). High resolution anatomical images were acquired using a standard SE-RARE sequence with FOV: 2.0x1.84; acquisition matrix: 256x256; in plane resolution of 78x72µm<sup>2</sup>; TE/TEff/TR: 12/36/3500ms; RARE factor: 8; NA:7; SLTH: 0.8mm; N° slices: 9. **Image analysis:** T2 and MTR maps were calculated using Biomap (M.Rausch, Novartis, Switzerland). All calculated parasagittal T2 maps from the same animal were coregistered and evolution of T2 values plotted over time for defined ROIs (frontal cortex: frctx; olfactory bulb: olfb; cerebellar white matter: cerbwm; brainstem: brnstm, Fig.2a). The effect of USPIO injection was calculated as the difference ( $\Delta T_2$ ) between T2(24h post) - T2(pre) injection of USPIOs for all timepoints (d4, 11, 18, 25, 32 and 39). Computed MTR maps were resliced and coregistered to the high-resolution images. ROIs were defined in brnstm, cerbwm and frctx. All MR data are given as mean±SEM. Time-courses were statistically analyzed using Repeated measures ANOVA (treatment: +/- TAM) and Fisher's PLSD post-hoc test (p<0.05).

**RESULTS** Analysis of T2 maps shows pronounced increase in T2 in +TAM vs -TAM animals in brain regions affected by demyelination as revealed by histology i.e. cerebellar white matter and brainstem (Fig.1). Significant differences between the two groups were observed for cerbwm (treatment x time interaction: F(6, 24) = 12.8, p<0.0001) and brnstm (treatment x time interaction: F(6, 24) = 3.79, p<0.0085). The temporal evolution of T2 changes (Fig.2b) in +TAM mice parallels the behavioral scores (indicated as demyelination score, Fig.2c). No change in T2 was observed in control brain regions not affected by the disease. The earliest time-point when a significant difference in T2 between -TAM and +TAM has been observed is d24 for the cerebellar ROI (p<0.05). We could not detect any difference between 24h post vs pre USPIO T2 values in +TAM mice for the regions analysed (shown for brnstm only, Fig.3a) indicating the integrity of the blood-brain-barrier (for USPIOs) and absence of pronounced active macrophage infiltration over the time-course of the experiments. Similarly only small decreases in MTR could be detected at a progressed pathology stage (Fig.4a). Fig. 4b shows colour coded MTR maps at the level of brnstm for baseline (d3) and end-stage (d42) for both groups.

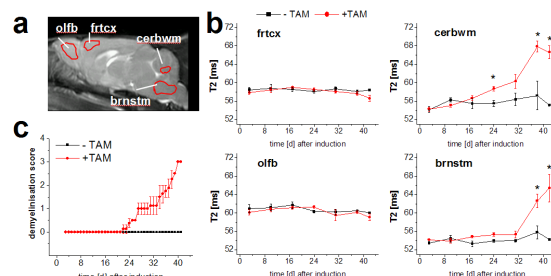
## DISCUSSION

T2 hyper-intensities become apparent at the timepoint of first clinical symptoms reflecting the massive, even lesion-like damage which occurs along/in white matter tracts of the CNS. Increased T2 values are indicative of edema formation. In view of this early non-invasive readout for this documented feature the assessment of T2 may serve as a valuable biomarker of the disease process. On the other hand a decrease in MTR could only be detected at a late stage in pathology probably reflecting the late loss of myelin macromolecules rather than their early structural disruption. No active or passive infiltration of macrophages could be measured reflecting the intact blood-brain-barrier (Fig.3.b) and the weak recruitment of blood-borne immune cells described for this intrinsically triggered model of demyelination.

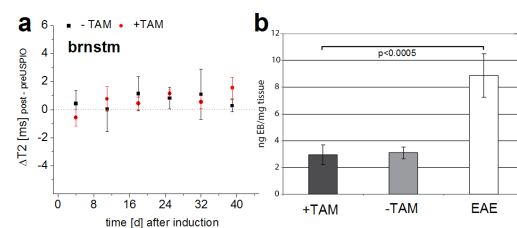
**REFERENCES** [1] Zaaraoui et al., (2008) MAGMA, 21(5):357-62. [2] Nessler et al., (2007) Brain, 130, 2186-98. [3] Berger et al., (2006) NMR Biomed., 19(1):101-7. [4] Leone et al., Mol. Cell Neurosci. (2003) 22(4):430-40. [5] Brockschneider et al., Mol. Cell Biol. (2004) 24(17):7636-42.



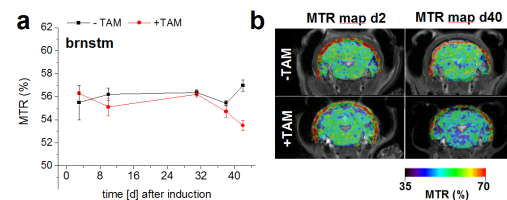
**Figure 1:** (a) Representative parasagittal T2 maps for -TAM and +TAM at day 3 and 38. Hyperintense areas are clearly visible in brain stem and cerebellum. Histological sections (luxol fast blue) at day 38 show the pronounced reduction in myelin in +TAM in cerbwm and brnstm (b) Hyperintense areas in T2 maps match cerebellar white matter demyelination which is visible on toluidine blue stained plastic sections (right image) as vacuolized lesion-like structures.



**Figure 2:** (a) Parasagittal view of the mouse brain indicating ROI selected for analysis. (b) Time-courses of T2 with significant differences (\*) p<0.05, Fisher's PLSD between +TAM (n=8) and -TAM group (n=4) which parallels behavioral scoring shown in (c).



**Figure 3:** (a) Time-course of  $\Delta T_2$  (post - preUSPIO) for -TAM and +TAM from ROI in brainstem. (b) Assessment of BBB leakage using Evans blue (EB) assay. Compared to the EAE model the intrinsic mouse model used in the current study shows significantly lower uptake (ngEB/mg tissue) and not due to myelination.



**Figure 4:** (a) Time-course of MTR (%) over 5 weeks in the brainstem for -TAM and +TAM. (b) Representative MTR (%) maps for the four different groups at baseline (d2) and endstage (d42). MTR decrease is visible in +TAM (d42).