dPFG MRI assessment of axonal beading in an injury model

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Introduction: Central Nervous System (CNS) neurites exhibit a beaded axonal morphology following mechanical, chemical, or metabolic insults[1]. Recently, this mechanism of axonal beading was advanced to explain both the reduction in the mean ADC during traumatic brain injury (TBI) and ischemia, and its re-elevation observed during recovery[2]. The ADC parallel and perpendicular to nerve fibers obtained by DTI showed demonstrable macroscopic changes associated with these injuries. While sensitive, these DTI-derived parameters do not provide specific microstructural or anatomical information about the origin of these changes in axonal dimensions. Here we use double Pulsed-Field Gradient (dPFG)[3,4] MRI to provide important new microstructural information that can help to characterize the beading process in axons. In a model of beading axons, we show that dPFG MRI furnishes estimates of the aspect ratio (shape) of axonal "beads" following injury. This unique information cannot be obtained by single PFG macroscopic diffusion MRI methods.

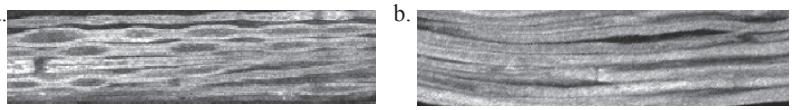
Materials and Methods: Rat sciatic nerves were excised and subjected to axial tension sufficient to induce beading or minimal tension to straighten out their macroscopic undulation (control). These nerves were immediately immersed in fixative and rehydrated in phosphate buffered saline (PBS) prior to acquiring MR data[2]. The d-PFG experiment was also performed with a glass capillary array (GCA) MRI phantom[4] consisting of a parallel pack of 10μm ID water-filled tubes. The samples were placed in a 7T vertical-bore Bruker AVANCE III MR microimager oriented along B_0 (Z axis). Diffusion tensor MRI (DTI) was performed prior to dPFG NMR to verify nerve fiber orientation. dPFG NMR parameters were: δ=3.15ms, Δ=30ms, G = 0-177 mTm^{-1}. The angle, ϕ, between the two consecutive PFG blocks was varied between 0 and 2π in the ZY plane. MRI parameters for the nerves were: TR/TE = 3000/7 ms, resolution = 468 x 468 x 5000 μm^3, while for the GCA they were: TR/TE = 7000/7.74 ms, resolution = 156 x 156 x 500 μm^3. A bi-compartmental model (free and restricted diffusion) was used to fit the data. The injured nerve was modeled as arrays of capped cylinders[5] while the control and GCA were modeled as arrays of infinite cylinders. The extracellular matrix compartment was approximated as free water diffusion.

Results: Figure 1 a and b show confocal microsope images of the injured and control nerves. The long axis of the beads in the injured nerves ranged between 17.2–35.6 μm with an average diameter, d, of 25.7 μm while the short axis ranged between 2.57–7.18 μm with d = 4.26 μm. The control nerve diameter ranged between 4.12–6.86 μm with d = 5.67 μm. Figure 2 a-c show the angular dependence of the signal in the dPFG experiments for the injured, control, the GCA (symbols) and their corresponding model fits (lines), respectively. Model fitting shows an average long axis of 79.1 μm, with d = 4.7 μm for the beaded nerve and d = 4.1 μm for the control nerve. For the GCA, d = 9.7 μm, which is very close to its nominal value.

Discussion: This study shows a significant change in the angular dependance of the dPFG MR signal between the injured and control nerves. The average long axis appears larger than the one obtained by optical microscopy. This discrepancy may be due to the fact that the model assumes the individual beads are capped while it is apparent in the optical microscopy images that they are connected. Alternatively, the dPFG signal is obtained from the entire nerve, whereas the small field-of-view (FOV) optical measurements reflect only a portion of the sample. Nonetheless, the estimated mean diameters of all samples however are resonable.

Conclusion: dPFG MRI shows potential for characterizing microstructural changes and their time-course in injured or ischemic white matter. The development of a more complex theoretical framework to represent other features of beaded axonal morphology is in progress.

Figure 1. a) and b) show confocal microscope images of beaded and control rat sciatic nerves respectively. Resolution=0.88 x 0.88 μm^2



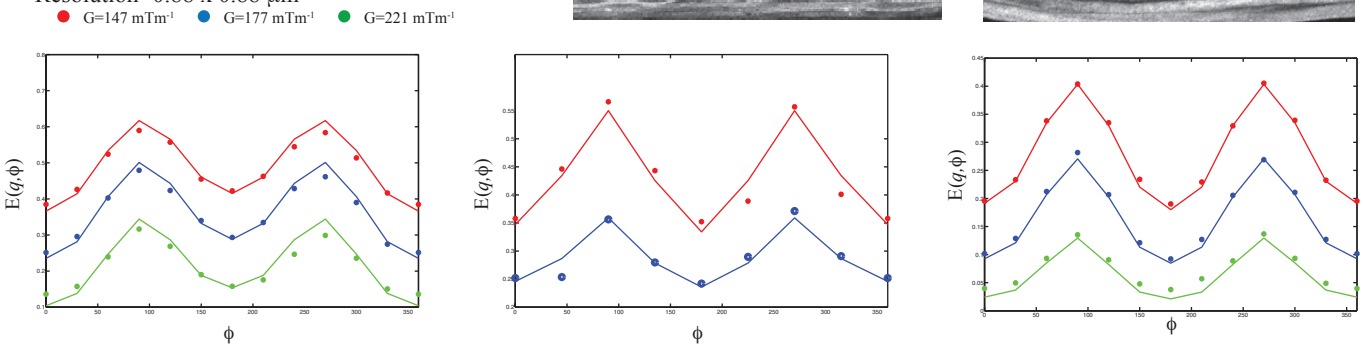


Figure 2. a), b) and c) show the the corresponding angular dependance of the dPFG signal (symbols) and simulations (lines) for the injured control and GCA sample respectively.

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