Improved Tissue Contrast of ex vivo Mouse Brain Using Magnetic Resonance Microscopy with Different MR contrast Agents

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Introduction: Magnetic resonance microscopy (MRM) has been widely used in various anatomical studies of normal, transgenic [1], or mutant mice [2] and developing mouse embryos [3]. Several groups have successfully established detailed mouse brain atlas based on high-resolution MRM images. In some of the previous studies, gadolinium-based MR contrast agents have been used to reduce imaging time and improve signal-to-noise ratio (SNR). On the other hand, multiple relaxation mechanisms and varying tissue-dependent affinity of different contrast agents may provide a wide range of tissue staining flexibility resembling that found in conventional histology. In this respect, the use of extrinsic contrast agents in MRM studies can be used not only to enhance overall image contrast but also to improve tissue and cellular specificity. The goal of this study was to investigate the possibility of manipulating tissue and cell contrast using two commonly used MR contrast agents: Gd-DTPA and $MnCl_2$, and to examine the neuro-chemical correlates of these different staining agents.

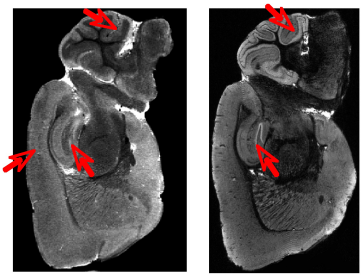

Figure 1: mouse brains stained with 0.36mM $MnCl_2$ (1a)and 5 mM Gd-DTPA (1b) showed different image contrast in different regions of the brain, such as Cerebellum, hippocampus, and cortex. In cerebellum, the granular cell layers is dark in MnCl2 stained brain. While it is brighter in Gd-DTPA stained brain.

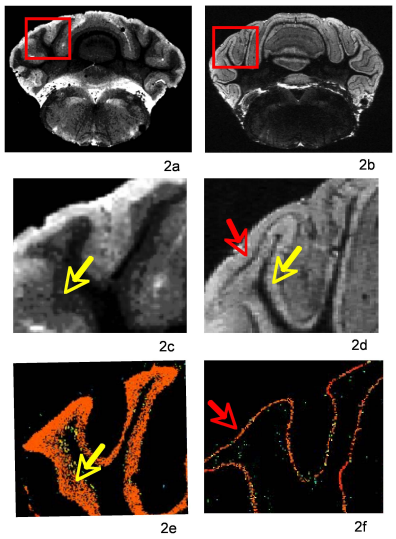

Figure 2: Regions in cerebellum of brain stained with $MnCl_2$ and Gd-DTPA showed correlation with zinc finger protein of the cerebellum 1 (Zinc1) expression and lectithin cholesterol acyltransferase (Lcat).ZInc1 and Lcat expression images are obtained from online Allen Brain Atlas.

Material and method: Male C57BL/6J mice (8 to 10 weeks old) were anesthetized and transcardial fixed through the left ventricle first with heparinized saline and then with 4% paraformaldehyde (PFA) solution mixed with MR contrast agent (5mM Gd-DTPA or 0.36 mM $MnCl_2$.). The brain was then removed and immersed in the same fixation solution overnight. All MRM images were acquired using a 14T magnet (Magnex Scientisfic, England) connected to a ParaVision console (Bruker Biospin) with a maximum gradient strength of 100 Gauss/cm. Bruker 10mm volume RF coil was used for image acquisition. Brains were placed in a glass tube filled with proton-free susceptibility matching agent (Perfluoro-compound FC-40, Fisher Scientific) during imaging to reduce the background signal. Before the high-resolution image acquisition, T_1, T_2, and T_2* of the brain were measured. 3D high-resolution images for MRM were obtained using a FLASH pulse sequence (TR/TE= 35/10 ms, FOV: 1.45x0.95x0.95 cm, Matrix: 400x256x256, with an isotropic resolution of 37 μm in each dimension).

Results and discussion: Table 1 shows the quantified relaxivity of $MnCl_2$ and Gd-DTPA in water and within ex vivo mouse brains. Their different relaxation and distribution properties led to significant different image contrasts observed in multiple regions throughout the brain, as shown in figure 1. For example, in cerebellum, the granular cell layer appears dark in the $MnCl_2$ stained brain but is bright in the Gd-DTPA stained brain (figure 2a and 2b). Interestingly, the staining patterns of $MnCl_2$ and Gd-DTPA showed differential staining which corresponded with genes highly expressed in neurons within the granular layer, for example the zinc finger protein1 (Zinc1) in cerebellum (figure 2c, 2d and 2e), a gene which plays an important role in cerebellar development. The dark staining of this layer using $MnCl_2$ indicates that Mn^{2+} may become compartmentalized into neurons in this layer enhancing T_2* relaxation, lowering the Mn signal intensity in T2*-weighted image. In addition, the distinctive dark line between the two layers in cerebellum cortex of our Gd-DTA stained brains shows remarkable correlation with lecithin cholesterol acyltransferase (Lcat) expression (figure 2d and 2f). Lcat, highly expressed in a distinct cell layer within the molecular layer adjacent to the Purkinje cell layer, is an important protein involved in lipid metabolism. Our study suggests that with the use of multiple MR "staining" agents in *ex vivo* MRM, we can manipulate image contrast to achieve differentiation MRM staining, which correlates strongly with detailed cytoarchitecture and may ultimately offer a window into patterns of gene expression. The use of MR contrast agents, therefore, offers us an MRM staining flexibility for further tissue delineation in addition to general improvement in SNR. With further biochemical manipulations of contrast agents as cell or tissue specific stains, high-resolution three-dimensional tissue/cell specific images can be acquired using MRM.

Relaxivity (S^{-1}• mM^{-1})	Gd-DTPA		$MnCl_2$	
	In water	In tissue	In water	In tissue
r_1	3.88 ± 0.12	2.813±0.26	6.3 ± 0.1	19.87±1.39
r_2	5.43 ± 0.11	9.96±0.21	162.44 ± 3.74	228.18±11.47
Ratio: r_2 /r_1	1.4	3.54	25.79	11.48

Table 1: Relaxivity of $MnCl_2$ and Gd-DTPA in water and ex vivo mouse brain at 14T

Reference: 1. Ma Y, et al. Neuroscience 2005. 2. Cry M, et al. NeuroImage 2005. 3. Schneider JE. Magnetic Resonance Materials in Physics, Biology and Medicine 2003**. 4.** Allen Brain Atlas www.brain-map.org

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