Validation of Diffusion Weighted Imaging of cortical anisotropy by means of a histological stain for myelin

In vitro cortical DWI shows layer-specific diffusion

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Introduction

To obtain a better understanding of anatomical connectivity, it would be very interesting to track fibers also in and into the cortex. However, the high isotropic component seen in the cortex by current low resolution in vivo Diffusion Weighted Imaging (DWI) makes the estimation of cortical connectivity by tractography very challenging. Recently, anisotropy has been shown in cortical grey matter [1,2]. In the cortex, fiber orientation was found to be predominantly radial, but more complex architectures were also observed in the deeper cortical layers of pigs [2]. In this study we aim to examine cortical fibers *in vitro*, as a cross-validation with histological techniques (i.e. myelin staining) as the current gold standard is possible.

Methods

Samples:

- Human brain tissue samples of primary visual cortex (V1) including underlying white matter.

MRI: 11.7T Bruker BioSpec system

- Diffusion Weighted Imaging (DWI) ➔ 0.3 mm isotropic

  DW-SE with segmented EPI readout; TR=13.75 s; TE=26.6 ms; 61 directions + 7 non-diffusion-weighted; 14 repetitions; b-value=4000 s/mm2; FOV=28.8x28.8 mm; matrix=96x96; 55 slices of 0.3 mm; scan time ~14 h.

- Multi-echo Gradient Echo (MGE) ➔ 0.1 mm isotropic

  3D FLASH; TR=40 ms; TE=3.36-38.36 ms; ΔTE=5 ms; flip angle=30°; matrix=256x256x256; FOV=28.8x28.8x28.8 mm; scan time 33 min

Histology:

- Tissue samples were stained *en bloc* for myelinated nerve fibers with Luxol Fast Blue (LFB).

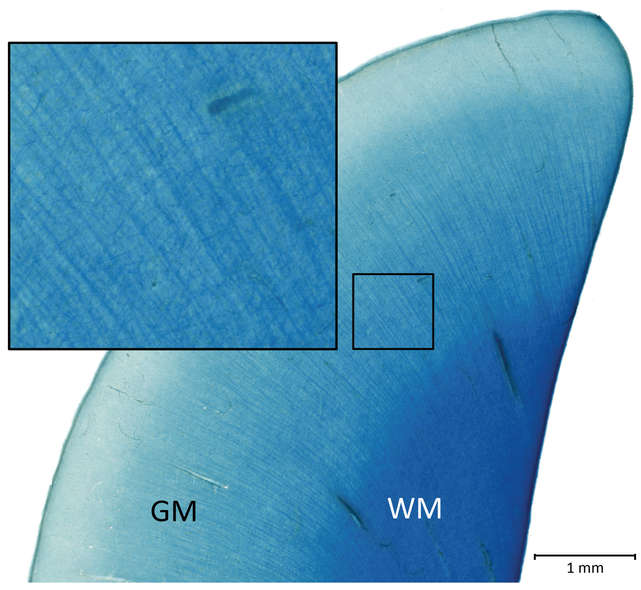

Figure 1: A histological virtual slice image at 20x magnification of LFB stained tissue of a 100 µm thick section. Directionality of myelinated axons is clearly visible in the gray matter. The inset shows radial and horizontal fibers in the gray matter.

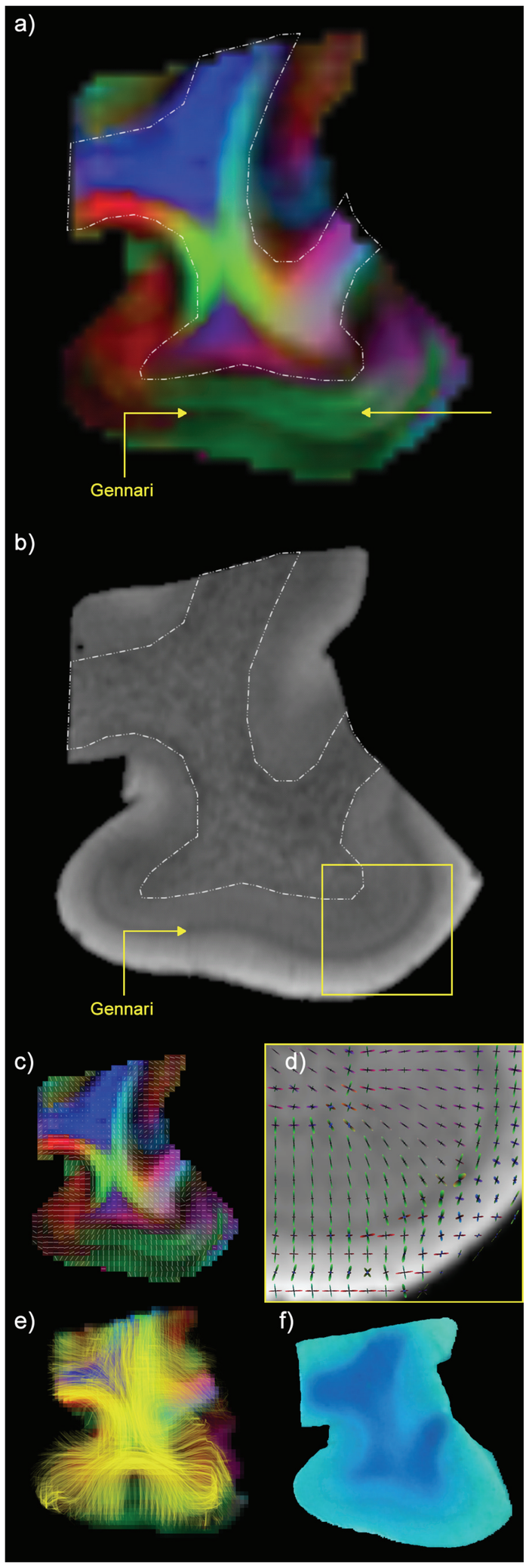

Figure 2: MR results. a) FA image showing layer-specific FA (yellow arrows) b) MGE image averaged over echoes. The line of Gennari (layer IVb) is visible as a low intensity band. c) Principal diffusion direction. d) Orientation density functions (PAS ODFs) of the voxels in the yellow square in b). Note crossing fibers at the WM-GM boundary, radial orientation in the deeper layers and multiple directions in the line of Gennari. e) DT tractography (seeding in all voxels; FA>0.02; tracts > 5 mm). f) LFB histology photograph

Results

MRI:

- Fractional Anisotropy is non-uniform over layers (Fig.2a).
- The line of Gennari shows reduced anisotropy and diffusivity (Fig.3). FA is also reduced in one of the deep layers.
- In the cortex fiber orientation is predominantly radial (Fig.2c,d), but multifiber reconstructions are seen in Gennari (Fig.2d) with fibers running horizontally within the layer.

Histology:

- Myelin-stained sections (Fig.1) clearly show fibers fanning out radially into the cortex and horizontal intracortical fibers.

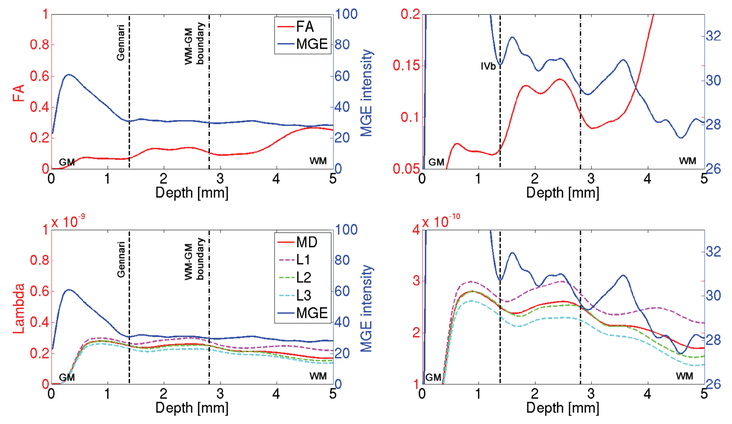

Figure 3: Cortical profiles from a small patch of V1 cortex (Ø 2.25 mm). MGE profiles (blue traces) are included in each panel for anatomical reference. Right panels are blow-outs of left panels. Upper panels: FA profile (red trace). Lower panels: MD and tensor eigenvalue profiles.

Conclusions

- Layer-specific diffusion parameters have been demonstrated in human primary visual cortex (V1) *in vitro.*
- The usefulness for connectivity research has to be investigated, as tractography within the cortex is challenged by an isotropic component within layers.
- Histological LFB staining successfully showed radial and horizontal cortical fibers and can therefore be used to quantitatively validate DWI results

Acknowledgements

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VIP Brain Networks

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