In vivo high-resolution diffusion tensor imaging shows progressive changes in hippocampal subfields after status epilepticus in rat

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Introduction. The identification and characterization of biomarkers for disease progression, as well as recovery and treatment efficiency, are highly important for the clinical management of neurological diseases. Diffusion tensor imaging (DTI) provides a high contrast based on tissue microstructure in both white and grey matter. This can be used in the detection of microstructural alterations caused by disease progression which can potentially serve as biomarkers. In our previous studies, we have utilized DTI in chronic stages of well-established animal models of epilepsy to identify potential biomarkers for epileptogenesis[1-4]. In this study, our hypothesis was that *in vivo* DTI is able to detect the progression of alterations in hippocampal subfields as well as in other white matter and grey matter areas during the early stages of epileptogenesis.

Methods. Status epilepticus (SE) was induced with kainic acid (KA) (i.p., 10mg/kg, n = 6) or pilocarpine (i.p., 320 mg/kg, n = 7) in adult male Wistar rats (no significant differences were found between KA and pilocarpine groups). All the animals were scanned under isoflurane anesthesia before (pre), and 10, 20, 34 and 79 days after induction of SE. Controls (n = 4) were scanned at pre and 79 days. *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). We used 21 diffusion weighting directions (δ = 4 ms, Δ = 11 ms and b-value = 1000 s/mm^2), 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. For data analysis, we performed a ROI analysis in the dentate gyrus (DG) and CA3bc in the hippocampus and Fourier analysis[5] in myelin stained sections to determine the fiber orientation in the above-mentioned subfields. We used tract-based spatial statistics (TBSS) analysis[6] to find statistical differences throughout the brain when comparing before and after induction time points. For characterization of DTI findings, we performed Nissl (cytoarchitecture) and myelin (myeloarchitecture) stainings, and glial fibrillary acidic protein (GFAP) (marker for astrocytes) and ox42 (marker for microglia) immunohistochemistry.

Results. In the DG, we detected an increase in fractional anisotropy (FA) (day 34, p<0.01 and day 79, p<0.001) along with an increase in axial diffusivity ($D_{||}$) (day 20, p<0.05; day 34, p<0.01 and day 79, p<0.01) (**Fig. 1A-B**). Linear diffusivity (CL) increased (day 10, p<0.05); day 34, p<0.01 and day 79, p<0.001) and spherical diffusivity (CS) decreased (day 34, p<0.05 and day 79, p<0.001) (**Fig. 1C-D**). Similarly in the CA3bc, we found an increase in FA (day 79, p<0.01) and CL (day 79, p<0.05), and a decrease in $D_{||}$ (day 20, p<0.05) and CS (day 79, p<0.05)) (**Fig. 1E-H**). These findings indicate that after status epilepticus tissue microstructure in the DG and CA3bc progressively increases the water diffusion along the principal direction. There was a progressive change in the diffusion orientation in the CA3bc from rostral-caudal to more dorsal-ventral during the observation period (**Table1, Fig. 2A-B**). However, in the DG, the diffusion orientation remained in dorsal-ventral orientation during the experimental time (**Fig. 2A-B**). Fourier analysis on myelin staining revealed changes in the fiber orientation in the both DG (**Table 1, Fig. 2C-D**) and CA3bc (**Table 1, Fig. 2E-F**), indicating that myelinated axons partially contribute to the diffusion orientation detected by DTI. TBSS analysis showed an increase in FA in the hippocampus and thalamus (p<0.05, FWE corrected) and a decrease in FA in the fimbria, external capsule and optic tract (p<0.05, FWE corrected) when compared control and SE animals at the latest time point (**Fig. 3A**). Histological examination of these areas revealed axonal injury in white matter areas as in the fimbria (**Fig. 3C**), and on-going inflammatory reactions in grey matter areas as in the thalamus (**Fig. 3E**).

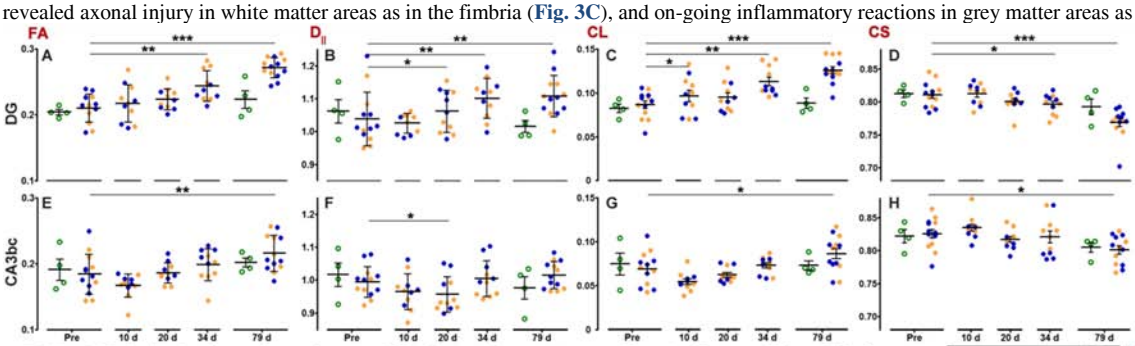

Fig. 1: FA (**A,E**), $D_{||}$ (10^{-3} mm^2/s) (**B,F**), CL (**C,G**) and CS (**D,H**) from the DG and CA3bc before the induction of SE (pre) and 10, 20, 34 and 79 days after SE. KA (orange), pilocarpine (blue), solid circles) and controls (green open circles). Black bars indicate mean ± SD. Wilcoxon test: *p<0.05, **p<0.01, ***p<0.001.

Table 1. Principal orientation of water diffusion and fiber orientation in histology (angles in °)

		DG	CA3bc
DTI	Pre	73.5 ± 5.1	51.9 ± 9.9
	10 d	75.2 ± 2.8	54.7 ± 9.8
	20 d	73.4 ± 3.5	54.8 ± 13.0
	34 d	74.0 ± 2.1	66.3 ± 5.9**
	79 d	74.3 ± 3.2	68.0 ± 6.7**
Histology	Controls	37.2 ± 12.6	23.6 ± 7.9
	SE	62.5 ± 8.0$^{\bullet}$	68.3 ± 9.2$^{\bullet\bullet}$

DTI angles are from x-y plane, and histological angles from 2D images. Values represent mean ± SD. Wilcoxon test: **p<0.0; Mann-Whitney test: $^{\bullet}$p<0.05, $^{\bullet\bullet}$p<0.01.

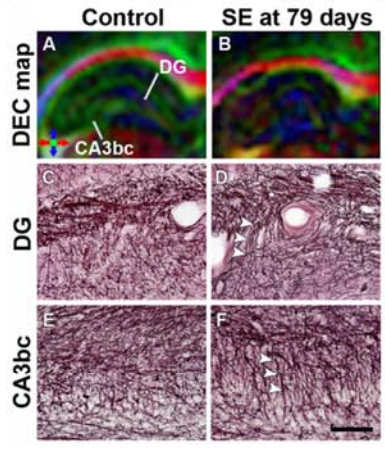

Fig. 2: Directionally encoded color (DEC) FA-maps of a control (**A**) and a pilocarpine (**B**) rats at 79 days after SE. Photomicrographs of myelin staining of the DG (**C-D**) and CA3bc (**E-F**) from the same rats. White arrowheads indicate changes in fiber orientation. Scale bar: 100 µm.

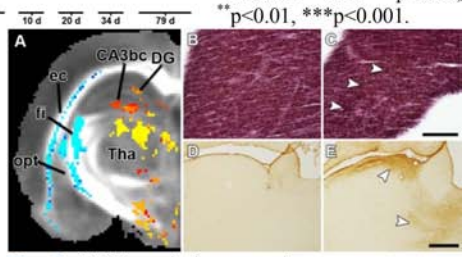

Fig. 3: TBSS analysis comparing same rats at pre and 79 days (red-yellow increased FA, blue decreased FA) (**A**). Photomicrographs of the fimbria (**B-C**) and thalamus (**D-E**) in a control (**B,D**) and KA (**C,E**) rats. White arrowheads indicate axonal alterations (myelin)(**C**) and reactive astrocytes (GFAP)(**E**). Scale bar: 100 (**B-C**) and 700 µm (**D-E**). *Abbreviations:* DG, dentate gyrus; ec, external capsule; fi, fimbria; opt, optic tract; Tha, thalamus.

Discussion. We were able to detect progressive microstructural alterations in different subfields of hippocampus and in other white and grey matter areas related to epileptogenesis using *in vivo* DTI. These results were consistent with our previous *ex vivo* studies in chronic time points[1-3,7]. Histological verification showed that these changes correlate with damage and plasticity during the early stages of the epileptogenic process. The value of these tissue changes as potential predictive biomarkers for epilepsy has to be tested in the future.

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. [4]Sierra et al. 21th *ISMRM* 2013; p1038. [5]Budde et al. *Brain* 2011;134(Pt 8):2248-60; [6]Smith S.M. et al. *NeuroImage* 2006;31:1487-1505; [7]Sierra et al. (under revision).
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