Cortical fiber insertions and automated layer classification in human motor cortex from 9.4T diffusion MRI

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INTRODUCTION

Ultra-high resolution diffusion weighted imaging (DWI) on *ex vivo* tissue represents a unique tool to investigate human brain anatomy at a microscopic scale [1, 2]. The combination of this technique with classic histology has proven to be very effective to describe both the short association pathways (u-fibers) and the microstructural properties of the brain, mostly in white matter [3]. Previously, we showed that multiple cortical layers are visible in high in-plane resolution diffusion weighted images when averaged over a few directions [4]. This study focuses on two separate aspects which can be derived from high isotropic resolution HARDI data analysis on *post mortem* human tissue: i) we reconstruct and analyze the short association fibers connecting human motor and premotor area and fanning cortical insertions in the gyral crown and ii) we distinguish the majority of cortical layers over large expanses of cortex by automatic clustering of their diffusion characteristics. Both aspects are validated using myelin stains of the sectioned tissue.

METHODS

Tissue preparation / Myelin stain

Studies were performed on a block of cortical tissue containing parts of primary motor and medial and lateral premotor cortex (Fig. 1). The tissue was obtained 6 hrs post mortem from the left hemisphere of a female subject, aged 38, without known neurological disorders. The tissue was fixated using 2.6% phosphate buffered paraformaldehyde and MR scans were performed after about 1 year of fixation. All procedures were approved by the local ethics committee in Frankfurt.

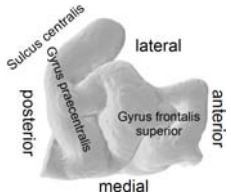

Figure 1

MR Data Acquisition and preprocessing

Measurements were performed on 9.4T animal scanner equipped with a 12cm ID, 600mT/m, 100μs rise time gradient coil and interfaced to a Siemens console. A 7cm transceiver loop-coil was used. A 2D spin-echo sequence was modified to include a diffusion preparation module. The measurement parameters included: FOV 53x53mm^2, matrix 160x160 (isotropic resolution of 330μm^2), 97 contiguous slices, TR=10000ms, TE=45ms, Δ=22.5ms, δ=3ms, flip angle=90^o, 4 averages, b=3000mm^{-2}s, 60 diffusion encoding directions (obtained by an electrostatic repulsion algorithm on the whole sphere) and six b=0 acquisitions. The temperature in the scanner was raised to 30°C using an in-bore animal warming system and constantly monitored with a temperature probe. FSL eddy current compensation was applied. All directional modeling and tractography was performed in 'native' diffusion data space.

U-fibers tractography

Diffusion tensors (DTs) were fitted to the data by standard OLS fitting on the log-signal. A deterministic streamline algorithm using a 4th order Runge–Kutta step integration approach, trilinear interpolation of the tensorial matrix, a fixed step size of 500μm and an angle threshold of 40 degrees was used.

Layer parcellation

Gray matter (GM) and white matter (WM) were segmented using the first b0 volume and 3D surface meshes of the outer GM ('pial') boundary and the WM/GM boundary were reconstructed in Brain Voyager QX. Nine intermediate internal GM surfaces were then obtained at fixed cortical thickness steps (10-90%, 10% step size). K-means clustering based on city block distance was used to cluster the different layers using different apparent diffusion coefficient (ADC) properties at every point of the reconstructed surfaces.

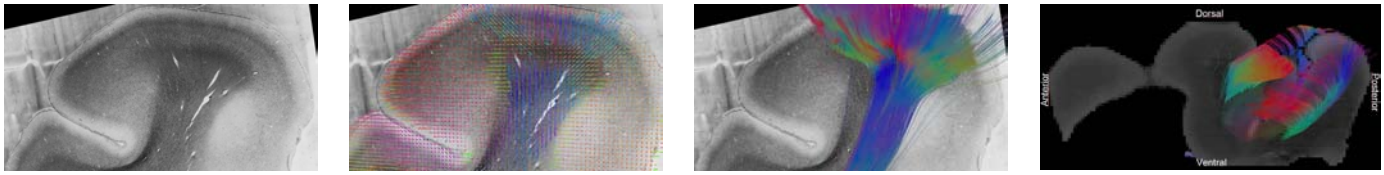

Figure 2 **A** **B** **C** **D**

RESULTS

Figure 2 shows (A) a myelin stained section of the excised tissue, highlighting the cortical insertion of the corticospinal tract in human pre-motor area (some tissue-slice tearing visible), (B) the main eigenvector of the DT superimposed on the histological section, (C) the reconstructed fiber tract inserting in cortical gray matter and (D) the u-fibers connecting the precentral gyrus with the superior frontal gyrus. Here we observe axonal bundles 'fanning' into the cortex in the crown of the precentral gyrus, reaching far into the gray matter, to be contrasted with fiber tracking *in vivo*, where cortical insertions cannot be easily achieved. Figure 3 shows (A) the result of the cortical layer clustering and (B) the same profile superimposed on the myelin stained section. The high degree of overlap between the clustering results and the histological section highlights the relationship between diffusion ADC and degree of myelination, which varies with cortical depth.

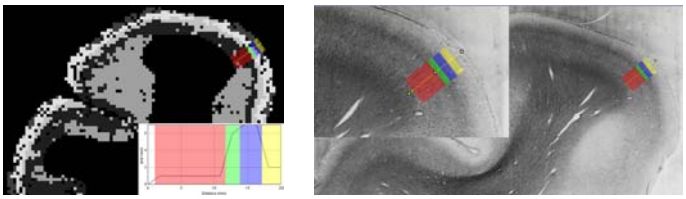
Figure 3 **A** **B**

DISCUSSION

DWI on *post mortem* tissue gives unique insights into human anatomy and can be used to help construct gold standards of cortical and sub-cortical connectivity which is of crucial importance to validate *in vivo* applications. We show here that at an isotropic resolution of 330μm, it is possible to investigate structural connectivity with high fidelity including cortical insertions of white matter fibers into the gray matter, even though discerning where (i.e. in which layer) the fiber really terminates remains uncertain with the current techniques. Moreover, with high spatial and angular resolution and moderate to high diffusion weighting, gray matter layer organization is shown to be accessible by DWI [5] and automated clustering algorithms. We show that almost all cortical layers in this particular cortical tissue can be successfully delineated using the ADC profile which correlates with the degree of myelinization at different cortical depths as validated by histology comparisons.

REFERENCES: [1] Roebroeck et al., NeuroImage 39(1):157-168 (2008). [2] Guilfoyle et al., NMR Biomed. 16:77–81 (2003). [3] Seehaus et al., Cereb. Cortex (2012). [4] A.M. Oros-Peusquens et al., 20th ISMRM, E-Poster #3241 (2012). [5] Leuze et al., Cereb. Cortex (2012).