In vivo measurement of inter-hemispheric axon diameters on a clinical scanner using multiple pulsed-field gradient (mPFG) diffusion MRI

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Introduction

Quantifying microanatomical features of neurons, such as mean axon diameters, can provide valuable neuropathological and functional information. Current biopspy-driven histological methods can only sample cells from a few tissue locations within a microscopic field-of-view (FOV) and are prone to bias due to deformation during tissue preparation.

Over the past decade diffusion tensor imaging DTI (Basser et al., 1994) has become the most sensitive clinical tool for non-invasive assessment of white matter microstructural changes (Pierpaoli and Basser, 1996) and mapping of brain connectivity (Basser et al., 2000). The anisotropy measured with DTI is modulated by both the ensemble coherence of white matter fibers (macroscopic anisotropy) and the geometry of microscopic restrictions such as myelinated axons (microscopic anisotropy). The coupling between these two mechanisms limits the specificity and selectivity of DTI, making it difficult to correlate observed changes in DTI-derived parameters, e.g., the fractional anisotropy (FA) and the mean apparent diffusion coefficient (ADC) with neuronal specific pathophysiology.

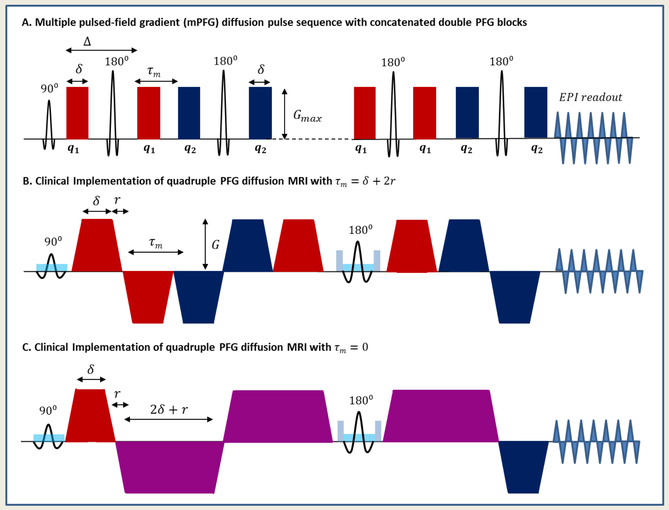
An extension of the single pulsed-field gradient (sPFG) sequence used in DTI is the double PFG sequence, in which a second diffusion block is applied. The diffusion signal can be quantified as a function the time τ_m and angle ψ between the two applied diffusion vectors $\boldsymbol{q_1}$ and $\boldsymbol{q_2}$ to exclusively characterize microscopic anisotropy with unique measures of average cell geometry. Even though the ability to measure axon diameters using dPFG MRI has been recently validated with histology (Komlosh, 2012), its translation to clinical scanners is challenging due to reduced gradient strength and limited scan duration.

Theory

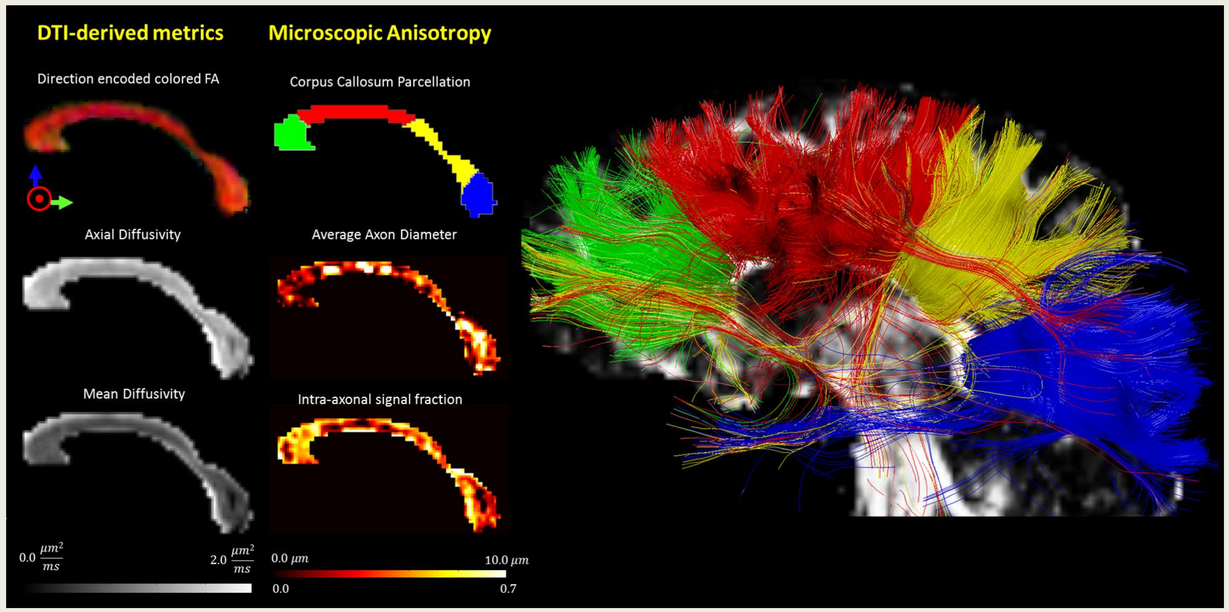
Theoretical studies indicate that concatenating several dPFG diffusion blocks can significantly improve the sensitivity to small axon diameters and reduce the overall diffusion attenuation (*b*-value) of the experiment (Finsterbusch, 2009). In the short diffusion gradient pulse approximation, the signal for $\tau_m \rightarrow 0$ becomes:

$$E_0(\psi) = 1 - 8n\pi^2 q^2 \langle A \rangle_{iso} - (2n-1)4n\pi^2 q^2 \langle A \rangle_{iso} \cos\psi$$

, where $\langle A \rangle_{iso}$ is the mean radius of gyration reflecting average cell size and shape characteristics (Özarslan, 2009). To evaluate $E(\psi)$ for arbitrary sequence parameters and pulse durations (e.g. τ_m , δ) used on clinical scanners it is necessary to employ numerical methods and a powerful mathematical framework, which has been recently developed (Özarslan and Basser, 2008). Moreover, to account for the relative orientation of the applied gradients with respect to local microstructure it is necessary to incorporate additional information. For example, a separate high-resolution DTI scan can be used to simplify the tissue model by approximating the fiber orientation $\boldsymbol{u_k}$, intra-axonal D, and extracellular diffusivities D_0 with measured diffusion tensor orientations, axial and mean diffusivities, respectively.



Results



Methods

We designed several clinical multiple PFG sequences and numerically evaluated their sensitivity and signal-to-noise ratio (SNR). We then implemented quadruple PFG sequences optimized for two different τ_m values that allow the use of full gradient strength for both $\boldsymbol{q_1}$ and $\boldsymbol{q_2}$ (Fig. 1B,C).

After validation in a calibrated free diffusion phantom, the qPFG pulse sequences were applied to measure inter-hemispheric axon diameters in 4 healthy volunteers. The following scan parameters were used:

1. Pulse Sequence: qPFG MRI with cardiac gating, TE=147ms, TR=7 R-R intervals
2. Acquisition: 7 sagittal slices through the medial corpus callosum, 2mm in-plane resolution, 6mm slice thickness, single-shot EPI with parallel imaging SENSE x2
3. Diffusion pulses: , G_{max}=50 mT/m, δ=11.8 ms, r=0.8 ms, $4q$=1,100 cm^{-1}, b=1,200 s/mm^2, 12 angles ψ from 0 to 360^0, τ_m=13.4 and 0.0 ms

A co-registered high-resolution DTI scan was acquired with 2mm isotropic resolution (three times smaller slice thickness) and 60 diffusion orientations and used to estimate the underlying fiber orientation. The diffusion signal in each voxel was modeled as a weighted sum of contributions from diffusion in restricted myelinated axons (infinite impermeable parallel cylinders) and extracellular space (hindered diffusion). Microscopic anisotropy parameters of average axonal diameter d and intra-axonal signal fraction f were estimated by fitting the following model to the measured angular qPFG MRI signal profiles:

$$E(\psi) = f\left[\sum_{k=1}^{3} E_k^{ax}(\psi, \mathbf{u}_k, D_k, d)\right] + (1-f)\left[\sum_{k=1}^{3} E_k^{out}(\psi, D_{k,0})\right]$$

Discussion

For all healthy human volunteers, DTI analysis in the corpus callosum produced standard values of mean apparent diffusion coefficient (ADC) and fractional anisotropy. Analysis of residual errors indicated good agreement between fitted and measured qPFG diffusion data, validating the applicability of our model for white matter tissue. Maps of trans-callosal average axon diameters showed significant heterogeneity along the anterior-posterior direction, with large axons in the midbody and posterior splenium corresponding to sensory-motor and visual pathways and small axons in the genu connecting prefrontal brain regions. This anterior-posterior variation was not observable with any DTI-derived metric suggesting that mPFG MRI provides microscopic restriction-specific information complementary to that obtained with conventional diffusion methods.

Moreover, the anterior-posterior organization adequately matched our functionally defined corpus callosum parcellation validated with fiber tracking of white matter pathways connecting brain regions in the prefrontal, sensory-motor, temporal/auditory and visual cortices. Average axon diameters measured in each of these regions-of-interest (3.85μm – prefrontal, 6.59μm – sensory-motor, 4.10μm – temporal/auditory, and 4.25μm – visual) are in remarkable topographical agreement with previous post-mortem studies (Aboitiz et al., 2003) and support the functional specialization for specific cognitive processing. The intra-axonal signal fractions estimated with qPFG diffusion MRI are in line with those reported in the literature (Barazany at al., 2009) and confirm the relatively large contribution from extracellular water signal at the echo time of our diffusion experiment.

Conclusions

Our results support the clinical feasibility of acquiring qPFG diffusion MRI *in vivo* using a conventional MRI scanners. Integration of high-resolution DTI data allows the extraction microscopic anisotropy parameters such as average axon diameter. The good agreement between axon diameters estimated with qPFG MRI and previous post-mortem studies provides a preliminary validation of our methodology.

Upon further technical refinement and additional clinical validation qPFG diffusion MRI could provide a histological assessment of white matter enabling a wide range of neuroimaging applications for improved diagnosis of neurodegenerative pathologies and treatment monitoring.

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