Early neuroanatomical development of the mouse brain characterized by diffusion tensor microimaging

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Introduction: The mouse is the most widely used model for studying vertebrate brain development. The short embryonic phase of 18-20 days is marked by very rapidly changing neuroanatomy. With the availability of genetically engineered mouse models of developmental disorders, many of which manifest in the embryonic stages, techniques for 3D anatomical imaging of the developing mouse brain are critically important. While conventional T_2-weighted MR contrasts during this phase are limited to visualizing gross changes in brain morphology, diffusion tensor imaging (DTI) has been shown to provide rich anisotropy-based anatomical contrasts in the premyelinated brain [1]. In order to capture the evolving embryonic neuroanatomy at miniature spatial scales, high spatial resolution is necessary which makes 3D anatomical imaging of the embryonic mouse brain challenging. Recent work has demonstrated the feasibility of high-resolution diffusion tensor microimaging (DTMI) [2, 3]. In this study, 3D DTMI of the developing mouse brain from embryonic through neonatal stages at an isotropic resolution of 50 µm is presented. At this resolution, DTMI enabled characterization of the evolving neuroanatomy at microstructural levels and delineating the spatiotemporal trajectories of evolving white matter tracts during embryonic brain development.

Methods: Brain specimens from C57Bl/6 mice at 24 h intervals from embryonic day 12 (E12) to postnatal day 3 (P3) were used in the study. Ex vivo DTMI was performed on an 11.7 T spectrometer using a diffusion-weighted gradient and spin echo (DW-GRASE) sequence with navigator echo phase correction previously developed by our group [3] (N_{rf} = 4, TE/TR = 32/700 ms , NA = 4, δ/Δ = 3/15 ms). Diffusion-weighted images in 12-16 directions (b-value ~1000 s/mm^2) and two non diffusion-weighted (b0) images were acquired at an isotropic spatial resolution of 50 µm^3 and scan time of 1.6 to 2.2 h per image, depending on the specimen size. The diffusion tensor was estimated by a multivariate linear fitting method. Direction-encoded color (DEC) maps were computed from the primary eigenvector scaled by Westin's linear index of anisotropy (CL). Red was assigned to the medial-lateral axis, green to the dorsal-ventral, and blue to the rostral-caudal axis. For analysis of morphological change over time, brain images at each age were nonlinearly mapped to the successive developmental stage using two-channel large deformation diffeomorphic metric mapping (LDDMM) based on fractional anisotropy (FA) and isotropic diffusion-weighted (iDW) contrasts. The FACT algorithm [4] was used for reconstruction of axonal tracts from the tensor data based on the primary eigenvector and FA maps.

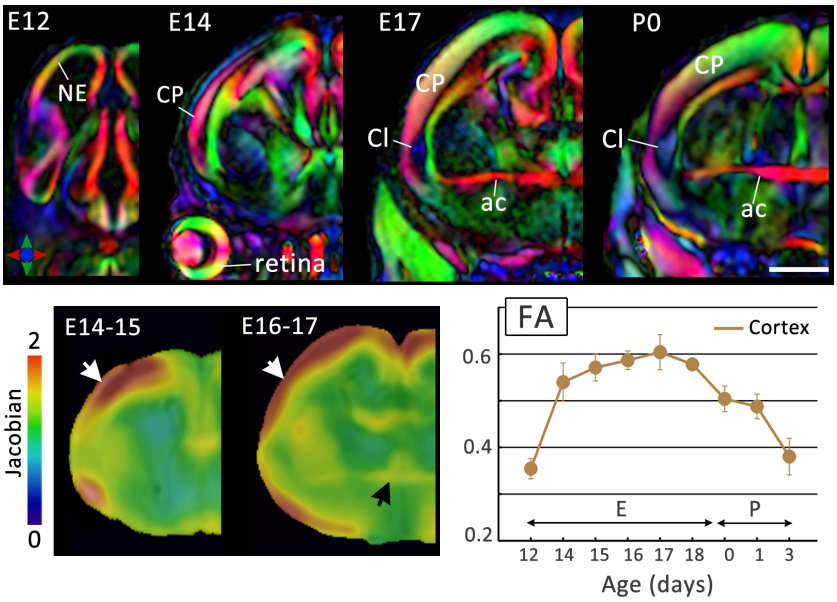

Fig. 1: Neuroanatomical details in the developing mouse brain resolved by DTMI at 50 µm resolution. Top panel) Anisotropy-based contrasts in the DEC maps showing the evolving grey and white matter structures at different developmental stages. NE: neuroepithelium, CP: cortical plate, Cl: claustrum, ac: anterior commissure. Bottom panel) Jacobian maps of deformations between E14-15 and E16-17 reveal the changing brain morphology. Volumetric growth of the CP (white arrows) and ac (black arrow) can be seen. Temporal profile of FA in the embryonic (E) and early postnatal (P) cortex shows a peak in the radially-oriented FA in the cortex, coinciding with the period of cortical neurogenesis and radial migration. Scale bar = 1 mm.

Results & Discussion: DTMI at 50 µm resolution revealed microstructural anatomical details in the developing mouse brain. Fig. 1 shows the temporal evolution of anisotropy-based DEC contrasts during embryonic development, that enabled delineation of evolving grey and white matter structures based on their unique structural orientations. Notably, the cerebral cortex in the embryonic brain was characterized by a high degree and distinct pattern of anisotropy. Cortical neurogenesis in the mouse occurs between E12.5 to E18, when neurons born in the neuroepithelium (NE) migrate along the radial glia to form the cortical plate (CP). At E12, the NE could be distinctly identified as a region of high radially-oriented FA. The CP emerged at E13, and continued to increase in thickness through the embryonic stages till P0 (DEC maps in Fig. 2). Plot of FA versus age showed a peak in the cortical FA (0.58 ± 0.03 from E14-E18) coinciding with the period of cortical neurogenesis and radial migration (FA plot in Fig. 2). These findings suggest that the time course of cortical development in the embryonic mouse brain can be uniquely visualized using DTMI. Further, the level of neuroanatomical detail resolved in the present study allowed LDDMM-based mapping of the embryonic images across different developmental stages in order to capture the time-dependent changes in brain morphology. Fig. 1 also shows the Jacobian maps of the resulting deformations from E14 to E15 and E16 to E17, revealing significant volumetric growth in the cortical plate (Fig. 1, white arrows) and the anterior commissure (Fig. 1, black arrow).

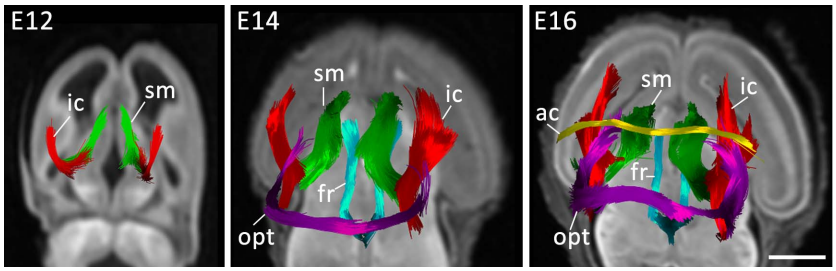

Fig. 2: Evolving spatiotemporal trajectories of early developing white matter tracts in the embryonic brain from E12 to E16 reconstructed from the DTMI data. 3D rendering of the internal capsule (ic, red), stria medullaris (sm, green), fasciculus retroflexus (fr, cyan), optic tract (opt, purple), and anterior commissure (ac, yellow) are shown. Scale bar = 1 mm.

Reconstruction of axonal fibers from the DTMI data at 50 µm resolution allowed tracing the emergence and 3D growth trajectories of white matter tracts in the embryonic brain, which are difficult to distinguish at lower resolutions. Fig. 2 shows the spatiotemporal evolution of developing axonal tracts in the embryonic brain, showing the evolving trajectories of early developing tracts such as the internal capsule and stria medullaris that appear at E12, and the development of commissural tracts such as the anterior commissure at later embryonic stages. The results of the present study demonstrate the level of microscopic neuroanatomical details in the developing mouse brain that can be resolved with high-resolution DTMI, and will be important for studies of embryonic brain development as well as screening of mouse models of developmental brain disorders.

References: [**1**] Mori *et al*, *MRM* 46, 2001 [**2**] Jiang & Johnson, *NeuroImage* 50, 2010 [**3**] Aggarwal *et al*, *Mag Res Med* 64, 2010 [**4**] Mori *et al*, *Ann Neurol* 45, 1999