Rapid Quantitative Magnetic Resonance Microscopy of Excised Anatomy in the Transgenic Mouse at 3.0 Tesla

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Introduction: Recently, significant advances have been made in the application of high field magnetic resonance imaging to small animal models in an attempt to more rapidly and accurately characterize anatomical and physiological differences in biological organisms. We report here a methodology for obtaining high spatial resolution images (100µm x 80µm x 80µm) of excised biological samples from transgenic mice. These images had a signal to noise ratio (SNR) of 25:1 or greater and were obtained in approximately 30 minutes using a standard 3.0 TESLA human clinical scanner. The acquired images were suitable for robust morphometry of the murine hippocampus, as well as visualization of cardiac arteries in the murine heart. The resolution and sensitivity were achieved through the use of 1) an optimized three-dimensional MP-RAGE pulse sequence, 2) specially designed sample preparation methods, and 3) modular, parallel wound solenoidal radiofrequency resonators. The results indicate that field strengths in the 3.0 Tesla range may be advantageous for studies of certain anatomy. The fact that images were obtained with high sensitivity and resolution within such a short time frame implies the utility of this experimental design for high throughput studies of animal models of disease, greater utility of clinical scanners, and accessibility and application of magnetic resonance microscopy to a broader group of researchers without access to high field scanners.

Figure 1

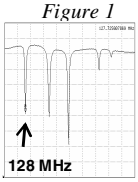

Figure 2

Methods: *Animals:* FoxG1 heterozygous "knock-in" lines were used in which the intron-less FoxG1 coding region was replaced with tetracycline transactivator (tTA). *Sample Preparation*: For brain and heart preparation, mice were deeply anesthetized with pentobarbital. Mice were intracardially perfused with 0.9% saline containing 0.1% sodium nitrite and 5% Gd-DTPA followed by a 4% paraformaldehyde and 5% Gd-DTPA in phosphate buffered saline (PBS). Perfusates were administered at a rate of 5mL per minute using a silastic pump. The brain and/or heart was postfixed and then stored for 3-7 days in a 0.1M PBS solution containing 5% Gd-DTPA until the time of imaging. *Radiofrequency Resonators:* A modular coil design was used, consisting of five inductively coupled, 10 mm diameter resonant loops arranged in a solenoidal geometry with an inductively coupled drive loop at one end (Fig. 1). The coil was wound directly on a 3cc syringe containing the sample, and was easily removed from the mounting platform when changing specimens. The lowest of five observed resonant modes was used for imaging (Fig. 2). The coil was tuned with mini trimmer capacitors on the loops and matched by optimizing the distance to the drive loop. *Magnetic Resonance Imaging*: All image data were acquired on a GE Medical Systems 3.0 Tesla EXCITE whole-body magnetic resonance imaging system. The MP-RAGE sequence had the following acquisition parameters: pulse repetition time = 20.3 ms, echo time = 9.6 ms, field-of-view = 1 cm X 2 cm, slice thickness = 100 µm, matrix size 128X256X86, flip angle = 60 degrees, and number of averages = 4, inversion time = 725 ms, and delay time = 1400 ms. These parameters yielded a total imaging time of approximately 30 minutes for complete brain or heart coverage. *Image Post-processing and Volumetrics*: 3D MRI dicom files were analyzed using Osirix software with the observer blind to the genotype of subjects. Anatomical boundaries for the hippocampus

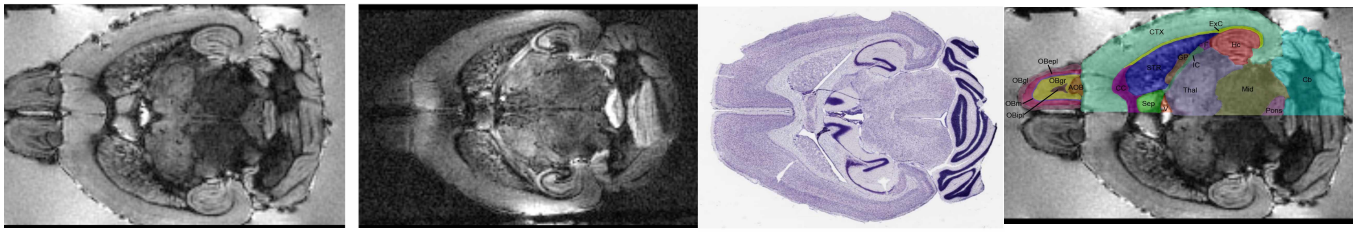

Figure 3 – Axial brain images (left to right): MRI in H_2O, MRI in D_20, Histology, Segmented Brain

were checked in reference to a mouse brain atlas. Slice preparation sections were analyzed using Steroinvestigator software. The entire volume of the hippocampus was measured at 4X objective magnification. The external capsule, alveus of hippocampus, and white matter were used as boundary landmarks. All sections throughout each hippocampus were traced and reconstructed. The Cavalieri estimator function was used to calculate the volume of each hippocampus.

Results: The use of a three-dimensional MP-RAGE pulse sequence at nearly isotropic resolution allowed the images to be reformatted in any spatial plane. Detailed imaging of anatomical structure was obtained at 30 minutes through the combination of a solenoidal coil design, MP-RAGE sequence *(1)*, a modified version of the "active stain" procedure *(2)*, and the use of different carrier media surrounding the specimen being imaged (Fig. 3). Image resolution and SNR allowed for the segmentation of multiple brain structures including substructural detail in the murine hippocampus. In addition, the inclusion of a vasodilator ($NaNO_4$) in the (1:20) gadolinium containing perfusates allowed for visualization of patent blood vessels (Fig. 4 and Fig. 5). To assay the utility of these techniques for magnetic resonance histology (MRH) we measured hippocampi of adult mice. Volumetric measurements conformed to previously published results at higher fields *(3)*. Further reliability of these measurements were assessed through direct comparison of brains measured first with MR segmentation techniques and then with standard histological procedures (Cavalieri estimation). We found a high degree of reliability between techniques, with MR volumes differing from standard histological procedures on average by 5% (+/-3.1% SEM). In order to assess whether these techniques would be sensitive enough to detect group differences in brain morphology of genetically modified mice, we used a recently described knock-in mouse in which expression of a single allele of the Foxg1 gene was replaced by tetracycline transactivator (tTA). These mice have been shown to have marked changes in hippocampal morphology, namely a loss of cell comprising the dentate gyrus of the hippocampus and general microencephaly. Brains of these animals were imaged and then segmented to assess changes in hippocampal volume. We found an 38.6% decrease in total volume of Foxg1+/- mice compared to wildtype controls.

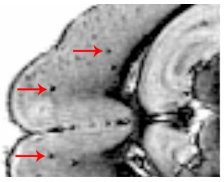

Figure 4

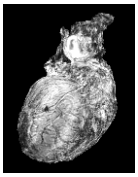

Figure 5

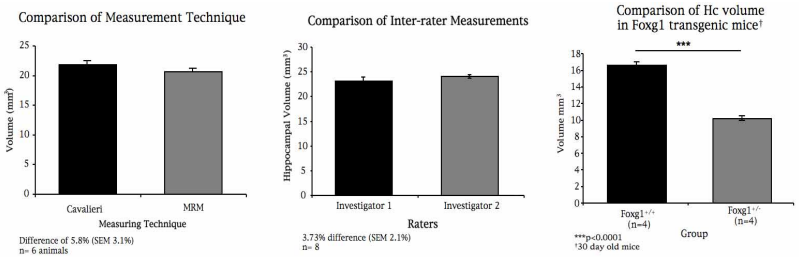

Figure 6

Conclusions: There is little doubt that the preparation methods are critical to the above results, as it was observed that the signal-to-noise ratios increased globally after immersion of the samples in Gd-DTPA for several days. Also, it is likely that the longer T_2 values of water *in vivo* as well as reduced magnetic susceptibility effects at 3.0 Tesla relative to higher field strengths had a significant impact on the quality of the above results. Finally, while the benefits of increased signal-to-noise ratios at high field strengths are well established, and many of the problems of high field work, including magnetic susceptibility issues, are currently under investigation, the purpose of this work is to point out that robust methods for quantitative morphometry of structures on 100 µm length scales do exist at 3.0 Tesla, and can therefore be widely disseminated in the MRI community.

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[2] *Johnson GA, Cofer GP, Gewalt SL, and Hedlund LW. Morphologic Phenotyping with MR Microscopy: The Visible Mouse. Radiology **222**, 789-793 (2002).*
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