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## Brain networks in people after a first unprovoked seizure

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

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## Abstract

**Background:** A single unprovoked seizure occurs in up to 10% of the population. Some develop epilepsy, but the majority do not. Brain network changes are observed in people with epilepsy, but it is unknown if they are present after this first seizure. This study examines network connectivity after the first seizure to determine if any changes exist.

**Methods:** Twelve patients after a single unprovoked seizure and twelve age- and sex-matched healthy controls were recruited. All underwent 7T resting-state fMRI scanning. Whole brain and limbic, default mode and salience network connectivity were analyzed with graph theory.

**Results:** Baseline characteristics were similar between groups. No network connectivity differences were observed between groups.

**Conclusions:** No network connectivity differences were found between patients and controls. This suggests that there are not inherent connectivity differences predisposing an individual to seizures; however, the small sample size and considerable variability could prevent realization of small group differences.

**Keywords:** first seizure, resting-state fMRI, graph theory, functional connectivity, epilepsy

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## List of Abbreviations

AAL: automated anatomical labelling

aCompCor: anatomical component-based noise correction

ART: quality assurance and artefact rejection software

BOLD: blood oxygen level dependent signal

CAE: childhood absence epilepsy

CMRO<sub>2</sub>: cerebral metabolic rate of oxygen

CSF: cerebrospinal fluid

DMN: default mode network

DTI: diffusion tensor imaging

EEG: electroencephalogram

EEG-fMRI: combined use of EEG and fMRI

fALFF: fractional amplitude of low frequency fluctuations

FC: functional connectivity

FDR: false discovery rate

fMRI: functional MRI

GGE: genetic generalized epilepsies

ICA: independent components analysis

IED: interictal epileptiform discharge

IGE: idiopathic generalized epilepsy

ILAE: International League Against Epilepsy

JME: juvenile myoclonic epilepsy

MEG: magnetoencephalography

MRI: magnetic resonance imaging

NBS: network based statistic

PCC: posterior cingulate cortex

PWE: people/person with epilepsy

ReHo: regional homogeneity

ROI: region of interest

rs-fMRI: resting-state fMRI

TLE: temporal lobe epilepsy

T: Tesla

## Preface

This thesis is submitted in a manuscript form, with chapter 2 constituting a publication-ready manuscript of this research. Chapters 1 and 3 provide additional background information and discussion not required for a single publication, and Appendix A also has additional information regarding some of the fMRI pre-processing methods.

Appendix B represents an analysis performed at the request of my advisory committee, and did not form part of the main research project. The data had been previously collected, and I used what was available as an analysis-validation procedure. This analysis was intended to assist in determine whether the negative results seen in the study population (first seizure) were truly negative, or merely a result of the analysis pipeline. It was assumed that given the pathology seen in temporal lobe epilepsy, analysis of this data should translate into significant differences between groups and suggest that the negative results in my study population were, in fact, real.

# Chapter 1

## Introduction

Epilepsy is one of the most common chronic neurological diseases, with 1 in every 26 people experiencing epilepsy at some point in their lifetime<sup>1</sup>. The hallmark feature of epilepsy is recurrent unprovoked seizures. Many other cognitive and psychological features can be prominent in people with epilepsy (PWE). However, the occurrence of a single seizure does not impart a diagnosis of epilepsy.

## 1.1 Seizures and Epilepsy

Up to 10% of the general population will have a single unprovoked seizure in their lifetime<sup>2</sup>, but less than half of these individuals will have recurrent seizures fulfilling a diagnosis of epilepsy<sup>3</sup>. After a second seizure, the risk of having a third seizure increases to 76%<sup>4</sup>.

Seizures must occur greater than 24 hours apart, as multiple seizures within a 24 hour time period does not increase the risk of developing epilepsy<sup>5</sup>.

It is also important to distinguish unprovoked seizures from provoked seizures, which occur in the presence of an acute identifiable cause, which can include toxins, medications, or metabolic disturbances such as hyponatremia, hypoglycaemia or hypomagnesemia, to name just a few<sup>3</sup>. These factors can decrease the seizure threshold, making an individual more prone to have a seizure. Seizures occurring in this context are not considered epilepsy as they

do not have the same risk of recurrence: although they may recur in a similar context, in the absence of this provoking feature, the person is not considered to be at higher risk of unprovoked seizures<sup>3</sup>. Acute symptomatic seizures, which occur in the context of an acute brain injury, such as stroke, intracerebral haemorrhage, central nervous system infection or trauma, also have a different natural history. These patients have an increased risk of mortality in the first 30 days following the seizure, compared to individuals with a single unprovoked seizure, but long-term recurrence risk and mortality were lower for the acute symptomatic group compared to the unprovoked group<sup>6</sup>.

### 1.1.1 Diagnosis of Epilepsy

Epilepsy is typically diagnosed after a person has two unprovoked seizures<sup>7</sup> due to the very high risk of further seizures (76%)<sup>4</sup>. However, if a person has a greater than 60% chance of seizure recurrence within the next 10 years, a diagnosis of epilepsy may be given after a single seizure<sup>7</sup>. This typically occurs if an epilepsy syndrome can be diagnosed clinically or if the ancillary investigations, usually electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) demonstrate epileptogenic abnormalities. However, the presence of interictal epileptiform discharges (IEDs) on EEG or an epileptogenic lesion on MRI does not automatically meet criteria for a diagnosis of epilepsy, and other circumstances should be considered before making a diagnosis of epilepsy<sup>7</sup>.

### 1.1.2 Classification of Epilepsy

There are many different types of epilepsy, all with different symptoms, disease trajectories and prognosis. The International League Against Epilepsy (ILAE) has recently updated their classification of seizures and epilepsy. Seizures can be generalized, originating in both cortical hemispheres, focal, where a limited area of cortex is involved in generating the seizure, or of unknown onset<sup>8</sup>. Similarly, the type of epilepsy a person has can be generalized, focal, generalized and focal, or unknown. This epilepsy type may be further classified into a particular epilepsy syndrome, such as Temporal Lobe Epilepsy (TLE), Sleep-Related Hypermotor Epilepsy<sup>9</sup>, or genetic generalized epilepsy (GGE)<sup>7</sup>. A select group of GGE can also be referred to as idiopathic generalized epilepsy (IGE)<sup>8</sup>. Numerous etiologies of epilepsy should also be considered in the classification, and include structural, metabolic, autoimmune, genetic, infectious and unknown<sup>8</sup>.

### 1.1.3 Treatment of Epilepsy

Once epilepsy is diagnosed, the standard of treatment is antiseizure medications to prevent recurrent seizures. As not everyone who has a single unprovoked seizure will develop epilepsy, treatment is not generally started until a diagnosis of epilepsy is made. Although early treatment after a single seizure increases the time to seizure recurrence, it does not change chances of seizure freedom over the long term<sup>10</sup>. Treatment with antiseizure medications is not without risks, and there are both short and long term side effects that should be considered before prescribing medication



after a single seizure. Seven to 31% of people will experience side effects from antiseizure medications, and while most of the time are mild, they can be more severe<sup>10</sup>. This is particularly important to consider when the cause is unknown, at the seizure recurrence risk in these patients is only 17% at 20 months<sup>11</sup>.

#### 1.1.4 Epilepsy Comorbidities

In addition to recurrent, unprovoked epileptic seizures, epilepsy is also recognized to have neurobiological, cognitive, psychological, and social effects<sup>12</sup>, which can significantly impact someone's life. Some of the most common psychiatric comorbidities include mood disorders, with up to 60% of people experiencing depression<sup>13</sup> and concomitant anxiety disorders may occur in 73% of people with depression and epilepsy<sup>14</sup>. Cognitive difficulties are also prevalent, particularly with childhood onset epilepsy, and even occur in children with benign epilepsies that they grow out of<sup>15</sup>. Certain types of epilepsy, such as Lennox-Gastaut syndrome, have more significant cognitive impairments. Their cognitive outcomes sometimes depend on the age of onset of epilepsy, with younger age of onset often resulting in greater cognitive difficulties<sup>13</sup>. Memory deficits are the most commonly reported cognitive impairments; however, deficits have also been demonstrated in language, executive function, intelligence and visuospatial function<sup>16</sup>. Children with epilepsy can also have impaired social development, and adults with epilepsy can suffer from significant social consequences, such as unemployment, lower socioeconomic status and isolation<sup>15</sup>.

In one study after a single unprovoked seizure, no differences in quality of life between these patients and those with well-controlled epilepsy or hypertension were found<sup>17</sup>. After one year

of follow-up, a large number of patients still were fearful of having another seizure (17%) or felt that the one seizure had a moderate to extreme impact on their quality of life<sup>17</sup>. These patients also had greater healthcare utilization, with increased number of visits to their primary care providers, not including visits required for testing<sup>17</sup>.

## 1.2 Functional MRI

Functional MRI (fMRI) relies on the blood oxygen level dependent signal (BOLD) as a surrogate measure of neuronal function. The BOLD response results from the magnetic properties of haemoglobin: oxygenated haemoglobin is diamagnetic whereas deoxygenated haemoglobin is paramagnetic<sup>18</sup>. This variable property results in temporal changes to the magnetic field that can be imaged. This was first exploited to image the brain while performing tasks. Cerebral blood flow increases to areas of increased activity, and thus changes to concentration of deoxygenated haemoglobin can be visualized. Cerebral blood flow increases greater than the consumption of oxygen or cerebral metabolic rate (CMRO<sub>2</sub>), and as such areas that are activated by a particular task actually show a decrease in the amount of deoxyhaemoglobin as a result of the increased cerebral perfusion of the activated area<sup>18</sup>. In task-based fMRI, the subject is provided with a stimulus, and then performs a task, typically motor or cognitive, based on the stimulus<sup>19</sup>. The fluctuations in BOLD signal temporally related to the task represent cortical activations required to complete the task<sup>19</sup>.

### 1.2.1 Resting-state fMRI

The field of resting-state fMRI (rs-fMRI), or task-negative fMRI, was born when low frequency fluctuations (<0.1 Hertz) noted in task-based fMRI studies were first described in 1995 as having potential physiologic bases<sup>20</sup>. These low frequency fluctuations corresponded in morphology to those generated by a task, and believed to relate to functional connectivity. Strong physiological fluctuations were also observed in areas now referred to as the default mode network (DMN) at rest, but deactivated during tasks<sup>20,21</sup>. The DMN consists of the posterior cingulate cortex/precuneus, medial frontal lobes, inferior lateral parietal cortex and medial temporal lobes<sup>21</sup>. Since the initial studies, the field has exploded and these fluctuations are well accepted to be physiological in origin.

Resting-state fMRI is performed while the subject is at rest, rather than performing a particular task. The subject will lie awake in the scanner with the eyes open or closed, and is asked not to think of anything in particular. An fMRI sequence is obtained during this time, and generally lasts between five and seven minutes, but increasing duration results in improved reliability<sup>22</sup>.

## 1.3 Applying Network Theories to Neuroscience

Neuroscience has classically been thought of a field where discrete brain areas perform distinct functions<sup>23</sup>. However, over time and with greater understanding of the brain, distinct brain areas are thought to work in concert with other areas in a more dynamic and integrative manner, like a network. Despite this predominating sentiment, early neuroscientists have

been proponents of a network theory of brain function, particularly Ramón y Cajal, whose microscopy techniques proposed that neurons were distinct cells that contacted others via close synaptic connections<sup>24</sup>.

Regions in the brain are both structurally and functionally connected. While structural connections have been studied for many years through tract-tracing pathology studies, animal models, cortical thickness analyses and diffusion tensor imaging (DTI), functional networks are just beginning to be explored. Different brain areas communicate with each other, combining information from each of these areas to assist with complex cognitive tasks.<sup>25</sup> These functional communications form the basis of functional connectivity analyses.

Functional connectivity (FC) is the temporal dependency between neurophysiological events that are spatially distant<sup>25</sup>. With rs-fMRI, FC analyses reflect the relationships of BOLD activations of spatially distinct areas. Other means of studying functional networks include magnetoencephalography (MEG), EEG, and combined EEG-fMRI<sup>12</sup>.

One of the simplest, and most frequently used methods of measuring FC is with seed-based correlational analysis<sup>25</sup>. In this method, a brain region, or seed, is chosen, and the fMRI time course is correlated with all other areas of the brain. If there is a high correlation between the seed region and another region, they are said to be functionally connected.

Many other methods have emerged to provide additional analyses of brain network data, moving towards more hypothesis free, data-driven analysis as well as analysis of the overall topology of the brain network<sup>25</sup>. Independent component analysis (ICA) is one frequently

used to isolate different brain regions that are functionally connected. ICA determines spatially independent component that are linear combinations of the original fMRI signal<sup>25</sup>.

### 1.3.1 Brain Networks

A number of consistent resting-state networks have been identified. These anatomically distinct areas show strong FC during rest. Many of these correspond to known functional networks, such as motor, auditory, visual, and executive control<sup>25</sup>. A salience network consisting of anterior insula, dorsal anterior cingulate and dorsomedial thalamus has also been identified, which is thought to be involved in processing of emotional information<sup>26</sup>. However, the main network identified, and subsequently studied, is the DMN<sup>21</sup>.

## 1.4 Graph theory

Graph theory is a powerful method of analysing brain networks. Graph theory has only been applied to neuroscience over the last 20 years, and can be applied at any level of study from a microscopic cellular level, to a macroscopic cortical areas level<sup>24</sup>. The fundamental tenets of graph theory are that graphs can be represented as nodes and edges. Any network can be conceptualized as a graph, with the nodes representing the areas being analyzed (e.g. neurons, cortical regions) and edges representing the connections between nodes. This provides a simple and elegant, yet mathematically robust means of characterizing complex networks such as the brain.

In fMRI, nodes may be defined based on structural landmarks, such as cytoarchitecture or macroscopic landmarks (such as the motor cortex, sensory cortex, frontal eye fields, etc.),

voxels or random parcels of the same size<sup>24</sup>. Once the nodes of a network have been defined, a connectivity matrix of the network<sup>27</sup> is computed using the correlation coefficient between nodes.

### 1.4.1 Thresholding

Once the connectivity matrix has been formed, it is typically then thresholded. Brain graphs have been found to be not fully connected<sup>28,29</sup>, and thresholding attempts to decrease the number of spurious connections in the connectivity matrix<sup>24</sup>. The connectivity matrix is often binarized prior to performing network calculations, as this provides a simpler analysis<sup>27</sup>. Many measures can then be employed to determine the network connectivity.

The most common means of thresholding a connectivity matrix is weight-based, where a threshold,  $\tau$ , is selected and all values below  $\tau$  are given a value of zero<sup>24</sup>.

Thresholding is also often commonly performed using cost, which is also referred to as connection density. Cost ( $k$ ) is defined as

$$k = \frac{\mathcal{E}_\tau}{\frac{N(N-1)}{2}}; \quad 0 \leq k \leq 1 \quad (1)$$

where  $\mathcal{E}_\tau$  is the number of edges at threshold  $\tau$  and  $N$  is the number of nodes in the network<sup>30</sup>.

## 1.4.2 Graph Theory Measures

Graph theory provides measures of both functional segregation, or the ability of the brain to perform specialized processing within densely interconnected brain regions, and functional integration, or the ability to combine this specialized information from different brain regions<sup>27</sup>. Measures of functional segregation include local efficiency and clustering coefficient and measures of functional integration include global efficiency and path length.

The most basic measure in graph theory is degree. Degree refers to the number of connections a node has, defined as

$$k_i = \sum_{j \in N} a_{ij} \quad (2)$$

where  $N$  is the set of nodes ( $n$ ) within a network,  $a_{ij}$  portrays whether a link is present ( $a_{ij}=1$ ) or absent ( $a_{ij}=0$ )<sup>27</sup>.

Degree is one of many measures of centrality. Centrality refers to the importance of a node within a network, and important nodes will have many connections with other nodes, or high centrality. Betweenness centrality is one of the more sensitive measures of centrality and refers to the fraction of all the shortest paths in a network that travel through a particular node<sup>27</sup>. Nodes with high betweenness centrality often link distant parts of a network<sup>27</sup>.

Betweenness centrality ( $b$ ) can be calculated as

$$b_i = \frac{1}{(n-1)(n-2)} \sum_{\substack{h, j \in N \\ h \neq i, i \neq j, j \neq h}} \frac{\rho_{hj}(i)}{\rho_{hj}} \quad (3)$$

where  $\rho_{hj}$  is the number of shortest paths between nodes  $h$  and  $j$  and  $\rho_{hj}(i)$  determines the paths of  $\rho_{hj}(i)$  which also pass through node  $i$ .

Shortest path length, from which the characteristic (average) path length is derived, refers to the shortest geodesic distance ( $g_i \leftrightarrow j$ ) between two nodes<sup>27</sup>.

$$d_{ij} = \sum_{a_{uv} \in g_i \leftrightarrow j} a_{uv} \quad (4)$$

The average path length ( $L$ ) can then be calculated as follows:

$$L = \frac{1}{n} \sum_{i \in N} L_i = \frac{1}{n} \sum_{i \in N} \frac{\sum_{j \in N, j \neq i} d_{ij}}{n-1} \quad (5)$$

Where  $L_i$  is the average distance between node  $i$  and all the other network nodes<sup>27</sup>.

Global efficiency ( $E$ ), or the total efficiency of a network, is the inverse of the characteristic path length<sup>27</sup>, or

$$E = \frac{1}{n} \sum_{i \in N} L_i = \frac{1}{n} \sum_{i \in N} \frac{\sum_{j \in N, j \neq i} d_{ij}^{-1}}{n-1} \quad (6)$$

The number of triangles ( $t$ ) is required to determine clustering coefficient. This refers to a group of adjacent nodes that are connected to each other and form a triangle<sup>27</sup>.

$$t_i = \frac{1}{2} \sum_{j, h \in N} a_{ij} a_{ih} a_{jh} \quad (7)$$

The clustering coefficient ( $C$ ), measuring how well connected a particular node is to surrounding nodes, can then be calculated<sup>27</sup>

$$C = \frac{1}{n} \sum_{i \in N} C_i = \frac{1}{n} \sum_{i \in N} \frac{2t_i}{k_i(k_i-1)} \quad (8)$$

where  $C_i = 0$  for  $k_i < 2$ .



Local efficiency ( $E_{loc}$ ), or regional efficiency of a subset of network nodes, is similar to the inverse of the clustering coefficient

$$E_{loc} = \frac{1}{n} \sum_{i \in N} E_{loc\ i} = \frac{1}{n} \sum_{i \in N} \frac{\sum_{j, h \in N, j \neq i} a_{ij} a_{ih} [d_{jh}(N_i)]^{-1}}{k_i(k_i-1)} \quad (9)$$

where  $d_{jh}(N_i)$  is the shortest path distance between  $j$  and  $h$  which only contains neighbours of  $i$ .

These measures can be applied for each node within the network, referred to as nodal-level measures, or to the brain or network of interest as a whole, where the measures for each node are averaged across the whole brain.

## 1.5 Epilepsy and Networks

The concept of epilepsy being a network disease is not new<sup>31,32</sup>, with the classic representation of seizures being an abnormal state of hypersynchronization in a circuit of neurons<sup>33</sup>. Previously, epilepsy was dichotomized into generalized or focal types, representing a generalized or localized process by which seizures arose<sup>34</sup>. More modern classifications of the epilepsies recognize the network nature of the disease and define focal epilepsies as originating in networks localized to one hemisphere and generalized epilepsies as originating in bilateral networks<sup>34</sup>. This classification recognizes network concepts in epilepsy that have emerged over the last 20 years as network theories become more broadly applied within neuroscience.

Many different types of epilepsies have been explored with network analyses of functional connectivity, most commonly TLE<sup>35-39</sup>, but also GGE<sup>40-44</sup>, extratemporal focal epilepsies<sup>45-47</sup>, epileptic encephalopathies such as Lennox-Gastaut syndrome<sup>48</sup> and benign childhood epilepsies, such as Benign Epilepsy with Centro-Temporal Spikes<sup>49,50</sup>. The types of connectivity alterations differ depending on the type of epilepsy, but functional connectivity changes have been seen in all populations with epilepsy.

Focal epilepsy is the most frequently studied group of epilepsies, and amongst the focal epilepsies, TLE is the most frequently studied disease. Although the GGEs are also common, they are not studied as frequently as TLE, possibly because they are usually well controlled with medications. However, GGE represents an important classification of epilepsy, and is distinct from focal epilepsies. The IGE's of JME, childhood absence epilepsy (CAE) and generalized epilepsy with tonic-clonic seizures only are the most common GGEs studied. The network studies outlined below will focus on these two entities.

## 1.5.1 Network Changes in Temporal Lobe Epilepsy

### 1.5.1.1 Functional Connectivity Measures

As TLE is the most common focal epilepsy, the most research has been performed in this population. Findings have not all been in agreement as to the distinct abnormalities found in TLE, but there are a wide variety of methodologies and modalities used. Hemispheric connectivity has been found to be altered depending on the laterality of the temporal lobe of onset. For example, one study of patients with right mesial TLE found that functional

connectivity was decreased in the ipsilateral (right) hemisphere, but increased in the contralateral (left) hemisphere<sup>51</sup>. The findings of decreased functional connectivity ipsilateral to the seizure focus are fairly stable across studies, which have mostly performed seed-based correlational analyses of the mesial temporal structures<sup>22,52</sup>. These changes may be more severe in patients with left compared to right TLE<sup>53</sup>. It has been suggested that the increased connectivity found in the contralateral hemisphere may reflect compensatory mechanisms<sup>54</sup>.

On a network level, a variety of different resting-state networks have been found to be altered in TLE, including the default mode, limbic, sensorimotor and thalamic networks<sup>22</sup>. In particular, the DMN has been found to have decreased connectivity<sup>51,55</sup>, particularly the hippocampi<sup>55</sup>. Component maps of the DMN generated from patients with TLE using ICA demonstrated smaller or absent clusters in the medial and lateral temporal cortex ipsilateral to the seizure focus<sup>40</sup>.

### 1.5.1.2 Graph Theory Measures

As with FC analyses, the results from graph theory studies of TLE have been quite variable. There was a meta-analysis of graph theory studies in patients with TLE included studies on both functional and structural networks: three rs-fMRI, three EEG, two MEG, two diffusion tensor imaging (DTI) and two based on cortical thickness of structural MRI. This meta-analysis demonstrated an increased average path length (nine studies) and clustering coefficient (12 studies) in the TLE group when analyzing the whole brain network<sup>56</sup>. Other graph theory measures were not explored in this analysis. Since this meta-analysis, additional studies have also examined additional graph theory measures and found decreased local

efficiency<sup>57</sup>, and decreased centrality on a variety of measures<sup>35,57,58</sup>. In particular, one study found the precuneus and posterior cingulate cortex (PCC) were weaker hub regions compared to controls<sup>57</sup>. Unlike the meta-analysis, two recent studies found decreased clustering coefficient in patients compared to controls<sup>35,58</sup>.

## 1.5.2 Network Changes in Idiopathic/Genetic Generalized Epilepsies

### 1.5.2.1 Functional Connectivity Measures

In the IGEs, abnormalities have principally been demonstrated in thalamocortical connections, particularly with the prefrontal cortex<sup>42,59</sup>, which agrees with the traditional concept that IGEs result from abnormal cortico-thalamic connections<sup>60</sup>.

Connectivity between the thalamus and prefrontal cortex was decreased in these studies<sup>42,59</sup>.

Another study in CAE performed a seed-based analysis with seeds located in the thalamus and medial occipital cortex<sup>42</sup>. The functional connectivity map from the seed in the thalamus was less extensive in the patients than controls, but greater for patients than controls when the seed was placed in the medial occipital cortex.

DMN abnormalities have also found to be prominent in IGE. In one study CAE, decreased connectivity was found in the DMN, cognitive control network and affective network<sup>61</sup>. The DMN abnormalities seen are similar to those in seed-based analyses, with decreased connectivity seen in the medial prefrontal cortex<sup>61,62</sup>. The DMN changes are greater in patients with TLE compared to those with IGE<sup>44</sup>.

### 1.5.2.2 Graph Theory Measures

Idiopathic generalized epilepsies have not been extensively studied with graph theory.

Patients with CAE demonstrated decreases in many network level measures including global and local efficiency, and absolute connection strength and clustering coefficient<sup>63</sup>.

Corresponding nodal measures, particularly in the orbitofrontal areas, were also decreased in patients with CAE. In patients with JME, no global network differences were found between patients and controls, but on the nodal level, nodal efficiency was increased in the left postcentral gyrus<sup>43</sup>. Another study in patients with generalized tonic-clonic seizures only found a decreased clustering coefficient for the whole network<sup>64</sup>.

### 1.5.3 Network Changes and Duration of Epilepsy

In general, the extent of network changes occurring in PWE worsens with the duration of disease, as well as frequency of seizures. This was demonstrated in people with TLE<sup>65,66</sup> and idiopathic generalized epilepsy<sup>41,62</sup>. In TLE, cross-hemispheric connections were decreased with increased duration of seizures, starting approximately 5 years after diagnosis in one rs-fMRI functional connectivity study<sup>66</sup>. Another MEG FC study found that whole-brain connectivity was decreased in patients as a function of time in both patients with TLE and extratemporal lobe epilepsy<sup>65</sup>. In generalized epilepsy, results are not consistent between studies, but seed-based analyses demonstrated decreased functional connectivity with increasing duration of disease in between the PCC and frontal cortex<sup>62</sup>, the right medial frontal cortex and bilateral prefrontal areas, left medial prefrontal area and right dorsal prefrontal area<sup>41</sup>. Additionally there were also areas of increased functional connectivity with

increasing duration of disease in the PCC and bilateral anterior temporal lobes<sup>62</sup> and left prefrontal cortex and left supplementary motor area<sup>41</sup>.

#### 1.5.4 Network Changes after a First Seizure

There is only one study of FC after a first seizure, which found that there were differences in FC between those who went on to develop epilepsy and those who did not<sup>67</sup>. This EEG-based study demonstrated that patients who developed epilepsy had increased synchronization likelihood, an EEG measure of FC, in the theta band. When this activity was combined with the presence of IEDs, the overall classification accuracy was 75%, with a specificity of 91% and sensitivity of 58%<sup>67</sup>. The classification accuracy deteriorated to a specificity of 70%, sensitivity of 58% and overall accuracy of 61% when EEGs with IEDs were excluded and only changes in the theta-band were present. The decrease in sensitivity, specificity and accuracy found with the exclusion of IEDs is not surprising, as these patients are more likely to develop epilepsy, and based on the newest ILAE definition of epilepsy, would now be considered to have epilepsy<sup>7</sup>. As many of the abnormalities in epilepsy are paroxysmal, their absence could be thought to influence results; however, a recent EEG-fMRI study demonstrated that network alteration in BOLD signal did not significantly differ with and without the presence of IEDs<sup>68</sup>, suggesting these network abnormalities exist even in the absence of abnormal brain activity during the scan.

No other study of network connectivity has been published in the first seizure population. One rs-fMRI has been performed in this population<sup>69</sup>; however, a network analysis was not performed. This study recruited patients from a first seizure clinic, and performed rs-fMRI in

patients with at least one confirmed seizure as well as healthy controls. They included both patients after a first seizure, and those in whom epilepsy could be diagnosed at the initial clinic visit. Resting-state functional connectivity using fractional amplitude of low frequency fluctuations (fALFF) was found to be altered in patients with newly diagnosed epilepsy compared to patients after a first seizure and healthy controls. The fALFF method of analysis is a power spectral analysis of low frequency oscillations. The time series is Fourier transformed into spectral bands between 10-250 mHz. These low frequency oscillations are thought to represent neuronal activity, but is not completely understood, and their sensitivity is not clear at this time<sup>69</sup>. In this group, seven patients in the first seizure group subsequently developed epilepsy, and four of them appeared to have these altered oscillations in the slow-3 (73-198 mHz) subband. The authors thus proposed that fALFF could be a biomarker used to help identify seizure recurrence<sup>69</sup>. This study also performed a regional homogeneity (ReHo) analysis, which did not produce any significant results. ReHo is a data-driven approach that compares the similarity between neighbouring voxels, assuming that voxels in close proximity are similar, and this similarity may be altered in a disease state<sup>69</sup>.

In looking specifically at the single seizure patients versus controls, an overall increase in fALFF was found in patients, but did not reach statistical significance<sup>69</sup>. The authors noted there was a step-wise increase in the fALFF values at the slow-3 frequency from healthy controls to single seizure to new-onset epilepsy. Of note, only 35% of patients in the single seizure group had increased fALFF values.

## 1.6 Motivation for Current Study

A single unprovoked seizure is common, and a significant source of anxiety in individuals and family members following its occurrence. People generally want to know the cause of the seizure, and in the absence of abnormalities on the EEG or structural MRI, clinicians are unable to provide a satisfying answer. Functional MRI may provide another diagnostic avenue to pursue to determine abnormalities that may lead to a diagnosis of epilepsy, or to reassure patients that there is nothing “wrong” functionally in their brain.

This study attempts to address the question of whether there are any functional network alterations that occur in patients following the first unprovoked seizure using a graph theoretical approach. It is performed using the 7 Tesla MRI in order to take advantage of the increased signal-to-noise ratio while investigating a population where changes, if present, could be subtle and more difficult to detect with lower field imaging.



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## Chapter 2

### 2.1 Abstract

**Background:** Epilepsy is characterized by recurrent unprovoked seizures, and is one of the most common neurological disorders. However, a single unprovoked seizure occurs in 10% of the general population, and not all of these people develop epilepsy. While many changes in brain network connectivity have been observed in people with epilepsy, it is not known if they are present in individuals who have experienced a single seizure.

**Methods:** Patients who have experienced a single, unprovoked, generalized tonic-clonic seizure and are between the ages of 16 and 65 were recruited and age- and sex matched with healthy controls. Patients could not have known EEG or neuroimaging abnormalities. Participants underwent MRI neuroimaging at 7 Tesla, acquiring structural and resting-state functional images. Data were pre-processed, thresholded and analyzed using graph theory measures.

**Results:** Twelve patients and healthy controls were recruited. There were no differences in baseline characteristics. Network-level measures were not different between groups for whole brain, default mode, salience and limbic networks. No consistent nodal-level network changes were observed.

**Conclusions:** No previous study has compared network connectivity after a single unprovoked seizure to controls. No network-level differences were found between people

who have had a single seizure and those who have not. This suggests that changes in network connectivity seen in people with epilepsy are not present after a single unprovoked seizure, and occur with disease progression and recurrent seizures. There are not inherent differences in network connectivity predisposing people to a single seizure. However, the small sample size and considerable variability could prevent realization of small, but significant, group differences.

## 2.2 Introduction

A single epileptic seizure occurs in up to 10% of the general population<sup>1</sup>. An epileptic seizure occurs when there are transient clinical manifestations due to abnormally excessive or synchronous neuronal activity<sup>2</sup>. Some, but not all, of these people will go on to develop epilepsy. Epilepsy is typically diagnosed after two unprovoked seizures, but based on the most recent International League Against Epilepsy (ILAE) definition, it may be diagnosed after a single seizure if the person has a greater than 60% recurrence risk over ten years, which is similar to the recurrence risk after two or more unprovoked seizures<sup>2</sup>. This high recurrence rate occurs if the EEG demonstrates epileptiform discharges, the MRI shows an epileptogenic lesion, or an epilepsy syndrome can be diagnosed clinically<sup>2</sup>. In patients who do not fulfill a diagnosis of epilepsy after the first seizure, predicting which of these individuals will develop epilepsy is difficult. Overall, the risk of a second seizure is 42% at five years, but the recurrence risk is greatest early after the seizure with the risk being 24% at six months and 32% at one year<sup>1</sup>.

Brain networks can be studied with modern neuroimaging techniques such as functional magnetic resonance imaging (fMRI). Resting-state fMRI (rs-fMRI), which determines brain functional connectivity (FC) in the absence of a task, is particularly useful in this regard. Several networks have been identified in resting-state studies and include motor, visual, frontal, and default mode<sup>3</sup>. Of particular interest is the default mode network (DMN), which is active primarily in the absence of tasks. The DMN functionally links the posterior cingulate cortex, precuneus, mesial frontal region, inferior parietal area, hippocampus and parahippocampal regions<sup>4</sup>.

There are many methods of analyzing network data, and graph theory has emerged as a powerful and eloquent way of modeling brain networks. Complex networks such as the brain can be represented as graphs and graph theory measures subsequently used to determine connections between different regions. In graph theory, graphs are broken down into components called nodes and edges, which in functional neuroimaging represent brain regions and connections, respectively<sup>5</sup>. The number of edges associated with a node is called degree, and the relative importance of a node in the network is called betweenness centrality<sup>5</sup>. Global and local efficiency are measures of functional integration and segregation, respectively<sup>6</sup>. The clustering coefficient is similar to local efficiency, and represents the probability that neighbouring nodes are connected, whereas path length is similar to global efficiency, reflecting the distance travelled from one node to another<sup>7</sup>. Local and global efficiency are inversely related to clustering coefficient and path length, respectively, and thus a higher local efficiency results in a lower clustering coefficient.

Graph theory has been applied to studying epileptic networks with a variety of methods, including rs-fMRI. In temporal lobe epilepsy, a meta-analysis including 12 studies (three using rs-fMRI) demonstrated an overall regularization of brain networks, reflecting less efficient network functioning compared to a healthy person<sup>8</sup>. This was reflected through an increased clustering coefficient and average path length.

Many studies have demonstrated network connectivity decreases with longer duration of epilepsy, as well as with increased frequency of seizures<sup>9-11</sup>. However, there has only been

one study looking at network connectivity following a single unprovoked seizure<sup>12</sup>. This study examined synchronization likelihood, an EEG measure of functional connectivity, after a single unprovoked seizure and compared those who developed epilepsy to those who did not. Patients who developed epilepsy had increased synchronization likelihood in the theta band. When this activity was combined with the presence of interictal epileptiform discharges (IEDs), the overall classification accuracy for developing epilepsy or not was 75%, with a specificity of 91% and sensitivity of 58%<sup>12</sup>. It is worth noting, that with the 2014 ILAE definition of epilepsy<sup>2</sup>, the patients with IEDs would now likely be considered to have epilepsy based on those EEG findings. When the patients with IEDs were excluded the specificity of the increased theta band synchronization likelihood to predict development of epilepsy became 70%, sensitivity 58% and overall accuracy 61%<sup>12</sup>.

Recently, the first paper using rs-fMRI in patients after consultation in a first seizure clinic was published<sup>13</sup>. This study used rs-fMRI in patients with at least one confirmed seizure. They included both patients after a first seizure, and those in whom epilepsy could be diagnosed at the initial clinic visit. The fractional amplitude of low frequency fluctuations (fALFF) was found to be altered in patients with newly diagnosed epilepsy compared to patients after a first seizure and healthy controls<sup>13</sup>. The fALFF method of analysis is a power spectral analysis of low frequency oscillations. These low frequency oscillations are thought to represent neuronal activity, but is not completely understood, and their sensitivity is not clear at this time<sup>13</sup>. In the single seizure group, seven patients in the first seizure group subsequently developed epilepsy, and four of them appeared to have these altered

oscillations<sup>13</sup>. The authors thus proposed that fALFF could be a biomarker used to help identify seizure recurrence<sup>13</sup>.

As there is only one study of network connectivity following a single unprovoked seizure, this remains a largely untapped area of research in which additional information to predict development of epilepsy would be helpful. Previous studies have demonstrated that network changes occur early after the development of epilepsy, but not whether they are present at the beginning of the disease, or even before a diagnosis of epilepsy is made. Additionally, it is not known if there are any differences between people who have a single unprovoked seizure and those who have never had a seizure, or those who eventually develop epilepsy.

## 2.3 Materials and Methods

### 2.3.1 Subjects

Patients having experienced a single, unprovoked, generalized tonic-clonic seizure, as determined by a neurologist, with no other significant medical comorbidities were recruited for the study. Participants were excluded if they had experienced a previous seizure, regardless of cause; therefore, no person with a remote provoked or febrile seizure was included. Patients in whom a diagnosis of epilepsy could be made at the initial clinic visit or with ancillary testing (epileptogenic MRI lesion or epileptiform discharges on EEG) were also excluded. Patients were age- and sex-matched with healthy controls. Patients were followed for development of epilepsy.

### 2.3.2 Imaging Protocol

All participants underwent neuroimaging using a 7 Tesla MR scanner (Siemens Medical, Erlangen, Germany). High-resolution 3D T1-weighted sagittal anatomical images were obtained with a gradient echo (MP2RAGE) sequence with a 6000 ms repetition time (TR), 2.83 ms echo time (TE), 4° flip angle, 240 mm field of view (FoV), 0.8 mm isotropic, and 208 contiguous slices. The flat-divided T1-weighted images were used for analysis. Two resting-state fMRI sessions were acquired with an echoplanar imaging sequence (1250 ms TR, 20 ms TE, 208 mm FoV, 45° flip angle), 2 mm isotropic, 60 slices, 300 volumes, with multiband acceleration factor of 3, GRAPPA acceleration factor of 3 and 7/8 phase partial Fourier. Participants were instructed to remain awake with their eyes closed for the resting state sequences.

### 2.3.3 Image Processing

Images were pre-processed using SPM12 (UCL, UK) within the CONN toolbox<sup>14</sup>. Functional and structural images were realigned and normalized. The functional images were realigned using a 6-parameter rigid body spatial transformation. Additional pre-processing steps to improve functional connectivity analyses included ART-based scrubbing for motion correction and anatomical component-based noise correction method (aCompCor) to correct for noise. The ART-based scrubbing technique allows for additional artefact detection that improves the validity of RSFC analyses, as motion artefact has been shown to result in spurious RSFC changes<sup>14</sup>. In addition, aCompCor improves both the validity and sensitivity and specificity of FC analyses<sup>14</sup>. This is achieved through modelling



the influence of noise as a voxel-specific linear combination of multiple noise sources estimated from the variability in BOLD responses within noise ROIs<sup>14</sup>.

Structural and functional images were co-registered, then registered to standard MNI space. The data was smoothed using a Gaussian smoothing kernel of 4 mm full width half maximum. Each participant's images were segmented into 100 regions of interest (ROIs) using the Harvard-Oxford Atlas, and mean time courses for each ROI were extracted using the CONN toolbox<sup>14</sup>. Network-specific ROIs were also created using the CONN network ROI template, which consists of coordinates derived from ICA analyses performed with CONN of 497 subjects from the Human Connectome Project. Eight common resting-state networks are identified through these ROIs (DMN, salience network, sensorimotor network, frontoparietal network, dorsal attention network, visual network, and cerebellar network). We used the ROIs for the DMN and salience networks for our analyses, as these have been shown to be altered in people with epilepsy<sup>15,16</sup>. A limbic network was also created using 16 ROIs from the Harvard-Oxford atlas, which consisted of the ROIs for bilateral hippocampus, anterior and posterior parahippocampal gyrus, anterior and posterior cingulate, orbitofrontal, insula, thalamus and nucleus accumbens. These three networks were analyzed separately and combined as one. The combined network was created to decrease the variability of the smaller parcellations<sup>17</sup> while still limiting the analysis to the networks of interest.

### 2.3.4 Data Analysis

Group analyses were performed for the first seizure and control subjects using graph theory measures. Local efficiency, global efficiency, average path length, clustering coefficient and

betweenness centrality were determined for each group using the CONN toolbox<sup>14</sup>. To calculate these measures, the correlation coefficient between each pair of ROIs was determined to create a connectivity matrix. Adjacency matrices were obtained at a variety of thresholds, as there is no standard threshold accepted in the literature. We applied thresholds of 0.15 to 0.8 in increments of 0.01 to the adjacency matrices. Each adjacency matrix was used to obtain graph theory measures of functional connectivity.

Adjacency matrices were also calculated using cost, where the matrix is determined based on a fixed number of edges. As many graph theory network measures are dependent on the number of edges in a graph, this analysis ensures graphs patient and control group graphs are of equal density<sup>18</sup>. It has been shown that there can be differing intrinsic levels of connectivity between individuals and patient groups, which can lead to unequal comparisons and possible spurious results<sup>18</sup>. Cost adjacency matrices were also thresholded at costs of 0.15 to 0.8. Cost and degree were not calculated for these adjacency matrices, as these measures are used to determine the thresholding of the network.

All measures were determined on a network and nodal level. For nodal level results, only the whole brain and combined networks were analyzed as the smaller networks are more prone to fragmentation.

The two resting state sequences were analyzed separately. The first resting state sequence used for initial analysis, and then compared to second for validation of results.

### 2.3.5 Statistical Analysis

All patient characteristics and graph theory measures were compared between groups using a two-sample t-test to determine differences between groups. Corrections for multiple comparisons were performed using the false-discovery rate (FDR). For network level results, the FDR was calculated with each measure (7) and threshold (65). For nodal level results, FDR was calculated for each measure, threshold and network node (100 for whole brain, 27 for combined networks). Standard deviation was calculated and plotted for the graph theory results, and effect sizes were calculated for any significant results.

The Network-Based Statistic (NBS)<sup>19</sup> was also calculated for the whole brain and combined networks. The NBS is a complementary tool to correct for family-wise error in network analysis. It makes the assumptions that there are connections between regions, and thus provides increased power to detect differences between groups<sup>19</sup>.

## 2.4 Results

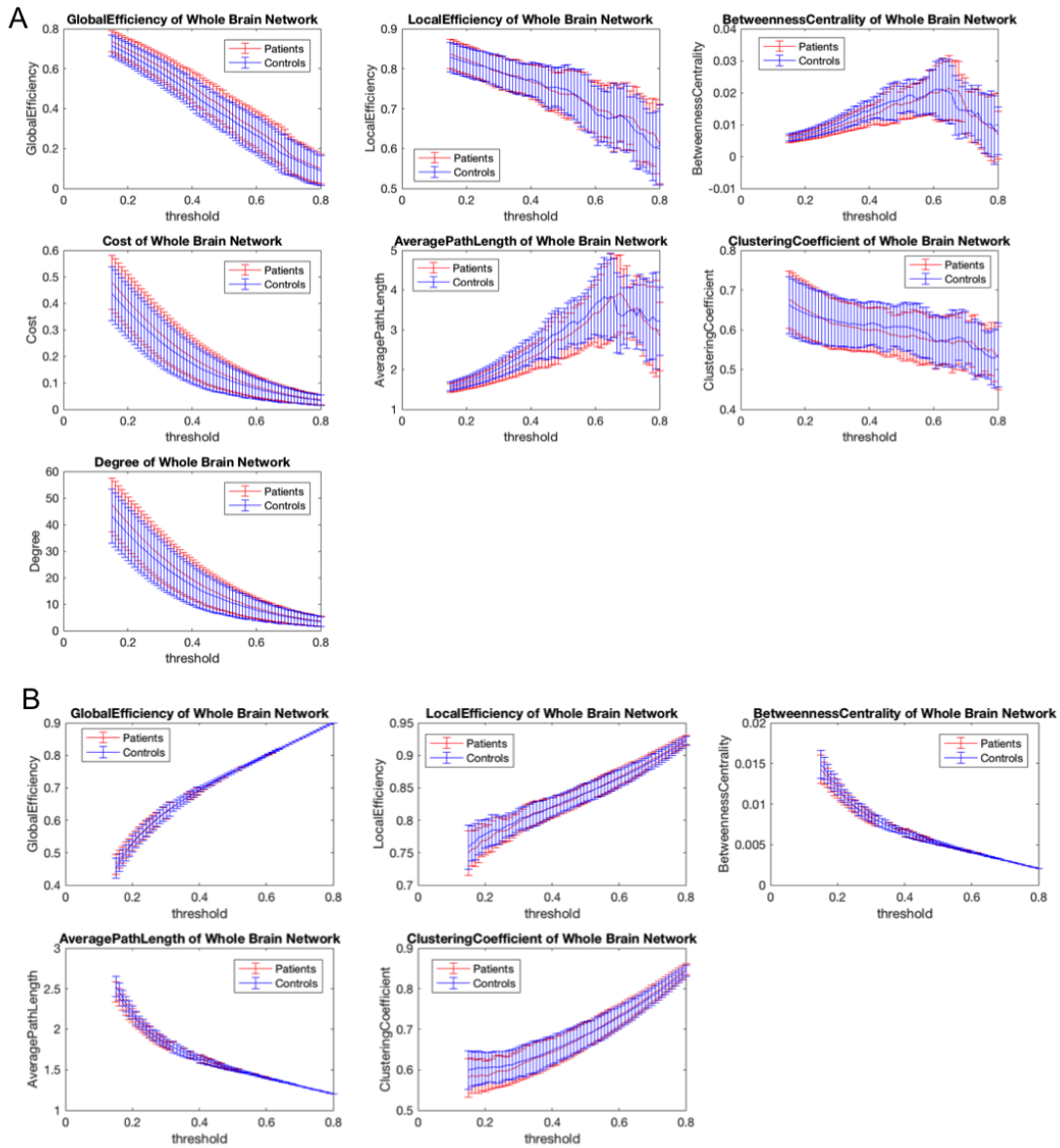
Twelve patients and twelve age- and sex-matched healthy controls were enrolled in the study between August 2016 and May 2017 (Table 2.1). There were no between group differences in demographic information. All patients participated in the study within 47 days of the seizure (mean 28 days). One patient developed epilepsy, which was diagnosed after a second EEG demonstrated generalized epileptiform discharges. The average duration of follow-up was 6.9 months.

**Table 2.1 Group Characteristics**

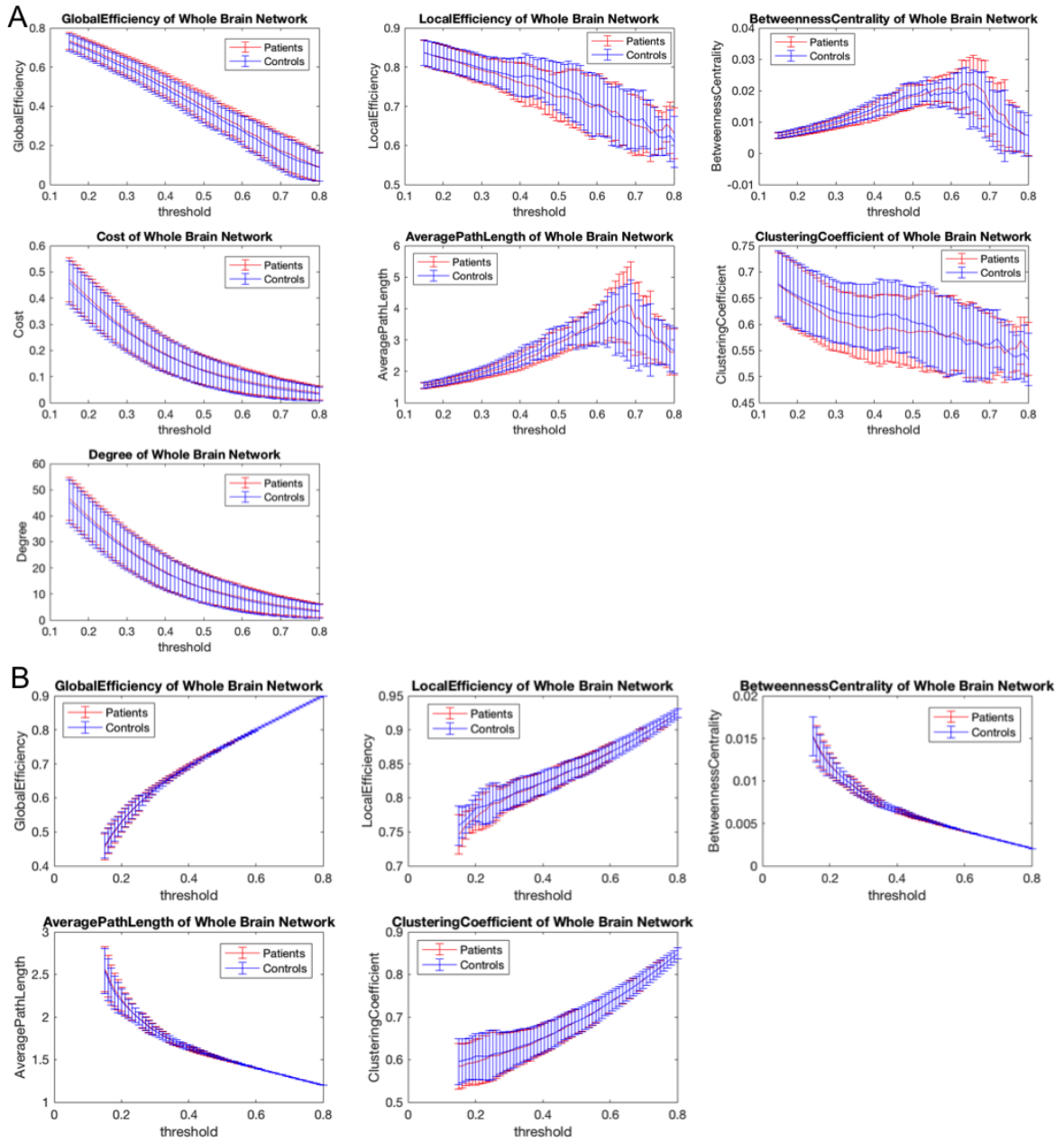
	First Seizure (n = 12)	Control (n = 12)	p
<b>Age (years, range)</b>	30.3 (18-56)	32.2 (21-59)	0.58
<b>Female (%)</b>	6 (50%)	6 (50%)	1
<b>Epilepsy</b>	1		
<b>Time from seizure to MRI (days)</b>	28 (6-47)		
<b>Time since seizure (months, range)</b>	6.9 (2-11)		

### 2.4.1 Global Connectivity Measures

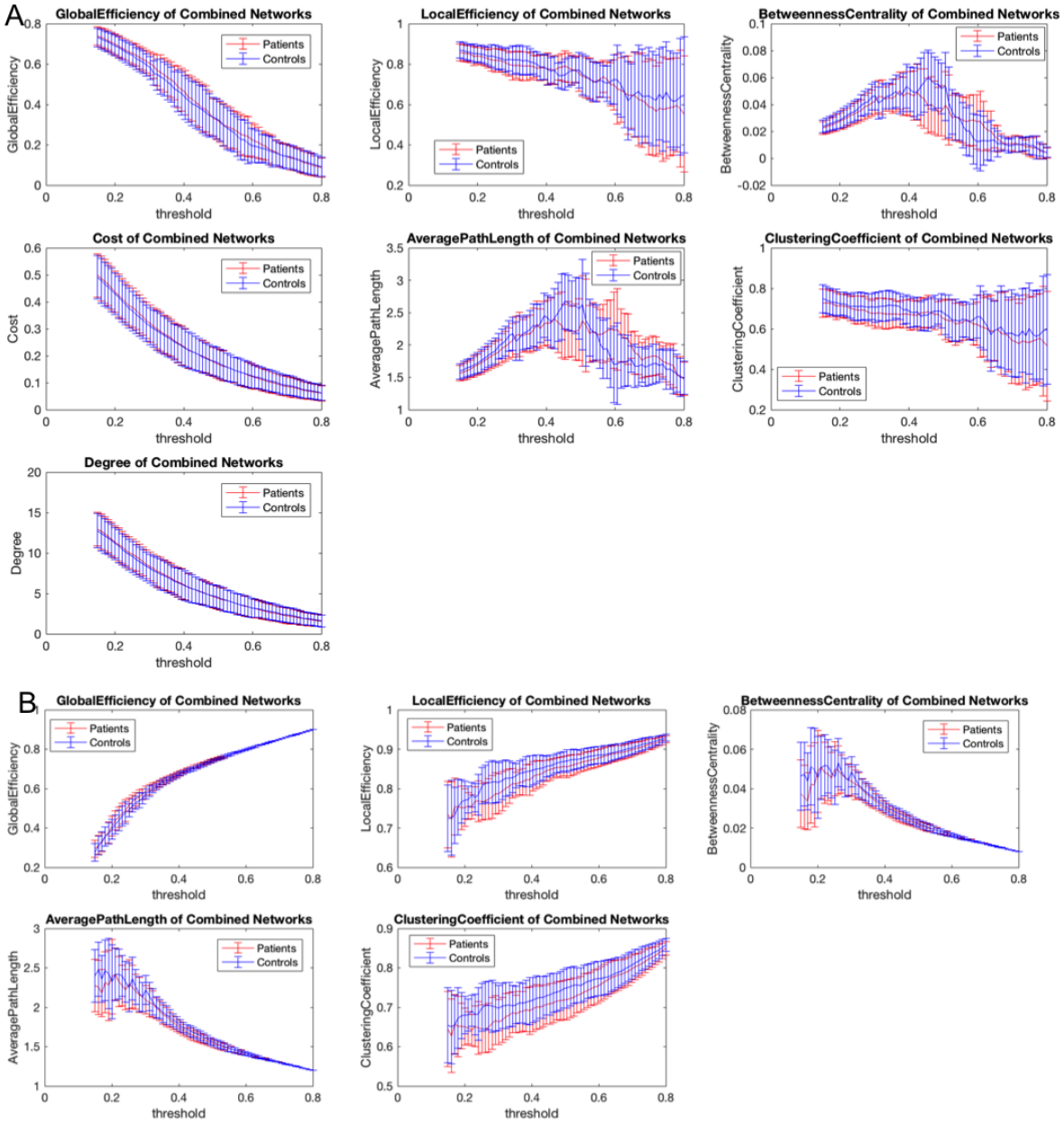
Global measures of whole-brain network connectivity were not different between patients and controls on any measure (Figure 2.1, Figure 2.2). On a functional network level, no significant differences occurred in the DMN, limbic or salience networks individually or combined (Figure 2.3, Figure 2.4). Additionally, no differences between groups were detected with the NBS.



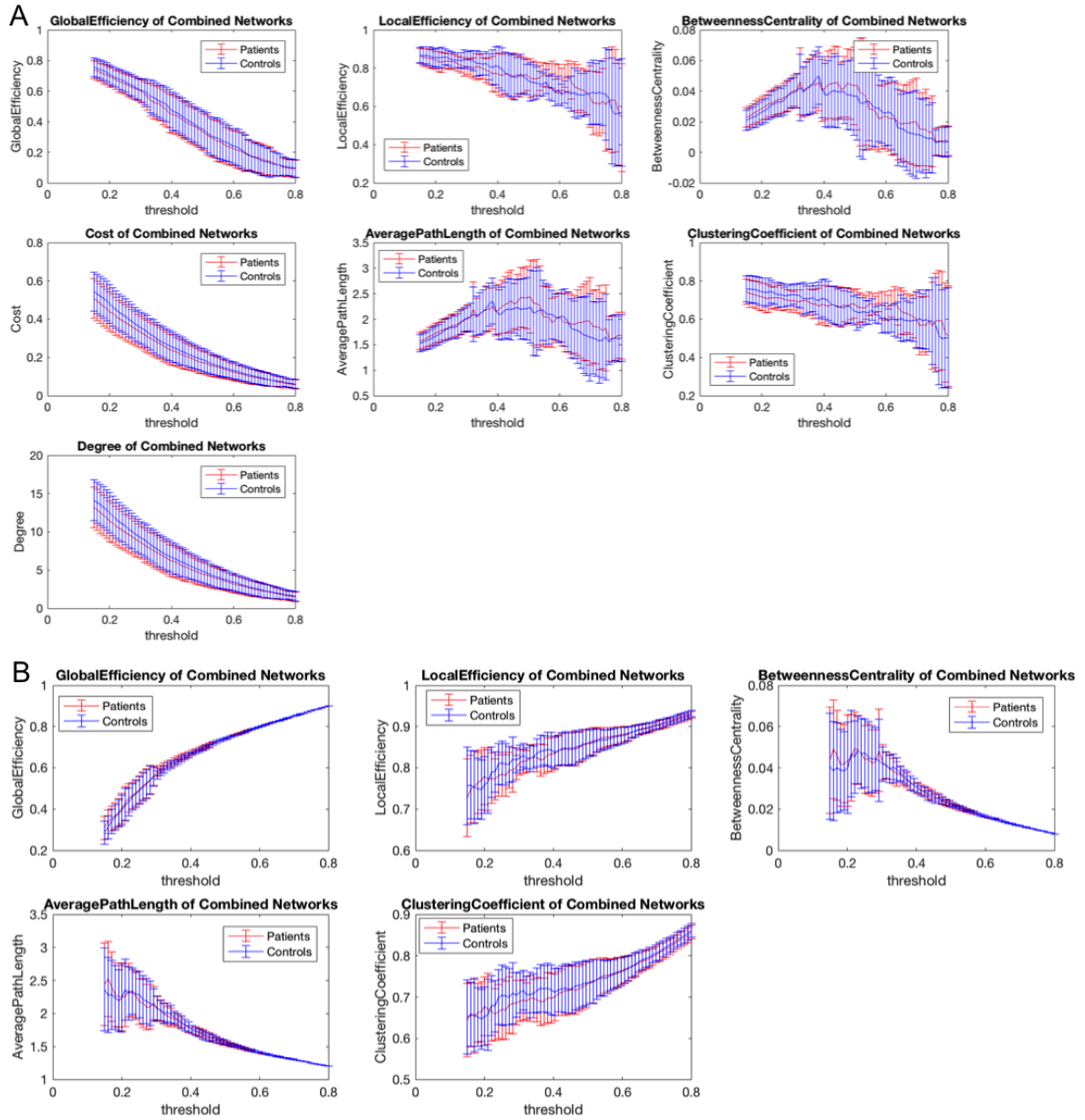
**Figure 2.1:** Network connectivity of the whole brain for the first resting-state sequence using correlation coefficient (A) and cost (B) to threshold.



**Figure 2.2:** Network connectivity of the whole brain for the second resting-state sequence using correlation coefficient (A) and cost (B) to threshold.



**Figure 2.3:** Network connectivity of the combined DMN, limbic and salience networks for the first resting-state sequence using correlation coefficient (A) and cost (B) to threshold.



**Figure 2.4:** Network connectivity of the combined DMN, limbic and salience networks for the first resting-state sequence using correlation coefficient (A) and cost (B) to threshold.

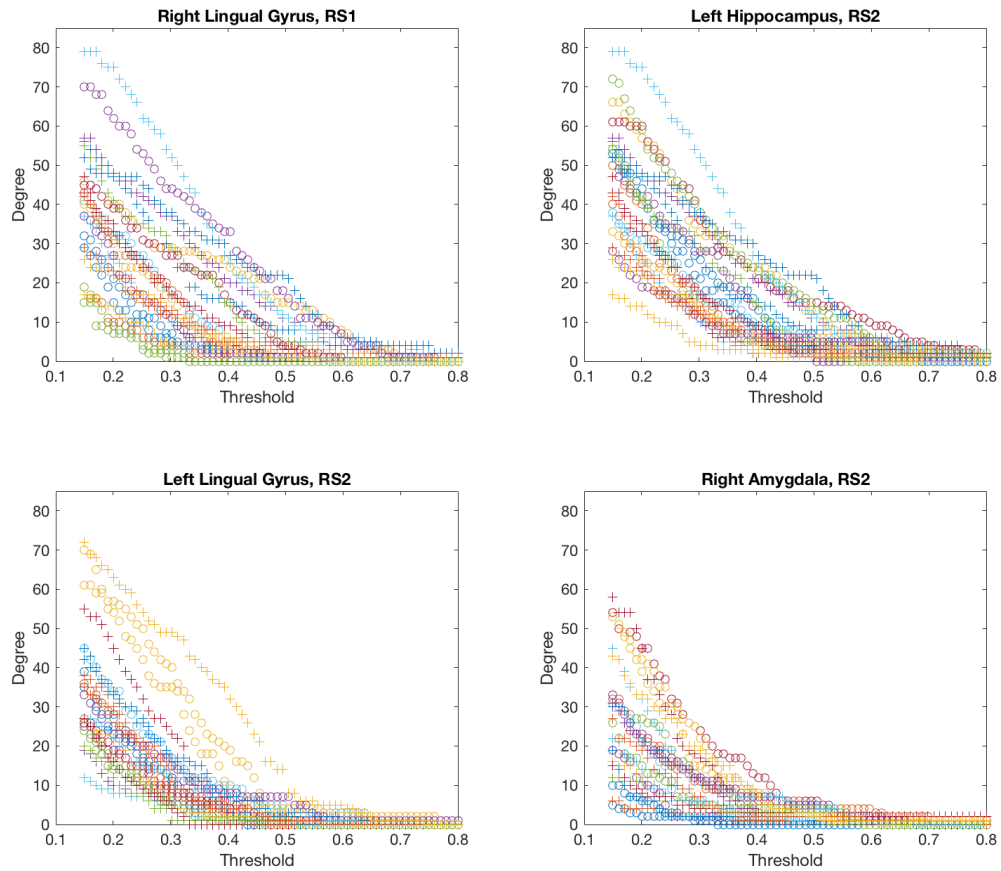


## 2.4.2 Nodal connectivity measures

Four individual nodal measures were significant after correction for multiple comparisons; however, most of these were limited to a single resting-state scan. Clustering coefficient and local efficiency were the only measures that were significantly different between patients and controls (**Table 2.2**). When examining these results more closely, clustering coefficient and local efficiency could not be calculated for most of the individual patients. After each node reached a threshold of approximately 0.4, multiple subjects had a degree of zero at the nodes with statistical significance (Figure 2.5). When the degree is zero, no connections exist between a node and a neighbouring node. Given that the majority of the subjects had a degree of zero after a threshold of 0.4, the statistically significant results obtained at higher thresholds are likely to be spurious.

**Table 2.2** Significant nodal level results for the whole brain network. All significant results were calculated with weight-based thresholding.

<b>Node</b>	<b>Measure</b>	<b>Threshold</b>	<b>p-unc</b>	<b>p-FDR</b>
<b>Scan 1</b>				
Right Lingual Gyrus	Local Efficiency	0.63	<0.0001	<0.0001
	Clustering Coefficient	0.61, 0.63	<0.0001	<0.0001
<b>Scan 2</b>				
Left Hippocampus	Local Efficiency	0.74-0.79	<0.0001	<0.0001
	Clustering Coefficient	0.74-0.79	<0.0001	<0.0001
Left Lingual Gyrus	Local Efficiency	0.6-0.61	<0.0001	<0.0001
	Clustering Coefficient	0.6-0.61	<0.0001	<0.0001
Right Amygdala	Local Efficiency	0.64	<0.0001	<0.0001
	Clustering Coefficient	0.64	<0.0001	<0.0001



**Figure 2.5:** Degree of nodes with significant clustering coefficient/local efficiency for patients (o) and controls (+), After thresholds of approximately 0.4, degree is zero in most subjects. Colours represent individual subjects.

## 2.5 Discussion

On a whole-brain level, no significant differences were seen between individuals after a first seizure or healthy controls. This suggests that there are no global alterations following a single unprovoked seizure. There were also no differences between groups in the combined networks. These networks were selected to be analyzed together as they are involved in epilepsy, and the combined analysis provided less fragmented graph theory measures over a

wider range of thresholds as more nodes were included in the analysis. The absence of differences suggests the interaction of these networks is not disturbed after a first seizure.

At the nodal level, a few nodes had significantly different clustering coefficient and local efficiency, but they lacked reproducibility, as they were different between resting-state scans. In addition, no significant differences were found between groups when using cost-based thresholding. Though statistically significant, many of the individual subject networks were fragmented in these analyses. Network fragmentation occurs when nodes within the network are disconnected and the network no longer functions as a unit<sup>20</sup>. Since no connections exist between some nodes, measures such as clustering coefficient and path length become infinity<sup>21</sup>. Network fragmentation typically occurs with very sparse networks, with low cost or high correlational thresholds. Fragmented nodes are excluded from the statistical analysis, and therefore a smaller number of subjects were analyzed in these sparse networks. There were only a few subjects whose clustering coefficient and local efficiency could be calculated at the significant thresholds. This makes between group comparisons difficult, as in some cases only one patient or control was being compared with one or two subjects from the other group, and could lead to falsely significant results.

Since only one patient developed epilepsy during the study, analyses were not performed on this individual, as any differences seen may result from individual differences alone. Single subject variability has been shown to be most significant in the frontoparietal control network and attentional networks, with moderate variability in the DMN and minimal variability in the visual and sensorimotor networks<sup>22</sup>. This study demonstrated that brain areas associated

with higher order functions were associated with more single subject variability. This suggests that applying this analysis, particularly as the networks of interest in epilepsy are primarily higher order networks, could produce spurious results based solely on this one subject's individual variability.

The lack of global alterations in network connectivity after a first seizure could also reflect that changes in network connectivity are not present in the approximately 60% of the population who experiences a single unprovoked seizure but does not go on to develop epilepsy. This suggests there is no inherent predisposition to seizures in these individuals, and network abnormalities may occur as the result of recurrent seizures. The Douw et al<sup>12</sup> study on functional connectivity following a single unprovoked seizure did not have a control group of people who have not had a seizure, and compared first seizure patients who did and did not develop epilepsy. We are unable to find similar significant differences in connectivity, as our group comparisons were different (first seizure vs. no seizure or first seizure with epilepsy vs. no epilepsy). If more patients develop epilepsy our patient group, a similar comparison may be able to be done.

The other first seizure study by Gupta et al.<sup>13</sup> did not look at functional connectivity, but rather BOLD frequency fluctuations. They found higher fluctuations in patients with new onset epilepsy compared to those with a single unprovoked seizure or healthy controls, which has been suggested to reflect a facilitation process of epileptic activity<sup>13</sup>. Similar to our results, there was no difference in patients with a single seizure compared to controls. In their cohort, seven patients developed epilepsy after the initial scan, and four of these had

increased fluctuations compared to the reference value. This should be explored further, and this group of patients may also have changes in functional connectivity, which could be used as a biomarker for subsequent development of epilepsy.

As epilepsy and recurring seizures are characterized by paroxysmal events, it is possible that network abnormalities may only occur in the presence of the abnormal IEDs or seizures. However, a recent EEG-fMRI study demonstrated that network alteration in BOLD signal did not significantly differ with and without the presence of IEDs<sup>23</sup>, suggesting network abnormalities exist even in the absence of abnormal brain activity. This allows the inference of any network changes seen in people with epilepsy to be a result of the disease, rather than the presence of IEDs. Additionally, absence of IEDs during a scan should not make results less reliable in detecting network abnormalities.

The small sample size of this study may preclude detection of any possible differences between groups. The two groups are likely quite similar, with minimal, if any, pathology in the patient group. This would require a large sample size to determine what is likely a small effect between groups. Other types of analyses, such as structurally based analysis using diffusion tensor imaging or voxel-based morphometry, among others, may also provide insight into this matter.

There was considerable variability between the two resting state scans, which limits reproducibility. However, it is well known that resting state networks are highly variable within and between subjects<sup>22,24</sup>. It has been suggested that averaging inter-session

fluctuations across individuals allows for improved comparisons<sup>24</sup>. Increasing the length of the resting-state scan may also improve reliability in determining resting-state functional connectivity<sup>25</sup>. However, the length of an individual sequence has to be balanced with the subject's ability to remain still and awake.

The ultimate question as to whether we can use rs-fMRI as a biomarker for the development of epilepsy after a first seizure was not answered in this study. Although a large number of the patients had at least six months of follow-up, only approximately 24% of patients will develop epilepsy at this time point. Further patient recruitment to increase the sample size, as well as longer follow-up may assist in answering this question more clearly. Enrolling patients who do go on to develop epilepsy will be an important step in order to determine if there are any changes that can distinguish between those who will or will not develop epilepsy. This has important clinical, social and psychological impacts.

## 2.6 Conclusions

We present the first study comparing network connectivity in patients after a single unprovoked seizure and healthy controls. This study demonstrates that no generalized changes in network connectivity occur after the first seizure. This suggests that there are no intrinsic brain connectivity differences predisposing an individual to a single unprovoked seizure. However, between-group differences in this population may be small, and difficult to detect with such a small sample size. Additional, larger studies would be helpful in elucidating this further.

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## Chapter 3

### Discussion

This is the first study of global network connectivity of patients after a single unprovoked seizure compared to healthy controls. It adds valuable information to the spectrum and development of network abnormalities in individuals experiencing seizures, whether it be a single unprovoked seizure or the eventual diagnosis of epilepsy. It also provides insight into possible timelines of epileptogenesis after a single seizure in people without any other seizure risk factors.

### 3.1 Network level results

The main finding of this study is the lack of network connectivity differences between individuals after a single unprovoked seizure and healthy controls on a whole-brain level. This suggests that there are no differences in brain function predisposing an individual to a seizure, or occurring as a result of the seizure.

The process of epileptogenesis, or the development of tissue capable of generating spontaneous seizures, plus the continued progression of disease and development of epilepsy<sup>1</sup>, is still poorly understood. There are many animal models of epileptogenesis, but all of them begin with an inciting factor to produce the seizure, and thus have not studied the spontaneous development of seizures such as that seen in non-lesional cases of epilepsy. The presence of a latent period after an epileptogenic lesion develops is a well-described

phenomenon. In patients, common examples of this include the development of TLE many years after experiencing febrile seizures or another neurological insult as a young child<sup>2</sup> or development of epilepsy after a stroke or traumatic brain injury. It also occurs commonly with malformations of cortical development, which are due to abnormal neuronal migration, organization or proliferation<sup>3</sup>. Unless the malformation is extensive, seizures do not begin at birth, and the average age of seizure onset is usually approximately five years<sup>4</sup>, but has been reported as late as 61 years<sup>5</sup>. It has now been well documented that patients with epilepsy have network alterations<sup>6-12</sup>; therefore, the process of epileptogenesis may produce widespread abnormalities that allow the spontaneous generation of seizures and the other associated effects. The lack of abnormalities in network connectivity after the first seizure could suggest that epileptogenesis has not taken hold at this time. This may not be the case with the one patient in this who was diagnosed with epilepsy, as the diagnosis was that of IGE whose etiology is believed to be genetic<sup>13</sup>. Due to the variability of single subject data (discussed further below), this could not be further explored. Alternatively, current imaging methods may not be sensitive enough to detect any changes occurring this early in the disease, or after only a single seizure. One study found that decreased FC was not noted until five years after epilepsy diagnosis; however, the minimum duration of epilepsy in this study was four years<sup>14</sup>.

The first seizure population studied here is at the lowest risk of recurrence, as no additional risk factors for developing epilepsy were present. It has been suggested that in this population, the risk of recurrence in 20 months is only 17%<sup>15</sup>, but this is still significantly higher than the general population risk of developing epilepsy, which is 3.8%<sup>16</sup>.

## 3.2 Nodal Level Results

The findings on a nodal level are highly variable depending on which of the two resting-state scans were used, and which analysis technique was performed. This could be due to a number of reasons, such as excessive fragmentation, inter- and intra-subject variability between scans, which are discussed further below. No stable result was found on the nodal level, suggesting that there are no reliable differences between patients and controls on any measure.

## 3.3 Methodological Considerations

In any functional connectivity study, there are many methodological decisions to be made regarding the analysis. There is not currently a gold standard of evaluation, and the ground truth of FC is not known, only inferred from functional imaging studies. Furthermore, more advanced mathematical theories such as graph theory are being applied to FC analyses as recognition that the brain behaves similarly to other networks with known properties increases. Applying these methods to neurosciences invokes some unique challenges<sup>17</sup>.

### 3.3.1 Parcellation Scheme and Scaling

Determining nodes for brain network analysis at the macrosystem level is variable, and influences the validity of the results<sup>17</sup>. There are two related factors to be considered in determining the size of a network: the parcellation scheme, or location and boundaries of a

node, and the scale, or number of nodes in the network<sup>18</sup>. There is no standard means of creating nodes<sup>19</sup>, and variety of techniques have been used based on structural, functional, voxel-based or random parcellation schemes<sup>17</sup>. Parcellations can also be created from ICA analyses of the data, where components derived from ICA are used for analysis<sup>20</sup>. The size of the parcellation can affect results, as the number of voxels can vary between ROIs, which can affect connectivity estimates, as they are based on mean time series of the ROI<sup>19</sup>. With larger ROIs, small, highly connected networks will be lost within the large ROI<sup>19</sup>. Recently, a multi-modal parcellation of 180 regions per hemisphere was created using information from T1-weighted/T2-weighted myelin content maps, cortical thickness, task-based fMRI and rs-fMRI, in which a machine-learning classifier was able to accurately detect 96.6% of new subject brain areas<sup>21</sup>.

Parcellation scheme also influences the scale of the network, with finer parcellations creating larger scale networks. The topological properties of a network varies significantly with its scale<sup>18</sup>. Large scale networks have less variance than networks with a smaller number of nodes<sup>17</sup>. Comparisons between networks of small and large scale can be quite different, with a network of 90 nodes having a discrepancy of 95% with a network created with 4000 nodes<sup>18</sup>. However, there are also concerns with increasing the number of nodes, as it was found that rs-fMRI analyses with high parcellation schemes had an increased signal-to-noise ratio<sup>19</sup>. The overall topological features of the graphs remained the same regardless of the parcellation scheme<sup>19</sup>, suggesting that the overall topology, such as whether the network exhibits small-world properties, may not be affected by scale, but most other measures are<sup>18</sup>.

Small-worldness is a measure of overall efficiency of the network, characterized by efficient information transfer over short distances<sup>19</sup>.

In this study, nodes for the main analysis were created on an anatomical scale using the Harvard-Oxford Atlas. From this atlas, 100 ROIs of cortical and subcortical grey matter were chosen, excluding the white matter, CSF and hemispheric labels. Smaller network sizes, particularly the DMN and Salience network, which each had fewer than ten nodes, are difficult to analyze with graph theory, and as such individual analyses were not pursued. In order to maintain evaluation of networks that have been shown to be altered in epilepsy, we chose to perform a combined analysis of these networks, as the larger number of ROIs provide a larger number of nodes, which is slightly better suited for evaluation with graph theory. The Harvard-Oxford atlas is a standard atlas segmented into 48 cortical and 21 subcortical areas created from manually segmented regions in MNI space ([http://www.cma.mgh.harvard.edu/fsl\\_atlas.html](http://www.cma.mgh.harvard.edu/fsl_atlas.html)). There are several other atlases used for analyses, but the most common one is the Automated Anatomical Labelling (AAL) atlas<sup>22</sup>. The AAL atlas is similar to the Harvard-Oxford atlas, and contains 90 cortical and subcortical regions.

### 3.3.2 Thresholding

Another early decision to be made when applying graph theory analyses to fMRI data regards thresholding. Most studies perform some sort of thresholding in their analysis as the raw connectivity matrix may harbour false or noisy connections<sup>23</sup>. Neural connectomes are sparse, that is, not all nodes are connected in some way to every other node. However, the

ideal threshold for performing analyses is unknown, and most studies employ a range of thresholds to test for the ideal connectivity matrix<sup>24</sup>. Connection densities have been estimated in a few species, and range from 6% in the nematode *Caenorhabditis elegans*<sup>25</sup>, to 66% in a macaque monkey<sup>26</sup>. It has been suggested that brain graphs have connection densities of less than 0.5, with a model density of approximately 0.3<sup>24</sup>. Many studies of brain networks in epilepsy have simply used a range of thresholds<sup>27-31</sup> in an attempt to find the most suitable one.

One method of thresholding is by using a weight-based approach where a threshold value is applied to the raw connectivity matrix. Values that are above this threshold remain as part of the connectivity matrix, and the remainder are set to zero<sup>23</sup>. Intrinsic connectivity differences between patient populations can make this method of thresholding challenging if there are between group differences in baseline connectivity, as the majority of network measures are affected by the number of edges in the network<sup>32</sup>.

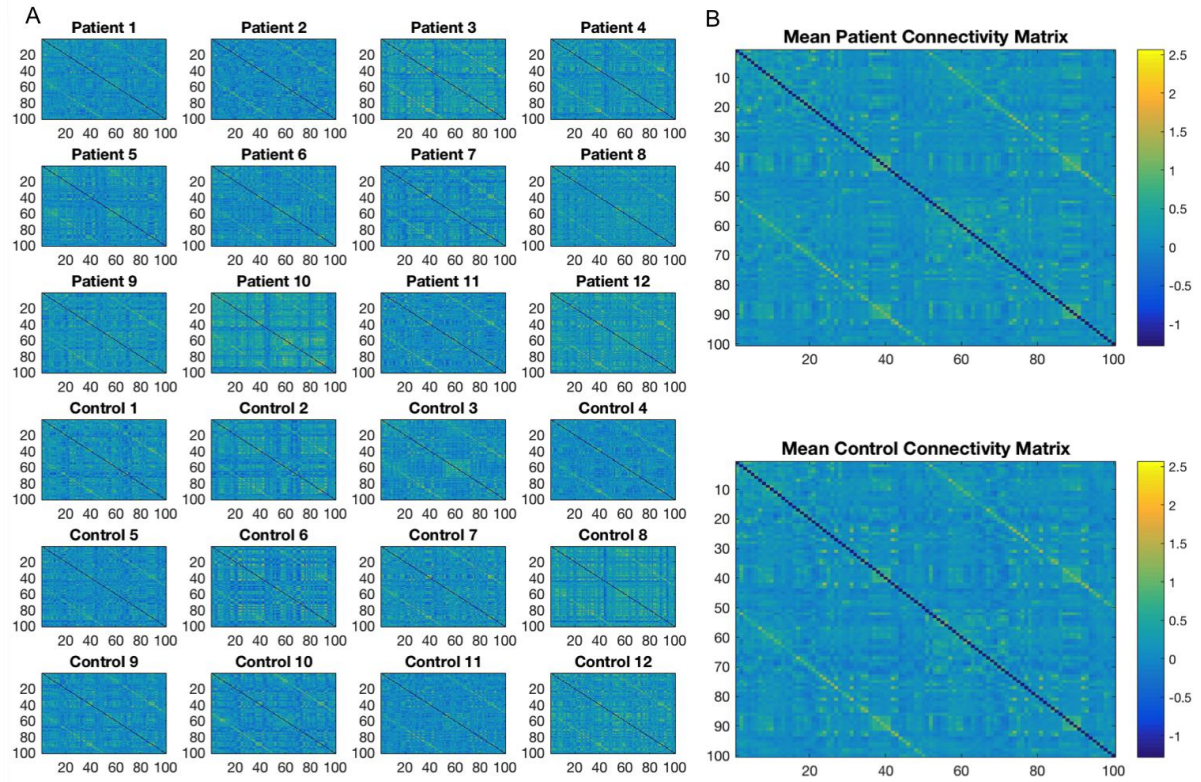
This can be addressed using a density-based approach, where the connection density (also called network density or cost) is fixed<sup>33</sup>. Density-based thresholding will result in connectivity matrices with a fixed number of edges, but different values for  $\tau$ <sup>23</sup>. This method also has limitations as a network with a low average connectivity may retain spurious edges to obtain the specified connection density<sup>32</sup>.

The most appropriate means of generating the connectivity matrix itself has been debated<sup>34,35</sup>, but most studies use Pearson correlation coefficients to determine correlations

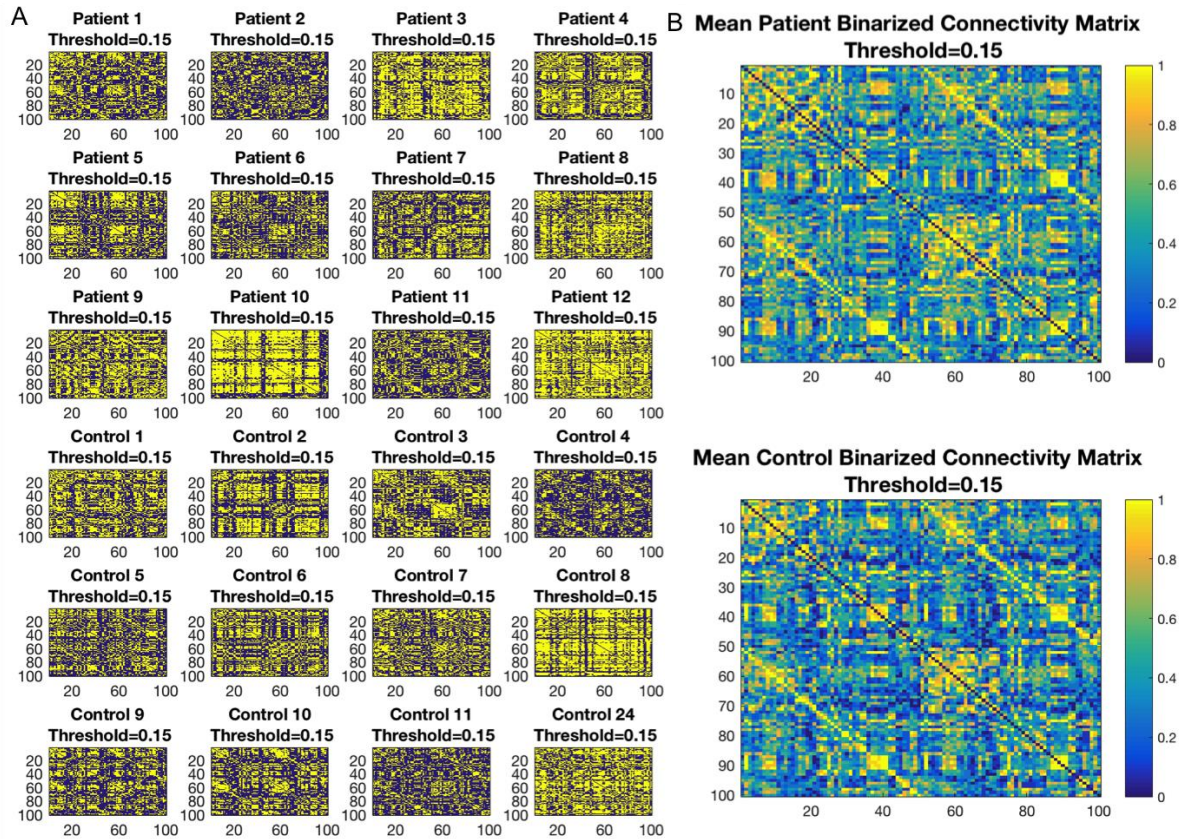


between mean timeseries of each ROI. Partial correlation, which is similar to Pearson correlation, but adjusts for the presence of other nodes in the network, has also been used with rs-fMRI data to generate connectivity matrices. A partial correlation derived matrix overall performed better than the Pearson correlation matrix in one study; however, once the simulated network reached 50 nodes, Pearson correlation performed better<sup>34</sup>. Another study using rs-fMRI data (parcellated into 90 nodes) instead of simulated data found Pearson correlation provided more reliable topological properties than partial correlation<sup>35</sup>. This likely occurs because as the size of the network increases, the overall fraction of indirect connections decreases, and there is greater adjustment in the partial correlation from an increased number of nodes potentially creating a larger effect on the signal<sup>34</sup>.

This study uses both weight-based and density-based methods of thresholding, acknowledging the limitations of both methods. There is pronounced variation between individuals in their connectivity matrices (Figure 3.1A), but appear quite similar when averaged (Figure 3.1B). Once the matrix has been binarized and thresholded, the differences between subjects become more obvious (Figure 3.2).



**Figure 3.1:** Unthresholded connectivity matrices for individual subjects (A) and averaged across patients and healthy controls (B).



**Figure 3.2:** Binarized connectivity matrices using a weight-based approach for individual subjects (A), and averaged across patient and healthy control groups.

### 3.3.3 Network Integrity

Brain graphs are inherently fragmented, as they do not have a connection density of 100%, as previously noted with *C. elegans*<sup>25</sup> and the macaque monkey<sup>26</sup>. This fragmentation suggests that there are multiple networks within the brain<sup>23</sup>. However, there is a critical point for these networks to be functional, after which they become over-fragmented and non-meaningful. In nodal-level analyses, network measures are calculated for each node independently, and some nodes can be disconnected from the network. When this happens, the average path length becomes infinity, making calculations of additional metrics problematic<sup>24</sup>. Measures such as local efficiency help with this matter, as it provides a

measure of fault-tolerance of the network<sup>24</sup>, or its ability to withstand losing edges. Defining the threshold at which fragmentation occurs is still challenging, as the true values of graph theory measures are not known.

### 3.4 Intra- and Inter-Subject Variation

It is well known that there is structural variation in brain structure across individuals<sup>36</sup>. Differences in volume, surface area and cortical thickness are often found to vary significantly between individuals, and this variance occurs in a coordinated fashion where certain brain regions have similar differences to others<sup>36</sup>. This has also been found to be true with functional neuroimaging studies<sup>37,38</sup>, including the current study.

One MEG based study looking at the sensorimotor, auditory and visual networks found that there was significant variability across subjects<sup>37</sup>. A spatial similarity of 0.7 or greater between patients was found in 58% of the sensorimotor maps, 29% of the visual maps and 14% of the auditory maps. In the intra-subject analysis, the sensorimotor and auditory networks were found to be robust, with the visual network somewhat more variable, and similarities between networks varied within individuals. The authors also suggested that there is an interaction between inter- and intra-subject variability because there was variability within subjects, but it was fairly predictable variations between subjects. The spatial similarities between and within subjects improved when optimized seed regions were used, rather than atlas based. Inter-subject variability also decreases when networks with larger scale (smaller parcellations) are used<sup>19</sup>.

Another study using rs-fMRI identified the inter-subject variation within seven resting-state networks and a set of seed ROIs<sup>38</sup>. The resting state networks included the frontoparietal control, ventral attention, dorsal attention, default mode, limbic, sensorimotor, and visual networks. A large degree of variation was noted in all networks; however, the sensorimotor and visual networks were the most robust, with an inter-subject variability of approximately 0.57. The frontoparietal control network, followed by the ventral attention network had the most variability, and the DMN and dorsal attention networks also had variability of greater than 0.6. These networks with the highest variability are ones of higher cognitive functions. These are also the networks that are most prominently affected in PWE and are of primary interest in this study.

### 3.4.1 Duration of fMRI Timeseries

The duration of the resting-state scan has also been found to influence reliability of results, with studies reporting various optimal scan lengths. One early study which helped determine scan length for rs-fMRI studies found that for seed-based FC analyses, spurious FC connections in individual subjects decrease through the timeseries approximately proportionally to the square root of the sampling time<sup>39</sup>. After approximately 5-6 minutes of sampling time these spurious connections are close to asymptotic<sup>39</sup>. However, a more recent study suggested that a scan length of 9-13 minutes was required to improve FC estimates and test-retest reliability within a subject<sup>40</sup>. Specifically, increasing the scan length from six to 12 minutes resulted in a 20% increase in the reliability<sup>40</sup>. This study also found that reliability of runs within the same scanning session was greater than the reliability of runs from different

scanning sessions<sup>40</sup>. For functional connectivity and ICA analyses, approximately 13.2 minutes of scanning was required to attenuate the effects of temporal dynamics on sensitivity, specificity, reproducibility, reliability and accuracy by at least 80%<sup>41</sup>. However, if individual subject identification is important, 15-25 minutes or more may be required to obtain reliable classification accuracies<sup>42</sup>. In order to become a useful biomarker for neurological disease, rs-fMRI must be able to distinguish affected individuals from unaffected ones to provide personalized information to a particular patient, which makes this longer scan length extremely relevant.

Graph theory measures seem to reach stabilization more quickly than seed-based ROI or ICA methods<sup>41,43</sup>, and shorter scan lengths may be sufficient to generate reliable results. In one study, measures of small-worldness, global efficiency and local efficiency were measured at costs of 0.1-0.5 and found to be stable after as little as 1.5-2 minutes of scanning time<sup>43</sup>. Another study found that the accuracy and sensitivity of the graph theory measure of local functional connectivity density converged around five minutes, specificity at 1.8 minutes and reproducibility at two minutes<sup>41</sup>. Overall, approximately 4.5 minutes of scanning was required to attenuate the effects of the temporal dynamics on the measure of local functional connectivity density for accuracy, sensitivity, specificity, reliability and reproducibility by at least 80%<sup>41</sup>.

This study used two resting state sequences of six minutes 40 seconds duration, and found variability between the two scans on a group and individual level (figures 2.1 and S1). These scans were analysed separately to assess for validity of the single scans, rather than

concatenated to an approximately 14 minute timeseries. Based on previous studies<sup>41,43</sup>, this scan length should be long enough to produce reliable results when doing a graph theoretical analysis. Network-level results were reasonably stable, with no significant differences between groups on any measure or either scan: these changes were only noted with the nodal-level results. Current studies on scan length have not addressed nodal-level results in graph theory specifically, and given the finding here, nodal-level results may benefit from the longer scan length similar to that required to obtain reliable FC measures. Additionally, these studies have all been performed at 3T, which may have different reliability measures than at 7T.

### 3.5 7T Dataset

One unique feature of this study is the use of the 7T MRI for data acquisition. Resting-state fMRI data is not commonly obtained at 7T in patient populations, with only two studies in patient populations currently published<sup>44,45</sup>. Imaging at 7T provides many advantages over lower field strengths, but the improved signal-to-noise ratio and shorter T2\* relaxation time are particularly valuable in fMRI studies. However, the higher field strength also has greater interference by physiological noise and is more sensitive to motion artefact<sup>46-49</sup>. In particular, motion associated with respiration such as head movement and alteration in the magnetic field, can interfere with image quality<sup>46</sup>. Acquisition of high-resolution images can improve the temporal SNR above what would ordinarily be expected for a particular field strength<sup>46</sup>. Additional motion-correction techniques and acquisition parameters can also help improve the SNR at higher field strengths<sup>46,47</sup>.

## 3.6 Statistical Testing

In graph theory, the problem of multiple comparisons is considerable. For example, in this study, networks consisted of 100 nodes, for each of which 5-7 graph theory measures were calculated at 65 different thresholds. Additionally, these values are not likely to be completely independent of each other<sup>23</sup>, compromising the validity of commonly used correction procedures such as the false-discovery rate (FDR) or Bonferroni correction. In particular, the FDR, which is a less stringent procedure for corrections allowing fewer false positives, may still be too conservative when dealing with brain network data<sup>23</sup>. It is therefore possible that a statistically significant result could be lost due to this procedure for correcting for multiple comparisons. The NBS attempts to overcome this issue by assuming that there are connections present in the data, and use them to improve the statistical power from what is possible with independent comparisons<sup>50</sup>. However, it does this at the cost of providing weaker control of the family-wise error, and cannot detect significance in individual connections, only network-level connections<sup>50</sup>.

## 3.7 Future Directions

A larger sample size would be ideal to determine if the absence of differences between groups is true or a function of an inadequate sample size. It may be worthwhile to consider an analysis with smaller parcellations, as this might provide more sensitive results. Quantitative analysis of the structural data with DTI, voxel-based morphometry, and cortical thickness



analyses may also provide insight into pathophysiology. In particular, DTI can provide information on structural connections between different brain areas, and thus more direct measures of connectivity can be calculated.

With these negative results, it could be interesting to study brain connectivity in people after a provoked seizure. Although the cause for the seizure is iatrogenic or metabolic and seizures will not recur providing the cause does not return, different people will become symptomatic at different levels of provocation. Thus, the seizure threshold in each individual is different, and could reflect individual connectivity differences, but this has not been explored.

Additionally, further follow-up of these patients may allow for the development of a biomarker for the development of epilepsy, if enough patients are recruited so that analyses can be performed comparing people who only have a single seizure compared to those who go on to develop epilepsy.

### 3.8 Conclusions

No differences in whole brain network connectivity were seen between patients after a single unprovoked seizure and healthy controls. This suggests that no inherent connectivity differences predisposing an individual to seizures are present between individuals who experience a single seizure and those who do not. However, these could be small and this study did not have the power to detect them.

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# Appendix A

## Supplemental Methods

Additional information is provided on noise correction techniques.

### A.1 ART-based scrubbing

([www.nitrc.org/projects/artifact\\_detect/](http://www.nitrc.org/projects/artifact_detect/))

The quality assurance and artefact rejection software (ART), also developed by the same authors as the CONN toolbox, is integrated into CONN for correction of head-motion artefacts<sup>1</sup>. ART identifies maximum outliers in head movement and saves them as a matrix which can subsequently be used as confounding variables when denoising the data<sup>1</sup>. The scrubbing procedure implemented by ART in CONN identifies motion outliers and effectively removes them from the original timeseries through the addition of dummy-coding regressors to maintain the continuity of the timeseries.

### A.2 Anatomical Component-Based Noise Correction

The anatomical component-based noise correction (aCompCor) method for noise correction was first described in 2007<sup>2</sup>. The underlying assumption in the aCompCor method is that the signal found in noise voxels, such as CSF or white matter, can be used to model physiological fluctuations in grey matter<sup>2</sup>. BOLD fluctuations representing neural activity only originate from grey matter, thus any fluctuations from CSF and white matter should represent physiological noise, such as respiratory and cardiac signals. Voxels containing CSF

or white matter determined from anatomical information, and then principal components analysis (PCA) is applied to further characterize the noise voxels.

The CSF and white matter masks applied for aCompCor are created through principal component decomposition during functional and structural segmentation steps of the SPM preprocessing.

A general linear model for grey matter voxels was applied with principal components determined from the noise timeseries being applied as regressors. This was found to accurately model physiological artefacts when compared to another noise correction algorithm, RETROICOR<sup>2</sup>. The application of aCompCor decreased the temporal standard deviation of the BOLD timeseries by 20% compared to no correction<sup>2</sup>.

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## Appendix B

### B.1 TLE Data

As a methodological validation, data previously collected from TLE patients was analyzed.

As this patient population has been shown to have network abnormalities, it was used to determine if the negative results obtained from the first seizure group was a true negative result, or the result of a methodological flaw. Eleven patients with TLE and healthy controls were selected for this analysis.

### B.2 Imaging Protocol

The functional images were acquired on the Agilent 7T magnet (Santa Clara, CA, USA).

Resting-state functional images were acquired with a repetition time of 2500 ms. One hundred twenty volumes were obtained. Structural T1-weighted images were acquired with a MPRAGE sequence with 8.1 ms TR, 2.8 ms TE, flip angle  $11^\circ$ , 1 mm isotropic.

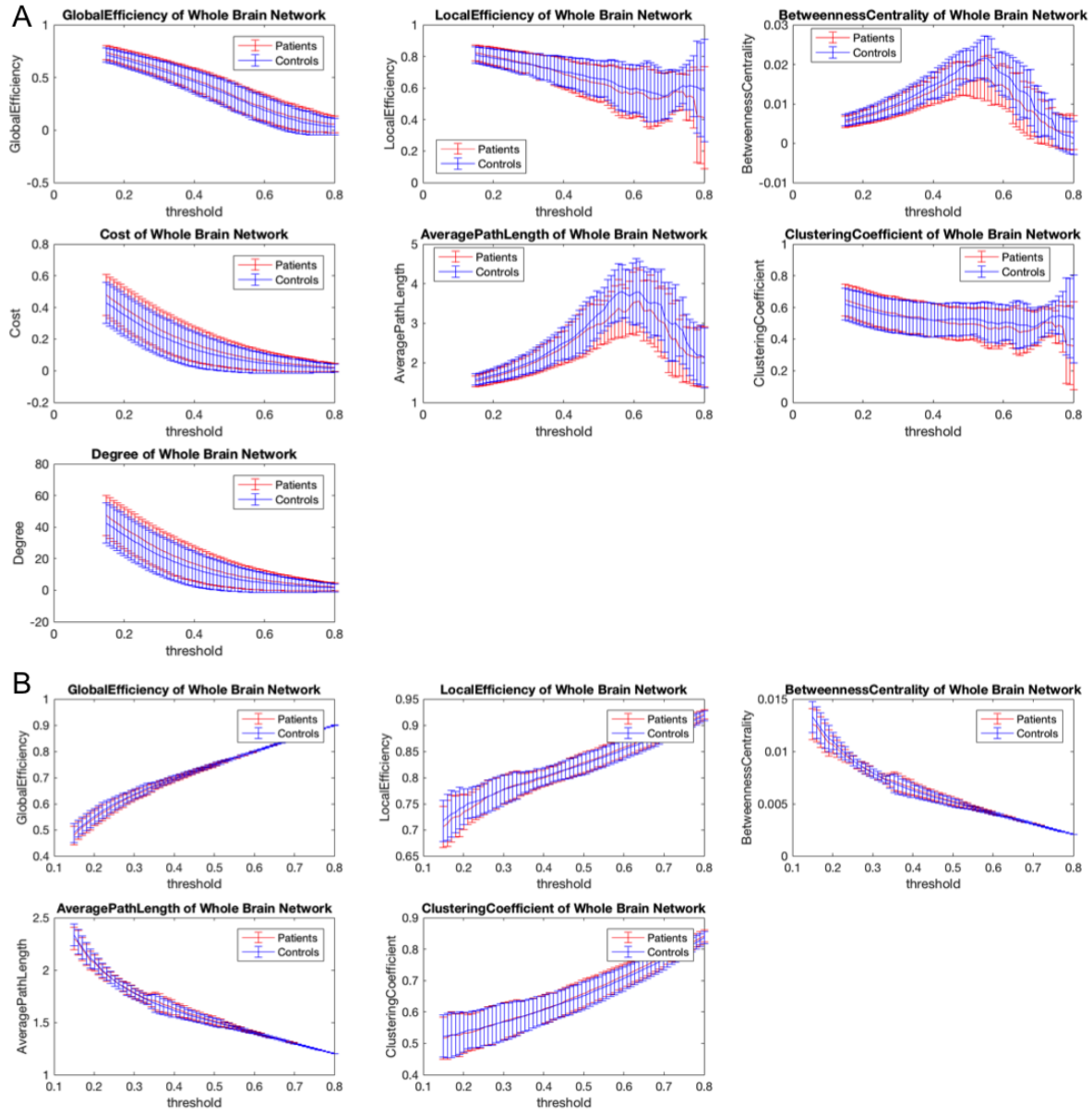
The preprocessing and analysis techniques used were identical to those described for the first seizure group, as discussed in chapter 2.

### B.3 Results

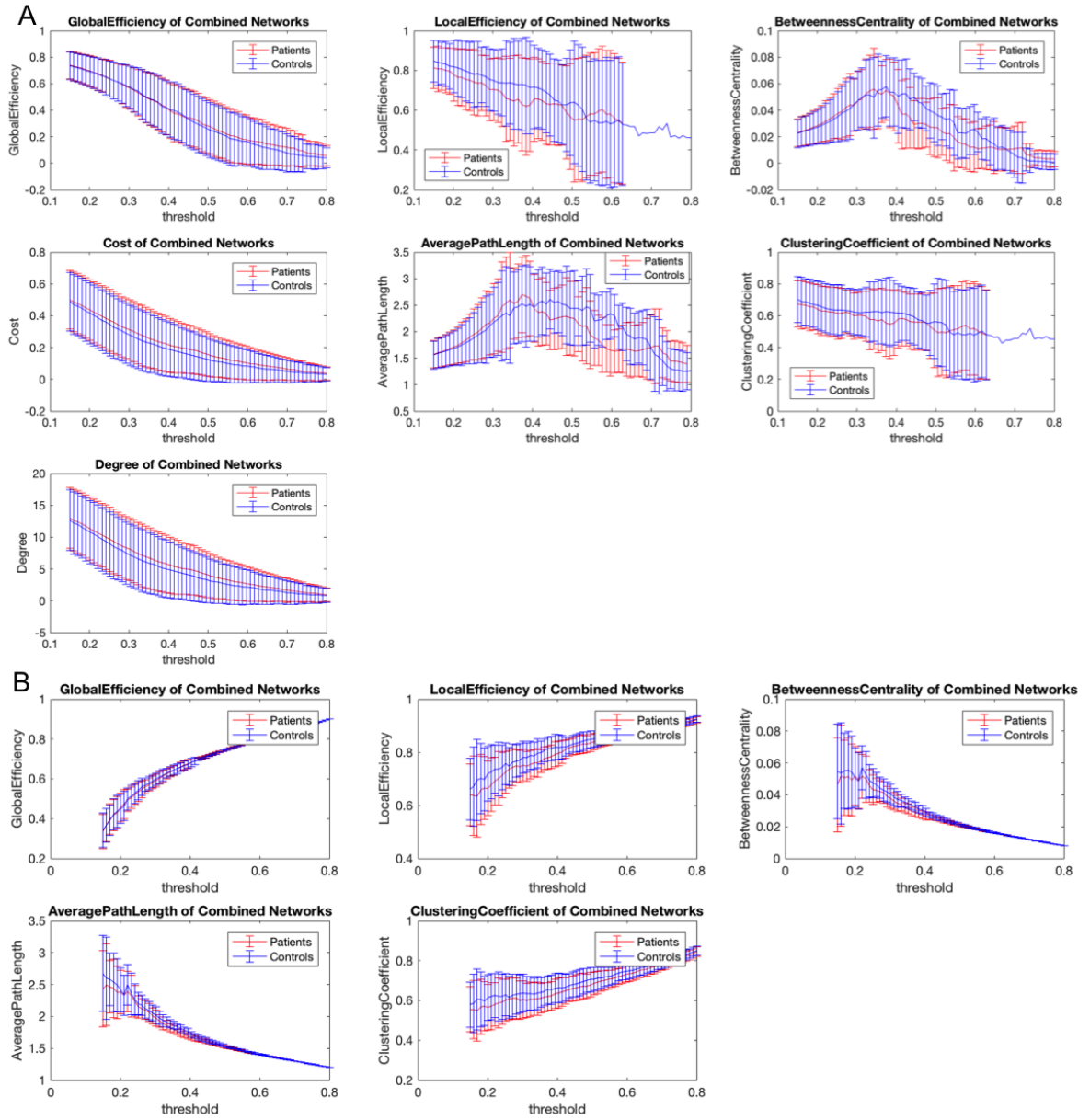
No significant differences were seen between TLE patients and healthy controls on any network-level measure (figure A1.1 & A1.2). On a nodal level, weight based, but not cost-based thresholding found a significantly decreased average path length and local efficiency in

the left parahippocampal gyrus in the combined networks analysis ( $p < 0.0001$ ). This occurred at thresholds of 0.60-0.67.

No significant results were found when using the Network Based Statistic in either the whole brain or combined networks.



**Figure B.1:** Network connectivity of the whole brain in TLE patients vs. healthy controls using correlation coefficient (A) and cost (B) to threshold.



**Figure B.2:** Network connectivity of the combined limbic, salience and default mode networks in TLE patients vs. healthy controls using correlation coefficient (A) and cost (B) to threshold.

## B.4 Discussion

In the analysis of networks most relevant in people with TLE, there was decreased connectivity in the left parahippocampal gyrus. This region is important in TLE, as most temporal lobe epilepsy involves the mesial temporal regions, of which the parahippocampal gyrus is a part, but particularly the hippocampus<sup>1,2</sup>. It has been shown that there is atrophy of extrahippocampal structures, including the parahippocampal gyrus, that correlates with the degree of atrophy in the hippocampus<sup>3</sup>. The parahippocampal gyrus along with the entorhinal cortex has been implicated in verbal memory decline following a temporal lobectomy<sup>4</sup>. These results reproduce previous findings of involvement of the parahippocampal in patients with TLE.

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# Appendix C

## C.1 Original Ethics Approval



Research Ethics

### Western University Health Science Research Ethics Board HSREB Delegated Initial Approval Notice

Principal Investigator: Dr. Seyed Mirsattari  
Department & Institution: Schulich School of Medicine and Dentistry/Clinical Neurological Sciences, London Health Sciences Centre

Review Type: Delegated  
HSREB File Number: 107246  
Study Title: Changes in network connectivity after an unprovoked first seizure and status epilepticus using resting state functional magnetic resonance imaging.  
Sponsor:

HSREB Initial Approval Date: February 17, 2016  
HSREB Expiry Date: September 17, 2017

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	RVDLT Images (Received 15Jan16)	
Instruments	WAIS Digit Span Recording Form (Received 15Jan16)	
Instruments	WAIS Vocabulary Test Recording Form (Received 15Jan16)	
Instruments	WAIS Matric Reasoning Recording Form (Received 15Jan16)	
Instruments	WAIS Vocabulary Test Stimuli (Received 15Jan16)	
Instruments	WAIS Matrix Reasoning Stimuli (Received 15Jan16)	
Instruments	Instructions for WAIS Matrix Reasoning and Vocabulary Tests (Received 15Jan16, received for information only)	
Instruments	Generalized Anxiety Disorder Scale 7-item (Received 15Jan16)	
Instruments	Beck Depression Inventory II (Received 15Jan16)	
Recruitment Items	Revised advertisement for normal volunteers clean copy	2015/12/13
Recruitment Items	Revised Recruitment information to Neurologists clean copy	2016/02/04
Instruments	RVDLT Administration (1) (Received 15Jan16, for information only)	
Instruments	RAVLT Administration (2) (Received 15Jan16, for information only)	
Instruments	WAIS Digit Span Instructions - Forwards and Backwards (Received 15Jan16 for information only)	
Letter of Information & Consent	Revised Control LOI clean copy	2016/02/04
Letter of Information & Consent	Revised LOI for Subjects clean copy	2016/02/04
Western University Protocol	Clean copy of revised Western protocol	2016/02/04

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

\_\_\_\_\_  
Ethics Officer, on behalf of Dr. Marcello Kremenchutzky, HSREB Vice Chair

Ethics Officer to Contact for Further Information: Erika Basile \_\_\_ Nicole Kaniki \_\_\_ Grace Kelly \_\_\_ Mrs. McAbell \_\_\_ Vikki Tran ✓

*This is an official document. Please retain the original in your files*

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## C.2 Ethics Approval after Protocol Revisions



**Western  
Research**

Research Ethics

**Western University Health Science Research Ethics Board  
HSREB Amendment Approval Notice**

**Principal Investigator:** Dr. Seyed Mirsattari

**Department & Institution:** Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,London Health Sciences Centre

**Review Type:** Delegated

**HSREB File Number:** 107246

**Study Title:** Changes in network connectivity after an unprovoked first seizure and status epilepticus using resting state functional magnetic resonance imaging.

**HSREB Amendment Approval Date:** June 08, 2016

**HSREB Expiry Date:** February 17, 2017

**Documents Approved and/or Received for Information:**

Document Name	Comments	Version Date
Revised Letter of Information & Consent	Clean copy of revised patient LOI	2016/04/26
Revised Letter of Information & Consent	Clean copy of revised control patient LOI	2016/04/26
Revised Western University Protocol		2016/06/07

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer: Erika Basile \_\_\_ Katelyn Harris \_\_\_ Nicole Kaniki \_\_\_ Grace Kelly \_\_\_ Vikki Traut \_\_\_ Karen Gopaul \_\_\_

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# Curriculum Vitae

**KRISTIN M IKEDA**

## **ACADEMIC APPOINTMENTS**

2017- Assistant Professor, Division of Neurology, Department of Medicine  
Dalhousie University  
Starting August 1, 2017

## **EDUCATION**

2015-2017 **Master of Neuroscience (candidate)**  
Western University  
Supervisors: Seyed Mirsattari and Ali Khan  
Thesis: Brain networks in people with a first unprovoked seizure

2015-2017 **Clinical Fellow**, EEG/Epilepsy, Department of Clinical Neurosciences,  
Western University

2010-2015 **Neurology Residency**  
Western University

2007-2010 **Doctor of Medicine**  
McMaster University

2005 **Music Therapy Internship**  
Park Nicollet Health Services, Minneapolis, MN

2000-2004 **Honours Bachelor of Music Therapy**  
Wilfrid Laurier University

## **PROFESSIONAL DESIGNATIONS**

2016 Canadian Society for Clinical Neurophysiologist Diplomate, EEG  
2015 Royal College of Physicians and Surgeons of Canada, Fellow in Neurology

## **COMMITTEES**

2016-2018 Canadian League Against Epilepsy  
Junior Member Representative  
Chair of Website Development Committee

2010-2015 Neurology Residency Training Committee  
2010-2015 Cohort Representative  
Resident Longitudinal Clinic Committee

## **AWARDS AND PRIZES:**

2017 Candian Neurological Society Andre Barbeau Prize for Basic Research  
\$1000, plus expenses to Canadian Neurological Sciences Federation Annual  
Congress

2016-2017 Canadian Society for Clinical Neurophysiologists Clinical Fellowship in  
EEG/Epilepsy  
\$65,000

2016-2017	Canadian League Against Epilepsy Post-Graduate Training Fellowship Award (for Clinical Fellows), declined \$65,000
2015	American Epilepsy Society Fellows Program
2014-2015	Best Neurology Grand Rounds Presentation, 3 <sup>rd</sup> place \$100
2014	Clinical Neurosciences Research Day, Best Neurology Platform Presentation \$300
2012-2013	Best Neurology Grand Rounds Presentation, 2 <sup>nd</sup> place \$300
2013	Western University Resident Travel Award \$750

### **SELECT PEER-REVIEWED PUBLICATIONS**

1. **Ikeda KM**, Aldosari MM, AlGhefari H, Hammond RR, Mirsattari SM. Focal cortical dysplasia type IIa manifesting as epilepsia partialis continua for 50 years. *Can J Neurol Sci.* 2017;In press.
2. Markovic N, **Ikeda KM**, Gob A, Burneo JG. Diffuse large B-cell lymphoma presenting as bilateral internal auditory canal lesions. *Can J Neurol Sci.* 2017; Apr 27:1-3. [epub ahead of print]. doi: 10.1017/cjn.2017.23.
3. **Ikeda KM**, Mirsattari SM. Evolution of epilepsy in hemimegalencephaly from infancy to adulthood: Case report and review of the literature. *Epilepsy & Beh Case Reports.* 2017;7:45-8.
4. **Ikeda KM**, Mirsattari SM. Emotional response of a preschooler to her mother's epileptic seizure. *Seizure.* 2017;45:62-3. <http://dx.doi.org/10.1016/j.seizure.2016.10.017>
5. **Ikeda KM**, Connors R, Lee DH, Khandji AG, Claassen J, Young GB. Isoflurane use in the treatment of super-refractory status epilepticus is associated with hippocampal changes on MRI. *Neurocrit Care.* 2017;26:420-7. doi: 10.1007/s12028-016-0340-0. [Epub ahead of print]
6. Rizek P, **Ikeda KM**, Mele T, Garcia B, Gofton TE. Bowel ischemia in the management of refractory status epilepticus: report of two cases and review of the literature. *Neurocrit Care.* 2016;24:128-31.
7. Young GB, **Ikeda KM**. Treatment of refractory status epilepticus: Better evidence is needed. *Can J Neurol Sci.* 2015;42:72-3.