Imaging the microstructure of the developing cerebral cortex in the mouse embryo with diffusion MR microscopy

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Purpose: We investigate the potential of diffusion MRI (dMRI) for 3D MR microscopy of cortical development in the mouse embryo. The mouse fetus is the primary focus in studies of cortical development, which have largely relied on 2D histological techniques to examine cortical structure. Three-dimensional imaging of cortical microstructure in the mouse embryo has the potential to significantly benefit studies of corticogenesis, but remains challenging. Corticogenesis involves the migration of neurons born in the ventricular zone (VZ) along radial glia to establish the cortical plate (CP)[1], forming the structural basis of diffusion anisotropy in embryonic cerebral tissue. Given that the cerebral wall in the mouse embryo is an exceedingly thin (0.1-0.3 mm) structure, 3D diffusion imaging of its laminar microstructure is technically challenging due to the low SNR and extremely high resolution required. Here, we achieved ultra-high resolution dMRI using a recently developed 3D microimaging sequence, to probe the microstructure of cortical tissue in the mouse embryo. The results of this work reveal unprecedented detail in the embryonic cortex using diffusion as an endogenous probe, resolving the transient embryonic cortical zones and their evolving microstructure during cortical formation.

Methods: MR experiments were performed on an 11.7 T spectrometer (Bruker Biospin) with a Micro5.0 gradient system (maximum strength 3000 mT/m) using a 10-mm saddle coil. Fixed C57Bl/6 mouse embryos at 7 developmental time points, from embryonic day 12 (E12) to E18 (n=3 at each age), were used in this study. dMRI data were acquired with a 3D diffusion-weighted gradient and spin echo (DW-GRASE) sequence[2] with navigator echo phase correction (N_{rf} = 4, three echoes per refocusing pulse, TE/TR = 34/650 ms, 4 averages, bandwidth of 100 kHz). Twin navigator echoes were implemented for motion and eddy current-induced phase correction[3]. For each diffusion dataset, two b0 images and 18 diffusion directions (b-value ~1420 s/mm^2) were acquired using double-refocusing bipolar diffusion-sensitizing gradients (δ/Δ = 3.2/12 ms), at an isotropic resolution of 52 x 52 x 52 µm^3. Total imaging time was 21 to 31.5 h for the E12 to E18 specimens with complete sampling of the k-space. Co-registered T2-weighted images were acquired at the same resolution using a 3D RARE sequence (rare-factor of 8, TE/TR = 40/1000 ms, 4 averages). Deformable diffeomorphic mapping was used to compute unbiased average dMR images of the mouse heads at each embryonic stage (n=3), to minimize sample-specific anatomic bias in the mapping of cortical development. Parametric fractional anisotropy (FA) and direction-encoded color (DEC) maps were calculated from diffusion tensor fitting. Constrained spherical deconvolution of the dMRI data was performed in MRtrix to generate track density images (TDI)[3] of the embryonic cerebral wall at each stage, based on probabilistic fiber-track density mapped to a 4-µm isotropic grid.

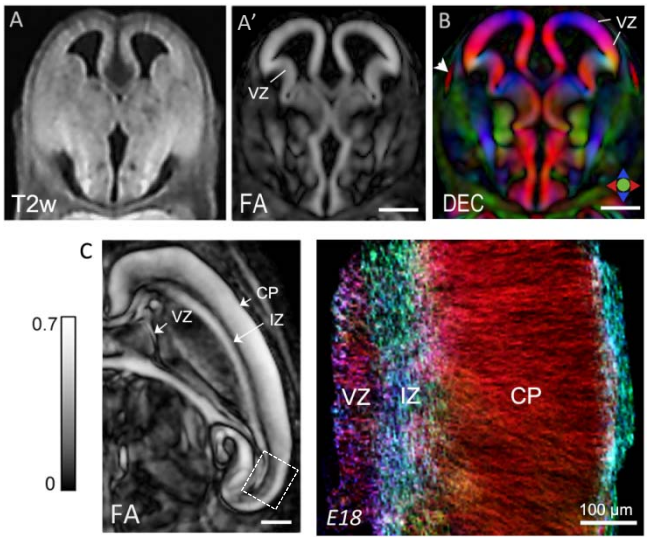
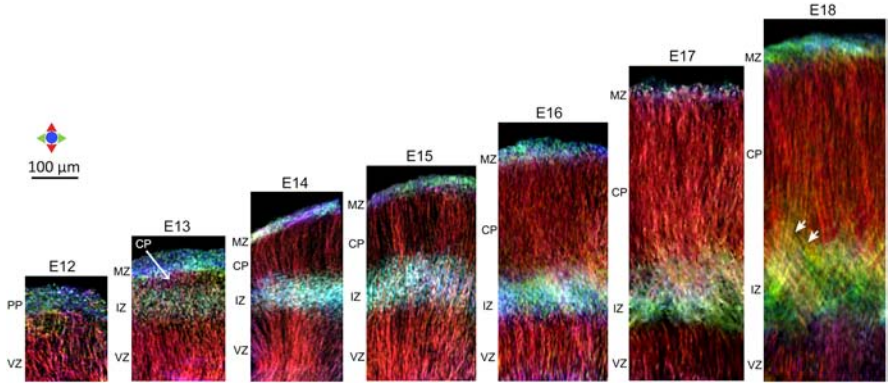


Fig. 1: Diffusion microimaging of the embryonic mouse cortex. **A-A')** Comparison of T2w and FA contrasts at E12. **B)** DEC map shows radial organization in the ventricular zone (VZ) and the early cortical plate (white arrowhead) resolved at E13. **C)** Track density image (area shown by white box) reveals the radially-arrayed microstructure of the CP by E18, reflecting the arrangement of fine radial glial fibers. Scale bars = 0.5 mm.

Results & Discussion: SNR values in the cortical region measured in b0 images were ~76 and ~87 at E12 to E18, respectively. The resulting dMRI data revealed microscopic detail in embryonic cortical tissue reflecting its cytoarchitectural organization. **Fig. 1A** compares T2w and FA contrasts at 52 µm resolution in the E12 embryo. While T2w images provided relatively limited contrast within embryonic cerebral tissue, the FA map resolved the ventricular zone (VZ) as a layer marked by high anisotropy along the ventricular wall (Fig. 1A). The VZ consists of radially-aligned processes of the radial glial cells, which form the scaffold for neuronal migration[1]. This radial organization of the VZ can be clearly resolved in DEC contrasts (**Fig. 1B**), which revealed the orientation of anisotropy to be perpendicular to the ventricular surface throughout the VZ. The first appearance of the microscopic cortical plate (70-80 µm thick) could be resolved in dMRI contrasts at E13 (white arrowhead in Fig. 1B). The intermediate zone (IZ), which contains axonal fibers running parallel to the pial surface, showed tangential orientation between the CP and VZ. The strong radially-arrayed microstructure of the CP reflecting the arrangement of radial glial fibers is further shown in TDI contrasts at E18 (**Fig. 1C**).

TDI maps of the cerebral wall from E12 to E18 revealed the embryonic cerebral zones and their evolving microstructure during corticogenesis (**Fig. 2**). At E12, the TDI map distinguished two separate zones, the VZ with radially-arranged microstructure (red in the TDI maps), and an outer preplate (PP) with low structural alignment. 24 hours later (at E13), the first appearance of the cortical plate (CP) with radial microstructure could be resolved, splitting the preplate into 2 layers - an outer marginal zone (MZ) and the deeper IZ. The emergence of the early CP with its radial cytoarchitecture appearing between the tangentially-oriented MZ and IZ is clearly resolved in Fig. 2 (white arrow at E13). Using diffusion as an endogenous probe, the TDI maps clearly revealed the resulting 4-layered structure of the embryonic cortex from E13, with the superficial MZ and intermediate IZ which contain tangentially-oriented horizontal bipolar cells and axons (blue-green in TDI maps), and the VZ and CP with the well-organized fibers of the radial glial cells (red in TDI maps). This structural arrangement is consistent with histological observations in the mouse embryo[1]. At later stages, the TDI maps revealed thickening of the IZ with tangentially-oriented fibers, corresponding to early efferent and afferent axons that infiltrate the IZ during this period. These fibers were delineated as tangential interdigitating processes in the IZ, and could be resolved innervating the gray matter of the CP in TDI contrasts at E17-E18 (white arrowheads at E18).



Conclusion: Our findings demonstrate the potential of dMR microimaging to resolve the microstructure of the developing cerebral cortex in the mouse embryo. The results of this study provide proof-of-principle for using dMR microscopy for 3D imaging of cortical tissue in the mouse embryo, and will be useful for studies of corticogenesis as well as its disruption in embryonic mouse models.

Fig. 2: TDI maps of the cerebral wall from E12 to E18 reveal the embryonic zones and their evolving microstructure during corticogenesis. Emergence of the thin cortical plate (CP, white arrow) with radial structure is seen at E13. White arrowheads indicate tangential fibers evident in the intermediate zone (IZ) at E17-E18, that could be resolved innervating the gray matter of the CP. VZ: ventricular zone, MZ: marginal zone, PP: preplate.

References: [**1**] Noctor *et al*, *Nature* 409, 2001 [**2**] Aggarwal *et al*, *Mag Res Med* 64, 2010 [**3**] Mori *et al*, *Mag Res Med* 40, 1998 [**4**] Calamante *et al*, *Neuroimg* 59, 2012

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