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Mapping postnatal mouse brain development with diffusion tensor microimaging

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While mouse brain development has been extensively studied using histology, quantitative characterization of morphological changes is still a challenging task. This paper presents how developing brain structures can be quantitatively characterized with magnetic resonance diffusion tensor microimaging coupled with techniques of computational anatomy. High resolution diffusion tensor images of ex vivo postnatal mouse brains provide excellent contrasts to reveal the evolutions of mouse forebrain structures. Using anatomical landmarks defined on diffusion tensor images, tissue level growth patterns of mouse brains were quantified. The results demonstrate the use of these techniques to three-dimensionally and quantitatively characterize brain growth.

Keywords: Mouse brain development; Diffusion tensor; Microimaging; Computational neuroanatomy

Introduction

In recent years, the number of studies on brain development using laboratory mice has increased enormously due to advances in gene engineering technologies and the increasing availability of various mutant mouse strains. These studies add to the information on the molecular and cellular mechanisms of mouse brain development. There are a number of sources describing embryonic mouse brain morphogenesis (Alvarez-Bolado and Swanson, 1996; Bayer and Altman, 1991; Bayer et al., 1994; Paxinos et al., 1994) and adult mouse brain anatomy (Paxinos and Franklin, 2000). However, quantitative three-dimensional information on the development of mouse brain morphology is still scarce.

Most studies on brain development have relied on histological examination, in which rich cellular and/or molecular information can be obtained with high spatial resolution. However, histological examination is not optimal for three-dimensional macroscopic characterization of anatomical changes because it requires large amounts of sectioned slides. Sectioning procedures can easily lead to complications such as tissue damage, deformation, and section-to-section variation. Consequently, histology-based studies are often confined to small areas and are hypothesis-driven or based on a priori knowledge so that optimum slice locations and staining methods can be designed. While histology-based studies remain the most valuable tool for neuroanatomical examination, methods of 3D imaging should greatly enhance our ability for phenotype characterization by providing accurate and efficient means for morphological delineation.

Several alternative techniques, including magnetic resonance imaging (MRI) (Jacobs and Fraser, 1994; Jacobs et al., 1999a,b; Johnson et al., 1993, 2002) and optical methods (Sharpe et al., 2002; Weninger and Mohun, 2002), have been developed and have shown great potential. Among them, three-dimensional MRI has the capability of delineating anatomical structures inside optically opaque samples with the potential of in vivo longitudinal studies. Although it has been successfully used in several developmental studies (Baratti et al., 1999; Jacobs and Fraser, 1994; Jacobs et al., 1999a,b; Mori et al., 2001), conventional relaxation-based MRI often fails to provide satisfactory tissue contrast for mouse brains at embryonic or paranatal stages, mostly due to lack of myelination.

Recently, we and other groups demonstrated that diffusion tensor imaging (DTI) can delineate detailed neuroanatomy of developing

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mouse brains with endogenous tissue contrast that has not been accessible by any other non-invasive, 3D imaging modalities (Mori et al., 2001; Zhang et al., 2003). This technique provides us an exciting new opportunity to accurately and quantitatively characterize 3D growth pattern of various brain structures.

With the availability of three-dimensional mouse brain images, quantitative measurement of evolving neuroanatomy becomes the next logical step. Following the early envisage of D'Arcy Thompson on using transformations to study biological forms, techniques of computational anatomy (CA) (Grenander and Miller, 1998; Miller et al., 1997, 2002; Toga and Thompson, 2003; van Pelt et al., 2001) are emerging for the quantitative metric study of growth and aging (Thompson et al., 2000, 2001a,b, 2003). Several groups have developed methodologies for the comparison of geometric structures based on maps on the dense continuum connecting anatomical configurations to extrinsic atlases (Bookstein, 1996; Chung et al., 2001; Dann et al., 1989; Evans et al., 1996; Good et al., 2001; Thompson et al., 2001a,b). The sheer complexity of brain anatomy requires the study of all submanifolds—landmarks, curves, surfaces, and subvolumes, all taken together forming the complete volume (Christensen et al., 1996; Miller and Younes, 2001; Miller et al., 1997). Using conventional MRI, we have been able to map the shape and structures of midbrain including the hippocampus and thalamus using diffeomorphisms generated via the differential equations of particle flows, in particular large deformation diffeomorphic metric mapping (LDDMM) (Beg et al., 2003; Miller et al., 2002). The transformations constructed by the LDDMM methods are diffeomorphisms so that connected structures remain connected, disjoint structures remain disjoint and submanifolds are preserved.

In this paper, we first investigated contrast change in DTI and conventional MR images throughout development. Identifiable structures were then manually segmented and their volume changes were observed. Morphometric changes of the neocortex due to growth were analyzed based on manual landmark placement followed by LDDMM. These results could quantitatively delineate normal developmental processes of male C57/BL mouse brains. This approach provides an important foundation for future applications to study of brain developmental abnormalities due to gene alteration (phenotype), induced lesions (e.g., ischemic or viral insults) or drug treatment (e.g., maternal drug addiction), by filling the gap between cellular/molecular changes and gross anatomical observation, and further our understanding of the underlying mechanisms involved.

Methods

Animal subjects

All experiments and procedures were approved by the Animal Research Committee of the Johns Hopkins University, School of Medicine. We used C57BL/6J male mice as the subject of our study. A total of 25 mice were used in this study, ranging from embryonic day 18 (E18) to postnatal day 80 (P80). Among them, 3 mice were at P0 and 16 mice were at P7, P20, P30 and P80 (4 mice at each stage). Specimens were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for over 1 month. Before imaging, we placed specimens in PBS for more than 24 h to washout the fixation solution and transferred them into home-built MR-compatible tubes. The tubes were then filled with fomblin (Fomblin Profludropolyether, Ausimont, Thorofare, New Jersey, USA) to prevent dehydration.

Image acquisition and processing

Imaging was performed using a GE Omega 400 (9.4T) spectrometer. Custom-made solenoid volume coils were used as both the radio frequency (RF) signal transmitter and receiver. The NMR sequence was based on a 3D multiple echo sequence with navigator-echo phase correction scheme and segmented k-space acquisition (Mori and van Zijl, 1998). Following each excitation, two imaging echoes were acquired followed by two navigator echoes to correct any instrumental instabilities and subsequent changes in signal phase and intensity during each scan. 3D diffusion-weighted images were acquired with a repetition time of 0.9 s, an echo time (TE) of 37 ms, and two signal averages (field of view 12 mm × 6 mm × 6 mm for the smallest sample (P0) and 17 mm × 11 mm × 8 mm for the largest, and others in between). The imaging matrix had dimensions from 128 × 70 × 64 to 128 × 84 × 80, which was zero-filled to double their sizes after the spectral data were apodized by a 10% trapezoidal function. The native imaging resolutions ranged from 94 μm × 86 μm × 93 μm to 133 μm × 131 μm × 100 μm. At least six diffusion weighted images with b values of 1000–1200 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]. We also acquired at least one image with a b value of 150 s/mm^2. The imaging time for DTI was approximate 24 h. T_2-weighted images were acquired with the same resolution as diffusion-weighted images, with a repetition time of 0.9 s, four TE values (37 ms, 60 ms, 80 ms, 100 ms), and 2 signal averages for an additional imaging time of approximately 9 h. The total imaging time for acquiring both DT and T_2 images was approximately 33 h.

The diffusion tensor was calculated using a multivariant linear fitting method, and three pairs of eigenvalues and eigenvectors were calculated for each pixel (Basser and Pierpaoli, 1996; Basser et al., 1994). The eigenvector associated with the largest eigenvalue was referred to as the primary eigenvector. For the quantification of anisotropy, fractional anisotropy (FA) was used. Color map images were generated by combining the images of primary eigenvector and FA into RGB images. In the color map images, the ratio among R(ed), G(reen), and B(lue) components of each pixel was defined by of the ratio of the absolute values of x, y, and z components of the primary eigenvector, and the intensity was proportional to the FA. Red was assigned to the anterior–posterior axis, green to the medial-to-lateral axis, and blue to the superior–inferior axis. T_2 map images at each stage were obtained by fitting T_2-weighted images of four different TE values to a mono-exponential model. Segmentation of cortex, hippocampus, caudate putamen, cerebellum, and superior colliculus were performed manually following the definition in the Paxinos atlas (Paxinos and Franklin, 2000).

Landmark definition

We followed a coarse-to-fine hierarchical procedure to generate 3D diffeomorphic maps between mouse brain images. The first step is based on operator-identified landmarks in diffusion tensor images. Nine landmarks placed on the anterior