Magnetic resonance characteristics of sucrose-infiltrated ex-vivo brain tissue preparations

Roger Jacob Mullins[1,2], Da Shi[1,3], Jiachen Zhuo[1], Su Xu[1], and Rao P Gullapalli[1]

[1]*Diagnostic Radiology and Nuclear Medicine, Core for Translational Research in Imaging @ University of Maryland, Baltimore, Maryland, United States,* [2]*Program in Neuroscience, University of Maryland, Baltimore, Maryland, United States,* [3]*Department of Biochemistry and Molecular Biology, University of Maryland, Baltimore, Maryland, United States*

Introduction

Magnetic resonance imaging of fixed brain tissue can be performed in conjunction with conventional tissue histology. Histology protocols often require fixation of ex vivo brain tissue in 4% paraformaldehyde (PFA) and then transfer to 30% sucrose for cryoprotection. It is well known that fixation in PFA alters MR characteristics[1,2,3], but the extent to which sucrose infiltration may affect the MR signal and image quality has not been examined. Sucrose is a hygroscopic molecule, which makes it very likely to perturb the outcome of MR methods reliant on the properties of water diffusion. Here, we compared the MR characteristics of ex vivo brain samples stored in 4% PFA or 30% Sucrose solution, and further relate these to in vivo mouse brains.

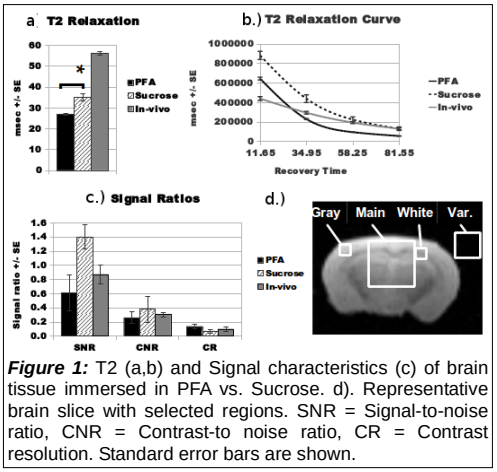

Figure 1: T2 (a,b) and Signal characteristics (c) of brain tissue immersed in PFA vs. Sucrose. d). Representative brain slice with selected regions. SNR = Signal-to-noise ratio, CNR = Contrast-to noise ratio, CR = Contrast resolution. Standard error bars are shown.

Materials and Methods

Animal model

Wild-type (JAX C57Bl/6J) mice were obtained from a breeding colony at the University of Maryland, Baltimore. All mice were weaned at day 21 with food and water given ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee at University of Maryland, Baltimore. Mice were anesthetized at PND 30 with 4% isoflurane and perfused with 0.1M phosphate buffered saline then 4% PFA. The whole brain was stored in 4% PFA for two days, then in 30% sucrose solution for a week. The fixed brain was placed in a customized conical tube filled with Fluorinert (3M, St. Paul, MN) to decrease background signal during scanning. Diffusion tensor images were obtained on six fixed brains previously in 4% PFA and six others that were in 30% sucrose. T2 relaxation data was obtained on three of the fixed brains from 4% PFA and 3 of those in sucrose. The same parameters were obtained on two mice in-vivo at PND 30.

MRI/MRS experiments

All experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner equipped with a BGA12S gradient system capable of producing pulse gradients of 300 mT/m in each of the three axes, and interfaced to a Bruker Paravision 5.1 console. A Bruker four-element ^{1}H surface coil array was used as the receiver and a Bruker 72mm linear-volume coil as the transmitter.

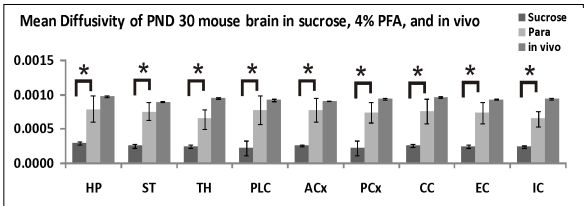

Figure. 2: Mean diffusivity values of PND 30 mouse brain immersed in sucrose, 4% PFA and in vivo in regions of white and gray matter. Standard error bars are shown. *p<0.05.

ex-vivo MRI: Diffusion tensor images (DTI) were acquired with the 4-shot spin echo echo-planar-imaging sequence in the axial plane. 30 diffusion directions were applied at b=700 s/mm^2, 2100 s/mm^2, and 4000 s/mm^2. Five images at b=0 s/mm^2 were acquired. The field of view was 1.88×1.50cm^2, with matrix resolution 64×64, TR/TE 6000/27.7msec, slice thickness 1mm, 12 slices and one average. T2 relaxation map images were acquired with a Bruker Rapid Acquisition with Relaxation Enhancement with Variable Repetition Time (RAREVTR) with FOV = 20×20 mm^2, matrix size = 100×100, slice thickness = 1 mm, number of slices = 10, and number of averages = 1, in the axial plane, TR = 5000.00 msec; TEs = 11.65, 34.95, 58.25, 81.55 msec.

in-vivo MRI: The mouse was anesthetized in an animal chamber using a gas mixture of 100% O_2 (1 L/min) and 4% isoflurane. The animal was then placed prone in an animal holder and the RF coil was positioned and fixed over the cranium. Diffusion weighted images (DWI) were acquired with a spin echo sequence with four segments in the axial plane. Three diffusion directions were applied with b=350, 700, and 1050 s/mm^2. Four images at b=0 s/mm^2 were acquired. Field of view was 2.50×2.50 cm^2, with matrix resolution 120×120, TR/TE 4500/26.9msec, slice thickness 1mm, 10 slices and four averages. In vivo T2 relaxation scans were acquired with the same sequence as the ex vivo scan, above.

Image processing: Regions of interest (ROI) in DTI and DWI images were drawn with FSLview (Analysis Group, FMRIB, Oxford, UK) including the hippocampus (HP), striatum (ST), thalamus (TH), prelimbic cortex (PLC), anterior cortex (ACx), and posterior cortex (PCx). White matter regions included the corpus callosum (CC), external capsule (EC), and internal capsule (IC). T2 map images were analyzed with Paravision 5.1, using the image sequence analysis and ROI functions. For overall T2, a 0.10 cm^2 voxel was chosen centered on the third ventricle. For gray and white matter contrast, the same slice was used with 0.0064 cm^2 ROI centered on the dorsal cerebral cortex and corpus callosum, respectively (fig 1d). An ROI placed at an empty corner of the axial slice was used to measure noise variance.

Results

Preliminary results on PND 30 mice revealed that brains immersed in 4% PFA have greater mean diffusivity (MD) than sucrose samples (n=6,p<0.05) (Fig.2). In vivo MD values (n=2) are closer to 4% PFA MD values (Fig.2). Radial and axial diffusivity is also greater in brains immersed in 4% PFA than sucrose (data not shown, n=6,p<0.05). The increased diffusion value of brains in 4% PFA compared to 30% sucrose was found in whole brain and specific gray and white matter regions. No difference in fractional anisotropy was observed between sucrose and 4% PFA immersed brains. Sucrose immersed brains (n=3) showed an increased signal intensity (p<0.01) and higher T2 relaxation (p<0.05) compared to those in 4% PFA (n=3, Fig. 1a).

Discussion and Conclusions

Understanding the MR characteristics of fixed tissue in different storage solutions enables the optimization of scans to improve the image. The low diffusion values in sucrose-infiltrated samples compared to 4% PFA samples could be caused by sucrose altering water diffusion properties by interacting with water or altering microstructures within the brain. The low diffusivity parameters indicate that sucrose-immersed brains may not be optimal for DTI, as higher b-values (stronger gradients) must be employed to induce the same diffusion effect. A previous study found no change in diffusion parameters between in vivo and ex vivo mouse brains[4], similar to our findings of in vivo and 4% PFA immersed brains. Despite the low number of subjects, the effects of sucrose immersion on T2 and signal characteristics appear to be particularly strong. Particularly striking is the significantly higher T2 relaxation time (Fig. 1a&b) and the unexpected trend towards a higher SNR and CNR in the sucrose immersed samples (Fig. 1c). Further experimentation along these lines would provide more reliable findings.

References: 1. Miller et al., Neuroimage 2011; 57(1-4). **2.** Tovi & Ericsson., Acta Radiologica, 1992: 33(5), **3.** Sun & Neil., Magnetic Resonance in Medicine, 2005, 53(6) **4.** Sun et al. Magn Reson Med. 2003 50(4):743-8.