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From in situ to ex vivo: the effect of autolysis and fixation on quantitative MRI markers for myelin

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Synopsis

***Ex vivo* histology remains the gold standard against which MRI biophysical models, e.g. the MR g-ratio which characterises the fraction of a fibre's diameter that is myelinated, are evaluated. The MR g-ratio model requires a measure of myelin density, for which magnetization transfer saturation (MT) has been used as a biomarker. However, changes occurring post mortem, e.g. autolysis, temperature changes and fixation, significantly alter the MRI signal. Here we investigate how these changes impact MT. We found that MT decreased post mortem but greatly increased upon fixation. These effects are similar to reported changes of other established MRI myelin-markers.**

Purpose

Validation of *in vivo* MRI-based biophysical models characterizing microstructure in the human brain tissue such as the myelin g-ratio (1) requires comparison to gold standard histology methods, which are mostly based on formalin fixed *ex vivo* tissue samples (2). However, the MRI signal and its parameters can significantly change from *in vivo* to *ex vivo* due to, e.g. (i) autolysis (varying post-mortem interval, PMI (3)), (ii) fixation (e.g. crosslinking of proteins (3,4)), and (iii) temperature changes (5). Consequently, it is necessary to characterize these changes for those specific MRI parameters used in biophysical models such as (1). The purpose of this pilot study is to investigate these changes for the magnetization transfer (MT) saturation (6), a semi-quantitative MRI (qMRI) myelin-marker that has been used for MR g-ratio mapping (7,8). Using a comprehensive qMRI protocol (9,10) in a longitudinal design (from *in vivo* via unfixed *in situ*, to fixed *ex vivo*), we compare the MT marker to previously tested qMRI parameters: (i) the clinically more established but less quantitative myelin marker, the MT ratio (11), as well as to the longitudinal (T1) and apparent transverse (T2*) relaxation times.

Methods

Animal preparation: A female Sprague-Dawley rat (380g) was sacrificed after in vivo measurement under deep general anesthesia (5% isoflurane) by employing carbon dioxide inhalation. Animal experiments were approved by the local authorities of the State of Hamburg and conform to the guidelines set by the European Union. A rectal temperature probe was used during *in situ* imaging. *MRI:* The qMRI data of the rat brain was measured longitudinally: we started with *in vivo* MRI, then after sacrifice, we carried out *in situ* MRI of the unfixed brain (in intervals of 1hour for 10 hours), and finally after 20 days of immersion fixation we performed *ex vivo* MRI. Data were acquired on a 7T Bruker Clinscan small animal MRI system using a multi-echo spoiled gradient echo (SPGR) sequence with predominantly proton density (PDw; flip angle = 60), T1w- (210), and magnetization transfer (MTw; 60 with a 4ms Gaussian pulse 2kHz off-resonance prior to excitation) weighting. Two protocols were used (i) for *in vivo* / *in situ* MRI, and (ii) for ex vivo MRI. Each had a TR of 30ms and echoes were acquired with 1.26ms echo-spacing from TE = 2ms to TE = 13.34ms (MTw, 10 echoes) or 18.6ms (PDw and T1w, 14 echoes). Protocol (i) had a resolution of 0.3 mm isotropic and a total scan time of about 18 min, whereas protocol (ii) had a resolution of 0.17 mm isotropic and a total scan time of about 50 min, and was acquired with 4 repetitions. *Processing:* Quantitative maps of MT, T1, T2*, as well as MTR maps and effective proton density (the latter is not used here) (6,9,10,12) were calculated using in-house software in Matlab. To define regions-of-interests within WM and GM (Figs. 2-5), we segmented the MT maps at each time point using SPM Mouse (13) and SPM12. The in vivo qMRI data are not used for figure 2-5 because of severe physiological artefacts (see Fig. 1).

Results and Discussion

Our most important result was that MT was increased after fixation (Fig. 2,3,4) (as opposed to all other qMRI parameters). MT is independent of underlying T1 and thus behaved similar to the more quantitative but time-consuming z-spectrum magnetization-transfer imaging (qMT, (14)) – a more established myelin marker (15), supporting previous findings that MT saturation is a good proxy for qMT (8). *In situ,* MT (and T2*) decreased (increased) with increasing PMI and decreasing temperature but greatly increased (decreased) upon fixation. T2* (GM and WM) and MT (WM) showed less strong correlation to temperature effects than MTR and T1 (Fig. 5), indicating temperature changes and autolysis processes are differently affecting these metrics. While the observed temperature-dependence of T1 agrees to a recent experiment (5), the observed increase in T2* with decreasing temperature contradicts their findings. This difference between Birkl's and our study might be caused by the varying environmental influence *ex vivo* and *in situ*.

Conclusion

The fact that fixation effects in MT are similar to reported changes in the myelin-marker obtained from quantitative z-spectrum magnetization-transfer imaging (14,15), is an indication that MT is an equally sensitive but more efficient biomarker and thus. should be more widely used.

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References

(1) Stikov N, Campbell JSW, Stroh T, Lavelée M, Frey S, Novek J, Nuara S, Ho M-K, Bedell BJ, Dougherty RF, Leppert IR, Boudreau M, Narayanan S, Duval T, Cohen-Adad J, Picard P-A, Gasecka A, Côté D, Pike GB. In Vivo Histology of the Myelin G-Ratio with Magnetic Resonance Imaging. NeuroImage 2015;118:397–405.

(2) Stikov N, Campbell JSW, Stroh T, Lavelée M, Frey S, Novek J, Nuara S, Ho M-K, Bedell BJ, Dougherty RF, Leppert IR, Boudreau M, Narayanan S, Duval

T, Cohen-Adad J, Picard P-A, Gasecka A, Côté D, Pike GB. Quantitative Analysis of the Myelin G-Ratio from Electron Microscopy Images of the Macaque Corpus Callosum. Data Brief 2015;4:368–373.

(3) Shepherd TM, Flint JJ, Thelwall PE, Stanisz GJ, Mareci TH, Yachnis AT, Blackband SJ. Postmortem Interval Alters the Water Relaxation and Diffusion Properties of Rat Nervous Tissue--Implications for MRI Studies of Human Autopsy Samples. NeuroImage 2009;44:820–826.

(4) Schmierer K, Wheeler-Kingshott CAM, Tozer DJ, Boulby PA, Parkes HG, Yousry TA, Scaravilli F, Barker GJ, Tofts PS, Miller DH. Quantitative Magnetic Resonance of Postmortem Multiple Sclerosis Brain before and after Fixation. Magn. Reson. Med. 2008;59:268–277.

(5) Birkl C, Langkammer C, Golob-Schwarzl N, Leoni M, Haybaeck J, Goessler W, Fazekas F, Ropele S. Effects of Formalin Fixation and Temperature on MR Relaxation Times in the Human Brain. NMR Biomed. 2016;29:458–465.

(6) Helms G, Dathe H, Kallenberg K, Dechent P. High-Resolution Maps of Magnetization Transfer with Inherent Correction for RF Inhomogeneity and T1 Relaxation Obtained from 3D FLASH MRI. Magn. Reson. Med. 2008;60:1396–1407.

(7) Mohammadi S, Carey D, Dick F, Diedrichsen J, Sereno MI, Reisert M, Callaghan MF, Weiskopf N. Whole-Brain In-Vivo Measurements of the Axonal G-Ratio in a Group of 37 Healthy Volunteers. Front. Neurosci. 2015;9:441.

(8) Campbell J, Leppert I, Boudreau M, Narayanan S, Duval T, Cohen-Adad J, Pike GB, STikov N. inProc Intl Soc Mag Reson Med 24 2016 Abstr. 1501,2016.

(9) Helms G, Dathe H, Dechent P. Quantitative FLASH MRI at 3T Using a Rational Approximation of the Ernst Equation. Magn. Reson. Med. 2008;59:667–672.

(10) Weiskopf N, Suckling J, Williams G, Correia MM, Inkster B, Tait R, Ooi C, Bullmore ET, Lutti A. Quantitative Multi-Parameter Mapping of R1, PD*, MT and R2* at 3T: A Multi-Center Validation. Front. Brain Imaging Methods 2013;7:95.

(11) Tofts P. Quantitative MRI of the Brain: Measuring Changes Caused by DiseaseJohn Wiley & Sons2004. p 650.

(12) Weiskopf N, Callaghan MF, Josephs O, Lutti A, Mohammadi S. Estimating the Apparent Transverse Relaxation Time (R2*) from Images with Different Contrasts (ESTATICS) Reduces Motion Artifacts. Front. Neurosci. 2014;8:1–10.

(13) Sawiak SJ, Wood NI, Williams GB, Morton AJ, Carpenter TA. Voxel-Based Morphometry in the R6/2 Transgenic Mouse Reveals Differences between Genotypes Not Seen with Manual 2D Morphometry. Neurobiol. Dis. 2009;33:20–27.

(14) Schmierer K, Thavarajah JR, An SF, Brandner S, Miller DH, Tozer DJ. Effects of Formalin Fixation on Magnetic Resonance Indices in Multiple Sclerosis Cortical Gray Matter. J. Magn. Reson. Imaging JMRI 2010;32:1054–1060.

(15) Tozer DJ, Davies GR, Altmann DR, Miller DH, Tofts PS. Correlation of Apparent Myelin Measures Obtained in Multiple Sclerosis Patients and Controls from Magnetization Transfer and Multicompartmental T2 Analysis. Magn. Reson. Med. 2005;53:1415–1422.

Figures

Figure 1: MT map measured with *in vivo*, *in situ*, and *ex vivo* MRI

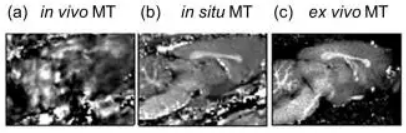

Figure 1: MT map acquired using *in vivo*, *in situ* (1 hour after scarification, unfixed), and *ex vivo* (after 20 days immersion fixation) MRI. Depicted are representative image quality in the three tissue status, indicating that the *in vivo* multi-parameter-mapping (MPM) protocol suffered from severe motion artifacts. The artifacts were probably due to residual cardiac pulsation and breathing that remained after anesthetizing the rat. Thus, we excluded the *in vivo* MPMs for the rest of the analysis.

Figure 2: Changes from in-situ to ex-vivo situation in gray matter

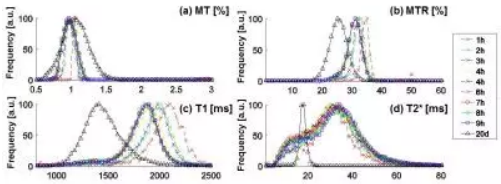

Figure 2: Detailed assessment of effects of autolysis and fixation in a gray matter (GM) mask is performed by depicting the histogram of the MT (a), MTR (b), T1 (c), and T2* (d) maps, longitudinally, using the time points 1 to 9 hours after scarifications, and 20 days after immersion fixation. The maximum of the histogram follows the same trend as the median of each metric in GM (for details see Fig. 4). The width of the histogram, however, provides additional information: it changes substantially after fixation (increased for MT, MTR, T1, and decreased for T2*).

Figure 3: Changes from in-situ to ex-vivo situation in white matter

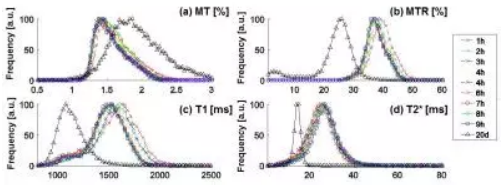

Figure 3: Detailed assessment of effects of autolysis and fixation in white-matter (WM). The same as in Fig. 3 is depicted using a WM mask. For the maximum of the histograms, the same trend as in Fig. 3 is observed. The width of the histograms of the MT and T2* values (a and d) show similar trends as in GM. The width of the histograms of the MTR and T1 values (b and c) behave differently as in GM: it stays the same or is even slightly decreases.

Figure 4: Relative change from *in situ* to *ex vivo* situation

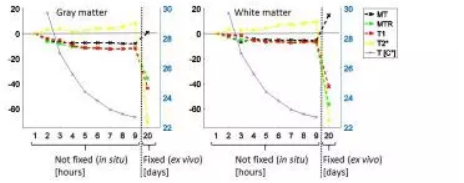

Figure 4: Fixation effects are assessed by relative change (to first time-point) of the median values of MT (black), MTR (green), T1 (red), and T2* (yellow) in GM (a) and WM (b), using longitudinal MRI of one unfixed rat brain (*in situ*) after scarification, and *ex vivo* MRI after immersion fixation (20 days). Changes of *in situ* metrics are small in GM and WM (T2*: +10%, other metrics: -10%). After fixation MTR, T1, and T2* decrease strongly (T1: -40%, T2*: -70%, MTR: -60%), while MT increases slightly (GM: +1%, WM: +15%). Temperature and autolysis effects are analyzed in Fig. 5.

Figure 5: Correlation with temperature

	MT	MTR	T1	T2*
Gray matter	R=0.900 P=0.003**	R=0.982 P<0.001**	R=0.959 P<0.001**	R=-0.500 P=0.209
White matter	R=0.573 P=0.137	R=0.982 P<0.001**	R=0.916 P=0.001**	R=-0.811 P=0.015*

Figure 5: To disentangle temperature (blue curve in Fig. 2) from other effects such as autolysis, the correlation between MT, MTR, T1, and T2* curves and temperature is evaluated for the time points 2h to 9h after scarification. In GM, MT, MRT, and T1 are strongly and significantly correlated to temperature, whereas T2* is not. In WM, MTR and T1 are strongly and significantly correlated to temperature, T2* is less strongly but still significantly correlated, and MT is not. These results indicate that T2* (GM and WM) and MT (WM) metrics are stronger affected by autolysis than temperature effects.