# *In vivo* characterization of microstructural changes during epileptogenesis by high resolution diffusion tensor imaging of rat hippocampal subfields

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**Introduction.** Biomarkers for disease progression and treatment efficiency are highly important for the clinical management of neurological diseases. Although, conventional magnetic resonance imaging (MRI) techniques are widely used in diagnosis and prognosis, they often provide insufficient contrast to detect microstructural tissue changes in early stages of the disease. High resolution diffusion tensor imaging (DTI) provides a high tissue contrast in both white and gray matter. In our previous studies, we utilized high resolution *ex vivo* DTI for the identification and characterization of potential biomarkers for epileptogenesis in animal models of epilepsy[1-3]. We found alterations of the tissue microstructure in different subfields of hippocampus and some other brain areas related to epileptogenesis, several months after status epilepticus (SE)[1-3]. In the present study, we tested a hypothesis that *in vivo* DTI can detect and follow progression of the changes in several hippocampal subfields.

**Methods.** SE was induced with kainic acid (KA) (i.p., 10mg/kg, n=6) or pilocarpine (i.p., 320 mg/kg, n=5) in adult male Wistar rats. All the animals were scanned under isoflurane anesthesia before, and 10, 20, 34 and 79 days after induction of SE. *In vivo* DTI was carried out in a 7T/30cm magnet interfaced to a Bruker PharmaScan console with a quadrature volume coil as transmitter and an actively decoupled quadrature rat head coil as reciever. Data were acquired using a diffusion-weighted segmented spin echo echo-planar imaging pulse sequence (TR = 2.5 s and TE = 30 ms, 4 segments). For DTI, 21 diffusion weighting directions were used with the following parameters: $\delta$ = 4 ms, $\Delta$ = 11 ms and b-value = 1000 s/mm$^2$. The FOV of 21.12 x 14.08 mm$^2$ was covered with a 192 x 128 points resulting in spatial resolution of 110 x 110 µm. Number of slices was 14, slice thickness 500 µm, and number of averages 32, resulting in 2 hours and 20 minutes scan time.

**Results.** Fig. 1 shows directionally encoded color (DEC) FA-maps from a representative animal before and after SE induced by pilocarpine. At 10 days after SE, the animals showed signs of degeneration in cortical areas and enlarged ventricles, progressing at 20, 34 and 79 days (Fig. 1A). Overall degeneration in the hippocampus was clearly visible starting at day 20 (Fig. 1B). In the dentate gyrus, FA increase could be detected at day 34 (17%, p<0.05) and day 79 (31%, p<0.01), as compared to the dentate gyrus before SE (Fig. 2A). Axial diffusivity ($D_{\parallel}$) was increased at day 34 (8%, p<0.05) (Table 1). Orientation of the principle eigenvector of the diffusion tensor stayed in dorsal-ventral orientation during the experimental time (Fig. 1C). This was consistent with our previous *ex vivo* studies[3], where FA and $D_{\parallel}$ increased, and no changes in water diffusion orientation or radial diffusivity ($D_{\perp}$) were found (6 months in Fig. 1 and Table 1). In CA3bc, FA was elevated 34 (7%, not significant) and 79 (17%, not significant) days after SE (Table 1, Fig. 2B). Differences in the principal orientation of water diffusion were found already at day 10 (Fig. 1D) as the orientation changed from rostral-caudal to more dorsal-ventral direction in a progressive manner during the observation period. These results were consistent with our previous *ex vivo* studies[3] (6 months in Fig. 1 and Table 1).

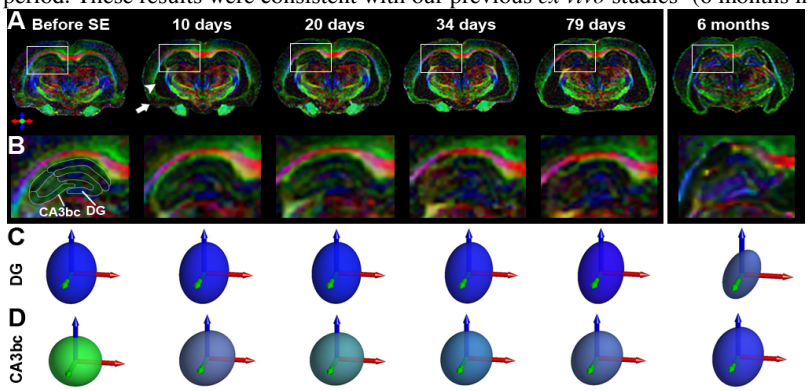


**Fig. 1:** Directionally encoded color (DEC) FA-maps of a rat before pilocarpine injection (pre) and 10 days, 20 days, 34 days and 79 days after SE (**A**). White arrow points at degeneration in cortical areas and white arrowhead at enlarged ventricles. White squares frame the hippocampus shown in panel **B**. Diffusion ellipsoids from two hippocampal subfields, dentate gyrus (**C**) and CA3bc (**D**), created to help visualize the changes in the diffusion tensor. For comparison, a representative animal from our previous *ex vivo* data[3] at 6 months after SE induced by pilocarpine is shown. Directions for directionally encoded colormaps: *green* rostral-caudal, *red* lateral-medial, and *blue* dorsal-ventral.

**Table 1. Relative changes of DTI parameters caused by SE**

| | Dentate gyrus | | | CA3bc | | |
|---|---|---|---|---|---|---|
| | FA | $D_{\parallel}$ | $D_{\perp}$ | FA | $D_{\parallel}$ | $D_{\perp}$ |
| **10 d** | 4% | 0.5% | -2% | -11% | -2% | 2% |
| **20 d** | 7% | 4% | 2% | -0.3% | -3% | -2% |
| **34 d** | 17%$^{\blacklozenge}$ | 8%$^{\blacklozenge}$ | 1% | 7% | 2% | -0.2% |
| **79 d** | 31%$^{\blacklozenge\blacklozenge}$ | 5% | -6% | 17% | 2% | -3% |
| **6 m** | 25%$^{***}$ | 18%$^{**}$ | -1% | 43%$^{***}$ | 17%$^{**}$ | -4% |

Relative changes (%) as compared to values before SE in the same animals (Kruskal-Wallis : $^{\blacklozenge}$p<0.05, $^{\blacklozenge\blacklozenge}$p<0.01). For *ex vivo* DTI, relative changes (%) as compared to controls at 6 months after SE[3] (Mann Whitney: **p<0.01, ***p<0.001).

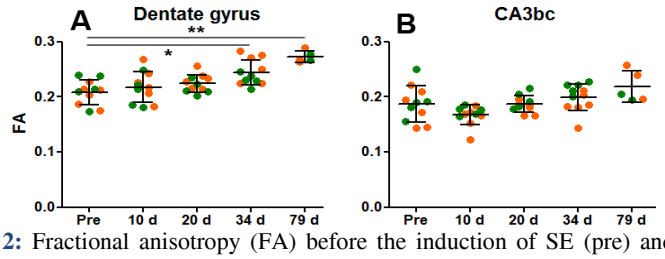


**Fig. 2:** Fractional anisotropy (FA) before the induction of SE (pre) and 10 days, 20 days, 34 days and 79 days after SE. No significant differences were found between KA (orange) and pilocarpine (green) groups (Mann Whitney analysis), mean and SD includes all the animals.

**Discussion.** We were able to detect progressive damage in different subfields of the hippocampus by *in vivo* DTI after induction of SE in rats. These results were consistent with our previous data obtained with *ex vivo* DTI together with histological verification showing that these changes correlate with progressive damage and plasticity in subfield specific manner. It remains to be shown if these microstructural tissue changes detected by DTI during epileptogenesis correlate with development of acute seizures in different etiologies and thus could be used as validated prognostic biomarkers for epilepsy.

**References.** [1]Laitinen et al. *Neuroimage* 2010;51(2):521-30. [2]Sierra et al. *Brain Struct Funct* 2011;216(2):123-35. [3]Sierra et al. 19th *ISMRM* 2011; p2513.

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