A longitudinal study of MR correlates during epileptogenesis in a mouse model of temporal lobe epilepsy

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Target Audience / Purpose: Epileptogenesis involves metabolic and structural alterations at the cellular level. Non-invasive tracking of these changes can reveal biomarkers, which are needed to test the effects of interventions. In humans epileptogenesis spans several years, therefore animal models offer the best opportunity to track longitudinal changes. In this study we demonstrate the ability of ^{1}H MR spectroscopy and high-resolution diffusion imaging combined with tractography to characterize the metabolic and structural developments during epileptogenesis in an established mouse model of temporal lobe epilepsy (TLE).

Methods: Unilateral hippocampal injection of kainic acid (KA) was used to induce epileptogenesis in C57BL/6N mice (n=3). Saline-injected animals (n=3) served as controls. The animals underwent MR scans under isoflurane anesthesia before and 1, 4, 8, 16 and 31 days after KA/saline injection. Subsequent in-vivo intra-hippocampal EEG recordings[1] and immunohistochemistry (IHC) were used to characterize pathological changes. The MR scans were performed with a 7T small animal system equipped with a CryoProbe (Bruker, Ettlingen, Germany). For diffusion imaging a spin-echo DTI-EPI sequence was applied: b=1000s/mm^2, 30 diffusion directions, TE=32.54ms, TR=2.5s, 3 segments, resolution 58x58x400µm^3, acquisition time 24min prolonged by respiratory triggering. A segmented mouse brain model[2] was registered to the diffusion images using FSL (FMRIB, Oxford, UK) to quantify alterations of fractional anisotropy (FA) and mean diffusivity (Trace) in hippocampal subfields. The diffusion images were also used to reconstruct the brain structure, for which we used a tractography method based on a global optimization approach[3]. ^{1}H MR spectra were acquired from two voxel located in the septal part of the ipsilateral and contralateral HC, respectively (voxel 2x1.4x1.4mm^3, PRESS: TE=20ms, TR=2.5s, NA=400). The spectra were quantified with LCModel (Provencher, Canada) and absolute concentrations were calculated using the unsuppressed water signal as reference.

Results / Discussion: In-vivo DTI and tractography revealed the development of strong dorso-ventral anisotropy in the dentate gyrus in the ipsilateral HC (Fig.1 a-c, also seen in Fig.2 Trace), which is not present in the contralateral HC (not shown here) or in control animals (Fig.1 e-g). Correspondingly, IHC staining for GFAP and GS revealed a strong hypertrophy of radially oriented astrocytes within the dispersed granule cell layer (GCL) (Fig.1 d).

^{1}H MRS revealed longitudinal metabolic changes related to epilepsy: The cell death following KA injection was reflected by a decrease in N-acetyl aspartate (NAA) and increase in lactate (Lac). Myo-Inositol (Ins), an indicator for glia activation, increased. The loss of glutamatergic neurons in hilus, CA1 and CA3 caused a reduction of the neurotransmitter glutamate (Glu). GABA was reduced shortly after KA injection, but this has to be elucidated by further measurements.

The amplitude of the observed metabolic changes correlates with the severity of the epileptiform discharges seen on the EEG (Fig 3, KA1 (green): single epileptic spikes only; KA2 (blue) and KA3 (red): distinct epileptic seizures). ^{1}H MRS thus not only allows for group comparison, but additionally distinguishes between individual animals from the first day after KA injection forth.

Conclusion: This study demonstrates that DTI and tractography are able to describe the progression of gliosis, a key feature of epilepsy. Together with ^{1}H MRS this allows the non-invasive monitoring of epileptogenesis in-vivo.

References: [1] Häussler et al., Cereb Cortex. 2012 [2] AMBMC Atlas [3] Reisert et al., NeuroImage 2011

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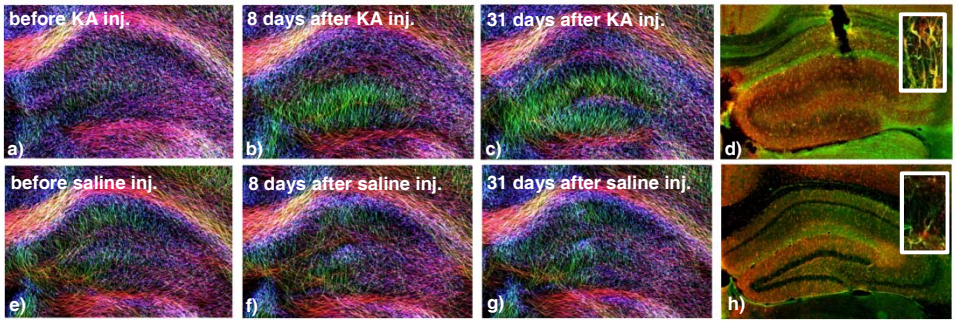
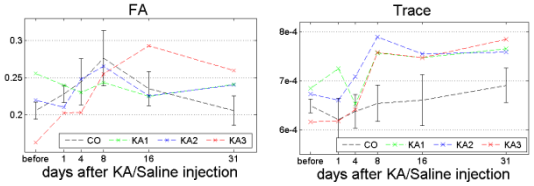


Figure 1: In-vivo DTI and tractography in a representative epilepsy mouse reveals the development of gliosis (a-c). Subsequent histology (GFAP red, GS green) of this animal shows the dispersion of the granule cell layer GCL (d) and a hypertrophy of radial glia cells (d, insert). This is not present in the control animals (e-h).

Figure 2 (right)**:** Alterations of FA and Trace within the dentate gyrus in the ipsilateral HC for control (black) and epilepsy animals (colored lines).



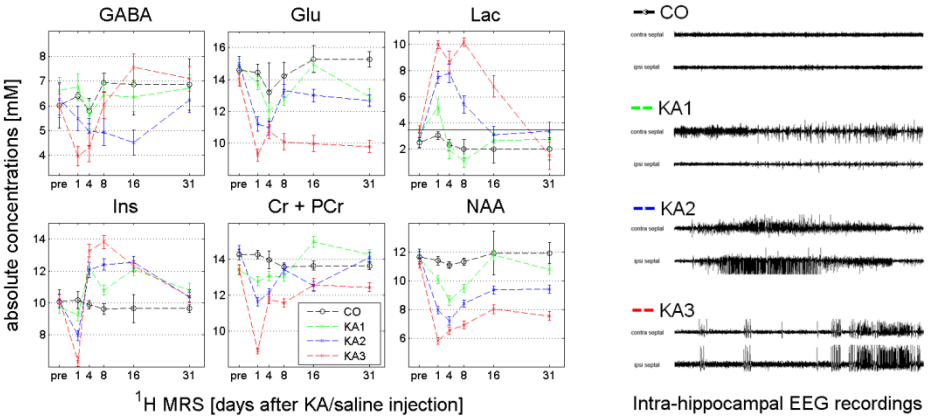


Figure 3: Longitudinal changes of metabolites in the septal part of the ipsilateral HC (figure left) correlates with the epileptic activity seen on intra-HC EEG recordings (figure right, contra- and ipsilateral EEG for control (CO) and kainate (KA) animals 32 days after KA/saline injection).