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Characterization of Mouse Brain and Its Development using Diffusion Tensor Imaging and Computational Techniques

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***Abstract*—Diffusion tensor magnetic resonance imaging (DTI) was used to study mouse brain development from early embryonic stage to adult. DTI provides necessary resolution and superb white matter and gray matter contrast in embryonic and neonatal brains for characterization of morphological changes during mouse brain development. A database and a digital atlas of developing mouse brains based on our DTI results are being constructed. To characterize the spatial and temporal patterns of mouse brain development, we applied landmark based computational techniques to analyze the database.**

I. Introduction

Development of mammalian central nervous system (CNS) from an embryonic neural tube to its mature form consists of a sequence of complex molecular and cellular events, e.g., neuronal migration, axonal path finding and myelination. These events, which are controlled by various genes and later affected by environment, result in considerable morphological changes of CNS[1, 2].

Magnetic resonance imaging (MRI) has been extensively used in the study of human diseases as well as in mouse models of human diseases. Compared to conventional histological methods, MRI can survey a subject in a nondestructive fashion, without the time consuming tissue slicing process required by histology[3]. It can acquire three dimensional (3D) images and therefore makes reconstruction of tissue morphology straightforward. MR images are digitized and are easy for quantitative analysis. Several groups have successfully demonstrated the use of MRI in characterizing animal brains in several animal models[3-7], and a MR atlas of developing mouse has been established[8].

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Diffusion tensor MRI (DTI), a more recent MRI technique, is especially suited for detection and characterization of morphological changes in the CNS during development. Conventional MRI is often limited by its lack of contrast between gray and white matters in the embryonic and neonatal periods, most likely due to lack of myelin in the early stage. Therefore, conventional MRI often fails to provide anatomical details in developing brains finer than the entire brain, ventricles and a few brain compartments. In comparison, DTI can provide rich anatomical information. DTI measures water molecule diffusion constants along a set of directions in tissue and fits the measurements into a tensor model[9, 10]. In axonal tracts, water diffusion is anisotropic, i.e., directionally dependent. Diffusion constants measured along the trajectory of an axonal tract are larger than perpendicular to the trajectory of the axonal tract. Using the tensor model, the degree of diffusion anisotropy and the direction of the maximal diffusion constant can be quantified. Such measurements of diffusion anisotropy and orientation provide useful image contrast to distinguish neuronal structures. Previous reports have shown that early embryonic structures can be successfully delineated in DTI images[11, 12].

With the image contrast provided by DTI, characterization of morphological change of the developing CNS becomes possible. Techniques of computational anatomy, which use transformations to study biological forms, have been established for the quantitative study of morphological changes in growth, aging and pathological conditions[13-15]. Several groups have developed techniques and digital atlases that facilitate the quantification of morphological configuration of human or animal brains[14, 16-22].

In this paper, we describe how we used DTI to monitor the evolution of brain structures from the early embryonic stage to adult. We first obtained high resolution diffusion tensor images of embryonic and postnatal mouse brains, and established a digital atlas. We then explored the possibility of combining DTI and landmark based large deformation diffeomorphic metric mapping (LDDMM) to quantitatively measure morphological variations and normal brain development.

II. Materials and Methods

A. Magnetic resonance imaging

C57BL mice at embryonic stages (E12 to E18) and postnatal stages (P0 to adult) were perfused-fixed using 4% paraformaldehyde and were left in fixation solution for at

least a month. Experiments were performed on either an 11.7 Tesla MRI scanner (Bruker Biospin Inc., Billerica, MA) or a 9.4 Tesla MRI scanner (GE omega). Samples were placed in plastic tubes filled with Fomblin (Perfluorinated Polyethers). Images were acquired using a multiple spin echo sequence. Imaging resolution ranged from 0.08mm to 0.12mm per pixel in all three directions, depending on the size of samples. Diffusion weighted images were acquired with TE of 37ms, TR of 900m. At least eight diffusion weighted images with different *b* values were acquired, with two images of minimum *b* value (150 s/mm^2) and the rest with maximum *b* value (1,000~1,200 s/mm^2). Diffusion sensitizing gradients were applied along six different orientations: [0.707, 0.707, 0], [0.707, 0, 0.707], [0, 0.707, 0.707], [-0.707, 0.707, 0], [0.707, 0, -0.707], [0, -0.707, 0.707]. High-resolution T_2-weighted images (0.04~0.06mm per pixel) were also acquired. Total imaging time for each sample was approximately 24 ~ 30 hours.

Average diffusion weighted images were obtained by adding all diffusion-weighted images. The diffusion tensor was calculated using a log-linear regression method, and three pairs of eigenvalues and eigenvectors were determined for each pixel[9]. The eigenvectors associated with the largest eigenvalue (λ_1) were referred to as the primary (V_1). Diffusion anisotropy was quantitatively measured by fractional anisotropy (FA)[10]. Color map images were generated by combining the images of primary eigenvector (V_1) and FA into RGB images (V_1·FA). In the color map images, the ratio among R(ed), G(reen), and B(lue) components of each pixel was defined by the ratio of the absolute values of x, y, and z components of V_1, and the intensity was proportional to the FA. Red was assigned to the medial-lateral axis, green to the rostral-caudal axis, and blue to the dorsal-ventral axis.

B. Digital atlas of mouse brain and database of developing mouse brain

Images from adult mouse brain samples were aligned to the standard orientation as defined by the Paxino's mouse brain atlas[23], and used as our template. Coronal, sagittal and horizontal sections of the brains were generated from the 3D image volume. White matter and gray matter structures identifiable in the MR images were labeled following the definition by the Paxino's mouse brain Atlas. Major white matter tracts were reconstructed using DtiStudio[24, 25]. Images from embryonic and postnatal mouse brains were rigidly aligned to the standard template.

C. Landmark based large deformation diffeomorphic metric mapping (LDDMM)

A total of 270 landmarks were defined in each set of mouse brain images from P7 to adult. Details on LDDMM have been described in a review by Miller et al. [14]. Using the LDDMM algorithm, we generated; 1) diffeomorphic maps between a landmark set and the average landmark set at P7, 2) diffeomorphic maps between two average landmark sets at different stages. The former maps were used to study individual variations within the same stage and the latter were used to study growth.

III. RESULTS

Fig. 1A shows a coronal DTI color map image from an adult mouse brain in our mouse brain atlas. Compared to a Nissl stained histological section, DTI highlights white matter structures due to their high diffusion anisotropy. Furthermore, DTI measures orientations of the axonal tracts, which is rendered by color in this figure and can be used to reconstruct axonal pathways. Axonal tracts identifiable in the DTI image are labeled in 2D images. The advantage of DTI over conventional histological method is demonstrated by visualizing 3D trajectories of axonal tracts reconstructed using fiber tracking (Fig. 1B)

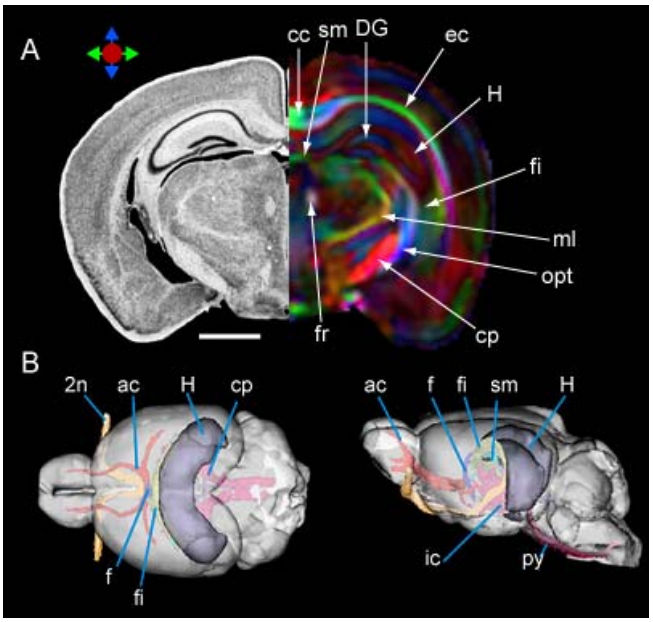

Fig. 1: Diffusion tensor microscopy of an adult mouse brain. A. Nissl stained histology (left) and diffusion tensor images of adult mouse brains. B. Reconstructed white matter tracts from the DTI results. Abbreviations are: 2n: optic nerve; ac: anterior commissure; cc: corpus callosum; cp: cerebral peduncle; DG: dentate gyrus; ec: external capsule; f: fornix; fi: fimbria; H: hippocampus; ml: medial lemniscus; opt: optic tract; py: pyramidal tract; sm: stria medularis. The scale bar represents 1 mm. The color arrows illustrate our color scheme. Red represents rostral-caudal, green for medial-lateral and blue for dorsal-ventral. The histological image is from the Mouse Brain Library[26].

Fig. 2 shows high resolution T_2 and color map images from embryonic (E14 – E18) and postnatal mouse brains (P0 – P45). Compared to conventional T_2 MRI, DTI provides sharp contrast between gray and white matter structures, especially in the early stages. The imaging contrast provided by DTI is consistent throughout development, which enables the tracking of the development of particular structures over time. The resolution achieved by our imaging technique enabled us to follow the development of major axonal tracts in the mouse brains.

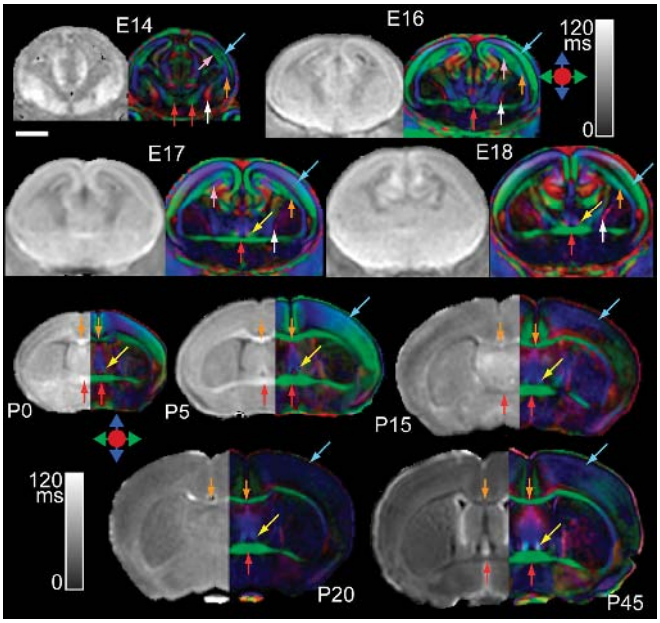

Fig. 2: DTI of embryonic (upper two panels, from E14 to E18) and postnatal (lower two panels, from P0 to P45) developing mouse brains. Three-dimensional volume images are carefully aligned to ensure proper orientation and position, and two-dimensional coronal images are selected at the level of anterior commissure. T_2 map images are scaled from 0 to 120 milliseconds (ms). Blue, orange and pink arrows indicate the location of the cortical plate (or cortex), the intermediate zone and the ventricular zone (neuroepithelium). White matter tracts shown here include the anterior commissure (red arrows), the corpus callosum (orange arrows), the fornix (yellow arrows), and the internal capsule (white arrows). The scale bars are 2 mm.

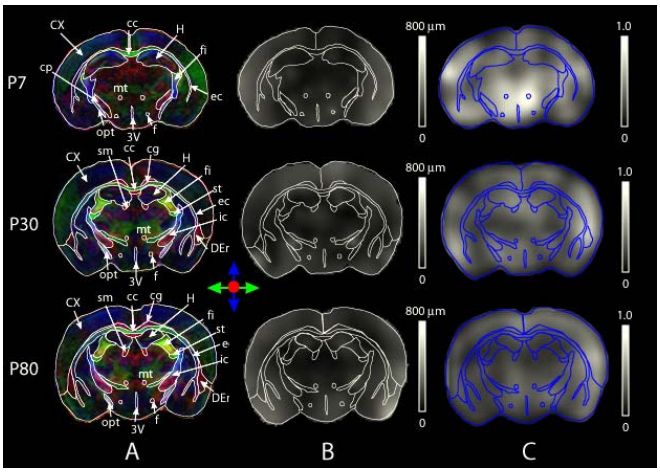

Fig. 3: Mouse brain morphological variations at three developmental stages (P7, P30 and P80): at each stage, with various structural compartments labeled in the left most images. Local variations of tissue displacement (B), and standard deviations of Jacobian fields (C) were displayed. Abbreviations are: cc: corpus callosum; cp: cerebral peduncle; cg: cingulum; CX: cortex; ec: external capsule; fi: fimbria; ic: internal capsule; sm: stria medullaris; st: stria terminalis; mt: mammolothalamic tract; opt: optic tract; 3V: third ventricle; H: hippocampus; f: fornix; DEn: dorsal endopiriform nucleus.

With the images in our mouse brain database, morphological variations of mouse brains were measured at P7, P30 and P80 and visualized here in Fig. 3. Coronal DTI mouse brain images at P7, P30 and P80 are shown with several structures labeled (A). Quantitative measurements of morphological variations of normal mouse brains show the magnitude of local variations at each stage, both in terms of tissue displacement (B) and local tissue volume (C). In these images, high intensity areas correspond to regions with large morphological variations. Boundaries of various tissue compartments are overlaid on these images, and the results show that cortical regions often demonstrated a higher degree of variations. These results provide quantitative measurements of normal variations and may form the basis for characterizing abnormal morphology.

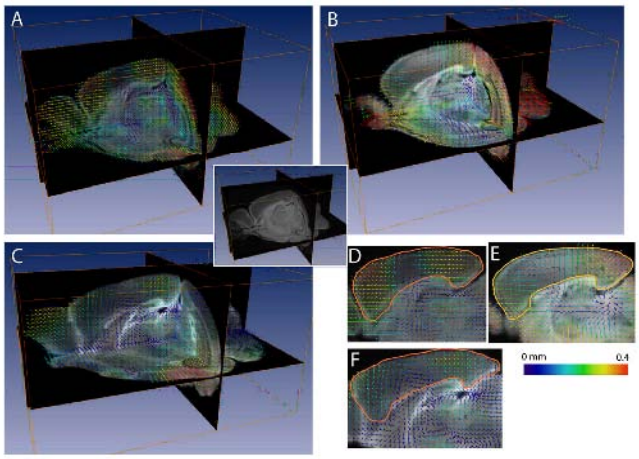

Fig. 4: Growth vector fields at P7 (A and D), P10 (B and E) and P20 (C and F) overlaid on average diffusion weighted images. Color represents the magnitude of growth rate.

Using landmark based LDDMM, growth vector fields during postnatal development were obtained. In Fig. 4. growth vector fields were overlaid on three orthogonal cross-sections of diffusion weighted images at P7 (A), P10 (B) and P20 (C). Vector colors reflect the rate of growth. These results suggest that the growth rates vary in different brain compartments. Cortical regions often had a higher growth rate than thalamic regions. Local growth vector fields in the cortical regions are shown in D (P7), E (P10), and F (P20). At P7, anterior and posterior regions of cortex showed rapid growth. At P10, the posterior region of cortex continued its rapid growth while anterior cortical regions showed slow growth. At P20, growth rates in both regions were slowed to approximately 0.2 mm/week.

IV. Discussion and Conclusion

Quantitative characterization of mouse brain development is a challenging task. In this paper, we have described imaging developing mouse brains using the DTI technique and using an image database and digital atlas to study mouse brain development. Using the images currently available in our database, morphological variations of mouse brains and changes associated with brain development could be quantified (Fig. 3). The spatial and temporal patterns of brain development measured by our techniques could be visualized (Fig. 4). However, it is necessary to note the limitations of our landmark based approach. To accurately capture developmental changes, a dense network of landmarks is often required, and this approach is time-consuming and

susceptible to human errors. For future studies with large populations, landmark based techniques may not be the optimal solution. Development of direct tensor or vector based image matching would greatly enhance the efficiency of the study.

In conclusion, DTI provides rich tissue contrast and the necessary resolution for characterizing changes in tissue morphological during mouse brain development. With these advanced techniques of computational anatomy, quantitative measurement of brain development is feasible.

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