The Effect of Age of Epilepsy Onset on Cognition, Brain Structure and Network Organization in People with Left and Right Temporal Lobe Epilepsy

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Psychology
University of Toronto

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Abstract

Temporal lobe epilepsy (TLE) is a characterized by recurrent seizures in the temporal lobes and is associated with memory impairments, widespread cortical abnormalities, and altered network properties. This disorder emerges at varying ages from perinatal periods to midlife. This thesis examines the relationship between the age of epilepsy onset with memory, cortical thickness and network properties. In the first study, I found that, in people with left TLE (LTLE), earlier onset was associated with worse verbal memory, visual memory, IQ, and thinner cortex in left frontal language regions, while onset in young adulthood was associated with peak memory and cortical thickness in these regions. Further, cortical thickness in these regions correlated with verbal memory ability. Conversely, in people with right TLE (RTLE), earlier onset was associated with better visual memory, but with thinner posterior cingulate cortex (PCC) and superior frontal cortex and thinner cortex in these regions was associated with worse visual memory. In study 2, I found that early onset was associated with higher network segregation in RTLE. Higher network segregation also related to better visual memory ability, suggesting that this network organization seen in early onset individuals is beneficial. In study 3, I used functional connectivity to parcellate the hippocampus into anterior and posterior divisions. In LTLE,
anterior hippocampus to PCC connectivity was related to verbal memory, while in RLTE, posterior hippocampus to PCC connectivity was related to visual memory, though age of onset was unrelated to these effects. Together, this dissertation presents evidence that early life onset of temporal lobe epilepsy affects cognition, brain structure and organization. Seizure laterality appears to play a major role in how this effect is expressed—early onset in the language dominant hemisphere is associated with a developmental hindrance, while early onset in the non-dominant hemisphere is associated with adaptive network organization.
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# Table of Contents

Acknowledgments ........................................................................................................ iv

Table of Contents ........................................................................................................ vi

List of Tables .................................................................................................................. x

List of Figures ................................................................................................................ xi

Chapter 1 Literature Review .......................................................................................... 1

1.1 Overview ................................................................................................................... 1

1.2 Summary of Diagnosis and Surgical Treatment ...................................................... 3

1.3 Cognition and brain abnormalities associated with TLE .................................... 5

1.3.1 Cognition ................................................................................................................. 5

1.3.2 Gray Matter Abnormalities .................................................................................... 6

1.3.3 White Matter Abnormalities .................................................................................. 7

1.3.4 Functional activation Abnormalities ..................................................................... 8

1.3.5 Brain Network Abnormalities ............................................................................... 9

1.4 Effects of Age of Onset on Cognition and the Brain ............................................. 15

1.5 Research Aims and Hypotheses ............................................................................. 17

Chapter 2 General Methods ......................................................................................... 20

2.1 Neuropsychological Testing .................................................................................... 20

2.1.1 Overview ................................................................................................................. 20

2.1.2 Visuospatial Memory Factor .................................................................................. 20

2.1.3 Verbal Memory Factor .......................................................................................... 21

2.2 Magnetic Resonance Imaging Parameters ............................................................. 21

2.2.1 T1-weighted Anatomical Images ......................................................................... 21

2.2.2 Resting state functional MRI ............................................................................... 21

Chapter 3 The effect of age of epilepsy onset on memory and cortical thickness in right and left temporal lobe epilepsy ................................................................. 22
### 3.1 Introduction

Chapter 4 Topological network properties are modulated by age of onset in right, but not left, temporal lobe epilepsy.

### 4.1 Introduction

### 4.2 Methods

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.1 Participants</td>
<td>52</td>
</tr>
<tr>
<td>4.2.2 Functional MRI preprocessing</td>
<td>52</td>
</tr>
<tr>
<td>4.2.3 Graph Theory Analysis</td>
<td>52</td>
</tr>
</tbody>
</table>

### 4.3 Results

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.1 Patient Demographics</td>
<td>59</td>
</tr>
<tr>
<td>4.3.2 Network Based Statistics</td>
<td>62</td>
</tr>
<tr>
<td>4.3.3 Whole Brain Graph Theory Results</td>
<td>64</td>
</tr>
<tr>
<td>4.3.4 Nodal Centrality Results</td>
<td>66</td>
</tr>
</tbody>
</table>
Chapter 5 Parcellation of the hippocampus using resting functional connectivity in temporal lobe epilepsy

5.1 Introduction

5.2 Methods

5.2.1 Participants

5.2.2 Functional MRI Preprocessing

5.2.3 \(k\)-means clustering

5.2.4 Region of interest connectivity analysis

5.3 Results

5.3.1 Patient Demographics

5.3.2 \(k\)-means clustering

5.3.3 Group Connectivity Differences

5.3.4 Hippocampal – PCC Connectivity and Memory

5.3.5 Hippocampal – PCC connectivity and age of onset

5.4 Discussion

5.5 Conclusion

Chapter 6 General Discussion

6.1 Overview

6.2 Summary of findings

6.3 Key Findings
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4 Alternative Explanations</td>
<td>105</td>
</tr>
<tr>
<td>6.4.1 Limitations</td>
<td>107</td>
</tr>
<tr>
<td>6.5 Future Directions</td>
<td>109</td>
</tr>
<tr>
<td>6.6 Conclusions</td>
<td>110</td>
</tr>
<tr>
<td>References</td>
<td>112</td>
</tr>
<tr>
<td>Copyright Acknowledgements</td>
<td>137</td>
</tr>
</tbody>
</table>
List of Tables

Table 3-1. Subject Demographic Data ........................................................................................................ 28
Table 3-2. Bootstrap ratios from partial least square analysis of cortical thickness in patient groups compared to a healthy control group ........................................................................................................ 33
Table 3-3. Regression slopes for predictors of cortical thickness for different regions in the brain with model explained variance ($R^2$) ........................................................................................................ 40
Table 4-1. List of graph theory measures and definitions ........................................................................... 58
Table 4-2. Patient Demographic Data ........................................................................................................ 61
Table 4-3. Spearman Correlation values relating age of epilepsy onset to whole brain graph theory network measures in right temporal lobe epilepsy across different percentile (%tile) thresholds ........................................................................................................ 71
Table 5-1. Cluster regions, peak coordinates, test statistic and cluster size for connectivity differences between people with LTLE and healthy controls ........................................................................ 90
Table 5-2. Cluster regions, peak coordinates, test statistic and cluster size for connectivity differences between people with RTLE and healthy controls ........................................................................ 91
List of Figures

Figure 1-1. A structural MRI scan of a patient who underwent an anterior temporal lobectomy, illustrating resected tissue in the epileptogenic hippocampus, and temporal neocortex. .......................... 5

Figure 3-1. Scatterplots demonstrating the relationship between age of onset and cognition in left and right temporal lobe epilepsy with modeled regression lines and regression line confidence intervals in dashed lines. MTS, mesial temporal sclerosis. ................................................................. 30

Figure 3-2. On the left, a scatterplot displaying age of onset and IQ factor score in people with left temporal lobe epilepsy with a regression line plotted in solid black with confidence intervals as dashed lines. Three influential cases are circled in red. On the right, a plot of each subject displaying their relative influence, measured by Cook’s Distance with a horizontal bar indicating a threshold of three times the mean Cook’s Distance of the sample. Three outliers are circled in red. MTS, mesial temporal sclerosis.............................................................................. 31

Figure 3-3. On the left, 3-dimensional renderings of the average FreeSurfer brain surface with brain regions highlighted corresponding to the regions plotted in those rows. Scatterplots with regression lines and confidence intervals as dashed lines depicting A, the relationship between age of onset and regional cortical thickness after regressing out variability explained by age and sex and B, the relationship between cortical thickness and verbal memory ability, in left temporal lobe epilepsy (LTLE). L, left; MFG, middle frontal gyrus; MTS, mesial temporal sclerosis. ..... 37

Figure 3-4. On the left, 3-dimensional renderings of the average FreeSurfer brain surface with brain regions highlighted corresponding to the regions plotted in those rows. Scatterplots with regression lines and confidence intervals as dashed lines depicting A, the relationship between age of onset and regional cortical thickness after regressing out variability explained by age and sex and B, the relationship between cortical thickness and verbal memory ability, in right temporal lobe epilepsy (RTLE). L, left; MTS, mesial temporal sclerosis; PCC, posterior cingulate cortex; SFG, superior frontal gyrus................................................................. 39

Figure 3-5. Scatterplots demonstrating the relationship between age of onset and regional cortical thickness in millimeters with regression line plotted showing the relationship between age of onset and thickness in black and a chronological age adjusted regression line demonstrating the relationship between age of onset and thickness in dashed red line. ................................................. 44
Figure 4-1. Group level, whole brain correlation matrices organized by community structure for healthy controls, and patients with left or right temporal lobe epilepsy (left) and centre points of nodes from the parcellation atlas, rendered on a standard brain surface, colour coded by community structure. Blue = Frontoparietal network, Red = Default Mode network, Orange = Limbic network, Yellow = Somatomotor network, Green = Visual network.

Figure 4-2. Graphical representation of topology measures.

Figure 4-3. On the left, group differences in network connectivity between patient groups and healthy controls are shown using network based statistics with 5000 permutations, p < .05, controlling for years of education. Blue cells represent reduced connectivity in TLE, red cells represent increased connectivity in TLE. On the left, centre points of nodes from the parcellation atlas are rendered on a standard brain surface, colour coded by community structure. Blue = Frontoparietal network, Red = Default Mode network, Orange = Limbic network, Yellow = Somatomotor network, Green = Visual network. Intra-module connectivity in the default mode network is reduced in TLE, while inter-module connectivity between the somatomotor network and the rest of the brain, increases.

Figure 4-4. Mean and standard error for global graph theory metrics for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds. * denote significant difference between LTLE and HC.

Figure 4-5. Mean and standard error for anterior (Ant) and posterior (Post) hippocampal degree for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds. * denote significant difference between LTLE and HC. † denotes significant difference between RTLE and HC.

Figure 4-6. Mean and standard error for anterior (Ant) and posterior (Post) hippocampal betweenness centrality (BC) for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds. * denote significant difference between LTLE and HC. † denotes significant difference between RTLE and HC.
Figure 4-7. Mean and standard error for dorsal and ventral, left (L) and right (R) Brodmann area 23 (BA23) degree for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds.

Figure 4-8. Mean and standard error for dorsal and ventral, left (L) and right (R) Brodmann area 23 (BA23) betweenness centrality (BC) for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds.

Figure 5-1. An example of a connectivity matrix from a subject from each hippocampal voxel to every other voxel in the brain outside of the hippocampus.

Figure 5-2. An example of a correlation matrix in which the whole brain voxel-wise connectivity patterns of each hippocampal voxel are correlated with each other. To make these matrices, each column of Figure 1 is correlated with every other column in a pairwise fashion. Strong correlations between hippocampal voxels indicate that those voxels have similar covariance in their connectivity with the rest of the brain.

Figure 5-3. An example of a sorted correlation matrix of whole brain voxel-wise connectivity pattern correlations between each hippocampal voxel following k-means clustering procedure. This matrix contains the same information as Figure 5.2, except it is reordered according to the k-means clustering.

Figure 5-4. Group level clusters from k-means clustering procedure, projected onto the standard MNI brain showing an anterior (yellow) and posterior (blue) cluster for both the left and right hippocampus derived from patients with left temporal lobe epilepsy (LTLE), healthy controls and patients with right temporal lobe epilepsy (RTLE).

Figure 5-5. Bilateral anterior hippocampal connectivity contrasted against bilateral posterior hippocampal connectivity in people with left temporal lobe epilepsy (LTLE), healthy controls and people with right temporal lobe epilepsy (RTLE) presented without statistical thresholding. The colour bars depict t values and warm colours depict areas of greater anterior versus posterior connectivity while cold colours depict areas of greater posterior versus anterior connectivity.
Figure 5-6. Contrast maps of differences in connectivity between patients with left temporal lobe epilepsy compared to controls, seeding from either the left anterior hippocampal seed (top) or the left posterior hippocampal seed (bottom), thresholded at $p < .01$ using 5000 permutations, corrected for multiple comparisons using FDR correction. Areas of increased connectivity in LTLE, depicted in warm colours were only significant using a small volume correction in the entorhinal cortex. The colour bars depict t values. .......................................................... 89

Figure 5-7. Contrast maps of differences in connectivity between patients with right temporal lobe epilepsy compared to controls, seeding from the right anterior hippocampal seed, thresholded at $p < .01$ using 5000 permutations, corrected for multiple comparisons using FDR correction. The colour bars depict t values. ........................................................................................................ 91

Figure 5-8. Scatterplot showing relationship between left anterior hippocampal to left posterior cingulate cortex connectivity with total number of words recalled on the Rey auditory verbal learning test over five trials, with a regression line drawn in black and regression line confidence intervals shown with dotted curves. ........................................................................................................ 93

Figure 5-9. Scatterplot showing relationship between posterior hippocampal connectivity to posterior cingulate cortex connectivity with memory performance in patients with right temporal lobe epilepsy, with a regression line drawn in black and regression line confidence intervals shown with dotted curves................................................................. 94
Chapter 1

Literature Review

1.1 Overview

Temporal lobe epilepsy (TLE) is a neurological disorder characterized by recurrent seizures mainly originating in the medial temporal lobes (MTL) and is strongly linked to deficits in episodic memory. While seizure management can be successful with medication, a subgroup of patients with TLE will not find seizure control with antiepileptic drugs. These people can be eligible for surgical resection of the epileptogenic focus to eliminate their seizures, which typically involves resection of the amygdala and hippocampus and varying amounts of anterior temporal neocortex. While surgical resection of this tissue is largely successful at ameliorating seizure occurrence (Josephson et al., 2013; Wiebe, Blume, Girvin, & Eliaziw, 2001), it can also result in post-surgical cognitive changes particularly in new learning and memory. Researchers have sought to predict which patients will take a cognitive ‘hit’ and which patients will not, using extensive presurgical neuropsychological testing (Chelune, 1995; Harvey et al., 2008; St-Laurent et al., 2014), assessment of hemispheric language dominance using a WADA procedure (Loring et al., 1995), positron emission tomography (PET; (Griffith et al., 2000; Leeman, Leveroni, & Johnson, 2009)), structural magnetic resonance imaging (MRI; (Reminger et al., 2004; Trenerry et al., 1993; Trenerry, Westerveld, & Meador, 1995)), and more recently functional MRI (fMRI; (Bonelli et al., 2010; McCormick, Quraan, Cohn, Valiante, & McAndrews, 2013; Mechanic-Hamilton et al., 2009)). While these studies have produced varied results in terms of successful prediction of pre-to-post surgical cognitive changes, they have demonstrated a general trend that resection of atrophied and inactive brain tissue results in fewer deficits compared to resection of active and structurally healthy tissue. These studies have also demonstrated extensive differences between patients with TLE and healthy controls in terms of memory performance, structural brain integrity and functional brain networks.
Importantly, seizure onset can occur at varying stages in life, from perinatal periods to midlife. Earlier seizure onset appears to be associated with different patterns of functional organization for both memory (Mechanic-Hamilton et al., 2009; Sidhu et al., 2015) and language (Bell et al., 2002; Springer et al., 1999), as well as altered resting state network properties (Doucet et al., 2014). Behaviourally, early onset has been related to reduced IQ (Kaaden & Helmstaedter, 2009), but also better memory outcome following surgical resection of the epileptogenic focus (Hermann, Seidenberg, Haltiner, & Wyler, 1995). Both types of changes could relate to the increased malleability of the developing brain. Increased malleability may reduce a function’s reliance on canonical networks that become disabled with later onset epilepsy allowing for adaptive plasticity. This can occur through increased reliance on degenerate components—distinct structural elements within a system that can carry out similar functions. Conversely, maladaptive plasticity may occur if early onset seizures hinder the development of key neurocognitive networks. Surprisingly, some studies report worse memory for individuals with early epilepsy onset (Hermann et al., 2002; Lespinet, Bresson, N’Kaoua, Rougier, & Claverie, 2002), while others report better memory associated with earlier epilepsy onset (Baxendale et al., 1998). These differences may come down to methodological decisions such as whether age of onset is treated as a continuous variable (Baxendale et al., 1998) or as a binary category with a particular cut-off for early onset (Hermann et al., 2002; Kaaden & Helmstaedter, 2009; Lespinet et al., 2002). This cut-off for early versus late also varies, sometimes being as early as 5 years old (Lespinet et al., 2002) based on evidence that language functioning can reorganize to the contralateral hemisphere prior to this point, or selected based on a median split of study participants (Hermann et al., 2002; Kaaden & Helmstaedter, 2009).

Another methodological issue is that age of epilepsy onset has been examined assuming a linear relationship for age as a continuous variable. Evidence from plasticity in rodents, however, has suggested a non-linear trend between age at which brain damage occurs and memory performance (Kolb, Mychasiuk, Williams, & Gibb, 2011; Kolb, Petrie, & Cioe, 1996). In this line of work, very early life lesions, during the first week post-birth, are very detrimental to performance as an adult, lesions during adulthood are also very detrimental to performance, but lesions in week two are related to control levels of performance. Thus, discrepancy among the age of onset findings in TLE may be due to a non-linear relationship between onset and memory ability.
This thesis contains three studies aimed at addressing questions of how age of epilepsy onset relates to memory performance, brain structure, and functional network integrity. To that end, I will first discuss the diagnosis and surgical treatment of TLE, memory differences in TLE, and the differences in brain structure, function, and network organization compared to healthy populations. I will then discuss the effects of age of seizure onset on cognition and the brain before outlining the specific studies. Critically, in this work I will treat age of epilepsy onset as a continuous variable, to take advantage of the full breadth of variance and examine both linear and non-linear relationships of onset age with memory performance, brain structure and functional brain network properties.

1.2 Summary of Diagnosis and Surgical Treatment

TLE often presents unilaterally, meaning that seizures originate from either the left or right MTL. Up to 30% of patients with epilepsy fail to find adequate control of seizures with medication and seek surgical treatment to remove the tissue that is generating seizures, namely the hippocampus and surrounding MTL cortex (Picot, Baldy-Moulinier, Daurès, Dujols, & Crespel, 2008; Sander, Genton, & Portera-Sanchez, 1993). Patients seeking surgery are admitted to an epilepsy medical unit (EMU) where their antiepileptic medication is gradually weaned while they are under continuous video and electroencephalography (EEG) monitoring. Epileptologists review these recordings to determine the seizure focus.

These patients also undergo extensive neuropsychological testing, evaluating their general intellectual functioning, memory, language, attention, and executive functioning, as well as a structural MRI. At Toronto Western Hospital (TWH) and some other epilepsy centres, patients will also receive fMRI scans to examine brain activity associated with language, memory and resting state networks.

In straightforward cases, scalp EEG measures will demonstrate seizure activity originating in a single, lateralized location in either the left or right hemisphere. When this activity can be corroborated with radiological evidence of hippocampal damage and neuropsychological
evidence of lateralized memory dysfunction, surgical teams can have reasonable confidence that resection of the suspected seizure focus will result in successful seizure management (Hennessy et al., 2001).

When scalp EEG measures of seizure activity demonstrate bilateral ictal abnormalities (seizures appearing to come from both the left and right temporal lobes), or multifocal abnormalities (seizures appearing to come from more than one location in the brain), invasive intracranial electrodes are implanted bilaterally into the temporal lobes (at TWH involving hippocampal depth electrodes and neocortical strips) to provide finer-grain diagnostic ictal recordings. These recordings provide critical diagnostic information directly from the suspected sources of seizure generation.

Identification of a discrete epileptogenic focus that can be removed without excessive morbidity provides the individual with the option of surgery. At many epilepsy surgery centres, including TWH, surgical resection in TLE includes removal of anterior temporal lobe structures including part of the anterior temporal neocortex, amygdala and anterior hippocampus. An example is showing the site of resection is presented in Figure 1.1. The extent to which tissues are removed may vary depending on whether a surgeon performs a standard anterior temporal lobectomy (ATL) or a selective amygdalohippocampectomy. Selective amygdalohippocampectomy involves resection of the parahippocampal gyrus, hippocampus, amygdala, with minimal invasion of neocortex serving only to approach the mesial structures. A standard ATL is similar to the selective approach, but involves an additional resection of the anterior temporal neocortex. Some studies have shown that ATL provides improved chance of seizure elimination above selective amygdalohippocampectomy (Josephson et al., 2013; Kollndorfer, Fischmeister, Kasprian, Prayer, & Schöpf, 2013), while others have shown that ATL does not differ from a selective amygdalohippocampectomy (Mansouri et al., 2014). Successful elimination of seizures occurs in 50 – 65% of cases, while significant reduction of seizures occurs in 86% of cases overall (Engel et al., 2003; McIntosh et al., 2004), but these numbers are improved by presence of mesial temporal sclerosis (MTS), the most common lesion in TLE characterized by neuronal cell loss and gliosis of the hippocampus (Engel et al., 2003).
Figure 1-1. A structural MRI scan of a patient who underwent an anterior temporal lobectomy, illustrating resected tissue in the epileptogenic hippocampus, and temporal neocortex.

1.3 Cognition and brain abnormalities associated with TLE

1.3.1 Cognition

Episodic memory is the most prominent deficit in TLE (Bell, Lin, Seidenberg, & Hermann, 2011). Patients with TLE tend to show material specific memory impairment — those who have left sided seizures (LTLE) demonstrate verbal memory impairment and those with right sided seizures (RTLE) show visual memory impairment (Hermann, Seidenberg, Schoenfeld, & Davies, 1997; Milner, 1968). These material specific impairment effects can increase following surgical resection of the seizure focus (Milner, 1968). In fact, neuropsychological examination of impairments provides valuable converging evidence to scalp EEG and MRI by characterizing focal functional deficits when clinicians are conferencing which patients should receive surgery (McAndrews & Cohn, 2012). Neuropsychologists conduct extensive testing on patients prior to surgery to compare visual memory performance to verbal memory performance within a given patient and how the patient performs relative to normative data to provide an estimate of that patient’s impairment. If someone has relatively weak visual memory compared to verbal memory functioning, but they are presenting with seizures generating in the left medial temporal lobes as measured by EEG, this may indicate greater likelihood of poor surgical outcome. When examining pre- to post-surgical changes, neuropsychologists have looked at the integrity of the to-be-resected tissue and the integrity of the spared, contralateral tissue. Typically, if the to-be-
resected tissue is dysfunctional and atrophied, resection of this tissue will have less impact on cognition following surgery. If the to-be-resected tissue is functional and shows little signs of atrophy or disconnection from other brain structures, then removal of this tissue may result in more impairment following removal. This type of phenomenon has been termed functional adequacy, since it examines the adequacy of the tissue being removed (Chelune, 1995). Clinicians and researchers also note that if the contralateral medial temporal structures are sufficiently able to support memory without the to-be-resected hippocampus, then resection of the epileptogenic hippocampus should lead to minimal impairments. This is most directly assessed by anesthetizing the hemisphere of the to-be-resected anterior temporal lobe, and administering a memory test to the patient. If the awake, to-be-spared hemisphere is capable of successfully completing the memory test, then catastrophic memory change is unlikely to occur. This type of examination is referred to as functional reserve, since it examines the integrity of the reserve tissue (Chelune, 1995).

Research has also shown that there are cognitive impacts outside of memory in individuals with TLE, including IQ, language and executive function, though these are less pronounced (Hermann et al., 1997). Language functioning has been particularly pertinent in TLE research, since there is a relationship between post-operative naming and fluency decline following ATL in patients with dominant hemisphere (typically left) TLE (Langfitt & Rausch, 1996; Seidenberg, Geary, & Hermann, 2005). These alterations in diffuse areas of cognition demonstrate that while seizures may be originating in a focal area related to episodic memory, seizure propagation may affect networks and cognition outside of memory and the MTL.

1.3.2 Gray Matter Abnormalities

TLE is associated with reduced focal MTL structural and functional integrity. The most common lesion in TLE, as mentioned above, is MTS, found in approximately two-thirds of cases (Berg, 2008). Since classification of MTS can only be done following surgical resection of the hippocampus and histological examination of the tissue, to evaluate the integrity of the hippocampus before surgery, radiologists will use MR images to estimate the extent of hippocampal atrophy and the extent of gliosis (based on increased hippocampal signal during a T2 weighted MRI scan). Volumetric analysis on the hippocampus, which is less common in clinical practice, can also reveal reductions in volume in the epileptogenic hippocampus when
compared to healthy controls and in terms of asymmetry with the non-epileptogenic hippocampus (Barnett, Park, Pipitone, Chakravarty, & McAndrews, 2015; Bernasconi et al., 2003; Mechanic-Hamilton et al., 2009). This damage appears to be more prominent in the head of the hippocampus (Bernasconi et al., 2003; Dam, 1980). Within the MTL, studies have also shown reduced volume of the amygdala, entorhinal cortex, and perirhinal cortex ipsilateral to the seizure focus compared to healthy controls (Bernasconi et al., 1999, 2001, 2003).

Outside of the MTL, semi-automated parcellation software, such as FreeSurfer (https://surfer.nmr.mgh.harvard.edu), allows for characterization of cortical thickness throughout the entire brain. Use of this technique has shown regional thinning in ipsilateral temporal pole, lateral temporal cortex, retrosplenial cortex, precuneus, insular cortex, middle frontal gyrus, superior frontal gyrus, and superior parietal regions in patients with TLE with and without hippocampal sclerosis compared to healthy age-matched controls (Bernhardt, Bernasconi, Concha, & Bernasconi, 2010; Mueller et al., 2009). While these patterns are similar between people with left and right TLE, there appears to be more thinning in people with left TLE particularly in ventrolateral prefrontal cortex (Kemmotsu et al., 2011). A meta-analysis of voxel-based morphometry studies (VBM), has also shown, in both LTLE and RTLE, reduced grey matter concentration in bilateral thalamus, and reduced middle cingulate grey matter in patients with LTLE (J. Li, Zhang, & Shang, 2012). Thus, even with a relatively focal epileptogenic zone there can be more widespread cortical and subcortical abnormalities, particularly in the affected hemisphere.

### 1.3.3 White Matter Abnormalities

Considerable differences in white matter between patients with TLE and healthy controls have also been observed. White matter is primarily examined using diffusion MRI, a technique that uses the diffusion of water to determine the degree to which tissues have high linear organization. If there is little or no tissue organization, then water molecules have Brownian motion like diffusion properties — the probability density of diffusion is spherical and uninhibited. If tissue has highly linear organization, then water will preferentially diffuse in one direction compared to other orthogonal directions. Fractional anisotropy (FA) is a measure of preferential diffusion along a primary direction and is often interpreted as reflecting underlying white matter integrity. Studies of patients with temporal lobe epilepsy have shown FA decreases
in fornix (Concha, Beaulieu, Collins, & Gross, 2009), external capsule (Arfanakis et al., 2002; Gross, Concha, & Beaulieu, 2006), genu of the corpus collosum (Gross et al., 2006), cingulum (Gross et al., 2006), uncinate fasciculus (Govindan, Makki, Sundaram, Juhász, & Chugani, 2008; Lin, Riley, Juranek, & Cramer, 2008), and arcuate fasciculus (Govindan et al., 2008; Lin et al., 2008) relative to healthy controls. These differences in FA are most prominent in the ipsilateral hemisphere and are thought to contribute to changes in cognition and functional network integration (Voets et al., 2012).

1.3.4 Functional activation Abnormalities

Functional integrity has also been measured, initially using positron emission tomography (PET) and, subsequently, with fMRI. PET involves the injection of radioactive isotopes attached to biologically relevant molecules, such as a glucose analogue, into the brain. As the radioisotope decays it will release pairs of gamma rays which can be detected and used to determine the location of the isotope and attached biological molecule in the brain. Theodore et al. (1992) found that there was significant metabolic asymmetry between the ipsilateral temporal lobe and contralateral temporal lobe in patients with temporal lobe epilepsy using \(^{18}F\) deoxyglucose and those patients showing the greatest asymmetry in temporal lobe metabolism tended to be the ones who were seizure free following surgery. Unfortunately, PET has several drawbacks that preclude its widespread use. First, it requires injection of isotopes that expose patients to radiation. Second, PET often requires the possession of a cyclotron to manufacture radioisotopes.

Another tool to investigate functional abnormalities is fMRI, which is more widely available and less invasive. Functional MRI detects differences in the blood oxygenated level dependency (BOLD) signal—the ratio of oxygenated to deoxygenated haemoglobin—in discrete voxels from scan to scan relying on the different profiles of magnetic properties held by oxy- and deoxyhaemoglobin. When patients with TLE are asked to complete an encoding task in the scanner, they tend to demonstrate less activation in the epileptogenic hippocampus compared to the contralateral hippocampus (Barnett et al., 2015; Powell et al., 2007; Vannest, Szaflarski, Privitera, Scheft, & Holland, 2008). Patients with TLE also show significantly less MTL activation during scene encoding tasks relative to healthy controls (Cheung, Chan, Chan, Lam, & Lam, 2006). This reduction in activation is thought to reflect dysfunction in the epileptic temporal lobe since the degree of activation in the MTL is related to memory performance on
clinical measures of memory (Barnett et al., 2015; Bonelli et al., 2010; Cheung et al., 2006; Powell et al., 2007). Thus, this research not only demonstrates differences between TLE groups and healthy controls, but also allows for the assessment of functional integrity.

### 1.3.5 Brain Network Abnormalities

The structural differences discussed above — subcortical volume, cortical thickness, grey matter concentration, and FA alterations — demonstrate that, even when the epileptic focus is confined to the hippocampus and mesial temporal structures, the effects of epilepsy are widespread, affecting the connections to the rest of the brain and regions outside the temporal lobes. Indeed, network changes have been found in terms of functional connectivity (Liao et al., 2011; Quraan, McCormick, Cohn, Valiante, & McAndrews, 2013; Waites, Briellmann, Saling, Abbott, & Jackson, 2006), diffusion-based measures of structural connectivity (Liao et al., 2011), and cortical thickness based measures of connectivity (Bernhardt, Chen, He, Evans, & Bernasconi, 2011). Further, some of these network differences have been shown to relate to cognitive ability (McCormick et al., 2014, 2013; Voets et al., 2014), and perform better than focal measures of integrity at predicting post-operative memory decline (McCormick et al., 2013).

#### 1.3.5.1 Network Analysis Methodology

Network analysis of the brain involves, centrally, characterizing the connections between brain regions. When describing the brain in network terminology, discrete brain regions are often termed as “nodes” while the connections between these nodes are called “edges”. Edges can be physical — such as axonal trajectories leading to synaptic connections — or they can be statistical — such as when two regions have a high level of signal covariance in fMRI.

A number of metrics can be extracted from network level analysis. The simplest measure is the strength of the connections between two regions. For example, if two regions have high temporal signal covariance in fMRI, one can assume that these regions are strongly connected relative to two regions that have low signal covariance. More complex metrics can be extracted using graph theory analysis—a method that treats the brain as a set of nodes and edges to create a simplified graph or network, from which topological characteristics can be calculated (Rubinov & Sporns, 2010). While graph theory is powerful in its ability to extract this topological information, it is
strongly dependent on several analysis choices such as how one defines the network nodes to construct the graph or how spurious connections will be treated.

When examining brain network organization, researchers must choose how to segregate the brain into disparate nodes. The smallest possible partition using neuroimaging is the voxel—a volumetric pixel that is the smallest unit of signal measurement. The size of a voxel will vary depending on the scanning parameters, but voxels do not have any inherent underlying biological meaning. Using individual voxels to represent nodes is also problematic because of signal redundancies that are introduced due to the nature of imaging technology (Sporns, 2012). For this reason, researchers attempt to partition the brain for connectivity studies using anatomically or functionally meaningful partitions. Though, no “gold standard” partition has been championed.

Once the brain is partitioned, whole brain tractography using diffusion weighted imaging or functional connectivity using fMRI can be performed, measuring the structural and functional connectivity of the brain, respectively. The structural or functional connectivity between regions is taken and used to create a connectivity matrix that contains the connectivity information (e.g. signal correlation, number of DTI-derived streamlines) from each node to every other node in the brain.

1.3.5.2 Healthy Brain Networks

Investigations of brain networks have found that, at rest, signals from disparate regions of the brain covary together forming reliable and highly replicable spatial patterns (Beckmann, 2005). The first investigation into resting state connectivity found that the left sensorimotor cortex had strong connectivity to the contralateral sensorimotor cortex, along with the supplementary motor and premotor cortex (Biswal, Yetkin, Haughton, & Hyde, 1995). Since then, researchers have speculated that this resting fluctuation may arise from rehearsal/learning, since these regions typically communicate and fire together during motor action (Buckner & Vincent, 2007). Further, a handful of canonical resting state networks (RSNs) have been identified that can be reliably extracted from resting state data: the somatomotor, auditory, visual, default mode, frontoparietal, and executive control network (Beckmann, 2005). These networks also overlap with functional networks activated during specific task demands (Smith et al., 2009). The default mode network (DMN) is of particular interest to the study of TLE because the hippocampus and
MTL cortex tends to fall within this network (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010). Furthermore, the spatial extent of the DMN is largely overlapping with the activity seen during episodic memory retrieval (Buckner, Andrews-Hanna, & Schacter, 2008).

Using graph theory, several other characteristics have been consistently found in the nervous system of healthy humans and deviations from this have been found in people that have TLE. The healthy brain has consistently been characterized by high network clustering, coupled with high network integration (Bassett & Bullmore, 2016; Bassett, Meyer-Lindenberg, Achar, Duke, & Bullmore, 2006) — a topology that allows for functional specialization of clusters with fast and efficient long range communication of the network, referred to as a small world topology (Watts & Strogatz, 1998). To extract these measures of topology, a connectivity matrix of pairwise connections in a network is typically thresholded so that only sufficiently strong connections remain, to exclude connections that may be spurious. The matrix is then often binarized so that connections either exist (edge = 1) or do not exist (edge = 0). From these thresholded and binarized matrices, graph metrics can be extracted to evaluate small worldness. Network clustering is the average clustering coefficient for each node in a network. The clustering coefficient for node $i$ is calculated by finding the proportion of nodes connected to node $i$ that are also connected to each other. If a high proportion of node $i$’s neighbours are also connected to each other, then node $i$ will have a higher clustering coefficient. Network integration is typically measured using a metric called characteristic path length which is the average shortest path length between any two nodes in a network. A given path between any two nodes may have to traverse other intervening nodes which is thought to take more time. If path length is short, with few intervening nodes, then network integration is thought to be high, since few steps are needed to send information between any two nodes in a network. Small world networks manage to balance both segregation and integration. Another reliable finding from graph theory analysis is that the healthy brain is modular, and can be divided into communities of nodes that have high within-community connectivity with low between-community connectivity (Chen, He, Rosa-Neto, Germann, & Evans, 2008; Meunier, Lambiotte, Fornito, Ersche, & Bullmore, 2009). Further, these communities tend to resemble canonical, RSNs (Beckmann, 2005). Below, I will describe how these network properties and characteristics are altered in TLE.
1.3.5.3 Structural Connectivity Alterations

Research investigating structural connectivity with diffusion weighted MRI, while limited, has demonstrated decreases in connection density between posterior cingulate cortex (PCC) and bilateral MTL in TLE compared to healthy controls (Liao et al., 2011). The PCC is a central hub of the DMN and disruption of network density between the MTL and PCC may explain some of the memory impairments seen in patients. Within the limbic system, another study found increased network clustering and nodal integration in the ipsilateral insula, thalamus and superior temporal cortex in patients with TLE relative to controls (Bonilha et al., 2012), further demonstrating that TLE has widespread network level alterations.

Another way to examine structural network properties is to examine the shared structural covariance of nodes across subjects often using cortical thickness. A positive correlation between two regions across subjects is assumed to indicate connectivity between these regions due to common maturational influences and adult neuroplasticity (Evans, 2013; Lerch et al., 2006; Sporns, 2012).

Bernhardt et al. (2011) parcellated the cortex of 122 TLE patients and 47 age and sex matched healthy controls. Based on the across-subject correlations of cortical thickness between 90 anatomically defined regions across the entire brain, correlation matrices were created which were analyzed using graph theory. Their analysis revealed a reduction in overall brain small-world topology. In a subset of their patient group, they performed a longitudinal analysis which revealed that this pattern worsened over time and that increased network disruptions were associated with worse postoperative seizure outcome. These results suggest that long term seizure exposure has detrimental effects on the network organization of the brain.

1.3.5.4 Functional Connectivity

While measures of structural connectivity are highly relevant to the expression of communication in the brain, functional connectivity provides a more direct examination of how communication is occurring. The following section will discuss the functional connectivity alterations in patients with TLE and how functional connectivity is related to presurgical cognition and post-surgical cognitive change.
Voets et al (2012) found that their LTLE group showed disconnection of the epileptogenic MTL from the DMN, while the RTLE group showed altered connectivity in bilateral MTL – DMN connectivity and alterations in the sensorimotor network. In a separate study, this group also showed that higher contralateral HC – PCC connectivity was associated with better episodic memory, and that connectivity between the ipsilateral or contralateral hippocampus and adjacent entorhinal cortex and parahippocampal gyrus was negatively associated with memory performance (Voets et al., 2014).

Further evidence demonstrating altered DMN connectivity comes from McCormick et al (2013) who found that connectivity from the PCC to the epileptogenic hippocampus and to the temporal pole on the epileptogenic side was disrupted in TLE during rsfMRI. Interestingly, they were able to show that connectivity from the left hippocampus to the PCC was related to presurgical verbal memory in LTLE and connectivity from the right hippocampus to the PCC was related to presurgical visual memory ability in RTLE. Since the PCC is a major hub of the DMN, this evidence demonstrates that a disconnection of the hippocampus from critical brain networks is related to deleterious effects on memory. To further support this, these authors also found that following surgery, patients who had strong connectivity between the epileptogenic hippocampus and the PCC had a greater loss of material specific memory compared to those who had weak connectivity. Finally, on a subsample of patients who received follow-up rsfMRI scans, the authors discovered that post-surgical connectivity between the PCC and the remaining hippocampus was related to better episodic memory performance for the ‘non-preferred’ material (e.g., better verbal memory for right PCC-HC), suggesting that the spared hippocampus has some compensatory ability provided that it can connect to the DMN.

In a subsequent study, McCormick et al. (2014) examined resting state network connectivity in 20 regions of the brain associated with the DMN and found that patients with TLE showed reduced interhemispheric connectivity relative to healthy controls and increased long-range anterior to posterior connectivity. This increased coupling appeared to connect posterior regions of the network to anterior regions, bypassing the medial temporal lobes. Within the patient group, altered connectivity (more disparate from controls) was related to worse material specific memory performance in patients with TLE (McCormick et al., 2014). This whole network pattern was more strongly related to material specific memory than simply the hippocampus to
PCC connectivity, suggesting that the integrity of the entire network provided meaningful information beyond just one link between nodes.

The studies mentioned here suggest a specific alteration in hippocampal to DMN connectivity, but have examined the hippocampus as a unitary, somewhat homogeneous structure. Evidence from the healthy brain has shown that the anterior and posterior hippocampus may have different network connectivity preferences and may contribute to memory expression in different ways (Adnan et al., 2016; Poppenk, Evensmoen, Moscovitch, & Nadel, 2013; Poppenk & Moscovitch, 2011). In the above mentioned study from Voets et al (2014), the authors sought to examine the connectivity profile along the long axis of the hippocampus using functional connectivity. The authors seeded from each voxel in the hippocampus, separately, and examined the strength of connectivity of that voxel to targets known to be connected to the anterior and posterior hippocampus in the healthy brain. The anterior memory network regions included the entorhinal cortex, orbitofrontal cortex, and temporal pole, while the posterior memory network included the parahippocampal gyrus, lingual/fusiform gyrus, dorsolateral prefrontal cortex, posterior cingulate cortex, precuneus, and thalamus. If a given voxel had a stronger magnitude of connectivity to one of these networks, it was labeled as being a part of that network, i.e. if a voxel in the hippocampus showed greater magnitude of connectivity to the anterior memory network, it was labeled as an anterior memory network voxel. Using this technique, they were able to show that both patients and healthy controls showed an anterior division and posterior division that was split along the long axis of the hippocampus. They also showed that the epileptogenic posterior hippocampus was less connected to the PCC, but was more connected with the parahippocampal gyrus in patients with TLE compared to healthy controls. Further, in LTLE, the epileptogenic anterior hippocampus showed increased connectivity to the entorhinal cortex When the authors examined how these connectivity changes related to memory ability (pooling LTLE and RTLE groups and using material specific measures of impairment), they found that stronger connectivity between the contralateral posterior hippocampus with the posterior cingulate cortex was related to spared memory ability, while increased connectivity between the ipsilateral anterior hippocampus and entorhinal cortex was associated with impaired memory ability.

Overall, these studies show that patients with TLE have reduced connectivity between the MTL and the DMN, largely demonstrated through a reduction in connectivity to the PCC. Furthermore, the greater the reduction in connectivity between the hippocampi and the PCC, the
worse memory performance is. However, those who do have this strong connectivity between the epileptic hippocampus and the PCC, presurgically, may have increased risk of postoperative memory decline.

1.4 Effects of Age of Onset on Cognition and the Brain

While the previous sections discussed differences in patients with TLE compared to neurologically healthy individuals, within the TLE population there is considerable variability in terms of cognitive ability, brain structure, and brain function. Some of this variability can be accounted for by age of onset. As noted before, TLE can present at varying points in life and in this section I will review the research demonstrating a connection between age of onset, cognition, and brain network organization. I will also discuss how early life plasticity can potentially serve as a double edged sword allowing for adaptive plasticity—reorganization of brain networks in order to spare cognitive functioning by engaging degenerate systems—and developmental hindrance—maladaptive or impaired network development which disrupts cognitive functioning.

Previous work has demonstrated that an earlier age of onset is associated with altered memory network organization (Sidhu et al., 2015), language reorganization (Bell et al., 2002; Cousin, Baciu, Pichat, Kahane, & Le Bas, 2008; Springer et al., 1999), better memory outcome following surgical resection of the epileptogenic focus (Griffin & Tranel, 2007; Hermann et al., 1995), greater activation asymmetry during encoding away from the epileptic hippocampus (Mechanic-Hamilton et al., 2009), altered resting state network properties (Doucet et al., 2014) and better memory presurgically (Baxendale et al., 1998). These changes may be indicative of some sort of adaptive plasticity is preferentially available in early onset patients. However, other work has shown that early onset epilepsy is associated with reductions in overall white matter (Hermann et al., 2002), reduced IQ (Kaaden & Helmstaedter, 2009), and worse memory (Lespinet et al., 2002). These changes may indicate that early life epilepsy hinders developmental processes leading to cognitive impairment.
Clearly, there is conflicting evidence for these theories, and the discrepancy across studies may be based on analysis decisions. One decision that needs to be made when examining the effects of age of onset is whether to treat onset age as a binary or continuous variable. While Baxendale et al. (1998) used age of epilepsy onset as a continuous variable in a linear regression, it has often been the case that age of onset is treated in a binary fashion, contrasting early onset groups with late onset groups (Bell et al., 2002; Doucet et al., 2014; Griffin & Tranel, 2007; Hermann et al., 1995; Kaaden et al., 2011; Lespinet et al., 2002; Mechanic-Hamilton et al., 2009). These binary contrasts require a threshold to determine what is considered *early onset* and what is considered *late onset* and a variety of cut-offs have been used ranging from 5 years old to 25 years old. Some cut-off thresholds are based on sample median split or database median split to determine their early vs. late cut-off (Hermann et al., 1995; Kaaden & Helmstaedter, 2009), while another study has used a cut-off based on independent evidence that language dominance can reorganize to the contralateral hemisphere, if damage occurs before this age (Lespinet et al., 2002). Deciding to use onset age as a continuous variable or a binary contrast and the further variability of cut-offs certainly may contribute to the conflicting findings with respect to age of onset.

Furthermore, these previous studies have assumed a linear relationship between age of onset and cognition and brain structure. However, research investigating plasticity in rodents suggests a non-linear relationship between age at which brain damage occurs and memory performance/synaptic arborisation in disparate networks (Kolb et al., 2011, 1996). In this series of investigations, very early lesions (in the first week of life) to the medial prefrontal cortex (mPFC) was associated with detrimental memory ability measured using the Morris water maze. Lesions to the mPFC in the second week of life were associated with minimal memory deficits relative to sham rats, but lesions to the mPFC during adulthood were severely detrimental to memory (Kolb et al., 1996). This suggests that adaptation can occur following early life damage, but damage too early still results in impairment. However, in follow up studies, certain factors such as environmental enrichment and exercise were able to improve performance in rats lesioned very early on and these animals saw the greatest benefit to recovery compared to animals who had lesions in the second week of life (Kolb & Elliott, 1987; Kolb et al., 1996). The performance benefits were associated with increased overall cortical thickness and dendritic branching in parietal cortex (Comeau, Gibb, Hastings, Cioe, & Kolb, 2008; Kolb et al., 1996).
healthy human development, there is also evidence for heterosynchronous, nonlinear development of the cerebral cortex (Giedd et al., 1999) and hippocampus (Gogtay et al., 2006), with a large amount of the cortex showing a cubic or quadratic trajectory (Shaw et al., 2008; Tamnes et al., 2010). Thus, I would argue that any examination of the effects of age of onset on cognition and the brain require examination of non-linear effects as well as linear effects.

1.5 Research Aims and Hypotheses

The current literature regarding the effect of age of onset on cognition reveals a somewhat complex pattern, with conflicting evidence regarding the impact of onset age on memory. This dissertation addresses several research aims. One aim was to examine the relationship between age of onset and material specific memory. For this, I analyzed data from 80 patients with TLE, with approximately equal numbers of patients with LTLE and RTLE, and examined their pre-operative performance on measures of verbal and visual memory in relation to their age of onset. Two competing hypotheses were examined — an adaptive plasticity hypothesis and a developmental hindrance hypothesis. The adaptive plasticity hypothesis posits that early age of onset affords increased neural plasticity allowing for advantageous re-wiring of networks. To the extent that degenerate processes are able to support memory ability, I expected to see better material specific memory performance in early onset individuals compared to individuals with later ages of epilepsy onset in whom the memory networks are more ‘fixed’. The developmental hindrance hypothesis would predict that early onset of TLE would cause a disruption in normal brain development of critical processing networks, leading to worse material specific memory compared to late onset of TLE. Of course, this relationship may also not be strictly linear and therefore nonlinear relationships will also be considered.

The second aim of this dissertation was to further characterize the differences in brain structure as it relates to age of onset. As mentioned above, there are many overall structural brain changes seen in patients with TLE. While some of these differences have been shown to relate to age of onset (Hermann et al., 2002), they have mostly been looked at in terms of TLE vs. healthy controls. This dissertation examines the effects of age of onset on regional cortical
thickness, while controlling for age at scan and sex. I also examined how regions that are modulated by age of onset relate to memory ability. Given that TLE is associated with widespread reductions in cortical thickness, I predicted that those individuals who have the thinnest cortex in DMN regions will have worse memory and this may be modulated by age of onset.

My third aim was to investigate functional network differences in TLE as a function of age of onset. I used a seed based functional connectivity analysis of resting state fMRI and extracted graph theory metrics across the brain. Specifically, I examined whole brain network integration and segregation and how they are modulated by age of onset in either a linear or non-linear fashion. I also extracted measures that represent ‘hubness’, such as betweenness centrality, from critical memory regions showing altered connectivity in TLE — the hippocampus and the posterior cingulate cortex. Importantly, I also examined the relationship between brain network properties and cognition as it relates to age of onset. In order to understand whether changes in network properties are adaptive or pathological it is important to investigate how they relate to behaviour. I hypothesized that early onset would result in “more healthy” whole brain network properties, as was shown in Doucet et al (2014), and that these network properties would be related to better material specific memory performance.

Finally, using resting state fMRI data, my fourth aim was to parcellate the hippocampus in healthy controls and patients with TLE using resting state functional connectivity and $k$-means clustering, examine whether the resulting clusters showed altered connectivity related to memory and examine whether age of onset modulated these effects. In Voets et al (2014), the authors used a very blunt approach of assigning a voxel to a particular category based on the magnitude of connectivity to an anterior or posterior memory network mask. In this final study, I parcellated the hippocampi into anterior and posterior divisions using a $k$-means clustering algorithm which clustered together voxels that have similar voxel-wise whole brain connectivity fingerprints. This approach does not make any assumptions about particular connections, and does not rely on anatomically defined targets. I then examined the differences in hippocampal whole brain voxel-wise connectivity between people with TLE and healthy controls, examined hippocampal to PCC connectivity as it relates to memory ability and, interrogated whether this relationship was modulated by age of onset. I hypothesized that $k$-means clustering would be able to cluster the hippocampus into anterior and posterior segments and that connectivity of these segments to the
PCC would be related to memory ability. I also hypothesized that age of onset would modulate this relationship.

The results of these experiments will provide insight into the possible plasticity processes in the brain. Plasticity is often considered in the context of one-off brain insults in terms of stroke, traumatic brain injury or lesions. In epilepsy, seizures are ongoing, throughout life and while each individual seizure may not induce a significant amount of damage, over the course of this disease, there is thought to be a build-up of structural damage (Bernhardt et al., 2009). How the brain reacts to this type of pathology in terms of plasticity is largely unknown and the results of these studies may open the door for generating hypotheses regarding the architectural processes that may subserve brain plasticity. Furthermore, these results may be significant to the field given the increased promise of network level measures in clinical utility. As studies are beginning to show the potential of MRI-based network measures in assisting in diagnosis, response to treatment, and surgical risk, it is important to understand how different disease variables, such as onset age affect these network measures. Given the increased malleability of the immature brain and subsequent alterations in network properties that have already been noted (Doucet et al., 2014), if we would like to use network information to predict surgical outcomes or cognitive changes following surgery, we need to understand if there is a modulating effect of age of onset on the relationship between cognition and brain networks.
Chapter 2

General Methods

2.1 Neuropsychological Testing

2.1.1 Overview

A comprehensive neuropsychological battery was administered to patients in Chapters 3, 4 and 5 that included assessment of intelligence, learning/memory, and language. Using factor loadings from a principle component analysis (PCA) performed by St-Laurent et al. (2014), raw scores of verbal memory, visual memory and IQ were transformed into factor scores for each patient. Our group had previously shown these factor score to (1) discriminate between patients with right and left TLE and (2) reliably predict the degree of material-specific memory change following anterior temporal lobe resection. We reasoned that the factor scores, which afforded data reduction over several measures, provided a more reliable representation of core abilities than single test scores. The IQ factor reflected loadings from verbal IQ and performance IQ from the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999).

2.1.2 Visuospatial Memory Factor

The visuospatial memory (VSM) factor was primarily based on loadings from correct responses on the Warrington recognition memory test for faces (RMF), total recall across trials one through five on the Rey visual design learning test (RVDL), and number of trials to criterion for the conditional associative learning test (CAL). The RMF test involves a study period in which 50 faces are viewed, followed by a delayed recognition test in which subjects are asked to make a forced choice recognition between previously studied faces and lures (Warrington, 1984). The RVDL consists of a study session for 15 visual line designs followed by an immediate recall session in which subjects are asked to draw the previously encountered visual designs (Spreen & Strauss, 1991). This is repeated five times. Finally, the CAL consists of having patients learn a
one-to-one association between four cards and four locations (A. E. Taylor, Saint-Cyr, & Lang, 1990).

2.1.3 Verbal Memory Factor

The verbal memory (VM) factor was based on loadings from correct responses on the Warrington recognition memory test for words (RMW), total recall (RAVLT-tot) over five study-test trials from the Rey auditory verbal learning test (RAVLT) and percent retention (RAVLT-ret) from the RAVLT. The RMW test consists of a study session for 50 words followed by a delayed forced choice recognition test between lures and studied words (Warrington, 1984). The RAVLT consists of a study session for 15 words followed by an immediate free recall period. This is repeated five times. Percent retention for the RAVLT is calculated by observing the percentage of words retained from the fifth session to an extra 20 minute delayed recall period (Strauss, Sherman, & Spreen, 2006).

2.2 Magnetic Resonance Imaging Parameters

2.2.1 T1-weighted Anatomical Images

For Chapter 3, 4 and 5 a high-resolution 3D anatomic scan was collected on a 3-T Signa MR system (GE Medical Systems, Milwaukee, WI, USA) for cortical thickness analysis (Chapter 3) or for normalization of functional images to standard MNI space (Chapters 4 and 5) for each subject (T1-weighted sequence, FOV 220 mm, 146 slices, flip angle = 12°, TE = 3 ms, TR = 8 ms, 256 x 256 matrix, resulting in voxel size of 0.86 x 0.86 x 1.0).

2.2.2 Resting state functional MRI

For Chapter 4 and Chapter 5, task-free resting state fMRI (T2*-weighted) scans with an echo-planar pulse imaging (EPI) sequence was also acquired (FOV 240 mm, 28–32 slices depending on head size, TR = 2000 ms, TE = 25 ms, 64 x 64 matrix, 3.75 x 3.75 x 5 voxels, 5-mm thick, for 180 volumes). Subjects were instructed to lie still, clear their thoughts and “not to think about anything in particular”, with their eyes open (their entire field of view was black).
Chapter 3

The effect of age of epilepsy onset on memory and cortical thickness in right and left temporal lobe epilepsy

3.1 Introduction

Temporal lobe epilepsy (TLE) is characterized by recurrent seizures originating in the temporal lobes. This disorder is associated with material specific memory impairments — people with left TLE (LTLE) typically have verbal memory impairments, while people with right TLE (RTLE) typically have visual memory impairments (Milner, 1968). There are also widespread cortical alterations in these patients (Mueller et al., 2009). Epilepsy can emerge at varying stages in life, from perinatal periods up through adulthood and, to date, there have been no investigations examining the relationship between age of epilepsy onset with cortical thickness and memory.

Two themes have emerged in the literature with regards to age of epilepsy onset. The first is that early onset affords privileged plasticity and reorganization of function that might lead to spared performance. In support of this, evidence has shown that early onset is related to language reorganization (Bell et al., 2002; Springer et al., 1999), less disrupted global resting network properties (Doucet et al., 2014), better memory prior to surgical resection of the epileptic focus (Baxendale et al., 1998) and less decline following resection of the epileptic focus (Griffin & Tranel, 2007; Hermann et al., 1995). The second theme is that an early onset age of epilepsy will hinder development. This has been supported by research showing that earlier onsets are associated with reduced whole brain white matter (Hermann et al., 2002), worse IQ (Kaaden & Helmstaedter, 2009), and, in contrast to Baxendale et al. (1998), worse presurgical memory (Lespinet et al., 2002).

These two lines of evidence may not be entirely contradictory. Reorganization of language and spared global network properties could be accompanied by reductions in IQ and reduced whole brain white matter. However, evidence from Baxendale et al. (1998) seems to be directly contrary to that of Lespinet et al. (2002). The disparity between these studies (and possibly the disparity among the other studies) could arise from analysis decisions, such as the decision to
analyze left and right TLE groups separately. In Baxendale et al. (1998), both left and right TLE patients were included in models predicting memory ability from various neuropsychological memory tests, while Lespinet et al. (2002) analyzed RTLE and LTLE with separate regressors. By combining LTLE and RTLE groups, there may be important material specific cognitive changes that are overlooked. This strategy also assumes that the pathophysiological effects of epilepsy are independent of seizure laterality, which may not be the case, as several studies have shown that LTLE is associated with more widespread and severe abnormalities in terms of cortical thickness (Kemmotsu et al., 2011), white matter integrity (Ahmadi et al., 2009). Further, in Baxendale et al (1998), age of onset was entered into regression models as a continuous linear variable among other predictors while, in Lespinet et al. (2002), age of onset was a binary variable split such that individuals were classified as having an early onset if seizure onset was at or before the age of 5 and classified as late onset if seizures began at or after age 10. The process of binarization requires a threshold and there is no agreed upon threshold for early vs. late onset in the literature; some studies use thresholds as young as 2 years old (Bell et al., 2002), and others using thresholds as old as 20-25 (Doucet et al., 2014). Finally, these studies typically assume that age of onset affects cognition and the brain in a linear fashion, which is unlikely to be the case given that the brain develops in a non-linear fashion (Shaw et al., 2008) and that research on plasticity does not show a strictly linear effect (Kolb & Gibb, 2014). Very early lesions to the medial prefrontal cortex of rodents, in the first week of life, results in severe memory deficits in adulthood. Early lesions in the second week of life results in spared memory ability, however, while lesions in adulthood, after week 4, are also severely detrimental, demonstrating a non-linear relationship between time of damage and adaptation (Kolb et al., 1996).

The current study investigates the relationship between age of epilepsy onset and material specific memory in patients with left and right TLE, analyzing both groups separately, with age of onset assessed as a continuous variable that may influence memory in a linear or nonlinear way. Further, I also examined the relationship between brain morphology and age of onset using regional cortical thickness. TLE is associated with widespread cortical thickness abnormalities (Bernhardt et al., 2010; Lin et al., 2007; Mueller et al., 2009), and the relationship between cortical thickness and age of onset may shed light on the neural correlates of memory deficits. To that end, 80 patients with TLE who had completed extensive neuropsychological assessment and
clinical anatomical MRI were interrogated along with 31 neurologically healthy controls who served to provide normative morphological data. I interrogated the relationship between age of epilepsy onset and material specific memory, and regional cortical thickness measured by FreeSurfer in the patients. Two hypotheses were tested against each other. A developmental hindrance hypothesis would predict that early life epilepsy onset would be associated with worse memory and aberrant cortical thickness that relates to this poorer memory ability while an adaptive plasticity hypothesis predicts that early life epilepsy will afford reorganization of function and thus better memory and altered cortical thickness that relates to this spared memory ability. I did not hypothesize any differences between the LTLE and RTLE groups in terms of demonstrating adaptive plasticity or developmental hindrance, but was open to the possibility that reorganization may not occur in the same way.

3.2 Methods
3.2.1 Participants

Ninety-two adult patients with pharmacologically intractable unilateral temporal lobe epilepsy were recruited from the Epilepsy Clinic at Toronto Western Hospital. Forty-nine patients presented with RTLE (26 females; mean age = 36.9; age range = 18 – 62) and 43 presented with LTLE (27 females; mean age = 37.8; age range = 18 – 62). Continuous recording of scalp EEG and video monitoring during an inpatient evaluation in our epilepsy monitoring unit were used to determine seizure focus. Thirty-one neurologically healthy controls (15 females; mean age = 33; age range = 22 – 59) were recruited to investigate cortical thickness differences. The study was approved by the UHN research ethics board. Due to incomplete data, poor cortical parcellation, or persistent seizures without reduction following anterior temporal lobectomy eleven patients with RTLE and one patient with LTLE were excluded from the analysis. Individuals with persistent seizures following surgery were excluded because the presence of seizures following resection of the anterior temporal lobe suggests that there are other sites of seizure generation in the brain which may be related to additional pathological changes and thus, these individuals were excluded to better characterize changes related to temporal lobe epilepsy.
3.2.2 Behavioural Analysis

All patients underwent an extensive neuropsychological battery of tests. Using factor loadings from a principle component analysis of our database, I calculated a verbal memory (VM), visual memory (VSM) and IQ factor scores for each patient using their neuropsychological performance. The details of this are presented in Chapter 2: General Methods. The VM factor, VSM factor, and IQ factor were separately used as dependent variables in three regression models using age of onset as a predictor. Separate model estimations were also performed to investigate a quadratic relationship between age of onset and neuropsychological factors. Importantly, patients with LTLE and RTLE were modeled separately. Age at the time of assessment, duration of epilepsy and proportion of life with epilepsy were interrogated as potential confounding variables in significant models. Since these variables are linear combinations of each other (e.g. duration is the difference between age and age of onset and proportion of life with epilepsy is the ratio or duration to age) and cannot all be included in the same regression model, I included the confounding variable that had the strongest relationship with the dependent variable. I also examined whether presence or absence of mesial temporal sclerosis interacted with age of onset effects. Bootstrapping with 1000 bootstraps were performed on models to estimate standard error.

3.2.3 Structural Analysis

FreeSurfer [Martino Center for biomedical Imaging, Harvard-MIT, Boston USA; http://surfer.nmr.mgh.harvard.edu] was used to calculate regional cortical thickness and has been previously described in detail (Fischl et al., 2004) and assessed in terms of validity and accuracy (Morey et al., 2009, 2010; Pardoe, Pell, Abbott, & Jackson, 2009). Each brain was preprocessed with intensity normalization, removal of non-brain tissue, Talaraich transformation and segmentation of tissue into grey matter, white matter and cerebral spinal fluid followed by subcortical segmentation of structures. Quality control was performed by examining white matter pial boundaries and manually adding in control points to correct segmentation in a semi-automated fashion. The cortex was then be parcellated into discrete regions using the Desikan-Killiany Atlas (Desikan et al., 2006) and cortical thickness measurements were extracted.
3.2.4 Cortical Thickness Statistical Analysis

A mean centred partial least squares (PLS) analysis was used to investigate the patterns of cortical thickness differences between LTLE versus healthy controls, and RTLE versus healthy controls. Cortical thickness measurements for 68 cortical regions in the Desikan-Killiany Atlas were entered into the PLS analysis which computed ranked latent variables from a singular value decomposition on a covariance matrix of the cortical thickness brain data and the study groups. The resulting latent variables express patterns of cortical thickness that relate to group membership. Significance of each latent variable was assessed using permutation testing with 1000 permutations and to test the reliability of each brain region to the overall pattern of the latent variable, a bootstrapping method with 500 bootstraps was used to produce a bootstrap ratio (BSR) for each region. A BSR is roughly proportional to a $z$ value and only regions with a BSR $> 2$ are reported as significant. The overall patterns of differences represent the multivariate difference between groups and thus reduces the prevalence of type I error that would occur using mass univariate t-tests.

Cortical thickness values from each cortical region were used as dependent variables in regression models using age of onset as a predictor linearly and quadratically. Given that older chronological age and biological sex is associated with altered cortical thickness (Pfefferbaum et al., 2013; Sowell et al., 2007), I also included age and sex as a predictor in these models to account for this variation as has been recommended by the literature (Barnes et al., 2010) and used false discovery rate correction for multiple comparisons (Benjamini & Yekutieli, 2001).

3.2.5 Brain – Behaviour Analysis

The VM factor, VSM factor, and IQ factor were separately used as dependent variables in regression models using cortical thickness from regions that were modulated by age of onset.

3.3 Results

3.3.1 Subject Demographics

There were no differences in age, $F(2, 108) = 1.79$, $p > 0.1$, sex distribution, $\chi^2(2, N = 111) = 1.44$, $p = .49$ or handedness, $\chi^2(4, N = 111) = 3.6$, $p = .46$. There was a significant difference
between groups in terms of education, $F(2, 108) = 34, p < .001$, with the control group having significantly higher education than both patient groups in post-hoc testing using Bonferroni correction. In the patient groups, I saw no differences in age of onset or duration between the LTLE and RTLE groups, all $t < .67, p > 0.5$, or presence of MTS, $\chi^2(1, N = 80) = 0.4, p = .53$. Patients with LTLE had worse verbal memory than patients with RTLE, $t(76) = 2.5, p = .015$, but there was no significant difference between groups for visual memory, $t(76) = 1.5, p = 0.15$, or IQ, $t(76) = 0.39, p = .69$. Demographic information and neuropsychological performance is reported in Table 3.1.
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>RTLE</th>
<th>LTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>31</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>33 (8.5)</td>
<td>36.8 (12.4)</td>
<td>37.5 (9.9)</td>
</tr>
<tr>
<td>Education, y (SD)</td>
<td>19.0 (2.7)</td>
<td>13.7 (3.0)</td>
<td>14.4 (2.9)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>16/15</td>
<td>18/20</td>
<td>16/26</td>
</tr>
<tr>
<td>Handedness, R/L/Bi</td>
<td>29/2/0</td>
<td>35/3/0</td>
<td>36/4/2</td>
</tr>
<tr>
<td>Language Dominance, R/L/Bi</td>
<td>--</td>
<td>0/37/0</td>
<td>0/42/0</td>
</tr>
<tr>
<td>Disease duration, y (SD)</td>
<td>--</td>
<td>19.0 (14.4)</td>
<td>20.1 (13.4)</td>
</tr>
<tr>
<td>Onset of seizures, y (SD)</td>
<td>--</td>
<td>17.9 (13.9)</td>
<td>17.6 (13.9)</td>
</tr>
<tr>
<td>Presence of MTS, Yes/No</td>
<td>--</td>
<td>22/15</td>
<td>22/20</td>
</tr>
<tr>
<td>Other Lesions</td>
<td>--</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Verbal Memory Factor</td>
<td>--</td>
<td>.31 (.99)</td>
<td>-.29 (1.2)</td>
</tr>
<tr>
<td>Visual Memory Factor</td>
<td>--</td>
<td>-.22 (1.1)</td>
<td>.15 (.97)</td>
</tr>
<tr>
<td>IQ Factor</td>
<td>--</td>
<td>.008 (1.5)</td>
<td>.1 (1.1)</td>
</tr>
</tbody>
</table>

RTLE, right temporal lobe epilepsy; LTLE, left temporal lobe epilepsy; y, years; SD, standard deviation; M, male; F, female; R, right; L, left; BI, bilateral; IQ, intelligence quotient. Characterization of MTS and other lesions was based on radiology (3T MRI protocol). In the RTLE group, other lesions included one individual with a small left posterior temporal cavernoma, one with right amygdala gliosis, one with a right amygdala ganglioma, one with a right amygdala dysembryoplastic neuroepithelial tumour and one individual with a right amygdala lesion. In the LTLE group, one individual had a left arteriovenous malformation, one had a left amygdala dysembryoplastic neuroepithelial tumour, and one individual had left amygdala ganglioma.
3.3.2 Behavioural Results

In patients with LTLE, age of onset was not linearly related to verbal memory, $R^2 = .023$, $F(1,42) = 1.0$, $p = .32$. However, when a quadratic term was added, the model was significantly predictive of verbal memory performance, $R^2 = .18$, $F(2,41) = 4.6$, $p = .02$ with each predictor being significant, $b_{\text{age of onset}} = 0.1$, $t(41) = 2.4$, $p = 0.02$, $b_{(\text{age of onset})^2} = -0.003$, $t(41) = -2.8$, $p = .007$. A similar finding was obtained in the relationships between age of onset and both visual memory and the IQ. For visual memory, a linear model was not significant, $R^2 = .001$, $F(1,42) = .001$, $p = .98$, but when a quadratic term was added, the model performed significantly better, $R^2 = 0.17$, $F(2,41) = 4.1$, $p = .02$, with each predictor being significant, $b_{\text{age of onset}} = .09$, $t(41) = 2.7$, $p = .009$, $b_{(\text{age of onset})^2} = -.002$, $t(41) = -2.9$, $p = .007$. For the verbal and visual memory models, a negative quadratic relationship was observed such that very early onset was associated with worse performance than adolescent onset and adolescent onset which, in turn, was associated with better performance than adult onset and these are shown in Figure 3.1. For the IQ factor, a linear relationship was significant, $R^2 = .15$ and a quadratic term significantly improved the model, $R^2 = .31$, $F_{\text{change}}(1,41) = 9.3$, $p = .004$. However, three data points showed considerable leverage with a Cook’s Distance greater than three times the mean Cook’s D, which caused considerable influence on the model. When these cases — shown in Figure 3.2 — are removed the linear relationship remained significant, $R^2 = .46$, $b_{\text{age of onset}} = .06$, $t(39) = 5.5$, $p < .001$, but the quadratic term did not significantly add to the model, $R^2_{\text{change}} < .001$, $F_{\text{change}} (1,38) = .006$, $p = .9$. Earlier onset of LTLE was associated with worse IQ factor scores as can be seen in Figure 3.1.

When the same outlier analysis was applied to verbal and visual memory, no outliers were found that significantly affected the models and removal of the three IQ outlier cases from the verbal and visual memory models did not eliminate the model effects.
Figure 3-1. Scatterplots demonstrating the relationship between age of onset and cognition in left and right temporal lobe epilepsy with modeled regression lines and regression line confidence intervals in dashed lines. MTS, mesial temporal sclerosis.
Figure 3-2. On the left, a scatterplot displaying age of onset and IQ factor score in people with left temporal lobe epilepsy with a regression line plotted in solid black with confidence intervals as dashed lines. Three influential cases are circled in red. On the right, a plot of each subject displaying their relative influence, measured by Cook’s Distance with a horizontal bar indicating a threshold of three times the mean Cook’s Distance of the sample. Three outliers are circled in red. MTS, mesial temporal sclerosis.

Among the potential confounds, age showed the strongest relationship with verbal memory (Age: $R^2 = .29$, $F(1,42) = 17.2$, $p < .001$; Duration: $R^2 = .06$, $p = .1$, Proportion of life with epilepsy: $R^2 = .009$, $p > .5$), and visual memory (Age: $R^2 = .22$, $F(1,42) = 11.8$, $p = .001$; Duration: $R^2 = 0.1$, $F(1,42) = 5.8$, $p = .02$; Proportion of life with epilepsy: $R^2 = .02$, $p > .1$), but proportion of life with epilepsy was most related to IQ (Proportion of life with epilepsy: $R^2 = .3$, $F(1,39) = 17.9$, $p < .001$; Duration: $R^2 = .2$, $F(1,39) = 8.2$, $p = .007$; Age: $R^2 = .06$, $p > .1$). Thus, chronological age was added to the models predicting verbal and visual memory and proportion of life with epilepsy was added to the model predicting IQ. When age was added to the model predicting verbal memory, the model was significant, $R^2 = .34$, $F(3,40) = 6.8$, $p < .001$, with older age associated with worse verbal memory, $b = -.06$, $t(40) = 3.3$, $p = .002$. In this model, the linear term for age of onset was only marginally significant, $b_{\text{age of onset}} = .07$, $t(40) = 1.9$, $p = .07$, but the quadratic term remained significant, $b_{(\text{age of onset})^2} = -.002$, $t(40) = -2.1$, $p = .04$. When age was entered in the model predicting visual memory, the model was significant, $R^2 = .31$, $F(3,40) = 5.9$, $p = .002$, with older age predicting worse memory, $b = -.04$, $t(40) = 2.6$, $p = .01$, with the linear term remaining significant, $b_{\text{age of onset}} = .07$, $t(40) = 2.3$, $p = .03$, and the quadratic term being marginally significant, $b_{(\text{age of onset})^2} = -.001$, $t(40) = -1.9$, $p = .06$. When proportion of life
with epilepsy was entered into the model predicting IQ, the model was significant, $R^2 = .44$, $F(2,38) = 14.9$, $p < .001$, though proportion of life with epilepsy was not a significant predictor in the model, $b = .5$, $t(39) = .6$, $p = .6$, while age of onset remained significant, $b_{\text{age of onset}} = .07$, $t(39) = 2.9$, $p = .006$. The presence or absence of MTS did not interact with age of onset effects in any of the models (all $t < 1$, $p > .1$).

In people with RTLE, age of onset was not linearly, or quadratically related to verbal memory ($R^2 = .01$ and $R^2 = .03$, $p > .1$), or IQ ($R^2 = .02$ and $R^2 = .02$, $p > .1$). Age of onset was, however, linearly related to visual memory $R^2 = .25$, $F(1,36) = 11.9$, $p = .001$, and adding a quadratic term did not significantly improve the model, $R^2$-change = .002, $p = 0.8$. In the linear model of age of onset, an early age of onset was related to better memory performance, $b_{\text{age of onset}} = -.04$, $t(36) = 3.5$, $p = .001$. These relationships are shown in Figure 3.1.

Since age was marginally related to visual memory performance, $R^2 = .09$, $F(1,36) = 3.6$, $p = .07$, it was added in to the model with age of onset to control for this effect. When age is included in the model, the model remains significant, $R^2 = .26$, $F(2,35) = 6$, $p = .006$, but age was not a significant predictor, $b = -.008$, $t(35) = .5$, $p = 0.6$, while age of onset remained significant, $b_{\text{age of onset}} = -.04$, $t(35) = 2.8$, $p = .009$. There was no interaction between presence or absence of MTS with age of onset ($t(34) = .7$, $p = 0.5$).

### 3.3.3 Cortical Thickness Analysis

A mean centred PLS with LTLE and healthy controls produced one significant latent variable at $p < .001$ estimated using 1000 permutations. There were widespread differences between the LTLE group and the healthy control group with large reductions in bilateral somatomotor and superior frontal regions, lateral and medial parietal regions, inferior frontal, lateral occipital, and left lateral temporal cortex in the LTLE group. There were no areas of increased cortical thickness in the LTLE group compared to healthy controls. These findings persisted after removing covariance related to years of education, but several occipital and right sided regions no longer contributed to the pattern. These results are presented in Table 3.2.
Table 3-2. Bootstrap ratios from partial least square analysis of cortical thickness in patient groups compared to a healthy control group

<table>
<thead>
<tr>
<th>Region</th>
<th>LTLE vs. Healthy Control</th>
<th>RTLE vs. Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH</td>
<td>RH</td>
</tr>
<tr>
<td>Temporal Lobe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bank STS</td>
<td>-1.9</td>
<td>-1.0</td>
</tr>
<tr>
<td>Trans Temporal</td>
<td>-3.8*</td>
<td>-2.7*</td>
</tr>
<tr>
<td>Inf Temporal</td>
<td>-3.9*</td>
<td>-1.0</td>
</tr>
<tr>
<td>Mid Temporal</td>
<td>-2.3*</td>
<td>-0.9</td>
</tr>
<tr>
<td>Sup Temporal</td>
<td>-1.6</td>
<td>-1.3</td>
</tr>
<tr>
<td>Temporal Pole</td>
<td>-0.8</td>
<td>-1.7</td>
</tr>
<tr>
<td>Entorhinal</td>
<td>-1.7</td>
<td>-1.3</td>
</tr>
<tr>
<td>Parahippocampal</td>
<td>-1.5</td>
<td>-3.1*</td>
</tr>
<tr>
<td>Fusiform</td>
<td>-1.8</td>
<td>-1.9</td>
</tr>
<tr>
<td>Frontal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precentral</td>
<td>-4.2*</td>
<td>-5.1*</td>
</tr>
<tr>
<td>Paracentral</td>
<td>-3.5*</td>
<td>-2.8*</td>
</tr>
<tr>
<td>Sup Frontal</td>
<td>-4.5*</td>
<td>-4.6*</td>
</tr>
<tr>
<td>Caudal Mid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>-3.8*</td>
<td>-4.9*</td>
</tr>
<tr>
<td>Location</td>
<td>Rostral Mid</td>
<td>Parietal Lobe</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Frontal</td>
<td>-2.9*</td>
<td>-4.0*</td>
</tr>
<tr>
<td>Pars Opercularis</td>
<td>-2.5*</td>
<td>-4.1*</td>
</tr>
<tr>
<td>Pars Orbitalis</td>
<td>-2.1*</td>
<td>-4.1*</td>
</tr>
<tr>
<td>Pars Triangularis</td>
<td>-1.5</td>
<td>-3.3*</td>
</tr>
<tr>
<td>Lat Orbitofrontal</td>
<td>0.2</td>
<td>-1.4</td>
</tr>
<tr>
<td>Med Orbitofrontal</td>
<td>-0.3</td>
<td>-0.8</td>
</tr>
<tr>
<td>Frontal Pole</td>
<td>-1.9</td>
<td>-2.5*</td>
</tr>
<tr>
<td>Postcentral</td>
<td>-4.0*</td>
<td>-3.6*</td>
</tr>
<tr>
<td>Inf Parietal</td>
<td>-4.1*</td>
<td>-2.9*</td>
</tr>
<tr>
<td>Sup Parietal</td>
<td>-3.0*</td>
<td>-4.5*</td>
</tr>
<tr>
<td>Supramarginal</td>
<td>-3.3*</td>
<td>-2.6*</td>
</tr>
<tr>
<td>Precuneus</td>
<td>-4.1*</td>
<td>-4.2*</td>
</tr>
<tr>
<td>Cuneus</td>
<td>-1.8</td>
<td>-3.6*</td>
</tr>
<tr>
<td>Lat Occipital</td>
<td>-3.3*</td>
<td>-2.5*</td>
</tr>
<tr>
<td>Lingual</td>
<td>-2.6*</td>
<td>-1.9</td>
</tr>
<tr>
<td>Pericalcarine</td>
<td>-2.5*</td>
<td>-2.7*</td>
</tr>
</tbody>
</table>
Rostral Ant  
Cingulate  -1.0  0.1  -0.3  -0.6  

Caudal Ant  
Cingulate  -1.5  -0.6  -0.6  -0.9  
Post Cingulate  -1.5  -4.1*  -1.3  -3.1*  
Isthmus Cingulate  -2.2*  -1.4  -2.8*  -2.0*  
Insula  0.6  0.1  0.4  1.2  

* indicates significance at p < .05. Bold text indicates areas that remained significantly reduced in LTLE, following correction for years of education.

A mean centred PLS with RTLE and healthy controls also produced one significant latent variable at p < .001, estimated using 1000 permutations, that characterized the differences between groups. Similar to the LTLE group differences, there were widespread reductions in cortical thickness in RTLE compared to healthy controls in bilateral somatomotor, superior frontal, middle frontal, inferior frontal, lateral and medial parietal, and lateral occipital cortex. There were no areas of increased cortical thickness in the RTLE group compared to healthy controls. After removing covariance associated with years of education, the pattern was no longer significant, p = 0.17. These results are also shown in Table 3.2.

Interestingly, medial temporal cortex was not consistently represented in these patterns. As this was unexpected, I interrogated group differences in medial temporal ROIs using univariate methods and, here, I report ANOVA results, uncorrected for multiple comparisons. Three way ANOVA’s revealed significant group differences for left entorhinal cortex, F(2, 110) = 3.3, p = .04, left parahippocampus, F(2, 110) = 3.3, p = .04 and right parahippocampus, F(2, 110) = 6.3, p = .002, but no significant differences for right entorhinal cortex (F(2, 110) = 1.1, p > .3). In the LTLE group compared to healthy controls, post hoc analysis revealed a smaller left entorhinal cortex, p = .02, left parahippocampal cortex, p = .01 and right parahippocampal cortex, p = .001.
There was no difference in right entorhinal cortex (p = .4) or right parahippocampal gyrus (p = .09) in RTLE compared to healthy controls.

In the LTLE and RTLE groups, separately, I modeled age of onset with cortical thickness controlling for age and biological sex. In the LTLE group, after corrected for multiple comparisons using FDR correction, 12 regions were significantly predicted by a model including age of onset, (age of onset)$^2$, age, and sex. These regions include left caudal middle frontal gyrus, rostral middle frontal, insula, pars orbitalis, posterior cingulate, precuneus, and supramarginal gyrus, right pars triangularis, and bilateral bank superior temporal sulcus and superior frontal gyrus and these models are summarized in Table 3.3. Most of these models were driven by age effects with older age being associated with thinner cortex. However, a subgroup, including the left rostral middle frontal, insula and pars orbitalis, showed significant terms for age of onset and (age of onset)$^2$. In these regions, after controlling for the effects of age and sex, later age of onset was associated with thicker cortex for onset ages between 0 and 25, after which, later onset is associated with a levelling off (pars orbitalis) or mild thinning (insula, rostral middle frontal). The cortical thickness of these regions, after removing the effect of age and sex, is shown in relation to age of onset in Figure 3.3A.
Figure 3-3. On the left, 3-dimensional renderings of the average FreeSurfer brain surface with brain regions highlighted corresponding to the regions plotted in those rows. Scatterplots with regression lines and confidence intervals as dashed lines depicting A, the relationship between age of onset and regional cortical thickness after regressing out variability explained by age and
sex and B, the relationship between cortical thickness and verbal memory ability, in left temporal lobe epilepsy (LTLE). L, left; MFG, middle frontal gyrus; MTS, mesial temporal sclerosis.

In the RTLE group, after corrected for multiple comparisons using FDR correction, 7 regions were significantly predicted by a model including age of onset, \((\text{age of onset})^2\), age, and sex. These regions include left post- and pre-central gyrus, bilateral posterior cingulate, and superior frontal cortex, and right superior parietal cortex. These models are also summarized in Table 3.3. Again, many of these regions were driven by age effects, with older age being associated with thinner cortex. Two regions, left posterior cingulate and right superior frontal cortex, showed significant effects of age of onset and \((\text{age of onset})^2\), with the right posterior cingulate showing marginal significance for these terms. Again, in these models, later ages of onset were associated with thicker cortices for onset ages between 0 and 25, after which, later onset was associated with a levelling off or mild thinning. The cortical thickness of these regions, after removing the effect of age and sex, is shown in relation to age of onset in Figure 3.4A.
Figure 3-4. On the left, 3-dimensional renderings of the average FreeSurfer brain surface with brain regions highlighted corresponding to the regions plotted in those rows. Scatterplots with regression lines and confidence intervals as dashed lines depicting A, the relationship between age of onset and regional cortical thickness after regressing out variability explained by age and sex and B, the relationship between cortical thickness and verbal memory ability, in right temporal lobe epilepsy (RTLE). L, left; MTS, mesial temporal sclerosis; PCC, posterior cingulate cortex; SFG, superior frontal gyrus.
I also examined a model in which I excluded \((\text{age of onset})^2\) given that, behaviourally, a linear relationship was found between age of onset and visual memory. While 14 regions showed significance after FDR correction with this model, none of these regions showed a significant age of onset relationship and the models were driven by the effect of age, with older age being related to thinner cortex. These regions included left pars triangularis, postcentral gyrus, superior frontal gyrus, superior temporal, supramarginal, and transverse temporal cortex, bilateral precentral and superior parietal cortex, and right lateral occipital, pars opercularis, posterior cingulate cortex, and precuneus.

**Table 3-3. Regression slopes for predictors of cortical thickness for different regions in the brain with model explained variance \((R^2)\)**

<table>
<thead>
<tr>
<th>LTLE</th>
<th>Age</th>
<th>Onset Age</th>
<th>((\text{Onset Age})^2)</th>
<th>Sex</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L Caudal MFG</td>
<td>-0.09</td>
<td>0.007</td>
<td>-0.0001</td>
<td>0.003</td>
<td>0.40</td>
</tr>
<tr>
<td>L Rostral MFG</td>
<td>-0.06</td>
<td>0.013</td>
<td>-0.0003</td>
<td>-0.001</td>
<td>0.33</td>
</tr>
<tr>
<td>L Insula</td>
<td>-0.05</td>
<td>0.013</td>
<td>-0.00034</td>
<td>0.02</td>
<td>0.38</td>
</tr>
<tr>
<td>L Pars Orbitalis</td>
<td>-0.09</td>
<td>0.022</td>
<td>-0.00042</td>
<td>0.03</td>
<td>0.27</td>
</tr>
<tr>
<td>L PCC</td>
<td>-0.09</td>
<td>0.004</td>
<td>0.00002</td>
<td>-0.02</td>
<td>0.30</td>
</tr>
<tr>
<td>L Precuneus</td>
<td>-0.08</td>
<td>0.003</td>
<td>-0.00007</td>
<td>0.01</td>
<td>0.32</td>
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<tr>
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<td>0.007</td>
<td>-0.001</td>
<td>0.02</td>
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</tr>
<tr>
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<td>-0.00007</td>
<td>-0.04</td>
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</tr>
<tr>
<td>L SFG</td>
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<td>-0.00004</td>
<td>-0.006</td>
<td>0.42</td>
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</table>
3.3.4 Brain – Behaviour Analysis

For the LTLE group, the left rostral middle frontal cortical thickness was positively related to verbal memory, r(40) = .46, p = .002, visual memory, r(40) = .39, p = .01, and the IQ factor, r(40) = .36, p = .02. The left inferior frontal gyrus, pars orbitalis was also significantly related to both verbal memory, r(40) = .52, p < .001 and visual memory, r(40) = .46, p = .003, but not the IQ factor, r(40) = .24, p = .1. Cortical thickness in the insula was related to visual memory performance, r(40) = .57, < .001, but not verbal memory, r(40) = .28, p = .07, or IQ, r(40) = .1, p = .5. Relationships between cortical thickness and verbal memory are shown in Figure 3.3B.

For the RTLE group, significant relationships with visual memory were seen for the left, r(36) = .35, p = .03 and right posterior cingulate, r(36) = .35, p = .03 and right superior frontal cortex, r(36) = .34, p = .04, but none were related to verbal memory or IQ (all r’s < .19). These relationships are shown in Figure 3.4B.
3.4 Discussion

This investigation has demonstrated that the effect of onset age and cognition differs in people with LTLE compared to RTLE. Furthermore, these relationships are not necessarily linear. In LTLE, a quadratic relationship best explained the data, demonstrating that earlier onset of epilepsy was associated with worse memory, improving up until an onset age of 17 or 25 years old, for verbal or visual memory, respectively. Later ages of onset after this point were related with worse memory. I also report a positive linear relationship between age of onset and the IQ factor in LTLE, with earlier onsets being associated with poorer IQ scores. In people with RTLE, age of onset was not related to verbal memory or IQ, but was linearly related to visual memory, with earlier ages of onset being related to better visual memory performance.

Using cortical thickness measures, I examined the neural correlates of this age of onset effect on memory. I found widespread cortical thinning across the cortex in line with previous studies (Bernhardt et al., 2010; Kemmotsu et al., 2011; McDonald et al., 2008). Furthermore, cortical thinning was related to age of onset a nonlinear way in subregions related to memory in both the LTLE and RTLE group. In the LTLE group, left frontal language regions, namely the pars orbitalis and rostral middle frontal gyrus (MFG) showed a negative quadratic relationship with age of onset. Very early onset was associated with thinner cortex in these regions, late adolescent and early adulthood onset was associated with the greatest cortical thickness which was closer to healthy control levels which levelled off or fell modestly at onset ages in the third and fourth decade of life. In these frontal language regions, those individuals who had thicker cortex (again, thickness closer to healthy controls) demonstrated better verbal memory ability. In the RTLE group, bilateral posterior cingulate cortex (PCC) and right superior frontal gyrus (SFG) thickness was nonlinearly related to age of onset in the same way and again, individuals who had thicker cortex here, closer to healthy control levels, showed better visual memory ability.

Previous reports of the effect of age of onset on cognition and the brain have shown mixed results with respect to support for an adaptive plasticity hypothesis — a theory suggesting that early onset affords increased plasticity and adaptability — versus a developmental hindrance hypothesis — a theory suggesting that early onset epilepsy impairs developmental processes.
Here, I report that individuals with early onset LTLE perform worse on memory and IQ tests compared to those who have onset in late adolescence and early adulthood. This is partially in line with previous reports from Lespinet et al (2002) who showed that early onset LTLE (before the age of 5) had worse performance on verbal paired associates immediate recall and immediate and delayed logical memory of the Wechsler memory scale subtests compared to late onset LTLE (designated as after the age of 10). Similarly, Kaaden and Helmsteadter (2009) reported a trend in LTLE showing that early onset (before the age of 14) patients perform worse on measures of verbal learning and IQ. Hermann et al. (2002) also report that an earlier age of onset was associated with overall reductions in brain white matter, which was in turn related to IQ.

Further, in a very large sample of over 2000 people with TLE and healthy controls, it was shown that the TLE group did not show significant reductions in verbal learning at age 5, but ultimately failed to reach a developmental memory peak that healthy controls achieved at age 23 (Helmstaedter & Elger, 2009). Instead, throughout development they had a shallow increase in verbal learning which peaked at around age 16. In combination with my work, this suggests that early onset epilepsy may hinder the development of verbal memory ability, particularly when onset is in the language dominant hemisphere. The cortex of key language areas such as the left pars orbitalis and rostral MFG was also thinner in this early onset group, and thin cortex here was associated with worse memory, in line with a developmental hindrance hypothesis. In healthy populations, the MFG and inferior frontal gyrus do not reach peak thickness until around the age of 10 years old (Shaw et al., 2008) and prune down in thickness until young adulthood (Tamnes et al., 2010). During this developmental period, epilepsy onset may lead to aberrant neuronal signalling and impairment of survival factor signalling, leading to loss of synapses resulting in reduced cortical thickness.

In the current study, individuals with later onsets in their 30s and 40s also had relatively worse visual and verbal memory compared to those who had onsets in their late adolescence up until mid 20s for the LTLE group. This was not previously captured by prior studies which simply had a binary distinction between early and late (Kaaden & Helmstaedter, 2009; Lespinet et al., 2002). Baxendale et al (1998), who analyzed age of onset as a continuous variable, showed that later onset seizures were associated with worse delayed story recall and worse immediate figure recall, but, in this study, people with LTLE and RTLE were pooled together which makes it difficult to disentangle these effects with respect to side of seizure onset. Baxendale et al (1998)
also modeled age of onset solely in a linear fashion which may not have captured relative performance deficits in very early onsets. This linear pattern reported by Baxendale et al. (1998) would suggest that earlier onset affords some mechanism of adaptation. The data presented here, however, depict a nonlinear pattern with onsets in late adolescence showing peak memory performance, with reduced performance seen for individuals with older onsets. This reduction of performance in people with older onset could be due to the fact that the individuals with a later onset are also chronologically older (given that onset age and chronological age covary in this sample, with a correlation of $r = .4$, $p < .001$) and are experiencing age-related declines in memory, that are also reflected in cortical thickness. Older age was associated with worse memory and thinner cortex and when it was included in a regression model with age of onset it accounted for some of the variance at later onset points which is reflected in a shift of the vertex of the regression curves to a later age of onset point, limiting the downward arc seen for late ages of onset (for example see Figure 3.5). Furthermore, after accounting for age, some of the age of onset effects became only marginally significant for verbal and visual memory prediction, which, together with the shifting peaks, suggests that chronological age accounts for some of the decline seen in the later age of onset cases.

Figure 3-5. Scatterplots demonstrating the relationship between age of onset and regional cortical thickness in millimeters with regression line plotted showing the relationship between
age of onset and thickness in black and a chronological age adjusted regression line demonstrating the relationship between age of onset and thickness in dashed red line.

When the relationship between cortical thickness and behaviour was investigated, those individuals with thicker cortex (thickness closer to healthy control levels) had better verbal and visual memory. Previous reports have shown that the inferior frontal gyrus is hyper-activated during successful encoding in patients with TLE compared to healthy controls (Guedj et al., 2011) and lateral PFC regions such as the rostral MFG are known to be important for processing item-specific information during encoding in healthy controls (Murray & Ranganath, 2007). Cortical thinning in this region may be indicative of functional compromise and potentially explain the effect of onset age and memory ability.

Given that this data is cross-sectional in nature, it is important to note that I cannot infer directionality. Thus, another explanation for these effects is that individuals who have very early ages of epilepsy onset have more compromised brains initially compared to those with onsets around adolescence and adulthood. However, hippocampal alterations are not reliably found at the time of initial diagnosis in children (Salmenperä et al., 2005) and widespread gray matter changes are very limited in pediatric TLE populations (Guimarães et al., 2007). This, and previous literature demonstrating impaired development of cognition (Korman et al., 2013), and reduced development of white matter volume (Hermann et al., 2010) in longitudinal cohorts, suggest some type of “failure to progress along expected developmental trajectory”.

Conversely, in people with RTLE, I report that an earlier onset is associated with better visual memory, but is unrelated to verbal memory or IQ. In Lespinet et al (2002), onset before the age of 5, was associated with worse immediate and delayed visual reproduction compared to onset after the age of 10 in the RTLE group. This is directly counter to evidence from Baxendale et al (1998) who showed that earlier ages of onset were associated with better immediate visual reproduction. In the current study, using the same binary cut-offs from Lespinet et al (2002) (early onset < 5 and late onset >10), I find that individuals with early onset perform better than late onset individuals, t(32) = 2.6, p = .01, although the number of patients with early onsets using this cut-off was quite low, n =7. Also, the early vs. late onset effect found by Lespinet et al (2002) was specific to the visual reproduction subtest and did not generalize to paired associate
figures of the Wechsler Memory Scale or the Rey complex figure performance. In the current study, the visual memory factor is a composite of multiple visual memory tests which we have previously found is sensitive to seizure laterality and predictive of post-operative memory change (St-Laurent et al., 2014). The results of the current study, and of Baxendale, suggest some sort of adaptive plasticity in early onset RTLE.

Given these behavioural findings, I expected to find mirrored effects in the brain. However, I again observed a quadratic relationship between age of onset and cortical thickness in bilateral PCC and right SFG. Those individuals with a very early onset had thinner cortex than those with an onset around young adulthood and following this point individuals with and older onset had levelled off or thinner cortex. Furthermore, thicker cortex was associated with better visual memory. This relationship logically follows with regard to later onset epilepsy. Those who showed the latest onsets tended to have worse visual memory and lower cortical thickness in the PCC and right SFG. However, in early onset cases, this set of relationships in RTLE is somewhat disconnected. Early age of onset is associated with better visual memory, but thinner cortex in regions where thinner cortex is associated with worse memory. This disconnect may be due to reorganization of structural or functional network changes, unmeasured in this study. For example, Doucet et al (2015) showed that early onset TLE (before the age of 20) was associated with higher network modularity compared to late onset TLE, and increased clustering of the PCC compared to healthy controls and patients with a late onset (after 20 years old). These types of functional changes may be indicative of network reorganization/compensation and future investigations should look into the link between these age of onset related network changes and memory ability.

My findings call into question whether LTLE and RTLE should be treated as simply mirrored disorders. Previous studies have reported morphometric differences between LTLE and RTLE groups (Ahmadi et al., 2009; Kemmotsu et al., 2011). Kemmotsu et al (2011) found more widespread cortical thinning in patients with LTLE and bilateral reductions in white matter integrity in fibre tracts associated with the temporal lobes using diffusion tensor imaging (DTI). These authors also found that, in the LTLE group, earlier onset was associated with more disrupted white matter integrity, but this relationship was not seen in the RTLE group, though the RTLE group seemed somewhat uncharacteristic since they showed minimal difference compared to controls which is contrary to other DTI studies of TLE (Ahmadi et al., 2009; Focke
et al., 2008). The authors take this as evidence that the left hemisphere may be more vulnerable to early insults in patients with TLE. Other studies have noted that the left hemisphere appears to be vulnerable to early life insult based on incident rates of stroke and epilepsy in early life (Kolk & Talvik, 2000; Njokiktjen, 2006; D. C. Taylor, 1969) and these authors have interpreted these findings as indicating that the left hemisphere undergoes a prolonged period of vulnerability compared to the right which might explain the varying effects of age of onset in left and right TLE. Further, developmental hemispheric asymmetries exists with grey matter development in left temporal language regions has been shown to have the most protracted maturation course compared to all other cortical regions (Sowell et al., 2003), while arcuate fasciculus maturation continues into late adolescence, but only in the left hemisphere (Ashtari et al., 2007).

Again, it is important to note that this study is cross-sectional and correlational and, thus, I cannot confirm the directionality of these effects. As mentioned above, previous evidence has shown that at time of diagnosis in childhood it is somewhat rare to find abnormalities of the hippocampus (Salmenperä et al., 2005), and there is limited cortical change in pediatric TLE populations, especially compared to adult populations (Guimarães et al., 2007). One study has also examined longitudinal gray matter and white matter changes in new/recent onset epilepsy in childhood compared to matched controls and found that both groups showed reductions in gray matter volume from baseline to a 2 year follow-up scan (Hermann et al., 2010). In the epilepsy group, volume reduction was slightly more rapid in frontal and parietal lobes, but this was not significant and unfortunately the analysis did not examine more specific anatomical demarcations. Interestingly though, the authors found that children with epilepsy had a delayed expansion of white matter volume compared to controls, driven by delays in the frontal lobe. The reduced white matter in these areas may be related to impaired development of intrinsic connectivity networks seen in childhood onset epilepsy (Ibrahim et al., 2014) and this loss of white matter may contribute to thinner cortex in frontal regions, due to reduced inputs, seen in the current study.

Another limitation of the current study is that I have used age of epilepsy onset to mark the initial disruption and I have also used duration to measure amount of time exposed to seizure activity. However, it is very likely that some pathological process may have occurred prior to the time of seizure emergence and it is very difficult to control for variables such as seizure frequency or severity as the reporting of these measures is unreliable (Hoppe, Poepel, & Elger, 2007). Another
limiting factor that is important to address is that epilepsy onset is often paired with administration of anti-epilepsy drugs (AEDs). The effects of AEDs on development are not well documented as it is difficult to tease apart the effect of the drug versus the effect of the disorder. In the same way, this investigation cannot tease apart the onset of epilepsy and onset of AED consumption. However, given that AED consumption is the reality of this patient population, the current investigation is still relevant to understanding the consequences of an early life diagnosis of TLE.

3.5 Conclusions

In this study, I found that age of epilepsy onset can have different effects on cortical thickness and memory ability depending on seizure focus laterality. In LTLE, very early onset is associated with worse verbal and visual memory and thinner left frontal language regions and insula relative to onset in adolescence and young adulthood and onset beyond this point, older ages of onset tends to be associated with worse memory and thinner cortex in these regions. In RTLE, earlier onset is associated with better visual memory ability than later onsets, but structurally, there is, again an inverted U relationship with cortical thickness and age of onset in the PCC and SFG. These findings suggest that age of onset does not affect left and right TLE in the same way and interrogation of the effects of age of onset should not be binary or assumed linear. It is important to understand the potential developmental impact of TLE on brain structure and function to assess whether early surgical intervention is beneficial to prevent impaired cognitive development and to understand what role reorganization may play in post-surgical memory outcome.
Chapter 4
Topological network properties are modulated by age of onset in right, but not left, temporal lobe epilepsy

4.1 Introduction

Temporal lobe epilepsy (TLE) is associated with widespread structural and functional alterations throughout the brain. There are also topological alterations that suggest suboptimal network resilience, but it remains unclear how these alterations relate to memory ability and whether this relationship is modulated by age of epilepsy onset.

The research using structural MRI has revealed significant bilateral grey matter alterations outside of the medial temporal lobe in widespread regions of prefrontal, somatomotor, lateral temporal, and medial and lateral parietal regions (Kemmotsu et al., 2011; Mueller et al., 2009), thought to be a consequence of seizure related damage. Furthermore, the underlying white matter that connects these regions is suspected of having compromised integrity based on diffusion MRI evidence (Concha et al., 2009; Kemmotsu et al., 2011; Riley et al., 2010). Reductions in integrity have been found in the cingulum bundle (Gross et al., 2006), uncinate and arcuate fasciculus (Govindan et al., 2008; Lin et al., 2008), and inferior longitudinal fasciculus (Otte et al., 2012). These widespread reductions in cortical and white matter integrity suggest that there may be network alterations and disrupted information flow in the epileptic brain.

Recent work has, in fact, shown disruptions in structural and functional networks in the brains of people with TLE, showing not only alterations in connectivity of the epileptogenic hippocampus, but also demonstrating whole brain structural and functional connectivity differences (Bernhardt et al., 2011; Bernhardt, Hong, Bernasconi, & Bernasconi, 2013; McCormick et al., 2014; Voets et al., 2012). Further, work from our lab has shown that altered network organization, measured during rest, is related to clinical memory ability (McCormick et al., 2014). People with TLE whose pattern of functional connectivity in the default mode network (DMN) more closely resembled healthy control DMN connectivity, tended to have better material specific memory performance—people with left TLE (LTLE) had better verbal
memory, and people with right LTE (RTLE) had better visual memory. However, we know that connectivity alterations extend beyond the DMN, with changes reported in temporo-limbic (Bettus et al., 2009; Doucet, Skidmore, Sharan, Sperling, & Tracy, 2013), sensorimotor (Haneef et al., 2014; Voets et al., 2012) and thalamic networks (Doucet, Osipowicz, Sharan, Sperling, & Tracy, 2012; Haneef et al., 2014).

Graph theory offers a framework for examining network connectivity of the whole brain that treats the brain as an abstract graph to interrogate topological network characteristics (Bullmore, Sporns, & Solla, 2009). In this type of analysis, disparate regions of the brain (nodes) are said to be connected to each other based on measures of structural connectivity such as diffusion tensor tractography, or across-subject grey matter covariance, or they can be connected based on measures of functional connectivity, such as the signal covariance during fMRI. The more strongly two regions covary in BOLD signal or grey matter thickness, the more strongly they are assumed to be connected. In healthy brains, networks tend to show a pattern of small-worldness which is a balance of high segregation with preserved whole network integration (Achard & Bullmore, 2007; Bassett et al., 2006). This topology allows for specialized local processing (segregation) and rapid long range communication (integration). In TLE, it appears as though an overall small world topology is preserved, but alterations have been reported in integration (Bernhardt et al., 2011; Horstmann et al., 2010; Liao et al., 2010), and segregation (Liao et al., 2010; Quraan et al., 2013). Reduced whole-network integration in TLE has been reported in structural connectivity (Bernhardt et al., 2011) and functional connectivity (Horstmann et al., 2010; Junjing Wang et al., 2014; Yasuda et al., 2015), while changes in network segregation have been less consistent. Structural and functional connectivity graph theory studies have shown both increased (Bernhardt et al., 2011; Bonilha et al., 2012; Junjing Wang et al., 2014) and decreased segregation (Chiang, Stern, Engel, Levin, & Haneef, 2014; Doucet et al., 2014; Vaessen et al., 2012). These inconsistencies are possibly due to sample heterogeneity and analysis decisions, such as node definition (Chiang & Haneef, 2014).

Only one study has investigated topological network characteristics in relation to age of onset in TLE. The authors found that patients showed reduced overall network segregation, measured by network clustering and modularity, together with increased network integration, but patients with early onset showed more normative clustering, modularity and integration compared to patients with a late onset for whole brain topology measures (Doucet et al., 2014).
When examining nodal graph theory metrics, they also showed that there was increased clustering in bilateral posterior cingulate cortex and reduced betweenness centrality of the epileptogenic hippocampus in the early onset group compared to both the late onset group and compared to healthy controls. The authors suggested that early onset may afford some type of reorganization that minimizes the aberrant influence of the epileptogenic hippocampus and increases the influence of posterior cingulate cortex in a compensatory fashion, given the known role of the PCC in memory networks (Buckner et al., 2008). However, the authors did not investigate the relationship between these effects and memory and thus it remains to be seen whether these changes are associated with a benefit to memory ability.

In this study, I aimed to investigate the relationship between network topology and age of onset in TLE, while also investigating the relationship between these measures and memory ability. To that end, I examined 46 adult patients with TLE (23 presenting with right lateralized seizures and 23 presenting with left) and 19 neurologically healthy control participants. Each subject underwent 6 minutes of resting state fMRI, from which I calculated whole brain network connectivity and extracted graph theory measures of topographic information. I hypothesized that this topological organization would be altered in people with TLE compared to healthy controls, with whole brain network integrity being compromised, and that nodal metrics would demonstrate deficits in the affected hippocampus. Furthermore, I examined whether graph theoretical measures of topology were related to age of epilepsy onset and memory ability. Unlike Doucet et al (2014), who treated age of onset as a binary categorical variable with a median split of their sample, I examined age of onset as a continuous variable as was done in the previous chapter. Further, given that in Chapter 1, I reported differences in age of onset effects for left and right TLE, I also analyzed left and right TLE separately, to interrogate laterality-specific effects and to examine whether age of onset related topology changes were associated with material specific memory ability.

4.2 Methods
4.2.1 Participants

Forty-six adult patients with pharmacologically intractable unilateral temporal lobe epilepsy were recruited from the Epilepsy Clinic at Toronto Western Hospital. Twenty-three patients presented with RTLE (Twenty-one of these patients overlap from Study 1) and 23 presented with LTLE (Seventeen of these patients overlap from Study 1). Continuous recording of scalp EEG and video monitoring during an inpatient evaluation in our epilepsy monitoring unit were used to determine seizure focus. Nineteen neurologically healthy control subjects were recruited to serve as comparison for our patient sample for resting-state fMRI networks.

4.2.2 Functional MRI preprocessing

Preprocessing was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) running in MATLABv8 12.0 (Mathworks). Anatomical and functional images were reoriented so that the origin falls on the anterior commissure. The functional images were then co-registered to the anatomical image before undergoing realignment and unwarping for motion correction. Anatomical images for each subject were segmented into grey matter, white matter and cerebral spinal fluid (CSF) and normalized into standard MNI space. Functional images were then normalized to standard space using the parameters from the anatomical transformation, before being smoothed with an 8-mm full-width half-max Gaussian kernel. Next, using the Artifact detection toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012), fluctuations in global signal greater than 3 standard deviations, translational motion greater than 1 mm, and rotational motion greater than 0.05 radians were identified and regressors were created to exclude these potentially confounding sources of variance. Finally, in the Conn toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012), temporal filtering was performed to exclude low (<0.008Hz) and high (>0.09Hz) frequency fluctuations, and aCompCor (Behzadi, Restom, Liau, & Liu, 2007) was used to exclude measures of physiological noise by regressing out the top five components of a PCA from the white matter and CSF masks produced from the SPM8 segmentation.

4.2.3 Graph Theory Analysis

The averaged BOLD time series from 246 ROIs from the Brainnetome atlas (Fan et al., 2016) were correlated with each other in a pairwise manner. This atlas was made by parcellating anatomical regions into subregions based on voxel-wise structural connectivity fingerprints, resulting in atlas nodes that are more fine grain than simple anatomical gyri, and have
homogeneous within-node network connectivity. Using an averaged healthy control connectivity matrix, I calculated the modular structure of the healthy brain by performing a spectral community detection with iterative fine tuning recommended by (Newman, 2006) implemented in the Brain Connectivity Toolbox (https://sites.google.com/site/bctnet/; (Rubinov & Sporns, 2010)). This procedure produced five community modules that roughly correspond to the frontoparietal network, default mode network, limbic network, somatomotor network and visual network (Yeo et al., 2011). Displayed in Figure 4.1 are the mean connectivity matrices of each group, organized by community module, and an average smoothed brain surface containing the centre points of each node colour coded by module.
Figure 4-1. Group level, whole brain correlation matrices organized by community structure for healthy controls, and patients with left or right temporal lobe epilepsy (left) and centre points of nodes from the parcellation atlas, rendered on a standard brain surface, colour coded by community structure. Blue = Frontoparietal network, Red = Default Mode network, Orange = Limbic network, Yellow = Somatomotor network, Green = Visual network.
The Network Based Statistics toolbox (Zalesky, Fornito, & Bullmore, 2010) was used to calculate significant group differences between the connectivity matrices of patient groups and healthy controls, using a cluster defining threshold of \( p < .01 \). Network Based Statistics performed a statistical test at every edge in the connectivity matrix and examined edges that were significantly different between groups at \( p < .01 \). It then identified topological clusters that occur at a greater than random chance. This was done by computing 5000 permutations of the data with the participant labels randomly rearranged, and calculating the largest cluster of significant nodes for each permutation. If the size of a topological cluster that was found using the correct participant labels occurred at a \( p < .05 \) chance over 5000 random permutations, then the cluster was considered significant. Given that education differed between healthy controls and the patient groups, I added years of education as a covariate of no interest.

I then examined the differences in topological network properties between groups. For each subject, I extracted graph theory metrics of whole brain characteristic path length, modularity, and network clustering from their subject specific connectivity matrix using the Brain Connectivity Toolbox. The derivatives of these metrics are displayed visually in Figure 4.2. Characteristic path length is the average of the shortest path lengths in a network. Path length is the number of nodes that need to be traversed to connect one node in the network to another and it describes the level of integration of each subject’s network. If fewer nodes need to be traversed to get from one node to another, then path length is short and integration is, thus, high. Higher integration means that information can be more rapidly combined from disparate regions across a network. Modularity describes how well nodes in a network group into communities or modules and whether this is greater than expected by chance. Communities have higher within community connections and lower connectivity to nodes outside of the community. The value of the modularity index, \( Q \), can range from -1 to 1 with values closer to -1 indicating that the network is less modular than expected by chance, and values closer to 1 being more modular than expected by chance. Modular structure was calculated for each subject using an spectral algorithm with iterative fine tuning as described above. The clustering coefficient is the proportion of a given node’s neighbours that are also connected to each other. The network clustering coefficient is the average clustering coefficient of every node in the network and is often taken as a measure of network segregation and describes how well nodes are topologically connected for specialized processing.
The human brain has been described as having a small-world topology that balances integration and segregation of nodes in the brain to control for wiring costs while still allowing for specialized clustering and long range integration (Bullmore & Sporns, 2012). Small worldness was calculated by taking the ratio of normalized clustering to normalized path length. Since clustering and path length are influenced by basic network characteristics, normalized measures are created to look at these measures in relation to null networks—networks that have the same basic characteristics, like degree distribution (the density of nodes that has a given degree), but are wired randomly (Rubinov & Sporns, 2010). Random networks have short characteristic path lengths, but almost no discernable clustering and these normalized measures reflect a given subject’s characteristic path length or network clustering relative to a set of individualized, null, random networks. Normalized path length for each subject was calculated by using the formula,

\[ \lambda = \frac{L}{L_{Rand}} \]

Where \( \lambda \) is normalized path length, \( L \) is the characteristic path length of a given subject’s network and \( L_{Rand} \) is the mean characteristic path length of 100 randomized networks that retain the same degree distribution of that subject. A \( \lambda \) of ~1 indicates that a given subject’s network

\[ \text{Modularity} \]

\[ \text{Clustering} \]

\[ \text{Shortest Path Length} \]
has approximately the same characteristic path length of a randomized network, indicating very efficient information transfer. A $\lambda$ value greater than 1 would indicate that a subject’s network has a higher path length than would be expected in a random network and, thus, has less efficient information transfer. Normalized clustering was calculated using the formula, 

$$\gamma = C/C_{Rand}$$

Where $\gamma$ is normalized clustering, $C$ is the mean clustering coefficient of a given subject’s network and $C_{Rand}$ is the mean clustering coefficient of 100 randomized networks that retain the same degree distribution of that subject. A $\gamma$ that is greater than 1 indicates that a given subject’s network has greater clustering than a random network which indicates that the network has specialized clusters of nodes for local processing. Using these, small worldness was calculated using the formula, 

$$\sigma = \gamma / \lambda$$

Where $\sigma$ represents the small world coefficient. Networks that have a $\sigma$ greater than 1 are considered to have small world properties since they have relatively greater clustering of nodes, with retained short path lengths compared to random networks (Humphries & Gurney, 2008).

Measures of hubness were calculated from bilateral hippocampus nodes as well as bilateral posterior cingulate cortex nodes, given that the PCC was modulated by age of onset in previous reports (Doucet et al., 2014). These measures include betweenness centrality which is the proportion of shortest paths between in the brain that pass through the node of interest. If there are many short paths that go through a particular node, then that node is thought to be of importance since it connects discrete regions in an efficient manner. Another measure of hubness is degree which is simply the number of connections a particular node has to the rest of the nodes in the brain. Nodes with higher number of connections are thought to transfer more information. A summary of the whole brain and nodal measures used is provided in Table 4.1.
### Table 4-1. List of graph theory measures and definitions.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
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<tr>
<td><strong>Network Integration</strong></td>
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<tr>
<td>Characteristic Path Length</td>
<td>The average of the shortest path lengths in a network. If fewer nodes need to be traversed to get from one node to another, then path length is short and integration is, thus, high.</td>
</tr>
<tr>
<td>Normalized Characteristic Path Length</td>
<td>The characteristic path length of a network normalized by the average characteristic path length of a large number of null networks that have the same degree distribution. A normalized characteristic path length that is close to 1 indicates that a given subject’s network has approximately the same characteristic path length of a randomized network, indicating efficient information transfer.</td>
</tr>
<tr>
<td><strong>Network Segregation</strong></td>
<td></td>
</tr>
<tr>
<td>Clustering Coefficient</td>
<td>The average clustering coefficient of every node in the network. Clustering coefficient of a node is the proportion of a given node’s neighbours that are also connected to each other. Higher clustering, indicates greater segregation and specialized processing.</td>
</tr>
<tr>
<td>Normalized Clustering Coefficient</td>
<td>The network clustering coefficient of a given network normalized by the average network clustering coefficient of a large number of null networks that have the same degree distribution. When this value is greater than 1, it indicates that a given subject’s network has greater clustering than a random network which reveals that the network has specialized clusters of nodes for local processing.</td>
</tr>
<tr>
<td>Modularity</td>
<td>How well nodes in a network group into communities or modules and whether this is greater than expected by chance. Can range from -1 to 1 with values near -1 indicating that the network is less modular than expected by chance, and values near 1 being much modular than expected by chance.</td>
</tr>
<tr>
<td><strong>Nodal Centrality</strong></td>
<td></td>
</tr>
<tr>
<td>Degree Centrality</td>
<td>The number of connections a particular node has to the rest of the nodes in the brain.</td>
</tr>
<tr>
<td>Betweenness Centrality</td>
<td>The proportion of shortest paths between in the brain that pass through the node of interest.</td>
</tr>
</tbody>
</table>
These measures are best interpreted and understood using binarized and thresholded matrices and thus, all measures were calculated across a range of binarized networks, thresholded from conservative (only selecting the top 5 percentile (%tile) of connections) to more liberal thresholds (selecting the top 35%tile of connections), as is common practice in the literature (Bernhardt et al., 2011; Bullmore et al., 2009; Doucet et al., 2014). Given that education was higher in the healthy control group, I investigated the relationship between each dependent variable and years of education using a nonparametric Spearman correlation and found that education was unrelated to these dependent variables, all \( r < 0.22, p > 0.09 \).

For these network characteristics, permutation tests were used to examine differences between TLE groups and healthy controls using 5000 permutations, since Gaussian distributions cannot be assumed for these measures. Given the number of tests performed, correction for multiple comparison was performed with a false discovery rate (FDR) correction for each network characteristic (Benjamini & Yekutieli, 2001) with an alpha of \( p < .05 \). Age of onset was modelled in regression linearly and non-linearly to investigate the influence of age of onset on graph theory metrics. Nonparametric Spearman correlations were used to characterize the relationship between network measures and age of onset and memory (measures as described in the previous chapter).

4.3 Results

4.3.1 Patient Demographics

There were no differences between the three groups in terms of age, \( F(2,62) = .51, p = .6 \), or handedness, Fisher’s exact test, \( p > 0.5 \). There was a significant difference between the three groups in terms of education, \( F(2,62) = 9.7, p < .01 \), with healthy controls having greater education than both the LTLE and RTLE group using a Bonferroni post-hoc test. There was a different proportion of male and females between the three groups, \( \chi^2(2, N = 65) = 10.2, p = .006 \). Specifically, there was a difference between the LTLE and RTLE group in terms of sex distribution, \( \chi^2(1, N = 46) = 8.7, p = .003 \). There were no differences in age of onset, duration of epilepsy, verbal memory, visual memory or IQ, between the LTLE and RTLE groups, all \( t < 1.5, p > 0.15 \), nor were there any differences between patient groups in presence or absence of MTS,
distribution $\chi^2(1, N = 46) = 0.37, p = 0.5$, in the presence of other lesions, $\chi^2(1, N = 46) = 1.1, p = 0.3$, or in language dominance using Fisher’s exact probability test, $p = 0.1$. Demographic information and neuropsychological performance is reported in Table 4.2.
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>RTLE</th>
<th>LTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>34 (10.7)</td>
<td>36.9 (13.2)</td>
<td>37.6 (9.2)</td>
</tr>
<tr>
<td>Education, y (SD)</td>
<td>18 (3.1)</td>
<td>14.2 (3.5)</td>
<td>14.2 (2.6)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>11/8</td>
<td>17/6</td>
<td>7/16</td>
</tr>
<tr>
<td>Handedness, R/L/B</td>
<td>17/2/0</td>
<td>22/1/0</td>
<td>20/2/1</td>
</tr>
<tr>
<td>Language Dominance, R/L/BI</td>
<td>--</td>
<td>0/23/0</td>
<td>1/20/2</td>
</tr>
<tr>
<td>Disease duration, y (SD)</td>
<td>--</td>
<td>15.6 (14.4)</td>
<td>18.4 (13.4)</td>
</tr>
<tr>
<td>Onset of seizures, y (SD)</td>
<td>--</td>
<td>21.0 (13.3)</td>
<td>19.2 (13.2)</td>
</tr>
<tr>
<td>Presence of MTS, Yes/No</td>
<td>--</td>
<td>15/8</td>
<td>13/10</td>
</tr>
<tr>
<td>Other Lesions</td>
<td>--</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Verbal Memory Factor</td>
<td>--</td>
<td>.23 (1.2)</td>
<td>.19 (1.1)</td>
</tr>
<tr>
<td>Visual Memory Factor</td>
<td>--</td>
<td>-.20 (1.1)</td>
<td>.20 (.77)</td>
</tr>
<tr>
<td>IQ Factor</td>
<td>--</td>
<td>-.14 (1.2)</td>
<td>.36 (1.0)</td>
</tr>
</tbody>
</table>

RTLE, right temporal lobe epilepsy; LTLE, left temporal lobe epilepsy; y, years; SD, standard deviation; M, male; F, female; R, right; L, left; BI, bilateral; IQ, intelligence quotient. Characterization of MTS and other lesions was based on radiology (3T MRI protocol). In the RTLE group, one individual had a right amygdala ganglioglioma, one
individual had a right amygdala dysembryoplastic neuroepithelial tumour, and one had a right amygdala harmatoma. In the LTLE group, one individual had a left amygdala dysembryoplastic neuroepithelial tumour.

### 4.3.2 Network Based Statistics

Network wide contrasts between LTLE and the healthy control group revealed significant reductions in within-module connectivity of the DMN module with increased inter-module connectivity, specifically in the edges connecting the somatomotor module with the DMN and frontoparietal network. In RTLE, I observed reduced connectivity within the DMN and the somatomotor modules, accompanied by increased inter-module connectivity in the edges connecting the somatomotor module to the frontoparietal, default mode and visual networks, p < .05. No significant differences were found between the two TLE groups. These group differences are shown in Figure 4.3.
Figure 4-3. On the left, group differences in network connectivity between patient groups and healthy controls are shown using network based statistics with 5000 permutations, p < .05, controlling for years of education. Blue cells represent reduced connectivity in TLE, red cells represent increased connectivity in TLE. On the right, centre points of nodes from the parcellation atlas are rendered on a standard brain surface, colour coded by community structure. Blue = Frontoparietal network, Red = Default Mode network, Orange = Limbic network, Yellow = Somatomotor network, Green = Visual network. Intra-module connectivity in the default mode network is reduced in TLE, while inter-module connectivity between the somatomotor network and the rest of the brain, increases.
4.3.3 Whole Brain Graph Theory Results

Permutation analysis revealed significant reductions in modularity across all thresholds for the LTLE group compared to healthy controls, but not the RTLE. No significant differences were found between people with left or right TLE in comparison to healthy controls for characteristic path length or clustering using nonparametric permutation tests with 5000 permutations (all \( p > .2 \)), even before correction for multiple comparisons. These results are shown in Figure 4.4.

When examining normalized metrics, however, I found reduced normalized clustering, \( \gamma \), and small worldness, \( \sigma \), in the LTLE group compared to healthy controls across a range of threshold from 10-35\%tile, after correcting for multiple comparisons, but found no differences in normalized characteristic path length, \( \lambda \). No significant differences were found between RTLE and healthy controls for any of the normalized metrics across any threshold, even before correcting for multiple comparisons. These results are shown in Figure 4.4. Given that sex was distributed differently across the two patient groups, I interrogated whether graph metrics varied by biological sex in TLE and found no significant differences, all \( p > .1 \).
Figure 4-4. Mean and standard error for global graph theory metrics for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds. * denote significant difference between LTLE and HC.
4.3.4 Nodal Centrality Results

The Brainnetome atlas has four hippocampal seeds—an anterior and posterior seed in each hemisphere—which were selected for analysis. The LTLE group had significantly lower degree in the left posterior hippocampus across a range of thresholds, from 15–35\% tile compared to healthy controls, demonstrating that fewer connections were made with the left posterior hippocampus in the LTLE group, likely due largely in part to the its hypoconnectivity with DMN nodes. There was a trend towards significantly reduced degree in the left anterior hippocampus using the 35\textsuperscript{th} (p = .08), 20\textsuperscript{th} (p = .06), 15\textsuperscript{th} (p = .05), and 10\% tile (p = .07) thresholds compared to healthy controls and, across all thresholds, degree was arithmetically lower in the LTLE group. There were no differences in degree for the right hippocampus across any thresholds for the LTLE group compared to healthy controls. These results are displayed in Figure 4.5.

When examining the RTLE group, reduced degree was seen in across all thresholds, 5-35\% tile in the right posterior hippocampus compared to healthy controls, indicating reduced numbers of connections to this region, also likely due largely in part to the its hypoconnectivity to DMN nodes. At uncorrected levels, there were reductions also seen in the left posterior hippocampus at the 30\textsuperscript{th} (p = .03) and 35\% tile (p = .02) thresholds. There were no areas of significant deficits in degree centrality in the anterior hippocampi in the RTLE group. These are also shown in Figure 4.5.

A similar pattern was seen for betweenness centrality in the hippocampi, with the LTLE group showing significantly reduced betweenness centrality in the left posterior hippocampus across a range of thresholds from 20-35\% tile and for the left anterior hippocampus at the 35\textsuperscript{th}, 30\textsuperscript{th}, and 5\% tile thresholds, compared to healthy controls. There were no differences in betweenness centrality in the right hippocampus across any thresholds for the LTLE group. These results are displayed in Figure 4.6.

For the RTLE group, reductions in betweenness centrality were seen the right anterior hippocampus across a range of thresholds from 25-35\% tile, compared to healthy controls and there was a trend for reduced betweenness centrality in the right posterior hippocampus, at uncorrected levels, at the 25\textsuperscript{th} (p = .02) and 35\% tile (p = .08).
Figure 4-5. Mean and standard error for anterior (Ant) and posterior (Post) hippocampal degree for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds. * denote significant difference between LTLE and HC. † denotes significant difference between RTLE and HC.
Figure 4-6. Mean and standard error for anterior (Ant) and posterior (Post) hippocampal betweenness centrality (BC) for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds. * denote significant difference between LTLE and HC. † denotes significant difference between RTLE and HC.

From the PCC, two seeds—a dorsal and ventral node from BA 23—from each hemisphere were selected based on community membership with the default mode network (represented in red on Figure 1) to interrogate. Betweenness centrality was increased in right ventral BA23 in LTLE compared to the healthy control group, at uncorrected levels for the 15th (p = .01), 20th (p = .01),
25th (p = .008) and 35%tile (p = .04) thresholds. No other differences were seen between the LTLE group and healthy controls for degree or betweenness centrality in the ventral or dorsal BA 23 seeds. In the RTLE group, the only difference with healthy controls was a reduction in betweenness centrality in the right ventral BA23 seed at the 5%tile threshold at uncorrected levels (p = .008). These findings are presented in Figure 4.7 (degree centrality) and Figure 4.8 (betweenness centrality). Given that sex was distributed differently across the two patient groups, I interrogated whether nodal characteristics varied by biological sex in TLE and found no significant differences.

Figure 4-7. Mean and standard error for dorsal and ventral, left (L) and right (R) Brodmann area 23 (BA23) degree for left temporal lobe epilepsy (LTLE), right temporal lobe epilepsy (RTLE), and healthy controls (HC) across different network proportional thresholds.
4.3.5 Relation to age of onset

The LTLE group did not show any relationship between age of onset with any of the whole brain graph theory measures, all $r^2 < 0.2$, $p > 0.05$, but in the RTLE group, older age of onset was associated with lower normalized clustering, $\gamma$, across all thresholds and lower small worldness and modularity across a range of thresholds (modularity: $5^{th}$ and 15-30%tile; small worldness: 10-35%tile). As noted above though, there were no differences between people with RTLE and
healthy controls for normalized clustering, small worldness, and modularity. As such, the older onset RTLE patients showed values on the low end of normal, while the earlier onset RTLE patients showed values on the high end of normal. These relationships were strictly linear, as regression models showed that there was no relationship with \((\text{age of onset})^2\) and any of the graph measures. Spearman correlation values for the linear relationships can be seen in Table 4.3. Neither age of the participant or duration of epilepsy were related to these measures (all \(r < .26, p > .1\)).

### Table 4-3. Spearman Correlation values relating age of epilepsy onset to whole brain graph theory network measures in right temporal lobe epilepsy across different percentile (%tile) thresholds

<table>
<thead>
<tr>
<th>Threshold</th>
<th>5%tile</th>
<th>10%tile</th>
<th>15%tile</th>
<th>20%tile</th>
<th>25%tile</th>
<th>30%tile</th>
<th>35%tile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized Clustering</td>
<td>-.43</td>
<td>-.46</td>
<td>-.55</td>
<td>-.54</td>
<td>-.53</td>
<td>-.54</td>
<td>-.52</td>
</tr>
<tr>
<td>Modularity</td>
<td>-.58</td>
<td>-.41</td>
<td>-.43</td>
<td>-.43</td>
<td>-.46</td>
<td>-.43</td>
<td>-.37</td>
</tr>
<tr>
<td>Small World Coefficient</td>
<td>-.38</td>
<td>-.47</td>
<td>-.52</td>
<td>-.52</td>
<td>-.52</td>
<td>-.51</td>
<td>-.52</td>
</tr>
</tbody>
</table>

Bold values indicate significance at \(p < .05\).

To see whether these measures were related to memory ability, Spearman correlations were run between memory scores and whole brain metrics that were related to age of onset. Greater normalized clustering was related to visual memory performance at the 30\(^{\text{th}}\) tile, \(r(22) = 0.42, p < .05\) and this effect was marginal at the 10-25\(^{\text{th}}\) and 35\(^{\text{th}}\) tile (\(r = .36 – .41\)). Greater modularity at the 5\(^{\text{th}}\) tile threshold was related to visual memory performance, \(r(22) = .51, p = .01\), as was greater small worldness at the 30\(^{\text{th}}\) and 35\(^{\text{th}}\) tile, \(r(22) = .42, p < .04\) and this effect was marginal at the 15-25\(^{\text{th}}\) tile (\(r = 0.36 – .4\)).

There was no consistent relationship between any nodal measures and age of onset, memory or IQ in LTLE or RTLE, all \(r < .4\).
4.4 Discussion

In this study, I report whole brain topology differences between people with LTLE compared to a group of healthy controls. Both TLE groups showed significant reductions in connectivity within the DMN, but increased connectivity for the inter-module connections of the somatomotor network to the DMN and frontoparietal networks. Further, people with LTLE had reduced modularity, reduced normalized clustering, and reduced small-worldness. Whole brain topology was not significantly different in RTLE compared to healthy controls, but earlier age of onset was associated with relatively greater modularity, normalized clustering, and small-worldness in this group compared to later age of onset—a relationship that was not seen in LTLE. Furthermore, those RTLE individuals who had greater normalized clustering and small-worldness also had better visual memory performance, suggesting earlier onsets were associated with network properties that were beneficial to memory ability, though this result did not achieve significance at all network thresholds. At the nodal level, I also found reductions in degree and betweenness centrality in the epileptogenic hippocampus in patients compared to healthy controls, but there were no differences between patient groups and controls for PCC nodes and none of the nodal measures were related to age of onset in a linear or quadratic way.

4.4.1 Whole Brain Graph Theory Measures

Previous reports have also found whole brain topological reductions in small-world measures and clustering in TLE in resting state fMRI (Doucet et al., 2014; Liao et al., 2010), and in the theta band using resting state EEG (Quraan et al., 2013). The loss of small-worldness in LTLE in the current study appears to be due to the reduction in normalized clustering, while path length is maintained. These parameters suggest that the LTLE network topology has reduced network segregation and is shifted towards a more random network topology, consistent with previous reports (Liao et al., 2010; Quraan et al., 2013). We also saw reduced modularity in LTLE that may be characterized by reduced DMN intra-module connectivity coupled with increased inter-module connectivity of the somatomotor network. While these findings suggest there is reduced specialized processing in LTLE compared to healthy controls, these altered network properties were not related to age of epilepsy onset, memory or IQ.

In RTLE, there were no whole brain topology changes in the overall group when compared to healthy controls. However, people with an earlier age of onset had higher modularity,
normalized clustering, and small-worldliness. Furthermore, increases in these graph characteristics were related to better visual memory in the RLTE group, though the statistical significance of this was less consistent across thresholds. This suggests that early onset in this patient group is associated with whole brain network organization that is beneficial to memory ability. The previous chapter demonstrated that early onset RTLE was associated with better visual memory, suggesting some sort of adaptive plasticity that may have been afforded to those people with an earlier onset. However, in that study, the morphometric relationship with age of onset was not indicative of visual memory ability. To explain this, I suggested that there may be some functional alterations of the brain that may better explain the age of onset relationship. Here, I report that, indeed, there are age of onset related network properties that are beneficial to visual memory ability. Previous studies have reported that earlier onset is related to better visual memory (Baxendale et al., 1998), and that earlier onset is associated with less aberrant network organization (Doucet et al., 2014), but this is the first study to link age of onset related network topology changes with visual memory ability in this patient group.

Epilepsy onset early in development may allow for meaningful reorganization of network topology. Studies of network topology across childhood in healthy populations demonstrate that small worldliness increases over the age range from 5 – 18 years (Wu et al., 2013), and connection preference in modules move from local to distributed (Fair et al., 2009). The age related increases in Wu et al (2013) were attributed to increases in global network segregation, while integration, measured by path length, was already established. Since segregation is ongoing across development, the formation of modules and clusters may be plastic, allowing for strategic reorganization of connections that may salvage efficient whole brain organization. One potential alternative—one that was also noted by Doucet et al (2014)—is that reorganization takes more time in the adult brain. Onset age and duration are correlated in this sample ($r = -.65$) and it is difficult to tease apart these effects. Nevertheless, duration itself was not correlated with modularity, normalized clustering or small worldliness.

As was found in the last chapter, the LTLE and RTLE groups differed in their relationship of functional characteristics with age of onset. In the previous chapter, I noted that research has demonstrated increased gray matter atrophy and reduced white matter integrity in people with LTLE compared to RTLE (Ahmadi et al., 2009; Kemmotsu et al., 2011). Given that the structural connections of the brain constrain functional connectivity (Honey et al., 2009), greater
alterations of network properties in the presence of reduced white matter integrity in this population may be expected. The specific network alterations in the LTLE group may also be related to epilepsy onset in the language dominant hemisphere. In the sample of people with LTLE in this study, 20 had left language dominance, two had bilateral representation and one had right language dominance. Those with atypical language representation had numerically higher measures of modularity, clustering and small worldness compared to those with left language dominance, though statistical tests could not be run on this due to small sample size.

4.4.2 Nodal Graph Theory Measures

Our nodal graph theory metrics showed differences in patients compared to healthy controls in the epileptogenic hemisphere in the hippocampus, but not in the posterior cingulate cortex. Given the multitude of regions and measures that can be extracted using graph theory, I focussed my analysis on these two regions based on a priori evidence that they have altered resting state properties in TLE (Doucet et al., 2014; Liao et al., 2011). Reductions in nodal degree in the epileptic hippocampus were significant only in the posterior region, which is thought to preferentially engage with posterior-medial aspects of the default mode network (Adnan et al., 2016; Poppenk & Moscovitch, 2011). The default mode network is a network of regions thought to be involved in internally guided thought and shows significant overlap with areas of the brain that become activated during recall of episodic memories (Buckner et al., 2008). Previous reports from our group have shown that, in TLE, reduced connectivity between the hippocampus and the PCC is associated with worse episodic memory in a material specific way (McCormick et al., 2014, 2013). Nodal degree in the posterior hippocampus, however, was not related to age of onset or memory, and may be capturing extra connectivity variability that is unrelated to memory performance. This suggests that the number of connections to the hippocampus with the rest of the brain may not be as important as the strength of connection to specific regions, like the PCC, shown in McCormick et al (2013). Degree in the PCC was not significantly different between patients and controls across a range of thresholds, which was also shown in a previous study from our lab (McCormick et al., 2013), nor was it related to any measures or memory or age of onset.

Differences in betweenness centrality were seen in both the anterior and posterior left hippocampi in the LTLE group, but were selective to the anterior hippocampus in the RTLE
group, though they did demonstrate some marginal differences in the posterior hippocampus. Again, no significant differences were seen in the posterior cingulate cortex for either group. Thus, the ipsilateral hippocampus appears to lose its importance as a hub of communication in the presence of TLE, while the PCC still retains its hub-like properties. A similar finding was observed by Addis, Moscovitch and McAndrews (2007), in which, during autobiographical memory retrieval, effective connectivity to and from the ipsilateral hippocampus was diminished, while the PCC retained its connectivity and even upregulated its communication to certain regions, like the medial prefrontal cortex in LTLE subjects compared to healthy controls. Despite the upregulated influence of the PCC in network communication, episodic autobiographical memory was impaired in these patients (Addis, Moscovitch, & McAndrews, 2007), suggesting that this alternative path is suboptimal for memory.

4.4.3 Limitations

One limitation of this study, and many graph theory studies, is that the results obtained are contingent upon methodological decisions such as thresholding, and choice of parcellation scheme. In the current study, I chose a range of thresholds that has previously been used in the TLE literature (Bernhardt et al., 2011; Doucet et al., 2014). However, I used a finer grain parcellation scheme (246 regions) than many of the previous graph theory studies which use between 52 and 116 regions. Defining the optimal parcellation scheme is an active area of research (Fornito, Zalesky, & Bullmore, 2016) which is attempting to utilize multimodal imaging techniques to parcellate the brain. The Brainnetome atlas used here provides meaningful anatomical and connectivity based divisions of the brain provided by anatomical MRI and FreeSurfer further divided into parcels of shared structural connectivity measured using diffusion MRI and clustered using spectral clustering. In brief, gross anatomical demarcations are broken down into subregions based on the shared structural connectivity of those subregions. This atlas should, thus, provide more meaningful divisions than those that are based purely off of anatomy such as the automatically anatomically labelled (AAL) atlas (Tzourio-Mazoyer et al., 2002). However, the values of graph theory metrics calculated here cannot be easily compared with those attained from studies using other atlases. Another limitation of this study is that all patients were on medication during resting state scanning and it is possible that antiepileptic drugs (AEDs) will have some effect on resting state network properties. Some AEDs have been shown to affect language networks and performance such as topiramate (Wandschneider et al.,
2017), but the degree to which they affect resting state properties has not been explored. Similarly, those individuals who have an earlier age of onset will also likely start taking AEDs at an earlier age. However, AED use is the reality for this patient group and the dynamics seen during the scan and the influence of the drugs during development reflect the regular state of these individuals. Further, due to the sample size, this study was unable to separate out effects that were dependent on presence or absence of mesial temporal sclerosis (MTS). A previous study by Doucet et al (2014) has investigated graph theoretical differences between MTS and non-MTS patients for early and late onset, but did not separate the TLE group by seizure laterality. Ideally, research in the future should interrogate the effect of onset age in both MTS vs. non-MTS and LTLE vs. RTLE, concurrently.

4.5 Conclusions

In conclusion, we showed that LTLE is associated with a reduction in network segregation resulting in reduced small-world properties compared to healthy control individuals. There was no difference in whole brain graph network properties in RTLE, but both left and right TLE had reductions of centrality measures in the epileptogenic hippocampus, with relatively spared hubness of the PCC. Early epilepsy onset in the RTLE group was associated with higher modularity, normalized clustering and small worldness and these network properties were partially related to better visual memory ability, suggesting adaptive network architecture.
Chapter 5
Parcellation of the hippocampus using resting functional connectivity in temporal lobe epilepsy

5.1 Introduction

Resting state functional connectivity has emerged as a potentially valuable tool for interrogating system integrity and predicting treatment outcome in neurological and psychiatric disease populations. Evidence from our group has demonstrated that resting connectivity among default mode network (DMN) nodes is useful for interrogating memory network integrity (McCormick et al., 2014) and for predicting pre- to post-operative memory change (McCormick et al., 2013) following surgery for temporal lobe epilepsy (TLE), that involves resection of the hippocampus, amygdala and anterior temporal lobes.

When evaluating resting state networks, multiple analysis choices need to be made which can affect the quantification of functional connectivity. Seed based measures, produced when the time course of a region of interest (ROI) is correlated with the time course of other voxels or regions, provide a straightforward metric for connectivity that can be performed in an individual subject and, thus, may be readily used in presurgical evaluation. In previous work from our group, McCormick et al (2013), seeded from the posterior cingulate cortex (PCC) to every voxel in the hippocampus and looked at where functional connectivity in the hippocampus to the PCC was correlated with clinical memory performance. They found that greater connectivity between the PCC seed and clusters of voxels in the body and tail of the ipsilateral hippocampus was related to presurgical memory performance in a material specific way—connectivity between the left hippocampus and the PCC in left TLE (LTLE) was related to verbal memory ability and connectivity between the right hippocampus and PCC in right TLE (RTLE) was related to visual memory ability. Further, in a subgroup of patients that went on to have surgical resection of the epileptogenic hippocampus, stronger connectivity in these hippocampi presurgically was related to greater memory decline following surgery, suggesting that strength of hippocampal-PCC connectivity can serve as an index for memory capacity ability, and predictor of post-surgical memory decline.
These results demonstrate the potential for the use of connectivity in presurgical clinical assessment of cognitive outcome. However, extracting a single meaningful connectivity value for an individual subject prospectively requires selection of a target ROI. Given the location of the significant clusters from McCormick et al. (2013) it would seem that the posterior hippocampus to PCC connectivity is of particular interest and thus selection of a posterior hippocampal ROI would seem ideal to extract this measure. Recent work in healthy adult populations have also highlighted a potential, preferred role of the posterior hippocampus in episodic memory retrieval (Poppenk et al., 2013; Poppenk & Moscovitch, 2011) as well as its preferred connectivity to the PCC (Adnan et al., 2016). In our previous work, we parcellated the hippocampus in 15 healthy adults using a k-means clustering algorithm based on the voxel-wise structural connectivity measured using diffusion tensor imaging (DTI) (Adnan et al., 2016). Voxels showing similar structural connectivity were clustered together and produced anterior and posterior clusters in both the left and right hippocampi. We examined the functional connectivity of these clusters and found that the anterior cluster was functionally connected more to the temporal poles and orbitofrontal cortex compared to the posterior, while the posterior cluster was more connected to the PCC and angular gyrus compared to the anterior.

In TLE, a similar attempt has been made to parcellate the hippocampus using functional connectivity, rather than structural connectivity. Voets et al (2014) seeded from each voxel in the hippocampus, separately, and examined the strength of connectivity of a given voxel to target masks that were constructed from regions known to be connected to the anterior and posterior hippocampus in the healthy brain. The anterior memory network mask included the entorhinal cortex, orbitofrontal cortex, and temporal pole, while the posterior memory network mask included the parahippocampal gyrus, lingual/fusiform gyrus, dorsolateral prefrontal cortex, posterior cingulate cortex, precuneus, and thalamus. Voxels were labelled according to which mask they had a stronger connectivity with i.e. if a voxel showed greater arithmetic magnitude of connectivity to the anterior memory network mask compared to the posterior memory network mask, it was labeled as an anterior memory network voxel. Using this technique, they demonstrated that both patients and healthy controls showed an anterior division and posterior division that was split along the long axis of the hippocampus. They also showed that the posterior epileptogenic hippocampus had decreased connectivity to the PCC, but increased connectivity to the parahippocampus, in patients compared to healthy controls. Further, when pooling LTLE and
RTLE groups and using material specific measures of impairment, they found that stronger connectivity between the contralateral posterior hippocampus with the posterior cingulate cortex was related to spared memory ability, while increased connectivity between the anterior hippocampus and entorhinal cortex was associated with impaired memory ability. The anterior hippocampus is often where mesial temporal sclerosis (MTS) is more strongly detected (O’Connor et al., 1996) and increased connectivity here may not be associated with viable communication.

The aim of the current study was to use resting state functional connectivity and $k$-means clustering to parcellate the hippocampus of healthy controls and patients with TLE and investigate whether connectivity of these parcels with the PCC was related to memory ability. Further, I sought to determine whether this relationship is influenced by the age at epilepsy onset. I hypothesize that the $k$-means clustering will produce anterior and posterior clusters, similar to what we found with structural measures (Adnan et al., 2016) and that their connectivity will relate to memory ability similar to previous work (McCormick et al., 2013; Voets et al., 2014). I also hypothesized that early onset in RTLE would be related to beneficial functional connectivity given the findings from Chapter 3 and Chapter 4, showing that early onset RTLE was associated with improved memory and adaptive whole brain network topology.

5.2 Methods

5.2.1 Participants

Refer to Chapter 4 for details on participant collection and inclusion criteria.

5.2.2 Functional MRI Preprocessing

Preprocessing was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8), a toolbox running in MATLABv8 12.0 (Mathworks). Anatomical and functional images were reoriented so that the origin fell on the anterior commissure. The functional images were then co-registered to the anatomical image before undergoing realignment and unwarping for motion correction. Anatomical images for each subject were segmented into grey matter, white matter and cerebral spinal fluid (CSF) and normalized into standard MNI space. Functional images were
then normalized to standard space using the parameters from the anatomical transformation. Two separate threads of processing then occurred with one thread undergoing spatial smoothing with a 4-mm full-width half-max Gaussian kernel and the other having no spatial smoothing performed. The unsmoothed files were used to extract hippocampal voxel timeseries which served as seeds for the $k$-means clustering analysis, while the 4-mm smoothed files were used to extract grey matter voxel timeseries which served as the targets. Next, using the Artifact detection toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012), fluctuations in global signal greater than 3 standard deviations, translational motion greater than 1 mm, and rotational motion greater than 0.05 radians were identified and regressors were created to exclude these potentially confounding sources of variance. Finally, in the Conn toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012), temporal filtering was performed to exclude low (<0.008Hz) and high (>0.09Hz) frequency fluctuations, and aCompCor (Behzadi et al., 2007) was used to exclude measures of physiological noise by regressing out the top five components of a PCA from the white matter and CSF masks produced from the SPM8 segmentation.

### 5.2.3 $k$-means clustering

To identify functionally distinct sub-regions of the hippocampus, we performed a functional connectivity-based parcellation using a $k$-means clustering algorithm. First, left and right whole-hippocampus masks were defined using the Harvard-Oxford subcortical structural probabilistic atlas in FSL. For each left and right hippocampus, we probed the functional organization of the ROI given its relation to every other grey matter voxel in the brain. We therefore used the segmented normalized gray matter images to create a whole-brain mask of the voxels for which the functional connectivity to the hippocampal ROIs would be computed. Critically, while the whole-brain gray matter mask was minimally smoothed with a 4-mm FWHM Gaussian kernel (see above), the hippocampal data were not smoothed, to ensure that spatial adjacency in the clustering results was minimally attributable to spatial correlation between neighbouring hippocampal voxels.

The parcellation procedure was conducted separately for each participant, and separately for each the right and left hippocampus. We computed the Pearson product-moment correlation coefficient (‘functional connectivity’) between the time-series of a given voxel within a hippocampal ROI and every other voxel in our whole-brain grey matter mask. This resulted in a
whole-brain gray matter functional connectivity profile for each hippocampal voxel (Figure 5.1). $k$-means clustering was performed on a second-order correlation matrix of each hippocampal voxel’s similarity in functional connectivity profiles (Figure 5.2), to reduce computational demand (this procedure is identical to performing a clustering analysis on the first-order functional connectivity profile vectors).
Figure 5-1. An example of a connectivity matrix from a subject from each hippocampal voxel to every other voxel in the brain outside of the hippocampus.

Figure 5-2. An example of a correlation matrix in which the whole brain voxel-wise connectivity patterns of each hippocampal voxel are correlated with each other. To make these matrices, each column of Figure 1 is correlated with every other column in a pairwise fashion. Strong correlations between hippocampal voxels indicate that those voxels have similar covariance in their connectivity with the rest of the brain.
Given the extensive literature on the functional organization of the hippocampus (Kahn, Andrews-Hanna, Vincent, Snyder, & Buckner, 2008; Poppenk et al., 2013; Voets et al., 2014) and parcellation work from our group (Adnan et al., 2016), we proceeded with subsequent analyses using the results from the $k=2$ parcellation. The squared Euclidean metric was used to define distance between clusters, and cluster centroid values were estimated using the $k$-means++ algorithm implemented in Matlab. This places random initial seeds for the analysis, and converges quickly to minimize within-cluster, point-to-centroid distance iteratively. We specified a max of 100 iterations for convergence, and 25 replications with random initial seeds were conducted to reduce the probability of convergence onto local minima. For each $k$-cluster solution, the results produced a cluster label for each voxel in the hippocampal ROI, as well as the centroid values of each cluster for $d$ dimensions, where $d$ equaled the number of grey matter voxels in the rest of the brain. Finally, the correlation matrix of functional connectivity profiles within each ROI was sorted according to the cluster labels derived from the $k$-means cluster analysis (Figure 5.3).

![Figure 5-3](image)

**Figure 5-3.** An example of a sorted correlation matrix of whole brain voxel-wise connectivity pattern correlations between each hippocampal voxel following $k$-means clustering procedure.
This matrix contains the same information as Figure 5.2, except it is reordered according to the k-means clustering.

To derive group-level parcellations, we averaged the within-ROI correlation matrices across participants after sorting the voxel sequences along the matrix dimensions identically. We then performed the same k-means clustering procedure described above, with the clustering parameters, the results of which were projected back to standard brain space at the group level.

### 5.2.4 Region of interest connectivity analysis

To interrogate the functional connectivity differences between the TLE and healthy control groups, we used the resulting masks from the k-means clustering analysis as regions of interest for the subsequent analyses. The mean time course from each ROI was correlated with the smoothed data from every other voxel in the brain with the regressors of no interest entered into the model to account for motion and global signal fluctuations. These correlations were then transformed using a Fisher’s z transformation. The resulting individual subject maps were entered into a group level between-subject analysis, to examine differences in voxel-wise whole brain connectivity of the anterior and posterior hippocampus from the left and right hemisphere. Analyses were performed separately for the LTLE group and RTLE group. Resulting contrast maps were corrected using permutation analysis with 5000 permutations, false discovery rate corrected. Years of education was entered for each subject and investigated as a covariate of no interest.

To examine the relationship between hippocampal–PCC connectivity and memory performance, we used two PCC seeds published by Andrews-Hanna et al (2010) that were used in previous studies by our group (McCormick et al., 2014, 2013). The left PCC seed is located at x = -8, y = -56, z = 26, while the right PCC seed is located at x = 8, y = -56, z = 26. The mean time course of each hippocampal cluster was extracted and correlated with the mean time course of the corresponding PCC seed. These correlation coefficients were then Fisher z-transformed and the resulting z-scores were correlated with memory scores using the verbal and visual memory factor scores described in Chapter 2: General Methods, with SPSS 21 (Chicago, IL). Significance for memory correlations was set at p < .05, uncorrected.
5.3 Results

5.3.1 Patient Demographics

Demographic variables are summarized and discussed in Chapter 4, Table 4.2.

5.3.2 k-means clustering

The $k = 2$ clustering procedure produced visually similar clusters for all three groups in both hemispheres, with anterior and posterior clusters divided along the long axis of the hippocampus. These clusters are displayed in Figure 5.4. A few voxels along the borders of the hippocampus seemed to misclassify. This could be due to noisy voxels that may represent white matter or cerebral spinal fluid that were encapsulated by the Harvard-Oxford hippocampal mask. Prior to group level connectivity analysis, these voxels were deleted from the clusters.

To examine the functional connectivity differences of each of these clusters in the groups, I contrasted the connectivity of the left and right anterior hippocampi with the left and right posterior hippocampi. Since these clusters were defined on this connectivity, I wanted to avoid statistical ‘double dipping’ and thus present the data unthresholded for visualization purposes in Figure 5.5. All groups show greater anterior hippocampal connectivity to the temporal pole, amygdala and ventral prefrontal cortices, while the posterior clusters showed preferred connectivity to the parahippocampal gyrus and thalamus. Visually, the pattern of anterior vs. posterior connectivity seemed aberrant in the RTLE group with respect to anterior hippocampal connectivity to early visual cortex and precuneus, while the LTLE and healthy control groups tend to show preferential posterior hippocampal connectivity to the precuneus.
Figure 5-4. Group level clusters from k-means clustering procedure, projected onto the standard MNI brain showing an anterior (yellow) and posterior (blue) cluster for both the left and right hippocampus derived from patients with left temporal lobe epilepsy (LTLE), healthy controls and patients with right temporal lobe epilepsy (RTLE).
Figure 5-5. Bilateral anterior hippocampal connectivity contrasted against bilateral posterior hippocampal connectivity in people with left temporal lobe epilepsy (LTLE), healthy controls and people with right temporal lobe epilepsy (RTLE) presented without statistical thresholding. The colour bars depict t values and warm colours depict areas of greater anterior versus posterior connectivity while cold colours depict areas of greater posterior versus anterior connectivity.

5.3.3 Group Connectivity Differences

When seeding from the left anterior hippocampal cluster, the LTLE group showed reduced connectivity to the parahippocampal cortex bilaterally, reduced connectivity to midline parietal and prefrontal cortex, bilaterally, and reduced connectivity to the left angular gyrus compared to the healthy control group. There were no areas of increased connectivity with the left anterior hippocampus in LTLE compared to controls when examining the whole brain. However, given that previous research had shown an increase in left anterior hippocampal connectivity to the
entorhinal cortex (Voets et al., 2014), I also explored this connection using a small volume correction with the entorhinal cortex mask from the Juelich histological atlas, thresholded at 35% (the same mask used by Voets et al. (2014)). Following this targeted analysis, I replicated their finding of increased connectivity between the left anterior hippocampus and the left entorhinal cortex, centred around xyz = -24, -14, -32, \( t(41) = 3.9, \ p < .001 \).

A similar pattern of reduction was seen for the left posterior hippocampal cluster, with reduced connectivity to midline parietal and prefrontal cortex, bilaterally, and reduced connectivity to the right medial temporal cortex. There were no areas of increased connectivity for the posterior hippocampus even when using a small volume correction with the parahippocampal mask from the Harvard-Oxford atlas, as was used by Voets et al. (2014). There were no differences between the LTLE group and healthy controls for either the anterior or posterior right hippocampal clusters. These results are displayed in Figure 5.6 and peak coordinates for these analyses are presented in Table 5.1.
Figure 5-6. Contrast maps of differences in connectivity between patients with left temporal lobe epilepsy compared to controls, seeding from either the left anterior hippocampal seed (top) or the left posterior hippocampal seed (bottom), thresholded at $p < .01$ using 5000 permutations, corrected for multiple comparisons using FDR correction. Areas of increased connectivity in LTLE, depicted in warm colours were only significant using a small volume correction in the entorhinal cortex. The colour bars depict $t$ values.
Table 5-1. Cluster regions, peak coordinates, test statistic and cluster size for connectivity differences between people with LTLE and healthy controls

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>Peak Coordinate</th>
<th>T</th>
<th>Cluster size (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Y</td>
<td>Z</td>
</tr>
<tr>
<td><strong>Left Anterior Hippocampus Seed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>R</td>
<td>24</td>
<td>-16</td>
<td>-16</td>
</tr>
<tr>
<td>LOC</td>
<td>L</td>
<td>-50</td>
<td>-66</td>
<td>28</td>
</tr>
<tr>
<td>Precuneus</td>
<td>B</td>
<td>-12</td>
<td>-52</td>
<td>16</td>
</tr>
<tr>
<td>Frontal Pole</td>
<td>B</td>
<td>-10</td>
<td>62</td>
<td>14</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>L</td>
<td>-24</td>
<td>-14</td>
<td>-32</td>
</tr>
<tr>
<td><strong>Left Posterior Hippocampus Seed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>R</td>
<td>26</td>
<td>-28</td>
<td>-14</td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td>B</td>
<td>12</td>
<td>-46</td>
<td>12</td>
</tr>
<tr>
<td>Anterior Cingulate</td>
<td>B</td>
<td>4</td>
<td>44</td>
<td>12</td>
</tr>
</tbody>
</table>

B, bilateral; L, left; LOC, lateral occipital cortex; R, right. Coordinates are presented in MNI space.

There were no significant differences between the RTLE group and the healthy control group when seeding from the right anterior hippocampal cluster. For the right posterior cluster, there was reduced connectivity to bilateral medial temporal cortex, right temporal pole, bilateral midline parietal and prefrontal cortex, right lateral orbitofrontal cortex and right somatomotor cortex in the RLTE group compared to controls. There were no areas of increased connectivity for the right posterior hippocampus, even when using a small volume correction in the parahippocampal gyrus. There were no differences between the groups for either the anterior or posterior left hippocampal clusters. These results are displayed in Figure 5.7 and peak coordinates for these analyses are presented in Table 5.2.
Figure 5-7. Contrast maps of differences in connectivity between patients with right temporal lobe epilepsy compared to controls, seeding from the right anterior hippocampal seed, thresholded at p < .01 using 5000 permutations, corrected for multiple comparisons using FDR correction. The colour bars depict t values.

Table 5-2. Cluster regions, peak coordinates, test statistic and cluster size for connectivity differences between people with RTLE and healthy controls

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>Peak Coordinate</th>
<th>T</th>
<th>Cluster size (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Posterior Hippocampus Seed</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>R</td>
<td>26</td>
<td>-18</td>
<td>-16</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>L</td>
<td>-24</td>
<td>-18</td>
<td>-22</td>
</tr>
<tr>
<td>Temporal Pole</td>
<td>R</td>
<td>34</td>
<td>12</td>
<td>-40</td>
</tr>
<tr>
<td>mPFC</td>
<td>B</td>
<td>2</td>
<td>52</td>
<td>-10</td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td>B</td>
<td>-10</td>
<td>-50</td>
<td>4</td>
</tr>
<tr>
<td>Precentral</td>
<td>B</td>
<td>-6</td>
<td>-30</td>
<td>54</td>
</tr>
</tbody>
</table>
B, bilateral; L, left; mPFC, medial prefrontal cortex; R, right. Coordinates are presented in MNI space.

For all groups, years of education and biological sex was unrelated to voxelwise hippocampal connectivity and did not influence group contrasts.

5.3.4 Hippocampal – PCC Connectivity and Memory

Connectivity between the hippocampal seeds and the PCC seeds were not related to verbal or visual memory in the LTLE group as a whole, all $R^2 < .08$, $p > .2$. When only the left language dominant group was selected, connectivity between anterior hippocampus and PCC significantly correlated with verbal memory factor score, all $R^2 = .2$, $F(1,18) = 4.5$, $p < .05$. Stronger connectivity between the left anterior hippocampus and the left PCC was associated with better verbal memory ability as shown in Figure 5.8. No other connections were related to memory ability, all $R^2 < .03$, $p > .4$. 
**Figure 5-8.** Scatterplot showing relationship between left anterior hippocampal to left posterior cingulate cortex connectivity with total number of words recalled on the Rey auditory verbal learning test over five trials, with a regression line drawn in black and regression line confidence intervals shown with dashed curves.

In the RTLE group, a marginal relationship was seen between the right posterior hippocampus–right PCC connectivity and the visual memory factor score, $R^2 = .13, p = .09$. When examining the visual memory neuropsychological scores individually, I observed that right posterior hippocampal connectivity to the right PCC was positively related to visual recall measured by RVDLT performance, $R^2 = .23, F(1,21) = 6.1, p = .02$, was marginally related to recognition memory for faces, $R^2 = .15, F(1,21) = 3.7, p = .07$, but was not related to number of trials to criterion for the conditional associative learning test, $R^2 = .06, p = .2$. The left posterior hippocampal connectivity to the left PCC was, however, positively related to recognition memory for faces, $R^2 = .31, F(1,21) = 9.5, p = .006$. These relationships are displayed in Figure 5.9.
5.3.5 Hippocampal – PCC connectivity and age of onset

The relationship between memory and HC – PCC connectivity was not influenced by age of onset, in a linear or quadratic fashion, in either LTLE or RTLE groups (all $R^2 < .02$, $p > .1$).
5.4 Discussion

Using a $k$-means clustering procedure, we were able to segment the left and right hippocampus into anterior and posterior divisions in people with left and right TLE and healthy controls. This demonstrates that the functional connectivity fingerprints of the hippocampal voxels are sufficiently distinguished along the long axis in the patient population, regardless of the effects of temporal lobe epilepsy. At the group level, the anterior clusters showed greater connectivity to the temporal pole, amygdala and ventral prefrontal cortices, while the posterior clusters showed increased connectivity to the parahippocampal gyrus and thalamus across all groups. Between group contrasts showed significant reductions in connectivity between the epileptogenic hippocampus and DMN regions in both LTLE and RTLE groups compared to healthy controls, but in the RTLE group this was limited to the posterior hippocampus. When interrogating the relationship of memory with hippocampal to PCC connectivity, I found that stronger connectivity between the left anterior hippocampus and PCC was related to better verbal memory ability in the LTLE group, while connectivity of the posterior hippocampus to the PCC was related to visual memory ability in the RTLE group. The connectivity of the regions related to memory were not influenced by age of onset.

Our findings are in agreement with previous work by Voets et al (2014) who similarly clustered the hippocampus into an anterior and posterior cluster using functional connectivity. Their methods involved giving a hard label to a voxel based on whether its magnitude of connectivity was arithmetically larger to either an anterior memory network mask (in which case it was given an anterior label) or a posterior memory network mask (in which case it was given a posterior label). This method is somewhat crude, but was very effective, producing results very similar to ours. In our $k$-means clustering of the hippocampus, we made no assumptions regarding what regions influence parcellation and, thus, we did not need to specify these regions 

&a priori with anatomical masks, that may not conform to the boundaries of resting connectivity networks. The authors went on to compare connectivity differences between patient groups and healthy controls and found reduced connectivity of the epileptogenic posterior hippocampus to the PCC, accompanied by an increase in connectivity to the parahippocampal gyrus, and—though only in the LTLE group—an increase in connectivity of the anterior hippocampus to the entorhinal cortex. We similarly found that the epileptogenic posterior hippocampus was less strongly connected to the PCC in the TLE groups compared to healthy controls, and in the LTLE
group, we also saw increases in left anterior hippocampal connectivity to the left entorhinal cortex after using a small volume correction. However, we did not observe increases in posterior hippocampal connectivity with the parahippocampus. There were differences in the approach to contrast ing patient and control hippocampal connectivity, with our study using voxel-wise testing compared to the region of interest approach used by Voets et al (2014). In the current study, I imposed a stringent threshold using permutation testing and a p < .01 cluster defining threshold, FDR corrected, given recent evidence that fMRI inference for spatial extent tends to inflate false positive rates (Eklund, Nichols, & Knutsson, 2016), and applied a small volume correction in regions that a priori prediction could be made. However, even at very liberal thresholds, our results did not suggest an increase in posterior hippocampal to parahippocampal connectivity. The disparity in these findings may come from the fact that Voets et al (2014) averaged the posterior hippocampal to parahippocampal gyrus connectivity across the two hemispheres in controls to compare against the patient groups, while in the current study, I did not average across hemispheres for the controls. When examining the data, I find that there is marginally stronger left posterior hippocampus – left parahippocampal gyrus connectivity compared to right posterior hippocampus – right parahippocampal gyrus connectivity in controls, t(18) = 2.07, p = .053. In Voets et al (2014), the authors report the individual connectivity values for each hemisphere and also show that the left hemisphere has higher posterior hippocampus – left parahippocampal gyrus connectivity compared to the right hemisphere in controls, though they state that this difference was not significant. The authors go on to average these values across hemispheres in healthy controls to serve as a comparison to people with TLE. By averaging these hemispheric values together, the authors may have introduced a bias in their comparison with the combined patient groups, as the right hemispheric values may have reduced the estimates of what is ‘normal’ for left posterior hippocampus – left parahippocampal gyrus.

Other than reduced connectivity to the PCC, I also saw reductions in connectivity to mPFC, temporal pole and lateral occipital cortex. In LTLE, the reductions were remarkably overlapping between the anterior and posterior left hippocampus, despite observations from previous research that these subregions have different preferred connectivity patterns (Adnan et al., 2016; Kahn et al., 2008; Voets et al., 2014). However, it is important to note that, while these regions will have connectivity preferences, both will tend to connect with DMN regions in the healthy brain. These alterations in connectivity in this network may be caused by interictal
epileptic discharges observed in TLE which are known to disrupt the functioning of the DMN (Laufs et al., 2007) and may lead to the deterioration seen in the cingulum bundle that connects the MTL to the PCC and mPFC (Liao et al., 2011). Reductions in white matter integrity of the uncinate and arcuate fasciculus have also been noted, which may be caused by seizure spread and may relate to reduced connectivity of MTL regions to frontal and parietal cortex (Lin et al., 2008). This reduced functional connectivity observed in LTLE was also seen quite strongly in the right posterior hippocampus of the RTLE group, but was not significant in the right anterior hippocampus.

Further, I replicated previous findings from our lab, and showed that connectivity of the hippocampus to the PCC is related to memory (McCormick et al., 2013). The PCC is a primary hub of the DMN (Andrews-Hanna et al., 2010), a network which shows strong overlap with autobiographical memory regions (Buckner et al., 2008), and has been implicated in episodic memory in TLE (McCormick et al., 2014). It is also thought to play an important role in information transfer across the entire brain (Bullmore & Sporns, 2012). In the LTLE group, connectivity of the left anterior hippocampus to the PCC was related to verbal memory performance outside of the scanner, on a separate day. This was true only for the subgroup of patients who had left language dominance. In RTLE, the posterior hippocampal connectivity to the PCC was related to visual memory performance in both the left and right hippocampus.

Previous studies have demonstrated differences (and similarities) between the anterior and posterior hippocampus based on preferential involvement of memory processing and connectivity. The anterior hippocampus is thought to be more involved in gist like or coarse grain representations in healthy brains (Gutchess & Schacter, 2012; Kjelstrup et al., 2008; Poppenk et al., 2008), though activity in this region during encoding is predictive of verbal free recall success at later time points (Addis & McAndrews, 2006; Staresina & Davachi, 2006). The posterior hippocampus is thought to contribute to fine grain representations (Kjelstrup et al., 2008; Poppenk & Moscovitch, 2011) and has been suggested to play a privileged role during episodic recall processes rather than encoding (Kim, 2015). Connectivity between the posterior hippocampus and the PCC following an encoding paradigm was shown to be predictive of subsequent recollection (Poppenk & Moscovitch, 2011). While these studies suggest that the hippocampus and PCC are involved in memory processes, they say little about how connectivity of these regions at rest is related to memory ability at an unrelated time. Previous work in TLE,
has shown that the ability to activate the hippocampus during some type of memory task is related to memory performance outside the scanner (Barnett et al., 2015; Powell et al., 2007) and activity of the MTL system during these types of tasks is thought to reflect the integrity or ability of the system. Along the same lines, connectivity of the hippocampus to the PCC at rest may reflect the integrity and potential capabilities of the memory network, as was shown and suggested in McCormick et al (2013).

Many of the limitations of this study are similar to those in Chapter 4. One limitation of is that all patients were taking anti-epileptic drugs during the scanning period and it is difficult to exclude the effect that this may have had on functional connectivity of the brain. However, these people rely on these drugs in their everyday life and thus, the state of their brain connectivity on these drugs is a reflection of their day to day experience. Furthermore, functional connectivity was related to individual variability in memory ability providing us with confidence that this measure is valuable in detecting the integrity of the memory system. Another limitation was that each subjects brain was transformed into standard space prior to \(k\)-means clustering which inherently leads to some signal blurring which could affect the parcellation at cluster boundaries. This step was performed to ensure each \(k\)-means clustering procedure was sampling from the same number of voxels in order to generate group level masks. While some small amount of smoothing may have occurred during transformation to standard space, we did not smooth with a Gaussian kernel inside the hippocampus, and performed minimal smoothing in the rest of the brain. Our results also produced clusters that replicate previous findings in the literature (Voets et al., 2014), including our previous work which performed clustering in native space (Adnan et al., 2016). Finally, we did not separate people that had MTS from those who did not have MTS. Individuals with MTS have more extensive reductions in hippocampal volume (Mumoli et al., 2013), reduced integrity of the fornix (Concha et al., 2009) and more aberrant network segregation (Doucet et al., 2014) than TLE patients without MTS. Unfortunately, given our sample size we were unable to separate those with MTS from those without to examine differences due to lesional status statistically, but we present the data in our scatter plots for visual inspection. There is no striking separation between those with MTS compared to those without in terms of hippocampal to PCC connectivity on the y-axis or with memory performance on the x-axes, but future studies could investigate whether there is a difference between these two groups in the relationship of connectivity with memory ability. This study also did not
examine whether these measures of connectivity were related to post-surgical memory change. Only a small portion of this patient group has had surgery and returned for follow-up neuropsychological evaluation, precluding the possibility for statistical analysis. Future studies should assess whether the connectivity in the anterior or posterior hippocampus to the PCC is related to post-operative memory change as this would help inform clinicians and patients of the risk for cognitive morbidity from the surgery.

5.5 Conclusion

We demonstrated that the hippocampus can be parcellated into an anterior and posterior component based on it functional connectivity fingerprint and this can be done in both healthy adults and in patients with TLE, suggesting that the hippocampus in TLE retains some preferential connectivity along its long axis. We also show that the epileptic hippocampus has reduced connectivity to the PCC, a key hub of the DMN, and that this connectivity is related to material specific memory ability. This suggests that hippocampal to PCC connectivity may be a useful marker for memory network integrity in people suffering from TLE.
Chapter 6
General Discussion

6.1 Overview

Across three studies in this thesis, I have interrogated a rich data set, performing structural MRI and resting state functional MRI analysis, and related these analysis measures to neuropsychological performance, in people with temporal lobe epilepsy (TLE). Critically, this allowed me to explore how the timing of seizure onset impacts cognition, the structure of the brain, and the functional organization of brain networks. For over 80 years, we have known that damage to the brain during youth differs from damage to the brain in adulthood (Kennard, 1936), initially with the discovery that early damage is associated with better recovery. We have since learned that the this relationship is not strictly linear (Kolb et al., 1996) and also depends on the kind of damage or functional domain examined (Kolb et al., 2011). Given that epilepsy is not a one-off event where damage occurs, that seizure activity can spread to remote areas of the brain, and that both memory and general intelligence can be impacted by onset age (Baxendale et al., 1998; Kaaden & Helmstaedter, 2009; Lespinet et al., 2002), I sought to examine how plasticity might affect the brain and cognition in TLE. While previous reports have explored the effects of age of onset on cognition (Baxendale et al., 1998; Kaaden & Helmstaedter, 2009; Lespinet et al., 2002), cortical thickness (Kemmotsu et al., 2011), and resting network properties (Doucet et al., 2014), separately, none have explored the interplay between these measures.

In this general discussion, I will summarize the findings of this thesis, highlight how the key findings are positioned in the broader literature with interpretation and describe the significance of these findings. I will then offer alternative explanations for the overarching results, describe the limitations of the studies performed, suggest future experiments, and provide concluding remarks.

6.2 Summary of findings

In Chapter 3, I investigated the relationship of age of onset with memory ability and cortical thickness in people with left or right TLE. Two hypotheses were tested. The first posited that
early life onset of epilepsy would be related to better memory performance due to adaptive plasticity and reorganization of cognitive networks. The second posited that early life onset of epilepsy would be related to worse memory performance due to hindrance of normal developmental processes by seizure activity and epileptic pathology. I found that early life epilepsy onset was related to worse memory performance and IQ in people with LTLE, though this effect was not strictly linear for memory and started to taper off in early adulthood. Early life onset in LTLE was also related to reduced cortical thickness in left frontal language processing regions, away from healthy control levels, and those individuals who had more normative thickness here, tended to have better verbal memory ability. These results were supportive of the second hypothesis, suggesting that early life epilepsy onset hinders typical developmental processes leading to a failure to achieve normative levels of memory ability. Conversely, in RTLE, I found that early life epilepsy onset was related to better visual memory ability, which is supportive of the first hypothesis that early onset may allow for adaptive plasticity. Interestingly, cortical thickness in the posterior cingulate cortex and superior frontal gyrus was thinner in those individuals who had early life onset, which was aberrant relative to healthy control thickness. Further, thinner cortex in these regions was related to worse visual memory ability. These structural results suggest that earlier onsets are related to detrimental cortical thickness changes and do not align with the behavioural results which led me to suggest that perhaps some functional adaptation could be occurring in early onset RTLE that may salvage performance.

In Chapter 4, I investigated the effects of age of epilepsy onset on brain network topology using graph theory and resting functional connectivity in people with left and right TLE. I hypothesized that people with LTLE would show detrimental network topology associated with earlier onset ages, in line with results from Chapter 3, while people with RTLE would have adaptive network topology associated with earlier onset age that contributed to better visual memory ability. I found that people with LTLE had reduced modularity, normalized clustering and small worldness relative to healthy controls and this was not affected by age of onset. The RTLE group showed no significant difference in global network topology relative to healthy controls, though modularity, normalized clustering and small worldness were all related to age of onset. Earlier ages of onset were related to higher than average modularity and normalized clustering, with maintained normalized path length, leading to increased small worldness. These increased levels of small worldness were related to better visual memory ability, suggesting that,
indeed, earlier onset in RTLE is associated with a beneficial network topology and visual memory. Nodal measures of hubness showed reductions in betweenness centrality in the epileptogenic hippocampus and reductions in degree centrality more specific the posterior epileptic hippocampus in both TLE groups. However, there were no differences in hubness of the PCC and none of the nodal measures from the hippocampus or PCC were modulated by age of epilepsy onset, nor were they related to memory.

In Chapter 5, I sought to extend previous findings from our lab showing that hippocampal – PCC connectivity was related to material specific memory in TLE by interrogating whether this effect was more specific to the anterior or posterior hippocampus and whether this effect was modulated by age of onset. Hippocampal – PCC connectivity could be a very useful tool in evaluating memory network integrity and predicting post-surgical memory change, but if this relationship is modulated by age of onset, it would be informative to know how and to what extent this relationship may differ in patients with earlier or later onsets. I was able to parcellate the hippocampus into an anterior and posterior component in healthy controls and both left and right TLE groups. Group level contrasts showed that both patient groups had reduced connectivity of the epileptic hippocampus to the default mode network (DMN), though this was specific to the posterior hippocampus in people with RTLE. In LTLE, stronger left anterior hippocampal connectivity to the PCC was related to better verbal memory ability while in RTLE, stronger posterior hippocampal connectivity to the PCC was related to better visual memory ability. Neither of these relationships were modulated by age of onset, suggesting that this connection may be useful for characterization of memory network integrity regardless of onset age. However, it is important to note that a true test of clinical usefulness would be to evaluate its ability to predict post-surgical cognitive morbidity.

6.3 Key Findings

One of the main—and unexpected—finding of these studies is that the effects of age of onset appear to be quite different for right and left TLE. Often, researchers have combined people with left and right TLE into the same group when investigating age of onset on memory (Helmstaedter & Elger, 2009; Hermann et al., 2002) and network organization (Doucet et al., 2014). However, a careful examination of the existing literature reveals that seizure laterality does not necessarily affect brain organization in symmetrical ways with some research
suggesting differences in brain structural connectivity (Ahmadi et al., 2009; Besson et al., 2014) and morphology (Kemmotsu et al., 2011) between people with LTLE and RTLE. In LTLE, earlier ages of onset were associated with worse verbal memory, visual memory and IQ, along with reduced cortical thickness in left pars orbitalis, left middle frontal gyrus and left insula. These results are in line with a developmental hindrance hypothesis that has been previously proposed by Kaaden and Helmsteadter (2009) which suggests that epilepsy related processes hinder normal development and that deficits are a result of a failure to acquire certain levels of cognitive ability rather than a loss of ability that was at some point present. Previous research investigating the effects of age of onset on cognition and the brain have found that early onset is associated with higher presence of MTS (Blümcke, Thom, & Wiestler, 2002), worse memory (Lespinet et al., 2002), lower IQ (Kaaden & Helmstaedter, 2009) and reduced whole brain white matter volume (Kaaden et al., 2011), although it should be noted that Kaaden et al. (2011) and Blümcke et al. (2002) did not separate left and right TLE groups. Functional network connectivity measured with graph theory was not related to age of onset in LTLE, nor was hippocampal to PCC connectivity.

Conversely, in RTLE, earlier epilepsy onset was associated with better visual memory and increased small world topology. This increased small world topology was characterized by increased normalized clustering, with retention of low normalized path length suggestive of higher functional specialization and efficient whole network integration. Doucet et al (2014) also demonstrated that earlier onset of epilepsy was associated with higher modularity and clustering, though they did not separate left and right TLE due to limited sample size. They did, however, separate people with MTS from those without and found that age of onset effects were more pronounced in the MTS group. In my investigations of age of onset and network properties, I also had a limited sample size and chose to separate LTLE and RTLE, but not MTS compared to no MTS and in this sense, our studies are complementary. When breaking down the LTLE and RLTE groups in my study into MTS and no MTS, I observed that the MTS subgroups, but not the no MTS subgroups, had a negative correlation between age of onset with modularity and clustering (range: $r = -.36$ – -.64), but statistical power was too low to make any inferences here. These findings are in line with Doucet et al. (2014) who showed a greater age of onset effect in the MTS group compared to the no MTS group.
When looking at the structural alterations related to early age of onset, I observed that there was reduced cortical thickness in superior frontal and the posterior body of the cingulate. Further, thinner cortex in these regions was related to worse visual memory. This was not concordant with the graph theory findings. This disparity suggests that multiple routes of plasticity may be co-occurring and that univariate cortical thickness analysis does not necessarily covary with topological brain organization.

One explanation for the disparity in functional adaptation between left and right TLE may involve the ability of degenerate pathways to sustain verbal versus visual memory processes. Language abilities required for verbal memory may rely on more fixed networks with fewer degenerate pathways. For example, very early life injuries to the left hemisphere language areas often involves reorganization of language functions to peri-lesional tissue (Rasmussen & Milner, 1977), suggesting that very circumscribed regions are capable of handling language processing. Memory for verbal materials likely heavily relies on sufficient output from language machinery. Memory for visual materials, however, may be able to rely on more degenerate pathways, especially since visual forms can be characterized by many dimensions, such as shape, size, colour, and, with some creativity, verbalization. Thus, if particular pathways are affected by pathological processes, a broader set of regions may be called on for reorganization. This may explain why the reorganization of topological network segregation seen in early onset RTLE was related to better visual memory.

Another key finding is that age of onset effects on cognition and the brain may not necessarily be linear. By comparing people with early onset versus late onset based on a cut-off age, researchers may miss non-linear effects and may miss differences all together depending on how the cut-off is chosen, which, at times, have been rather arbitrary. The effect of age of onset on cortical thickness showed that childhood onset was associated with thinner cortex in certain areas, with individuals having an adolescent or young adult onset showing thicker cortex. This trend, however levelled off or reversed for onset ages in middle adulthood. In the current sample, this effect may have been missed if early and late onset were determined by a simple median split given that the median age of onset was around 18 years old. Given that the cerebral cortex does not develop in a linear or homosynchronous manner (Gogtay et al., 2004; Shaw et al., 2008), and that memory and cognition over the lifespan do not follow a linear trajectory (Craik & Bialystok, 2006; Helmstaedter & Elger, 2009), analysis of age of onset effects should not assume linearity.
My results also speak to the widespread nature of brain alterations in TLE. In the introduction, I noted that there are distributed alterations in grey matter, white matter, and network integrity. The results I present here also demonstrate widespread reductions in cortical thickness across all lobes of the brain, with particularly strong effects in the parietal and frontal lobes. Of note, the cortical thickness reductions that were associated with early epilepsy onset were not in temporal lobe regions. Rather, they were more focussed in the frontal lobes and parietal lobes. This may be due to the failure to expand white matter in these regions, that is seen in childhood onset epilepsy (Hermann et al., 2010). Along with these structural alterations, I demonstrated reductions in small world network organization in LTLE and reduced hippocampal integration with the DMN in both LTLE and RTLE. Not only do these widespread alterations exist, but they have functional consequences for cognition, despite being distal to the site of seizure generation and lesion. While it is unsurprising that damage and dysfunction in the hippocampus is related to memory impairments, it is quite interesting that whole brain network topology can relate to memory. Previous studies have found that whole brain network measures such as low characteristic path length (Y. Li et al., 2009; van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009; Zalesky et al., 2011) and higher clustering coefficient (Zalesky et al., 2011) relate to higher order cognitive abilities like IQ. Similarly, work from our group has shown that connectivity patterns among DMN regions is related to episodic memory in people with LTLE and RTLE (McCormick et al., 2014). These findings all speak to the distributed nature of higher cognition and memory in the brain and for the need to take a network/connectivity approach to understanding memory ability in people with TLE.

6.4 Alternative Explanations

There are several factors that are linked with age of epilepsy onset. Later onsets are significantly correlated with older ages in these studies and childhood onset is typically associated with longer durations, considering these investigations focussed on adult patients. Great care was taken to statistically control for these variables and explore their potential effect on memory and brain structure and function. Age is known to relate to episodic memory ability (Scott & Small, 2001) and cortical thickness (Storsve et al., 2014; Tamnes et al., 2010) with increasing duration also linked with cortical thinning (Bernhardt et al., 2009), and white matter alteration (Riley et al.,
In the series of studies presented here, the findings related to age of onset were statistically controlled for the influence of these confounding variables, where appropriate. While this may not be able to completely eliminate the influences of all the potential confounds, it should provide a reasonable estimation of the independent impact of age of onset.

As was mentioned in the Chapter 3 and Chapter 4 discussion, earlier epilepsy onset is also often associated with earlier and, possibly, longer courses of anti-epilepsy drug (AED) use. The relative contribution of earlier epilepsy onset compared with earlier AED use are unknown and there is considerable heterogeneity of AED type in our samples of TLE that limit our ability to examine their effects. As such, I cannot exclude the possibility that long term and early life AED use does not contribute to some of the age of onset effects seen. Specific AEDs, such as topiramate and zonisamide, for example, reduce language-related task activation in fMRI (Wandschneider et al., 2017), but the effect of AEDs on resting network properties has not yet been determined. However, memory ability, brain structure, and brain dynamics during AED use reflect the reality for these patients in everyday life and thus, I have presented, here, valid descriptions of the alterations that occur in TLE with standard drug treatment.

Another explanation for the effects of age of onset that were reported here, is that the differences seen in earlier age of onset, existed at the time of epilepsy onset. It is possible that, rather than adaptive plasticity processes or hindered development, the differences in early onset memory and brain structure/function existed at or even before the time of unprovoked seizure emergence. The findings I presented in this dissertation are correlational and cross-sectional in nature and thus, directionality cannot be inferred, but, to better unpack these explanations, I will review the literature examining differences between pediatric populations of TLE and typically developing children. The studies investigating functional connectivity have shown alterations in the anterior DMN characterized by reduced connectivity in frontal and lateral temporal regions (Mankinen et al., 2012), as well as reduced intranetwork connectivity of the DMN and salience network coupled with increased internetwork connectivity between the DMN and salience network (Ibrahim et al., 2014) in children with TLE compared to typical developing children. These results suggest that even in childhood there are some connectivity alterations in TLE, though, right and left TLE groups were not separated. These individuals were also not exclusively recent onset cases. Whether, over time, these alterations exacerbate and lead to impairments, or whether these alterations lead to some reorganization of function and spared cognitive ability is unknown,
but my findings suggest that in RTLE, those with an early onset will have better memory and beneficial network organization compared to those with a later onset. Further, hippocampal alterations are not reliably found at the time of initial diagnosis in children (Salmenperä et al., 2005), the constellation of grey matter alterations seen in adult TLE patients are relatively minimal in children with TLE (Guimarães et al., 2007), and material-specific memory impairment is less common (Gonzalez, Anderson, Wood, Mitchell, & Harvey, 2007). Longitudinal studies are needed to fully understand the plastic processes that occur during development in children with TLE.

6.4.1 Limitations

Several limitations potentially impact the findings presented in this dissertation. For example, I have attempted to control for the cumulative effect of epilepsy (such as lifetime number of seizures) by investigating the role of epilepsy duration on my dependent variables. However, duration, at best, serves only as a proxy for lifetime number of seizures since seizure frequency can vary substantially patient to patient. Further, the severity of seizures is also not taken into account in my investigations. Approximations of seizure frequency, severity and lifetime number of seizures are difficult to assess and thought to be somewhat unreliable since people can have seizures in the absence of awareness (Hoppe et al., 2007). Further, the people seen in our clinic do not keep comprehensive records such as seizure diaries, and so our clinical histories record, at best, general estimates of frequency at a given point in time. However, these variables have not been found to systematically co-vary with age of onset in a study that did attempt to quantify these variables using medical chart reviews and patient self-reports (Szaflarski, Meckler, Privitera, & Szaflarski, 2006). Given that these variables are not suspected to systematically co-vary across my primary independent variable, individual variability on these measures likely exists in model error terms.

Another limitation of this work is that many of the analysis techniques I used produce measures that heavily rely on analysis choices. Cortical thickness values extracted for given regions are dependent my use of the Desikan-Killany atlas. Use of this atlas removed spatial specificity for effects that could occur in a subregion of the atlas-defined cortical labels or effects that may span the boundaries of cortical labels. However, this atlas provides familiar and widely available
labels that can be used by other groups and allowed for fewer multiple comparison corrections compared to a vertex-wise approach that can be performed with cortical thickness meshes.

Along similar lines, the metrics produced by graph theory analysis are dependent on the definition of a parcellation scheme (Fornito, Zalesky, & Bullmore, 2010; Jinhui Wang et al., 2009; Zalesky, Fornito, Harding, et al., 2010). An ideal parcellation scheme should distinguish and group brain regions based on “coherent patterns of extrinsic anatomical or functional connections” (Rubinov & Sporns, 2010). That is to say, voxels within a region should have homogeneous connectivity to other regions in the network. In the graph theory analysis presented in Chapter 4, the Brainnetome atlas was used which has anatomically meaningful regions, parcellated to optimize homogeneity of connectivity among clusters (Fan et al., 2016). However, the findings and specific values of the metrics reported here will not likely generalize to other studies which may have used coarser parcellation schemes such as the automatically anatomically labelled atlas (Tzourio-Mazoyer et al., 2002) which has fewer defined regions and different region boundaries. The exact numbers for the metrics, however are less important than the individual variability that is associated with the metrics. If the individual variability that is associated with the metrics is preserved, then it is still viable to compare these metrics between groups and examine across subject relationships with covariates such as age of onset. Fornito, Zalesky and Bullmore (2010) investigated whether the number of nodes in a parcellation scheme altered the inter-individual variability of graph theory metrics and found that consistency of individual variability could be seen at parcellation schemes of ~200 nodes and up. Thus, given that the Brainnetome atlas has 246 nodes, I feel confident that the individual variability in graph theory metrics found here reflect actual variability, that would be stable across different parcellation schemes, rather than inconsistent variation that can occur across coarser atlases.

Lastly, as mentioned, the results presented here are cross-sectional and are unable to speak to the trajectory of developmental hindrance or adaptive plasticity. My results present a clear relationship between age of onset and cortical thickness, memory and network properties, but only in conjunction with other findings in the literature can these relationships can be seen as supporting theories regarding brain organization and development. Directionality of these effects, cannot be directly observed in these data and, ultimately, longitudinal examinations will be necessary to fully explore memory, cortical thickness and network trajectories in people with TLE.
6.5 Future Directions

One interesting avenue for exploring the relationship of age of onset and the brain would be to examine white matter structure via diffusion imaging. In the introduction, I mentioned that there are considerable white matter alterations in people with TLE (Concha et al., 2009; Focke et al., 2008; Riley et al., 2010), and while one study was able to a relationship between age of onset and white matter volume (Hermann et al., 2002), few have used DTI measures in understanding these effects (Kemmotsu et al., 2011). Furthermore, plastic processes (adaptive or pathological) may involve an interplay of grey matter structure, white matter structure and network organization. Large consortiums to collect and store multicentre neuroimaging data in accessible databases could allow for the sharing of data, leading to large sample sizes and examination of effects over different imaging modalities. These databases exist for other disorders such as Alzheimer’s Disease and Parkinson’s disease, but are limited for TLE.

The altered network properties seen in TLE also open up questions about how large scale cognitive networks interact in TLE. In Chapter 4, I observed that people with TLE had reduced connectivity within the DMN nodes and increased connectivity between the somatomotor network with the DMN, frontoparietal network and visual network. During task performance, it has been shown that deactivation of task-negative networks is associated with healthy brain organization (Pihlajamäki & Sperling, 2009), and task driven coupling/decoupling of networks supports goal-directed cognitive processes (Spreng, Stevens, Chamberlain, Gilmore, & Schacter, 2010). Thus, an interesting avenue of future research, may be to investigate whether network coupling and decoupling is altered in TLE under differing task demands. For example, in Spreng et al (2010) participants were asked to perform either an autobiographical retrieval (internally directed) or complete a visuospatial planning task (directed to the external world). During the internally directed task, the frontoparietal control network coupled with the DMN, and decoupled with the dorsal attention network. During the externally directed task, the frontoparietal control network showed the reverse coupling. Given that network modularity was deficient in people with LTLE, future research should examine whether task dependent network coupling is altered in this population and how that dynamic coupling may affect cognitive performance.
Additionally, my findings have been macroscopic in nature, relying solely on MRI for imaging. It has been suggested that alterations in cortical thickness are due to neuronal loss—from either excitotoxic effects of seizure spread or from loss of neuronal input (Mueller et al., 2009) — but these effects are difficult to characterize in living humans with standard clinical MRI. Histological findings in hippocampal resections may also lend microscopic insight while functional recordings with faster time scales such as EEG, MEG and intracranial recordings may provide new ways of exploring age of onset effects. Early onset ages have been associated with altered hippocampal architecture in the dentate gyrus (Blümcke et al., 2002), but the effects of this altered architecture on memory ability has not been explored. In rodents, there have been reports of microscopic changes in terms of altered dendritic structure following plastic processes that are modulated by age of onset (Kolb, 1999) and these are also affected by exposure to enriched environments and exercise. This also raises the questions as to whether there are particular factors in TLE that might bias people towards adaptive plasticity and sparing of cognitive abilities. This was also not explored in this thesis, but would be an interesting avenue for future research.

Finally, a primary goal of clinical neuroimaging is to predict post-operative cognitive morbidity. Several key neuroimaging indicators have emerged to serve this purposes, including assessment of hippocampal sclerosis via neuroradiological assessment, hippocampal activation levels during an fMRI memory task (Bonelli et al., 2010), and functional connectivity (Doucet et al., 2015; McCormick et al., 2013). Functional connectivity is still relatively new and since graph theory metrics appear to be influenced by age of epilepsy onset, demonstrated by findings in this dissertation and in Doucet et al (2014), it may prove important to determine whether onset age has a modulating effect on post-operative cognitive morbidity.

### 6.6 Conclusions

In this dissertation I have shown that age of onset is related to memory ability, brain structure, brain network organization and that these relationships are not the same in both LTLE and RTLE. Earlier onset ages in childhood in LTLE are related to lower verbal memory, visual memory and IQ along with reduced cortical thickness in frontal language regions. Across the sample of LTLE, those with thinner cortex in these language regions have poorer verbal memory
ability, suggesting that early onset is associated with some detrimental processes that may hinder development. Earlier onset ages in RTLE are associated with better visual memory and increased small-world network topology and network segregation. This increased small world topology and segregation in turn is related to better visual memory, suggesting that early onset in RTLE is associated with some adaptive network organization relative to those with a later onset. These findings highlight the heterogeneity that exists between left and right TLE and generate questions regarding why age of onset has such disparate effects between these two groups. These findings also may have clinical implications regarding whether age of epilepsy onset modulates the use of connectivity derived metrics in predicting post-surgical cognitive morbidity. Finally, these findings promote the approach of treating age of onset as a continuous variable, that may be modelled linearly or non-linearly, so that researchers can view the relationships revealed by the data with fewer assumptions and arbitrary groupings.
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112


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