SPATIOTEMPORAL NETWORK ALTERATIONS IN EXPERIMENTAL FOCAL CORTICAL EPILEPSY: MRI-BASED LONGITUDINAL FUNCTIONAL CONNECTIVITY AND WEIGHTED GRAPH ANALYSIS

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ABSTRACT
There is increasing evidence that the topology of brain networks may be changed in epilepsy. In particular, a random topology has been suggested as an explanation for lower seizure thresholds. To test this hypothesis, we assessed focal epileptic and healthy networks over time using resting state functional MRI and weighted graph theoretical analysis in a rat model. Brain networks in focal epilepsy were globally affected, toward a more ordered network topology. Networks largely normalized at ten weeks after epilepsy induction. Graph analysis provides a promising method to explore dynamical network alterations in epilepsy.

Index Terms— experimental focal epilepsy; longitudinal resting state functional MRI; graph theoretical analysis; small-world networks; rat brain

1. 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, 2]. An example of a pathophysiologic neuronal synchronization disease is epilepsy [3], in which unprovoked recurrent seizures result from a complex interaction between distributed neuronal populations. The underlying pathophysiology of epilepsy is still poorly understood [4]. 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 [3].

The importance of network organization for seizure spread has been emphasized in several modeling studies [5, 6, 7, 8]. 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 [9, 10]. During seizures, the neuronal network moves in the direction of a more ordered topology as compared to the interictal state. As random networks are more synchronizable than small-world networks [11, 12], it has therefore been hypothesized that interictal networks in patients with epilepsy are characterized by a (relatively) more random organization [13]. However, until now, comparison of the interictal epileptic brain with a non-epileptic control situation has only been reported, in a clinical cross-sectional temporal lobe epilepsy study [14].

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) and graph theoretical analyses. Rs-fMRI enables quantification of functional connectivity by computing temporal correlations between blood oxygenation level-dependent (BOLD) fMRI signals in different regions. Spontaneous low-frequency BOLD fluctuations are highly synchronous between homologous brain areas and have been associated with neuronal communication [15].

2. METHODS
Chronic focal epilepsy was induced in adult male Sprague-Dawley rats by injection of tetanus toxin (0.6 µL; 120 µg tetanus toxin/mL; 0.5 nL/min) into the right primary motor cortex, which is known to induce frequent, mild facial seizures [16, 17, 18]. The animal experimental protocol was approved by the Utrecht University Ethical Committee on Animal Experiments. Rs-fMRI was performed at 1, 3, 7 and 10 weeks post epilepsy induction, both in epileptic (n = 12) and age-matched control rats (n = 12) on a 4.7T MR system under isoflurane anesthesia. Exactly ten minutes prior to rs-fMRI acquisition, end-tidal isoflurane anesthesia concentration was reduced to 1% and maintained at this level.

Rs-fMRI of seven 1.5 mm coronal brain slices were acquired using gradient echo planar imaging (TR=0.5 s, TE=19 ms, 64 × 64 data matrix, field of view (FOV) 3.2 × 3.2 cm², 1200 volumes, 10 minutes acquisition). In addition, a gradient echo 3D image (ge3d) was collected for registration purposes (TR=10 ms; TE=2.57 ms, pulse angle=20°, 128 × 128 × 256 data matrix, FOV=4 × 4 × 6 cm³).

In a subset of animals (five controls and six epileptic rats) offline EEG activity was recorded following rs-fMRI acquisition under 1.0% and 0% isoflurane anesthesia (band pass filtering: 0.1 < f < 250 Hz; sampling rate: 1000 Hz/channel).

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 stereotactic rat brain atlas [19]. 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, to reduce the computational burden of the graph analysis and to prevent resampling artifacts. All registrations were performed with elastix (version 4.2; http://elastix.isi.uu.nl) [20]. Rs-fMRI pre-processing included spatial smoothing with an isotropic Gaussian kernel of 1.0 mm full width at half-maximum; 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 [21].
Bilateral ROIs were selected within the sensorimotor network, i.e. the primary and secondary motor cortex (M1, M2), fore- and hindlimb region of the primary somatosensory cortex (S1FL and S1HL, respectively), secondary somatosensory cortex (S2), caudate putamen (CPu) and thalamus (Th). 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 $z' = \ln((1 + r)/(1 - r))/2$.

Interregional FC between cortical and subcortical ROIs was calculated as the correlation coefficient between mean BOLD fMRI signals. FC maps were obtained by calculating the voxel-wise correlation with the mean signal of a seed ROI. Group mean FC maps were constructed using nonparametric 1-sample, permutation-based, $t$-tests (false discovery rate (fdr) corrected) and were overlaid on an anatomical template image.

For each functional dataset a weighted graph $G = (V, E)$ was constructed, with $V$ the collection of $N$ bilateral cortical and subcortical gray matter voxels (projected from atlas onto functional data), and $E$ the collection of functional edges defined between any pair of voxel time series $i$ and $j$ using the Fisher-transformed zero-lag correlation coefficient $z'$. Self connections and negative edges were excluded. We quantified the local and global graph structures via the weighted clustering coefficient $C$ and the weighted characteristic path length $L$ [22].

$C$ is defined as the mean of the local clustering coefficients of all nodes:

$$C = \frac{1}{n} \sum_{i \in N} 2t_i k_i(k_i - 1)$$

where $t_i$, the weighted geometric mean of triangles around a node $i$:

$$t_i = \frac{1}{2} \sum_{j,h \in N} (w_{ij} w_{ih} w_{jh})^{1/3}$$

and $k_i$, the weighted degree, in which $w_{ij}$ equals the correlation strength between two rs-fMRI signals:

$$k_i = \sum_{j \in N} w_{ij}$$

$L$ is defined as the mean geodesic lengths over all couples of nodes:

$$L = \frac{1}{N(N-1)} \sum_{i,j \in N, i \neq j} \frac{1}{w_{ij}}$$

Usage of the harmonic mean circumvented the problem of disconnected nodes in calculating $L$ and resembles the global efficiency measure (i.e. $1/\infty \rightarrow 0$) [23].

For each functional dataset, $L$ and $C$ were normalized using 10 surrogate, or null-hypothesis, networks to enable statistical comparison within and between animals [24]. These networks were constructed by randomly shuffling of edges, while preserving the degree and graph symmetry. Normalized weighted $L$ and $C$ were defined as: $\bar{L} = L / \langle L_{\text{surrogate}} \rangle$ and $\bar{C} = C / \langle C_{\text{surrogate}} \rangle$.

Linear mixed-effect modeling with repeated measures was performed for (interaction) effects (SPSS, version 15). Three factors were added: group (control or epilepsy), time (1, 3, 7 or 10 weeks) and their interaction. All measures were tested separately. Between group post-hoc $t$-tests were performed at the separate time-points (Sidak corrected). Significance level was set at $\alpha = 0.05$.

3. RESULTS

The tetanus toxin treatment induced frequent (on average ten / hour), mild, but persistent facial motor seizures in all treated animals, approximately starting 1 week after tetanus toxin injection.

A status epilepticus occurred in four out of 12 animals in the second week after treatment, necessitating termination and exclusion of these animals from further analysis. EEG recordings at 1.0% isoflurane confirmed frequent interictal spikes, and 5 sec discharges with discrete focal motor signs were measured at 0% isoflurane in epileptic rats as compared to controls. EEG patterns at 1% were normal.

We detected increased FC between intrahemispheric sensorimotor (sub)cortical regions and brain voxels in epileptic rats at 1, 3 and 7 weeks after epilepsy induction as compared to controls, but decreased FC between interhemispheric cortical regions. At 10 weeks, FC normalized, despite persistent but less frequent seizures in all six animals. FC maps of the left M1 (non-injected hemisphere) are shown in figure 3. Similar patterns were found for M2, S1FL, S1HL and S2.

Interregional FC between cortical regions and subcortical CPu and thalamus revealed increased connectivity between bilateral sensorimotor areas and the CPu, but no increased connectivity with the thalamus. As an example the interregional connectivity between the left M1 and left CPu (significant group difference: $F(1,20) = 22.88$, $P < 0.0005$ and significant post-hoc difference at time-point 7 days and 49 days: $t(80) = 3.86$, $P < 0.0005$ and $t(80) = 3.35$, $P = 0.001$, respectively) is shown in figure 3.

Normalized clustering coefficients ($\bar{C}$) and normalized weighted characteristic path lengths ($\bar{L}$) are shown in figure 3.

The repeated measures linear mixed model showed a signifi-
Fig. 2: Example of interregional FC.

cant two-way interaction between group and time for $L$ ($F(3, 60) = 3.60, P = 0.022$). Post-hoc testing indicated time-point 21 days as different ($t(73.48) = 2.88, P = 0.005$). A significant group effect was found for $\hat{C}$ ($F(1, 20.14) = 5.22, P = 0.033$) with differences between groups at time-points 7 and 49 days ($t(77.13) = 2.12, P = 0.037$ and $t(77.13) = 2.31, P = 0.023$, respectively).

The main effect time was also significant different for both $\hat{C}$ and $L$ ($F(3, 59.50) = 5.61, P = 0.002$; $F(3, 59.50) = 4.45, P = 0.007$, respectively): increased at 1.3 and 7 weeks and decreased at 10 weeks.

4. DISCUSSION / CONCLUSION

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.

The hypothesis, that interictal brain networks in focal epilepsy have a more random topology as compared to similar control networks, was tested using weighted graph theoretical analysis.

First, our results point out that functional networks are affected after focal cortical epilepsy induction, but tend to normalize over time, although seizure activity remained, and is reported to continue up to 5 months [18]. Second, up to 7 weeks after induction the epileptic brain is characterized by a more ordered configuration, with higher $\hat{C}$ and $L$, 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 serial 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. We are currently investigating the existence of (pathological) hubs, which could possible explain our findings. Furthermore, changes in the underlying structural network could be an explanation for changes in global efficiency. Focal temporal lobe epilepsy is known to introduce remote white matter changes, probably as a result of frequent seizure propagation [25]. The temporal correspondence between the evolution of FC of (sub)cortical regions within the sensorimotor network and graph analytical measures ($\hat{L}$ and $\hat{C}$) 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.

In conclusion, functional changes extend beyond the seizure onset zone in focal epilepsy in rats, and are detectable using functional resting state MRI and concepts from graph theory. We found a deviation from a normal configuration towards a more regular topology in the interictal epileptic brain.

5. REFERENCES

Fig. 3: Normalized weighted clustering coefficient (a) and weighted characteristic path length (b) of epileptic rats and in controls.


