

DTI abnormalities in anterior corpus callosum of rats with spike-wave epilepsy

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INTRODUCTION

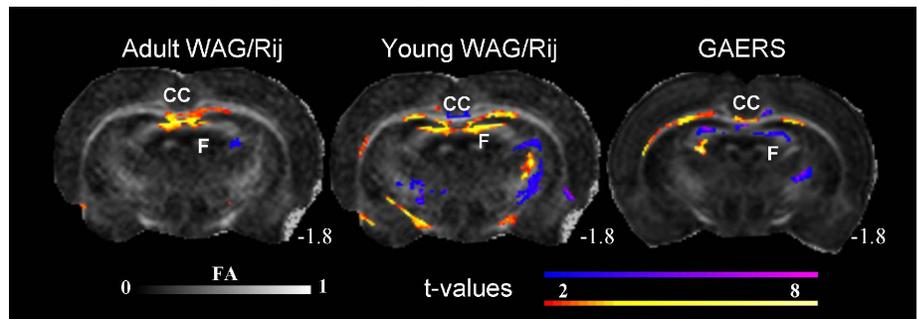
Absence epilepsy is a common seizure disorder that can significantly impair quality of life in childhood [1, 2]. It is associated with 5-10 second episodes of unconsciousness with a rhythmic 3-4 Hz “spike-wave” discharge (SWD) as detected by electroencephalogram (EEG). By use of various neuroimaging techniques in conjunction with electrophysiology, both in human and animal models of epilepsy, involvement of heterogeneous brain regions and/or alteration of anatomical structures have been implicated. More recently, diffusion tensor imaging (DTI) has provided unique insights into human epilepsy [3, 4], and albeit to a lesser extent, animal models of seizure disorder [5]. The aims of this study were to use *ex-vivo* DTI in WAG/Rij an animal model of absence epilepsy at two different developmental stages to first identify DTI changes related to epileptogenesis, and to then use a different animal model of absence (GAERS) to determine the specificity of these changes.

MATERIALS and METHODS

Animal preparation: Animals studied were Wistar albino Glaxo rats of Rijswijk (WAG/Rij) (a genetic model of absence epilepsy) at 1.7 and 8 months and age-matched nonepileptic (control) Wistar rats, as well as genetic absence epilepsy rats of Strasbourg (GAERS) epileptic and its nonepileptic control at 4.16 months. SWD frequency was measured for 3 hours in all 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 brains were harvested 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 into a home built MRI compatible tube. The tubes were then filled with Fluorinert (3M Corp., St Paul, MN), 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 = 128x64 (zero filled to 256x128); FOV = 30x15 mm; slice thickness = 1 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/ μ m²). The Eigenvalues λ_1 λ_2 λ_3 were derived from the diffusion tensor matrix. Quantitative maps of fractional anisotropy (FA) were then obtained. The coregistration of all animals to the same template was performed using rigid body transformations followed by a non-linear coregistration. The t-maps for adult WAG/Rij, young WAG/Rij and GAERS, all vs. matched nonepileptic controls were generated and overlaid onto FA images. ROIs were drawn after viewing the regions in the t-maps that showed the strongest changes. The anterior corpus callosum and the fornix were examined by ROI analysis.

RESULTS and DISCUSSION

EEG recordings confirmed frequent SWD in adult WAG/Rij and GAERS, but not in young WAG/Rij and not in controls. t-maps for the whole brain of adult and young WAG/Rij and GAERS vs. nonepileptic controls were generated to illustrate the tissues anisotropy changes. Fig. 1 shows t-maps in a single coronal slice at the AP level -1.8 from bregma. Warm colors indicate reduced FA in the epileptic animals. While Adult WAG/Rij exhibited a strong localized decrease in FA in the regions of the fornix (F) and corpus callosum (cc), the young WAG/Rij rats experienced a decrease only in the fornix. The GAERS exhibit a decrease in FA the in the corpus callosum but not in the fornix. ROI analysis was performed in the regions showing the strongest differences. The FA values were significantly decreased in the anterior corpus callosum in adult WAG/Rij ($P < 0.003$) and GAERS ($P < 0.03$) compared to controls. In the young WAG/Rij, there were no significant FA differences in the anterior corpus callosum. The reduced FA in the adult epileptic animals originated from an increase of the radial diffusivity ($\lambda_{\perp} = 0.5 \times (\lambda_2 + \lambda_3)$). Thus, λ_{\perp} was significantly higher in the corpus callosum of adult WAG/Rij ($P < 0.003$) and GAERS ($P < 0.03$), while young WAG/Rij showed no significant difference from controls. FA changes in the fornix were not clearly associated with the epileptic phenotype. Thus, both adult (epileptic) and young (not epileptic) WAG/Rij rats had reduced FA in the fornix ($P < 0.003$, $P < 0.04$, respectively), while epileptic GAERS did not, and in fact had increased FA in the fornix ($P < 0.006$). A significant elevation of λ_{\perp} in the corpus callosum of epileptic brain may imply that myelin integrity of the white matter is affected by absence epilepsy. 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. This study demonstrates that there was no impairment in the anterior corpus callosum in young animal before the onset of seizure. Impairment of the white matter was not specific to one animal model of spike-wave epilepsy as both adults WAG/Rij and GAERS exhibited impairment in corpus callosum. In conclusion, we have shown that DTI is sensitive for the detection of white matter impairment in animal models of epilepsy and that these abnormalities are not present early in life before the development of seizures. These *ex vivo* DTI results in spike-wave epilepsy models are important for understanding neurological difficulties in children suffering from epilepsy and may be useful for developing noninvasive methods to evaluate beneficial effects of treatment.



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