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***In vivo* high-resolution diffusion tensor imaging of the mouse brain**

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Abstract

Diffusion tensor imaging (DTI) of the laboratory mouse brain provides important macroscopic information for anatomical characterization of mouse models in basic research. Currently, *in vivo* DTI of the mouse brain is often limited by the available resolution. In this study, we demonstrate *in vivo* high-resolution DTI of the mouse brain using a cryogenic probe and a modified diffusion-weighted gradient and spin echo (GRASE) imaging sequence at 11.7 Tesla. Three-dimensional (3D) DTI of the entire mouse brain at 0.125 mm isotropic resolution could be obtained in approximately two hours. The high spatial resolution, which was previously only available with *ex vivo* imaging, enabled non-invasive examination of small structures in the adult and neonatal mouse brains. Based on data acquired from eight adult mice, a group-averaged DTI atlas of the *in vivo* adult mouse brain with 60 structure segmentations was developed. Comparisons between *in vivo* and *ex vivo* mouse brain DTI data showed significant differences in brain morphology and tissue contrasts, which indicate the importance of the *in vivo* DTI based mouse brain atlas.

Keywords

diffusion tensor imaging; mouse brain; *in vivo*; high-resolution; mouse brain; brain atlas; magnetic resonance imaging

1. Introduction

Diffusion tensor imaging (DTI) (Basser et al., 1994) is a useful tool for studying brain anatomy and pathology. Based on a three-dimensional (3D) Gaussian model of water

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molecule diffusion, DTI provides several unique image contrasts that can reveal the spatial arrangement and structural integrity of major white matter tracts in the brain (Horsfield and Jones, 2002; Le Bihan, 2003; Lori et al., 2002; Mori et al., 1999; Mori and van Zijl, 2002). Besides its utility in the clinic, DTI has also been frequently applied in basic research to examine the laboratory mouse brain (Mori and Zhang, 2006). It has been widely used to characterize white matter injuries in mouse models of neurological disease, e.g., traumatic brain injury (Mac Donald et al., 2007), multiple sclerosis (Budde et al., 2007), and stroke (Wang et al., 2009). DTI has also been used to study brain development and its genetic control mechanisms through various mutant mouse models (Baloch et al., 2009; Chahboune et al., 2007; Ren et al., 2007), as the macroscopic information about neuro-anatomy provided by DTI complements the cellular and molecular information from histology. The wide applicability of DTI to studies on mouse brain anatomy and pathology makes it useful to have DTI based mouse brain atlases to assist structure delineation and lesion detection in DTI data. To fulfill this demand, several *ex vivo* DTI based mouse brain atlases have been established, which provided exquisite anatomical details within the mouse brain (Aggarwal et al., 2009; Chuang et al., 2011; Jiang and Johnson, 2011). However, currently no high-resolution *in vivo* atlas is available.

While *in vivo* mouse brain DTI can now be routinely performed, its application to a broader range of mouse models is still limited by the available imaging resolution and speed. The reason is that several complex structures in the mouse brain, e.g., the small white matter tracts in the thalamus, can only be visualized properly with high-resolution imaging (Aggarwal et al., 2010; Jiang and Johnson, 2010). Such high-resolution DTI of the live mouse brain, however, requires lengthy acquisition times, which limits the throughput and makes it challenging to use DTI to examine neonatal or injured mice that may not survive long MRI scans. Even though *ex vivo* DTI can be used to acquire high-resolution images from postmortem samples (Aggarwal et al., 2010; Flint et al., 2010; Jiang and Johnson, 2010), it cannot substitute for *in vivo* DTI when longitudinal monitoring is necessary. Recent reports have also shown that *ex vivo* DTI is less sensitive to certain white matter injuries than *in vivo* DTI due to changes in tissue microstructure associated with death and chemical fixation (Shepherd et al., 2009; Sun et al., 2009; Sun et al., 2005; Zhang et al., 2011).

Because signal-to-noise ratio (SNR) is the main factor dictating the available resolution and speed in MRI, improvement in SNR can be translated into improvements in imaging resolution and speed. In the past few years, high-field magnets and sensitive radio frequency coils in combination with fast imaging sequences have greatly enhanced our ability to use DTI to examine the mouse brain (Aggarwal et al., 2010; Cai et al., 2011; Chahboune et al., 2007; Harsan et al., 2010; Sun et al., 2006). The recent advent of the cryogenic probes (cryoprobes), which can significantly improve SNR by reducing the thermal noise of the electronics, has opened new opportunities to further improve the resolution and speed of *in vivo* mouse brain DTI. Several recent reports have shown that a cryoprobe can improve SNR by a factor of 2-3 over similar room temperature coils under comparable experimental conditions (Baltes et al., 2009; Ratering et al., 2008). The use of fast imaging sequence is also critical for *in vivo* mouse imaging. Compared to conventional diffusion-weighted spin-echo (DW-SE) or echo-planar (DW-EPI) sequences, the diffusion-weighted gradient-spin-echo (DW-GRASE) sequence (Aggarwal et al., 2010) provides imaging speed comparable to multi-shot DW-EPI with reduced image distortion. In this study, we demonstrate high-resolution *in vivo* DTI of the mouse brain using a transmit-receive cryoprobe and the DW-GRASE sequence at 11.7 Tesla. Based on the results, an *in vivo* group-averaged DTI atlas of the adult mouse brain with structural segmentation was developed, together with a detailed analysis of the differences in structural morphology and tissue contrasts between the *in vivo* atlas and a previous *ex vivo* atlas.

2. Material and methods

2.1 Animals and experimental setup

All experimental procedures were approved by the Animal Use and Care Committee at the Johns Hopkins University School of Medicine. Both adult (C57BL/6, two-month old, female, $n = 13$, from five litters) and neonatal (postnatal day 7, 11 and 14, $n = 3$) C57BL/6 mice were used.

2.2 Image acquisition

In vivo imaging was performed on a horizontal 11.7 Tesla MR scanner (Bruker Biospin, Billerica, MA, USA) with a triple-axis gradient (maximum gradient strength = 74 Gauss/cm) and a quadrature surface transmit/receive cryoprobe with a size of 20 mm $\times$ 16 mm for each of two RF elements. The surface temperature of the cryoprobe was maintained at 37 °C. During imaging, mice were anesthetized with isoflurane (1%) together with air and oxygen mixed at 3:1 ratio via a vaporizer and positioned in an animal holder (Bruker Biospin, Billerica, MA, USA). Custom-designed mouse beds with different sizes were built for positioning the neonatal mice. Respiration was monitored via a pressure sensor (SAII, Stony Brook, NY, USA) and maintained at 40-60 breaths per minute. After imaging, animals recovered within 5 minutes.

In vivo DTI of the adult mouse brains ($n = 8$) was performed using a modified 3D diffusion-weighted gradient and spin echo (DW-GRASE) sequence (Aggarwal et al., 2010) with the following parameters: TE/TR = 33/500 ms, 2 signal averages, 20 imaging echoes (4 spin echoes distributed along the phase encoding direction and 16 gradient echoes distributed along the slice selection direction) after each excitation with twin navigator echoes in the end for motion and phase corrections, 12 diffusion directions, $b = 1000$ s/mm², field of view (FOV) = 16 mm $\times$ 16 mm $\times$ 17.6 mm, a matrix size of 128 $\times$ 128 $\times$ 140, and a native imaging resolution = 0.125 mm $\times$ 0.125 mm $\times$ 0.125 mm. BIR4 adiabatic pulses (Tannus and Garwood, 1997) were used to achieve uniform excitation and refocusing over the 3D volume of interest. With respiratory gating, the total imaging time was approximately 2-2.5 hours. The same protocol was used for 3D DTI of the neonatal mouse brains and a separate testing group of adult mouse brains ($n=5$). For the neonatal mice, the FOV was reduced to 16 mm $\times$ 13 mm $\times$ 15 mm and the matrix size was reduced to 128 $\times$ 104 $\times$ 120. The total imaging time was within 1.5 hours with respiration gating. For the testing adult mouse brains, the imaging resolution was 0.125 mm $\times$ 0.125 mm $\times$ 0.2 mm, and the total imaging time was 1.5 hours. Reference scans, which used the same DW-GRASE sequence but had the phase encoding gradients set to zero, were performed before each experiment to evaluate T2 and T2* decays of the spin and gradient echo signals. Effects of the T2 and T2* decays on image resolutions were evaluated using the point spread functions, obtained by Fourier transforms of the signal decay curves along the phase encoding (T2 decay) and slice selection (T2* decay) directions (Farzaneh et al., 1990). The average FWHM (full width at half maximum) of the PSFs measured in our experiments were approximately 0.082 mm and 0.098 mm along the phase encoding and slice selection directions, respectively. The SNR was evaluated as the ratio of the mean signal intensity against the standard deviation of noise, where signals were averaged from 1 mm² boxes within the regions of interest (ROIs) and the noise was chosen from the background of the image in an area of about 4 mm².

We compared our *in vivo* adult mouse brain DTI data with previous acquired *ex vivo* data (Chuang et al., 2011). The *ex vivo* mouse brain specimen were obtained after perfusion-fixation with 4% paraformaldehyde (PFA) and preserved inside the skull. Before imaging, the specimen were immersed in phosphate buffer saline (PBS) with 0.1mM gadopentetate dimeglumine (Magnevist, Berlex Imaging, Wayne, NJ, USA) for more than 48 hours to

enhance MR signals. Ex vivo DTI was performed on an 11.7 Tesla vertical-bore NMR spectrometer (Bruker Biospin, Billerica, MA, USA) with a 15 mm diameter Bird-cage transceiver RF coil and a 3D diffusion-weighted multiple spin echo sequence (4 spin echoes, TR/TE = 35/700ms, 2 signal average, 6 diffusion directions, $b = 1200 \text{ s/mm}^2$) at $0.125 \text{ mm} \times 0.125 \text{ mm} \times 0.125 \text{ mm}$ resolution. The total imaging time was approximately 24 hours.

2.3 Data processing

The 3D images acquired using the DW-GRASE sequence were reconstructed from raw data in MATLAB (www.mathworks.com) with navigator-based motion and phase correction (Mori and van Zijl, 1998). Using the log-linear fitting method implemented in DTIStudio (<http://www.mristudio.org>) (Jiang et al., 2006), diffusion tensor was calculated at each pixel along with the apparent diffusion coefficient (ADC), fractional anisotropy (FA), primary eigenvector, axial diffusivity ($\lambda_{\parallel}$, the primary eigenvalue), and radial diffusivity ($\lambda_{\perp}$, the average of the secondary and tertiary eigenvalues). The adult mouse brain images were rigidly aligned to *ex vivo* mouse brain images in our MRI based mouse brain atlas (Chuang et al., 2011) using the landmark based rigid transformation implemented in the DiffeoMap software (www.mristudio.org). 3D reconstruction of white matter tracts was performed in DTIStudio using the multi-ROI fiber assignment by continuous tracking (FACT) method (Conturo et al., 1999; Huang et al., 2004; Mori et al., 1999).

Based on the 3D adult mouse brain images ($n = 8$), group-averaged mouse brain images were generated using the iterative procedure described in (Chuang et al., 2011; Kovacevic et al., 2005), first using intensity based linear affine transformation (Woods et al., 1998) and then dual-channel (ADC+FA) large deformation diffeomorphic metric mapping (LDDMM) (Ceritoglu et al., 2009) implemented in Diffeomap. ADC and FA maps were used for image registration because they provide complimentary contrasts that define the brain and ventricular boundaries (from the ADC image) and internal white matter tracts (from the FA image) and are not directly affected by the inhomogeneous intensity profile associated with the transceiver (Ratering et al., 2008). Sixty brain structures were manually segmented in the group-averaged mouse brain images as described in (Chuang et al., 2011), following the Paxinos's mouse brain atlas (Paxinos and Franklin, 2003; Paxinos et al., 2006). The group-averaged mouse brain images and structural segmentation together formed the *in vivo* group-averaged adult mouse brain atlas.

For comparisons between *in vivo* and *ex vivo* mouse brain DTI data, a previously published *ex vivo* DTI based mouse brain atlas (Chuang et al., 2011) and datasets (C57BL/6, 2-3 months old female, $n = 10$) were used. Using dual-channel (ADC+FA) LDDMM, *in vivo* and *ex vivo* diffusion tensor images of each mouse brain were registered to the *in vivo* and *ex vivo* mouse brain atlases, respectively. Using the maps derived in this process, the structural segmentations in the atlases were transformed to the rigidly aligned individual mouse brain data (automated structural segmentation) and refined by manual segmentation. Based on these segmentation results, volumes and diffusion parameters (ADC, FA, $\lambda_{\parallel}$, and $\lambda_{\perp}$) of major structures were obtained from both datasets. Differences in structural volume and diffusion parameters between the *in vivo* and *ex vivo* datasets were tested using the nonparametric Wilcoxon test. The five adult mouse brain images acquired separately were registered to both the *in vivo* and *ex vivo* mouse brain atlases using dual-channel (ADC+FA) LDDMM, and results of automated structural segmentation based on the *in vivo* and *ex vivo* atlases were compared to the manual segmentation by examining the kappa values, which measure the agreement between the segmentation approaches (Landis and Koch, 1977). To further quantify the morphological differences between the *in vivo* and *ex vivo* mouse brain atlases, a mapping between the two atlases was also generated using the dual channel (ADC+FA) LDDMM. By concatenating this mapping with the mapping from individual *ex vivo* mouse brain to the *ex vivo* template, a combined mapping from

individual *ex vivo* mouse brain to the *in vivo* atlas was obtained. Based on the mappings from individual *in vivo* and *ex vivo* mouse brains to the *in vivo* mouse brain atlas, Jacobian maps (Toga and Thompson, 2003) were calculated, and voxel-wise comparisons of the Jacobian maps from the *in vivo* and *ex vivo* datasets were performed (Matlab, Mathworks, www.mathworks.com) after correction for multiple comparisons with a false discovery rate of 0.05 to locate regions with significant changes in local tissue volume. A tissue displacement map was also calculated between the group-average *in vivo* and *ex vivo* mouse brain images to characterize morphological differences.

3. Results

In vivo high-resolution DTI enhanced our ability to non-invasively examine microstructures in the mouse brain. As is shown in Figs. 1A-B, when an isotropic resolution of 0.125 mm is used, small white matter structures in the adult mouse brain (e.g., the stria medullaris, stria terminalis, and fasciculus retroflexus) could be delineated in the *in vivo* DTI data. As is also shown in Fig. 1D-E, complex 3D trajectories of these white matter tracts could be reconstructed and visualized with high spatial resolution in all three dimensions. Certain gray matter regions that possess unique DTI contrast patterns, e.g., the axon-rich layers in the hippocampus and the cerebellar cortex, could also be resolved. The *in vivo* data, however, showed a small but noticeable loss of sharpness attributed to T2/T2* decay and physiological motions when compared to our previous *ex vivo* 3D DTI data (Fig. 1C), which were acquired at the same spatial resolution using a diffusion-weighted spin echo sequence for over 20 hours (Chuang et al., 2011).

The relatively short acquisition time and high resolution made it possible to acquire *in vivo* DTI data from neonatal mouse brains. Fig. 2 shows high-resolution 3D DTI data acquired from normal P7, P11 and P14 mouse brains. Even though the sizes of the neonatal mouse brains were significantly smaller than the size of the adult mouse brain, the high-resolution 3D DTI data allowed us to delineate immature white matter (e.g., the corpus callosum and stria medullaris) and gray matter structures (e.g., the cerebellar cortex) in the neonatal mouse brain. It is necessary to note that due to the geometry of the cryoprobe (dual surface coil quadrature setup), the *in vivo* data have decreasing SNR along the dorsal-ventral axis of the brain (Table 1).

A group-averaged *in vivo* high-resolution DTI dataset of the adult mouse brain was created to study group-averaged morphological features and tissue contrasts (Fig. 3). Although a loss in image sharpness due to residual mismatch after registration among the subject images could be observed in the averaged images, small white matter tracts in the thalamus and cerebellar cortex/white matter remained well defined. A segmentation map of 60 brain structures of the *in vivo* mouse brain atlas was generated (Fig. 3, superimposed on direction-encoded colormap images). The group-averaged atlas and datasets can be downloaded at (<http://cmrm.med.jhmi.edu>). Several differences were observed in structure morphology and tissue water diffusion properties between the *in vivo* and *ex vivo* mouse brain DTI data. Although the volumes of major brain structures did not change significantly between *in vivo* and *ex vivo* data (Table 2), the 60% reduction in the ventricular volumes after death and fixation was accompanied by large deformations in its neighboring structures. For example, in the tissue displacement and Jacobian maps (Fig. 4), the hippocampus showed a large displacement accompanied by significant ($p < 0.05$) expansion at its caudal portion (indicated by the white arrows in Fig. 4) in the *ex vivo* mouse brains. The dorsal cortex also showed a relatively large tissue displacement and significant expansion in local tissue volume (indicated by the yellow arrows in Fig. 4). While the FA changed significantly in gray matter (Table 2), FA values measured in major white matter tracts *in vivo* and *ex vivo* show no apparent difference (Table 3), which agrees with previous reports (Sun et al.,

2005). ADC measured in major white matter tracts were significantly reduced in the *ex vivo* data (Fig. 5A and Table 3), with both axial diffusivity and radial diffusivity reduced proportionally with ADC (Table 3). In major gray matter structures, ADC values were also reduced in the *ex vivo* data, but less than white matter structures (e.g., approximately 20% reduction in the cortex vs. 47% reduction in the corpus callosum, Tables 2 and 3), and FA also showed significant reduction, e.g., in the cortex, cerebellum, and thalamus (Table 2). As a result of these changes, the ADC images of the *in vivo* and *ex vivo* mouse brains showed different contrasts, with the *ex vivo* ADC image showed stronger contrast between the white and gray matter structures than the *in vivo* ADC image (Fig 5A). In the axial diffusivity images, the contrast between the cortex and corpus callosum observed *in vivo* mostly disappeared *ex vivo*, whereas in the radial diffusivity images, the contrast was preserved (Fig. 5B). The standard deviations of ADC measured in the *ex vivo* mouse brains ($n=10$) were larger than those measured *in vivo* ($n=8$), whereas standard deviations of FA measured *ex vivo* were mostly less than those measured *in vivo* (Fig. 5C). Table 4 compares the results of automated segmentation of a different set of *in vivo* mouse brain DTI data ($n = 5$) using the *in vivo* and *ex vivo* DTI-based atlases as templates. For major gray matter structures, the performance of the *in vivo* atlas was on par with the *ex vivo* atlas, whereas for major white matter structures and the lateral ventricles, the results generated using the *in vivo* atlas showed better agreement with manual segmentation results than the *ex vivo* atlas.

4. Discussion

In this study, high-resolution DTI data were acquired using a 3D imaging sequence, while previous *in vivo* DTI studies mostly used multi-slice sequences, e.g., multi-slice spin echo or multi-slice EPI. Multi-slice DTI is more time efficient than 3D DTI, and has been commonly used to study the structural integrity of major white matter pathways, such as the corpus callosum, in mouse models (Sun et al., 2006; Wang et al., 2008). With four-segment EPI acquisition, Harsan et al., reported *in vivo* mouse brain DTI (30 diffusion directions) with an in-plane resolution of $0.156 \text{ mm} \times 0.156 \text{ mm}$ and 0.5 mm slice thickness (Harsan et al., 2010). Multi-slice DTI, however, has limited through-plane resolution ($\sim 0.5 \text{ mm}$) depending on available gradient strength and SNR. The partial volume effect due to this limitation makes it difficult to delineate complex white matter or gray matter structures along the slice direction. The limited through-plane resolution also is not ideal for co-registering images from multiple animals, as interpolations in the slice direction can often cause visible degradation in image resolution. Compared to multi-slice acquisition, 3D acquisition allows high spatial resolution along all three dimensions at the cost of longer acquisition times, which often limits its application for imaging live mice. Previous studies using 3D DTI were mostly *ex vivo* anatomical studies (Aggarwal et al., 2010; Jiang and Johnson, 2010; Zhang et al., 2003). Recently, Cai et al. demonstrated *in vivo* 3D DTI of the neonatal rat brain using a 3D diffusion weighted fast spin echo sequence with a spatial resolution of $0.15 \text{ mm} \times 0.15 \text{ mm} \times 0.2 \text{ mm}$ in 3 hours (Cai et al., 2011).

By using a cryoprobe, we were able to speed up 3D acquisition by reducing the repetition time while still maintaining an acceptable SNR. Two recent reports demonstrated high-resolution *in vivo* mouse brain DTI using a cryoprobe and multi-slice sequences (Lemke et al., 2010; Muller et al., 2012). With 11.7 T cryoprobe and a 3D DW-GRASE imaging sequence, we were able to acquire 3D images from the adult and neonatal mouse brain with an isotropic resolution of 0.125 mm within 2-2.5 hours. Even though the *in vivo* 3D DTI results shown here still could not match our previous *ex vivo* 3D DTI in term of SNR or image sharpness (Table 1 and Fig. 1), the results nonetheless showed considerable improvements in our ability to resolve small structures in the live mouse brain. The spatial resolution in this study was chosen so that the *in vivo* mouse brain data could be compared to our previous *ex vivo* mouse brain data at similar levels of partial volume effects. In

practice, if a reduced resolution is deemed satisfactory to delineate the target structures or lesions, the acquisition time can be shortened. While the cryoprobe offers significant advantages over conventional room temperature coils for imaging the mouse brain at high resolution, it also has its own technical constraints. First, the cryoprobe used in this study operated in transceiver mode. As a result, the intensity profile of the diffusion-weighted images and the SNR measured in different parts of the brain were not uniform (Table 1), which may affect the quantification of the measured diffusion parameters, e.g., FA and diffusivities (Farrell et al., 2007; Pierpaoli and Basser, 1996). The non-uniform excitation and refocusing profiles can also result in imaging artifacts for multiple RF sequences such as the GRASE sequence used here. To resolve this problem, we applied adiabatic excitation and refocusing pulses to achieve near uniform excitation and refocusing profiles at the expense of high radio-frequency power deposition. Second, compared to existing phased array coils with 4 or more channels (Gareis et al., 2007), the current cryoprobe has two channels and is not suitable for parallel imaging, which can further reduce imaging time. Improved coil design such as discussed in (Baltes et al., 2009) or the use of room temperature body coil transmission with phased array cryocoil receive is expected to offer more uniform excitation profiles and parallel imaging capability in the future.

Using the high-resolution *in vivo* mouse brain DTI data, we constructed a group-averaged DTI atlas of the live mouse brain. Mouse brain atlases are important resources for basic neuroscience research because they contain useful anatomical information for identifying brain structures and understanding their spatial relationships. While there are several histology-based mouse brain atlases (e.g., (Paxinos and Franklin, 2003)), whose structural definitions are commonly used as the gold standard, MR-based atlases have been established in recent years, with increasing resolution and sophistication (i.e., multiple MR contrasts, detailed structural segmentation, and creation of population averaged and multi-modality brain atlases) (Aggarwal et al., 2009; Badea et al., 2007; Benveniste et al., 2000; Chan et al., 2007; Chuang et al., 2011; Dorr et al., 2008; Jacobs et al., 1999; Jiang and Johnson, 2011; Ma et al., 2005; Ma et al., 2008; MacKenzie-Graham et al., 2004; Natt et al., 2002; Petiet et al., 2008). Compared to histology-based atlases, MR-based atlases are inherently three-dimensional, and the ability to construct group-averaged atlas makes it possible to present group-averaged anatomical features and quantify inter-subject variability. MR-based mouse brain atlases are useful in analyzing MRI data acquired from mouse brains, for example, automated structural segmentation and volume measurements (Ali et al., 2005; Badea et al., 2007; Bock et al., 2006; Zhang et al., 2010). They can also be used as templates to perform voxel-based analysis to examine changes in structural morphology and tissue properties (Aggarwal et al., 2012; Lau et al., 2008; Lerch et al., 2008; Sawiak et al., 2009; Tyszkiewicz et al., 2006). Many of these MR-based atlases, especially DTI based mouse brain atlases, were previously generated from post-mortem brain specimens in order to achieve high spatial resolution and image quality. Post-mortem data, however, may not accurately capture the properties of the brain under normal physiological conditions as death and chemical fixation can alter structural morphology and tissue properties (Ma et al., 2008; Shepherd et al., 2009; Sun et al., 2009; Sun et al., 2005; Zhang et al., 2011). By comparing *in vivo* T2-weighted images with *ex vivo* T2*-weighted images acquired from brain specimens dissected out of the skull, Ma et al. (Ma et al., 2008) reported significant differences in structural volume and surface areas between *in vivo* and *ex vivo* data. Keeping the brain inside the skull could reduce but not completely remove the structural deformation associated with death and fixation (Aggarwal et al., 2009). In terms of tissue diffusion characteristics, Sun et al. (Sun et al., 2005) showed significant differences in tissue ADC values after death and fixation but no apparent change in white matter FA values. For studies that involve *in vivo* monitoring of structural morphology and pathology using DTI and perform atlas or voxel-based image analysis of *in vivo* mouse brain DTI data, it is beneficial to have an *in vivo* mouse brain atlas

and a good understanding of the differences between *in vivo* and *ex vivo* DTI-based outcome measures.

In this study, using the structural segmentation embedded in the atlas, differences between the *in vivo* and *ex vivo* mouse brain datasets were systematically characterized. Compared to previous studies based on T2-weighted images (Aggarwal et al., 2009; Ma et al., 2008), the rich DTI contrasts allowed accurate registration for white matter structures (Ceritoglu et al., 2009), which in turn improved the overall image registration quality and enhanced our ability to detect subtle morphological differences between *in vivo* and *ex vivo* mouse brain images. In addition to the well-documented collapse of the ventricles in post-mortem samples and deformations of surrounding structures, voxel-based analysis revealed additional regions with significant morphological differences between the two datasets, mainly located in the dorsal part of the brain. Several factors, e.g., fixation related non-uniform tissue shrinkage (Aggarwal et al., 2009; Cahill et al., 2012), changes in tissue osmotic pressure after death and fixation, and shear stresses associated with the collapse of the ventricles (Ma et al., 2008), may contribute to these deformations and their effects remain to be investigated. In terms of changes in diffusion parameters after death and fixation, changes in gray and white matter contrasts in ADC maps, and to a lesser degree, in FA maps (Tables 2 and 3, and Fig. 5) suggest that the effects of death and chemical fixation on tissue ADC values were not uniform throughout the brain. As expected, FA values measured *in vivo* showed larger variability, both inter-subject and inter-scan, than FA measured *ex vivo*, because the *ex vivo* DTI data had higher SNR than the *in vivo* data and were free of motion-related artifacts. Interestingly, we found that the ADC values measured *in vivo* showed less variability than the ADC values measured *ex vivo*. Potential causes of this difference include variations due to perfusion fixation, e.g., differences in fixation related changes in cell membrane permeability and the ratio between intracellular and extracellular space, and precision of temperature control during *ex vivo* imaging. Even though the number of animals studied here is limited, these results indicate that some morphological and diffusion properties from existing *ex vivo* atlases cannot be taken to accurately represent the properties that exist under normal physiological conditions *in vivo*.

Knowing the differences between the *in vivo* and *ex vivo* atlases may guide their applications in analyzing *in vivo* mouse brain DTI data. For example, while both atlases could be used to segment major gray matter structures with similar levels of precision, the *in vivo* atlas based results showed better agreement with the manual segmentation results of the lateral ventricles and major white matter structures than the *ex vivo* atlas (Table 4). While the actual improvement from using the *in vivo* atlas compared to the *ex vivo* atlas depend on several factors, e.g., the choice of image registration method and parameters, the similar brain morphology and tissue contrasts shared between the *in vivo* atlas and the test data could potentially benefit automated segmentation as it would reduce the challenge for the registration methods to accommodate large tissue deformations or differences in tissue contrast (e.g., the changes in ventricular volumes and cortical FA values between *in vivo* data and the *ex vivo* atlas). More rigorous examinations that consider multiple image registration methods and involve mouse models of diseases will likely reveal the exact benefits of *in vivo* mouse brain atlas.

4. Conclusions

In summary, our study demonstrates the use of a cryoprobe in combination with 3D DW-GRASE sequence to achieve *in vivo* 3D high-resolution DTI of the mouse brain. The high resolution and relatively short acquisition time allowed detailed examination of neuroanatomy in the mouse brain. A high-resolution DTI atlas of the live mouse brain was developed with structure segmentations. Detailed analysis showed volumetric and contrast

differences between *in vivo* and *ex vivo* DTI of the mouse brains, indicating the importance of the *in vivo* DTI atlas.

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Highlights

1. *In vivo* high-resolution mouse brain diffusion tensor imaging (DTI) was achieved.
2. A group-averaged adult mouse brain DTI atlas was developed.
3. Differences between *in vivo* and *ex vivo* mouse brain DTI data were examined.

Fig. 1.

In vivo 3D high-resolution DTI and tractography of a representative adult mouse brain at 0.125 mm isotropic resolution. Direction-encoded colormaps (DECs) at several coronal slices (A) and sagittal slices (B) of the mouse brain are compared to matching images from our previous *ex vivo* data (C) of the same resolution after rigid alignment of the two datasets. The high resolution of the *in vivo* data allows reconstruction of both major and small white matter tracts (D & E) in the brain. The red-green-blue color scheme used in the DEC images is: red: left-right axis; green: rostral-caudal axis; blue: superior-inferior axis. Abbreviations: ac – anterior commissure; bsc: brachium sup colliculus; cp – cerebral peduncle; opt – optical tract; fi – fimbria; ec – external capsule; f – fornix; ml: medial

lemniscus; sm – stria medullaris; st – stria terminalis; fr – fasciculus retroflexus; LV – lateral ventricle; 3V – third ventricle; 4V – fourth ventricle. Scale bar = 1 mm.

Fig. 2.

In vivo DTI of normal neonatal mouse brains at 0.125mm isotropic resolution. Coronal and sagittal FA and DEC images at postnatal day 7 (P7), 11 (P11), and 14 (P14). Abbreviations: cc – corpus callosum; ac – anterior commissure; cp – cerebral peduncle; opt – optical tract; fi – fimbria; sm – stria medullaris; st – stria terminalis; fr – fasciculus retroflexus. Scale bar = 1 mm.

Fig. 3. Group-averaged FA and DEC images and structural segmentations in the in vivo mouse brain DTI atlas. Average FA and DEC images from eight adult mouse brains are shown in coronal and horizontal views. Inset figures magnify complex structures from the hippocampus and cerebellum. Outlines of structural segmentations are overlaid on the DEC images.

Fig.4.

Morphological differences between the *in vivo* ($n=8$) and *ex vivo* ($n=10$) mouse brains. (Top row) Color-coded Log-Jacobian maps of the transformation between the *in vivo* and *ex vivo* mouse brains superimposed on average *in vivo* FA maps). Only the voxels with significant changes in Log-Jacobian values ($p < 0.05$ after corrections for multiple comparisons) are shown. A Log-Jacobian value greater than 0 indicates expansion of the *ex vivo* mouse brains with respect to the *in vivo* mouse brains, and shrinkage otherwise. (Bottom row) Color-coded maps of estimated tissue displacement between the average *ex vivo* and *in vivo* mouse brain images superimposed on average *in vivo* FA maps. Cortical and hippocampal regions that show large morphological changes between the *in vivo* and *ex vivo* images are indicated by the yellow and white arrows, respectively.

Fig. 5. Morphological and diffusion properties differ between the average *in vivo* and *ex vivo* mouse brains. (a) ADC measured in the *in vivo* mouse brain (left) is much higher than that in the *ex vivo* mouse brain (right). (b) Parallel diffusivity ($\lambda_{||}$) and radial diffusivity ($\lambda_{\perp}$) show different white matter – gray matter contrasts in the *in vivo* and *ex vivo* mouse brains. (c) Standard deviation of ADC and FA maps in the *in vivo* ($n=8$) and *ex vivo* ($n=10$) groups.

Table 1

SNR values in five dorsal to ventral brain regions of in vivo ($n=8$) and ex vivo ($n=10$) mouse brain DTI images. The in vivo images were acquired with the cryoprobe and the described DW-GRASE sequence, and the ex vivo images were acquired with a 15 mm diameter bird-cage coil and a diffusion weighted multiple spin echo sequence. The mean SNR values and standard deviations were measured on the non-diffusion-weighted images.

Structures	In vivo	Ex vivo
Dorsal neocortex	161.5±8.8	175.8±21.3
Hippocampus	133.3±11.9	203.1±21.3
Thalamus	90.3±12.1	148.5±12.9
Hypothalamus	73.3±10.5	199.8±30.7
Amygdala	69.8±9.4	196.4±24.4

ROI-based measurements in gray matter structures of the *in vivo* and *ex vivo* mouse brain DTI datasets. The volume (mm³), ADC (mm²/s) and FA in several major gray matter structures are presented as mean ± standard deviation from *in vivo* (*n*=8) and *ex vivo* (*n*=10) DTI data. Gray matter segmentations defined in the *in vivo* atlas were transformed to individual mouse brain data and used as ROIs. * *p* < 0.01, ** *p* < 0.005. The ADC and FA values of the ventricles are not shown because the low signal-to-ratio of diffusion-weighted signals in the ventricles resulted in unreliable estimates of diffusion parameters in this region.

Table 2

Structures	Volume (mm ³)		ADC (×10 ⁻³ mm ² /s)		FA	
	<i>In vivo</i>	<i>Ex vivo</i>	<i>In vivo</i>	<i>Ex vivo</i>	<i>In vivo</i>	<i>Ex vivo</i>
Neocortex	100.4±5.1	99.5±4.9	0.55±0.02	0.45±0.10	0.21±0.01**	0.19±0.01
Hippocampus	25.0±1.4	26.5±2.4	0.56±0.03**	0.45±0.10	0.25±0.02	0.25±0.02
Striatum	21.0±0.9*	23.0±1.8	0.52±0.03*	0.41±0.10	0.27±0.02**	0.18±0.01
Thalamus	20.6±0.9	20.7±1.4	0.53±0.03**	0.39±0.07	0.37±0.03**	0.27±0.01
Septum	1.6±0.1	1.8±0.2	0.63±0.05**	0.41±0.10	0.35±0.03**	0.25±0.01
Cerebellum	54.9±3.0	55.0±1.6	0.50±0.03**	0.37±0.09	0.35±0.02**	0.29±0.02
Ventricles	8.42±0.71**	3.36±0.47	---	---	---	---

ROI-based comparison of white matter diffusion properties in the *in vivo* and *ex vivo* mouse brains. The FA, ADC (mm²/s), parallel diffusivity ($\lambda_{\parallel}$, mm²/s) and radial diffusivity ($\lambda_{\perp}$, mm²/s) of several white matter tracts are presented as mean $\pm$ standard deviation from *in vivo* (*n*=8) and *ex vivo* (*n*=10) DTI data. White matter segmentations defined in the *in vivo* atlas were transformed to individual mouse brain data and used as ROIs. * *p* < 0.01, ** *p* < 0.005. Abbreviations: cc –corpus callosum; ac –anterior commissure; opt –anterior commissure; ic –internal capsule; fi –fimbria; fx –fornix.

Table 3

Structures	FA		ADC ($\times 10^{-3}$ mm ² /s)		$\lambda_{\parallel}$ ($\times 10^{-3}$ mm ² /s)		$\lambda_{\perp}$ ($\times 10^{-3}$ mm ² /s)	
	In vivo	Ex vivo	In vivo	Ex vivo	In vivo	Ex vivo	In vivo	Ex vivo
cc	0.52 $\pm$ 0.02	0.53 $\pm$ 0.02	0.58 $\pm$ 0.03**	0.31 $\pm$ 0.08	0.95 $\pm$ 0.04**	0.52 $\pm$ 0.11	0.79 $\pm$ 0.05**	0.44 $\pm$ 0.10
ac	0.57 $\pm$ 0.03	0.54 $\pm$ 0.02	0.56 $\pm$ 0.03**	0.32 $\pm$ 0.09	0.97 $\pm$ 0.05**	0.56 $\pm$ 0.13	0.70 $\pm$ 0.06**	0.44 $\pm$ 0.10
opt	0.66 $\pm$ 0.04	0.64 $\pm$ 0.03	0.63 $\pm$ 0.04**	0.31 $\pm$ 0.06	1.22 $\pm$ 0.12**	0.61 $\pm$ 0.10	0.67 $\pm$ 0.05**	0.34 $\pm$ 0.05
ic	0.60 $\pm$ 0.03	0.57 $\pm$ 0.02	0.55 $\pm$ 0.04**	0.33 $\pm$ 0.07	0.98 $\pm$ 0.06**	0.59 $\pm$ 0.10	0.66 $\pm$ 0.08**	0.42 $\pm$ 0.07
fi	0.68 $\pm$ 0.02**	0.64 $\pm$ 0.02	0.69 $\pm$ 0.05**	0.26 $\pm$ 0.06	1.33 $\pm$ 0.06**	0.47 $\pm$ 0.10	0.73 $\pm$ 0.08**	0.31 $\pm$ 0.06
f	0.49 $\pm$ 0.04	0.44 $\pm$ 0.03	0.52 $\pm$ 0.04**	0.30 $\pm$ 0.07	0.83 $\pm$ 0.06**	0.46 $\pm$ 0.10	0.74 $\pm$ 0.08**	0.45 $\pm$ 0.09

Table 4

Comparisons of automated structural segmentation results based on the *in vivo* and *ex vivo* atlases in a separate group of mouse brain DTI data ($n=5$). The accuracy of segmentation was evaluated by relative volume differences and *kappa* coefficients (K) between the automated segmentation and manual segmentation results of selected structures.

	Neocortex	Hippocampus	Cerebellum	Corpus callosum	Fimbria	Lateral ventricles
Manual segmentation (mm ³)	98.86±4.2 ₃	24.75±1.17	52.58±4.43	12.80±0.65	2.64±0.32	5.61±0.7
Segmentation based on the <i>in vivo</i> atlas (mm ³)	98.05±3.9 ₉	25.88±1.13	53.29±4.37	12.39±0.58	2.85±0.34	6.03±0.89
Segmentation based on the <i>ex vivo</i> atlas (mm ³)	103.68±5.5 ₅₀	25.80±1.62	55.39±4.81	12.33±0.60	2.90±0.19	4.13±0.29
Relative volume difference (<i>in vivo</i> v.s. manual) (%)	1.27±0.58	4.58±1.06	1.35±0.33	3.17±0.82	7.92±3.23	7.96±4.54
Relative volume difference (<i>ex vivo</i> v.s. manual) (%)	4.89±3.73	4.22±2.33	5.32±1.44	6.80±2.43	15.17±8.7 ₂	25.21±9.34
K (<i>in vivo</i> v.s. manual)	0.985±0.002	0.973±0.005	0.987±0.001	0.943±0.039	0.922±0.010	0.932±0.010
K (<i>ex vivo</i> v.s. manual)	0.950±0.012	0.916±0.012	0.962±0.008	0.725±0.016	0.715±0.067	0.537±0.063