Magnetic Resonance Microscopy of ex vivo Mouse Brains: Contrast and Structure

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Introduction: With the advances in magnetic resonance imaging (MRI) techniques, MR imaging resolution has approached to a microscopic level, comparable to that of light microscope. Magnetic resonance microscopy (MRM) has now become an important tool to study brain structures in healthy and diseased animal models [1-4]. The inherent motion free nature of *ex vivo* MRM and the ability to manipulate tissue contrast by "staining" samples with different contrast agents enable researchers to investigate the differences and changes in brain structure and tissue content across different mouse strains and transgenic mouse models. In addition, further tissue preparation for histologyical and pathological purpose is also possible, as the brain stays intact during MRM acquisition. Therefore, multiple data sets from different imaging modalities can be obtained for the same animal. This allows co-registration, direction comparison, and across validation of images obtained from different imaging methods on the same animal, and helps neuroscientists to better understand differences in different mutant mice and to draw meaningful conclusions. The goal of this study is to investigate the changes of tissue contrast of ex vivo mouse brain by using Gd-DTPA and $MnCl_2$ as MRI contrast agents. Our preliminary results showed that both Gd-DTPA and $MnCl_2$ could improve tissue contrast, however, with different tissue differentiation abilities.

Methods and Results: Male C57BL/6J mice (8 to 10 weeks old) were anesthetized and transcardial fixed through the left ventricle. The brain was first perfused with heparinized saline, followed by 4% paraformaldehyde (PFA) with or without contrast agent. The brain was then taken out and post fixed in the same PFA buffer overnight. It was then transferred to PBS buffer with same contrast agent of the same concentration used during fixation. The brain was imaged one week after soaking in PBS buffer to ensure full penetration of contrast agent in the brain. We used 5 mM Gd-DTPA (Magevist) and 0.24 mM $MnCl_2$ (Sigma) in 4% PFA and PBS buffer, respectively.

Magnetic resonance microscopy was done at 14T system (Bruker Biospin). Quick scan at three day and one week after soaking in PBS was done to test contrast agent penetration using gradient echo sequence with the same imaging parameter (FLASH sequence: TR/TE: 400/3.4 7.1 10.8 14.6 ms, 37x37 μm^2 in plane resolution). We found that three day soaking was not enough; therefore, we choose to image brains one week after soaking in PBS. T1 and T2* of the brain were also measured using IR_RARE and multi-gradient echo sequences, respectively. Table 1 shows the T1 and R2* change brain tissue after soaking in different contrast agent. High-resolution 3D images were acquired using a gradient echo sequence (FLASH). The imaging parameters are as following: TR/TE: 35/10 ms, FOV: 1.3x1.3x0.95 cm, Matrix: 512x512x256, resolution: 25x25x37 μm^3, average: 10, imaging time: about 10 hours. The flip angle was chosen to maximize the signal. Figure 1 shows high-resolution images of two mouse brains stained with different contrast agents. Figure 1B is the brain stained with 0.24 mM $MnCl_2$, and figure 1C is the brain stained with 5 mM Gd-DTPA. Both contrast agents can enhance tissue contrast and help reveal detailed brain structures. However, they showed different tissue specificity, especially shown in cerebellum. From figure 1 we can clearly see that granular layer is stained dark in $MnCl_2$ staining, but is bright in Gd-DTPA staining.

Discussion: In summary, (1) we have shown that both contrast agents help enhance image contrast and increase image SNR. (2) There are tissue specific differences in the resultant contrast between the two contrast agents especially in cerebellum. This may help us to delineate brain cytoarchitecture. Our protocol of $MnCl_2$ administration in the perfusate appears to lead to activity induced uptake, and along with Mn^{2+} scalor-coupling relaxivity may account for the observed tissue differentiation. (3) The two MRM staining methods may complement each other and enable a better understanding of the phenotypic changes observed in transgenic mouse models. Future work will include fully characterize tissue MR parameters after MR contrast agent staining, histology comparison, and application to transgenic mouse model.

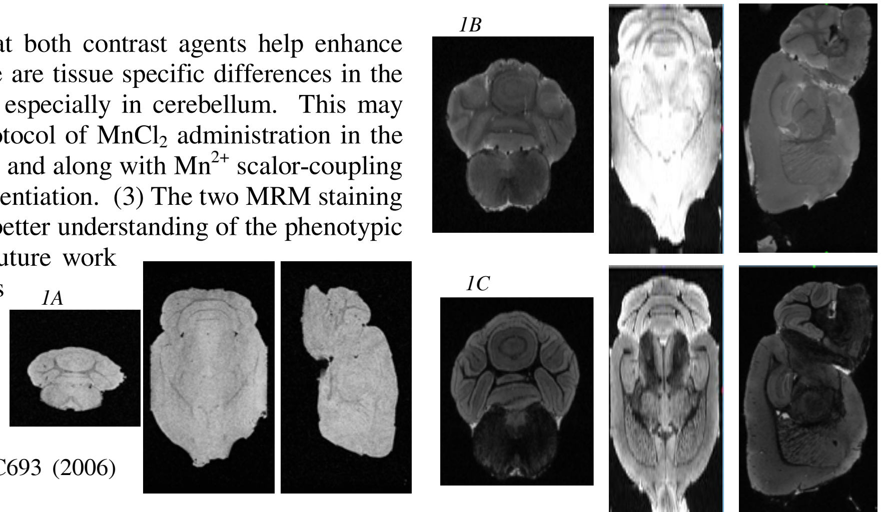

Figure 1: High-resolution images of unstained (2A), stained with 0.24 mM MnCl2 (2B) and 5 mM Gd-DTPA (2C) reaveal different tissue contrast. Unstained brain was acquired at a a resolution of 37 µm isotropic.

References: **1.** J. Magn. Reson. Imaging, 16, 423-429, 2002 **2.** Magn. Reson. Q. 9, 1-30, 1993 **3.** NeuroImage 26 (2005) 744C: 754 **4.** MRM 55:687C693 (2006)

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	Brain in 0.24 mM $MnCl_2$		Brain in 5 mM Gd-DTPA	
	T1 (ms)	R2* (1/s)	T1 (ms)	R2* (1/s)
cortex	166.3409	145.8991	62.923	181.3029
striatum	172.4568	126.8839	63.5178	198.3527
thalamus	172.3263	122.047	67.6622	199.6143
hippocampus	188.5096	129.7029	61.7058	175.9285

Talbe 1: T1 and R2 in selected regions of the brains soaking in different MR contrast agetns*