# Spatiotemporal Network Alterations in Experimental Focal Cortical Epilepsy: MRI-based Longitudinal Functional Connectivity and Weighted Graph Analysis

**W. M. Otte[1,2], R. M. Dijkhuizen[2], C. J. Stam[3], K. van der Marel[2], M. P. van Meer[1,2], M. A. Viergever[2], and K. P. Braun[1]**

[1]Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, Netherlands, [2]Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands, [3]Department of Clinical Neurophysiology, VU University Medical Center, Amsterdam, Netherlands

## Introduction

The brain is increasingly recognized as a complex network of dynamically interacting subsystems with numerous functional interactions between local and more remote regions, where synchronization plays an important role in its (dys)functioning [1]. An example of a pathophysiologic neuronal synchronization disease is epilepsy, in which unprovoked recurrent seizures result from a complex interaction between distributed neural populations. The old concept of a localized epileptic focus, may possibly be extended with that of an epileptic network, which functional interactions extend to numerous remote brain areas. The importance of network organization for seizure spread has been emphasized in several modeling studies. Recently it has been shown, using complex network analysis, that epilepsy in functional brain networks produces specific patterns of altered functional connectivity (FC) among distant cortical regions [2]. During seizures, the neuronal network moves in the direction of a more ordered topology as compared to the interictal state, it has therefore been hypothesized that interictal networks in epilepsy are characterized by a more random organizations. In this study we aimed to evaluate experimental interictal focal epilepsy brain networks in rats longitudinally, and compare their spatiotemporal evolution with healthy control brains. To this end we assessed functional brain connectivity using longitudinal resting state functional MRI (rs-fMRI) [3] and graph theoretical analyses.

## Methods

Chronic, mild, focal neocortical epilepsy was induced by intracortical injection of 120 ng tetanus toxin in the right motor cortex in male Sprague-Dawley rats (n=10) [4]. Resting state fMRI was acquired in epileptic rats and age-matched controls (n=10) at 7, 21, 49 and 70 days after epilepsy induction. Rats were mechanically ventilated with 2.5% isoflurane in air/O2 (2:1) during MRI. MRI measurements were performed on a 4.7 T horizontal bore Varian MR system with use of a Helmholtz volume coil (90-mm diameter) and an inductively coupled surface coil (35-mm diameter) for signal excitation and detection, respectively. Then, for at least 10 minutes end-tidal isoflurane was reduced to 1%. Subsequently, 10 minutes of resting state fMRI was performed using a T2*-weighted gradient echo EPI sequence (35° flip angle, TR/TE=500/19 ms, 64×64 matrix; 0.5×0.5×1.5 $mm^3$ voxels, 1200 BOLD images). In addition, a gradient echo 3D image (ge3d) was collected for registration purposes (TR/TE=10/2.6 ms; 20° flip angle, 128x128x256 matrix, 0.3x0.3x0.3 mm3 voxels $mm^3$). Functional time series were non-rigidly aligned to the high-resolution ge3d image. The ge3d anatomical images were registered non-linearly to a 3D model of a stereotaxic rat brain atlas [5]. The inverse b-spline transformations were estimated, which enabled mapping of atlas regions of interest (ROIs) to original functional time series space. The functional data were kept at the original resolution. Rs-fMRI preprocessing included spatial smoothing (1 mm FWHM kernel); rigid-body motion correction; band-pass filtering (0.01<f<0.1 Hz); and linear regression against rigid-body realignment parameters, deep white matter signals, ventricular system signals and global signal. Bilateral ROIs were selected within the sensorimotor network. The ROIs were projected from the atlas onto the functional time series. Functional connectivity was measured as the zero-lag correlation coefficient *r* and Fisher-transformed according to $z' = \ln( (1+r) / (1-r) ) / 2$. FC maps were obtained by calculating the voxel-wise correlation with the mean signal of a seed ROI. Averaging across subjects yielded group mean FC maps. For each functional dataset a weighted graph was constructed with bilateral cortical and subcortical gray matter voxels. Edges were defined between any pair of voxel time series using Fisher-transformed correlation coefficients. We quantified the local and global graph structures via the weighted clustering coefficient *C* and the weighted characteristic path length *L* [2]. Self connections and negative edges were excluded. For each functional dataset, *L* and *C* were normalized using 10 surrogate networks. Normalized weighted *L* and *C* were defined as: Lratio = $L$ / ‹ $L_{surrogate}$ › and Cratio = $C$ / ‹ $C_{surrogate}$ ›.

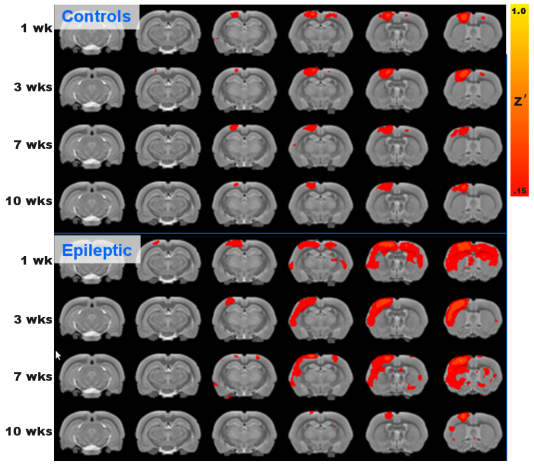
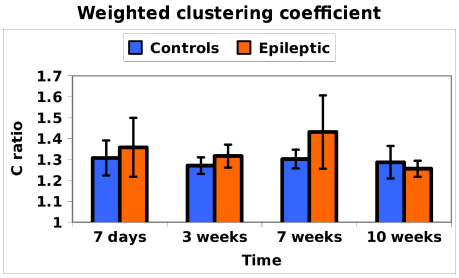



**Figure 1.** Left M1 functional connectivity (0.15 < *z'* < 1.0).



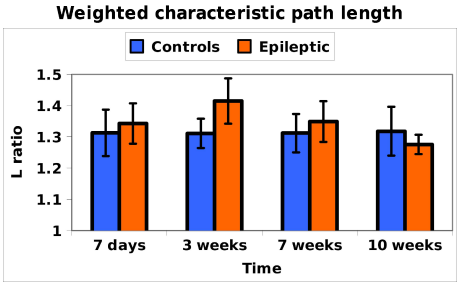




**Figure 2**. Normalized weighted clustering coefficient and weighted characteristic path length of epileptic rats and in controls (mean ± SD).

## Results

The tetanus toxin treatment induced frequent, mild, but persistent facial motor seizures in all treated animals. Four animals died due to a status epilepticus and were excluded from the study. Only EEG recordings at 0% isoflurane confirmed 5 sec discharges with discrete focal motor signs. We detected increased FC between bilateral sensorimotor (sub)cortical regions in epileptic rats at 1, 3 and 7 weeks after epilepsy induction as compared to controls. At 10 weeks, FC normalized, despite persistent but less frequent seizures in all six animals. FC maps of the left primary motor cortex (M1) (non-injected hemisphere) are shown in figure 1. Similar patterns were found in other cortical ROIs. Cratios and Lratios are shown in figure 2. The repeated measures linear mixed model (SPSS, v15) showed a significant interaction between group and time for both $C_{ratio}$ and $L_{ratio}$ ($F(3, 48) = 3.22$, $P = 0.031$; $F(3, 48) = 4.58$, $P = 0.007$, respectively).

## Discussion

In this study we assessed changes in longitudinal resting state functional MRI in a rat model of focal cortical epilepsy and compared local and global networks properties with healthy controls. First, our results point out that functional changes extend beyond the seizure onset zone. Second, up to 7 weeks after induction the epileptic brain is characterized by a more *ordered* configuration, with higher $C_{ratios}$ and $L_{ratios}$, compared to the healthy brain. We repeated our analysis with binary graph analyses (data not shown) resulting in similar shifts to more ordered network configuration. This is the first study to compare the functional interictal epileptic network organization with the healthy brain, suggesting the previously suggested hypothesis to be false, at least in this animal model of focal epilepsy. The underlying pathophysiology of this epileptic network shift is not known. The temporal correspondence between the evolution of FC of (sub)cortical regions within the sensorimotor network and graph analytical measures ($L_{ratio}$ and $C_{ratio}$) emphasizes the potential of resting state functional MRI to assess spatiotemporal characteristics of functional brain alterations in relation to pathophysiological disorders such as focal epilepsy.

## References

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