Enhancement, De-enhancement and Contrast Locking of Fixed Rodent Brains

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Introduction: Magnetic resonance histology is now well established as a non-destructive tool for morphological studies which preserves the geometric integrity of tissue samples, allows high-resolution, high SNR estimation of microstructure [2,3]. Effective design of MR histology experiments generally requires accurate knowledge of tissue relaxation times. Diffusion-weighted MRI for example can be severely compromised by over-enhancement which potentially reduces T_2 relaxation times to impractically short levels, particularly in white matter[1]. The lack of information in the literature regarding the rate of ingress and egress of typical Gd-chelate contrast agents from fixed tissue motivated this study, which attempts to quantify time constants for the enhancement and also address the practical issue of locking enhancement at a given level for interim storage prior to MR imaging.

Method: Three C57BL/6 mice (mean age = 6 weeks) were deeply anesthetized with .3ml avertin and transcardiac perfused with 4% paraformaldehyde following exsanguination with phosphate buffered saline. Following perfusion fixation, mice heads were removed and stripped of superficial tissue and jaw prior to overnight post-fixation in 4% PFA at 4C. Four phases of a typical enhancement protocol were studied: (1) post-fixation washing for 5 days in PBS (2) contrast enhancement for 8 days in 2mM, 5mM and 10mM gadoteridol (Prohance, Bracco Diagnostics) (3) immersion in a polyfluropolyether oil (Galden, Solvay Solexis) for 15 days and (4) final washout of contrast agent in PBS. In all cases 0.01% sodium azide was added to aqueous solutions to suppress mold and bacterial growth. Both ROI-based global estimates and spatial maps of T_1 and T_2 were calculated for each time point and contrast agent concentration.

Results and Discussion: T_1 relaxation enhancement by immersion in millimolar concentrations of gadolinium chelate is an effective and straightforward method for improving the efficiency of high-resolution MR histology. The time-course of the enhancement suggests that an equilibrium would be reached approximately 8 days and that gadoteridol concentration in the 1-5 mM range is ideal for fixed rodent brain, resulting in general T_1 relaxation times of 100-200ms and T_2 times of 20-30ms (Fig.1). Replacing the surrounding medium with fluorinated oil effectively stops any further ingress of contrast agent, though some residual spatial equilibration of enhancement is observed over the course of a week (shown in Fig.2). Further soaking of the sample in PBS, gradually removes the contrast agent from the sample at a comparable rate to that seen during enhancement. The use of an oil to lock contrast enhancement benefits large scale sample preparation for MR histology without requiring a staggered contrast enhancement start.

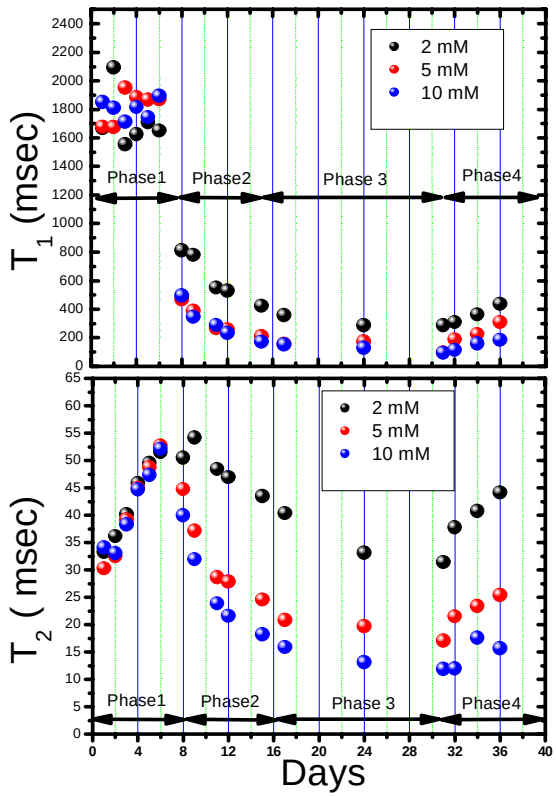

Fig1. Time course of relaxation times for each mouse brain during each phase of the enhancement, locking and de-enhancement

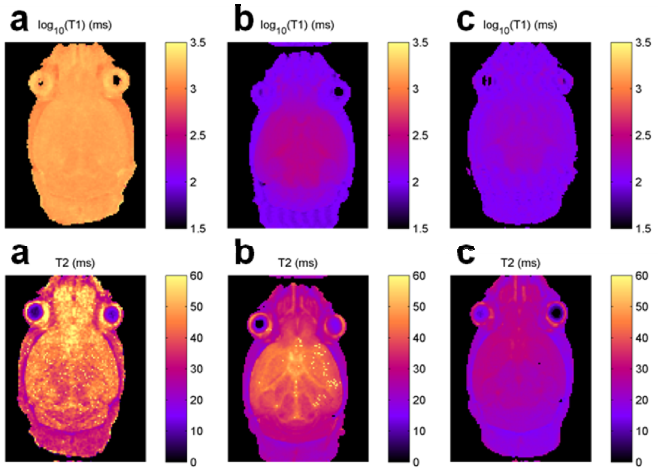

Fig 2. T_1 (top,log10 scale) and T_2 (bottom) maps of 2mM mouse brain at (a) day 6 (b) day 15 and (c) day 24. There is a clear distinction in enhancement across brain, particularly in caudal regions where gadoteridol enhancement appears to proceed more rapidly..

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