Region-specific microstructure of cortical areas revealed with high angular resolution diffusion MR microimaging

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Target audience: Researchers who are interested in high-resolution diffusion MRI acquisition, and imaging of cortical microstructure.

Purpose: The cortical gray matter has a heterogeneous layered architecture. Regional variation in cortical microstructure has been widely investigated using conventional histological methods, and forms the basis of ongoing efforts on structural mapping of cortical areas[1]. 3D microimaging of cortical gray matter has the potential to reveal further insights into underlying microstructure and connectivity across layers compared to in-plane histology, but remains technically challenging. Diffusion MRI (dMRI) is a powerful technique to probe brain tissue structure, but its application to microscopic imaging of cortical tissue is technically hindered, by both the significantly low anisotropy in gray matter microenvironments and the resolution level necessary to resolve the layered architecture. In this work, we demonstrate 3D dMR microimaging to investigate the microstructure of cortical gray matter in the fixed human brain. High angular resolution dMRI with micro-imaging spatial resolution was achieved using 3D diffusion-weighted gradient and spin echo acquisition, which revealed the laminar microstructure of cortical areas with unprecedented detail. Our findings show 3D imaging of cortical microstructure that closely reflects the underlying myeloarchitecture revealed with histological staining.

Methods: Cortical specimens (n=3 each) from the prefrontal (Brodmann area 9), primary motor (area 4), and primary visual (area 17) cortex were obtained from three adult PFA-fixed human brains. MR micro-imaging was performed on an 11.7T scanner (Bruker Biospin) with a Micro2.5 gradient system. High angular resolution diffusion imaging (HARDI) data of cortical areas were acquired using 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= 35/600 ms, 2 averages, bandwidth of 90 kHz). 30 diffusion directions were acquired with a *b*-value of 2000 s/mm^2 using double-refocusing bipolar diffusion gradients (δ/Δ = 3.2/14 ms), at a native isotropic resolution of 90 x 90 x 90 µm^3 in ~28 h with fully sampled *k*-space. Twin navigator echoes[3] were implemented for motion and eddy current induced phase correction. T2-weighted images were acquired using a 3D RARE sequence (rare-factor of 4, TE/TR = 40/1000 ms, 8 averages). Parametric fractional anisotropy (FA) and direction-encoded color (DEC) maps were calculated from tensor fitting. HARDI data were reconstructed using constrained spherical deconvolution in MRtrix with a maximum harmonic order of 6, and track-density images (TDI)[4] were generated using probabilistic fiber track density mapped to grid size of 10 µm isotropic. After dMR micro-imaging, the same specimens were histologically sectioned at 40 µm and stained with Bielschowky's silver stain for myelin, for direct *in situ* histological comparison of dMRI findings.

Results & Discussion: Diffusion MR microimaging contrasts revealed the distinct layered microstructure of cortical areas. **Fig. 1** shows DEC maps of the primary visual (V1) cortex, which revealed a striated appearance based on diffusion orientation in specific layers. The V1 cortex is marked by tangential fibers in layer 4 identified as the stria of Gennari (SoG), which can also be delineated in T2-w images. The DEC contrasts revealed the tangential orientation of fibers in the SoG running parallel to the pial surface throughout the V1 cortex (white arrow in Fig. 1). In addition, a second band of tangentially-oriented fibers corresponding to layer 5 could be resolved, apposed orthogonally to the radial orientation of adjacent layers. The resulting six-layered architecture of V1 is clearly seen in DEC contrasts in sagittal and transverse views in Fig. 1. The structural transition from V1 to the secondary visual (V2) cortex was delineated by the marked absence of the tangential bands in V2 (dashed lines in Fig. 1). In the prefrontal cortex (area 9), the inner and outer bands of Baillarger (IB and OB), which form thin fascicles of myelinated axons tangential to the cortical surface, could be resolved in DEC contrasts (**Fig. 2A**). Quantitative mapping of FA measurements across cortical depth revealed distinct region-specific profiles for areas 4, 9, and 17. In area 9, the FA profile demarcated two distinct zones with significantly ($p<0.005$) low anisotropy relative to adjacent layers, corresponding to the IB and OB (**Fig. 2B**).

Regional microstructure resolved with the dMR micro-imaging data is further shown in TDI maps of cortical gray matter, that are compared to corresponding myelin-stained sections from the same tissue in **Fig. 3.** The motor cortex (area 4) revealed radial processes with dispersed tangential fibers seen in inner cortical layers (5/6), without the presence of distinct coherent tangential bands corresponding to the IB and OB (**Fig. 3A**) These findings were consistent with the myeloarchitecture of area 4 seen with silver staining (Fig. 3A). In V1, the tangentially-oriented bands of intra-cortical fibers in Layers 4b and 5 could be reconstructed interspersed with orthogonal radial processes, reflecting the microscopic arrangement of axonal fibers and neurites seen in the myelin-stained section (white arrows in **Fig. 3B**). TDI maps of cortical gray matter also clearly resolved the outer molecular layer (layer 1, marked by asterisks in TDI maps) with horizontal intra-cortical fibers. These findings demonstrate the structural detail and layered architecture of cortical areas resolved with dMR microimaging, which was consistent with underlying cortical myeloarchitecture seen in histological sections.

Conclusion: With the high angular and spatial resolution dMRI achieved in this study, regional microstructure of cortical areas could be resolved in microscopic detail, which closely reflects the underlying myeloarchitecture revealed with in-plane silver staining. These findings will provide insights into understanding the microstructural basis of diffusion MR contrasts in cortical gray matter, and will be important for studies investigating the myeloarchitectonic mapping of areas across the human cortex.

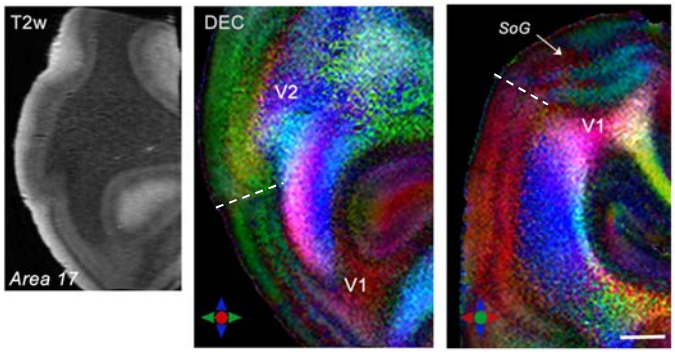

Fig.1: Diffusion MR microimaging of the primary visual cortex (V1, area 17). DEC contrasts in sagittal (left) and transverse (right) sections reveal two distinct layers of tangential fibers resolved in the V1 cortex, corresponding to the stria of Gennari (SoG, layer 4b) and cortical layer 5. The marked transition in the layered-structure between V1 and the secondary visual (V2) cortex can be clearly delineated (indicated by dashed lines). Scale bar = 2 mm.

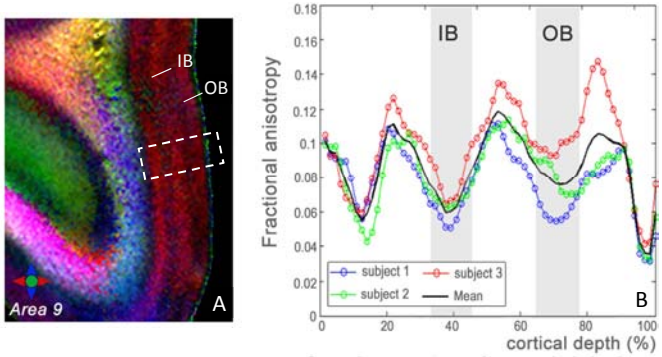

Fig. 2: A) DEC contrasts in the prefrontal cortex (area 9) revealed the inner (IB) and outer (OB) bands of Baillarger. B) Plot of FA profile across cortical depth (within dashed box) clearly shows two distinct zones of low anisotropy, corresponding to the bands of crossing tangential fibers in the IB and OB.

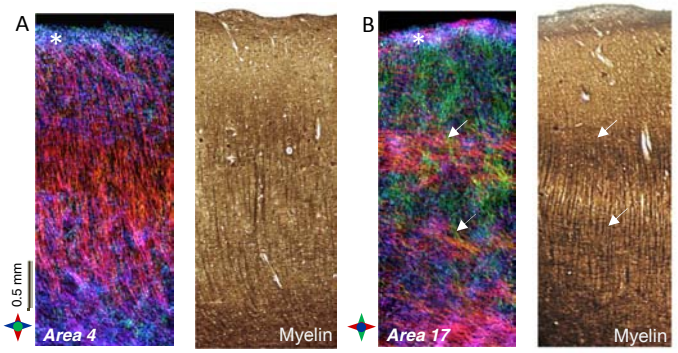

Fig. 3: TDI maps from dMRI compared with myelin-stained sections. A) Transverse view of the motor cortex (area 4). B) Coronal view through the visual cortex (area 17) show the regional microstructure resolved with dMRI, which closely reflects cortical myeloarchitecture shown by silver-staining.

References: [**1**] Zilles *et al*, *Nature Rev* 11, 2010 [**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.

Acknowledgements: NIH grants R01AG020012, R01EB003543.