MRI Contrasts Generated Using Fictitious Fields in High-Rank Rotating Frames Correlate With Myelin Content in Normal Rat Brain ex vivo

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Introduction: A unique feature of relaxation experiments in high rotating frames of ranks n > 2 is the possibility to probe slow to ultra-slow motional regimes, as demonstrated in the pioneering works by Mefed and co-workers who measuring a significant dipolar line narrowing of CaF_2 single crystal in the 3rd rotating frame [1]. Recently, the method entitled RAFFn (Relaxation Along a Fictitious Field, where n indicates the rank of the rotating frame) was introduced and used for generating relaxation dispersion in living tissue [2-4]. With this method, a correlation between relaxation time constant T_{RAFFn} and histologically derived cell density in rat glioma gene therapy model was shown [5]. Here, we demonstrate the advantage of RAFFn [3,4] to probe slow motion by quantitatively comparing the MRI relaxation time constants T_{RAFFn}, free precession T_1 and T_2, magnetization transfer (MT) ratio (MTR), and adiabatic $T_{1\rho}$ and $T_{2\rho}$ with histologically derived myelin content in the rat brain *ex vivo*.

Materials and Methods: The amplitude and frequency modulations functions of RAFFn were generated as discussed in prior works [3,4] and the signal intensity decay was measured as a function of the number of pulses in the pulse train, assembled in $PP^{-1}P_{\pi}P_{\pi}^{-1}$ packets [2]. Five rat brains were perfused with 4% paraformaldehyde and washed out with saline prior to the imaging. MRI experiments of Galden immersed brains were carried out in a 9.4 T magnet using a quadrature volume transceiver. Fast spin echo readout (TR=5s and effective TE=7ms, 512x256 matrix size in 15x15 mm^2 FOV) was used for all experiments. T_2 was measured with adiabatic double spin echo preparation, T_1 with inversion recovery, and MTR was obtained using ZAPISM method (ω_1^{max}= 150 Hz, duration of irradiation 2s, and saturation bands 5 kHz off-resonance) [6]. After MRI measurements the brains were washed out in saline, cryoprotected and frozen. 30 µm sections were stained with gold chloride for myelin. Optical density was calculated and correlated with MRI parameters.

Results and Discussion

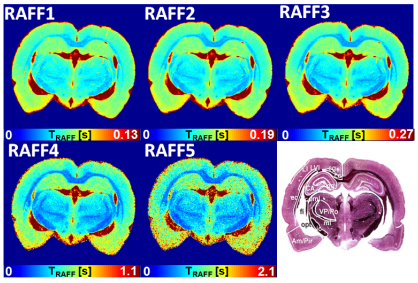

Fig. 1 Representative example of RAFFn relaxation time constant maps and Gold chloride stained section from MRI matched slice. The ROIs used for correlation analysis are superimposed on the histological section.

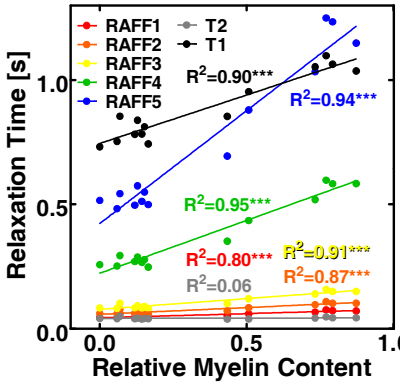

Fig. 2 Linear regression between MRI time constants and myelin content. (*** p < 0.0001)

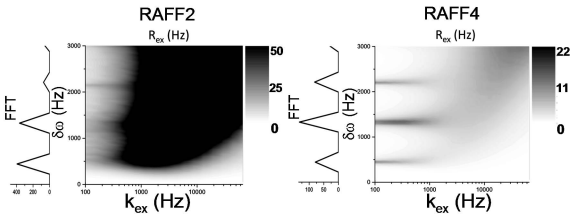

Fig. 3 Product operator calculations [7] of the exchange-induced relaxation rate constants of RAFF2 and RAFF4 as a function of chemical shift difference and exchange rate for two-site exchange (2SX). The fractional population of sites A and B used in calculations were P_A=0.9 and P_B=0.1. The corresponding FFT of the RAFF2 and RAFF4 pulse patterns are shown on the left of each image.

The measurements demonstrate an increase of T_{RAFFn} values with the rank n (**Fig. 1**). In all T_{RAFFn} maps the tissue contrast was sufficient to delineate areas of high myelin content (blue areas) which corresponded well with the areas of high myelin content in histological sections (dark purple areas). Linear regression analysis reveals higher correlation between T_{RAFF4} and T_{RAFF5} with myelin content than other MR relaxation methods including T_1, T_2, continuous wave CW $T_{1\rho}$,, adiabatic $T_{1\rho}$ and $T_{2\rho}$ measured with hyperbolic secant pulses of the HSn family, n=1 or 4 [8], and MTR (**Fig. 2**). In white matter, the anisotropy of water motion as well its restriction are likely the main reasons for higher correlation observed between $T_{RAFF4\ and\ 5}$ and myelin than with other MRI methods, suggesting greater sensitivity of rotating frame experiments with ranks 4 and 5 to slow/restricted molecular motion. In **Fig. 3**, the calculations of exchange-induced relaxations for 2SX during RAFF2 and RAFF4 are shown. It can be seen that the presence of the sidebands in RAFF4 leads to enhanced relaxation in the slow exchange regime ($k_{ex}/\delta\omega << 1$). The simulations show enhanced relaxations for the chemical shifts which correspond well to the sidebands seen in the FFT of the pulses (**Fig. 3**). The averaged tip angle during the pulse duration of RAFFn decrease with the increase of n and the contrast increase. For example, for RAFF4 the tip angle is smaller than that of RAFF2 (6° vs. 54°). This could lead to greater contribution of slow exchange induced relaxation to RAFF4 than to RAFF2. Because the R^2 between measured MTR and myelin content was fairly low (0.46, p<0.01), the MT contribution cannot explain high R^2 between $T_{RAFF4,5}$ with myelin content. Therefore, we attribute high correlation between $T_{RAFF4,5}$ with myelin content to the sensitivity of RAFFn to slow motion. Spin-echo readout with nominal echo time of 9 ms likely negatively influenced the relaxation mapping assessment. Therefore, future implementation of the readout with no echo time (i.e., SWIFT [9]) will be beneficial. The data presented strongly indicates correlation between high rank rotating frame relaxation measurements and myelin content in the brain. **Acknowledgments:** Academy of Finland, Sigrid Juselius Foundation, NIH Grants BTRC P41 EB015894, P30 NS057091, R01 NS061866. **References:** [1] A.E. Mefed, Appl. Magn. Reson., 21, 127-145, 2001. [2] T. Liimatainen et al. MRM 2010 [3] T. Liimatainen et al. ENC abstract 2012, [4] T. Liimatainen et al. ISMRM abstract 2012, [5] T. Liimatainen et al. MRM 2012, [6] J Närväinen et al. JMR 2010, [7] D Idiyatullin et al. JMR 2004, [8] Michaeli et al. CAC 2008, [9] Idiyatullin et al. JMR 2006.