In vitro layer-specific Diffusion Weighted Imaging in human primary visual cortex

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Objective Recently, with the advent of Diffusion Weighted Imaging (DWI) at high field strength, cortical anisotropy has been shown in humans indicating a radial orientation of fibers (Heidemann et al., MRM 2010). More complex configurations have been shown in the deeper cortical layers *ex vivo* in pigs (Dyrby et al., HBM 2010). The variable fiber density and configurations over the layers of the human cortex (Nieuwenhuys et al., 2007) is also likely to be reflected in diffusion properties. Primary visual cortex (V1) is an excellent candidate area to investigate this, because it features the line of Gennari: the prominent layer IVb consisting of horizontal myelinated fibers. In the present study, we therefore image human V1 samples *in vitro* with diffusion MRI at ultra-high field strength, as this allows for the spatial resolution necessary and validation with histological techniques can be performed.

Methods Human brain tissue samples of V1 including underlying white matter were fixed in 10% buffered formalin and stored at 4°C at autopsy (15 h post-mortem). Before MRI, samples were rehydrated in phosphate buffered saline (> 2weeks). MRI was performed on an 11.7T Bruker BioSpec system. Diffusion-weighted images were acquired in a DW-SE protocol using a segmented EPI readout (TR/TE=13750/26.6 ms; 61 directions + 7 non-diffusion-weighted; 14 repetitions; b-value=4000 s/mm^2; FOV=28.8×28.8 mm; matrix=96×96; 55 slices of 0.3 mm). Multi-echo gradient-echo images (MGE) were acquired for anatomical reference of cortical cytoarchitecture (3D FLASH; TR=40 ms; TE=3.36-38.36 ms; ΔTE=5 ms; flip angle=30°; matrix size 256×256×256; FOV 28.8×28.8×28.8 mm). DWI and MGE volumes were realigned and coregistered. Calculation of diffusion tensors (DT), fractional anisotropy (FA) and mean diffusivity (MD) and tractography were performed with diffusion MRI toolkit Camino. MGE images were averaged over echoes.
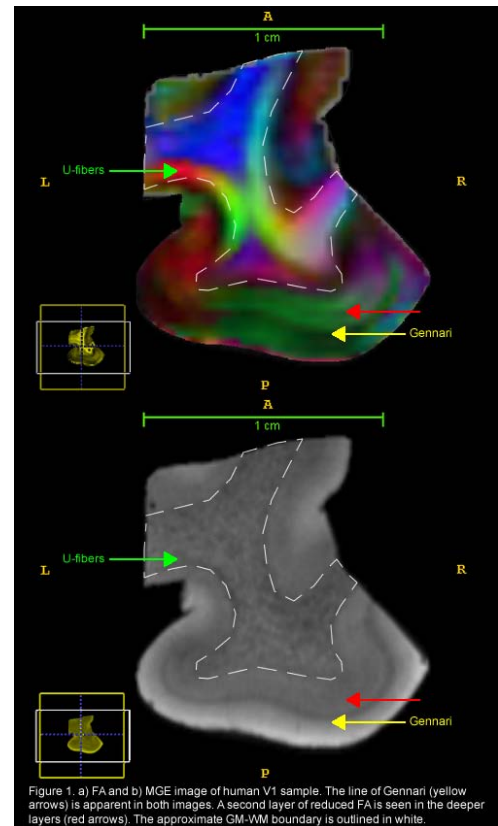

Figure 1. a) FA and b) MGE image of human V1 sample. The line of Gennari (yellow arrows) is apparent in both images. A second layer of reduced FA is seen in the deeper layers (red arrows). The approximate GM-WM boundary is outlined in white.

Results In the cortex FA and MD were non-uniform over layers. In particular, a layer of decreased FA (Fig.1; yellow arrow) and MD is evident throughout most of the sample that coincides well with the line of Gennari in the MGE image. A second layer of reduced FA in the deeper layers of the cortex is less pronounced but clearly visible (red arrow). DT tractography results show predominant radial fiber tracts in the cortex and many u-fibers spanning most of the gray-white matter boundary.

Conclusions We have provided a clear demonstration of layer-specific diffusion parameters in the human neocortex. DT tractography results show anatomically plausible fiber reconstructions. The usefulness for connectivity research has to be investigated further, as tractography within cortical layers is challenged by a probably isotropic diffusion component within the layer (i.e. horizontal fibers are likely to be equally distributed over all within-layer directions). To elucidate this, reconstruction of the orientation density functions is a topic of active investigation, as is validation with histological methods.

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