Three dimensional rapid diffusion tensor microimaging for anatomical characterization and gene expression mapping in the mouse brain

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Introduction: Imaging the developing mouse brain at microscopic levels is critically important for the ongoing effort to integrate the evolving neuroanatomy with spatiotemporally-varying gene expression patterns, to understand the mechanisms of genetic control during development. Diffusion tensor imaging (DTI) can provide far superior anatomical contrasts than conventional MRI in the premyelinated developing mouse brain [1], but is limited by currently achievable spatial resolution. DTI is an inherently low signal-to-noise-ratio (SNR) technique, and its implementation at higher resolutions is faced with significant technical challenges. Existing studies have reported resolutions up to 80 µm, which is still too coarse to study fine structures in the embryonic mouse brain. In this study, we present ultra high resolution (up to 50 - 60 µm) rapid DT-microimaging (DTMI) of the embryonic and adult mouse brains based on a 3D diffusion-weighted gradient and spin echo (DW-GRASE) scheme with twin-navigator echo phase correction. The DTMI data provide unprecedented amount of anatomical information in the mouse brain. We also demonstrate successful 3D mappings of gene expression data from *in situ* hybridization (ISH) to DTMI images in an early embryonic mouse brain.

Methods: In current mouse brain DTI protocols, echo planar imaging at high fields is susceptible to B0 inhomogeneity, and diffusion-weighted fast spin echo is limited by breakdown of the CPMG condition, which prohibits the use of long echo trains. In this study, a 3D DW-GRASE sequence with twin-navigator echo phase correction was developed for high-field DTMI, with N_{rf} refocusing pulses per echo train, and 3 echoes per refocusing. For 3D acquisition, the SORT phase encoding strategy was used to separate the T_2-dependent amplitude modulation and the phase modulation due to off-resonance spins along different Fourier encoding axes [2]. A twin-navigator echo scheme was implemented to minimize artifacts caused by phase modulation from off-resonance spins and phase oscillations between echoes from odd- and even-numbered refocusing pulses due to breakdown of the CPMG condition [3]. DTMI of adult and embryonic day 12 (E12) mouse brains was performed on an 11.7 T spectrometer using the DW-GRASE sequence (N_{rf} = 4, TE/TR = 32/800 ms, NA = 4, δ/Δ = 3/15 ms). For the adult samples, 12 DW directions (b~1700 s/mm^2) were acquired at 55 x 55 x 55 µm^3 and scan time of 3 h 15 min per DWI. For the E12 samples, 16 DW directions (b~1200 s/mm^2) were acquired at 60 x 60 x 60 µm^3 and scan time of 1 h 50 min per DWI. Direction-encoded color (DEC) maps were computed from the primary eigenvector and fractional anisotropy (FA) images. Red was assigned to the medial-lateral axis, green to rostral-caudal, and blue to the dorsal-ventral axis. For mapping of gene expression data, serial ISH sections from an E12 mouse brain were reconstructed into a 3D volume and coregistered to the DTMI images using landmark-based rigid and nonlinear deformation [4].

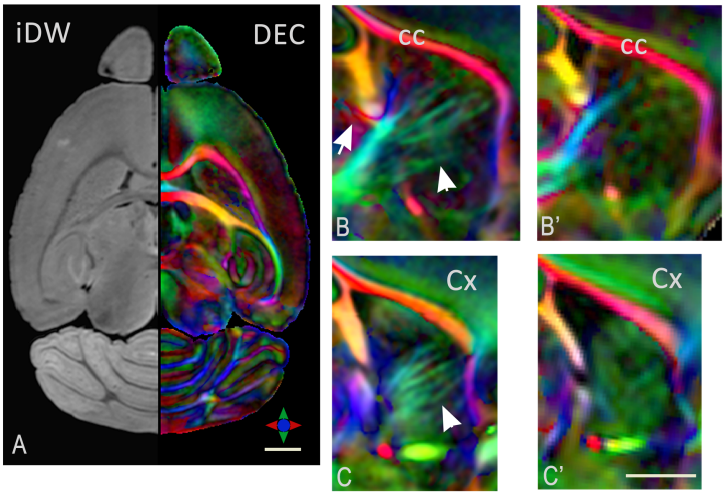

Fig. 1: DTMI of an adult mouse brain at 55 µm resolution. A) iDW and DEC contrasts in a horizontal brain section. B-C) Comparison of anatomical details revealed by DTI at 55 µm (B,C) and 125 µm (B',C') resolutions. DTMI at 55 µm revealed the microstructural organization of white matter fibers in the striatum (white arrows), that were not seen at lower resolutions (right). Scale bars=1mm.

Results & Discussion: Comparison of mouse brain DTI from multiple spin echo (MSE) and GRASE acquisitions revealed no significant differences in anisotropy mapping (6.2 ± 2.1% difference in FA in voxels with FA > 0.2). For the same resolution and SNR, the GRASE technique resulted in a threefold reduction in scan time compared to the MSE acquisition. In DEC maps of the adult mouse brain, important white matter structures could be distinctly delineated based on their structural orientation, but were not discernible in conventional T2-w or isotropically diffusion-weighted (iDW) images (Fig. 1A). To assess the efficacy of high resolution DTI for detailed anatomical characterization of the brain, we compared the DTMI results from the present study to a DTI-based mouse brain atlas (125 µm resolution) previously developed by our group [5]. The most striking difference at the two resolutions was seen in the striatum. DTMI at 55 µm resolution revealed the microstructural organization of a mesh of sagittally-oriented fine fibers traversing through the striatum (white arrows, Fig. 1B,C), that could not be resolved at lower resolutions (Fig. 1B',C'). In the premyelinated embryonic brain, T2-w and iDW images provide severely limited contrasts. In comparison, DTMI revealed rich anatomical contrasts for characterization of gray and white matter organization at the microscopic level. The neuroepithelium could be clearly differentiated as a region marked by high anisotropy and radial orientation (not shown). 3D reconstructions of early axonal fibers in the E12 brain revealed developing white matter tracts such as the internal capsule and stria medularis (Fig. 2), which are not distinguishable at lower resolutions. Fig. 3 illustrates the results of mapping Lhx8 and Shh gene expression data in an E12 mouse brain from sagittal two-color ISH sections (Fig. 3a) to the DTMI data. Mapping of gene expression data enabled inspection of the 3D expression patterns, and the spatial distribution of these genes could be visualized and compared with specific anatomical structures (Fig. 3c). These results provide proof-of-principle for using rapid DTMI for microanatomical characterization of the mouse brain to enable comprehensive studies of development and gene expression analysis.

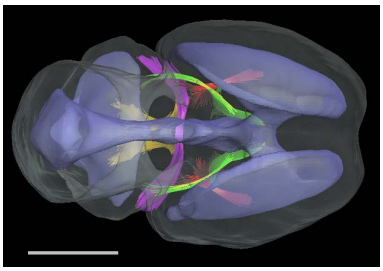

Fig. 2: Reconstruction of developing axonal tracts in the E12 mouse brain from DTMI at 60 µm resolution. internal capsule (red), stria medularis (green), transverse pontine fibers (magenta), and medial longitudinal fasciculus (yellow). Scale bar=1mm.

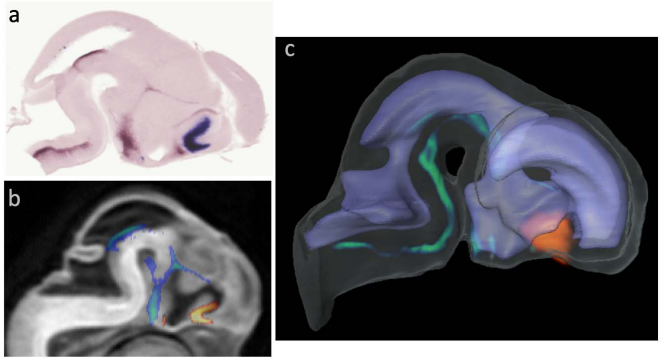

Fig. 3: Gene expression data mapping to high resolution MRI of an E12 mouse embryo. In original 2D ISH sections (a), it is difficult to comprehend the distributions of Lhx8 (blue) and Shh (brown) genes relative to each other and to brain structures. After mapping, coregistered Lhx8 (orange) and Shh (green) expression data are overlaid on the iDW image (b), and inspected in 3D with brain structures e.g. ventricles (blue) to appreciate the spatial expression patterns of the two genes (c).

References: [**1**] Zhang *et al*, *NeuroImage* 20, 2003 [**2**] Mugler, *J Mag Res Imag* 9, 1999 [**3**] Mori et al, Mag Res Med 40, 1998 [**4**] Zhang *et al*, *NeuroImage* 44, 2009 [**5**] Aggarwal *et al*, *Neuroscience* 162, 2009