White matter changes in a model of temporal lobe epileptogenesis: a combined diffusion tensor imaging and histopathology study

W. M. Otte[1,2], P. van Eijsden[1], W. S. van der Hel[1], O. van Nieuwenhuizen[1], R. M. Dijkhuizen[2], R. A. de Graaf[3], and K. P. Braun[1]

[1]Rudolf Magnus Institute of Neuroscience, UMC Utrecht, Utrecht, Utrecht, Netherlands, [2]Image Sciences Institute, UMC Utrecht, Utrecht, Utrecht, Netherlands, [3]Department of Diagnostic Radiology, Magnetic Resonance Research Center, Yale School of Medicine, New Haven, CT, United States

Introduction: Although epilepsy is historically considered to be a grey matter disease, recent diffusion tensor imaging (DTI) studies, enabling the quantification of tissue water diffusion properties, have demonstrated that white matter structures adjacent to and distant from the primary epileptogenic zone are structurally affected in patients with focal epilepsies. A bilateral reduction in fractional anisotropy (FA) is described in corpus callosum, fornix, cingulum and external capsules in adults with temporal lobe epilepsy (TLE). Mean diffusivity and perpendicular diffusivity (λ2–3) are increased in fornix, cingulum, and external capsules[1,2]. The exact meaning of these diffusion alterations remains to be elucidated, and correlating histopathological studies are lacking. We aim to longitudinally characterize white matter changes during epileptogenesis by combining high field DTI with dedicated histological staining for white matter pathology in a rat model of TLE epilepsy model. We hypothesize that corpus callosum white matter is affected as part of a widespread and complex pathophysiological process that underlies epileptogenesis in TLE.

Methods: Status epilepticus (SE) was induced in 21-day old male Wistar rats by injection of lithium and pilocarpine. Dubé et al extensively characterized this model and showed that 24% of animals developed spontaneous recurrent seizures after an average of ten weeks[3]. In an additional 43% of animals seizures could be provoked by stress or handling. All these animals showed ictal and interictal epileptic activity in EEG recordings. We video-monitored a separate group of 18 pilocarpine-treated animals. This group showed spontaneous recurrent seizures in 44% of animals, which is in agreement with the results found by Dubé and colleagues.

MR experiments were performed on a 9.4T animal MR system with a 9 cm diameter gradient coil insert (490 mT/m, 175 µs). DTI was carried out at 4 and 8 weeks after pilocarpine (+SE) or saline injection (n=8) under halothane anesthesia. DTI (21 slices; 0.5 mm; TR 3.5s; matrix 128x128; 8 acquisitions including 6 diffusion-weighted directions (b 1200 s/mm^2) and 2 b-zero images), quantitative T2 (TR 2.5s; TE 10-85 ms; matrix 256x256) and quantitative T1 (TR; inversion times 10 - 7000 ms; matrix 128x128) images were acquired (FOV of 25.6x25.6 mm^2). The diffusion tensor for each voxel was calculated based on the eigenvectors and eigenvalues, using multivariate fitting and diagonalization. Data was coregistered using the Elastix toolkit (http://elastix.isi.uu.nl) using nonlinear registration preceded by affine-only registration. The derived fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity (λ1) and perpendicular diffusivity (λ2–3) data were further analyzed using *a priori* regions-of-interest (ROIs) analysis. Two ROIs, the medial and lateral corpus callosum (CC) were bilaterally outlined on the first echo T2w images. Differences between ROIs were analyzed using ANOVA with LSD post-hoc testing. Data are expressed as mean ± SD. Statistical significance was set to $P < 0.05$. For histology, animals were perfused with paraformaldehyde at 4 and 8 weeks. A Klüver-Barrera myelin stain and a Bielschowsky stain for axonal integrity were performed on 7 µ-thick paraffin coronal sections of rat brain including hippocampus.

Results: Of the animals injected with lithium and pilocarpine, 77% progressed to Racine's final stage[4] of SE. We studied five animals at four and five at eight weeks after SE with MRI, twelve additional animals were used for histology (six per group). No mortality or abnormal behavior was observed in the age-matched control animals (n=8). No significant differences were observed in body weights between controls and +SE animals at 4 weeks (240 ± 37 g vs. 243 ± 19 g, respectively) or at 8 weeks (346 ± 24 gr. vs. 323 ± 16 g, respectively). Typical MD and FA maps are shown in Fig 1. ROI analysis demonstrated a significant decrease in MD in the medial CC in the 4 weeks +SE animals, recovering at 8 weeks. The lateral CC showed similar changes without reaching statistical significance. A significant reduction was found in the λ1 of the medial CC at 4 and 8 weeks +SE. λ2–3 values were not significantly altered. T1 was reduced in the medial CC 4 weeks +SE. Fig 1, left, shows myelin-stained sections from control animals (left) and +SE animals (right) at 4 weeks and 8 weeks. Decreased myelin staining was evident in the CC at 4 weeks which normalized at 8 weeks. No differences were found between controls and +SE for the axonal staining at both 4 and 8 weeks (fig 1, left, bottom).

Discussion: Reported reduction in FA and λ1 and increase in λ2–3 is interpreted to reflect myelin degeneration, reduced axonal density, as a result of axonal fragmentation, or both[5, 6]. However, a recent electron microscopy studies has nuanced this view[7]. Myelin appears to play a role in the modulation of λ2–3, although this could be the reflection of axonal density and not myelin per se. Our results show a reduction in λ1 of the medial and, to a lesser degree, lateral CC after four weeks before spontaneous seizures occur. There was a tendency to normalization at eight weeks. Histology confirmed that myelin in the CC was affected at four weeks and to a lesser degree at eight weeks. In contrast, the axonal component did not seem to be affected. These findings contrast with the common DTI interpretation that gliosis and axonal loss lead to decreases in λ1 and FA, while myelin breakdown leads to an increase in λ2–3 and MD. Our data suggest that, prior to seizures, white matter tracts are affected, but to a different extent than after onset of spontaneous seizures. We detected alterations in λ1 and myelination, but not in axonal integrity. A possible explanation for our observation is delayed myelination of CC white matter in +SE animals, as myelin staining normalizes at 8 weeks +SE together with λ1 normalization in the medial CC. This normalization makes generalized white matter damage, as a result of initial SE, unlikely. Our DTI and histology findings show that the one-to-one relation between decreased FA/λ1 and axonal damage and increased MD/λ2–3, as described in TLE patients, is not as consistent in this process of epileptogenesis. Recently a disagreement between TLE patients white matter diffusion changes, correlated to the presence or absence of mesial temporal sclerosis, was reported[8]. Additional longitudinal DTI and histology experiments are needed to get a better understanding of the exact relationship between white matter diffusion properties and histopathological alterations before spontaneous seizures occur.

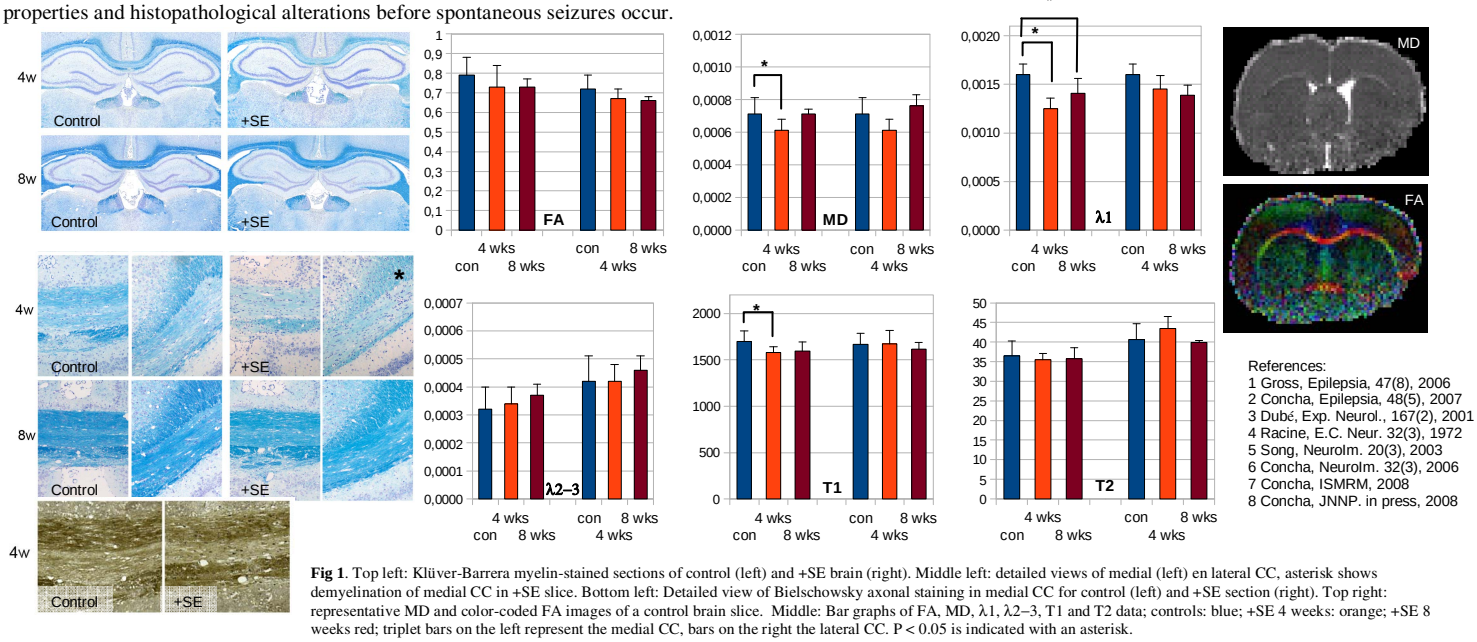

Fig 1. Top left: Klüver-Barrera myelin-stained sections of control (left) and +SE brain (right). Middle left: detailed views of medial (left) en lateral CC, asterisk shows demyelination of medial CC in +SE slice. Bottom left: Detailed view of Bielschowsky axonal staining in medial CC for control (left) and +SE section (right). Top right: representative MD and color-coded FA images of a control brain slice. Middle: Bar graphs of FA, MD, λ1, λ2–3, T1 and T2 data; controls: blue; +SE 4 weeks: orange; +SE 8 weeks red; triplet bars on the left represent the medial CC, bars on the right the lateral CC. $P < 0.05$ is indicated with an asterisk.