Direct correlation between diffusion tensor imaging and electron microscopy of the fornix in humans with temporal lobe epilepsy

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Introduction: Diffusion tensor imaging (DTI) is a sensitive technique for the detection of abnormalities of white matter. Based on animal models, it is generally accepted that the degree of diffusion anisotropy in the highly ordered tissue architecture of white matter is caused by axonal membranes [1] and is modulated by myelin [2, 3]. In a *post mortem* study of the brains of patients with multiple sclerosis, myelin content and axonal counts were correlated with diffusion anisotropy and mean diffusivity [4]. To this day, however, a direct correlation of DTI obtained *in-vivo* with fresh tissue specimens of human white matter has not been performed. Medically intractable temporal lobe epilepsy (TLE) patients who undergo temporal lobe resection as a treatment of their epilepsy provide a unique opportunity to perform such a correlative analysis. Previous DTI studies have shown reduced anisotropy and increased perpendicular diffusivity in the fornix of TLE patients with mesial temporal sclerosis (MTS, characterized by neuronal loss and gliosis of the mesial temporal structures) [5]. In contrast, TLE patients without such a lesion have normal diffusion parameters of the fornix [6]. The purpose of this study is to compare electron microscopy–derived measures of extra-axonal fraction, number of axons, and myelin area and thickness of the resected fimbria/fornix with prior *in vivo* DTI parameters of the fornix in two groups of TLE patients, those with or without MTS.

Methods: Seven patients with temporal lobe epilepsy (TLE) (36-57 years old; 1 male, 6 female) were scanned on a 1.5T Siemens Sonata scanner and separated into two groups: four with MTS (abnormally elevated hippocampal T2) and three without MTS (normal hippocampal T2). CSF-suppressed DTI (2×2×2 mm^3, 9:30 min) and tractography using the FACT algorithm of the fornix on the same hemisphere as TLE were performed prior to temporal lobe resection surgery (<13 months), yielding four diffusion parameters: fractional anisotropy, FA; mean diffusivity, MD; parallel diffusivity, $\lambda_{\parallel} = \lambda_1$; and perpendicular diffusivity, $\lambda_{\perp} = (\lambda_2+\lambda_3)/2$. A segment of the fimbria/fornix was excised during the anterior temporal resection with the aid of a surgical microscope. The specimen was immediately fixed in 4% paraformaldehyde in 0.1M phosphate buffer and processed for electron microscopy (EM). Tissue processing and subsequent EM analyses were performed blinded to diagnosis of with/without MTS. Ten microphotographs were randomly acquired per patient using a FEI Morgagni transmission electron microscope at a magnification of 3500x. The number of axons, as well as their inner and outer diameter (diameter of the axon with or without including the myelin sheaths, respectively), was manually assessed for all ten EMs per patient. The outer diameter was used to calculate axonal area per axon (including myelin, Ax_{area}), assuming circular axonal profiles. Extra-axonal fraction was defined as $1 - ([\Sigma Ax_{area}]/F_{area})$, where F_{area} is the area of the counting frame. Inner diameter was used to derive intra-axonal fraction. *In vivo* derived DTI parameters were plotted versus EM derived structural indices.

Results: In line with previous reports [5, 6], TLE patients with MTS show lower FA (FA=0.50±0.01) and higher $\lambda_{\perp}$ of the ipsilateral fornix as compared with TLE patients without MTS (FA=0.55±0.03) (Figure 1 A,B). Electron microscopy showed interesting differences between the two TLE groups, with higher extra-axonal fraction and lower axonal density in patients with MTS than in patients without MTS (Figure 1C,D). Extra-axonal fraction is related to diffusion parameters, with larger extra-axonal fractions translating into lower FA values and higher $\lambda_{\perp}$ (Figure 1E,F), but not affecting MD or $\lambda_{\parallel}$. Similarly, larger number of axons per field relate to higher FA and lower $\lambda_{\perp}$. Mean axonal diameter did not appear to influence diffusion parameters. Myelin thickness did not differentiate between the two TLE groups, but myelin area appears to influence diffusion parameters to a lesser degree, with higher myelin area relating to higher FA values and lower $\lambda_{\perp}$ (data not shown). However, myelin area and axonal count show a strong positive correlation (r=0.79, $p=4\times10^{-14}$).

Conclusions: Extra-axonal fraction and axonal density are closely related to the degree of diffusion anisotropy, mostly by reducing the diffusivity perpendicular to the tracts. Myelin content appears to play a role in the modulation of perpendicular diffusivity, although this could be the reflection of axonal density and not myelin per se. The present results from human white matter are in line with previous reports of animal models [7] and appear to be the root of the diffusion abnormalities of the fornix measured *in vivo* in patients with MTS. Our findings provide support of the widely held assumption that, as in animal models, abnormalities of DTI parameters correspond to changes in axonal/myelin integrity in humans.

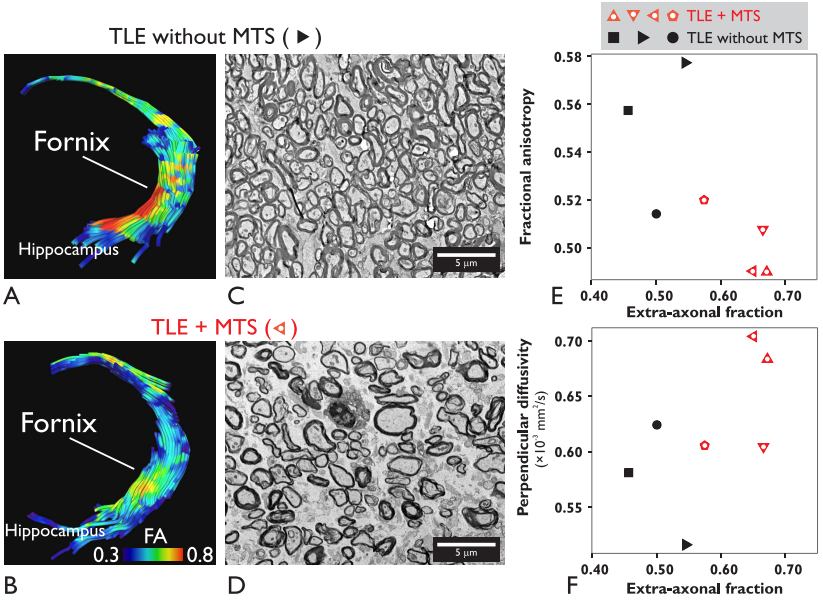

Figure 1. Tissue correlates of DTI parameters. Pre-operative tractography-defined left fornix of two TLE patients with and without MTS (A,B). Patients with TLE and MTS have lower diffusion anisotropy of this tract, characterized by the cool colors in B, as compared to TLE patients who lack MTS (A). Electron microscopy of the resected tissue shows that the microstructure of the fimbria/fornix is abnormal in patients with TLE and MTS (D), mostly due to fewer axons than patients without MTS (C). Lower diffusion anisotropy is related to an increase of extra-axonal fraction, characteristic of TLE patients with MTS (E). The low FA seen in patients with MTS is secondary to high perpendicular diffusivity, which is also related to extra-axonal fraction (F).

References:

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