Corpus callosum injury and seizures in WAG/Rij rats: Correlation between DTI and disease phenotype

H. Chahboune[1,2], A. M. Mishra[3], X. Papademetris[1,4], F. Hyder[1,2], and H. Blumenfeld[3,5]

[1]Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut, United States, [2]MRRC/QNMR, Yale University School of Medicine, New Haven, Connecticut, United States, [3]Neurology, Yale University School of Medicine, New Haven, Connecticut, United States, [4]Biomedical Engineering, Yale University School of Medicine, New Haven, Connecticut, United States, [5]Neurosurgery, Yale University School of Medicine, New Haven, Connecticut, United States

INTRODUCTION

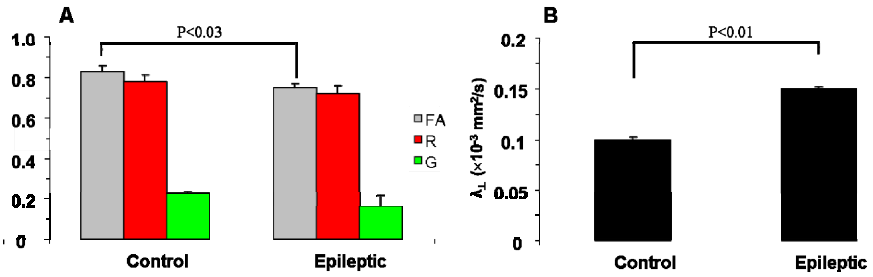
DTI has shown promising results in detecting early changes in tissues microstructural organization due to neurological disease in animal model such as Alzheimer disease [1,2], Multiple Sclerosis [3], and Parkinson [4]. The purpose here was to use DTI to assess potential morphological changes in a well-established animal model for absence epilepsy characterized by the presence of spike-wave discharges (SWDs) [5] on electroencephalograph, where rats also show behavioral arrest during seizures, analogous to those in humans.

MATERIALS and METHODS

Animal preparation: Wistar albino Glaxo rats of Rijswijk (WAG/Rij) (a genetic model of absence epilepsy) and nonepileptic (control) Wistar rats age-matched (28-30 weeks) were studied. SWD frequency was measured for 3 hours in all WAG/Rij and control animals by epicortical EEG to confirm phenotype. Rats were then deeply anesthetized with pentobarbital and perfused with saline through the left ventricle followed by 4% paraformaldehyde (PFA) in PBS (PBS, pH=7.4). The intact brain were excised from the cranium and stored in 4% paraformaldehyde in PBS at 4°C. Before imaging the brains were placed in PBS for 24 h to wash out the fixation solution and transferred them to into home built MRI compatible tube. The tubes were then filled with fluorinert an MRI susceptibility matching fluid. **DTI**: MRI experiments were performed on a 9.4T Bruker horizontal-bore system with custom-made Cosine coils. DTI experiments were performed using a modified Stejskal-Tanner spin-echo diffusion-weighted sequence = 5 ms; Δ = 8 ms; TR/TE = 1000/18; NEX = 24; matrix = 128×64 (zero filled to 256×128); FOV = 30×15 mm; slice thickness = 0.5 mm; number of slices = 15. Images were obtained with diffusion gradients applied in sixteen orientations with two diffusion sensitizing factors (approximately 0 and 1 ms/μm2). The Eigenvalues $\lambda1$ $\lambda2$ $\lambda3$ were driven from the diffusion tensor matrix. Quantitative maps of fractional anisotropy (FA) were calculated and the primary eigenvectors were used to calculate directionally encoded color (DEC) maps to highlight the orientation of anisotropic tissues using medial-lateral (R for red), dorsal-ventral (G for green), and anterior-posterior (B for blue) color maps [6]. Four regions were examined: corpus callosum, internal capsule, motor fibers and forelimb cortex.

RESULTS and DISCUSSION

The brain regions quantified by the current ex vivo DTI studies indicate that there were no changes in fractional anisotropy (FA) in the regions of internal capsule, forelimb and motor fibers in epileptic rats when compared to control. However a difference was found in the corpus callosum. In the epileptic rats, the FA was decreased ($p<0.03$) which was dominant in the medial-lateral and dorsal ventral directions (Fig. A). The radial diffusivity ($\lambda_\perp = 0.5\times(\lambda2 + \lambda3)$) was increased ($p<0.03$) in the epileptic rats (Fig. B), with no significant change in the axial diffusivity ($\lambda_\parallel = \lambda1$). A significant elevation of $\lambda_\perp$ with the preservation of $\lambda_\parallel$ in the epileptic brain may imply that myelin integrity of the white matter is affected by absence epilepsy while axonal integrity is not impaired. Alteration in myelin may be either a result of damage from seizures or a reflection of a primary underlying pathology as the cause of absence seizures. Since comparison of the DTI results with histology will be extremely valuable to study the alteration found in the corpus callosum, further work is in progress by using histological techniques to asses the severity of the damage. In conclusion, we have shown that DTI is sensitive for the detection of white matter changes in the WAG/Rij rat model of absence epilepsy. These ex vivo DTI results in the absence epilepsy model are important for understanding neurological difficulties in children suffering from absence epilepsy, and similar *in vivo* studies may enable DTI to serve as a non-invasive biomarker for disease progression.



References

[1] Song et al (2004) Neurobiology of Disease. 15: 640– 647
[2] Sun et al (2005) Experimental Neurology. 191: 77– 85
[3] Bude et al (2007) MRM 57:688–695
[4] Boska et al (2007) Neurobiology of Disease 26: 590–596
[5] Coenen et al (2003). Behav. Genet. 33:635-55
[6] Pajevic & Pierpaoli (1999) Magn Reson Med. 42:526–540

ACKNOWLEDGEMENTS

Supported by R01 DC-003710 (FH), R01 MH-067528 (FH), R01 NS-049307 (to HB), R01 EB-006494 (to XP), P30 NS-052519 (FH).