Disrupted Brain Functional Organization in Epilepsy Revealed by Graph Theory Analysis

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Running head: Disrupted brain functional organization in epilepsy
Key Words: epilepsy, functional networks, graph theory, network analysis
Abstract

Objective: The human brain is a complex and dynamic system that can be modeled as a large-scale brain network to better understand the reorganizational changes secondary to epilepsy. In this study, we developed a brain functional network model using graph theory methods applied to resting-state fMRI data acquired from a group of epilepsy patients and age- and gender-matched healthy controls.

Methods: A brain functional network model was constructed based on resting-state functional connectivity. A minimum spanning tree combined with proportional thresholding approach was used to obtain sparse connectivity matrices for each subject, which formed the basis of brain networks. We examined the brain reorganizational changes in epilepsy thoroughly at the level of the whole brain, the functional network and individual brain regions.

Results: At the whole brain level, local efficiency was significantly decreased in epilepsy patients compared with the healthy controls. However, global efficiency was significantly increased in epilepsy due to increased number of functional connections between networks (albeit weakly connected). At the functional network level, there were significant proportions of newly-formed connections between the default mode network and other networks, and between subcortical network and other networks. There was a significant proportion of decreasing connections between the cingulo-opercular task-control network and other networks. Individual brain regions from different functional networks, however, showed distinct pattern of reorganizational changes in epilepsy.

Significance: These findings suggest that epilepsy alters brain efficiency in a consistent pattern at the whole brain level yet alters brain functional networks and individual brain regions differently.
Introduction

The human brain can be modeled as a network or a graph represented by a collection of nodes (i.e., cortical and subcortical brain regions) and links (i.e., associations between nodes) (Sporns et al., 2005). This approach is based on graph theory which provides a powerful way of examining the dynamic interactions among multiple brain regions and how these interactions produce complex behaviors in human beings. This approach has provided insights into many neurological disorders including epilepsy, and has the potential to provide useful biomarkers for diagnostic and prognostic purposes (Haneef & Chiang, 2014). By definition, an epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005). This regional abnormal neuronal activity could potentially disrupt the base connectivity of individual brain regions as well as brain networks.

Previous studies on epilepsy have shown topological changes of whole brain functional networks in temporal lobe epilepsy (TLE) (Wang et al., 2014, Doucet et al., 2014) and in idiopathic generalized epilepsy (IGE) (Zhang et al., 2011). These studies showed epilepsy-related impairments in the whole brain functional networks including reduced clustering coefficient and increased global efficiency in in TLE and IGE patients relative to healthy controls.

In the current study, we developed a network model based on resting-state functional connectivity using graph theory to examine the epileptic reorganizational changes in brain functional networks at whole-brain level and regional level. Higher-order graph theoretic measures such as local efficiency and global efficiency along with lower-order measures such as strength and degree are estimated to quantify brain network organization and to compare epileptic brains with age- and gender-matched healthy controls. We hypothesize that the local
and global efficiency can be affected by epilepsy and that epileptic disruptions may display
distinct pattern across brain regions and networks. Here, we examined brain functional network
reorganization at the whole brain level, individual functional networks, and individual brain
regions to better understand the underlying mechanism of these reorganizational changes.

Methods

Subjects

Current findings are based on data acquired from 9 epilepsy patients (33.8 ± 9.4 years, 6 F/3 M)
and 9 age- and gender-matched healthy control subjects (33 ± 10.4 years, 6 F/3 M) (Table S1).
No significant (p < 0.05) age (2-sample t-test, two-tailed, p-value = 0.87) or gender differences
were found between the two groups. This study was approved by the University of Wisconsin-
Madison’s Institutional Review Board. All subjects provided written informed consent. Subject
profiles are shown in Table S1.

Neuroimaging data acquisition

Each epilepsy patient received a 5-minute resting-state fMRI scan as part of the clinical
examination on a 3.0 Tesla whole-body MRI scanner (DISCOVERY MR750, General Electric
Medical Systems, Waukesha, WI, USA) with an 8-channel head coil. MR imaging parameters
were: single-shot Echo Planar Imaging (EPI), axial plane, TR = 2000 ms, TE = 30 ms, flip angle
= 75°, slice thickness = 5 mm, number of slice = 28, acquisition matrix = 64 × 64, field of view
(FOV) = 240 × 240 mm², voxel size = 3.75 × 3.75 × 5 mm³, single average (NEX = 1). T1-
weighted structural images were acquired axially (TR = 8.688 ms, TE = 3.468 ms, TI = 450 ms,
flip angle = 12°, FOV = 240 × 240 mm², voxel size = 0.94 × 0.94 × 1.2 mm³, number of slices = 136).

Each age- and gender-matched healthy control subject received a 10-minute resting-state fMRI scan on the same scanner (only the first 5 minutes were used for analyses). MR imaging parameters were: single shot EPI, sagittal plane, TR = 2600 ms, TE = 22 ms, flip angle = 60°, slice thickness = 3.5 mm, number of slice = 40, acquisition matrix = 64 × 64, FOV = 224 × 224 mm², voxel size = 3.5 × 3.5 × 3.5 mm³, NEX = 1. T1-weighted structural images with 1 mm isotropic voxels were acquired axially (TR = 8.132 ms, TE = 3.18 ms, TI = 450 ms, flip angle = 12°, FOV = 256 × 256 mm², number of slices = 156).

**Imaging data pre-processing**

Resting-state fMRI data was processed in AFNI (Cox, 1996) following the processing pipeline (Jo et al., 2013) for controlling head motion. The preprocessing steps included: 1) despiking to remove extreme outliers in the signal intensity time courses, 2) correcting for motion and slice-timing, 3) removing first three time points of the scan. T1-weighted structural images were warped to standard MNI-space using a 12-parameter affine transformation. This transformation was combined with T1-to-EPI alignment and used to map the functional EPI scans to MNI space with a resampling of 3mm resolution. The resulting structural images were later skull stripped and segmented into gray matter, white matter (WM) and cerebrospinal fluid (CSF) masks using FSL (Smith et al. 2004). The average signal time course from the WM and CSF masks and the 6 rigid body motion parameters were normalized and regressed out. The residuals from the functional data were spatially smoothed with a 4 × 4 mm² full width half maximum isotropic Gaussian kernel in AFNI and then temporally filtered with a band-pass from 0.01 to 0.1 Hz.
Head motion has been shown to significantly affect the resting-state functional connectivity measures (Saad et al., 2009; Satterthwaite et al., 2012; Van Dijk et al., 2012). In the current study, a secondary motion correction was performed to exclude fMRI volumes (i.e., time frames/points) with motion above a threshold. A score of motion measurement corresponding to each volume was calculated as the square root of the sum of squares of the derivatives (SSD) of the six time courses of the motion parameters (Birn et al., 2013; Jones et al., 2010; Meier et al., 2012). A stringent threshold of SSD was set at 0.2 mm; in other words, any time frame associated with a score of SSD greater than 0.2 mm was censored and later excluded using the –censor option provided in the AFNI program, 3dDeconvolve. This option essentially performed zero-filling to maintain the same sampling rate for each subject. Head motion evaluated with the average SSD scores per subject showed no significant group difference between epilepsy patients (0.056 ± 0.021 mm) and healthy controls (0.048 ± 0.015 mm) after correcting for motion (2-sample t-test, two-tailed, p-value = 0.36).

To avoid the potentially confounding effect of the different rs-fMRI scan lengths (i.e., 5 minutes for epilepsy patients but 10 minutes for healthy controls), we only used the first 5 minutes from each age- and gender-matched healthy control subject.

**Brain network construction**

Brain networks were constructed based on resting-state functional connectivity (RSFC). A total of 187 brain regions of interest (ROIs) (Figure S1) were predefined within the sensorimotor, cingulo-opercular task control, fronto-parietal task control, dorsal/ventral attention, default mode, salience and subcortical/cerebellar networks (Power et al., 2011). An averaged time series signal resulting from the preprocessed rs-fMRI data was extracted from each of these 187
regions with a radius of 4mm. Pearson correlation coefficients ($r_{ij}$) were calculated for the $i$th and $j$th ROI. This process generated a $187 \times 187$ connectivity matrix for each subject within each group. We then thresholded each of these connectivity matrices using a minimum spanning tree (MST) approach in order to obtain a sparse connectivity matrix with optimal functional connections for detecting epileptic alterations of the brain networks (Song et al., 2014). Each MST per subject is a spanning tree of a weighted sub-graph that is fully connected with all nodes containing maximum total weights of all links, which can be considered as the “skeleton structure” of the brain network for each subject. However, these MSTs do not form clusters or loops among individual brain regions which keeps it from a biologically meaningful sparse representation of brain networks (Alexander-Bloch et al., 2010). In order to obtain a sparse yet biologically meaningful connectivity matrix, proportions of the remaining connections were added to the MST to form clusters or loops among brain regions. To do so, the top 2, 4, 6 and 8 percent of the remaining connections from the MST-extracted connectivity matrix were added to the MST, respectively. This resulted in 4 sparse connectivity matrices with four levels of highest proportion of functional connections from the original connectivity matrix for each subject. This approach ensured that 1) each sparse connectivity matrix for each epilepsy patient and healthy control subject contained an equal number of functional connections, and that 2) a range of different proportional thresholds was examined to examine the effect of thresholding on group comparisons.

Network analysis

Graph theoretic analyses were applied to the sparse connectivity matrices. Graph metrics, including local efficiency, global efficiency and strength, were estimated using the Brain
Connectivity Toolbox (Rubinov & Sporns, 2010). The Brain Connectivity Toolbox provided calculations of these metrics at whole-brain level across all regions. In the current study, we made adaptation to the original scripts to calculate these metrics for individual brain regions. The epilepsy patients and control subjects were compared across 187 brain regions with each graph-theoretic measure estimated for each brain region and averaged within the group.

Local efficiency is a measure of information transmission among locally connected regions, such as in a module or a sub-network, whereas global efficiency is a measure of system-wide (i.e., over the whole brain) information transmission. Local efficiency quantifies the extent that connections are being segregated into local clusters or sub-networks (Achard et al., 2012), whereas global efficiency quantifies the extent that connections are being integrated into a system-wide network (Rubinov & Sporns, 2010). These two higher-level graph metrics were estimated on unweighted graphs (Achard et al., 2012). Strength and degree are two lower-level graph metrics that measure how strongly connections are formed over the whole brain or at individual brain regions. Strength is estimated as the mean value of weights (i.e., Pearson’s cross-correlation coefficients) of all functional connections linking to a particular brain region or across multiple brain regions and is measured on weighted graphs. It is worth pointing out that the use of negative connections (such as functional anti-correlations with negative correlation coefficients) in graph theory measures remains highly debated (Achard & Bullmore, 2007; Fair et al., 2009; Rubinov & Sporns, 2010) and may tend to decrease test-retest reliability of global network properties (Wang et al., 2011). In the current study, all self-connections or negative connections were removed from the networks prior to analysis. Degree, an unweighted measure of functional connections, is estimated as the number of functional connections linking to a particular brain region that survived thresholding on unweighted graphs.
Estimating brain networks disruptions in epilepsy

Epilepsy may disrupt brain network organization differently across regional locations and functional networks. We adopted a similar approach that was originally used for detecting functional network changes in comatose patients and referred to as the hub disruption index, $\kappa$ (Achard et al., 2012). The hub disruption index for a given graph metric, e.g., strength, was constructed by subtracting the mean regional strength of the healthy control group from the mean strength of the corresponding brain region in the epilepsy group and plotting this group mean difference against the healthy control group mean. The slope of a straight line fitted to the data was referred to as hub disruption index, $\kappa$. This approach essentially provided a visualization as well as estimation for examining network and brain regional disruptions combined from a single plot.

Statistical analysis

Group comparison was conducted for each graph metric at each threshold level using the non-parametric Wilcoxon rank sum test (one-sided) to avoid the invalid assumption of Gaussian distribution of these metric values in each group. As group comparison was conducted at each threshold of connection density (i.e., top 2, 4, 6 and 8 percent), an adjusted Bonferroni method was applied to correct for multiple comparisons (Holm, 1979). One-sided binomial proportion tests were later used to compare the proportions of strength and degree of each network to the total strength and degree of whole-brain networks, respectively between the two groups (Song et al., 2012). The same Bonferroni method was applied to correct for multiple comparisons made at
different networks (i.e., sensorimotor network, default mode network, cingulo-opercular network, etc). All statistical tests were evaluated at a significance level of 0.05.

**Results**

*Disrupted local efficiency in epilepsy*

At whole-brain level, we found that local efficiency was significantly decreased in epilepsy across a range of thresholds (Figure 1). Group-mean local efficiency increased monotonically as a function of increasing connection density (i.e., proportional thresholds). The measure of local efficiency estimated for individual brain regions on average over all subjects in each group also showed decrease in regions such as the medial temporal lobe of the default mode network (DMN) and postcentral gyrus (PCG) of the sensorimotor network. However, increased local efficiency was observed in some regions such as the posterior cingulate cortex (PCC) and angular gyrus from the DMN, thalamus from the subcortical network, and cerebellar vermis from the cerebellar network. A negative hub disruption index ($\kappa < -0.6$) was observed across all thresholds (Figure 2). In other words, brain regions with high information processing efficiency in healthy control subjects showed great reduction in epilepsy patients (i.e., medial temporal lobe), whereas brain regions with normal information processing efficiency in healthy controls showed abnormal increase in patients (i.e., PCC).

*Disrupted global efficiency in epilepsy*

At whole-brain level, global efficiency was significantly increased in epilepsy across a range of thresholds (Figure 3). Group-mean global efficiency increased monotonically as a function of increasing connection density. A negative hub disruption index of global efficiency ($\kappa < -0.4$)
was observed across all thresholds (Figure S2). Similar to what was found in local efficiency, distinct pattern of changes in global efficiency across different brain regions were observed. The medial temporal lobe and PCG continued to show decreased global efficiency, whereas the PCC, angular gyrus, thalamus and cerebellar vermis continued to show increased global efficiency.

**Increased yet weakly connected between-network functional connections**

At whole-brain level, both groups had similar functional connection strength that increased monotonically as a function of increasing connection density (Figure 4). A negative hub disruption index of functional strength ($\kappa < -0.4$) was observed across all thresholds (Figure S3). To further examine the reorganizational changes for each functional network, we conducted a second-level analysis based on two basic graph metrics—the strength and degree. We found that within each functional network, there was no significant difference between the two groups in terms of the number or strength of connections (binomial proportional tests, $p$-value > 0.05; Figure S4). However, there was an increased number of between-network connections in the epilepsy patients (Figure 5). Three functional networks showed significant group differences (Table 1). Epilepsy patients had significantly decreased proportion of between-network connections in cingulo-opercular task control network (binomial proportional tests, corrected $p$-value < 0.001) and significantly increased proportion of between-network connections in the DMN and subcortical network (binomial proportional tests, corrected $p$-values < 0.001).

However, the corresponding functional strength of these newly-formed between-network connections was not significantly different between the two groups (Table S2), suggesting that these connections were weakly connected.
Discussion

Previous studies have examined brain efficiency in both functional networks (Vlooswijk et al., 2011; Wang et al., 2014; Zhang et al., 2011) and structural networks in epilepsy (Bernhardt et al., 2011; Liu et al., 2014). However, these findings have remained conflicting potentially due to various factors such as different data processing approach and heterogeneous study population. In the present study, we matched each epilepsy patient with an age- and gender-matched healthy control subject and have taken stringent steps to safeguard against head motion artifact following the recommended data processing pipeline (Jo et al., 2013). Furthermore, graph-theory metrics were assessed at the regional level for each ROI following consideration of different thresholds upon network calculations. Besides these traditional graph metrics, a “hub-disruption” index was calculated for these metrics to better visualize the epileptic disruptions across different networks and brain regions. Only a small number of patients were included into the current study, which might limit the power, but can also be regarded as strength, as we still showed a significant difference between epilepsy patients and healthy control subjects.

Disrupted network efficiency

We observed a whole-brain disruption of both local and global efficiency in epilepsy patients, consistent with previous studies (Vlooswijk et al., 2011; Doucet et al., 2014). Local efficiency was significantly decreased in patients with epilepsy (Figure 1), suggesting impaired regional information transmission and a disruption of network segregation (Rubinov & Sporns, 2010). In contrast, global efficiency was significantly increased in epilepsy (Figure 3), suggesting a higher efficiency for global information transmission, which may be a marker of abnormal transmission such as seizure propagation (Rubinov & Sporns, 2010). Both local and global efficiency tend to
decline with a negative hub-disruption index compared with healthy controls when examined at functional network level (Figures 2 and S1), indicating an exchange of higher-efficiency regions to lower-efficiency regions. In addition, these regional reorganizations reveal an alteration of functional importance of individual regions within the same functional network due to epilepsy, which may not be easily observed from a brain-wide network analysis.

We observed that the medial temporal lobe from the DMN and postcentral gyrus from the sensorimotor network consistently showed decreased efficiency, whereas the PCC and angular gyrus from the DMN, thalamus from the subcortical network, and cerebellar vermis from the cerebellar network showed increased efficiency. These regions are critical areas in corresponding functional networks. We conducted secondary network analyses for each functional network to further examine if these changes are adaptive or maladaptive.

Abnormally increased weak connections between networks

A finer-grained network analysis was performed to examine the changes in the number and strength of connections within and between networks. Besides similar whole-brain functional strength (Figure 4), there was no significant differences in terms of the number or strength of connections within each functional network between the two groups (Figure S4). However, there was a markedly increased number of between-network connections (Figure 5) in the epilepsy group. Further statistical analysis demonstrated that the DMN and subcortical networks had a significantly increased number of connections to all other networks (Table 1). There was also a small yet significant amount of connections decreased between cingulo-opercular network and other networks. Overall there was an increase in between-network connections in the epilepsy group. Nevertheless, these newly-formed connections between networks were shown to be
weakly connected as the functional strength of between-network connections was not significantly different between the epilepsy and healthy control subjects (Table S2). This finding further supports the observation of increased global efficiency which is potentially elevated by the increased amount of shortest path length for long-distance information processing (Rubinov & Sporns, 2010).

**Limitations**

The small sample size (n = 9) and the heterogeneity of seizure location and duration were the primary limitations of this study.

First, we used a convenient sample comprising previously collected data without prior coordination of acquisition parameters such as different echo time, repetition time and voxel size. Although a previous study showed that failure to hold these acquisition parameters constant might lead to systematic differences when studied in the context of multicenter fMRI studies (Glover et al., 2012), one of our previous studies showed high level of reliability of resting-state functional connectivity in normal healthy subjects with different acquisition parameters (Song et al., 2012). Further interpretations based on findings reported here should proceed with caution.

Second, it was reported that the presence of head motion tends to bias the correlations (Saad et al., 2009; Satterthwaite et al., 2012; Van Dijk et al., 2012). Taken these factors into account, in the present study, we had 9 epilepsy patients with minimal head motion and matched these patients with control subjects with no significant differences in terms of age, gender and head motion (described in *Imaging data pre-processing* section). We also ensure that these patients and the control subjects had fMRI data of the same time length. To further test our method in a relatively larger sample, we added another 4 epilepsy patients and 4 age-, gender- and motion-
matched control subjects to the study group and applied the same method and analyses on 13 epilepsy patients vs. 13 control subjects (see Supplemental Data). These patients and their matched control subjects had the same number of time points of fMRI data after correction for motion but with variation in terms of the time length of fMRI data. We performed analyses on these 13 patients and 13 control subjects and observed similar findings. This further suggests the robustness of this method based on graph-theoretic measures made at regional or nodal level.

Essentially, we compared the two groups across 187 brain regions with each graph-theoretic measure estimated for each brain region and averaged within the group.

In addition, seven of nine patients were identified to have temporal lobe epilepsy, while one patient had different seizure location, and one patient was idiopathic. There are differences in treatments between these different patients in terms of medications as well as two of our patients were status post focal resection which could also influence brain reorganization (See Table S1).

A recent study (Doucet, 2014) suggests that age of seizure onset has an impact on whole-brain and regional RSFC in temporal lobe epilepsy patients. It would be important to examine brain functional reorganization in a larger and homogenous subset of epilepsy patients (e.g., temporal lobe epilepsy or mesial temporal sclerosis patients) and evaluate the potential relationship between graph theoretic and clinical measures.

Another limitation is the robustness of network estimation depending on the applied parcellation method (ICA and anatomic). For future study, we plan to investigate the robustness of different parcellation methods in terms of network estimation as well as group differences.

**Conclusion**
In the current study, graph theoretic analysis was applied to resting-state functional connectivity to examine the brain functional network reorganization in the presence of epilepsy. It provided direct evidence of large-scale network disruption in epilepsy patients. Compared with healthy control subjects, these patients demonstrated impaired local efficiency and increased global efficiency at brain-wide level. When examined at functional network level, these patients showed an increased number of connections between networks that are weakly connected, supporting the observations of abnormally high global efficiency. At the individual brain regional level, however, epilepsy patients exhibited distinct pattern of alterations. Our findings suggest that brain-wide network efficiency is affected in the presence of epilepsy. With a finer-grained network analysis at network and individual brain regional level, epileptic disruption on functional properties may be better understood.

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Disclosure of Conflict of Interest
The authors have no conflicts of interest to report, as this research was conducted in the absence of commercial and financial relationships that might compromise the integrity of the results reported herein.

**Figure/Table Legends**

Figure 1 Local efficiency compared between epilepsy and healthy control subjects

Figure 2 Hub disruption index of local efficiency

Figure 3 Global efficiency compared between epilepsy and healthy control subjects

Figure 4 Connection strength compared between epilepsy and healthy control subjects

Figure 5 Between-network functional connections in epilepsy

Table 1 Degree of functional connections between networks
Supplemental Data:

Figure S1 Illustration of the 187 ROIs from sagittal, axial and coronal views.

Figure S2 Hub disruption index of global efficiency

Figure S3 Hub disruption index of strength

Figure S4 Strength and degree of within-network functional connections

Figure S5 Local efficiency compared between 13 epilepsy and 13 healthy control subjects

Figure S6 Global efficiency compared between 13 epilepsy and 13 healthy control subjects

Figure S7 Hub disruption index of local efficiency

Figure S8 Connection strength compared between 13 epilepsy and 13 healthy control subjects

Table S1 Characteristics of epilepsy patients

Table S2 Strength of functional connections between networks

Table S3 Characteristics of 4 added epilepsy patients

References:


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Graph Theory Analysis

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Figure 1 Local efficiency compared between epilepsy and healthy control subjects

Shown are group comparison based on the Wilcoxon rank sum tests. The epilepsy patients show significantly decreased local efficiency across a range of thresholds/connection density (p-values listed are corrected for multiple comparisons.) (Epilepsy-blue line, Healthy control-red line).
The hub disruption index of local efficiency is plotted at each threshold of connection density. Each data point is color coded representing a node belonging to a particular functional network (i.e., red dots represent nodes belonging to the sensorimotor network, and blue dots represent nodes belonging to the DMN). The mean value of local efficiency of each node in the healthy control group \(<\text{Healthy Control}>\) (x-axis) is plotted against the difference between groups in mean local efficiency of each node \(<\text{Epilepsy}> - <\text{Healthy Control}>\) (y-axis). The hub disruption index of local efficiency is then estimated as the slope of the solid black line fitted to the scatterplots. Negative hub disruption indices are observed across different thresholds, indicating an overall disruption of local efficiency in the epilepsy group. Compared to the healthy control group, the epilepsy patients show distinct pattern of regional changes in local efficiency. The posterior cingulate cortex (PCC) has increased local efficiency, whereas the medial temporal lobe shows decreased local efficiency.
Figure 3 Global efficiency compared between epilepsy and healthy control subjects

Global efficiency is significantly increased in the epilepsy group across a range of different connection density (Wilcoxon rank sum tests, p-values < 0.001 with multiple comparison correction) (Epilepsy-blue line, Healthy control-red line).
Figure 4 Connection strength compared between epilepsy and healthy control

Functional connection strength is statistically similar between the epilepsy and healthy control groups across a range of different connection density (Wilcoxon rank sum tests, p-values > 0.05) (Epilepsy-blue line, Healthy control-red line).
Overall, there is an increased number of functional connections between networks in the epilepsy patients.
### Table 1 Degree of functional connections between networks

<table>
<thead>
<tr>
<th>Functional Network</th>
<th>Proportion of degree of between-network connections</th>
<th>Corrected p-value</th>
<th>Directional change</th>
</tr>
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<tbody>
<tr>
<td>Healthy</td>
<td>Epilepsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensorimotor</td>
<td>0.179</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>cingulo-opercular</td>
<td><strong>0.132</strong></td>
<td><strong>0.093</strong></td>
<td>&lt;0.001</td>
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<tr>
<td>fronto-parietal</td>
<td>0.153</td>
<td>0.148</td>
<td></td>
</tr>
<tr>
<td>dorsal/ventral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>attention</td>
<td>0.167</td>
<td>0.150</td>
<td>0.06</td>
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<tr>
<td>default mode</td>
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<td><strong>0.235</strong></td>
<td>&lt;0.001</td>
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<tr>
<td>salience</td>
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<td>0.122</td>
<td></td>
</tr>
<tr>
<td>subcortical</td>
<td><strong>0.037</strong></td>
<td><strong>0.055</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cerebellar</td>
<td>0.012</td>
<td>0.018</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Binomial proportional test was used to compare the proportion of between-network connections for each functional network followed by multiple comparison correction. The cingulo-opercular network showed significantly decreased between-network connections (p-value < 0.001), whereas the default mode and subcortical network showed significantly increased between-network connections (p-value < 0.001).
Supplemental Data: Disrupted Brain Functional Organization in Epilepsy Revealed by Graph Theory Analysis

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Figure S1 Illustration of the 187 ROIs from sagittal, axial and coronal views.
Illustration of the 187 ROIs from sagittal (top), axial (middle) and coronal (bottom) views. ROIs within each functional network are color-coded. Functional networks include the sensorimotor (red; 35 regions), cingulo-opercular task control (orange; 14 regions), fronto-parietal task control (gold; 25 regions), dorsal/ventral attention (magenta, 20 regions), default mode network (DMN; medium blue, 58 regions), salience network (royal blue; 18 regions), and subcortical/cerebellar (green; 17 regions) network.
The hub disruption index of global efficiency is plotted at each threshold of connection density. Each data point is color coded representing a node belonging to a particular functional network (i.e., red dots represent nodes belonging to the sensorimotor network, and blue dots represent nodes belonging to the DMN). The mean value of global efficiency of each node in the healthy control group <Healthy Control> (x-axis) is plotted against the difference between groups in mean global efficiency of each node <Epilepsy> - <Healthy Control> (y-axis). The hub disruption index of global efficiency is estimated as the slope of the solid black line fitted to the scatterplots. Negative hub disruption indices are observed across different thresholds, indicating an overall disruption of global efficiency in the epilepsy group.
The hub disruption index of strength is plotted at each threshold of connection density. Each data point is color coded representing a node belonging to a particular functional network (i.e., red dots represent nodes belonging to the sensorimotor network, and blue dots represent nodes belonging to the DMN). The mean value of nodal strength in the healthy control group <Healthy Control> (x-axis) is plotted against the difference between groups in the mean nodal strength <Epilepsy> - <Healthy Control> (y-axis). The hub disruption index of strength is estimated as the slope of the solid black line fitted to the scatterplots. Negative hub disruption indices are observed across different thresholds, indicating an overall disruption of connection strength in the epilepsy group.
Within each functional network, both groups had statistically similar strength and degree of connections (Binomial proportion test, p-values > 0.05 after multiple comparison correction).
Table S1: Characteristics of epilepsy patients

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age at study participation (years)</th>
<th>Gender</th>
<th>Seizure Side</th>
<th>Seizure location (EEG)</th>
<th>Start of syndrome</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>F</td>
<td>L</td>
<td>status post amygdala/tumor resection</td>
<td>8-9 years old</td>
<td>Keppra 1500mg BID, Carbamazepine 200mgBID</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>F</td>
<td>R</td>
<td>mesial temporal onset</td>
<td>Reportedly encephalitis at 3 years old leading to seizure disorders</td>
<td>Lamotrigine 300mg QAM/QPM, Topiramate 100mg QAM / 200mg QPM</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>F</td>
<td></td>
<td>no definite epileptiform abnormalities on EEG. EEG video suggests an area of neurophysiological dysfunction in the left occipital lobe</td>
<td>17 years old</td>
<td>None at time of fMRI (Previous were zonisamide, levetiracetam, lamotrigine and carbamazepine)</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>F</td>
<td>L</td>
<td>anterior temporal lobe / mesial temporal sclerosis</td>
<td>14 years old; generalized tonic-clonic</td>
<td>Zonisamide 300mg, Carbamazepine 500mgBID</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>F</td>
<td>rare left temporal disorganization which has an uncertain clinical significance. There are certainly no frank epileptiform abnormalities present</td>
<td>7th-8th grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lamotrigine, Citalopram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>M</td>
<td>centro-parietal region/parasagittal region</td>
<td>early teens</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>Oxcarbazepine 1800mg/600mg, Keppra 1000mg/2000mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>F</td>
<td>mesial temporal lobe</td>
<td>31 years old</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>Keppra 1500mgBID, Zonisamide200mgQA M/400QPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>M</td>
<td>status post temporal cavernoma resection; partial temporal lobectomy</td>
<td>Staring spells as a child and had trouble in school because of it</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Lamotrigine 100 mgBID, levetiracetam 1500 mgBID, topiramate 200 mgBID, Nortryptiline 25mgPRN migraines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>M</td>
<td>focal temporal/mesial temporal sclerosis</td>
<td>49 years old</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Several medication combinations including: Keppra, lamotrigine, gabapentin, pregabalin, zonisamide, lorazepam</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table S2 Strength of functional connections between networks

<table>
<thead>
<tr>
<th>Functional Network</th>
<th>Proportion of strength in each network</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy Control</td>
</tr>
<tr>
<td>sensorimotor</td>
<td>0.185</td>
</tr>
<tr>
<td>cingulo-opercular</td>
<td>0.150</td>
</tr>
<tr>
<td>fronto-parietal</td>
<td>0.142</td>
</tr>
<tr>
<td>dorsal/ventral attention</td>
<td>0.181</td>
</tr>
<tr>
<td>default mode</td>
<td>0.168</td>
</tr>
<tr>
<td>salience</td>
<td>0.138</td>
</tr>
<tr>
<td>subcortical</td>
<td>0.029</td>
</tr>
<tr>
<td>cerebellar</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Shown here are the proportions of between-network connection strength in each individual network relative to the total between-network connection strength across all networks in each group. Both groups had similar between-network connection strength (Binomial proportion test, corrected p-values > 0.05).

Additional analyses on group differences in brain functional organization

In the present study, we used a convenience sample comprising previously collected data without prior coordination of acquisition parameters (i.e., different TRs). In addition, head-motion was suggested to have a significant impact on resting-state brain functional connectivity (Saad et al., 2009; Satterthwaite et al., 2012; Van Dijk et al., 2012). We carefully conducted 2-level head-motion correction and excluded patients who had head-motion greater than threshold (i.e., SSD = 0.2 mm). To mitigate the bias of different TRs and the impact of head-motion on correlations, we
performed analyses on 9 patients and 9 control subjects with the same time length of fMRI data and with minimal motion contamination, for which the findings were reported in the manuscript. To further test our method in a relatively larger sample, we added another 4 epilepsy patients and 4 age- and motion-matched control subjects to the study group and applied the same method and analyses on 13 epilepsy patients vs. 13 control subjects (Table S3).

There was no significant group difference in age (2-sample t-test, one-tail, p-value = 0.42) or gender (9 F/4M in both groups) between the two groups. Each patient and the age-matched control subject had the same number of time points of fMRI data with variation in total time length after correction for motion. The epilepsy patient group had average motion SSD of $0.055 \pm 0.020$ mm and the healthy control group had average motion SSD of $0.047 \pm 0.015$ mm. There was no significant group difference in head motion between the two groups (2-sample t-test, one-tail, p-value = 0.13).

Consistent with the main findings from 9 epilepsy and 9 age-matched control subjects, we observed that regardless of the factors of age of seizure onset, seizure location and duration of illness, at whole-brain level, the epilepsy patients showed consistent decreases in local efficiency (i.e., a measure of functional segregation; Figure S5) and increases in global efficiency (i.e., a measure of functional integration; Figure S6) relative to the control subjects.

We again observed a negative hub-disruption index in local efficiency examined at regional level across different functional networks, indicating an exchange of higher-efficiency regions to lower-efficiency regions (Figure S7). Within the default mode network (DMN), the posterior cingulate cortex (PCC) consistently showed increased local efficiency while the medial temporal lobe showed decreased local efficiency in the epilepsy group relative to the control group (Figure S7).
Functional strength at the whole-brain level was statistically similar between the two groups (Figure S8).

Figure S5 Local efficiency compared between 13 epilepsy and 13 healthy control subjects
Shown are group comparison based on the Wilcoxon rank sum tests. The epilepsy patients show significantly decreased local efficiency across a range of different connection densities (p-values listed are corrected for multiple comparisons.) (Epilepsy-blue line, Healthy control-red line).

Figure S6 Global efficiency compared between 13 epilepsy and 13 healthy control subjects

Global efficiency is significantly increased in the epilepsy group across a range of different connection densities (Wilcoxon rank sum tests, p-values < 0.001 with multiple comparison correction) (Epilepsy-blue line, Healthy control-red line).
Figure S7 Hub disruption index of local efficiency

The hub disruption index of local efficiency is plotted at each threshold of connection density. The mean value of local efficiency of each node in the healthy control group <Healthy Control> (x-axis) is plotted against the difference between groups in mean local efficiency of each node <Epilepsy> - <Healthy Control> (y-axis). The hub disruption index of local efficiency is then estimated as the slope of the solid black line fitted to the scatterplots. Negative hub disruption indices are observed across different thresholds, indicating an overall disruption of local efficiency in the epilepsy group. Compared to the healthy control group, epilepsy patients show distinct pattern of regional changes in local efficiency. The posterior cingulate cortex (PCC) has increased local efficiency, whereas the medial temporal lobe shows decreased local efficiency.

Figure S8 Connection strength compared between 13 epilepsy and 13 healthy control subjects

Functional connection strength is statistically similar between epilepsy and healthy control subjects across a range of different connection densities (Wilcoxon rank sum tests, p-values > 0.05) (Epilepsy-blue line, Healthy control-red line).
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age at study participation (years)</th>
<th>Gender</th>
<th>Seizure Side</th>
<th>Seizure location (EEG)</th>
<th>Start of syndrome</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>19</td>
<td>F</td>
<td>L</td>
<td>left hemisphere in origin; possibly insular seizure</td>
<td>2 years old</td>
<td>Keppra 1000mgBID, Zonisamide 300mgQBed, Adderall 30mgAM</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>F</td>
<td>L</td>
<td>mesial temporal onset of seizures</td>
<td>25 years old</td>
<td>Keppra750mgBID, Lamotrigine200mgBID, Zonisamide300mgQBed</td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>F</td>
<td>L</td>
<td>temporal region consistent with left mesial temporal sclerosis</td>
<td>Febrile seizures in 1st year of life, complex partial automatism thereafter, and</td>
<td>Keppra 1000mgBID, Carbamazepine 200mgBID,</td>
</tr>
<tr>
<td>13</td>
<td>51</td>
<td>M</td>
<td>L</td>
<td>All seizures electrographically started in the left anterior quadrant</td>
<td>14 months old</td>
<td>Lamotrigine 400 mgBID, Citalopram 60 mg, Levetiracetam 2000 mgBID</td>
</tr>
</tbody>
</table>