DOI: 10.1111/epi.13225

FULL-LENGTH ORIGINAL RESEARCH

Aberrant topological patterns of brain structural network in temporal lobe epilepsy

*†[1]Clarissa Lin Yasuda, ‡[1]Zhang Chen, †§Guilherme Coco Beltramini, †Ana Carolina Coan, †Marcia Elisabete Morita, †Bruno Kubota, †Felipe Bergo, ‡Christian Beaulieu, †Fernando Cendes, and *Donald William Gross

Epilepsia, 56(12):1992–2002, 2015
doi: 10.1111/epi.13225

Clarissa Yasuda is currently an assistant professor of neurology at University of Campinas, Brazil.

Dr. Chen is a research associate at the Department of Biomedical Engineering of University of Alberta, Alberta, Canada.

Summary

Objective: **Although altered large-scale brain network organization in patients with temporal lobe epilepsy (TLE) has been shown using morphologic measurements such as cortical thickness, these studies, have not included critical subcortical structures (such as hippocampus and amygdala) and have had relatively small sample sizes. Here, we investigated differences in topological organization of the brain volumetric networks between patients with right TLE (RTLE) and left TLE (LTLE) with unilateral hippocampal atrophy.**

Methods: **We performed a cross-sectional analysis of 86 LTLE patients, 70 RTLE patients, and 116 controls. RTLE and LTLE groups were balanced for gender ($p = 0.64$), seizure frequency (Mann-Whitney *U* test, $p = 0.94$), age ($p = 0.39$), age of seizure onset ($p = 0.21$), and duration of disease ($p = 0.69$). Brain networks were constructed by thresholding correlation matrices of volumes from 80 cortical/subcortical regions (parcellated with Freesurfer v5.3 https://surfer.nmr.mgh.harvard.edu/) that were then analyzed using graph theoretical approaches.**

Results: **We identified reduced cortical/subcortical connectivity including bilateral hippocampus in both TLE groups, with the most significant interregional correlation increases occurring within the limbic system in LTLE and contralateral hemisphere in RTLE. Both TLE groups demonstrated less optimal topological organization, with decreased global efficiency and increased local efficiency and clustering coefficient. LTLE also displayed a more pronounced network disruption. Contrary to controls, hub nodes in both TLE groups were not distributed across whole brain, but rather found primarily in the paralimbic/limbic and temporal association cortices. Regions with increased centrality were concentrated in occipital lobes for LTLE and contralateral limbic/temporal areas for RTLE.**

Significance: **These findings provide first evidence of altered topological organization of the whole brain volumetric network in TLE, with disruption of the coordinated patterns of cortical/subcortical morphology.**

KEY WORDS: **Mesial temporal lobe epilepsy, Hippocampal atrophy, Graph theory.**

The most commonly observed structural change in temporal lobe epilepsy (TLE) is atrophy of hippocampus and other mesial temporal structures.[1] However, studies using quantitative analysis have consistently revealed a more widespread pattern of both structural and functional brain abnormalities in TLE.[2,3] Alterations in gray matter (GM) have been assessed using regional volumetry, voxel-based morphometry (VBM), and cortical thickness,[2,4] whereas

Key Points

- The inclusion of hippocampal volume in the analysis of whole brain network topology with graph theory revealed distinct patterns of abnormalities for RTLE and LTLE
- Both groups show significant decreases in global efficiency of network organization
- RTLE had increased local efficiency (not observed in LTLE), consistent with a compensational mechanism to global decreased efficiency
- Controls had a more even distribution of hub nodes than TLE, whose hub concentration was primarily in the paralimbic/limbic and temporal association cortices

diffusion tensor imaging (DTI) has demonstrated abnormalities of white matter (WM).[5] These studies deepen the understanding of the extent of brain abnormalities in TLE. However, although they depict an ensemble of pathologically affected areas, these studies have been constrained to report regional abnormalities without providing information about the dynamic interactions among these regions.[6]

As emphasized by Evans,[7] the last decade witnessed a growing interest in understanding the typical relationship among different brain areas (regarding both structural and functional connectivity) in healthy subjects and the pathologic changes imposed by different neuropsychiatric disorders, including epilepsy.[8] To depict large-scale abnormal connectivity between different regions in the brain, the connectome analysis can provide details about the topological features (integration and segregation aspects) of the whole brain network.[9,10] This technique has been applied to investigate both functional and structural connectivity in epilepsy. *Functional connectivity* is based on the measurements of interdependencies or statistical association between pairs of regions, and uses data from neurophysiologic signals (electroencephalography [EEG], functional magnetic resonance imaging [fMRI], or magnetoencephalography [MEG]).[11] *Structural connectivity* can be modeled using either DTI data,[5,11] which evaluate connections, or metrics from structural MRI (i.e., cortical thickness, GM volume),[7,12] which can be used to study structural associations (e.g., statistical dependence or correlation between brain areas). The brain connectome is "a comprehensive structural description of the network of elements and connections forming the human brain."[13] Mathematically, brain networks can be portrayed as graphs encompassing arrays of nodes (neuronal elements or brain regions) and edges (the interconnections between nodes). In the framework of structural covariance, a network edge is defined by the identification of high morphometric correlation (e.g., volume) between two regions. An edge can exist even in the absence of direct fiber connection, as indirect connection through a third region can give rise to the structural correlation.[7] These graphs can be examined with graph theory analysis, which provides a straightforward and powerful mathematical framework for characterizing topological properties of these complex brain networks[7,10]; therefore, allowing the comparison between healthy subjects and patients. It is commonly recognized that normal brain networks are *small-world networks*, with high clustering coefficients and low shortest path lengths, resulting in a highly efficient network that is capable of simultaneously integrating and segregating information processes.[10]

Previous network studies in TLE on either structural or functional data have demonstrated alterations of network properties, including global and local efficiency.[5,12,14] Most of the whole brain structural network studies have focused on DTI data,[5,9,14] with a few others on cortical thickness.[12] In addition to abnormalities of local and global network properties, these studies also suggested that left TLE (LTLE) is associated with more severe disruption of network properties than right TLE (RTLE).[9,12] Despite the key involvement of the subcortical structures in TLE (i.e., hippocampus, amygdala, thalamus, and others),[1,2] there is a lack of their inclusion in most network studies, although Besson et al.[9] showed disconnections of hippocampus and thalamus, with more pronounced reduction of connectivity in patients with LTLE, compared to RTLE. Another limitation of previous studies is a small sample size, which can limit the statistical power of this type of analysis.

This is the first study to apply graph theory analysis to the GM volumetric network that includes both cortical and subcortical structures in a large group of TLE patients (70 right TLE and 86 left TLE) and 116 healthy volunteers. Left and right TLE patients were investigated separately to assess changes in the network efficiency, interregional correlations, and hub distribution that may differ based on the hemisphere of hippocampal atrophy (HA). The aim of this study was to examine the alterations in whole brain network topology associated with HA, both within and outside the

Accepted September 28, 2015; Early View publication November 4, 2015.

*Division of Neurology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; †Laboratory of Neuroimaging, Department of Neurology, University of Campinas, Campinas, Brazil; ‡Department of Biomedical Engineering, University of Alberta, Edmonton, Alberta, Canada; and §Institute of Physics "Gleb Wataghin," University of Campinas, Campinas, Brazil

Address correspondence to Donald William Gross, Associate Professor, Division of Neurology, Director of Adult Epilepsy Program, Department of Medicine, University of Alberta, 7-112N Clinical Sciences Building, 11350 83 Ave, Edmonton, AB, Canada, T6G 2G3. E-mail: dwgross@ualberta.ca

[1]First and second authors have equal contribution.

ipsilateral temporal lobe, comparing RTLE and LTLE. The underlying hypothesis was that the presence of HA is associated with disruption of the global and local network properties in TLE, although LTLE patients will display a more severe impact on the network organization.

Materials and Methods

Subjects

Consecutive patients (n = 170) with TLE and unilateral HA were recruited from the outpatient epilepsy clinic at University of Campinas, Brazil (2009–2013). The diagnosis of TLE was obtained after comprehensive investigation including detailed clinical history, serial EEG studies (video-EEG was performed for patients with unclear origin of ictal activity), and MRI.[15] Determination of unilateral HA was based on hippocampal volumetry and signal quantification.[16] To obtain a group of homogenous patients, subjects with normal MRI, bilateral atrophy, or other lesions were excluded. From the 170 subjects enrolled, we excluded 14 subjects due to inaccuracies of automated hippocampal segmentation, leading to a final sample of 156 subjects: RTLE, n = 70 (45 women, mean age [standard deviation, SD] 48.4 [10.5] years); and LTLE, n = 86 (51 women, mean age [SD] 46.8 [11.1] years). The two groups were balanced for gender (p = 0.64), seizure frequency (Mann-Whitney *U* test, p = 0.94), age (p = 0.39), age of seizure onset (p = 0.21), and duration of disease (p = 0.69) (Table 1).

The control group included 116 healthy subjects without history of neurologic or psychiatric disease (77 women, 45.7 [12.3] years). Patients and controls were balanced for age (p = 0.2) and gender (p = 0.49).

All subjects signed an informed consent form prior to MRI scan, approved by the ethics committee of University of Campinas.

Table 1. Clinical characteristics of patients with left (86 LTLE) and right (70 RTLE) unilateral hippocampal sclerosis

	Side	Mean (SD)	Mean diff	95% Confidence interval Lower bound	95% Confidence interval Upper bound	p-Value
Age	RTLE	48.4 (10.5)	1.51	−1.95	4.96	0.39
	LTLE	46.8 (11.1)				
Age of first seizure	RTLE	14.5 (13.0)	2.37	−1.29	6.02	0.21
	LTLE	12.1 (10.1)				
Duration	RTLE	33.9 (13.8)	−0.86	−5.18	3.46	0.69
	LTLE	34.5 (13.4)				

		Median	Interquartile range	p-Value (Mann-Whitney)
Seizure frequency (per month)	RTLE	2	3.4	0.94
	LTLE	2	4.1	

		Female	Male	p (χ^2)
Gender	RTLE	51	26	0.64
	LTLE	55	38	

		Positive	Negative	p (χ^2)
Febrile seizures	RTLE	7	63	1
	LTLE	9	77	
IPI	RTLE	17	53	0.12
	LTLE	32	54	
Seizure control	RTLE	10	60	0.39
	LTLE	18	68	

		Monotherapy	Polytherapy	
AEDs	RTLE	10	60	0.6
	LTLE	16	70	

Both groups were balanced for age, gender, seizure onset, seizure frequency, duration, occurrence of initial precipitating injuries (IPI), seizure control (with or without seizures), and AEDs (mono or polytherapy). SD, standard deviation; mean Diff, mean difference (RTLE−LTLE); AEDs, antiepileptic drugs; IPI, initial precipitating injuries such as head trauma, meningitis, and other infections.

MRI acquisition

All subjects were scanned on a Philips 3T with a protocol for epilepsy described elsewhere.[16] For this study, the analysis was performed on three-dimensional (3D) sagittal T_1-weighted image (isotropic voxels of 1 mm, no gap, flip angle = 8 degrees, repetition time [TR] = 7.0 msec, echo time [TE] = 3.2 msec, matrix = 240 × 240, field of view [FOV] = 240 × 240 mm).[2]

Image processing—volumetric parcellation

Brain parcellation of 3D T_1-weighted images was obtained with Freesurfer software package (v5.3 https://surfer.nmr.mgh.harvard.edu/). This suite provides a reliable, fully automated segmentation of brain structures based on probabilistic information obtained from a manually labeled training set. In total, 80 regions of interest (40 from each hemisphere), including 34 cortical and 6 subcortical regions, were extracted and applied to the network analysis. The anatomical labeling of those regions is provided in Table S1.[17]

Construction of structural brain network and graph theoretical analysis

Detailed procedures used for characterizing human brain networks using brain morphologic features are well known and have been published previously.[7,8] Comprehensive descriptions of structural brain network construction and graph theoretical analysis are included in the Supplementary Methods section (Data S1). In summary, the structural connections between brain regions were defined as statistical associations (Pearson correlation with age, gender, and total GM volume effects removed using linear regression) of cortical and subcortical volumes between all regions (80 × 80), with the final structural brain network for each group containing a total of

80 nodes and 3,160 connections. Correlation differences between groups were examined using Fisher's r-to-z transform followed by both false discovery rate (FDR)–corrected and uncorrected analysis, with a p-value of 0.003 being used in the exploratory uncorrected analysis to demonstrate the top 15 changes between TLE and control groups. Binary network properties including global network efficiency (gE), local network efficiency (locE), and clustering coefficient (CC) were analyzed over a fixed range of network sparsity (0.05–0.40) for all groups. *Global network efficiency* (gE) derives from the inverse shortest *path length* in the network, whereas short path length is the minimum number of edges that must be traversed to go from one node to another. Therefore, as the shortest path length increases, the global efficiency decreases as more edges have to be crossed for transmission between every pair of nodes.[18,19] *Local network efficiency* (locE) of a node is calculated from the average of the inverse shortest path length among the neighboring nodes of the node.[20] *Clustering coefficient* (CC) relates to local network efficiency, as it measures the degree of the neighboring nodes of a specific node. In a network, a cluster is formed when the nearest neighbors of a node are also connected to each other. The CC measures the connectivity of a local neighborhood, based on the number of triangular connections between groups of three nodes.[21]

Regional *betweenness centrality* (BC) and *hubs* were examined at a fixed network sparsity of 0.16 for all groups. *Betweenness centrality* quantifies the number of short paths (between all other node pairs) that pass through a specific node divided by the total number of short paths in the entire network. It is an attribute of the importance of a specific node, as high BC means that it is part of "highly traveled paths"[22]); *Hubs* are the topologically important nodes with high centrality (nodes with regional BC >group mean + group SD in each group). These are the strategic nodes that are highly connected and mediate many of the short path lengths between other nodes. Topological parameter differences between groups were examined using a nonparametric permutation test method[8] that is consistent with previous studies.

Results

Network properties in TLE

Compared with controls, both LTLE and RTLE groups demonstrated significantly decreased gE, with the LTLE group being decreased over a much wider sparsity range (Fig. 1A). In addition, RTLE demonstrated significant increases in both locE and CC, between sparsity range of 15–20%, whereas LTLE showed a much narrower sparsity range for increased locE and only a trend for increased CC (Fig. 1B,C).

Altered interregional structural connections

Similar patterns of connections were observed from the correlation matrices at 16% sparsity in the three groups, with strong correlations between bilateral homologous regions and within the same lobe (Fig. 2, left). However, the comparison of interregional analysis of connectivity between controls and both TLE groups revealed some peculiarities (Table 2). Compared with controls, both TLE groups showed lower cortical/subcortical connectivity, including the bilateral hippocampi (Fig. 1D). For normal controls, the interregional correlation between bilateral hippocampal volumes demonstrated a highly significant linear correlation (adjusted R^2 0.518, $p = 5 \times 10^{-20}$); this correlation was weaker for RTLE (adjusted R^2 0.165, $p < 0.001$), but still significant, whereas for LTLE it displayed only a trend (adjusted R^2 0.0355, $p = 0.05$). The comparisons of bilateral hippocampal correlations between controls and both TLE patient groups resulted in significant differences, indicating weaker correlations for TLE, more severe for LTLE ($p < 0.003$). It is important to note that the hippocampus was not the only subcortical structure with reduced connectivity; the LTLE group also showed reduced correlation between bilateral amygdalae, in addition to changes in correlations between the putamen and pallidum with other areas. Of interest, the most significant interregional correlation increases occurred within the limbic system in LTLE and the contralateral hemisphere in the RTLE group (Table 2).

Hub regions of the three volumetric networks

The nodal centrality of the anatomic network was examined separately for each group at a sparsity of 16% (Table S2). Hub nodes in controls were mostly association regions and distributed over all four lobes (e.g., superior frontal gyrus [SFG], superior temporal gyrus [STG], precuneus [PCU], lateral occipital [LO]) (Fig. 2). In contrast, in TLE groups, the pattern of distribution of hub nodes was disrupted, as the hub nodes were concentrated primarily in the paralimbic/limbic (including ipsilateral hippocampus as a new hub) and temporal association cortices (Fig. 2). The LTLE group also presented with a more disrupted pattern of hub node distribution compared with controls, as only a single region (right lingual) overlapped with the control group. In the RTLE group, three key hub regions overlapped with the control group (LO.R, SFG.R, and PCU.L), with most of the other regions located in the temporal cortices (Fig. 2).

Altered nodal centrality in patients with TLE

In addition to hub distribution for each group, we investigate which nodes presented significant alterations in BC compared to controls. LTLE demonstrated regions with increased centrality, located mostly in the occipital lobes (70% of the nodes coincide with the new hubs), whereas in RTLE, areas were concentrated in contralateral limbic/temporal association (50% coincide with the new hubs)

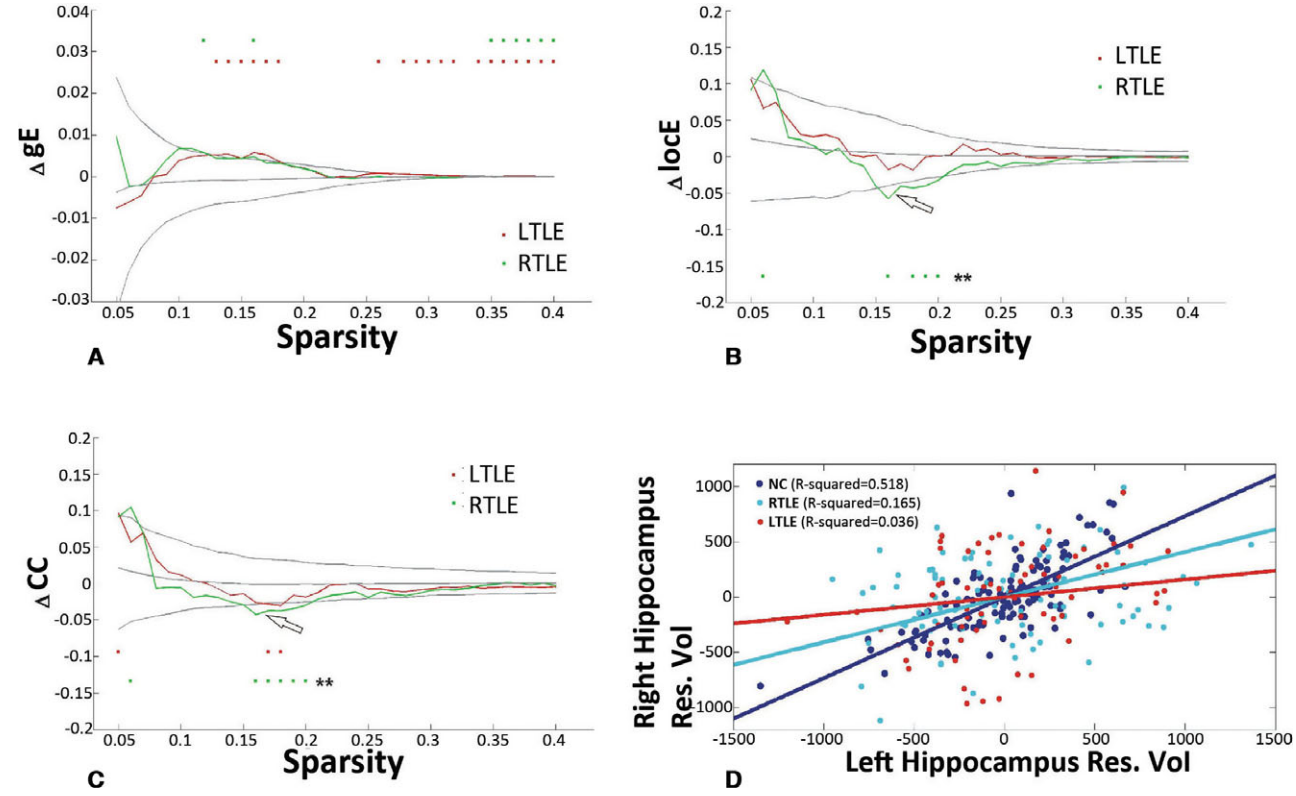

Figure 1.

Between-group differences (controls vs. patients) in global efficiency (gE) (**A**), local efficiency (locE) (**B**), and clustering coefficient (CC) (**C**) between the controls and both LTLE (red) and RTLE (green) as a function of sparsity thresholds. The gray lines represent the mean values and 95% confidence intervals of the between-group differences obtained for 1,000 permutation tests at each sparsity value. The red/green dots indicate statistically significant ($p < 0.05$) difference in gE, locE, and CC between the controls and the two groups. $\Delta > 0$/ $\Delta < 0$ indicates a decrease/increase in the network properties of TLE patients relative to controls. Note that both TLE patient groups show decreased gE; however, only RTLE shows significant increased locE and CC (arrows) over a wider range of sparsity. (**D**) Interregional correlation between bilateral hippocampus volumes (residual) in controls (purple), LTLE (red), and RTLE (blue). Controls (adjusted R^2 0.518, $p = 5e\text{-}20$) and RTLE (adjusted R^2 0.165, $p = 0.0003$) demonstrated significant linear correlation, as LTLE displayed only a trend (adjusted R^2 0.0355, $p = 0.045$).

(Table S3, Fig. 3). As expected, part of the nodes with altered BC coincided with the new hubs in both groups.

Discussion

We applied graph theoretical analysis to a macroscale brain structural network constructed from correlations among cortical and subcortical volumes. The inclusion of key structures such as hippocampus and amygdala yielded a more complete network analysis, given the importance of these structures in the pathophysiology of TLE. The main strength of this study is the novelty of the network analysis approach, which can provide information that cannot be obtained with a conventional structural data analysis such as VBM. Network analysis applied a systematic model for the description of complex brain networks because it supports both segregated and integrated information process. Graph theory analysis of anatomic covariance relies on morphologic correlations of GM volume.[7] Although neurobiologic mechanisms of such correlations remain unknown, structural covariance analyses have been applied to investigate development, aging, and other diseases.[7] In normal brain, homotopic regions preserve GM morphologic correlations,[23] allowing brain areas to organize into highly efficient networks, combining global and local efficiency. Because chronic TLE typically presents widespread, asymmetric GM atrophy,[24] one can expect abnormal GM correlations, yielding atypical network connections, and finally reducing network efficiency. For example, increased local efficiency in RTLE might be indicative of increased integration of local regions, thus providing a compensational mechanism that is associated with disrupted connectivity between long-distance regions, whereas LTLE showed impairments in both segregation and integration competences. It also allowed us to identify/confirm a pathologic distribution of hubs (paralimbic/limbic/temporal) in both TLE groups, pro-

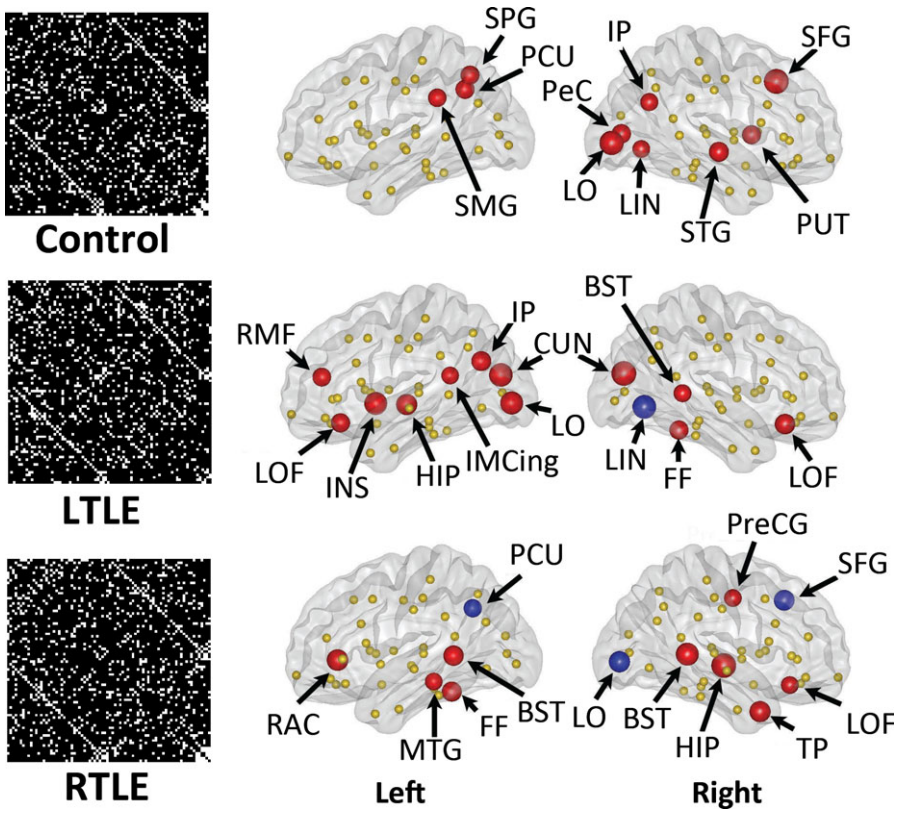

Figure 2.
Left: volumetric networks of control, LTLE, and RTLE groups at a network sparsity of 0.16. Right: Distribution of hub regions (red and blue dots with their sizes indicating their relative betweenness centrality) of control, LTLE, and RTLE brain volumetric networks according to regional betweenness centrality (mean + 1 SD) for all groups, whereas blue dots indicate overlapping hubs between controls and patient groups. Note that controls present widespread distribution of hub nodes, while both LTLE and RTLE concentrate the hub nodes mainly in the temporal lobe and limbic/paralimbic regions. For both groups of patients, the atrophic hippocampus became hub.
Epilepsia © ILAE

viding evidence of which regions are more vulnerable as a result of anatomic abnormality in the epileptic brain network.

By comparing a large group of patients and controls, we observed that both patient groups had altered network efficiency, disrupted cortical/subcortical connectivity, and different hub distributions. In addition to characterizing subtle differences between RTLE and LTLE, there was evidence showing more severe abnormalities in the LTLE group.

Network properties in TLE

Compared to controls, TLE groups demonstrated small-world configuration with significantly decreased gE, with LTLE presenting decreases over a much wider sparsity range (Fig. 1A). Because decreased gE reflects an abnormal, increased distance between nodes, we infer that long-range communications in the network are slower (and consequently less efficient) than in controls. Previous studies with TLE have shown decreased global network efficiency in functional networks,[25] anatomic networks using fiber tractography,[5,14] and structural networks using morphologic measurements.[12,20] Of interest, Bernhardt et al.[12] were able to identify only a trend of increased mean path length (decreased gE) across network densities (5–40%) for both LTLE and RTLE groups. The discrepancy between this result and ours might be attributed to the inclusion of important subcortical regions and larger number of subjects in our study (they analyzed 122 TLE patients and 47 controls), which could increase the statistical power.

The examination of localized properties such as clustering coefficient (CC) and local efficiency (locE) provides information regarding the level of local connectedness within a network, as a high level of local organization is associated with high levels of clustering. The association of decreased gE with increased CC suggests a more regular network organization, characterized by increased network efficiency within clusters of nodes, although marked by reduced global efficiency in information transmission between them.[5,26] Our results have suggested better locE for RTLE, as we observed significant increases in both locE and CC for RTLE between network sparsity of 0.15–0.2, whereas LTLE showed only a trend in the CC over a narrow sparsity range (Fig. 1B,C). These alterations suggest that greater local connectivity is associated with poor integration with remote areas for RLTE, whereas LTLE presents disadvantages of both segregation and integration. In the framework of GM covariance, we speculate that widespread GM atrophy triggers abnormal nodal development, combined with nonstandard edge formation, thereby increasing the length (reducing global efficiency). It is possible that compensational increases in local connectivity arising in RTLE require some degree of GM/WM integrity (for appropriate linkage of nodes), which is not possible in LTLE.[27,28]

The results are in accordance with recent studies of resting-state fMRI[19] and anatomic covariance networks that

Table 2. Significant changes of interregional correlations in LTLE and RTLE groups ($p < 0.003$), compared to controls

LTLE		r (NC)	r (LTLE)	z-Score
Left-hippocampus	Right-hippocampus	0.72	0.22	4.79[a]
Left-amygdala	Right-amygdala	0.69	0.36	3.23
Left-hippocampus	Right-cuneus	0.09	−0.36	3.21
Left-pallidum	Right-rostral anterior cingulate	0.1	−0.34	3.17
Left-precentral	Left-cuneus	0.28	−0.19	3.35
Left-entorhinal	Right-superior temporal	0.21	−0.26	3.33
Left-isthmus cingulate	Right-lateral orbitofrontal	0.19	−0.27	3.26
Left-superior temporal	Right-insula	0.25	−0.2	3.15
Left-lingual	Right-medial orbitofrontal	0.27	−0.17	3.13
Left-putamen	Left-lateral orbitofrontal	−0.24	0.22	−3.23
Left-putamen	Right-lateral orbitofrontal	−0.29	0.33	−4.45
Left-insula	Left-entorhinal	−0.09	0.4	−3.52
Right-lingual	Right-cuneus	0.1	0.52	−3.3
Right-putamen	Right-lateral orbitofrontal	0.3	0.23	−3.8
Right-isthmus cingulate	Right-superior parietal	−0.3	0.19	−3.47
RTLE		**r (NC)**	**r (RTLE)**	**z-Score**
Right-hippocampus	Left-hippocampus	0.72	0.22	3.01
Right-amygdala	Left-precuneus	0.1	−0.35	2.99
Right-postcentral	Left-superior frontal	0.12	−0.45	3.86
Right-middle temporal	Right-fusiform	0.14	−0.45	4.03
Right-superior frontal	Left-insula	0.32	−0.12	2.96
Right-supramarginal	Right-rostral anterior cingulate	0.2	−0.26	3.06
Right-lingual	Right-inferior parietal	0.01	−0.43	3.04
Right-postcentral	Left-thalamus-proper	−0.12	0.33	−3.01
Left-precuneus	Left-amygdala	0.18	−0.33	3.39
Left-precentral	Left-cuneus	0.28	−0.2	3.17
Left-superior parietal	Left-pars triangularis	0.17	−0.29	3.06
Left-supramarginal	Left-inferior parietal	−0.17	0.36	−3.5
Left-superior frontal	Left-pars opercularis	−0.01	0.47	−3.39
Left-supramarginal	Left-bankstss	−0.26	0.31	−3.83
Left-superior frontal	Left-rostral anterior cingulate	−0.26	0.24	−3.33

NC, normal controls; bankstss, banks of the superior temporal sulcus; r, Pearson correlation coefficient.
Gray cells show decrease, while the others show increase.
[a]FDR-corrected.

described increased CC in TLE groups.[12,29] Using the same framework of anatomic covariance in children with epilepsy, a recent study also detected an increase in CC.[20] Graph-theory analysis of other imaging modalities have yielded different results such as decreased CC observed in functional[30] and anatomic networks[31] of patients with TLE. The divergences observed across different studies are likely to result from various connectivity measurements (e.g., fMRI, DTI, and EEG), as different imaging modality may represent different underlying brain systems.

The differences between network efficiency of LTLE, RTLE, and controls suggest that the epileptogenic process may affect LTLE and RTLE patients in different ways, as RTLE tends to be less severely affected. A recent study of whole-brain DTI connectivity described a more severe pattern of loss of connectivity for the LTLE group.[9] Structurally, the pattern of abnormalities in LTLE could be related to a more severe and widespread pattern of abnormalities in both GM/WM.[4] From the functional perspective, these findings could also be associated with the more severe cognitive dysfunction observed in LTLE.[32] The mechanisms underlying more severe, bilateral structural changes in LTLE are not fully understood. Brain functional asymmetry starts in fetal stages with hand preference[33] and is later reinforced by language development.[34] Structural asymmetries are also apparent,[35] including a great number of large pyramidal cells within language-associated regions of left temporal lobe,[36] as well as deeper left central sulcus for right handers (and vice versa for left-handers).[37] Based on the evidence of brain asymmetry, it is tempting to speculate that the left (dominant) temporal lobe develops stronger ipsilateral and contralateral connections than the right temporal lobe. Following this hypothesis, the development of hippocampal sclerosis in the dominant hemisphere would be expected to trigger a more widespread disruption of network properties through either deafferentation or cytotoxic damage.[27,28] Thus, it is logical to expect a greater disorganization in the volumetric brain network of LTLE, as patients with RTLE demonstrated less severity in the loss of global efficiency associated with a better compensational mechanism based on increased locE and CC.

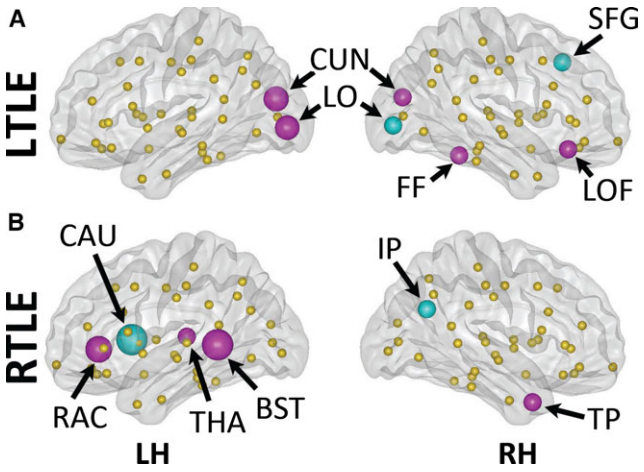

Figure 3.
Results from comparisons of BC between TLE groups and controls. Nodes with increased centrality were located primarily in occipital lobes for (**A**) LTLE patients and (**B**) contralateral limbic/temporal–associated areas for RTLE patients. It is notable that some of these nodes coincided with the new hubs for each group. Node sizes indicate differences in nodal centrality between controls and LTLE/RTLE patients. Pink dots, increased centrality; blue dots, decreased centrality; LH, left hemisphere; RH, right hemisphere; CUN, cuneus; LO, lateral occipital; FF, fusiform; LOF, lateral orbitofrontal; SFG, superior frontal gyrus; CAU, caudate; THA, thalamus, BST, banks of superior temporal gyrus; IP, inferior parietal; TP, temporal pole; RAC, rostral anterior cingulate.

The more regularized network topologic configuration (decreased gE and increased CC) we identified in TLE suggests a derangement of long-distance integrative ability,[7] which could be secondary to a complex interaction of factors. For example, the impact of antiepileptic drugs on brain[38] could interact with GM/WM tissue abnormalities.[15] In addition, episodes of acute, repeated regularization of functional networks reported after analysis of seizures recorded with scalp EEG[39] and intracranial recordings[40] could initiate a chronic process of remodeling structural networks.[6] Given the impact of global efficiency of functional brain networks on cognitive performance,[41] we are tempted to speculate that less-efficient brain networks detected in TLE are associated with cognitive impairment observed in these patients.

Pathologic changes in hub node distribution of TLE patients

Brain regions (nodes) are subject to incoming (afferent) and outgoing (efferent) connections; those strategically positioned in the highly traversed short-paths of network possess a high number of connections and are classified as *hubs*.[8] *Hubs* possess high topological value, are critical for efficient interactions, and are, consequently, associated with both integrative information processing and adaptive behavior.[8] Recently, hubs have been considered "biologically costly," associated with longer spatial distance edges (compared to distance edges connecting peripheral nodes) and may have higher metabolic rate, especially for cortical hubs.[8] The combination of high topological value with its biologic cost makes them vulnerable to several disease processes.[8]

We investigated changes associated with key subcortical structures (nodes) such as the hippocampus. In contrast to controls, which displayed hub nodes distributed over hemispheres with most in association cortices, TLE patients presented a distribution of hubs concentrated in the paralimbic/limbic and temporal association cortices as previously described.[12,42] The normal distribution of hubs is apparently disrupted in both groups, which may result in an impaired flow of information through the whole brain network. The novel finding is that, given the large sample, each ipsilateral atrophic hippocampus became hubs for RTLE and LTLE. The recent analysis of a small sample of 44 MTLE patients with resting state MEG data also showed changes in the pattern of network hubs, which included left hippocampus for left MTLE.[42] These findings are in accordance with previous studies that report an association between pathologic hubs (or areas with highly synchronized areas) and the capacity of the epileptogenic zone to generate seizures.[43] More specifically, Palmigiano et al.[40] analyzed intraoperative electrocorticography (ECoG) from TLE patients, and identified stable interictal synchronization clusters in both mesial and lateral areas of ipsilateral temporal lobe, suggesting a central role for these highly synchronized clusters in the epileptic network. As well, a recent study demonstrated a "hub-concentrated lesion distribution" for right and left TLE, emphasizing the idea that more valuable hubs are vulnerable and more likely to be anatomically abnormal in some brain pathologies.[8]

Another possible factor involved in the patterns of hubs for TLE may relate to the abnormal anatomic connectivity between extratemporal and temporolimbic regions.[44] This idea is supported by the report of an increased connectivity within the limbic network, associated with reduction of efficiency and increased nodal degree of the ipsilateral hippocampus.[45]

It is also interesting to note the lack of precuneus as a hub in TLE patients, given that it is a pivotal hub in most control networks. This finding is consistent with a recent DTI study of LTLE,[5] which also showed that the left precuneus was a non-hub. Another study revealed that reduction in GM of the left precuneus was associated with decreases in resting state functional connectivity in LTLE.[46] These findings are supported by the findings from an fMRI network analysis in TLE,[47] in which some hubs (including areas of the default mode network [DMN] such as, posterior cingulate and precuneus) became less prominent. It is also possible that the reduced functional connectivity in the DMN of TLE patients[3] can be related to the decrease of connectivity of precuneus, which prevented it from being classified as a

hub. These concordant findings provide more evidence in support of a loss of significance for the precuneus in the TLE brain networks.

We also examined alteration in regional centrality between patients and controls and identified different patterns for LTLE (regions with increased centrality were mostly located in occipital lobes) and RTLE (areas with increased centrality were in contralateral limbic/temporal associated areas), whereas most of these nodes coincided with the new hubs. These findings indicate a disruption of the normal pattern of short paths in TLE, with deviations toward occipital and temporal lobes, suggesting local excess connectivity, which may impair the normal flow of information through nodes in the global network. Increased functional connectivity within visual cortex has been reported,[48] as well as in the contralateral temporal lobe,[3] in patients with TLE. Our findings of increased centrality in those regions could be indicative of an underlying compensational mechanism in TLE structural brain networks.

Changes in interregional connections in TLE

The investigation of interregional morphometric correlations revealed differences between TLE groups and controls. For the interpretation of these interregional correlations, it is important to note that the existence of fibers directly connecting regions is not mandatory as, similar to functional correlation, the morphometric correlation may result from an indirect connection facilitated by a third party.[7] Changes in covariance patterns may result from a complex combination of different factors such as genetic influence, maturational and aging effects, reciprocal trophic reinforcement, and pathologic processes.[6,7,10]

Based on the hippocampal correlation obtained from controls, and from the studies of covariance in human cortex,[23,49] our findings suggest that patients with TLE have decreased homotopic effect for the hippocampi. In addition to a more severely decreased hippocampal correlation in the LTLE group, LTLE patients had decreased connectivity between the amygdalae and in other cross-hemispheric correlations. RTLE had decreased cross-hemispheric correlations, along with several ipsilateral and contralateral connections. Because functional and structural brain networks seem to be closely related,[50] it is not surprising that the reductions identified in TLE coincide with other studies that used different modalities for graph theory and connectivity analysis. A recent DTI study, revealed "bi-hemispheric network pathology" for both groups, but pointed to a more severe reduction of connectivity in LTLE as well as increased disconnection degree for ipsilateral hippocampus in both groups.[9] Similarly, a functional connectivity study in TLE[51] described a disruption of cross-hemispheric networks, with reduction of connectivity between right and left hippocampus. In accordance with these results, other recent studies also showed decreased functional interhemispheric connectivity, not only between hippocampi, but also with temporal neocortex.[52]

Increases in interregional correlations identified in the limbic system have been observed previously.[45] A recent DTI study revealed a pathologic increase in connectivity within the ipsilateral mesial and limbic structures as well as in the contralateral hemisphere, which was associated with persistent seizures after surgery.[53] We also identified increased interregional correlations in the contralateral hemisphere, more specifically in the RTLE group, which is in accordance with a resting state functional connectivity study of TLE patients[54] that reported decreased basal functional connectivity in both hemispheres, but an increase almost exclusively in the contralateral temporal lobe.

Limitations

Limitations are related to the method itself, as the origins of neuroanatomic covariance at cellular and molecular levels are not completely characterized.[7] Plausible mechanisms involve interactions between genetic influence (i.e., the importance of genes in cortical/gyri development), mutual trophic factors modeling synapses (leading to synchronized maturation), and environmental-related plasticity.[7,55] Recent studies suggest that patterns of anatomic covariance are less correlated to patterns of WM connections,[56] but more closely related to patterns of functional connectivity[57] in which some areas can be strongly correlated without direct, structural connection.[58] Future studies of structural covariance may investigate the complex interactions between genetic influence, experiential factor, and environmental impact (such as initial precipitating injury) in epilepsy. The combination of innovative and advanced techniques such as 3D immunohistochemistry and digital histology may further the comprehension of mechanisms underlying the anatomic covariance at the macroscopic level.[7]

One other limitation of this study is that currently there is no suitable approach for analyzing the correlation between binarized brain morphologic network properties and clinical measurements, which is one of the most important aspects of clinical imaging studies due to the binary and sparsity dependent nature of the network analysis. In our future work, we plan to overcome these limitations by using a weighted network analysis, which allows us to describe the network properties without thresholding, and more importantly, alterations of the network properties in patients with different clinical measurements.

Conclusions

We used the structural covariance from regional GM volumes[7] to perform graph theory analysis of a large group of TLE patients and controls. The novelty of this study was the inclusion of subcortical structures in our model of the whole

brain connectome, which allowed us to disclose particular features of network reorganization, thereby differentiating LTLE and RTLE. With a comprehensive approach, more severe abnormalities in LTLE were observed, including an accentuated disruption of homotopic effect for hippocampi. The impact of altered network efficiency on cognition and mood dysfunction is of great importance and will be explored in a future analysis.

Acknowledgments

This study was supported by the Canadian Institute of Health Research (DWG, CB, and CLY); CEPID–FAPESP: "The Brazilian Institute of Neuroscience and Neurotechnology (BRAINN)," grant 2013/07559-3; and Brazilian National Council for Scientific and Technological Development (CNPq)–(GCB).

Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Cendes F, Andermann F, Gloor P, et al. Atrophy of mesial structures in patients with temporal lobe epilepsy: cause or consequence of repeated seizures? *Ann Neurol* 1993;34:795–801.
2. Coan AC, Campos BM, Yasuda CL, et al. Frequent seizures are associated with a network of gray matter atrophy in temporal lobe epilepsy with or without hippocampal sclerosis. *PLoS ONE* 2014;9:e85843.
3. Voets NL, Beckmann CF, Cole DM, et al. Structural substrates for resting network disruption in temporal lobe epilepsy. *Brain* 2012;135:2350–2357.
4. Kemmotsu N, Girard HM, Bernhardt BC, et al. MRI analysis in temporal lobe epilepsy: cortical thinning and white matter disruptions are related to side of seizure onset. *Epilepsia* 2011;52:2257–2266.
5. Liu M, Chen Z, Beaulieu C, et al. Disrupted anatomic white matter network in left mesial temporal lobe epilepsy. *Epilepsia* 2014;55:674–682.
6. Bernhardt BC, Hong S, Bernasconi A, et al. Imaging structural and functional brain networks in temporal lobe epilepsy. *Front Hum Neurosci* 2013;7:624.
7. Evans AC. Networks of anatomical covariance. *NeuroImage* 2013;80:489–504.
8. Crossley NA, Mechelli A, Scott J, et al. The hubs of the human connectome are generally implicated in the anatomy of brain disorders. *Brain* 2014;137:2382–2395.
9. Besson P, Dinkelacker V, Valabregue R, et al. Structural connectivity differences in left and right temporal lobe epilepsy. *NeuroImage* 2014;100C:135–144.
10. Sporns O. The human connectome: a complex network. *Ann N Y Acad Sci* 2011;1224:109–125.
11. Richardson MP. Large scale brain models of epilepsy: dynamics meets connectomics. *J Neurol Neurosurg Psychiatry* 2012;83:1238–1248.
12. Bernhardt BC, Chen Z, He Y, et al. Graph-theoretical analysis reveals disrupted small-world organization of cortical thickness correlation networks in temporal lobe epilepsy. *Cereb Cortex* 2011;21:2147–2157.
13. Sporns O, Tononi G, Kotter R. The human connectome: a structural description of the human brain. *PLoS Comput Biol* 2005;1:e42.
14. Vaessen MJ, Jansen JF, Vlooswijk MC, et al. White matter network abnormalities are associated with cognitive decline in chronic epilepsy. *Cereb Cortex* 2012;22:2139–2147.
15. Yasuda CL, Morita ME, Alessio A, et al. Relationship between environmental factors and gray matter atrophy in refractory MTLE. *Neurology* 2010;74:1062–1068.
16. Coan AC, Kubota B, Bergo FP, et al. 3T MRI quantification of hippocampal volume and signal in mesial temporal lobe epilepsy improves detection of hippocampal sclerosis. *AJNR Am J Neuroradiol* 2014;35:77–83.
17. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically parcellating the human cerebral cortex. *Cereb Cortex* 2004;14:11–22.
18. van Diessen E, Zweiphenning WJ, Jansen FE, et al. Brain network organization in focal epilepsy: a systematic review and meta-analysis. *PLoS ONE* 2014;9:e114606.
19. Haneef Z, Levin HS, Chiang S. Brain graph topology changes associated with anti-epileptic drug use. *Brain Connect* 2015;5:284–291.
20. Bonilha L, Tabesh A, Dabbs K, et al. Neurodevelopmental alterations of large-scale structural networks in children with new-onset epilepsy. *Hum Brain Mapp* 2014;35:3661–72.
21. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci* 2009;10:186–198.
22. Chiang S, Haneef Z. Graph theory findings in the pathophysiology of temporal lobe epilepsy. *Clin Neurophysiol* 2014;125:1295–1305.
23. Mechelli A, Friston KJ, Frackowiak RS, et al. Structural covariance in the human cortex. *J Neurosci* 2005;25:8303–8310.
24. Coan AC, Campos BM, Bergo FP, et al. Patterns of seizure control in patients with mesial temporal lobe epilepsy with and without hippocampus sclerosis. *Arq Neuropsiquiatr* 2015;73:79–82.
25. Vlooswijk MC, Vaessen MJ, Jansen JF, et al. Loss of network efficiency associated with cognitive decline in chronic epilepsy. *Neurology* 2011;77:938–944.
26. Bernhardt BC, Bonilha L, Gross DW. Network analysis for a network disorder: the emerging role of graph theory in the study of epilepsy. *Epilepsy Behav* 2015;50:162–70.
27. Coan AC, Appenzeller S, Bonilha L, et al. Seizure frequency and lateralization affect progression of atrophy in temporal lobe epilepsy. *Neurology* 2009;73:834–842.
28. Bonilha L, Rorden C, Halford JJ, et al. Asymmetrical extra-hippocampal grey matter loss related to hippocampal atrophy in patients with medial temporal lobe epilepsy. *J Neurol Neurosurg Psychiatry* 2007;78:286–294.
29. Bernhardt BC, Bernasconi N, Hong SJ, et al. Subregional mesiotemporal network topology is altered in temporal lobe epilepsy. *Cereb Cortex* 2015 Jul 28 [Epub ahead of print].
30. Vlooswijk MC, Jansen JF, de Krom MC, et al. Functional MRI in chronic epilepsy: associations with cognitive impairment. *Lancet Neurol* 2010;9:1018–1027.
31. Vaessen MJ, Hofman PA, Tijssen HN, et al. The effect and reproducibility of different clinical DTI gradient sets on small world brain connectivity measures. *NeuroImage* 2010;51:1106–1116.
32. Fernandes DA, Yasuda CL, Lopes TM, et al. Long-term postoperative atrophy of contralateral hippocampus and cognitive function in unilateral refractory MTLE with unilateral hippocampal sclerosis. *Epilepsy Behav* 2014;36:108–114.
33. Hepper PG. The developmental origins of laterality: fetal handedness. *Dev Psychobiol* 2013;55:588–595.
34. Chiron C, Jambaque I, Nabbout R, et al. The right brain hemisphere is dominant in human infants. *Brain* 1997;120(Pt 6):1057–1065.
35. Sun T, Walsh CA. Molecular approaches to brain asymmetry and handedness. *Nat Rev Neurosci* 2006;7:655–662.
36. Hutsler JJ. The specialized structure of human language cortex: pyramidal cell size asymmetries within auditory and language-associated regions of the temporal lobes. *Brain Lang* 2003;86:226–242.
37. Amunts K, Schlaug G, Schleicher A, et al. Asymmetry in the human motor cortex and handedness. *NeuroImage* 1996;4:216–222.
38. Pardoe HR, Berg AT, Jackson GD. Sodium valproate use is associated with reduced parietal lobe thickness and brain volume. *Neurology* 2013;80:1895–1900.
39. Schindler KA, Bialonski S, Horstmann MT, et al. Evolving functional network properties and synchronizability during human epileptic seizures. *Chaos* 2008;18:033119.
40. Palmigiano A, Pastor J, Garcia de Sola R, et al. Stability of synchronization clusters and seizurability in temporal lobe epilepsy. *PLoS ONE* 2012;7:e41799.

41. Giessing C, Thiel CM, Alexander-Bloch AF, et al. Human brain functional network changes associated with enhanced and impaired attentional task performance. *J Neurosci* 2013;33:5903–5914.
42. Jin SH, Jeong W, Chung CK. Mesial temporal lobe epilepsy with hippocampal sclerosis is a network disorder with altered cortical hubs. *Epilepsia* 2015;56:772–9.
43. Wilke C, Worrell G, He B. Graph analysis of epileptogenic networks in human partial epilepsy. *Epilepsia* 2011;52:84–93.
44. Concha L, Beaulieu C, Gross DW. Bilateral limbic diffusion abnormalities in unilateral temporal lobe epilepsy. *Ann Neurol* 2005;57:188–196.
45. Bonilha L, Nesland T, Martz GU, et al. Medial temporal lobe epilepsy is associated with neuronal fibre loss and paradoxical increase in structural connectivity of limbic structures. *J Neurol Neurosurg Psychiatry* 2012;83:903–909.
46. Holmes MJ, Yang X, Landman BA, et al. Functional networks in temporal-lobe epilepsy: a voxel-wise study of resting-state functional connectivity and gray-matter concentration. *Brain Connect* 2013;3:22–30.
47. Liao W, Zhang Z, Pan Z, et al. Altered functional connectivity and small-world in mesial temporal lobe epilepsy. *PLoS ONE* 2010;5:e8525.
48. Zhang Z, Lu G, Zhong Y, et al. Impaired perceptual networks in temporal lobe epilepsy revealed by resting fMRI. *J Neurol* 2009;256:1705–1713.
49. Seeley WW, Crawford RK, Zhou J, et al. Neurodegenerative diseases target large-scale human brain networks. *Neuron* 2009;62:42–52.
50. Kramer MA, Cash SS. Epilepsy as a disorder of cortical network organization. *Neuroscientist* 2012;18:360–372.
51. Morgan VL, Abou-Khalil B, Rogers B. Evolution of functional connectivity of brain networks and their dynamic interaction in temporal lobe epilepsy. *Brain Connect* 2015;5:35–44.
52. Maccotta L, He BJ, Snyder AZ, et al. Impaired and facilitated functional networks in temporal lobe epilepsy. *Neuroimage Clin* 2013;2:862–872.
53. Bonilha L, Helpern JA, Sainju R, et al. Presurgical connectome and postsurgical seizure control in temporal lobe epilepsy. *Neurology* 2013;81:1704–1710.
54. Bettus G, Bartolomei F, Confort-Gouny S, et al. Role of resting state functional connectivity MRI in presurgical investigation of mesial temporal lobe epilepsy. *J Neurol Neurosurg Psychiatry* 2010;81:1147–1154.
55. Alexander-Bloch A, Giedd JN, Bullmore E. Imaging structural co-variance between human brain regions. *Nat Rev Neurosci* 2013;14:322–336.
56. Gong G, He Y, Chen ZJ, et al. Convergence and divergence of thickness correlations with diffusion connections across the human cerebral cortex. *NeuroImage* 2012;59:1239–1248.
57. Segall JM, Allen EA, Jung RE, et al. Correspondence between structure and function in the human brain at rest. *Front Neuroinform* 2012;6:10.
58. Honey CJ, Sporns O, Cammoun L, et al. Predicting human resting-state functional connectivity from structural connectivity. *Proc Natl Acad Sci USA* 2009;106:2035–2040.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Parcellation of 80 cortical and subcortical regions and their abbreviations (1–40: left hemisphere, 41–80: right hemisphere).

Table S2. Characterization of hub nodes for each group. RTLE presents three hubs in common with the control group, whereas LTLE has only one hub in common.

Table S3. Regions with increased and decreased centrality in patients with TLE, as compared to normal controls. LTLE presented increased centrality in nodes located mostly in the occipital areas, whereas those from RTLE were located in the contralateral limbic/temporal regions.

Data S1. Supplementary methods.