Microscopic Determinates of Anisotropy in the Injured Rodent Brain Using Histological Fourier Analysis

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Introduction

Diffusion tensor imaging (DTI) is sensitive to microstructural changes following traumatic brain injury (TBI), and it is hoped that this sensitivity will improve prognostication[1]. Many human[2] and animal[3] studies have demonstrated a decrease of white matter fractional anisotropy (FA) consistent with axonal injury, myelin injury, or both, but increased FA has occasionally been observed after TBI[4,5]. In animal models, increased FA has been argued to be associated with axonal reorganization and regrowth following stroke[6] or TBI[7], but the histological evidence for such changes remains tenuous. We performed DTI on rats in the chronic stage following TBI and implemented a novel histological image processing technique to relate FA to specific pathological features of TBI.

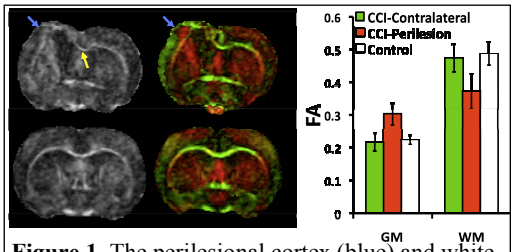
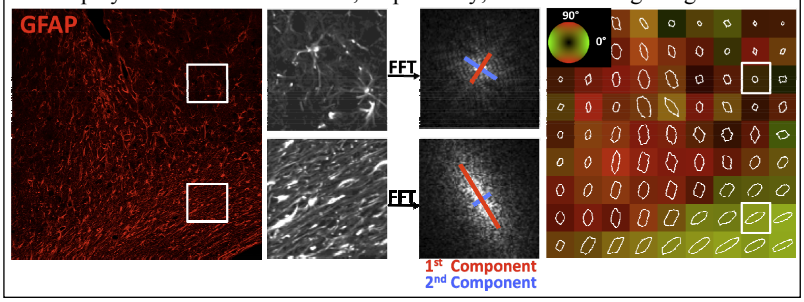


Figure 1. The perilesional cortex (blue) and white matter (yellow) had increased and decreased FA, respectively, in the injured brain (top) compared to the contralateral and control brain (bottom).

Methods and Materials

Controlled cortical impact (CCI) was delivered to the motor cortex (+1.0 anterior, 2.5 lateral) using a 5 mm diameter impact tip attached to an electromagnetic piston at a depth of 2 mm and a velocity of 5 m/s. Six injured and three control rats underwent in vivo DTI 2 months post-injury using a multiple-echo spin-echo sequence and the following parameters: TR=2250 ms, TE=20 ms (first echo; 4 ms echo spacing, 4 echoes), Δ=10 ms, δ=4 ms, b=0 & 1000 s/mm^2, 15 directions, 500 μm thickness, 240 μm^2 in-plane resolution. Histological sections were stained for GFAP (astrocytes), SMI31 & SMI32 (neurofilaments), MAP2 (microtubules), and MBP (myelin). Each confocal image was processed as shown in Figure 2.

Figure 2. The 2D Fourier transform and Principle Component Analysis[8] was applied to each local region of the grayscale histological image. The 2D orientation (eigenvector) and anisotropy index (AI=1–PC2/PC1) was calculated and displayed as hue and saturation, respectively, on the resulting images.



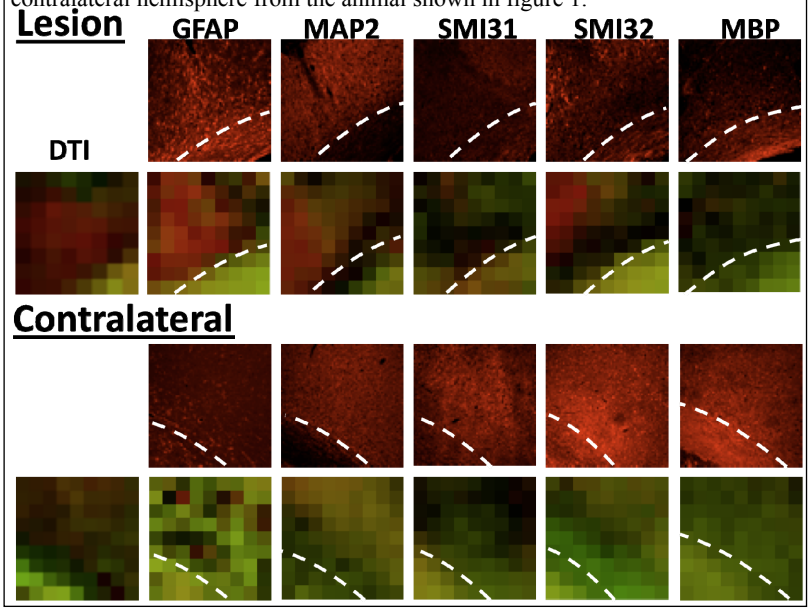
Results

The histological-based Fourier analysis captured the microscopic anisotropy and orientation as measured by DTI. In the CCI injured white matter (WM), FA decreased and was consistent with axonal (SMI31 & SMI32) and myelin (MBP) injury, although astrocytes (GFAP) also had high anisotropy. In gray matter (GM), FA increased and this was consistent with coherent astrogliosis (GFAP) and to a lesser extent, dendrite organization (MAP2 & SMI32). The results highlight that the microscopic cellular and subcellular elements that contribute to anisotropy in the normal brain, including axons and myelin, are not necessarily the only contributors to anisotropy in the injured brain. Astrocyte remodeling and migration also appears consistent with the DTI-measured anisotropy.

Discussion and Conclusions

The increased FA measured with DTI following TBI appears to be largely a consequence of astrogliosis, not axonal regeneration as previously suggested. One limitation of the results is the difficulty in aligning images from such disparate modalities as well as the obvious size disparity between the thickness of the histological sections and that of the DTI slice. Nonetheless, the method helps elucidate a quantitative and specific relationship between the microscopic anisotropy and the DTI-derived anisotropy. The proposed method can be easily extended to validate anisotropy in both normal and injured tissues.

Figure 3. The anisotropy and orientation maps derived from histology and DTI are shown for a region encompassing the GM-WM interface in the CCI and contralateral hemisphere from the animal shown in figure 1.



References: [1]Niogi SN et al. 2010. *J Head Trauma Rehab*. 25; [2]Arfanakis K, et al. 2002. *AJNR*. 23; [3]Mac Donald C, et al. *J Neurosci*, 27; [4]Wilde L, et al. 2008 *Neurol*, 70. [5]Mayer AR, et al. 2010. *Neurol*. 74; [6]Jaing Q, et al. 2006. *Neuroimage*. 32; [7]Jaing Q, et al. 2010. *Proc ISMRM*. 4495; [8]Josso B, et al. 2005. *Mech. Systems and Sig. Proc.* 19(5);