The generation of epileptic seizures requires interaction of sclerotic and intact networks

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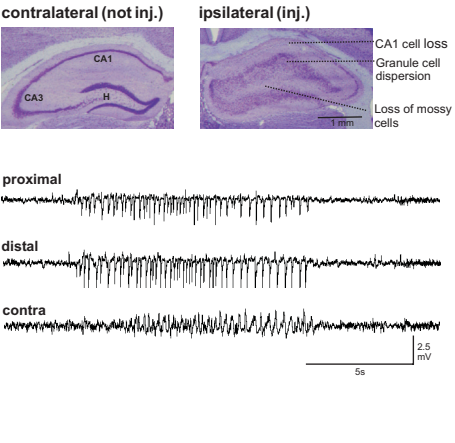
Objective

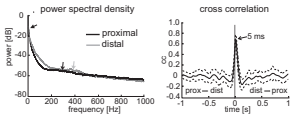
Mesial Temporal Lobe Epilepsy (MTLE) is associated with severe changes in the hippocampal histology, including cell loss in CA3, CA1 and the hilus, dispersion of the granule cells and the sprouting of mossy fibers. It is, however, unclear, whether this degenerated network structure can generate epileptiform activity (EA) or whether this requires the interaction with further brain areas. In the current study we aimed at characterizing the network structure responsible for the generation of EA and at clarifying the underlying mechanisms.
Therefore, we used a model for MTLE in mice, in which a single unilateral injection of kainate into the dorsal hippocampus induces histological and physiological changes and pharmacoresistance, which is comparable to human MTLE. The histological changes are mainly restricted to the dorsal hippocampus. It is, thus, possible to investigate the role of networks with different degrees of reorganization on the generation of EA.

Epileptiform activity spreads along the hippocampal length axis and to the contralateral hippocampus

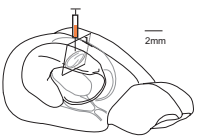


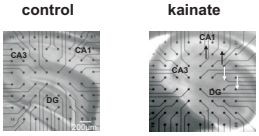
Kainate induced cell loss and granule cell dispersion in the ipsilateral hippocampus close to the injection site (here: 8 weeks after inj.).
The contralateral hippocampus was histologically unchanged.

EA was not restricted to proximal sclerotic hippocampal regions but occurred also distally in the ipsilateral hippocampus and propagated to the contralateral hippocampus. A positive delay in the cross-correlation suggests the propagation from distal to proximal sites.

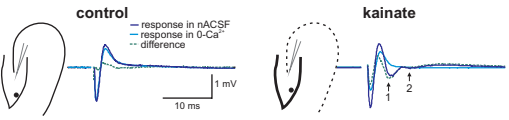


Excitability in slices proximal to the injection site is severely decreased

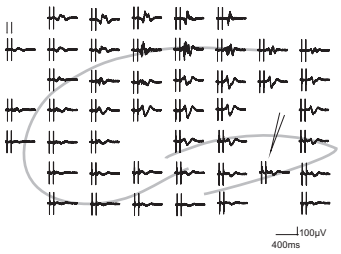




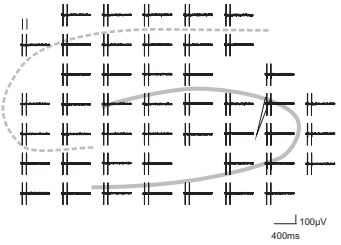
Slices were prepared from sites proximal to the kainate injection. Hippocampal slices covered th MEA electrodes completely.
Kainate slices showed salient degeneration.



Kainate induced **recurrent connectivity** in the dentate gyrus elicits reverberating post-synaptic responses only in proximal slices from kainate mice.



EA in control slice:
Application of the GABA-blocker bicuculline (here 20µM) induced EA in slices from uninjected control mice. EA had large amplitudes in the dentate gyrus and CA1 and smaller amplitudes in CA3.
EA occurred spontaneously and upon stimulation with comparable waveforms.



EA in kainate slices:
EA could not be induced in kainate slices from the most sclerotic regions, despite recurrent connectivity through mossy fiber sprouting.
Neither blocking the inhibition (bicuculline, picrotoxin) with or without stimulation, nor increasing the excitation (low Mg^{2+}, high K^+) induced EA.

*The most sclerotic regions therefore show **decreased excitability** and are unlikely to be the sole generator of EA in vivo.*

Conclusions

1. Intrahippocampal kainate injections cause unilateral hippocampal sclerosis and recurrent EEs.
2. EEs are not generated in the most sclerotic areas independently, despite recurrent connectivity
3. Distant hippocampal regions without salient histological changes show a functional decoupling within hippocampal areas, partially due to decreased function of gap junctions
4. We hypothesize that complex networks consisting of sclerotic and less or non-sclerotic sub-networks are needed for the generation of epileptiform activity.

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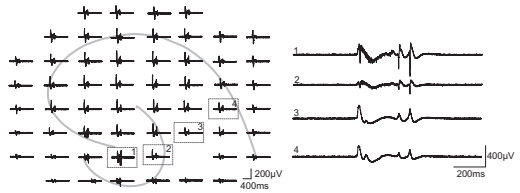
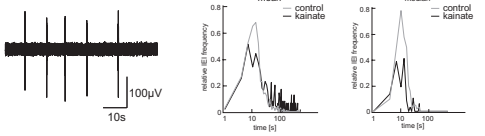
EA in kainate slices distal to the injection site had comparable waveform and duration as controls



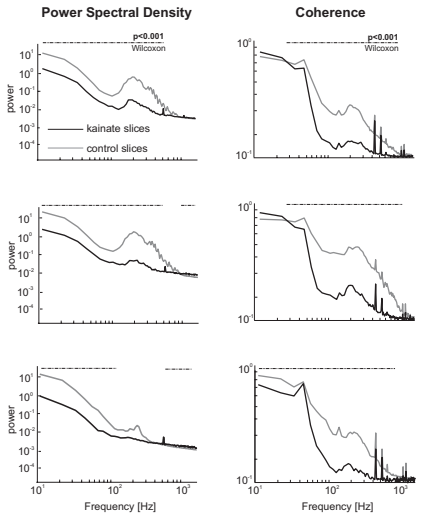
EA could be induced with disinhibition in distal kainate slices (2), which showed no or only light sclerosis. EA in kainate and control slices had comparable waveforms and durations.



EA in kainate and control slices occurred with regular intervals. Inter-event-intervals in kainate and control slices were comparable (mean &median).

Excitability in slices from epileptic was thus not increased.

Decreased power spectral density and coherence indicate a reduction in synchronization of activity in kainate slices



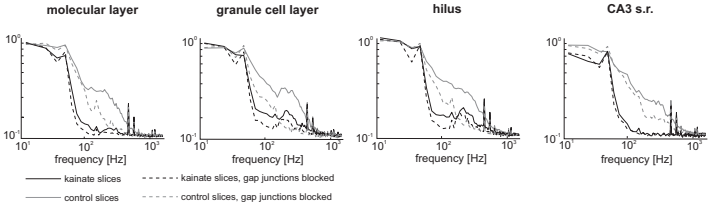
molecular layer:
Power spectral density (PSD) of EA in distal slices was significantly reduced in kainate slices (dashed line: Wilcoxon rank order test, p<0.001).
Inter-electrode coherence in kainate slices was significantly reduced at frequencies >30 Hz.

granule cell layer:
PSD and coherence were significantly reduced in kainate slices. This also accounts for the **hilus**.

CA3 stratum radiatum:
PSD and coherence are significantly reduced.

Decreased synchronization in all hippocampal layers in kainate slices indicates severe changes in network dynamics, although the histology is not saliently changed.

The decrease in coherence depends (partially) on decreased gap junction coupling

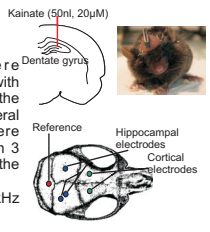


Blocking the gap junctions (2-APB, 10µM) reduced the coherence in normal slices in the dentate gyrus almost to the level of kainate slices at frequencies >50 Hz. 2-APB had only marginal effect on kainate slices. Reduced synchronization at high frequencies in kainate slices was therefore due to decreased coupling via reduced gap junction expression or impaired functionality.

Can these seemingly anti-epileptic changes have a pro-epileptic effect?

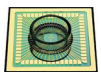
Methods

Injection & in-vivo recordings
Male C57Bl/6 mice were anaesthetized and injected with kainic acid (50nl, 20µM) into the right dorsal hippocampus.Bilateral hippocampal electrodes were implanted in 16 mice, of which 3 with an electrode distal in the ipsilat. hippocampus.
Recordings:Spike2, CED, 20kHz sampling, bandpass 1Hz-5kHz).



Slice preparation
Hippocampal slices (400µm) were prepared 6-8 weeks after kainate injection from different hippocampal levels (proximal to injection site (N=40 slices) and distal (N=8 slices)) and from untreated control mice (N=10 and N=8 resp.). Activity was recorded with planar, substrate-integrated micro-electrode arrays (60 electrodes, diameter 30µm, 200µm spacing; MEA1060 system, Multi-Channel-Systems).

Slices were perfused with oxgenated ACSF (K^+, 5mM) and recorded at 33°C. Stimulation with tungsten electrodes with double pulses (40/-40µA to 100/-100µA). Bicuculline (Bic, 5-100µM), Picrotoxin (50µM), high K^+-ACSF (up to 12 mM) and 2-APB (10µM) was applied to the bath.



Data analysis
Data were analyzed in Matlab using the data analysis package MEA-tools (Egert et al. 2002) and custom programs . Coherence was calculated as cross-power spectral density function normalized by individual auto-spectral density functions between electrode pairs for cutouts of EA (2^{11} data points for the FFT, periodic Hamming windowing (window 0.15s, 50% overlap). Same windows for average power spectral density. Cross-correlation was calculated for windows 2s before to 2s after EA onset.

Histology
Cresyl violet stainings to identify the electrode position and verify hippocampal sclerosis.