Morphological Difference between In Vivo and Ex Vivo Adult C57BL/6J Mouse Brain by Magnetic Resonance Microscopy

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Introduction: Mouse brain morphometry is of high interest since mouse models are commonly used in translational studies of human diseases and genetic variations. During past decade, many research groups have made the effort to develop 3D digital mouse brain atlases and gather quantitative brain structure information [1]. However, due to the major challenges of high resolution and noninvasive imaging techniques, most of the recent available digital atlases are derived from ex vivo mouse brain images through high field MRI imaging or histology techniques [1]. These brains went through sample handling and fixation which caused unrealistic deformations. Tissue morphology is also affected by the significant physiology change between ex vivo and in vivo condition. In this regard, in vivo mouse brain atlases are more valuable for longitudinal phenotyping and functional mapping. To our knowledge, there has been no in vivo mouse brain atlas available and the significance of the morphological differences between in vivo and ex vivo brains has not been quantified. In this work, we constructed an in vivo adult male C57BL/6J mouse brain atlas database directly from T2-weighted 3D micro-MRI images (acquired on a 9.4T Brucker magnet). This ongoing database offers three different atlas formats (individualized brain atlases, a minimal deformation atlas, and a probabilistic atlas) and associated quantitative information of 12 in vivo adult C57BL/6J mouse brains. The structural volume difference between the groups of in vivo and ex vivo mouse brains is quantitatively compared.

Methods: Normal male C57BL/6J (12-14 week old, Jackson Lab) mice were scanned on a 9.4T (400MHz) horizontal bore Brucker magnet. T2-weighted MR data were generated with 3D large flip angle spin echo sequence which shortens TR and the total scan time (NEX = 1; TE = 7.5 ms; TR = 400 ms, FA=145°)[2]. The mice were anesthetized with an initial dose of intraperitoneal injection of a mixture of Nembutal (50mg/kg), glycopyrrolate (0.01-0.02 mg/kg) and saline. A mixture gas of oxygen and isoflurane was used as sustaining anesthesia during later stage of the MRI scan. The MRM images were segmented into 20 neuro-anatomical structures using a semiautomatic procedure based on image registration [1]. The ex vivo data we compared to were based on MRI images of the excised brains in [1].

	Volume Difference
Ventricles	77.5%
Inferior colliculi	17.7%
Hypothalamus	14.8%
Basal forebrain-septem	11.6%
Cerebellum	11.1%
Superior colliculi	10.8%
Olfactory Bulb	10.5%
Hippocampus	9.8%
Neocortex	6.4%
Total Brain *	7.1%

Table 1. Volume difference between in vivo and ex vivo brains.

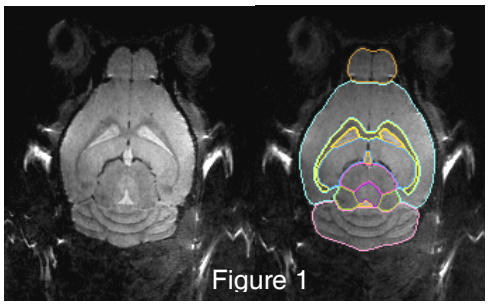

Figure 1. An original in vivo MRI image of a mouse brain (left) and the superimposed segmentation (right).

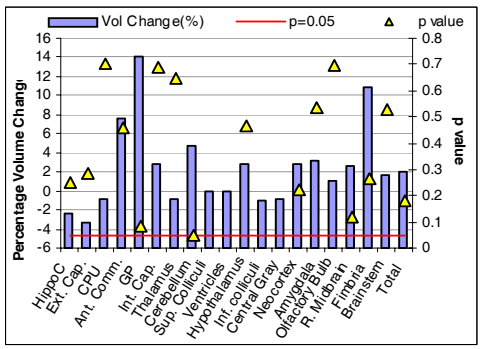

Figure 2. Volume difference between high resolution(n=12) and low resolution (n=12)

Results and Discussion: Figure 1 shows a slice of the original in vivo MRI image (left) and the superimposed segmentation(right). The 3D in vivo image provides enough details and contrast of the 20 structures we segmented.

I. In vivo vs. ex vivo: Table 1 shows the significant percentage volume increase ($p<0.05$) between the in vivo and ex vivo group. Ventricles underwent the most shrinkage at ex vivo condition (77% volume reduction). Most other ex vivo structures also showed various degree of shrinkage possibly due to the lost of fluids.

II. Faster image acquisition: a. Lower acceptable resolution: Animal control in in vivo imaging of the mouse brain is very challenging. A mouse under hours of anesthesia is prone to be unstable and create motion artifacts which could easily destroy hours of scan. So the possibility of using lower resolution to shorten scan time was investigated. Figure 2 shows the *p* values and the percentage volume difference measured from images of 100µm isotropic resolution (3hr scan) and another set of images of 200µm slice thickness and 100µm in plane resolution (1.5hr scan). No significant change was detected by 2 tailed t-test, which shows the feasibility of using the lower resolution as a practical approach in general morphological phenotyping. The structures with bigger volume variations are usually smaller and associated with higher segmentation errors in lower resolution image.

b. Other imaging sequence: We also tested an alternative imaging technique for comparison to the high flip angle Spin Echo sequence described above: The use of RARE sequence with a Rare Factor of 8, TR = 1200 ms and TE effective of 64 ms provided improved gray/white matter contrast and reduced image acquisition time. However, this sequence has increased sensitivity to motion artifacts. We are in the process of evaluating the optimized MRI sequences.

III. Summary: Our study quantifies the detailed structure volume difference between in vivo and ex vivo mouse brains using high field MRI. It also provides normal variation of adult C57BL/6J mouse, which is important in morphological phenotyping. Our results showed that through appropriately reducing the resolution, higher productivity with acceptable accuracy can be achieved through faster imaging.

References: [1]. Ma Y, Hof PR, Grant SC, Blackband SJ, Bennett R, Slatest L, McGuigan MD and Benveniste H., Neuroscience 2005;135(4):1203-15. [2]. Ali A.A., Dale A.M., Badea A., Johnson G.A., 2005 Automated segmentation of neuroanantomical structures in multispectral MR microscopy of the mouse brain. NeuroImage 27, 425-435.