Memory functioning in patients with unilateral temporal lobe epilepsy: Neuroimaging indicators of functional integrity in the hippocampus and beyond.

by

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A thesis submitted in conformity with the requirements for the degree of Master of Arts

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Abstract

Temporal lobe epilepsy (TLE) is a common form of intractable epilepsy that can be treated with surgical resection of the epileptogenic medial temporal lobe tissue, specifically the hippocampus. This resection can lead to a variable degree of memory deficit and considerable research has been directed at identifying predictors of these deficits. This thesis explores the relationship between structural predictors and functional predictors in TLE. I looked at fMRI activation asymmetry produced by a scene encoding task as well as volume asymmetry ratios within the hippocampus and the relationship of these predictors to memory performance in patients with TLE. Mediation analysis was performed according to Baron and Kenny (1986) and showed that fMRI activation asymmetry mediated the relationship between volume asymmetry and memory asymmetry in patients with TLE. This suggests that activation asymmetry may be a preferred variable for assessing functional adequacy in the medial temporal region.
Acknowledgments

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Table of Contents

Chapter 1: Introduction 1
  1 Predictors of Decline 3
    1.1 Neuropsychological Testing 3
    1.2 Neuroimaging 4
    1.3 Multimodal 6

Chapter 2: Methods 10
  1 Participants 10
  2 Neuropsychological Testing 12
  3 fMRI Data Acquisition 12
  4 fMRI Task 13
  5 Functional Data Processing 13
  6 Data Analysis 14
  7 Structural Data Analysis 16
  8 Group Comparison 16
  9 Neuroimaging Measures and Behaviour 17

Chapter 3: Results 19
  1 Demographics 19
  2 Task Activation 19
  3 Asymmetry Ratios 22
  4 Neuroimaging Measures and Behaviour 22

Chapter 4: Discussion 26
List of Tables

Table 1  ..............................................  11
Table 2  ..............................................  24
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>15</td>
</tr>
<tr>
<td>Figure 2</td>
<td>18</td>
</tr>
<tr>
<td>Figure 3</td>
<td>20</td>
</tr>
<tr>
<td>Figure 4</td>
<td>21</td>
</tr>
<tr>
<td>Figure 5</td>
<td>23</td>
</tr>
<tr>
<td>Figure 6</td>
<td>25</td>
</tr>
<tr>
<td>Figure 7</td>
<td>27</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

Epilepsy is a prevalent neurological disorder. In 2001 the World Health Organization estimated that approximately 50 million people worldwide were affected by epilepsy (Levav & Rutz, 2002). Temporal lobe epilepsy (TLE) specifically is one of the most common presentations of this disorder (Bell, Lin, Seidenberg, & Hermann, 2011). Epilepsy is characterized by recurrent seizures, but also involves a profile of cognitive deficits, the most apparent being memory. Medication is prescribed to reduce the frequency of seizures, but within this population can sometimes be ineffective (Kwan & Brodie, 2000). Surgical interventions have been able to successfully eliminate or greatly reduce the occurrence of seizures in patients with TLE (Engel, 1993; Engel et al., 2003). This surgery involves the resection of the varying amounts of anterior portion of the hippocampus and the amygdala. Despite the overwhelming success of these surgical procedures, post-surgical memory declines are very common (Gleissner, Helmstaedter, Schramm, & Elger, 2004; Hermann, Seidenberg, Haltiner, & Wyler, 1995; Martin et al., 1998). The canonical example of this effect is the case of Henry Molaison, who was unable to form any new long term memory following bilateral resection of the anterior hippocampus (Scoville & Milner, 1957). Since surgical treatment involves the resection of critical memory structures, particularly the hippocampus, post-surgical memory decline is highly prevalent and variable (Bell & Davies, 1998; Chelune, 1995). The case of Henry Molaison launched an extensive body of research investigating the relationship between the hippocampus and memory as well the investigation of predictors of post-surgical memory change.

As part of standard procedure in epilepsy surgery centres, all patients undergo an extensive battery of neuropsychological testing pre- and post-surgically which allows clinicians to understand the specific deficits of this patient population. This battery of testing also provides
Anatomical pathology has also been characterized within this population by examining excised and post-mortem hippocampi taken from patients with TLE. Patients with TLE are very likely to show signs of hippocampal sclerosis (HS), which is characterized by cell loss and synaptic reorganization in the hippocampus and is the most common lesion in patients with TLE (Blümcke, Thom, & Wiestler, 2002; Shamim et al., 2004). This lesion has been shown to be related to memory deficits, with HS associated with poorer memory scores (Hermann et al.,
1992; Kneebone, Lee, Wade, & Loring, 2007; Marques et al., 2007; Saling et al., 1993; Trencerry et al., 1993) and, with the advent of MRI, HS is typically diagnosed pre-operatively (Berkovic et al., 1991). Of importance, structural neuroimaging studies using MRI for region of interest (ROI) volumetry and voxel-based morphometry have shown atrophy not only in the hippocampus, but also in a broader extent of medial temporal lobe including parahippocampal gyrus, fornix, amygdala and entorhinal cortex (Bernasconi, 2003; Bernasconi et al., 1999, 2001; Kuzniecky et al., 1999). The literature focuses heavily upon hippocampal changes in patients with TLE, but there are clearly structural differences that occur in the MTL.

1 Predictors of Memory Decline

1.1 Neuropsychological Testing

Since the variability of memory decline is so high, researchers and clinicians have focused on identifying factors that affect or predict the degree of memory decline due to surgery (Bell et al., 2011). One of the first trends noted was that there is an inverse relationship between preoperative memory functioning and postoperative memory change, with higher pre-operative scores associated with greater post-operative declines (Chelune, Naugle, Lüders, & Awad, 1991; Elshorst et al., 2009; Helmstaedter & Elger, 1996; Hermann et al., 1995). Chelune (1995) looked at a sample of 181 patients with TLE and observed that higher verbal memory presurgically on the Wechsler Memory Scale Revised (Wechsler, 1987) resulted in greater postsurgical decline of verbal memory in patients LTLE. They also found that higher visual memory presurgically resulted in greater postsurgical decline of visual memory in patients with RTLE. Chelune (1995) explains this by reasoning that if a patient has high presurgical functioning they may have a greater amount of functioning to lose after surgical resection.
1.2 Neuroimaging

Neuroimaging advances provide additional opportunities for predictive markers of post-surgical memory decline. Structural MRI has allowed us to examine hippocampal atrophy. As discussed earlier, hippocampal sclerosis has been shown to relate to cognitive deficits (Hermann et al., 1992; Marques et al., 2007; Saling et al., 1993) and thus MRI measures of presurgical structural integrity of the hippocampus may relate to the post-surgical outcome of memory. Several studies have linked hippocampal volume in patients with TLE to post-surgical memory change (Kneebone et al., 2007; Martin et al., 2001; Martin et al., 2002; Mechanic-Hamilton et al., 2009). The results indicate that larger ipsilateral volumes, indicating a higher degree of intact tissue, are related to greater declines in memory post-surgery. These results are often best seen using asymmetry ratios (AR) \([\text{contralateral volume} - \text{ipsilateral volume}] / [\text{contralateral volume} + \text{ipsilateral volume}]\) since ARs tend to provide a greater spread separating patients with LTLE and RTLE (Mechanic-Hamilton et al., 2009). The understanding here is that if the epileptogenic hippocampus has little atrophy then it is more likely to support memory processes as it does in the healthy brain. Therefore, removal of a more intact and useful hippocampal piece will result in greater decline.

Functional MRI (fMRI) has allowed researchers to examine the functional integrity of the to-be-resected tissue. Studies have attempted to use memory tasks during fMRI to allow for quantification of hippocampal activity. Analysis of this activity, which is taken as a measure of functional integrity, has been linked with memory changes following surgery (Bonelli et al., 2010; Mechanic-Hamilton et al., 2009; Rabin et al., 2004; Richardson et al., 2004; Richardson, Strange, Duncan, & Dolan, 2006). Rabin et al. (2004) examined 35 unilateral TLE patients (20 RTLE, 15 LTLE) and 30 healthy controls. This group used a scene encoding task (Stern et al., 1996) to elicit medial temporal lobe activation in participants. All subjects were shown a series
of novel visual scenes in block design with a control scene that was degraded and retiled. Contrasts between novel scenes and the control block were created for each individual and activation was extracted from the hippocampus in the left and right hemispheres. Activation was used to make an asymmetry ratio using the formula \([L_{activity} - R_{activity}]/(L_{activity} + R_{activity})\). This method was also used for a larger ROI encompassing the hippocampus, parahippocampus and fusiform (HPF ROI). The ARs created from the larger HPF ROI were shown to significantly correlate with post-surgical change in visual memory in patients with RTLE such that patients having more right activation compared to left activation showed greater post-surgical decline. The authors did not find significant correlations in their LTLE patients however and attribute this to the scene encoding potentially being more sensitive to right hemisphere dysfunction.

Richardson et al. (2004) examined 10 LTLE patients presurgically on a verbal encoding task in which patients were presented with a list of words and asked to make “living” or “nonliving” judgments on the words, though not asked to memorize the words in the scanner. Following the scan patients were given a surprise memory test on the words and asked to make recognition judgments on the words. This judgment involved subjects giving a recollection response (R), a familiarity response (K), or a new response (N) as described by Tulving (1985). Recognition responses that are associated with recollection responses specifically activate the hippocampus compared to recognition responses associated with familiarity (Eldridge, Knowlton, Furmanski, Bookheimer, & Engel, 2000). They then contrasted the words for each subject labeled correctly as R against the words labeled correctly as K. Activation from this contrast was then used to create “encoding asymmetry” indices for each subject by subtracting left activation from right activation using a small volume correction on the hippocampus. The researchers then were able to find that this presurgical encoding asymmetry was able to significantly predict post-surgical memory decline. They found that the greater the activation in
the left compared to the right hippocampus was linked with larger declines in memory following surgical resection of the anterior portion of the left hippocampus in these patients.

Much of the data relating presurgical measures to predicting memory outcome support a model called the functional adequacy theory. This theory posits that the amount of memory decline seen in patients with TLE postsurgery is dependent upon the presurgical integrity of the to-be-resected tissue. If the tissue is intact, and functionally involved in memory processes, then removal of this tissue will presumably cause a larger reduction in memory functioning than if the tissue was atrophied and not functionally involved in memory (Chelune, 1995).

### 1.3 Multimodal
Several studies have attempted to use multimodal methods for predicting post-surgical memory change in patients with TLE. Elshorst et al. (2009) looked at presurgical neuropsychological memory scores, MRI grading of hippocampal integrity and WADA memory performance as predictors for post-surgical memory change in 59 patients with LTLE. They used the California Verbal Learning Test (CVLT) and the Rey Auditory Verbal Learning Test (RAVLT) as determinants of memory change and as predictors. They also used a ranking for MTS which was based on a combination of anatomical scans to identify size (T1-weighted) and MR signal increase (T2-weighted scans) which are indicators of neural atrophy. Finally, patients underwent an intracarotid amytal procedure (IAP) in which one hemisphere of the brain is anesthetized while the other hemisphere is given a memory task. This is used to identify the ability of one hemisphere without the assistance of the anesthetized hemisphere. Each patient was shown a series of 18 objects to remember on a subsequent recognition test that took place after clearance of the drug that include the original 18 objects with 36 lures. Scores for each hemisphere were determined in a pass/fail manner whereby the patient had to recognize at least 60% of the items encoded at optimal anesthetization. Elshorst et al. (2009) found that presurgical
neuropsychological memory best predicted post-surgical memory outcome along with MRI classification of hippocampal atrophy to explain around 45% of the variance in memory change. In contrast, memory as assessed via the IAP did no add any predictive power to explaining post-surgical memory outcome.

Mechanic-Hamilton et al. (2009) looked at the relationship between neuropsychological functioning and WADA with fMRI as well as examining (in a separate analysis) the predictive power of hippocampal volume and fMRI activation with memory outcome following surgery. The study examined 49 patients with TLE (21 LTLE, 24 RTLE, 4 bilateral) and 25 controls. Participants performed a visual scene encoding task while in the MRI scanner. Scenes were presented in a block design fashion, alternating a series of novel scenes in the encoding blocks with a single randomly retiled scene in the control blocks. Participants performed a self-paced forced choice recognition test following the scan. After surgery, the majority of patients returned to perform an alternative version of the visual scene encoding task outside the scanner and a pre-to post-recognition change score was calculated. Activation was calculated by contrasting novel blocks against the control block. ROI analysis yielded voxel counts for the hippocampus and a mask that included the hippocampus, parahippocampus cortex and fusiform gyrus (HPF). The resultant voxel counts were used to create ARs using the formula \((\text{voxelcount}_{\text{Contra}} - \text{voxelcount}_{\text{Ipsi}}) / (\text{voxelcount}_{\text{Contra}} + \text{voxelcount}_{\text{Ipsi}})\). These ARs were used as predictors for changes in clinical neuropsychological measures from pre- to post-surgery. Mechanic-Hamilton et al. (2009) also used signal change within the ROIs as predictors of changes in clinical neuropsychological measures.

Hippocampal volumes were calculated by manual tracing using published guidelines (Watson et al., 1992). Volumes were also used to create ARs. In addition to examining changes on the scene task, the study included pre- and post-operative measures from clinical memory
tests. For verbal memory, tasks included the CVLT and the Logical Memory subtest of the WMS-III as measures of verbal memory. To measure visuospatial memory, they used the Faces and visual reproduction subtests of the WMS-III. They calculated change scores for each subtest by subtracting presurgical scores from postsurgical scores.

This study demonstrated a correlation between fMRI activation in the hippocampus in the left hemisphere of LTLE patients and memory change determined by scene recognition. Activation ARs did not however relate to changes in neuropsychological measures of memory following surgery in either patients with LTLE or those with RTLE. Volume ARs were able to correlate with several of the neuropsychological measures of memory following surgical resection. Interestingly and in accord with Elshorst et al (2009), the strongest predictor of post-surgical memory change was pre-surgical clinical memory scores.

Although these results provide important information regarding predictive values of preoperative functional and structural measures, the authors did not directly explore a relationship between functional ARs and volumetric ARs. If volumetric ARs are presumed to provide us with some index of structural integrity we would expect that this would reflect the functionality of the tissue, whether it be that ipsilateral atrophy results in hypo-activation of the afflicted tissue or that ipsilateral atrophy results in a hyper-activation of the afflicted tissue for compensation. Indeed, there are important reasons to expect that there may be a mediating relationship between functional and structural integrity such that by understanding how much tissue is available, and how efficiently the tissue is being used, we can better understand the link between the brain and memory. In the literature on functional neuroimaging in neurodegenerative conditions, Dickerson et al. (2004) examined the relationship between clinical impairment and functional activation in individuals with Mild Cognitive Impairment (MCI). They found that greater impairment (patients with ‘severe’ rather than ‘milder’ symptoms; all of
them non-demented by clinical criteria) was linked with increased MTL activation. Furthermore, those individuals who activated a larger extent of the MTL showed accelerated cognitive decline. In a later study the investigators reported that older adults with MCI showed greater MTL activation compared to controls, whereas those with Alzheimer’s Disease showed significantly less MTL activation (Dickerson et al., 2005). This evidence was taken to demonstrate that increased activation may be a compensatory mechanism or might reflect a pathophysiological mechanism leading towards Alzheimer’s disease (AD). The authors suggested that there may be a transitional continuum between normal aging and AD in which individuals with early MCI show increased activation compared to controls, but as damage accrues in the MTL resulting in more severe deficits, MTL activation also decreases. Such a relationship between volume, activation and impairment may exist in TLE and is the driving force behind this study. We, however, are not looking for a transitional continuum between healthy brain function and epilepsy since our patient population has intractable epilepsy. Rather, we are looking at the continuum between patients that have milder deficits to severe deficits in memory domains.

In summary, this study intends to build off of past research to better understand how neuroimaging measures relate to memory in TLE. As discussed earlier, several studies have shown that patients with TLE tend to have decreased volume in medial temporal lobe regions beyond the hippocampus, including parahippocampal gyrus, fornix, amygdala and entorhinal cortex (Bernasconi et al., 2003; Kuzniecky et al., 1999). Therefore, this thesis will attempt to look at activation and volume, not solely in the hippocampus but also the parahippocampal gyrus. Not only will this thesis look at an extended view of the MTL, but also it will examine both volume and functional activation and how they relate to memory in this clinical population. With this in mind we will employ the use of a scene encoding task which has been shown to produce robust activation in the hippocampus and parahippocampal gyrus (Stern et al., 1996).
within the MRI scanner to allow for visualization of MTL functionality. We hypothesize that the functional integrity, as measured by weighted voxel count in our ROIs, will correlate significantly with presurgical neuropsychological measures of memory. We also hypothesize that volume measures for the hippocampus and parahippocampal gyrus will correlate with presurgical neuropsychological measures of memory. Finally, this thesis proposes that measures of MTL activation mediate the relationship between volume and memory. In the current study we are using presurgical memory performance so that we may examine the relationship between volume and activation with memory when the MTL is intact. The implications of understanding the relationship between functional activation and volume extend beyond TLE and may help explain phenomenon that occur other disease populations with altered hippocampal functioning such as MCI, AD, or depression.

Chapter 2
Methods

1 Participants
Twenty three patients with pharmacologically intractable unilateral TLE were recruited from the Neuropsychology and Epilepsy Surgery program at Toronto Western Hospital. Thirteen presented with RTLE (5 men, 7 women; mean age = 35.5 years, range = 22-58 years) and 11 presented with LTLE (4 men, 7 women; mean age = 36 years, range = 19-53 years). Seizure focus was determined using scalp EEG, and (if necessary) intracranial EEG. Twelve controls were also recruited (8 men, 4 women; mean age = 29.4 years, range = 23-38). Exclusion criteria included any history of neurological disorders or severe head trauma. Consent was obtained from all participants. A list of demographics can be found in Table 1.
Table 1 Group Demographics, range in brackets

<table>
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<th>Controls</th>
<th>RTLE</th>
<th>LTLE</th>
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<tr>
<td>Age (years)</td>
<td>29.4 (23-38)</td>
<td>35.5 (22-58)</td>
<td>36 (19-53)</td>
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<tr>
<td>Years of education</td>
<td>17.9 (10-26)</td>
<td>14.4 (8-20)</td>
<td>14.6 (10-20)</td>
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<td>Gender</td>
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<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Seizure Duration</td>
<td>--</td>
<td>16.2 (4-39)</td>
<td>18.4 (3-32)</td>
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</table>
2  Neuropsychological Testing

Patients were administered a standard neuropsychological battery as part of the clinic’s standard pre-operative investigation which included several tests assessing learning/memory. These include verbal and visual long term memory tests. Two verbal tests were available and included the Rey Auditory Verbal Learning Test (RAVLT) and Warrington Recognition Memory Test for words. The visual long term memory tests included the Rey Visual Design Learning Test (RVDLT) and Warrington Recognition Memory Test for faces. These measures were used to create behavioural ARs according to the formula \[ \left( \frac{\text{Verbal memory} - \text{Visual memory}}{\text{Verbal memory} + \text{Visual memory}} \right) \]. One behavioural AR was calculated using scores from the RAVLT and the RVDLT (REY-AR) and one was calculated using scores from the Warrington memory test for words and the Warrington memory test for faces (WARR-AR). These tests were used to examine the relationship of recall (via REY-AR) and recognition (via WARR-AR) on other neuroimaging measurements. Both are clinically relevant, but there is no consensus on which may be superior for characterizing hippocampal function (McAndrews & Cohn, 2012).

3  fMRI Data Acquisition

Data was collected on a 3-T Signa MR system (GE Medical Systems, Milwaukee WI). A high-resolution 3D anatomic scan was collected first for visualization, normalization of fMRI data to a common anatomic template and volumetric analysis (T1-weighted sequence, FOV 220mm, 146 slices, flip angle=12°, TE=3ms, TR=8ms, 256 x 256 matrix, resulting in voxel size of .85939 x .85939 x 1.0). Echo-Planar Imaging sequences (TE=20 ms, TR=2000 msec, 32 5mm oblique slices angled to be orthogonal to the long axis of the hippocampus to maximize signal and minimize partial volume effects in the MTL) were run during the 6 ½ min functional scan.
4  fMRI Task

Participants performed a commonly used scene encoding task during an fMRI scan. While in the scanner they passively viewed a series of 60 complex visual scenes for 3500ms each. Two of these visual scenes were exposed repeatedly immediately prior to the fMRI run and were presented 15 times each during the critical run to serve as ‘baseline’ trials while the other 30 were novel. Presentation order of scenes was randomized for each subject. Previous literature suggests that hippocampal activation will be seen for the novel>repeated contrast (Stern et al., 1996).

5  Functional Data Preprocessing

Preprocessing was done using SPM8 (Statistical Parameter Mapping 8; Wellcome Department of Imaging, London) run through Matlab 7.9 (Mathworks, Inc). The first three images of the functional scan were dropped to remove acquisition artifact. Preprocessing first included realignment of anatomical and functional scans to the anterior commissure and then co-registration of the functional scans to the anatomical scan. The functional scans were then realigned and unwarped to correct to scanner motion artifact and subject movement. The anatomical scan was segmented into gray matter, white matter and cerebral spinal fluid. The resulting parameter files of this segmentation were then used to normalize functional and anatomical scans into MNI space using affine registration. Finally, the functional images were smoothed using a full-width at half maximum Gaussian kernel to 8 x 8 x 8 mm resolution. Data went through a high-pass filter to account for low-frequency drift. Each stimulus event was then modeled by SPM8’s canonical haemodynamic response function.
6 Data Analysis

Novel encoding scenes were contrasted against repeated events using a general linear model (GLM) in SPM8 for each subject. The resulting contrast images were used for group level analyses to compare LTLE vs. controls, RTLE vs. controls and LTLE vs. RTLE. Healthy individuals were used as a positive control to ensure implementation of the scene encoding paradigm was successful. Group differences between healthy controls and our patient groups were performed to see global changes between healthy and disease state.

ROI masks derived from MARINA (Walter et al., 2003) were applied to each patient’s contrast to find voxel counts and t-values at a threshold of $p<0.15$, uncorrected. This liberal threshold was chosen to allow for an inclusive representation of activated voxels, while excluding those that are very likely to be due to chance. The application of low thresholding for this purpose has been applied in the past (Branco et al., 2006; Mechanic-Hamilton et al., 2009). Individual masks were made for left and right hippocampus and left and right parahippocampus, as shown in Figure 1. Voxel counts were weighted by their t-scores using these masks. Weighted voxel counts have been shown to provide a better measure than voxel count alone (Chlebus et al., 2007). These weighted voxel counts (WVC) were used to create activation ARs using the formula $[(\text{Left WVC} - \text{Right WVC}) / (\text{Left WVC} + \text{Right WVC})]$. Larger activation ARs indicate greater activity in the left ROIs compared to right ROIs. An AR of zero indicates equal activity bilaterally in the ROIs and negative ARs indicate greater activity in the right ROIs. This resulted in two activation ARs for each patient (one for the hippocampus and one for the parahippocampus). If there was no activation above threshold in either the left or right ROI, then that data point was discarded for that patient. At the $p<0.15$ threshold there was 5 discarded (1 RTLE, 4 LTLE) for the hippocampal analysis.
Figure 1. These coronal MRI images depict the masks used for small volume correction in each patient to produce activation asymmetry ratios (ARs). Right hippocampus in blue, left hippocampus in red, right parahippocampal gyrus in purple, left hippocampal gyrus in green.
7 Structural Data Analysis
FreeSurfer [Martino Center for biomedical Imaging, Harvard-MIT, Boston USA; http://surfer.nmr.mgh.harvard.edu] was used to find volumetric values for the hippocampi, and cortical thickness for the parahippocampal gyrus. Cortical thickness rather than volume is used for the parahippocampal gyrus because the methods used by FreeSurfer tend to underestimate cortical volume due to its surface based measurement procedures. FreeSurfer has been previously described in detail (Fischl et al., 2004) and has been assessed in terms of validity and accuracy (Dickerson et al., 2008; Han et al., 2006; Lee et al., 2006; Morey et al., 2010; Pardoe, Pell, Abbott, & Jackson, 2009). Preprocessing included intensity normalization, removal of non-brain tissue, Talaraich transformation and segmentation of tissue into grey matter, white matter and cerebral spinal fluid. Subcortical structures are then segmented. For cortical thickness, the white and grey matter boundaries are identified and the distance between the two surfaces are calculated. The cortex is then automatically parcellated. Thickness is then calculated for a structure based on this cortical parcellation. Structural ARs were calculated for the hippocampus according to the formula \([((\text{left volume} - \text{right volume}) / (\text{left volume} + \text{right volume}))\] while ARs for the parahippocampal gyrus used the formula \([(\text{left thickness}^+ - \text{right thickness}^-) / (\text{left thickness}^+ + \text{right thickness}^-)]\).

8 Group Comparison
Independent samples t-tests were used to look at group differences between left and right TLE patients on neuropsychological memory test ARs, activation ARs, and structural ARs using SYSTAT 13 (Systat Software Inc. Chicago IL).
9 Neuroimaging Measures and Behaviour

Mediation analysis was performed following the methods outlined in Baron and Kenny (1986) using linear regression in SYSTAT 13 (Systat Software Inc. Chicago IL). The hippocampal activation AR (acting as the predicted mediator) was regressed onto the hippocampal volume AR (acting as the independent variable). Each behavioural AR (the dependent variables) was then regressed onto the hippocampal volume ARs, separately. Finally, each behavioural AR was regressed onto both the hippocampal activation ARs and the hippocampal volume ARs. A mediation relationship could exist if there is a significant decrease in structural volume ARs ability to predict memory once activation ARs are added into the regression model. A model of this is shown in Figure 2. In Figure 2, if $c'$ is significantly smaller than $c$, then this suggests a mediation interaction is present. This significance can be assessed by testing the indirect effect as outlined by Sobel (1982) which is as follows:

$$z = \frac{a \times b}{\sqrt{b^2 \times s_a^2 + a^2 \times s_b^2}}$$

Where $b$ is the slope of the mediator for predicting the dependent, in this case structural AR predicting memory scores, $s_b$ is the standard error of the slope represented by $b$, $a$ represents the slope of the independent variable on the mediator, in this case structural AR on activation AR and $s_a$ is the standard error of the slope represented by $a$. This test produces a z-score which can be used to test for significance. Additionally, behavioural ARs scores were regressed onto the parahippocampal activation AR and parahippocampal thickness ARs (separately) for each subject to test the contribution of the parahippocampus to understanding memory presurgically.
Figure 2. A model of the interaction between structural volume, fMRI activation and memory. A. The influence of volume on memory is shown as path c. B. Shows the model of influence provided a mediation relationship exists. Volume now indirectly influences memory through activation and the direct path (c’) should be significantly smaller than the previous direct path (c).
Chapter 3
Results

1 Demographics
There was no difference in age between the RTLE, the LTLE group, and the control group, \( F(32,2) = 1.63, p = 0.21 \). There was a significant group level difference in years of education between patients with RTLE, patients with LTLE and healthy controls, \( F(32,2) = 3.5, p = .042 \). Post-hoc tests revealed no difference in years of education between patients with RTLE and those with LTLE, \( t(21) = .16, p = .87 \), no difference in years of education between patients with RTLE and healthy controls, \( t(22) = 2.2, p = .12 \) and no difference between patients with LTLE and healthy controls, \( t(21) = 2.15, p = .13 \). There was no difference in seizure duration between patients with RTLE and those with LTLE, \( t(21) = .384, p = .7 \). These demographic variables are summarized in Table 1.

2 Task Activation
As shown in Figure 3, all groups showed bilateral activation in occipital, fusiform gyrus, parahippocampal gyrus and hippocampus (p<0.005, uncorrected). Group level analysis, shown in Figure 4, controls activated bilateral parahippocampal to a greater extent than patients with LTLE (p<0.005, uncorrected). There were no voxels in the MTL that activated greater in patients with LTLE compared to healthy controls subjects. Controls had greater activation in the left parahippocampal and compared to patients with RTLE (p<0.005, uncorrected). There were no voxels in the MTL that activated more strongly in patients with RTLE compared to healthy control subjects. Patients with RTLE had greater left parahippocampal and greater left hippocampal activation compared to patients with LTLE patients (p<0.005, uncorrected). There
Figure 3. Whole brain analysis of functional activation for the contrast novel > repeated scenes for LTLE, RTLE and control groups at p<0.005, uncorrected, showing activation bilaterally in occipital, fusiform, parahippocampal and hippocampal regions. Colour bars display t-values.
**Figure 4.** Whole brain group analysis of functional activation for the contrast novel>repeat scenes contrasting LTLE>RTLE, Controls>LTLE and Controls>RTLE at $p<0.005$, uncorrected. Colour bars display t-values.
were no voxels in the MTL that demonstrated greater activation in patients with LTLE compared to those with RTLE at this threshold.

3 Asymmetry Ratios
There was no significant difference for behavioural ARs between the LTLE and RTLE groups. Rey Test AR $t(21) = 1.85, p = .078$, Warrington test AR $t(21) = 1.62, p = .12)$. These null results are likely a function of small sample size as the Cohen’s d value for REY-AR was 0.79, which is considered a large effect size, while the Cohen’s d value for the WARR-AR was 0.70 which is considered to be medium-large. Turning to functional activation, patients with RTLE had significantly more left lateralized activity compared to LTLE in the hippocampal activation ARs, $t(16) = 3.11, p = .007$, but this was not observed for the parahippocampal activation ARs, $t(16) = 1.56, p = 0.14$. Patients with RTLE also had a significantly different hippocampal volume ARs compared to patients with LTLE, $t(21) = 4.97, p < 0.001$ but there was no significant difference in parahippocampal thickness ARs, $t(21) = 0.42, p = .68$ (See Figure 5 and Table 2).

4 Neuroimaging Measures and Behaviour
Hippocampal volume ARs were able to explain a significant proportion of variance in hippocampal activation ARs, $R^2 = .31, F(1,16) = 7.31, p = .016$, establishing a link between the independent variable and the mediator. This regression is shown in Figure 6A. Hippocampal volume ARs were not able to significantly explain the variance in WARR-AR, $R^2 = .061, F(1,21) = 1.37, p = .26$, nor was it able to significantly explain the variance in REY-AR, $R^2 = .004, F(1,21) = .094, p = .762$. This failed to produce a link between the independent variable and the dependent variable. Hippocampal activation ARs were able to explain a significant proportion of variance in the WARR-ARs (Figure 6B), $R^2 = .28, F(1,16) = 6.26, p = .024$, but not REY-ARs, $R^2 = .003, F(1,16) = .046, p = .833$. This established the link between the mediator
Figure 5. Behavioural ARs, activation ARs, hippocampal volume ARs and parahippocampal cortical thickness ARs using “box-and-whisker” plots between patients with RTLE and LTLE. The centre horizontal line represents the group median, while the edges of the box represent the 25th and 75th percentiles. The ends of the whiskers represent the 1.5 interquartile range (IQR) of the distribution. Asterisks represent values that fall beyond the 1.5 (IQR). * denotes significant group differences (p < .05) in ARs after correcting for multiple comparisons.
Table 2. A summary of the means and standard deviations of the asymmetry ratios (ARs) produced for the LTLE and RTLE groups.

<table>
<thead>
<tr>
<th></th>
<th>RTLE</th>
<th>LTLE</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>REY-AR</td>
<td>.22</td>
<td>.22</td>
</tr>
<tr>
<td>WARR-AR</td>
<td>.073</td>
<td>.051</td>
</tr>
<tr>
<td>Hippo activation AR</td>
<td>-.050</td>
<td>.24</td>
</tr>
<tr>
<td>Hippo volume AR</td>
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<td>.11</td>
</tr>
<tr>
<td>Para activation AR</td>
<td>.29</td>
<td>.21</td>
</tr>
<tr>
<td>Para thickness AR</td>
<td>-.011</td>
<td>.031</td>
</tr>
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</table>
Figure 6. Regression models for the mediation analysis. A. hippocampal volume ARs shown as a predictor for hippocampal activation ARs. B. Hippocampal activation ARs shown as a predictor for WARR-ARs. Patients who did not show activation in both hemispheres were excluded.
and one of the behavioural ARs. When both hippocampal volume ARs and hippocampal
text ar were included as predictors of WARR-ARs the overall model failed to explain a
significant amount of variance in WARR-ARs, $R^2 = .29$, $F(1,16) = 3.04$, $p = .078$. Activations
ARs were still significant predictors, $\beta = .586$, $t(16) = 2.23$, $p = .042$, and Volume ARs were not,
$\beta = -.099$, $t(16) = -.378$, $p = .711$.

Despite the failure to establish a link between volume ARs and a behavioural AR, we
proceeded to test the indirect effect of a mediation relationship using Sobel’s Test (1982), since
the link between volume ARs and behaviour has been reported previously in the literature
(Mechanic-Hamilton et al., 2009). According the Sobel’s Test (1982) there is a significantly
large indirect effect of this mediation model, $z = 1.74$, $p = 0.041$, which indicates that the effect
of volume on memory flows through activation. The model is summarized in Figure 7.

Parahippocampal activation ARs and Parahippocampal thickness ARs did not correlate
significantly with any behavioural asymmetry ratios.

Chapter 4
Discussion

This thesis examined the relationship between volume, functional activation during
encoding, and behavioural measures of memory. During the novel scene encoding task all
patients and healthy controls activated bilateral MTL regions including the hippocampus and the
parahippocampal gyrus. This was in support of previous work (Rabin et al., 2004; Stern et al.,
1996). Healthy controls showed greater parahippocampal activation bilaterally compared to
patients with LTLE. This could be due to the widespread structural changes in the MTL that are
seen in TLE (Bell et al., 2011; Bernasconi et al., 2003). The opposite contrast did not reveal
Figure 7. A depiction of the proposed mediation model accompanied by path correlations showing a significant correlation between volume ARs and activation ARs for path a, a significant correlations between activation ARs and WARR-ARs when controlling for volume ARs in path b and a significant reduction in path c to path c'.

Path c: $r = .247$
controlling for Activation AR Path $c' = .054$

Hippo Volume AR $\rightarrow$ Hippo Activation AR $\rightarrow$ WARR-AR

controlling for Volume AR $r = .499^*$
any significant regions in the MTL that were activated in LTLE compared to controls.
Interestingly, healthy controls also had greater left parahippocampal activation compared to patients with RTLE. Patients with RTLE did not activate any MTL regions to a greater degree than healthy controls. Patients with RTLE activated the left hippocampus and parahippocampus greater than patients with LTLE during novel scene encoding. This could be due to the fact that patients with LTLE have damaged left hippocampi leading to weaker activation. There were no regions in the MTL that were significantly more active in patients with LTLE compared to those in RTLE, even at very liberal thresholds.

This study was unable to find a significant difference between neuropsychological asymmetry ratios between patients with LTLE and RTLE though there was a trend shown in which RTLE patients tended to perform better on verbal memory compared to visual memory and LTLE patients tended to perform better on visual memory tests than verbal memory tests. We would expect that an increase in sample size would produce a significant difference that is shown in the literature in line with material specificity (Marques et al., 2007; McAndrews & Cohn, 2012; Milner, 1968). The effect sizes between groups for both the Rey memory tests and the Warrington memory tests were substantial suggesting that a larger sample would yield results more consistent with the literature.

Functional activation asymmetry within the hippocampus during a novel scene encoding task differed significantly between our RTLE and LTLE group. As predicted, patients with LTLE displayed a greater proportion of right hippocampal activation compared to left hippocampal activation, while patients with RTLE displayed a greater amount of left hippocampal activation compared to right hippocampal activation. This is in support of previous findings (Detre et al., 1998; Mechanic-Hamilton et al., 2009). However, there was not a significant difference in parahippocampal activation asymmetry between patients with LTLE and
those with RTLE. Rather, there was a non-significant trend that showed patients with LTLE preferentially activating the right parahippocampus compared to the left and patients with RTLE preferentially activating the left parahippocampus compared to the right. Several studies have included the parahippocampus in ROI analysis, but as a part of a large ROI including the hippocampus and the fusiform (Mechanic-Hamilton et al., 2009; Rabin et al., 2004) but none have included a separate mask for the parahippocampal gyri specifically. We expect that a larger sample would reveal that a significant difference between these two patient groups does in fact exist.

This study also saw a significant difference in hippocampal volume asymmetry between patients with LTLE and those with RTLE. This is in line with past studies (Bernasconi et al., 2003; Mechanic-Hamilton et al., 2009) and showed that patients with LTLE had smaller left hippocampi compared to their right hippocampus and those with RTLE had smaller right hippocampi compared to their left hippocampus. There was not a significant asymmetry in parahippocampal thickness between patients with RTLE and those with LTLE. This could be due to the extensive boundaries considered for parahippocampal cortex in thickness analysis. It has been previously shown that parahippocampal atrophy may be more restricted to anterior portion, specifically the entorhinal and to a lesser degree the perirhinal cortices whereas the posterior portion of the parahippocampal cortex does not show much atrophy when comparing patients with TLE to healthy controls (Bernasconi et al., 1999, 2003). Our boundaries for measuring cortical thickness included entorhinal, perirhinal and posterior parahippocampal cortices together as one measurement. This may have diluted any differences that may exist in this patient group. Future studies would therefore be encouraged to use a finer method of examining parahippocampal cortical measurements that differentiate entorhinal, perirhinal and posterior parahippocampal cortices.
The results of this study also demonstrated that the link between hippocampal volume and memory is mediated by hippocampal activation. The link between volume and memory was not found to be significant in this study, but has been previously shown (Mechanic-Hamilton et al., 2009; Reminger et al., 2004). Despite not finding a significant link between volume and memory, the influence of hippocampal volume on memory was found to decrease significantly following the introduction of hippocampal activation asymmetry. Hippocampal volume asymmetry was correlated with hippocampal activation asymmetry, which in turn was correlated with memory asymmetry in Warrington words and faces. This relationship leaves hippocampal activation as the middle link between volume and memory. This finding represents a link that has not previously been explored in the literature and suggests that the relationship between hippocampal volume and memory is partially explained through the activation (extent and magnitude in this case) that tissue is able to generate during encoding. This may suggest that hippocampal activation asymmetry may be a better marker of hippocampal adequacy than hippocampal volume is. Two individuals with TLE may both have significant ipsilateral hippocampal damage, but if one of those individuals has activation biased to their ipsilateral side and shows relatively unimpaired presurgical memory, that individual may be at greater risk of post-surgical memory loss than their counterpart who may have activation biased more towards the contralateral side. The first individual has a decrease in hippocampal volume, yet is still using their ipsilateral hippocampus to its best ability, whereas the second individual has a decrease in hippocampal volume, and is relying on their contralateral hippocampus, suggesting that ipsilateral removal would not cause as much of a disruption.

There was very little overlap between volume asymmetry scores for patients with LTLE and those with RTLE. This lack of variance however may restrict the ability of volume asymmetry to correlate with memory. It may have better clinical utility therefore in serving to aid
in the assessment of seizure laterality. Conversely, activation asymmetry has a larger variance that correlates strongly with memory asymmetry. This would allow activation asymmetry to better assess presurgical functional adequacy of the to-be-removed hippocampus. According to the functional adequacy theory (Chelune, 1995), individuals who demonstrate a higher degree of hippocampal adequacy presurgically are at greater risk of post-surgical memory decline.

The relationship between MRI measures of structural and functional integrity is one that has not been investigated extensively but has more recently become explored (Poppenk & Moscovitch, 2011; Voets et al., 2012). Poppenk & Moscovitch (2011) looked at healthy controls across multiple studies to find a relationship between posterior-anterior hippocampal volume ratios and recollective memory. They went on to find that this relationship between hippocampal volume and recollective memory was mediated by post-encoding resting-state connectivity of the posterior hippocampus to precuneus, inferior parietal and lateral frontal cortex. Voets et al. 2012 used resting-state connectivity, voxel-based morphometry and diffusion tensor imaging (DTI) to look at patients with TLE to identify voxel-wise associations between resting state connectivity and white matter coherence (determined by fractional anisotropy from DTI). They found the functional connectivity between parahippocampal cortex, frontal and temporal neocortex was associated with white matter coherence in the pathways connecting these regions. Their overall findings suggest that cortical atrophy and white matter coherence are associated with functional connectivity. Now that neuroimaging has developed tools to look at structural integrity measures via DTI, volumetry, voxel based morphometry and at functional integrity via activation contrasts, functional connectivity and multivoxel pattern analysis, we are better prepared to identify and exploit connections between structure integrity, activation and connectivity. Within the hippocampus, we have shown a link between volume asymmetry and activation asymmetry.
and this may generalize to other disorders that may have MTL disruption such as AD, schizophrenia and depression.

This study attempted to find a link between activation asymmetry and thickness asymmetry in the parahippocampal gyrus, but was unsuccessful. This may be explained through having a somewhat small sample size, and having overly inclusive boundaries that would have been better restricted to certain regions of parahippocampal gyrus such as entorhinal cortex. Despite the failure of finding significant correlations or differences in parahippocampal regions, I maintain that exploring parahippocampal parameters as potential predictors post-surgical memory change may still be of interest, since parahippocampal cortex is also removed during temporal lobectomy procedures. There have been changes in volume seen in regions of the parahippocampal cortex (Bernasconi et al., 1999, 2001, 2003) some of which are independent of hippocampal volume changes, specifically the entorhinal cortex. The entorhinal cortex is thought to be a gate to the hippocampus and an independent change in the entorhinal cortex could therefore affect memory performance. This study therefore suggests a more precise look at entorhinal cortex structure and function measures as potential predictors of post-surgical memory in future studies following this thesis.

Several limitations to this study exist. Firstly, all patients were using anticonvulsive medication during all stages of the study. It is unclear as to how this medication may have affected the memory performance or functional activation during fMRI scanning. For example, we do not know how these medications may affect vasodilation which would ultimately alter the BOLD signal. This limitation exists in most studies of this nature. The use of ARs may temper part of this limitation because it measures a ratio of activation between the hemispheres. Also, the control group used was not age matched with the patient sample. The control group however was used to ensure proper task implementation, while the true results of this thesis focusses
specifically on differences between LTLE and RTLE groups and on correlations between the
neuroimaging measures with patient memory performance.

Future work should follow up on the reported patients following surgery, to correlate
post-surgical memory change with presurgical activation and volume asymmetries in both the
hippocampus and parahippocampal cortices.

The main goal of this thesis was to explore the relationship between volume, activation
and memory in patients with TLE as well as exploring any influence the parahippocampus may
have on memory. We discovered that activation asymmetry mediates the relationship between
volume asymmetry and memory asymmetry in patients with TLE. This is in line with recent
research that has shown that underlying brain processes mediate the relationship between volume
and memory (Poppenk & Moscovitch, 2011) and suggests the need for more structure-activation
based analyses, such as DTI and resting-state connectivity. Based on our findings we conclude
that hippocampal activation asymmetry is indeed a good predictor of hippocampal adequacy,
perhaps even more so than volume asymmetry. Despite our concerns about the independent
value of activation measures (given the patterns seen in MCI and AD), current results make us
more confident in interpreting regional activation as a viable ‘biomarker’.
References


of no added value to preoperative neuropsychological assessment and MRI. *Epilepsy & behavior : E&B, 16*(2), 335-40. Elsevier Inc. doi:10.1016/j.yebeh.2009.08.003


