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## Interictal Epileptogenic Networks and Endophenotypes in Mesial Temporal Lobe Epilepsy

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# **Interictal Epileptogenic Networks and Endophenotypes in Mesial Temporal Lobe Epilepsy**

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**A Thesis Submitted for the Degree of**

**Doctor of Philosophy (Clinical Neuroscience)**

**March 2017**

## Abstract

Mesial temporal lobe epilepsy (MTLE) is the most common adult focal epilepsy and is traditionally thought to involve focal-onset seizures arising from the affected hippocampus. Recent neuroimaging studies however have shown widespread bilateral structural and functional abnormalities in MTLE, providing evidence for MTLE as a network disorder. There has also been some evidence that neuroimaging-derived traits could be potential endophenotypes for MTLE.

This thesis aimed to identify abnormalities in MTLE and to investigate the suitability of these traits as potential endophenotypes through magnetic resonance imaging- (MRI) and electroencephalography- (EEG) based investigations in MTLE patients, their first-degree unaffected relatives and healthy control participants.

Temporal lobe morphology alterations were detected in MTLE patients and unaffected relatives using structural MRI morphometry. Diffusion MRI tractography of the fornix identified alterations in MTLE patients but not their relatives, although tract indices were correlated with hippocampal volumes in both groups but not in healthy controls. A reduction in the peak alpha frequency (PAF) of the EEG data was observed in MTLE patients, with a trend toward decreased PAF in unaffected relatives. The anterior insular and frontal opercular regions were implicated in a network associated with the occipital alpha rhythm in MTLE patients from EEG-correlated MRI and fMRI. Finally, there was a trend toward network reorganisation at the whole-brain level and in hippocampal sub-networks, and evidence for altered hubs in MTLE patients.

This thesis presents evidence indicating structural and functional abnormalities in MTLE extend beyond the affected hippocampus and that some subtle alterations may be present in unaffected relatives. This suggests possible candidate MRI- and EEG-based endophenotypes for MTLE for further investigation.

## Contributions

Recruitment and clinical data collection of MTLE patients and first-degree relatives from King's College Hospital, St Thomas' Hospital, St George's Hospital, Croydon University Hospital and Princess Royal University Hospital was a joint effort between myself, MRC Programme Manager Miss Amber Collingwood, and Post-Doctoral Research Fellow Dr Sarah Carr through referrals and clinic lists from the Epilepsy Consultant Neurologists at the respective hospitals. Two patients and one relative were recruited by Research Nurse Ms Helen Wooldridge at Darent Valley Hospital.

I collected all imaging and electroencephalography data, with the exception of 2 healthy control datasets collected by Dr Carr. Neuroradiographers at the IoPPN Centre for Neuroimaging Sciences assisted with running the MRI scanner, and Dr Carr and several other members of the epilepsy research group assisted with preparing the EEG cap for most participants.

The hypotheses and plan of investigation were formulated after discussions with my supervisor, Professor Mark Richardson. I carried out all data processing and analyses reported here.

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## Acknowledgements

Thanks go first and foremost to my supervisors, Professor Mark Richardson and Professor Gareth Barker. They were always enthusiastic, encouraging and available for questions, even with their busy schedules, and I always came away from our meetings feeling more optimistic about my PhD. Their excellent guidance in all aspects of my PhD, from the initial MRI study set-up through to patient recruitment and data analysis and interpretation, has made my PhD experience a fantastic one.

I am also extremely grateful to the epilepsy research group, and fellow researchers in the Clinical Neuroscience department and at the IoPPN. I have been very fortunate to have been in the company of such brilliant and helpful people. To Colm, Adam, Chin, Sarah and Eugenio, thanks for answering my many questions (no matter how little or silly), for our many helpful and interesting discussions and for hearing out my complaints and keeping me sane throughout. Amber helped immensely with the initial setup of the study and was always available for help with patient recruitment and the EEG cap. Sharon and Stuart taught me all I know about clinical EEGs, Paul taught me about DTI tract reconstructions, and Isabella and Suejen helped with the in-scanner EEG setup and were always on hand to solve problems with the EEG equipment and cap. The wonderful folks at the Clinical Research Facility and radiographers at the Centre for Neuroimaging Sciences made scans go smoothly and scan days enjoyable, and Rodney at the CNS sorted out all my computing issues. Thanks also go to the patients, their families, and all the participants, without whom this study would not have been possible.

Finally, my deepest gratitude goes to my family, my husband Nick, our cat Momo, and our hens, Alpha, Floppy and Squawky. To my parents I am eternally indebted for giving me the opportunities and experiences that have made me who I am. To my siblings for being my closest friends. And to my husband Nick, for his continuous and unquestioning support and love. This journey would not have been possible if not for them, and I dedicate this milestone to them.

## Abbreviations

| Abbreviation | Meaning                                                |
|--------------|--------------------------------------------------------|
| ANCOVA       | Analysis of Covariance                                 |
| ANOVA        | Analysis of Variance                                   |
| AED          | Anti-epileptic Drug                                    |
| ADC          | Apparent Diffusion Coefficient                         |
| AUC          | Area Under the Curve                                   |
| AAL          | Automated Anatomical Labelling                         |
| BCG          | Ballistocardiogram                                     |
| BOLD         | Blood Oxygenation Level Dependent                      |
| CSF          | Cerebrospinal Fluid                                    |
| CSD          | Constrained Spherical Deconvolution                    |
| CA           | Cornu Ammonis                                          |
| CritC        | Critical Coupling Parameter                            |
| DMN          | Default Mode Network                                   |
| DT           | Diffusion Tensor                                       |
| DTI          | Diffusion Tensor Imaging                               |
| DWI          | Diffusion Weighted Imaging                             |
| EPI          | Echo Planar Imaging                                    |
| ECoG         | Electrocorticography                                   |
| EEG          | Electroencephalography                                 |
| ENIGMA       | Enhancing Neuro Imaging Genetics through Meta-Analysis |
| ERP          | Event Related Potential                                |
| EPSP         | Excitatory Post-Synaptic Potential                     |
| EV           | Explanatory Variable                                   |
| FDR          | False Discovery Rate                                   |
| FMTLE        | Familial Mesial Temporal Lobe Epilepsy                 |
| FWE          | Family-Wise Error                                      |
| FOD          | Fibre Orientation Distribution                         |
| FLAIR        | Fluid Attenuated Inversion Recovery                    |
| FSL          | FMRIB Software Library                                 |
| FA           | Fractional Anisotropy                                  |
| FWHM         | Full Width at Half Maximum                             |
| FC           | Functional Connectivity                                |

|         |                                                      |
|---------|------------------------------------------------------|
| fMRI    | Functional Magnetic Resonance Imaging                |
| GLM     | General Linear Model                                 |
| GE      | Gradient Echo                                        |
| GM      | Grey Matter                                          |
| HRF     | Haemodynamic Response Function                       |
| HS      | Hippocampal Sclerosis                                |
| IGE     | Idiopathic Generalised Epilepsy                      |
| IPSP    | Inhibitory Post-Synaptic Potential                   |
| IQR     | Inter-Quartile Range                                 |
| IED     | Interictal Epileptiform Discharge                    |
| ICBM    | International Consortium for Brain Mapping           |
| ILAE    | International League Against Epilepsy                |
| ICC     | Intraclass Correlation Coefficient                   |
| ICV     | Intracranial Volume                                  |
| IR-SPGR | Inversion Recovery-Prepared Spoiled Gradient-Echo    |
| LFP     | Local Field Potential                                |
| MR      | Magnetic Resonance                                   |
| MRI     | Magnetic Resonance Imaging                           |
| MD      | Mean Diffusivity                                     |
| MTLE    | Mesial Temporal Lobe Epilepsy                        |
| MTS     | Mesial Temporal Sclerosis                            |
| MNI     | Montreal Neurological Institute                      |
| MANCOVA | Multivariate Analysis of Covariance                  |
| MANOVA  | Multivariate Analysis of Variance                    |
| NART    | National Adult Reading Test                          |
| PHC     | Parahippocampal Cingulum                             |
| PAF     | Peak Alpha Power                                     |
| PLF     | Phase Locking Factor                                 |
| PET     | Positron Emission Tomography                         |
| PSD     | Power Spectral Density                               |
| RF      | Radiofrequency                                       |
| RGB     | Red-Blue-Green                                       |
| ROI     | Region of Interest                                   |
| SPECT   | Single Photon Emission Computed Tomography           |
| SCN1A   | Sodium Channel, Voltage-Gated, Type 1, Alpha Subunit |

|      |                                       |
|------|---------------------------------------|
| SE   | Spin Echo                             |
| SPM  | Statistical Parametric Mapping        |
| SC   | Structural Connectivity               |
| sMRI | Structural Magnetic Resonance Imaging |
| TLE  | Temporal Lobe Epilepsy                |
| TR   | Time of Repetition                    |
| TMS  | Transcranial Magnetic Stimulation     |
| UNC  | Uncinate Fasciculus                   |
| VBM  | Voxel-Based Morphometry               |
| WM   | White Matter                          |

## PART I: Introduction

# Chapter 1 Epilepsy

The term *epilepsy* is derived from the ancient Greek verb *epilambanein*, meaning “to seize, to take hold of, or to attack”. Epilepsy as a neurological disorder has probably existed since human life began. While it may not have been recognised in its present form, the first reference to a seizure dates back to Assyrian texts almost 4000 years ago (Magiorkinis et al., 2010). In fact, the origin of seizures was shrouded in superstitions and epilepsy was thought of as a sacred disease until Hippocrates first provided a medical description for epilepsy in 400 B.C.

Epilepsy is now understood as a heterogeneous group of neurological disorders characterised by recurrent, unprovoked and unpredictable epileptic seizures, with seizures being brain states characterised by abnormal, excessive and synchronous neuronal depolarisation (Fisher et al., 2014).

The International League Against Epilepsy (ILAE) has defined epilepsy as:

*“A disease of the brain defined by any of the following conditions:*

- 1) At least two unprovoked (or reflex<sup>1</sup>) seizures occurring >24 hours apart;*
- 2) One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years;*
- 3) Diagnosis of an epilepsy syndrome.”* (Fisher et al., 2014)

## 1.1 Epidemiology

The World Health Organisation estimates the number of people suffering from epilepsy worldwide at about 50 million (ILAE/IBE/WHO, 2003), making it one of the most common neurological disorders. The incidence of a first unprovoked epileptic seizure is in the range of 50

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<sup>1</sup> Reflex seizures refer to seizures that are caused as a result of a specific trigger or stimulus.

to 70 per 100,000 in developed countries (Hauser & Beghi, 2008), and while there are no consistent racial or national differences, the incidence is higher in developing countries and rural communities with increased risk of central nervous system infections and limited access to healthcare (Meinardi et al., 2001; Ngugi et al., 2010).

Epilepsy affects people of all ages, although most studies show a bimodal distribution, with a higher incidence in the first 20 years (highest in the first year), lowest incidence between 20 and 40 years, increasing incidence after age 50 and a marked increase after age 80 (Neligan & Sander, 2009).

About 70% of patients with newly diagnosed epilepsy will enter remission, but this means a large proportion still do not achieve seizure freedom (Kwan & Brodie, 2000). Based on UK primary care electronic health records, the prevalence of active epilepsy is around 1.2% of the population (Ridsdale et al., 2011). Epilepsy often requires continuing assessments and hospital treatments, sometimes including surgery, placing a significant burden on health services. Mortality rates are also higher in patients with epilepsy, and among neurological disorders, only stroke leads to more years of life lost than epilepsy (Thurman et al., 2014). Mortality from epilepsy is also on the rise in the UK unlike mortality from other causes (Ridsdale et al., 2011). The economic cost of epilepsy is also significant, with the total cost of epilepsy in Europe estimated at €15.5 billion in 2004 (Pugliatti 2007).

## **1.2 Classification**

Two main schemes are used in the classification of epilepsies: a classification based on epilepsy syndrome (organised by typical clinical presentation, underlying aetiology, prognosis, etc.) and a classification by seizure type (broadly classified as focal or generalised) (Richardson et al., 2015).

The 1981 operational seizure classification schemes from the ILAE divided seizures into two main types, partial seizures and generalised seizures. Partial seizures start in a localised area of the cortex and are generally divided into simple partial seizures (with no impairment of consciousness) or complex partial seizures (with impairment of consciousness). Generalised seizures start almost synchronously in both hemispheres of the brain and some partial seizures can evolve into secondarily generalised seizures. The 1989 epilepsy classification scheme from the ILAE divided epilepsy syndromes based on aetiology: symptomatic epilepsies, in which there is a known cause, such as a lesion, tumour or cortical malformation; idiopathic epilepsies, in which there is no known cause and a genetic predisposition is presumed; and cryptogenic epilepsies, in which a symptomatic cause is suspected but not proven.

These classification schemes have been much debated in the 30 years since, as many diagnosed epilepsy cases do not fit clearly into the classification categories and these schemes do not take into account evidence from advances in neuroimaging and genetic research (Manford et al., 1992). The ILAE has since recognised the importance and need for revising the classification scheme in line with these developments, and have proposed a new classification scheme in 2010 (Berg et al., 2010). Under the new proposed scheme, networks of brain regions are implicated in both generalised seizures (where a bilateral network of regions is implicated at seizure onset) and focal seizures (where networks are thought to be localised or involve a more widespread network of regions within one hemisphere), emphasising the importance of investigating networks in focal epilepsies. Very recently, two new position papers on the classification of seizures and epilepsies were published to clarify this shift in the way epilepsies and seizures are viewed and understood (Fisher et al., 2017; Scheffer et al., 2017).

### **1.3 Mesial Temporal Lobe Epilepsy**

Temporal lobe epilepsy (TLE) is the most common type of adult focal epilepsy and accounts for around 60-70% of focal epilepsy cases (Diehl & Duncan, 2013). Seizures can be of lateral or

mesial temporal origin, with mesial temporal lobe epilepsy being more common. Hippocampal sclerosis (HS) accounts for about 60 to 70% of MTLE cases, making MTLE+HS one of the most common and most distinct of epilepsy syndromes (Tatum IV, 2012). The present work focuses on MTLE.

### **1.3.1 Clinical presentation and characteristics**

Clinical features of MTLE are widely documented and observed in the majority of patients. Patients with MTLE often present with a past history of febrile convulsions, trauma, or cerebral infection in the early years of life. Prolonged febrile seizures are thought to be a risk factor for MTLE+HS, with around 60 to 70% of patients having had a history of prolonged febrile seizures (French et al., 1993). With improvements in neonatal care, some epileptologists have observed that the incidence of classic MTLE+HS is reducing (Helmstaedter et al., 2014; Rossi, 2015).

The primary type of seizures in MTLE are focal seizures with impairment of consciousness (Tatum IV, 2012), although other seizure types are also observed, with MTLE patients sometimes having focal seizures without impairment of consciousness, or focal seizures that evolve into bilateral convulsive seizures. The seizure semiology of TLE may include an aura, which is an ictal event typically involving subjective sensory or psychic phenomena (typically rising epigastric sensations and déjà vu) but no motor symptoms, lasting several seconds or minutes before seizure onset. This is followed by focal seizures with altered consciousness characterised by unilateral motor signs, with oro-facial automatisms, contralateral arm and hand dystonia, and ipsilateral manual automatisms usually observed. Post-ictal confusion often follows, although not all patients will experience all the above stages (Blair, 2012).

MTLE can affect people of all ages, although studies have found an early age of onset (before 17 years) to often be related to TLE with mesial temporal origin, while later onset (18 years and

over) is often related to TLE with neocortical origin (Villanueva & Serratosa, 2005), although this is not always the case (O'Brien et al., 1996; Wieser, 2004).

As seizures represent periods of hyper synchronous activity in the brain, scalp electroencephalography (EEG), a method that records electrical activity from the brain<sup>2</sup>, is often used to assess both ictal (during seizures) and interictal (periods between seizures) electrical brain activity in epilepsy. In MTLE, scalp interictal EEG often shows anterior or mid-temporal spikes or sharp waves. Spikes usually appear ipsilateral to the seizure onset lobe, but can also appear bilaterally, and are often visible during periods of drowsiness and stage I and II of sleep. Temporal slow waves might also be seen and could be a lateralising feature indicating the presence of a lesion (Wieser, 2004). Ictal activity is characterised by unilateral anterior temporal theta or alpha activity in the range of 5 to 9 Hz (Tatum IV, 2012). On clinical depth and grid electrode recordings<sup>3</sup>, pathological high-frequency oscillations in the range of 250-600 Hz are also seen in the entorhinal cortex and hippocampus in MTLE patients (Bragin et al., 1999) and is believed to always be associated with the epileptogenic activity in these regions (Köhling & Staley, 2011).

Hippocampal sclerosis is the most common pathology in refractory focal epilepsy and is usually detected with a magnetic resonance imaging (MRI) fluid attenuated inversion recovery (FLAIR) sequence showing an increase in the T2-weighted signal<sup>4</sup>, in most cases associated with atrophy in the hippocampus. Consistent structural abnormalities have also been detected outside of the affected hippocampus, in the contralateral hippocampus, ipsilateral limbic system and neocortical regions (Wieser, 2004). Other pathologies detected with neuroimaging include malformations of cortical development, benign tumours, gliomas and vascular lesions (Diehl & Duncan, 2013). Classical HS, as described by Corsellis and Bruton (Bruton, 1988; Margerison &

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<sup>2</sup> The EEG and normal EEG features, as well as epileptiform activity is further described in Chapter 2.

<sup>3</sup> Depth and grid electrode recordings are often performed on MTLE patients prior to the surgical resection of the hippocampus. Depth microelectrodes are able to measure electrical brain activity closer to the source as they are implanted in the brain.

<sup>4</sup> MRI is further described in Chapter 2.

Corsellis, 1966), involves neuronal loss in the cornu ammonis (CA) 1 subfield of the hippocampus, with less neuronal loss in the CA3 and CA4 subfields. The CA2 subfield is relatively spared. Often there is also severe gliosis found in the CA1, CA3 and CA4 subfields and mossy fibre sprouting and reorganization is observed in the dentate gyrus (Sutula et al., 1989). The hippocampus is involved in a range of cognitive functions, including learning, memory formation, emotion regulation and spatial navigation (O'Keefe & Nadel, 1978; Tulving & Markowitsch, 1998; Vargha-Khadem et al., 1997) and patients with MTLE often present with cognitive co-morbidities including learning difficulties and memory impairment (Helmstaedter et al., 2003; Hermann et al., 2008) as well as psychiatric co-morbidities including a higher rate of depression (Garcia, 2012).

### **1.3.2 Treatment for MTLE /epilepsy surgery**

Medical tractability in MTLE is generally low, with studies citing a range of 11-42% of patients achieving seizure freedom on medication (Kumlien et al., 2002; Tatum IV, 2012), compared with about 60-70% 2 years post-surgery (Bonilha & Keller, 2015; Engel, 1996). MTLE is the most common drug resistant epilepsy referred for surgery (Téllez-Zenteno & Hernández-Ronquillo, 2012). Although surgery is significantly better at controlling seizures than prolonged medical therapy (Wiebe et al., 2001), up to 60% of patients do not achieve long-term post-surgical seizure freedom (McIntosh et al., 2004; Spencer & Huh, 2008), and even among those whose seizures remit, 72% continue to be reliant on antiepileptic medication (de Tisi et al., 2011).

It is unknown why some patients continue to have seizures post-surgery. Although some factors such as a clear unilateral temporal pathology on MRI, unilateral interictal epileptiform discharges (IEDs) ipsilaterally, younger age at operation and shorter epilepsy duration have been cited as factors for favourable surgery outcome (Janszky et al., 2005), they do not consistently predict outcome in all cases and most patients with poor outcome present with the same clinical features as those with favourable outcome (Bonilha & Keller, 2015). Incomplete

resection of the putative epileptic focus does not account for all cases of poor outcome (Hennessy et al., 2000) and it has been suggested that generation and propagation of seizures in MTLE involves not just the seizure focus, but a network of temporal and extra-temporal brain regions. Structural morphometric changes in the bilateral thalamus and contralateral hippocampus have been implicated in persistent post-operative seizures in a group of 115 MTLE patients (Keller, Richardson, O'Muircheartaigh, et al., 2015). A study of 87 MTLE patients with unilateral HS found that bilateral thalamo-temporal structural network alterations were more prevalent in patients who continued to have post-operative seizures (Keller, Richardson, Schoene-Bake, et al., 2015). Another recent study found that patients who were seizure free post-surgery had pre-surgical functional MRI networks that were more resilient to damage (Liao et al., 2016). These studies all suggest that poor post-surgical outcome may be related to an abnormal widespread network of regions further supporting the network hypothesis in MTLE.

### **1.3.3 Heritability in MTLE**

Focal epilepsies are generally considered symptomatic, however, genetic factors may be important in some focal epilepsies including MTLE (Salzmann & Malafosse, 2012). Relatives of patients with focal epilepsy show a lower risk of epilepsy than relatives of those with generalised epilepsy, but an increased risk compared to healthy controls (Ottman, 1989). Offspring of patients with focal temporal EEG abnormalities have also been shown to have a higher chance of having temporal EEG abnormalities themselves and the inheritance of this gene may be autosomal dominant (Ottman, 1989).

Familial MTLE (FMTLE) is a benign syndrome with an autosomal dominant inheritance, is usually not associated with hippocampal sclerosis or febrile convulsions (Berkovic et al., 1996), and usually has a good outcome (Salzmann & Malafosse, 2012). Its relationship with febrile convulsions appears to vary between different families (Crompton et al., 2010; Depondt et al., 2002). In a study of 52 asymptomatic relatives of patients with FMTLE, 34% had reduced

hippocampal volumes suggesting this is a strongly heritable trait (Kobayashi et al., 2002). Mutations in the DEPDC5 gene have been identified as a cause of familial focal epilepsies (Dibbens et al., 2013; Ishida et al., 2013).

The heritability and genetics of sporadic MTLE is less clear. While a gene hasn't been found that is clearly associated with sporadic cases of TLE in general (Salzmann & Malafosse, 2012), a gene has recently been found that relates to a specific type of MTLE. In a genome-wide association study of 1018 individuals with MTLE with HS, a strong association was found with the sodium channel, voltage-gated, type 1, alpha subunit (SCN1A) gene, which was specific to patients who had MTLE with HS and a history of febrile seizures (Kasperavičiute et al., 2013). This suggests that MTLE with HS may be a condition requiring a genetic predisposition plus an additional environmental exposure, in this case an infection causing fever and leading to febrile seizures. It is unclear whether HS is a cause or consequence of febrile seizures with studies showing conflicting results (Auer et al., 2008; Bower, 2000; Cendes et al., 1993; Fernández et al., 1998), but we could speculate that there may be an endophenotype brain network that predisposes to seizures, causing some individuals to have febrile seizures that may eventually lead to HS.

Another gene recently identified was the Sestrin 3 gene, responsible for regulating a gene network related to local inflammation in the epileptic hippocampus of MTLE patients, suggesting that on-going local inflammation may be an important aspect of epileptogenesis (Johnson et al., 2015).

### **1.3.4 Endophenotypes**

The genotype is the complete set of genetic information of an individual, while the phenotype is the set of observable traits, or observable gene expression, which is influenced by both the genotype and the individual's environment. The distinction between these concepts have been established since the early 1900s through the work of Danish botanist Wilhelm Johanssen (Allen,

1979; Churchill, 1974). A more recent concept that has emerged is that of the *endophenotype*. The latter was first described by Bernard John and Kenneth R. Lewis who postulated that the geographic distribution of grasshoppers cannot be explained by the obvious and external “exophenotype” of the grasshoppers, but must be due to an internal “endophenotype” (John & Lewis, 1966). This concept was adapted and applied to the study of schizophrenia genetics in 1973 (Gottesman & Shields, 1973) and since the early 2000s has gained traction in the field of neuroscience and neuroimaging to aid in the investigation of the genetic underpinnings of complex psychiatric disorders (Flint & Munafò, 2007).

An endophenotype is an intermediate phenotype that lies between the genotype and the disease state. It may be any trait, or biomarker, that can be measured or observed in an individual, including cognitive, neuropsychological, neurophysiological, endocrinological or neuroanatomical measures (Gottesman & Gould, 2003). Several criteria have been suggested for the identification of a biomarker as an endophenotype (Gottesman & Gould, 2003) and I list them here in the context of epilepsy: 1) it should be associated with epilepsy, 2) it should be heritable, 3) it should be state-independent, and 4) it should co-segregate within families, and present at higher rate in unaffected family members than in the general population.

The rationale behind using endophenotypes is that they are thought to be more proximal to the genotype than phenotypes are, and may provide a starting point in identifying susceptibility genes associated with a complex polygenic disorder. In practice, studies of psychiatric disorders have identified several candidate endophenotypes but while some studies have come close, none have yet been successful at identifying specific susceptibility genes for psychiatric disorders (Iacono et al., 2014; Miller & Rockstroh, 2013). In Alzheimer’s disease, for example, neuroimaging endophenotypes and genome-wide association studies have shown some promise in identifying risk genes for late onset Alzheimer’s (Braskie et al., 2011). Many of these individual studies lack the statistical power to identify risk genes due to small sample sizes, so several initiatives have been set up to collaboratively investigate new genetic risk variants

associated with illnesses using large datasets and meta-analysis techniques. Two such initiatives are the Alzheimer's Disease Neuroimaging Initiative (ADNI; <http://www.loni.ucla.edu/ADNI>) and Enhancing Neuro Imaging Genetics through Meta-Analysis project (ENIGMA; <http://enigma.loni.ucla.edu/>). The ENIGMA epilepsy consortium has been working on translating endophenotypes into large MRI-based genetic mapping studies, consolidating data from multiple groups internationally.

In epilepsy research, interictal epileptiform discharges on the EEG have been identified as endophenotypes in "EEG affected" but clinically asymptomatic relatives of patients with juvenile myoclonic epilepsy, and these EEG endophenotypes were found to have a high genetic linkage to JME (Delgado-Escueta et al., 1989). Endophenotypes have successfully aided in the discovery of susceptibility genes for Rolandic epilepsy (also known as benign epilepsy of childhood with centro-temporal spikes (BECTS)), thought to be a highly heritable childhood epilepsy. Evidence for endophenotypes have been found in neurocognitive measures (Smith et al., 2012), centro-temporal sharp waves and verbal dyspraxia on EEG in relation to variation in the Elongator Protein Complex 4 gene (Pal et al., 2010; Strug et al., 2009), and migraine in relation to chromosome 17q12-22 (Addis et al., 2014). These findings suggest that endophenotypes have a promising role in the identification of susceptibility genes in epilepsy.

Several studies have also begun to investigate endophenotypes in adult epilepsies using neurophysiological and neuroimaging techniques. Potential endophenotypes have been identified using transcranial magnetic stimulation (TMS) motor evoked potentials and brain networks derived from EEG in idiopathic generalised epilepsy (Chowdhury et al., 2014; Chowdhury et al., 2015), functional MRI brain connectivity in juvenile myoclonic epilepsy (Wandschneider et al., 2014) and structural and diffusion MRI features in MTLE (Alhusaini et al., 2013; Alhusaini, Whelan, Doherty, et al., 2016; Scanlon, Ronan, et al., 2013; Whelan et al., 2015).

## **Chapter 2 EEG & MRI Techniques**

EEG and MRI are two modalities widely used in the clinical and research setting for evaluating brain activity and alterations in MTLE. This chapter describes the two techniques in general terms and their use in the study of epilepsy.

### **2.1 Electroencephalography (EEG)**

First recorded in humans by Hans Berger in 1929, EEG has become a mainstay in the diagnosis and study of epilepsy. EEG is an electrophysiological technique that records electrical activity related to neuronal activity in the brain. Neuronal communication and interaction induce electrical fields that can be detected by electrodes placed on the scalp, on the surface of the cortex or intracranially (e.g. depth electrodes). EEG with a simultaneous video recording is an important tool for recording paroxysmal events in patients with epilepsy and is one of the most widely used tools for epilepsy diagnosis (Panayiotopoulos, 2010).

#### **2.1.1 Signal generation**

Neurons communicate through a combination of electrical and chemical signals. Typically, intracellular communication involves an action potential that is conducted along the neuronal axon until it reaches the synapse. Intercellular communication at the synapse occurs through neurotransmitters that are released from the pre-synaptic cell to bind to either ionotropic or metabotropic receptors at the post-synaptic cell causing ion channels to open or close. In the case of ionotropic receptors, depending on the type of neurotransmitter, cations or anions then flow in or out of the post-synaptic cell creating a current dipole at the post-synaptic membrane. Excitatory neurotransmitters produce Excitatory Post-Synaptic Potentials (EPSPs) that increase the chance of a post-synaptic cell producing another action potential, while inhibitory neurotransmitters produce Inhibitory Post-Synaptic Potentials (IPSPs) that reduce the probability that another action potential is generated. (Purves et al., 2004).

Action potentials themselves are fast activities and although they make up the bulk of multi-unit activity recorded extracellularly, their spatial specificity and short duration relative to the typical sampling rate on scalp EEGs doesn't allow them to be measured on scalp EEG. Instead the electrical activity recorded by scalp EEG is thought to represent the summation of much slower post-synaptic activity (EPSPs and IPSPs) that gives rise to slower local field potentials (LFPs). LFPs are often generated by many neurons simultaneously and the bulk of this slower activity is thought to originate in pyramidal cells, which are aligned in parallel to each other and perpendicular to the cortical surface. These relatively slow and large signals from spatially aligned cells allow for greater spatial and temporal summation and easier detection on EEG (Michel & Brandeis, 2010). It is estimated that 1 million synapses synchronously activated in a cortical area of 4-6cm<sup>2</sup> are required to produce activity that can be measured with EEG (Hämäläinen et al., 1993).

### **2.1.2 General EEG acquisition**

In scalp EEG, 21 electrodes (19 active electrodes, plus a ground and reference electrode) are typically placed on the surface of the scalp according to the 10-20 system (see Figure 5.1 for this set-up in the context of a 64-channel array). This system is the international standard for the locations of electrodes and distances between electrodes on the scalp to make them comparable between recordings and studies. Each electrode location is labelled with a letter and a number. Odd numbers denote the left side of the brain, while even numbers denote the right side. The letters refer to the underlying brain area with F for Frontal, P for parietal, T for temporal, O for occipital, C for central and combinations thereof.

The scalp is usually prepared by lightly abrading the skin at each electrode location to remove dead skin cells and reduce signal impedance. Electrodes are then applied or adhered to the scalp using a conductive gel or paste. If many electrodes are required in the recording (e.g. in high-

density arrays of 64 channels or higher typically in research studies<sup>5</sup>), a cap is used with electrodes already embedded in it in order to avoid the need to measure each electrode location.

Electrical activity is typically measured as the voltage difference between two electrodes. As the EEG activity measured on the scalp has a very low amplitude (around 10-100 $\mu$ V) a differential amplifier is used to amplify the voltage difference between pairs of electrodes. Electrodes are displayed in two main ways: using a referential montage where the potential difference between an active scalp electrode and a common reference electrode (or an average of all electrodes) is displayed, or using a bipolar montage where the potential difference between two adjacent active scalp electrodes is displayed (Hughes, 1994). Clinical EEG is typically recorded at a sampling rate of around 256Hz, while higher sampling rates (up to around 5000Hz) could be used in research, depending on the application.

In the clinical telemetry setting, a typical EEG measurement could last anywhere between 20 minutes to several days in order to capture events of interest. A series of “activation procedures” is typically performed, including hyperventilation, photic stimulation, eye closure, sleep and sleep deprivation, to elicit normal or abnormal EEG responses that are sensitive to these stimuli. Besides spontaneous EEG activity, event related potentials (ERPs) are also typically used in research to study EEG responses to a given stimulus.

For display and analysis, filters are typically used to reduce noise or artefact in the EEG signal. A high-pass filter at 0.5Hz and a low-pass filter at 70Hz are typically used to limit the EEG signal to the frequencies of interest, and a notch filter is applied to remove 50Hz line noise (in the EU/UK).

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<sup>5</sup> High-density arrays usually have upwards of 64 channels and electrodes are typically placed either using an interpolated 10-20 system or equidistant to each other.

### 2.1.3 Normal and abnormal EEG activity

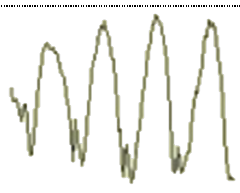
The EEG signal is traditionally divided into 5 main frequency bands – delta (<3.5Hz), theta (4-7.5Hz), alpha (8-13Hz), beta (14-30Hz) and gamma (>30Hz) – that characterise different brain states and functions. Combinations of these rhythms are found in the background EEG. The background rhythm typically has a higher frequency in adults than in children and slow frequencies dominate during sleep. The main rhythms and their functions and features are described in Table 2.1.

**Table 2.1** Conventional EEG frequency bands (adapted from Malmivuo and Plonsey, 1995).

| Band name (range) | Example trace                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Functions and features                                                                                                                                       |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Delta (<3.5Hz)    | <p>A line graph showing a Delta wave over a 4-second period. The x-axis is labeled 'Time [s]' with markers at 0, 1, 2, 3, and 4. The wave has a period of approximately 3-4 seconds, with peaks around 0.5s, 1.8s, and 3.2s.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Typically found in infants and in moderate to deep sleep in adults. Indicative of dysfunction if found in the awake state in adults.                         |
| Theta (4-7Hz)     | <p>A line graph showing a Theta wave over a 4-second period. The wave has a period of approximately 0.5-0.7 seconds, with peaks around 0.5s, 1.0s, 1.5s, 2.0s, 2.5s, 3.0s, and 3.5s.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Typically found in children and sleeping or drowsy adults. May indicate dysfunction if found in alert and awake adults. Often associated with focal lesions. |
| Alpha (8-13Hz)    | <p>A line graph showing an Alpha wave over a 4-second period. The wave has a period of approximately 0.1-0.15 seconds, with peaks around 0.1s, 0.2s, 0.3s, 0.4s, 0.5s, 0.6s, 0.7s, 0.8s, 0.9s, 1.0s, 1.1s, 1.2s, 1.3s, 1.4s, 1.5s, 1.6s, 1.7s, 1.8s, 1.9s, 2.0s, 2.1s, 2.2s, 2.3s, 2.4s, 2.5s, 2.6s, 2.7s, 2.8s, 2.9s, 3.0s, 3.1s, 3.2s, 3.3s, 3.4s, 3.5s, 3.6s, 3.7s, 3.8s, 3.9s, and 4.0s.</p>                                                                                                                                                                                                                                                                                 | Normal activity found in relaxed waking adults with eyes closed, predominantly in occipital channels. Should attenuate or disappear when eyes are open.      |
| Beta (13-30Hz)    | <p>A line graph showing a Beta wave over a 4-second period. The wave has a period of approximately 0.05-0.07 seconds, with peaks around 0.05s, 0.1s, 0.15s, 0.2s, 0.25s, 0.3s, 0.35s, 0.4s, 0.45s, 0.5s, 0.55s, 0.6s, 0.65s, 0.7s, 0.75s, 0.8s, 0.85s, 0.9s, 0.95s, 1.0s, 1.05s, 1.1s, 1.15s, 1.2s, 1.25s, 1.3s, 1.35s, 1.4s, 1.45s, 1.5s, 1.55s, 1.6s, 1.65s, 1.7s, 1.75s, 1.8s, 1.85s, 1.9s, 1.95s, 2.0s, 2.05s, 2.1s, 2.15s, 2.2s, 2.25s, 2.3s, 2.35s, 2.4s, 2.45s, 2.5s, 2.55s, 2.6s, 2.65s, 2.7s, 2.75s, 2.8s, 2.85s, 2.9s, 2.95s, 3.0s, 3.05s, 3.1s, 3.15s, 3.2s, 3.25s, 3.3s, 3.35s, 3.4s, 3.45s, 3.5s, 3.55s, 3.6s, 3.65s, 3.7s, 3.75s, 3.8s, 3.85s, 3.9s, and 4.0s.</p> | Normal activity found in eyes open or closed waking adults, predominantly in frontal or midline channels. Associated with active thinking or focus.          |
| Gamma (>30Hz)     | n.a.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Associated with high-level information processing.                                                                                                           |

Abnormal patterns such as background slowing, spikes, spike and wave discharges and sharp waves may occur in EEG recordings. For example, the background rhythm shows a decreased frequency in patients with focal lesions, tumours, head injury, and haemorrhages. Patients with MTLE frequently show IEDs in the form of spikes or sharp waves over the anterior-temporal electrodes on the side of the lesion. Seizures are characterised by abnormally synchronised high-amplitude rhythmic activity on the EEG. Importantly, abnormal EEG patterns are not entirely specific or sensitive to epilepsy, and while the EEG is an important tool in diagnosing epilepsy, interpretations have to be taken in the context of the clinical presentation of the patients (Smith & Kennet, 2011). The main EEG abnormalities found in epilepsy patients are described in Table 2.2.

**Table 2.2** Epileptiform activity on EEG (adapted from Blume et al., 2002).

| Epileptiform activity | Example trace                                                                       | Features                                                                                                 |
|-----------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Spike                 |  | Transient with pointed peak distinguishable from background with a duration of <80ms.                    |
| Sharp wave            |  | As above but with duration of 80-200ms.                                                                  |
| Spike and slow-wave   |  | Consisting of a pointed peak followed by a slow-wave.                                                    |
| Focal slowing         | n.a.                                                                                | Rhythm appearing at a lower frequency than in a normal EEG, e.g. theta or delta waves in a waking adult. |

#### 2.1.4 EEG analysis

Clinical EEG recordings are qualitatively reported by trained electrophysiologists. In research, methods for analysing EEG data vary widely depending on application. The most common

methods include analysis of the power spectrum of the EEG to determine the constituents and relative power and frequency of the main EEG bands, and analysis of averaged ERPs to investigate EEG responses to stimuli.

The scalp EEG presumably consists of activity from many independent sources. Ideally, we would like to know everything about the sources (where they are, what rhythms/activities the source generates etc.), however the estimation of independent cortical sources that give rise to scalp EEG is not trivial. Known as the inverse problem, it has no unique solution as there are a much larger number of possible sources compared to data sampling points, or EEG electrodes (Lopes Da Silva, 2010). Much work has been done in source localisation and some of this will be mentioned in the next chapter, but this method is not considered further in this thesis.

## **2.2 Magnetic resonance imaging**

Magnetic resonance imaging (MRI) produces detailed images of internal anatomy using the principles of nuclear magnetic resonance. MRI typically uses the properties of the hydrogen nucleus in a magnetic field, taking advantage of the abundance and different concentrations of water, as well as other molecules containing hydrogen nuclei such as fat, in different tissue types.

### **2.2.1 Signal generation**

The hydrogen nucleus contains a positively charged proton and acts as though spinning on its own axis, producing a tiny magnetic field called a magnetic moment. A spinning proton (often referred to as a “spin”) placed in an external magnetic field ( $B_0$ ), as in an MRI scanner, will experience a torque which makes it try to align itself with the direction of the field, although it cannot align perfectly with  $B_0$  and instead continues to move around this axis. This motion of the spin around the  $B_0$  axis is known as precession and can be compared to the motion of a spinning top under the influence of gravity. In reality, some protons may align in the opposite direction to

the field (anti-parallel), but many more will favour the slightly lower energy state of aligning with the external field (parallel) so that the net magnetisation effect ( $M_0$ ) is in the direction  $B_0$ . All the protons in the magnetic field will precess at the Larmor frequency which is proportional to  $B_0$ , a feature which is known as resonance (McRobbie et al., 2006).

$M_0$  can be rotated through  $90^\circ$  using a radiofrequency (RF) pulse ( $B_1$ ) applied at the Larmor frequency. This generates a signal that can be detected by a receiver coil that is sensitive to magnetic fields in the transverse plane. However, the signal disappears very quickly after applied due to various tissue and scanner properties, so an “echo” signal is usually collected instead. This can be in the form of a gradient echo (GE) or a spin echo (SE) pulse sequence.

### **2.2.2 Producing the MR image**

Relaxation times are the time it takes for the protons to return to equilibrium after a perturbation;  $T_2$  (and  $T_2^*$ ) refer to how long it takes for the detected signal to decay away, while  $T_1$  refers to the time it takes for the magnetisation to return to its original orientation (aligned with  $B_0$ ). Fluids tend to have longer  $T_1$  and  $T_2$  relaxation times, followed by water-based tissues, with fat-based tissues having the shortest relaxation times.

In acquiring an MRI image, there are a range of pulse sequences that can be used, the timings of which determine exactly what signal is recovered from particular tissues. The above allows us to detect a signal, and by applying magnetic field gradients we can arrange that the Larmor frequency will be different at different points in the magnet, allowing us to determine where the signal is coming from and therefore to create an image.

### **2.2.3 Structural MRI**

Structural MRI (sMRI) produces images of anatomical structure with high spatial resolution taking advantage of differences in the  $T_1$  and  $T_2$  relaxation times of different tissues.  $T_1$ - and

T2-weighted images are the most common images acquired in the clinical MRI setting and form the bulk of what is referred to as “structural MRI”. These imaging techniques produce images of the anatomical structure of the brain with high spatial resolution, signal to noise ratio and tissue contrast, allowing for the identification of anatomical location and morphology of specific structures in the brain (Symms et al., 2004).

T1-weighted imaging produces images of the brain with excellent grey/white matter contrast allowing for the qualitative and quantitative analysis of volumes and shapes of structures of interest. In a T1-weighted image, the tissues with highest water content have the lowest signal intensity, so cerebrospinal fluid (CSF) appears darkest, followed by grey matter (GM), and white matter (WM) appears brightest (McRobbie et al., 2006). T1-weighted images are widely used in assessing volumetric atrophy in patients with hippocampal sclerosis (Panayiotopoulos, 2010). Figure 2.1 shows an example of hippocampal volume loss in a patient with epilepsy.

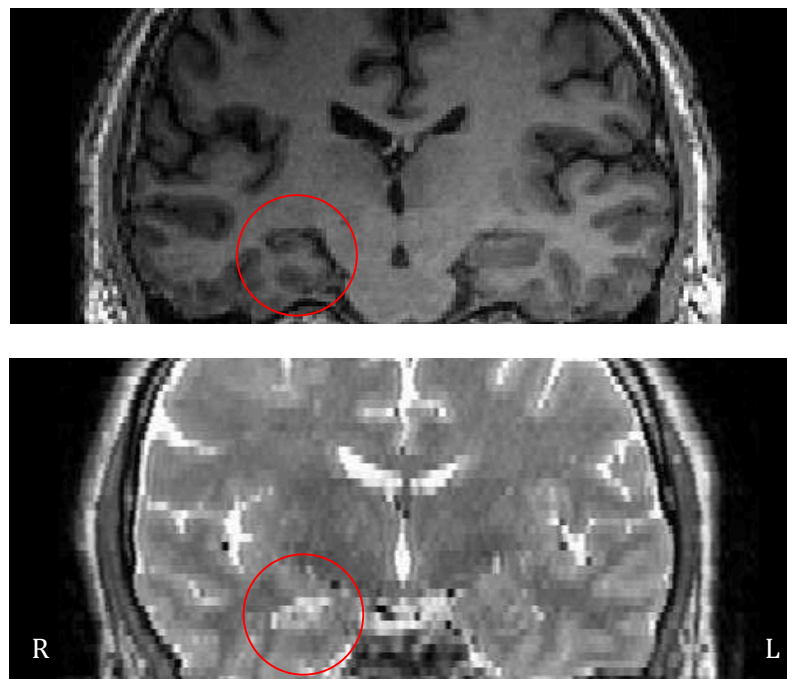


**Figure 2.1** Example MRI images from a patient with right hippocampal sclerosis who participated in the study reported in this thesis (TLE/P/027). Top: Coronal slice of a T1-weighted image showing right hippocampal atrophy. Bottom: Coronal slice of a T2-weighted image with the sclerotic hippocampus appearing brighter than surrounding normal tissue. Ipsilateral thalamic atrophy, an enlarged ipsilateral lateral ventricle and small ipsilateral fornix are also evident.

T2-weighted imaging is particularly sensitive to fluid and is used for detecting pathology. The signal in a T2-weighted image is a combination of the T2 relaxation properties of the tissue and its proton density and most T2-weighted scans are optimised to highlight the former. The Fluid Attenuated Inversion Recovery (FLAIR) sequence is a type of T2-weighted scan that suppresses signal from CSF (McRobbie et al., 2006). It is particularly good for identifying lesions on MRI and commonly used in the identification of focal brain lesions in patients with epilepsy, with lesions appearing brighter than surrounding normal tissue on the images (Panayiotopoulos, 2010).

In research, T1-weighted sMRI data are often used in volumetry- and morphometry-based analyses. Volumetry is the study of volumes of brain regions of interest, with brain regions usually defined either by manual delineation or automatic segmentation methods. Morphometry studies the morphometric properties of cortical surfaces (e.g. shape, cortical thickness). Commonly used imaging analysis software for such MRI research include FreeSurfer (Laboratory for Computational Neuroimaging, Athinoula A. Martinos Center for Biomedical Imaging, MA, USA; <http://surfer.nmr.mgh.harvard.edu>), the FMRIB Software Library (FSL; FMRIB, University of Oxford, UK; <https://fsl.fmrib.ox.ac.uk>), and the Statistical Parametric Mapping suite (SPM; Wellcome Trust Centre for Neuroimaging, University College London, UK; <http://www.fil.ion.ucl.ac.uk/spm>).

## **2.2.4 Diffusion-weighted MRI**

Diffusion weighted imaging (DWI) is a technique that applies diffusion encoding gradients in several different directions to measure the direction and extent of the random movement of water molecules in brain tissues. This method produces detailed images that enable us to study white matter architecture and allows for the inference of white matter connections in the brain in vivo. DWI is a relatively new method, having been developed in the last 20 years or so, and is primarily used in research in epilepsy in assessing white matter microstructure during the interictal state and not commonly used in the clinical environment (Symms et al., 2004).

In an isotropic medium, water molecules are free to move and collide, or diffuse, in any direction in a process called Brownian motion. The movement of water molecules in an anisotropic medium (such as biological tissue) can be described by the diffusion tensor (DT) model (Basser et al., 1994). Diffusion tensor imaging (DTI) in its most basic form fits an ellipsoid, or tensor, at each voxel whose shape and size depict the estimated direction of water movement in each direction (x, y and z) and any relationships between these directions. The DT image is often visualised using the Red-Blue-Green (RGB) colourmap, which encodes the principal direction of the tensor at each voxel (Pajevic and Pierpaoli, 1999; Figure 2.2).

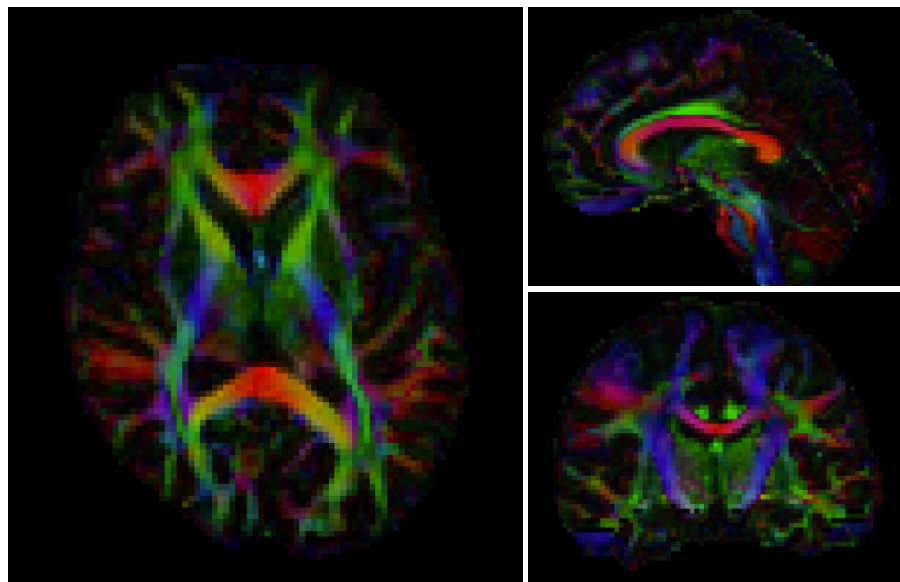


**Figure 2.2** Example of DTI RGB colourmap from patient TLE/P/027. Voxels in red represent fibres in the left-right direction, voxels in green represent fibres in the anterior-posterior direction and voxels in blue represent fibres going superiorly or inferiorly.

Parameters that can be extracted from DTI data include the apparent diffusion coefficient (ADC), mean diffusivity (MD), and fractional anisotropy (FA) of the particular voxel. The ADC is an index of water mobility, or how easily water molecules can diffuse in a particular direction within different tissue types. The MD is the average of the ADCs across different directions, therefore representing the average mobility of water molecules. Higher diffusivity values are seen in the cerebrospinal fluid where there is less obstruction to movement, while lower diffusivity values are seen in white matter and grey matter. FA measures the degree of diffusion anisotropy in a

tissue. A tissue is said to be anisotropic if water diffusivity in a particular direction is preferred in that tissue type. For example, in white matter, tissue microstructure encourages the diffusion of water along axonal bundles but restricts diffusion perpendicular to tracts. These measures are indirectly related to a number of tissue features including myelination of nerve fibre bundles.

Modelling the direction of white matter fibres is not trivial and the DTI model is unable to resolve multiple overlapping fibres or more complex fibre orientations (like fanning, bending and crossing fibres). Higher-order models have been suggested to overcome these limitations and include constrained spherical deconvolution (CSD), which estimates the fibre orientation distribution (FOD) using a response function, rather than estimating a single tensor at each voxel (Tournier et al., 2004).

Diffusion MRI tractography is a method for estimating white matter fibre bundles or pathways in the brain. In deterministic fibre tractography, fibres are “tracked” from a seed voxel, following the direction of maximum diffusivity in each subsequent voxel to produce a “streamline” that can be terminated when it reaches a tissue of low anisotropy (i.e. in CSF or GM) or if the angle of curvature of the fibre tract exceeds a predefined value that is unlikely to reflect actual tract curvature. This streamline represents the most likely path of white matter fibre connecting two voxels (Behrens & Jbabdi, 2013). Streamline tractography is subject to errors from a wide range of sources including imaging noise, participant motion and model errors and these errors are compounded with the length of streamline. Probabilistic tractography is a method that builds probability maps of tract trajectories from each seed voxel, with the final image representing some measure of the certainty with which a tract might be present between two voxels (Behrens & Jbabdi, 2013).

A particularly useful application of streamline tractography is the 3D in vivo dissection of known white matter tracts, a method that has been shown to very closely match the underlying white matter anatomy obtained using blunt dissection of the post-mortem brain (Lawes et al., 2008).

Particular tracts of interest in MTLE are those starting and terminating in the hippocampus and limbic regions (Figure 2.3).

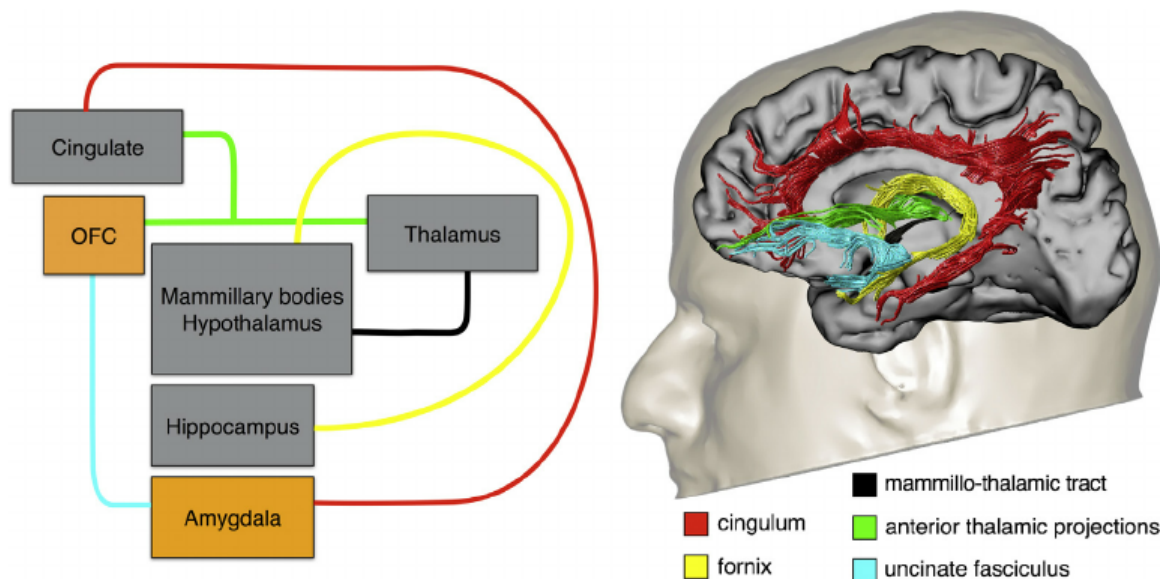


**Figure 2.3** Left: A pictorial representation of the connections between different regions of the limbic system. Right: White matter tracts of the limbic system dissected using in vivo tract tracing methods. Figure taken from Catani and Thiebaut de Schotten, 2012.

## 2.2.5 Functional MRI

First described by Seiji Ogawa in 1990, blood oxygenation level dependent (BOLD) functional MRI (fMRI) is a type of T2\*-weighted image that gives an indirect measurement of neural activity related to changes in blood oxygenation in the brain (Buxton, 2002). T2\*-weighted images are sensitive to magnetic susceptibility, and take advantage of the paramagnetic properties of deoxyhaemoglobin.

The BOLD effect is a combination of changes in blood flow and oxygen metabolism following neural activation. Neuronal activity requires energy in the form of sugar and oxygen, supplied by blood to surrounding cells. An increase in activity causes an increase in the local consumption of oxygen accompanied by an increase in the supply of oxygenated blood. The oxygenated blood supplied to an activated region exceeds that utilised by the tissue, resulting in a reduction in the concentration of deoxyhaemoglobin in the veins leaving the activation site. This causes an

increase in the  $T2^*$  and the BOLD signal increases (McRobbie et al., 2006). Several studies have shown that the increase in the BOLD response is linearly related to local increases in neural activity, as assessed by simultaneously acquired electrophysiological data (Logothetis, 2002, 2003).

BOLD-fMRI (from here on referred to as fMRI), although indirect, allows for non-invasive mapping of brain activity at a higher spatial resolution than available with scalp EEG, albeit at a lower temporal resolution, due in part to the so-called “haemodynamic delay”. The neural activity detected with fMRI typically lags behind the actual neuronal activity by as much as 6s, and the effect observed also lasts much longer. The haemodynamic response function (HRF) is a mathematical representation of the estimated BOLD signal in response to an impulse stimulation. It is used in the generalised linear model analysis of fMRI data to identify brain activity related to specific stimuli (Buxton et al., 2004). The HRF is typically modelled as two gamma functions, with a delay, a steep increase, slower decrease and post-stimulus undershoot (Figure 2.4).

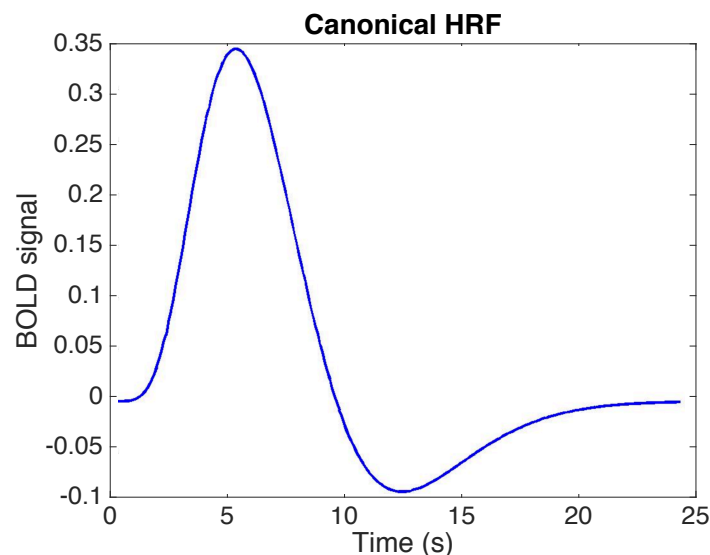


**Figure 2.4** Canonical haemodynamic response function (plot in Matlab using SPM’s canonical HRF).

Unlike sMRI, fMRI is not as widely used in the clinical environment, however it does have utility as a non-invasive alternative to the Wada test in pre-surgical lateralisation of language and memory (Richardson, 2010; Richardson et al., 2004). fMRI is also widely used in the epilepsy research setting with applications in the study of abnormalities in relation to cognition and memory (Vlooswijk et al., 2010), and interictal connectivity networks at rest (Cataldi et al., 2013).

### **2.2.6 Simultaneous EEG and fMRI**

EEG generally has higher temporal resolution but lower spatial resolution than fMRI, while fMRI benefits from a high spatial resolution but has low temporal resolution. Each modality measures slightly different aspects of changes related to neural activity at different temporal and spatial resolutions, and leveraging the strengths of the two methods allows us to build a better picture of neural activity (Goense & Logothetis, 2010). Primarily developed as a tool to investigate activity related to epileptiform discharges, EEG was first recorded within the MRI scanner by Ives and colleagues in 1993 (Ives et al., 1993). This was followed by “interleaved” EEG-triggered fMRI in 1996 (Warach et al., 1996), and more recently, the development of simultaneously recorded EEG and fMRI (EEG-fMRI) (Bonmassar et al., 1999; Lemieux et al., 2001). These methods have allowed the localisation of haemodynamic correlates of EEG activity.

The acquisition of simultaneous EEG-fMRI data has seen much development over recent years and is now an established and safe technique, albeit it is not without its challenges (Huster et al., 2012). The two key issues in acquiring EEG-fMRI data are ensuring high EEG and fMRI data quality and patient safety. There are currently several major manufacturers of commercially available simultaneous EEG-fMRI systems and the system used in this thesis was developed by Brain Products (Brain Products GmbH, Munich, Germany). The setup involves placing MR-compatible EEG equipment in the scanner bore, which is a non-trivial setup as the equipment has to be specially designed to ensure patient safety and data quality (e.g. use of non-ferrous

materials, ensuring electrodes are not in direct contact with the skin, the inclusion of current-limiting resistors on each electrode lead, reducing current induction due to loops and wire lengths, reducing equipment motion with the use of tapes or sandbags etc.). The EEG signal is recorded through EEG electrodes connected to the MR-compatible amplifiers, which amplify and digitise the signals within the MR suite. These signals are then passed on to the recording equipment located in the MR control room through fibre-optic cables. The EEG clock is also synchronised with the MR clock using a SyncBox from Brain Products, to ensure that both sets of data are acquired in sync and to improve MR artefact removal (see below) from the EEG signal.

The interactions of the EEG electrodes, the MR magnetic fields (due to the static magnetic field, switching magnetic field gradients and radio frequency pulses), and pulse-related (ballistocardiogram; BCG) and participant motion produce artefacts due to induced currents on the EEG signal. These artefacts cause degradation of the EEG signal and need to be addressed to recover the underlying EEG signal from data recorded in the scanner. Due to the approximately constant nature of these artefacts, they can usually be removed using template subtraction algorithms provided with the EEG-fMRI system. These methods are not always perfect and may sometimes be complicated due to practicalities of data acquisition including participant motion and drift in electrode impedances.

EEG-fMRI is currently used mainly as a research tool, but has applications as a non-invasive tool in pre-surgical management. It is used in the localisation of epileptiform activity and some studies have shown that its accuracy may be comparable to more established methods like intracranial EEG in identifying the seizure foci and epileptic zones in patients with epilepsy (van Graan et al., 2015).

## **Chapter 3 The Network Concept in MTLE: State of Current Research**

### **3.1 Pathology within and beyond the ipsilateral hippocampus**

Hippocampal sclerosis (HS) is the most common structural pathology found in patients with MTLE. Based on histology, classic hippocampal sclerosis is characterised by neuronal loss and gliosis in the Ammon's horn region of the hippocampus, in particular in the cornu ammonis 1 (CA1) subfield, and to a lesser extent CA4 and CA3 as described by Sommer in 1880 and then Bratz in 1899 (Margerison and Corsellis, 1966) a finding that has been also confirmed in post-mortem cell-counting studies (Steve et al., 2014).

Hippocampal sclerosis can presently be reliably identified on MRI. On T1-weighted scans, atrophy, reduced signal intensity, and alterations of the internal structure of the hippocampus are seen, while on T2-weighted scans, an increased signal is observed (Van Paesschen, 2004). Besides a qualitative assessment of hippocampal integrity and signal, quantification of hippocampal volume in vivo is possible by manual delineation of MRI images or with automated MRI analysis software. Quantitative methods are especially useful in cases where hippocampal atrophy is not large enough to be immediately apparent, there is bilateral hippocampal pathology, varying degrees of atrophy along the hippocampus, or atrophy in neighbouring brain regions (Duncan, 2008). In an early study using a combination of hippocampal T2 relaxometry and hippocampal volume ratio, hippocampal sclerosis, amygdala sclerosis and end-folium sclerosis were identified in a group of intractable MTLE patients (Van Paesschen et al., 1995). With further advances in high-resolution MRI imaging, in vivo quantification of hippocampal sub-field abnormalities have more recently been performed using manual segmentation (Mueller et al., 2009). These methods, however, are laborious requiring a high level of expertise in imaging anatomy, and recent developments in MRI analysis and segmentation techniques have resulted in automatic segmentation techniques that have shown high concordance to underlying histopathology (Schoene-Bake et al., 2014).

### 3.1.1 Extra-hippocampal structural abnormalities

Using advanced whole brain volumetric and morphometric analysis techniques on imaging data, structural abnormalities outside of the affected hippocampus have also been reported in patients with MTLE. Local atrophy in mesial temporal regions, predominantly in the hemisphere ipsilateral to the affected hippocampus (Keller & Roberts, 2008), have been reported in the parahippocampal gyrus, entorhinal cortex, perirhinal cortex and amygdala (Bernasconi et al., 2003, 2004; Bonilha et al., 2004; Keller et al., 2002). Structural changes also extend beyond the temporal lobes and have been reported in extra-temporal and subcortical structures, including the cingulate gyrus, frontal and parietal lobes, thalamus, and cerebellum (Bernasconi et al., 2004; Bernhardt et al., 2010; Bonilha et al., 2004; Cormack et al., 2005; Keller et al., 2014, 2009; Sandok et al., 2000).

Of these regions, the most common change found outside of the hippocampus is the bilateral atrophy of the thalamus in voxel-based morphometry (VBM)<sup>6</sup> studies (Keller & Roberts, 2008), in particular the medial dorsal nucleus of the thalamus (Barron et al., 2013). This atrophy appears to be correlated with different aspects of memory and cognition than the hippocampus (Seidenberg et al., 2008; Stewart et al., 2009). Using MRI volumetry and DTI tractography, abnormalities of the thalamus have been found to be specific to regions that have connections to the epileptic temporal lobe. A structural MRI and DTI study of thalamo-temporal impairment found bilateral volume loss, streamline reduction and increase in the T2 signal in the anterior, dorsomedial and pulvinar regions of the thalamus (Keller et al., 2014). These abnormalities were found to be correlated with the extent of MTL damage in the same study. Another study found atrophy of the medial surface of the ipsilateral thalamus in MTLE patients correlated to a wider network of structural alterations in bilateral frontocentral, lateral and mesial temporal regions. These alterations were also more severe with increasing epilepsy duration and in patients with a

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<sup>6</sup> Voxel-based morphometry (VBM) is an automated technique that allows for voxel-wise quantification of the amount of a specific tissue type (Ashburner and Friston, 2000) and is described further in Chapter 5.

history of febrile convulsions (Bernhardt et al., 2012). These findings suggest a role for the thalamus in the epileptogenic network.

### **3.1.2 White matter alterations**

Accompanying the widespread grey matter changes found in MTLE are changes in the underlying white matter architecture, or the pathways that connect these regions, as assessed by DTI. Widespread changes in white matter MD and FA have been consistently found primarily in the ipsilateral temporal lobes and limbic system in patients with MTLE, but also in the contralateral temporal lobe and bilateral regions outside of the temporal lobes (Arfanakis et al., 2002; de la Roque et al., 2005; Focke et al., 2008; Rodríguez-Cruces & Concha, 2015; Thivard et al., 2005). A meta-analysis of white matter changes in MTLE compared to controls found that these changes were more pronounced in the ipsilateral than the contralateral hemisphere, and that the degree of change depended on how closely the region was connected (whether directly or indirectly) to the affected hippocampus (Otte et al., 2012).

DTI tractography has also been used to dissect white matter tracts in vivo to assess for abnormalities of known tract bundles in MTLE. The most severely affected white matter tracts in MTLE, as measured by reduced FA, increased MD, or reduced tract volume, are found bilaterally in the temporal, parahippocampal and limbic system fibers, particularly the fornix, cingulum and uncinate fasciculus (Ahmadi et al., 2009; Concha, Beaulieu, et al., 2005; Diehl et al., 2008; Focke et al., 2008). Abnormalities were bilateral and symmetrical in the fornix and cingulum (Concha, Beaulieu, et al., 2005) and these abnormalities in the contralateral tracts persist after surgery (Concha et al., 2007).

In many of the above studies, the degree of abnormality relates to the duration of epilepsy (Richardson, 2012), and may reflect a combined effect of neuronal loss and synaptic reorganisation due to prolonged seizures (Bernhardt et al., 2013). However, there have also

been conflicting reports on whether these changes are related to disease duration or other clinical variables, so the mechanisms of the propagation of white matter changes outside of the affected hippocampus are still unclear (Otte et al., 2012).

The above morphometric & diffusion techniques are important for patient lateralisation & identifying targets for surgical resection, since the identification of a clear epileptogenic lesion significantly improves post-surgical outcome. However, the widespread abnormalities found both within and outside of the temporal lobe suggest that MTLE is not a focal disorder, but involves a focal lesion with a network of surrounding white matter and grey matter changes, and highlights the importance of the concept of the epileptogenic network in understanding, diagnosing and treating epilepsy.

## **3.2 Epileptogenic networks in MTLE**

The network hypothesis in MTLE, while not a new concept, is now more widely accepted. With increasing evidence of widespread bilateral grey and white matter abnormalities from neuroimaging research in MTLE, the notion that a single pathological region, the hippocampus, is involved in the generation of MTLE seizures has shifted to that of a complex network of regions being involved in the generation and propagation of focal epileptic seizures (Berg et al., 2010; Richardson, 2012; Spencer, 2002; van Diessen et al., 2013).

### **3.2.1 Networks involved in ictal onset and propagation**

Ictal single photon emission computed tomography (SPECT) imaging is a technique that uses a tracer to provide a picture of blood flow. This technique has allowed for the investigation of networks during ictal events by subtracting an interictal SPECT from an ictal SPECT study (in which the tracer was injected at a specific time related to seizure onset). During a seizure, there is an increase in neuronal metabolic activity and consequently an increase in cerebral blood flow in regions involved in the seizure. The general sequence of blood flow changes seen in complex

partial seizures in MTLE begins with a hyperperfusion of the entire temporal lobe at ictal onset. This is followed by hyperperfusion of the MTL regions and hypoperfusion of the lateral TL regions up to 2 minutes post-ictally, and then up to 15 minutes post-ictally there is hypoperfusion of the whole temporal lobe. A return to baseline levels of perfusion are seen between 10 to 30 minutes later (Duncan et al., 1993; Newton et al., 1995). This method has reliably been used to localise the seizure onset region in MTLE patients (McNally et al., 2005; O'Brien et al., 1998; Van Paesschen et al., 2000).

Studies have also shown regions with perfusion changes outside of the temporal lobes. In addition to ictal hyperperfusion in the ipsilateral temporal lobe regions, hyperperfusion has also been found in the contralateral temporal lobe, cerebellum, thalamus and basal ganglia (Bohnen et al., 1998; Shin et al., 2001; Spencer, 2002). Hypoperfusion is commonly found in the frontal lobes (Nelissen et al., 2006; Rabinowicz et al., 1997; Van Paesschen et al., 2003), and is thought to be a surround inhibition response against seizure propagation. Contralateral hyperperfusion is often seen to mirror ipsilateral regions, possibly reflecting regions of seizure propagation (Huberfeld et al., 2006). A study comparing relative differences in ictal and interictal perfusion within patients suggested an altered network of regions involved in seizure generation and propagation including the hippocampus, thalamus, insular cortex and paracentral lobules, with ictal hypo perfusion in the anterior frontal lobes (Tae et al., 2005).

Using depth electrodes, studies have been able to identify several mesial temporal lobe regions showing synchronous oscillations at seizure onset, suggesting that the epileptogenic zone does not consist of just a single focus. The most commonly reported regions include a network involving the hippocampus, amygdala, entorhinal cortex and temporal pole (Bartolomei et al., 2008), with some studies finding that activity in the temporal pole preceded or occurred concurrently with the hippocampus at seizure onset in some patients (Chabardès et al., 2005; Kahane et al., 2002). Studies were also able to distinguish between several sub-types of TLE based on seizure onset and propagation patterns (Bartolomei et al., 1999, 2004, 2001; Gotman &

Levtova, 1996). Most of these studies seem to indicate a strong coherence between several temporal lobe leads at the start and end of seizure activity, but the maintenance of this interaction is not required for sustained seizure activity (Duckrow & Spencer, 1992; Le Van Quyen et al., 1998). From the analysis of coherence of inter-hemispheric intracranial signals, the major commissures are thought to be involved in the initial contralateral spread of TL seizures, but are do not play a role in sustaining the seizures contralaterally (Gotman, 1987; Lieb et al., 1987). These studies indicate that ictal initiation and propagation involve a network of regions both within and outside of the epileptogenic hippocampus.

### **3.2.2 Interictal sub-networks associated with MTLE**

EEG-fMRI studies have enabled the localisation of metabolic changes related to interictal epileptiform discharges (IEDs) observed on scalp EEG (Salek-Haddadi et al., 2003). Although they tend to be highly variable between individuals, BOLD activation related to IEDs are generally spread across the ipsilateral temporal neocortex, with some contralateral activation and extratemporal activation, with few patients showing exclusively mesial TL activation (Kobayashi et al., 2006; Lemieux et al., 2008; Salek-Haddadi et al., 2006). Interactions between interictal epileptogenic networks and large-scale brain networks have also been studied and IEDs have been shown to deactivate the default mode network (DMN), possibly reflecting altered consciousness during IEDs indicating a direct relationship between the DMN and EEG activity (Fahoum et al., 2013; Laufs et al., 2007).

Alterations in interictal sub-networks have also been shown to have some value in predicting surgical outcome of MTLE patients. A review of quantitative MRI alterations in MTLE patients found that pre-surgical abnormalities in the connectivity of extra-temporal brain regions may be linked to a higher chance of post-surgical seizure persistence (Bonilha & Keller, 2015). In a study of 87 pre-surgical MTLE patients, the 40 patients who subsequently continued to have seizures were found to have bilateral thalamotemporal structural network alterations compared to those

who were seizure free (Keller, Richardson, Schoene-Bake, et al., 2015). Studies have also shown that specific network architectures and network “hubs”<sup>7</sup>, may also be involved in post-surgical seizure persistence. In a study of pre-surgical fMRI brain networks of 37 MTLE patients, those who were subsequently seizure free showed a pre-surgical network that was more resilient to targeted attack than those who were not seizure free (Liao et al., 2016). These findings suggest there may be components of an extra-temporal network capable of generating seizures autonomously, even after surgical removal of the epileptogenic focus.

### **3.2.3 Evidence for large-scale network abnormalities in MTLE**

There has been a growing body of evidence to suggest that network alterations in MTLE extend beyond the immediate temporal lobe and involve large-scale structural and functional network abnormalities and interactions. In imaging and electrophysiology, whole-brain network alterations are usually studied using network connectivity approaches.

Functional connectivity assessed using resting-state fMRI has consistently shown networks of brain regions with spontaneous correlated activity in healthy controls (Smith et al., 2009). Particular interest has been shown in activity and interactions with the DMN, the primary network showing increased activity at rest, centred on the posterior cingulate cortex and medial prefrontal cortex, and involving a mesiotemporal sub-system including the hippocampus (Greicius et al., 2003). Reduced functional connectivity has been reported in mesial temporal regions as well as in the DMN in patients with MTLE (Liao et al., 2011; Zhang et al., 2010). The decreases in functional connectivity are related to the duration of epilepsy (Zhang et al., 2010) with suggestions that they are caused by white matter damage associated with seizures (Voets et al., 2012). The bilateral hippocampus and amygdala have also shown decreased functional connectivity with DMN regions, with the epileptic side being less connected than the contralateral side (Pittau et al., 2012).

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<sup>7</sup> Network hubs are regions of the brain that have a high influence on the connectivity and integration of the network. It is a concept in the graph theoretical analysis of networks, further described in Chapter 5.

Graph network analysis<sup>8</sup> has been applied to resting fMRI data in MTLE, although relatively few studies have performed whole-brain resting fMRI studies of networks in patients with MTLE. In a whole-brain network analysis of 90 cortical and subcortical regions, increased connectivity within the mesial temporal lobe was observed, while widespread decreases in connectivity were seen in the rest of the brain (Liao et al., 2010). Patients showed altered small-world network properties including decreased node degree, clustering coefficients and path lengths. In another study examining the connectivity in 36 limbic and mesial temporal regions in MTLE patients, five network measures – degree, strength, closeness, clustering coefficient and betweenness centrality – were used in a classification analysis to distinguish MTLE patients from controls (Zhang et al., 2011). In a relatively small study of 6 medically refractory epilepsy patients, whole-brain voxel-wise networks were constructed and regions with increased fMRI connectivity coincided with epileptogenic regions identified using invasive EEG (Stufflebeam et al., 2011).

DTI structural connectivity networks in TLE have been investigated using tractography to define network connections. Using deterministic tractography on DTI data segmented into 1015 regions of interest (ROIs), left TLE patients showed reduced long-range connectivity, but increased local connectivity in several default mode network (DMN) regions (Desalvo et al., 2014). Another study of left MTLE patients using a 78 ROI parcellation and probabilistic tractography showed reduced connectivity both globally and locally in patients compared to controls (Liu et al., 2014). A study of networks in the limbic system of MTLE patients showed increased clustering within the limbic system with regions showing higher clustering, efficiency and degree (Bonilha et al., 2012). White matter network abnormalities may also be related to cognitive comorbidities in patients (Vaessen et al., 2012).

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<sup>8</sup> A mathematical framework for the analysis of networks. Network measures including degree, clustering, path length, efficiency and small-worldness are described in Chapter 5.

Network theory has also been applied to electrophysiological data in MTLE patients. In an electrocorticography (ECoG) study of the temporal lobe in medically intractable TLE patients, a longer duration of illness was related to decreased functional connectivity, clustering and small worldness (van Dellen et al., 2009). In a study of intracerebral EEG networks during seizures, networks in mTLE patients were seen to become more regular with increased clustering and path length, suggesting functional connectivity is altered during seizures (Ponten et al., 2007). Another study that investigated networks in interictal intracerebral EEG also found a more regular network configuration across a broad range of frequencies in the mesial temporal lobe of mTLE patients and this was found to be related to epilepsy duration (Bartolomei et al., 2013). In functional networks obtained from scalp EEG recordings, networks in the theta band tended to show more regular architecture while networks in the high alpha band tended to show more random lattices (Quraan et al., 2013).

The above studies all demonstrate that large-scale pathological networks are altered in MTLE patients and that in some cases these network alterations are capable of sustaining seizures even after the removal of the epileptic focus. It has been suggested that the “spread” of the epileptogenic network outside of the seizure focus could be a result of repeated seizures in the seizure propagation network (Bernhardt et al., 2009). In mouse models of MTLE, large-scale networks have been shown to emerge after the initial induction of HS, which cannot be controlled by silencing the primary epileptogenic focus in the chronic stages of the disease (Arabadzisz et al., 2005; Riban et al., 2002). More recent evidence has shown that early intervention may have some promise for reducing seizure activity within this extended network (Sheybani, 2016).

The mechanisms of the initial emergence of hippocampal sclerosis and the susceptibility of large-scale networks to abnormalities resulting from the initial insult is still not fully understood, although the literature suggests a relationship to seizure propagation and that early intervention may help reduce seizure activity.

### **3.3 Heritability of brain abnormalities in TLE**

Brain network properties have been shown to be heritable. Functional connectivity networks derived from EEG data in twins have shown that several network parameters – clustering, path length and small-worldness – and network efficiency are heritable and may reflect genetic variability in brain network organisation (Fornito et al., 2011; Smit et al., 2008). Structural brain properties have also been shown to be heritable in TLE. Hippocampal abnormalities have been found in unaffected relatives of FMTLE patients (Kobayashi et al., 2002; Suemitsu et al., 2014), and there has been some mixed evidence for structural abnormalities in unaffected relatives of non-familial MTLE patients.

In a preliminary structural MRI study, both MTLE patients and their unaffected siblings showed a reduced total cerebral WM volume and ipsilateral entorhinal cortex volume, not correcting for multiple comparisons (Scanlon, Ronan, et al., 2013). However, these findings were not corrected for multiple comparisons and only 12 relatives were studied. In a larger study of 101 MTLE patients (with and without HS) and 83 unaffected siblings, no subcortical volume differences were found in the siblings (Alhusaini et al., 2013). White matter alterations have also been reported in the left superior longitudinal fasciculus and corticospinal tracts in unaffected siblings of MRI-negative MTLE patients (Whelan et al., 2015). Perhaps the most convincing structural changes demonstrated so far are morphology changes of the ipsilateral temporal lobe in unaffected siblings of MTLE patients (Alhusaini, Whelan, Doherty, et al., 2016). Alterations of temporal cortex morphology were found in the entorhinal cortex, parahippocampal gyrus and temporal pole in MTLE patients and similar, but more subtle changes were identified in siblings. These changes were driven by contractions of cortical surface area in both groups. To date there have been no studies of functional brain alterations in unaffected relatives of MTLE patients. Identification of alterations in unaffected relatives of MTLE patients may help in the understanding of the mechanisms of the disorder, and whether abnormalities are secondary to repeated seizures or a predisposing factor for seizures.

## **Chapter 4 Aims and Objectives**

### **4.1 Aims and objectives**

This thesis aims to investigate the suitability of neuroimaging and electrophysiological measures as endophenotypes for MTLE in order to improve our understanding of the underlying mechanisms of the disorder. To achieve these aims, structural and functional imaging and electrophysiological data from MTLE patients and their first-degree unaffected relatives will be compared to data from healthy controls to characterise alterations in MTLE patients and investigate whether similar alterations are present in unaffected relatives.

Alterations present in both MTLE patients and in their unaffected relatives may indicate suitable candidate endophenotypes that represent genetic liability for the disorder that is heritable and independent of epilepsy itself, including any treatment, severity or duration of disease. These markers could prove useful in gene discovery to help us understand the genetic mechanisms of MTLE.

### **4.2 Planned investigations**

Patients with MTLE, first-degree unaffected relatives of patients with MTLE and a group of healthy control comparison participants will undergo a series of neuroimaging and electrophysiological examinations, including structural MRI, diffusion-weighted MRI, functional MRI, EEG and simultaneous EEG and functional MRI. The following investigations are planned:

Chapter 6: Structural MRI endophenotypes in MTLE

Chapter 7: Alterations in fornix microstructure in MTLE

Chapter 8: EEG-fMRI: Alterations in networks related to alpha activity

Chapter 9: Large-scale DWI and fMRI connectivity networks in MTLE

Structural abnormalities are first characterised in Chapter 6, using sMRI data. This is followed by the characterisation of abnormalities in white matter tracts connected to the affected hippocampus using DTI data in Chapter 7. Chapter 8 investigates functional and structural abnormalities (on fMRI and sMRI) related to the specific network related to alpha activity on EEG. Finally, Chapter 9 investigates large-scale functional and structural networks using fMRI and DWI modalities.

## PART II: Original Research Studies

## **Chapter 5 General Methods**

### **5.1 Ethical approval and participant recruitment**

#### **5.1.1 Ethical approval**

The study was performed at the National Institute for Health Research (NIHR)/Wellcome Trust King's Clinical Research Facility at King's College Hospital. Ethical approval was obtained from the National Research Ethics Service (NRES) Committee London – Bromley, Ethics Approval Number 14/LO/0193, and locally approved by the King's College Hospital NHS Foundation Trust Research Office. Written informed consent was obtained from each participant after all procedures were fully explained. Study consent forms are given in Appendix A.

#### **5.1.2 Participant recruitment**

Both male and female participants between 17 to 60 years old, with no contra-indications to MRI and full-scale IQ >70 were included in this study. As this study will only consider MTLE in adults, the lower age limit was set to be within the lower limit of the consent age, according to research ethics guidelines, and the upper limit was set to minimise possible neuroimaging changes due to age effects. Three groups of study participants were recruited: patients with MTLE, first-degree unaffected relatives of patients with MTLE, and healthy control comparison participants.

Patients were recruited from outpatient epilepsy and neurology clinics at King's College Hospital, St Thomas' Hospital, Darent Valley Hospital, Princess Royal University Hospital and St George's Hospital. Patients were included if they had a diagnosis of temporal lobe epilepsy, with evidence for mesial temporal sclerosis (MTS) on MRI (e.g. atrophy of the hippocampus). If patients had no evidence of MTS, they were also included if they had any other abnormality on imaging, or scalp EEG abnormalities consistent with a mesial temporal origin. Only pre-surgical patients and those who had not recently had any invasive brain investigations (including depth electrode recordings) were included in the study.

Clinically unaffected first-degree relatives were recruited either through patients in the study or through patients who have a clinical diagnosis of MTS but were not suitable for the study for any of the abovementioned reasons. This resulted in the group of unaffected relatives and patients in this study not necessarily being case-controlled, but representing a group of unaffected relatives drawn from the general population of MTLE patients. There were 7 matched pairs of patients and relatives in the study. Relatives had no current or previous clinical diagnosis of neurological disorders and a review of history confirmed no events compatible with seizures in relatives. Relatives with a current or previous history of clinical diagnosis of neurological or psychiatric disorders were excluded from the study.

Healthy control participants were recruited through spouses of patients, university staff and students, and the general population through email advertisements and word of mouth and had no current or previous clinically diagnosed personal or family history of neurological or psychiatric disorders. A summary of the participant characteristics is described in Table 5.1 and full details are provided in Appendix Tables B1 to B3.

**Table 5.1** Summary of participant characteristics.

|                                          | MTLE Patients | Relatives   | Controls    |
|------------------------------------------|---------------|-------------|-------------|
| Number recruited                         | 27            | 26          | 33          |
| Age in years: mean (SD)                  | 39.8 (11.8)   | 35.9 (13.5) | 36.3 (10.6) |
| Gender: Female (%)                       | 13 (48.2%)    | 15 (57.7%)  | 17 (51.5%)  |
| <b>Clinical Characteristics</b>          |               |             |             |
| Age of onset in years: mean (SD)         | 23.7 (10.1)   | -           | -           |
| Duration of epilepsy in years: mean (SD) | 15.9 (13.3)   | -           | -           |

## **5.2 Experimental procedure**

All participants underwent at least 20 minutes of EEG recording outside of the MRI scanner, during which they were asked to stay awake and relaxed with their eyes closed. This was followed by a short break (up to 30 minutes) and a series of MRI scans including a T1-weighted structural MRI scan, 2 runs of 10-minute simultaneous EEG-fMRI scans with eyes closed, and a DWI scan. In addition to the EEG and MRI scanning, the National Adult Reading Test (NART) was administered to obtain an estimated measure of premorbid IQ.

Not all participants completed the full study protocol (for a variety of reasons) and some data had to be excluded due to incidental findings or poor data quality. The list of complete procedures for each participant and reasons for exclusion are detailed in Appendix C.

## **5.3 EEG acquisition and data processing**

### **5.3.1 EEG acquisition**

EEG data were recorded using a 64 channel MRI-compatible EEG system (Brain Products GmbH, Munich, Germany; Figure 5.1). Participants were fitted with a BrainCap MR EEG cap with 63 Ag/AgCl electrodes arranged according to the extended international 10-20 system with the reference electrode placed between Fz and Cz and the ground between Fz and Fpz. An electrocardiogram (ECG) electrode was placed on the left collar bone to record pulse data for correction of BCG artefacts. EEG was recorded at a sampling frequency of 5kHz using the BrainVision Recorder software (Brain Products). EEG recordings outside the scanner were performed in an electrically shielded Faraday cage room.

In the MRI scanner, participants underwent 2 eyes-closed resting-state fMRI runs with simultaneous EEG recording lasting 10 minutes 48 seconds each. A SyncBox device (BrainProducts) was used to synchronise the EEG amplifier clocks and the MR gradient system.

The start of each TR in the fMRI acquisition was synchronised to an EEG data sampling point and marked in the EEG data timeseries for removal of MR gradient artefact in the EEG data.

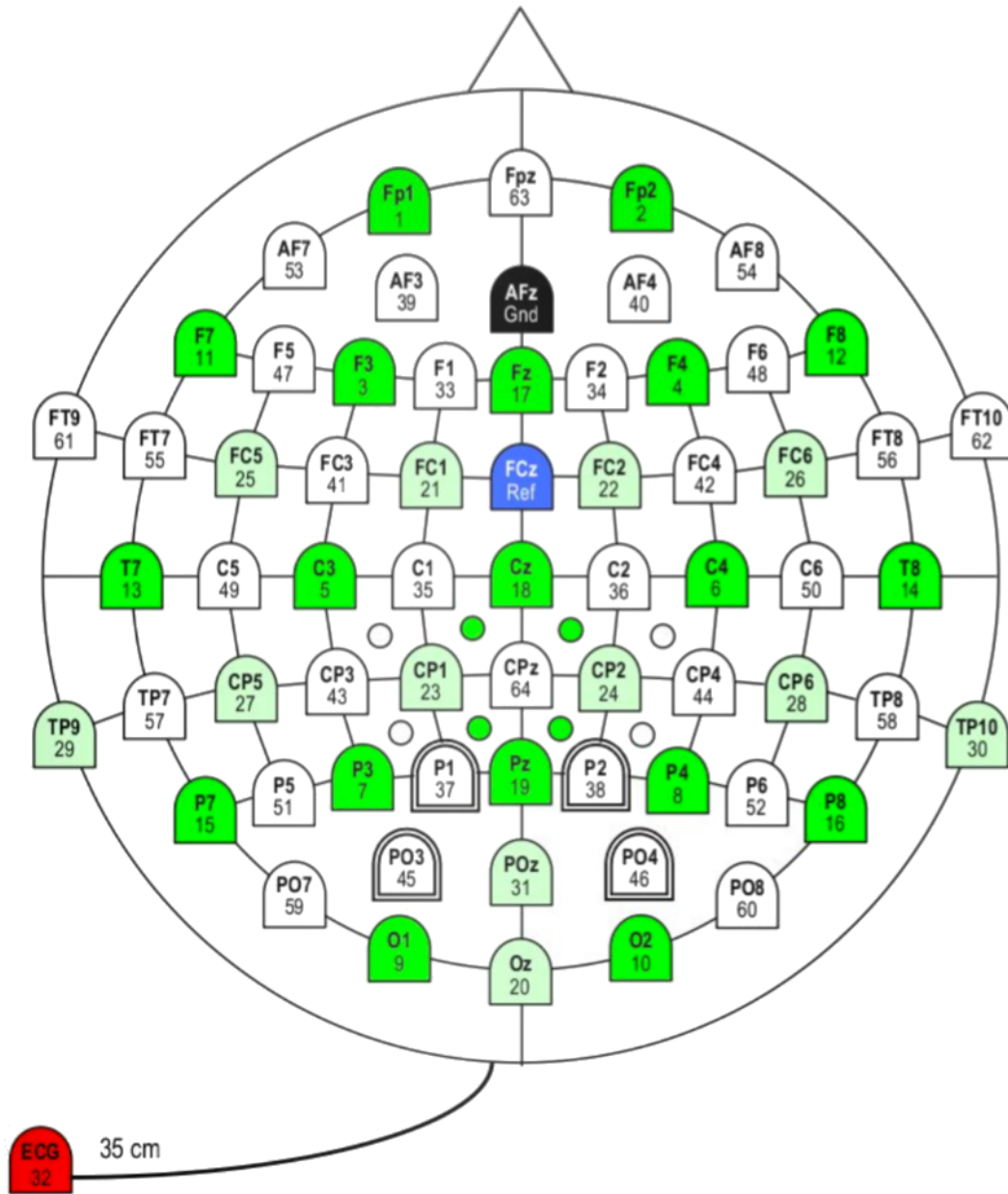


**Figure 5.1** Locations of EEG electrodes on BrainCap MR. Electrodes in bright green are the 19 electrodes in the conventional 10-20 system. Ground is at AFz (black), reference is at FCz (blue), and ECG is shown in red. Picture taken from accompanying information provided with the BrainCap MR (Brain Products GmbH & Easycap GmbH, Germany).

### 5.3.2 EEG data processing

EEG data recorded in the scanner were processed using BrainVision Analyzer (version 2.0, Brain Products) to remove MR gradient and BCG artefacts from the simultaneous EEG-fMRI runs. A sliding average template of MRI scanner artefacts using the average of 21 TR intervals identified by the gradient onset markers was subtracted from the EEG signal to correct for MR gradient artefacts. Data were then downsampled to 500Hz. The peaks of the R-waves were identified from the ECG signal in a semi-automated manner using a template pulse wave, and subsequently manually checked and adjusted by eye. (Manual checks were included alongside the automated ones, as the algorithm did not perform well in some subjects where the ECG signal was either not very clear, irregular or had a low amplitude. All checks were performed by me to avoid any inter-rater differences. BCG artefacts were then corrected by subtracting a sliding average template for the R-waves from the EEG signal).

## 5.4 MRI acquisition and data processing

### 5.4.1 Image acquisition parameters and scanning protocol

MRI data collection was performed on a General Electric 3.0T scanner (GE Discovery MR750, GE Healthcare Systems, Chicago, USA) using the body coil for RF transmission and a 12-channel head coil (part of the manufacturer's "head neck spine" array) for signal reception.

#### ***T1-weighted image:***

A three-dimensional inversion recovery-prepared spoiled gradient-echo (IR-SPGR) image was acquired in the sagittal plane with FoV 270mm,  $256 \times 256$  matrix (resulting in an in-plane voxel size of  $1.05 \times 1.05$ mm), 196 sagittal slices, slice thickness 1.2mm, repetition time 7.312ms, inversion time 400ms, echo time 3.016ms, excitation flip angle  $11^\circ$ .

***T2\*-weighted images:***

Participants underwent 2 resting-state fMRI scans where they were asked to close their eyes and try to stay awake. The resting-state scans were acquired using a gradient echo echo planar imaging (EPI) sequence in a plane parallel to the AC-PC line, with TR=2160ms, TE=25ms, flip angle 75°, 36 interleaved slices of  $64 \times 64$  matrix size, giving a  $211 \times 211$  mm field of view with a voxel size of  $3.3 \times 3.3 \times 3.3$  mm. These parameters were deliberately chosen to ensure that the time for individual slices was a whole number of ms (and therefore a multiple of the EEG sampling rate), to allow easy matching to the EEG data (for gradient artefact removal). Each of the resting-state runs lasted approximately 10 minutes and 57 seconds, with 300 EPI volumes acquired and 4 dummy volumes at the start of each scan. A finger-clipped pulse oximeter and pneumatic respiration belt were used to acquire cardiac and respiratory data sampled at 40Hz during each fMRI scan.

Participants were given a short break before the T2-weighted, fluid attenuated inversion recovery (FLAIR) and DTI scans, before which the EEG cap was removed in order to avoid potential heating effects from these higher Specific Absorption Rate sequences. The T2-weighted and FLAIR images were used for evaluation of incidental findings and not analysed in the study.

***Diffusion weighted images:***

Diffusion weighted images were acquired using a cardiac-gated, multi-slice, peripherally-gated singly refocused spin echo EPI sequence with 72 axial slices of 2mm thickness with no slice gap, FoV 256mm,  $128 \times 128$  matrix size, giving an isotropic voxel size of  $2 \times 2 \times 2$  mm<sup>3</sup>. The echo time was 72.9ms and repetition time varied between subjects in the range of 12 and 20 RR intervals.

Six images were acquired with no diffusion weighting followed by 60 diffusion-weighted images with maximum  $b = 1500$ s/mm<sup>2</sup> and encoding directions distributed over a sphere, with phase encoding direction P-A. Reversed phase-encoded data was also acquired consisting of 3 images

with no diffusion weighting and 6 diffusion-weighted images (the smallest number allowed) with phase encoding direction A-P. This resulted in 3 pairs of images with opposite distortion directions, which were used to allow for the estimation and correction of susceptibility induced distortions.

#### 5.4.2 Imaging data quality control

All images were retrieved from the scanner in DICOM format and converted to NIFTI format for further pre-processing and analysis, as this was the format compatible with most neuroimaging software packages.

Data were visually inspected to identify any gross motion or other artefact. T1-weighted data in some participants showed an artefact in the frontal region of the brain (Figure 5.2) thought to be related to the EEG equipment. All images were used for registration to standard space and for structural analyses. The artefact did not prove problematic as it occurred sporadically across all participant groups and the extent of the artefact was not very large or within areas of interest in the present study. However, as a precaution, any findings in the frontal regions in the analyses were treated with caution.

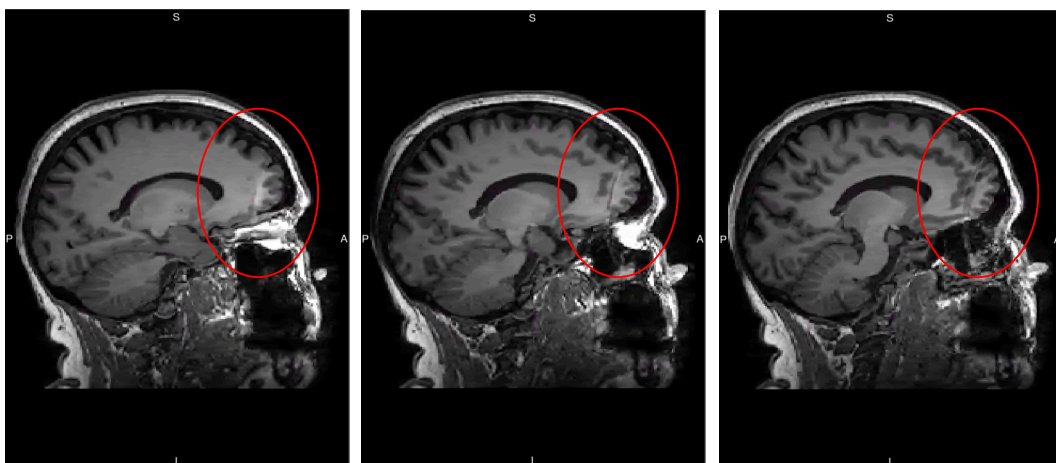


**Figure 5.2** Three sagittal sections showing the artefact in a typical participant.

One patient with right-sided TLE had brain atrophy not typical in a TLE patient with no other co-morbidities (Figure 5.3) and was not included in further analyses.

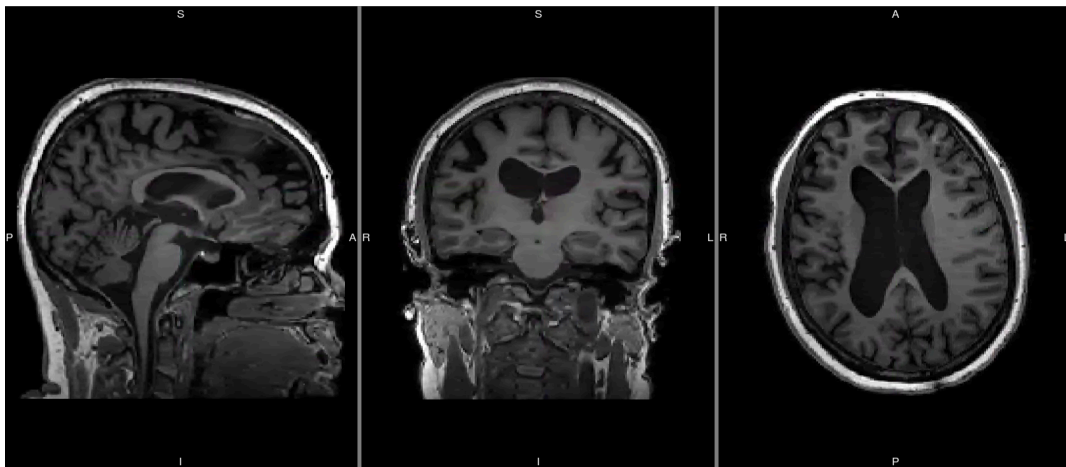


**Figure 5.3** Sagittal, coronal and axial sections showing extent of atrophy in the MTLE patient who was excluded from further analyses (TLE/P/DVH1).

#### 5.4.3 Imaging data pre-processing

Data pre-processing common to multiple analyses are described below and data processing steps specific to each data type and analysis are described in later chapters.

To increase sample size and power to detect pathological differences in the MTLE group, all imaging data from right MTLE patients were left-to-right flipped so that the ipsilateral side is on the left for all patients and all differences were considered as ipsilateral or contralateral to the pathological hippocampus. Since there is previous evidence for smaller and more asymmetric hippocampi in unaffected relatives of TLE patients (Tsai et al., 2013), and to maintain a similar proportion of flipped images as in the patient group, imaging data from unaffected relatives of MTLE patients were left-to-right flipped if the ipsilateral side of the affected family member with MTLE was on the right. For comparison, the same proportion of healthy control data was side-flipped with subjects chosen at random. Subjects whose data were left-to-right flipped are indicated in Appendix C.

#### 5.4.3.1 fMRI data pre-processing

fMRI data were pre-processed using FEAT (fMRI Expert Analysis Tool, Version 6.0.0, part of FSL) software and custom scripts in bash. fMRI data pre-processing involved motion correction with MCFLIRT, spatial smoothing with Gaussian filter with a 6mm full-width at half maximum (FWHM; chosen to be approximately twice the voxel size), and bandpass filtering with a highpass filter sigma of 100s and a lowpass filter sigma of 2.8s giving frequencies between 0.01Hz and 0.12Hz. Each subject's native space data were co-registered to the Montreal Neurological Institute (MNI) standard space image by way of a linear transform to the subject's high-resolution T1-weighted image and then a non-linear registration to standard space.

To model and exclude effects of noise within the data, several nuisance regressors were used as explanatory variables (EVs) of non-interest in the general linear model (GLM). These included an average signal each from the white matter (WM) and cerebrospinal fluid (CSF), the 6 motion parameters, the square of each of these regressors, the temporal difference of the regressors and the square of the difference values. This is an autoregression-moving average model useful for accounting for spin history effects and variation not otherwise accounted for by motion correction (Friston et al., 1996). The 6 motion parameters were obtained from the output of MCFLIRT and the average signals from the WM and CSF were obtained by segmenting each subject's high-resolution T1 image using FSL's FAST, setting a threshold of 95% probability on the resulting segmentation probability image, and using this as a binary mask from which to extract an average time series from the fMRI image. In addition, the *fsl\_motion\_outliers* tool was used to detect any outliers in motion (defined using the standard box plot outlier threshold as described above) for each subject giving one regressor for each instance of a motion outlier with a value of 1 at the timepoint where the outlier was detected and zero otherwise.

## 5.5 General analysis methods

### 5.5.1 FreeSurfer recon-all processing stream

A widely used method for processing of anatomical MRI images is the FreeSurfer *recon-all* processing stream (Dale et al., 1999; Fischl et al., 2002, 2004, 1999). It is a fully automated processing stream that takes a T1-weighted image as the input and gives the segmented image, together with volumes and surface statistics as output. The processing stream can be divided into two parts: volumetric processing and surface processing; and the steps will be described briefly here and shown in Figure 5.4.

Volumetric processing is performed first. The input T1-weighted MRI is first corrected for any slowly varying intensity changes (known as “bias field”) across the brain and the transform for registration to the MNI305 template is computed. Images are then “skull-stripped” to remove non-brain voxels (e.g. skull, eyes, neck, and dura) in the image. Automatic volume labelling is then performed based on a template segmentation of sub-cortical regions. Voxels are given labels based on their known intensity profiles, nearest neighbours and label probabilities at a given location. Finally, white matter segmentation is performed to obtain the cortical surface.

Next, surface processing is performed. The surface atlas is registered and the cortical white matter and pial surfaces are extracted from the image. The cortical surface is modelled as a mesh with properties like surface area, cortical thickness and curvature measured at each vertex. The cortex is then parcellated and each vertex given a label.

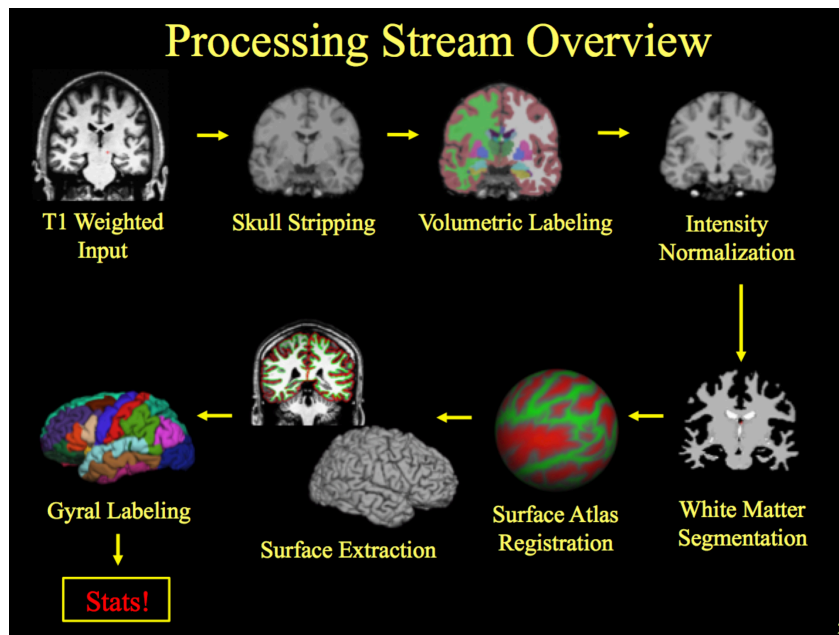


**Figure 5.4** FreeSurfer Processing Stream. Image taken from slide 2 of the “Introduction to FreeSurfer Output” tutorial on the FreeSurfer webpage (<http://freesurfer.net/fswiki/Tutorials>).

### 5.5.2 Voxel-based morphometry

Voxel-based morphometry (VBM) is another commonly used method for comparing voxel-wise local grey matter concentration differences between groups of subjects (Ashburner & Friston, 2000). The four main steps involved are: 1) spatial normalisation, 2) tissue segmentation, 3) spatial smoothing, and 4) statistical analysis.

The input is a T1-weighted MR image. In order to compare images between subjects, each subject’s image must first be transformed into a common atlas space in the spatial normalisation step. This involves an affine transformation (12 degrees of freedom: 3 each of translations, rotations, expansions/contractions and shears), followed by a non-linear basis function normalisation. The aim of this registration step is to broadly match brain shapes while allowing for individual differences in brain structures. Next, images are automatically segmented into white matter, grey matter and CSF. This is based on template “priors” that have information about the typical distribution of these different tissue types in the brain (from the International Consortium for Brain Mapping, ICBM), along with the image intensity differences in the individual images. Images are then spatially smoothed with a Gaussian kernel with FWHM

typically between 8 to 12mm to account for inter-individual variation in the brain morphology and compensates for errors in spatial normalisation. Each voxel is now a representation of the local concentration of grey matter, white matter and CSF. This is followed by image “modulation” whereby the grey matter voxel values are multiplied by the deformation parameters (Jacobian determinants) from the spatial normalisation step, which allows for the comparison of voxel-wise grey matter volumes rather than concentrations. Finally, voxel-wise parametric statistical tests are used to compare the smoothed grey matter images between groups of subjects (Keller & Roberts, 2008).

### **5.5.3 Graph theory applied to brain networks**

Graph theory is a mathematical description of graphs, and provides a framework for their analysis. The two basic components of a mathematical graph are vertices (nodes) and edges (relationships between vertices).

The human brain can be contextualised as a graph, with vertices being defined using functional or anatomical brain regions or recording sites in EEG, and edges described by functional or structural connections between these brain regions (Bullmore & Sporns, 2009; Stam & Reijneveld, 2007). The edges in the graph can be directed, where the direction of information flow is known, or undirected. Edges can also be weighted by the strength (or some other quantifiable measure) of connections, or be binary, where the presence of a connection is indicated by a one and a zero indicates no connection between nodes. A graph is usually represented by a square matrix, called an adjacency matrix, where the number of rows and columns are equal to the number of nodes and each value in the matrix represents the connection between two nodes.

### **5.5.3.1 Choice of nodes and edges**

The choice of nodes and edges are dependent on the modality used and these are described in general here and in more detail in later chapters.

For scalp EEG data, the simplest model is to treat each electrode as a node, while edges can be any measure of connection between the timeseries from each electrode. Commonly used measures in EEG are correlation, cross-covariance and phase synchrony. Of course, more complex models could involve source localisation on the EEG data to identify source nodes.

In imaging data, there is no standard method to parcellate the brain. This poses a major difficulty in many studies of whole-brain networks. The choice of nodes makes a difference in the analysis and interpretation of brain networks, and global network properties such as small-worldness have been shown to vary greatly with the parcellation scheme used (Wang et al., 2009; Zalesky et al., 2010). The nodes in the graph need to accurately reflect the structure and function of the brain in the underlying regions, and the challenge in the analysis presented here was to choose a parcellation scheme that would be suitable for both the functional and diffusion-weighted datasets. This is further discussed in Chapter 9.

In fMRI data, edges could again be any measure of connection and the commonly used measures are correlations and partial correlations. In DWI data, tractography is usually used to identify streamlines connecting two nodes and measures like the number of tracts, length of tracts or diffusivity or FA measures of tracts form the edges.

### **5.5.3.2 Network measures**

The topology of a graph network can be described using a wide variety of global (at the whole network level) and local (at the node level) measures. In the study of the brain, there is no consensus on which measures are most appropriate or important, but measures should be able to be interpreted in a biologically meaningful way. An example network is shown in Figure 5.5,

and used to describe some of the more common measures used in the study of brain networks listed below (adapted from Bullmore & Sporns, 2009). Mathematical formulae for network measures used in this thesis are given in Appendix D.

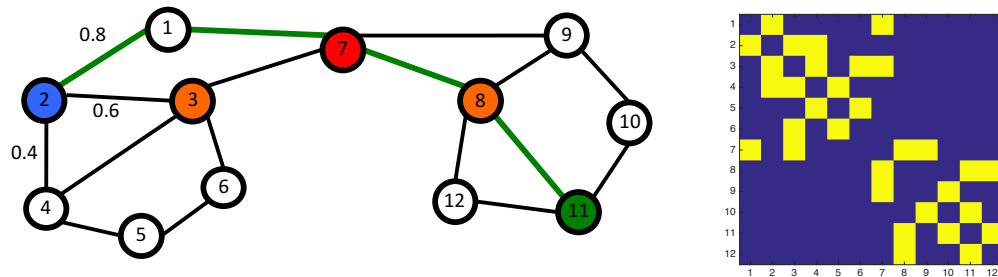


**Figure 5.5** Example graph network with circles representing nodes lines representing edges, and its binary adjacency matrix. Example weights are given to the edges connecting node 2.

### ***Node degree, node degree distribution and node degree strength***

The node degree is the number of connections between a node and all other nodes in the network. The node degree strength is the node degree weighted by the connectivity measure. In Figure 5.5, the node in blue has a node degree of 3 and a node degree strength of 1.8. The degrees of all the network's nodes form a degree distribution. This is the most basic of network measures and most other measures are linked to it.

### ***Clustering coefficient***

The tendency of a node to form local, fully connected clusters (or triangles) with its nearest neighbours is called the clustering coefficient. It is the proportion of the number of connections between a node and its nearest neighbours and the total number of possible connections. Random networks have a low average clustering, while complex networks have high clustering. In Figure 5.5, the node in blue has a clustering coefficient of 0.33.

### ***Path length and efficiency***

The path length is the shortest distance between two nodes, or the minimum number of edges that must be traversed to get from one node to another. A node's local efficiency is the inverse of

average shortest path connecting it to all its nearest neighbours. A network's characteristic path length is an average shortest path length in the whole network and its global efficiency is inversely related to this measure. In Figure 5.5, one of the shortest paths between the blue and the green node is shown in green and represents a path length of 4. The characteristic path length of the network is 2.3 and its global efficiency is 0.5.

### ***Connection density or network cost***

The connection density is the number of edges in the graph as a proportion of the total number of possible edges. It is an estimate of the network cost. The network in Figure 5.5 has a density of 0.2.

### ***Node centrality and network hubs***

The centrality of a node measures how important it is to the overall information transfer and efficiency of a network. Node centrality measures may include degree centrality, betweenness centrality, and eigenvector centrality. Degree centrality is usually equal to node degree or node degree strength and measures the number or strength of connections a node has to other nodes. Betweenness centrality is number of shortest paths in the network that pass through a particular node. Eigenvector centrality is the vector component of the largest eigenvector of the adjacency matrix, and is a self-referential measure where nodes have a high eigenvector centrality if they connect to other nodes that also have high eigenvector centrality. Nodes with a high degree centrality may not necessarily have a high eigenvector centrality if it is not connected to other nodes with high centrality.

The network hubs are the nodes with the highest centrality in the network and are the most important nodes for information transfer and efficiency in the network. In Figure 5.5, the node in red can be considered a network hub as it has high degree, betweenness and eigenvector centrality.

## ***Modularity***

Modules are sub-graphs that contain nodes that are more strongly connected to each other than to the rest of the network. There are different algorithms used to estimate modules in a network, usually involving some sort of hierarchical clustering. A network can have multiple modules and modules can also be defined within modules (e.g. hierarchical modules). Hubs can be described in terms of their roles within the modular network structure, with provincial hubs having more connections to nodes within their own modules (e.g. orange nodes in Figure 5.5) and connector hubs being the nodes connecting one module to another (e.g. red node in Figure 5.5).

## ***Network topology***

Depending on the connections between nodes, networks can have configurations along the scale between regular and random. Regular networks have higher clustering and longer path lengths, while random networks have short path lengths and lower clustering. Small-world networks lie in between, and are considered the most efficient network topology due to the short path lengths and high clustering. Most biological networks fall within the small-world regime (Achard et al., 2006; Watts & Strogatz, 1998).

The “small-worldness” of a network can be calculated using the small-world index, which is the ratio of the clustering coefficient over path length of a network to the clustering coefficient over path length of a random network with the same number of nodes and node distribution. The formula for this is given in Appendix D.

An additional characteristic of brain networks is their scale-free organisation, where there is a low number of connections per node and a small number of highly connected nodes known as “hubs”, forming a network that is robust, highly efficient and integrated (van den Heuvel et al., 2008).

#### 5.5.4 Kuramoto oscillator model & brain network ictogenicity

Brain network ictogenicity is the propensity for the brain network architecture to spontaneously produce highly-synchronised oscillations often associated with seizures. To investigate the propensity for the network architecture to drive synchronisation, the Kuramoto oscillator model was applied using scripts from Dr Helmut Schmidt, a collaborator from the University of Exeter (Schmidt et al., 2014).

The Kuramoto oscillator model is a computational model of a network of oscillators. In the model applied here, each node consists of  $N$  oscillators with uniform coupling strength and phase, and the connectivity matrix (either structural connectivity, SC, or functional connectivity, FC) represents the coupling matrix that describes the interactions between nodes, modulated by a global coupling parameter  $C$ . When  $C$  is below the critical value, the oscillators are not synchronised, but when the critical  $C$  value is reached, oscillators become phase-locked and large amplitude synchronised activity emerges, akin to what is observed with the onset of seizure activity in patients with epilepsy.

Within this framework, differences in nodes or connections between nodes contribute to the overall network synchrony and the model applied here allows us to estimate the critical coupling parameter (CritC) for a network based on empirical SC or FC data and to quantify the tendency for a particular network structure to support whole-brain synchronous activity.

## Chapter 6 Structural MRI endophenotypes in MTLE

### 6.1 Introduction

Quantitative sMRI studies have demonstrated a wide range of structural alterations in patients with MTLE. The most common structural alteration found in MTLE is atrophy of the hippocampus, associated with hippocampal sclerosis, but extra-hippocampal alterations have also been widely reported. Outside of the hippocampus, imaging studies have found atrophy in other mesial and limbic structures (Bernasconi et al., 1999, 2000, 2003; Bonilha et al., 2003), subcortical structures including the thalamus (Dreifuss et al., 2001; Seidenberg et al., 2008), the cerebellum and white matter (Bernasconi et al., 2004; Whelan et al., 2015). In a VBM review, bilateral thalamic atrophy has been shown to be the most consistently found structural abnormality outside the temporal lobe (Keller & Roberts, 2008). Most of these structural changes, particularly in the hippocampus, appear to be correlated with epilepsy duration and age of onset (Richardson, 2010), but it is still unclear whether these changes result from recurrent seizures, pre-date seizures and represent a predisposition to epilepsy, or are a combination of the two effects (Bonilha & Keller, 2015).

Structural changes have also been identified in familial forms of MTLE, suggesting some evidence for a genetic contribution to hippocampal atrophy. There have been several studies quantifying structural differences in unaffected relatives of MTLE patients using MRI (Alhusaini, Whelan, Sisodiya, et al., 2016). Two of these studies investigated FMTLE and found a non-significant trend toward increased T2 relaxometry in the right hippocampi of 9 unaffected relatives (Suemitsu et al., 2014), and hippocampal atrophy in 34% of relatives studied (Kobayashi et al., 2002). Another study found asymmetric hippocampal volumes in 17 members of 2 families with familial febrile convulsions (Fernández et al., 1998). Using manual hippocampal segmentation, another study found smaller and more asymmetric hippocampi in relatives compared to controls, in a group of 35 relatives of 15 probands (Tsai et al., 2013). Importantly, no evidence of HS was found in the relatives, and the hippocampus was more

abnormal in relatives where there was a family history of epilepsy (family history was present in 5 of the 15 probands). These findings suggest an inherited abnormality of the hippocampus and the possibility for identifying suitable sMRI endophenotypes of MTLE.

In this chapter, structural abnormalities will be characterised in the present cohort of MTLE patients and their first-degree relatives representing, to my knowledge, only the second such investigation, in an independent sample, of these differences in relatives of sporadic MTLE cases. Hippocampal abnormalities will be characterised using volumetry (whole-volume and subfield volumes) and surface morphometry approaches (see below for details of analysis) to enable the identification of subtle subfield differences in the hippocampus. As the thalamus is the most consistent subcortical region (other than the hippocampus) showing abnormalities in MTLE patients, volumetric and morphometric alterations in this region will also be investigated in MTLE patients and their first-degree unaffected relatives.

Similarities in structural abnormalities in patient and relative groups could point to candidate structural imaging endophenotypes that represent a high genetic risk for MTLE and could be potential biomarkers for further validation and investigation in genetic studies.

## **6.2 Aims and hypotheses**

This chapter aims to characterise sMRI differences in the present sample of MTLE patients and to investigate structural alterations in first-degree unaffected relatives to identify possible imaging endophenotypes for MTLE.

This will be done using four analyses applied to MTLE patients and their first-degree unaffected relatives compared to a group of healthy controls:

- 1) Region-based cortical and subcortical volume characterisation using an automated segmentation method.

- 2) Whole-brain vertex-wise cortical surface metric distortion analysis and identification of surface area and cortical thickness contributions to cortical surface distortion.
- 3) Sub-cortical shape analysis of the hippocampus and thalamus using an automated segmentation method.
- 4) Hippocampal subfield volume analysis using an automated segmentation method to characterise more subtle changes specific to certain subfields in the hippocampus.

The hypotheses are that MTLE patients will show reduced volume and surface shape contraction in the ipsilateral hippocampus compared to healthy controls, and that unaffected relatives will show a subtle difference in similar regions of the hippocampus. In the hippocampal subfield analysis, volumes of CA1, CA3, CA4 and dentate gyrus subfields are hypothesised to be reduced in MTLE patients, with unaffected relatives hypothesised to show values intermediate to MTLE patients and healthy controls. Bilateral thalamus volume reduction and surface contraction are hypothesised in MTLE patients compared to healthy controls, with unaffected relatives hypothesised to have intermediate values. No specific hypotheses are made regarding the location or form of extra-hippocampal abnormalities due to inconsistencies in findings outside the hippocampus in prior studies, but it is hypothesised that patients will show structural alterations outside the hippocampus and relatives will show subtle differences in a similar set of regions.

## **6.3 Method**

The general study protocol and acquisition methods have been described in Chapter 5. The methods described below are specific to the analyses reported in this chapter.

### 6.3.1 Participants

Data from 25 participants with MTLE, 22 relatives of patients with MTLE and 32 healthy controls were used in the analysis of structural changes in MTLE<sup>9</sup>. Of the 25 MTLE patients, 16 had left-lateralised MTLE and 9 had right-lateralised MTLE, from MRI and EEG assessments. The sample characteristics are shown in Table 6.1. Age was compared between groups using an analysis of variance (ANOVA) and gender was entered into a  $\chi^2$  test to assess for group differences.

**Table 6.1** Sample characteristics

|                                          | MTLE Patients | Relatives   | Controls    |
|------------------------------------------|---------------|-------------|-------------|
| Number                                   | 25            | 22          | 32          |
| Age in years: mean (SD)                  | 39.2 (12.0)   | 33.5 (13.2) | 36.1 (10.7) |
| Gender: Female (%)                       | 12 (48%)      | 13 (59%)    | 17 (53%)    |
| <b>Clinical Characteristics</b>          |               |             |             |
| Age of onset in years: mean (SD)         | 23.7 (10.1)   | -           | -           |
| Duration of epilepsy in years: mean (SD) | 15.9 (13.3)   | -           | -           |

### 6.3.2 Image acquisition

High-resolution T1-weighted IR-SPGR images were acquired on a 3.0T scanner, with parameters and details described in Chapter 5, Section 5.4.1. As described in Section 5.4.3, data were left-to-right flipped and the left side is considered ipsilateral to the pathological hippocampus. Details of subjects whose data are side-flipped are given in Appendix C.

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<sup>9</sup> This sub-set of data was used because not all participants had complete datasets across all modalities; this is fully detailed in Appendix C.

### 6.3.3 Image processing & analysis

#### *FreeSurfer: volumetric analysis*

MRI images were processed using the FreeSurfer image analysis suite, which is documented and freely available for download online (version 5.3.0, <https://surfer.nmr.mgh.harvard.edu>). The details of the surface and volumetric processing stream have been described in several publications (Dale et al., 1999; Fischl et al., 2002, 2004, 1999) and briefly described in Chapter 5, Section 5.5.1. Here the automated *recon-all* processing stream was applied for cortical reconstruction and volumetric segmentation. The output from this processing stream includes cortical and subcortical volumes summarised according to anatomical labels, and vertex-wise cortical surface areas and cortical thickness values.

Segmentations were visually inspected for any gross errors and a set of summary statistics identifying outliers in the volumes of the data was generated according to the quality checking pipeline from the ENIGMA consortium (Stein *et al.*, 2012; protocols can be downloaded from <http://enigma.usc.edu>). This procedure involved visual quality control with guidelines as to where most frequent errors of segmentation occur and any subjects with outlier data were visually inspected to determine whether segmentation errors occurred in those volumes. There were some subjects identified as outliers, but whose segmentations were nevertheless deemed within acceptable limits upon visual inspection.

An exploratory analysis was first performed to investigate gross differences in cortical volumes between groups. Total region volumes obtained from the FreeSurfer summary statistics files were compared. FreeSurfer uses the Desikan-Killiany Atlas (Desikan et al., 2006) to automatically assign anatomical labels to each vertex. The summary statistics files (*aparc.stats* and *aseg.stats*) provide the total volumes for each of the cortical and subcortical anatomical labels. These values were entered into 3 separate multivariate analyses of covariance (MANCOVA) in SPSS Statistics (IBM SPSS Statistics for Mac, Version 23.0, IBM Corp, Armonk, NY) with age, gender and intracranial volume (ICV) as covariates. In the first MANCOVA I

investigated whole-brain group differences in total grey and white matter volumes, total cortical grey and white matter volumes, and cerebellar grey and white matter volumes. In the second MANCOVA I investigated group differences in volumes of each of the main lobes, frontal, parietal, temporal and occipital lobe volumes.

Sub-cortical regions were of particular interest in this analysis, as the most consistently reported differences in previous literature of MTLE is the reduced volumes of the ipsilateral hippocampus and bilateral thalamus. The volumes of six bilateral subcortical regions<sup>10</sup> (hippocampus, amygdala, thalamus, caudate, putamen and accumbens), obtained from the FreeSurfer sub-cortical segmentation pipeline were compared between groups in a third MANCOVA with age, gender and ICV as covariates.

All MANCOVAs were assessed for significance using Pillai's trace and further univariate ANCOVAs were performed on significant effects. Follow-up t-tests were performed on significant univariate ANCOVAs and adjustment for 18 multiple comparisons (6 regions and 3 group comparisons) was done using Bonferroni correction. Planned linear contrasts were also performed for each measure to test the hypothesis that volumes in unaffected relatives lie in between MTLE patients and healthy controls. The partial eta squared ( $\eta^2$ ) effect size was computed for the MANOVAs and each linear contrast. A rough guideline for small, medium and large effect sizes using  $\eta^2$  are 0.01, 0.06 and 0.14 respectively (Cohen, 1988).

### ***FreeSurfer: morphometric analysis***

As the cortical differences reported in previous literature are subtler and more varied and thus less likely to be detected in a total region cortical volume analysis, a vertex-wise approach to test for metric distortion, surface area and cortical thickness differences was applied. Metric distortion is the amount of distortion required to achieve optimal spherical registration of the subject's cortical surface to the template surface at each vertex (Fischl et al., 1999; Wisco et al.,

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<sup>10</sup> These regions were the default segmentations given by FreeSurfer.

2007). It has been used in previous studies assessing temporal lobe morphology in MTLE patients and their relatives (Alhusaini, Whelan, Doherty, et al., 2016) and is thought to be more sensitive to overall morphometric differences as compared to conventional volume or thickness-based analyses (Wisco et al., 2007). To identify the specific mechanisms that drive the differences in metric distortion, where a group difference in metric distortion was found, group differences in surface area and cortical thickness were also assessed.

Following *recon-all* processing, all subjects were mapped to an average template subject (the default FreeSurfer subject: *fsaverage*) and smoothed using a Gaussian kernel (10mm FWHM) as a compromise between SNR and resolution. The analysis was performed using the Query, Design, Estimate, Contrast (Qdec) tool available as part of the FreeSurfer package. A general linear model (GLM) was applied at each vertex of the cortical surface to assess for group differences in the data, with the measure of interest (i.e. metric distortion, surface area, or cortical thickness) as a dependent variable, group and participant gender as fixed independent factors, and participant age as a nuisance variable. Since surface area and brain volumes have been shown to vary with head size (Barnes et al., 2010), ICV was additionally included as a nuisance variable in the metric distortion and surface area analysis.

Three separate group comparisons were made: 1) between MTLE patients and healthy controls, 2) between relatives and healthy controls, and 3) between MTLE patients and relatives. Z-maps of group differences were corrected for multiple comparisons using the Monte Carlo simulation cluster correction with a two-tailed cluster-wise threshold of  $p < 0.05$ .

### ***FSL FIRST: subcortical shape analysis***

A subcortical shape analysis was performed to identify subtle vertex-wise differences in shape in subcortical regions. Images were processed using the FMRIB Software Library (FSL; <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL>) FIRST software (Patenaude et al., 2011). FIRST uses manually segmented subcortical images from the Center for Morphometric Analysis, MGH,

Boston, as models and performs segmentation of grey and white matter and subcortical regions. The automated processing pipeline, *run\_first\_all*, was applied to each subject's T1-weighted image to segment the subcortical structures and produce mesh and volumetric outputs. Each segmentation was then visually checked for gross errors, to ensure that segmentations and registrations were successfully run.

To investigate shape differences between groups in subcortical structures, a vertex-wise analysis of local differences in shape was then applied. The vertices for each subcortical structure from each subject were projected onto the average surface of the group and the resulting projection values represent the signed perpendicular distance from the average surface, with positive values indicating a local expansion and negative indicating a contraction in the surface for a particular subject. The vertex-wise output for each region was entered into a GLM with age and gender as covariates. FSL *randomise* (Winkler et al., 2014), a non-parametric permutation method, was used to generate a null distribution of the data and the False Discovery Rate (FDR) implemented in FSL was used to control for multiple comparisons. Differences were considered significant at  $p < 0.05$  FDR corrected. The subcortical structures examined in this analysis were the bilateral hippocampus and thalamus. Group comparisons were made between MTLE patients and healthy controls, MTLE patients and unaffected relatives, and unaffected relatives and healthy controls.

### ***FreeSurfer: hippocampal subfield volumes analysis***

Since the atrophy patterns in hippocampal sclerosis are very specific to certain subfields (Steve et al., 2014), analysis of hippocampal subfield volumes was performed to investigate differences in the patient cohort as well as in their unaffected relatives. After the initial *recon-all* was run in FreeSurfer, automated segmentation of hippocampal subfields on T1-weighted images was performed using the hippocampal subfield functionality of FreeSurfer 6.0 (Development Version, Iglesias et al., 2015). This automated segmentation is based on the statistical atlas of ultra-high resolution ( $\sim 0.1$  mm isotropic) ex vivo MRI data and has been shown to produce subfield

segmentations that fit underlying hippocampal subfields from histological studies well (Iglesias et al., 2015). The subfields segmented included the parasubiculum, presubiculum, subiculum, cornu ammonis (CA) subregions 1, CA2/CA3 (CA2 and CA3 were merged because of insufficient in vivo structural contrast between the 2 subfields) and CA4, granule cell layer of the dentate gyrus (GC-DG), hippocampus-amygdala transition area (HATA), fimbria, molecular layer, hippocampal fissure and hippocampal tail. A sample segmentation from one of the control participants is shown in Figure 6.1.

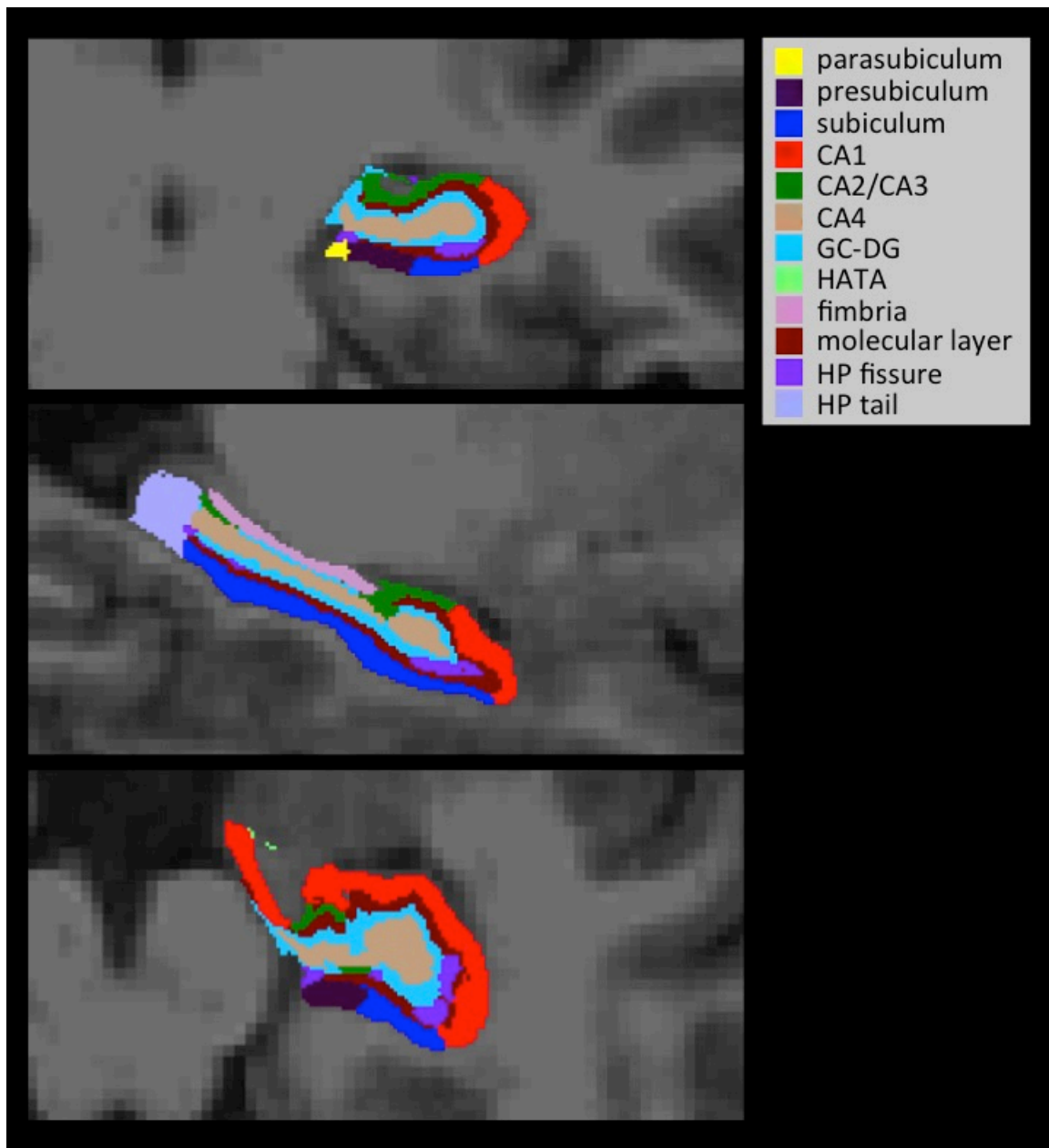


**Figure 6.1** Sample segmentation of hippocampal subfields in a control participant.

Subfield volumes were entered into a MANCOVA in SPSS, with age, gender and ICV as covariates, to identify group differences in volumes. Group effects were assessed for significance using Pillai's trace and further univariate ANCOVAs were performed on significant effects. Follow-up t-tests were performed on significant univariate ANCOVAs and adjustment for multiple comparisons was done using Bonferroni correction. Planned linear contrasts were also performed for each measure to test the hypothesis that volumes in unaffected relatives lie in between MTLE patients and healthy controls. The partial eta squared ( $\eta^2$ ) effect size was computed for the MANOVA and each linear contrast.

## **6.4 Results**

The participant groups did not differ significantly in age or gender when assessed using a one-way ANOVA and chi-square test.

### **6.4.1 FreeSurfer volumetric & morphometric analysis**

#### **6.4.1.1 Volumetric analysis: cortical volumes**

Overall there were no significant differences between groups in total ICV or total cortical white and grey matter volumes and total cerebellar white and grey matter volumes. The groups also did not differ significantly in volumes of the main cortical lobes (Figure 6.2).

#### ***Volumetric analysis: subcortical volumes***

Figure 6.3 shows the average subcortical volumes in each group. Using Pillai's trace, there was a significant effect of group on subcortical brain volumes,  $F(24,122) = 1.64$ ,  $p = 0.042$ ,  $\eta^2 = 0.24$ , suggesting a large effect size. Separate univariate ANCOVAs on subcortical volumes revealed a significant effect of group on ipsilateral hippocampal volumes:  $F(2,71) = 9.68$ ,  $p < 0.001$ ,  $\eta^2 = 0.21$ , indicating a large effect size. Follow-up tests showed significant differences between MTLE patients and healthy controls ( $p < 0.001$ ), and MTLE patients and unaffected relatives ( $p = 0.007$ ). The difference between unaffected relatives and healthy controls was not statistically significant. Patients had smaller ipsilateral hippocampal volumes than relatives and controls.

Planned linear contrasts revealed a significant linear trend in the ipsilateral hippocampus only:  $p < 0.001$ ,  $\eta^2 = 0.20$ , indicating the linear trend had a large effect size.

For the other comparisons with a priori interest (contralateral hippocampus and bilateral thalami), no significant differences were found and effect sizes were small ( $\eta^2 < 0.02$ ) for all comparisons except the ipsilateral thalamus, where a small to medium effect size was found for the linear contrast:  $F(2,71) = 1.76$ ,  $p = 0.079$ ,  $\eta^2 = 0.05$ , and patients had smaller ipsilateral thalamus volumes than controls, with relatives intermediate. No significant differences were found between relatives and controls.

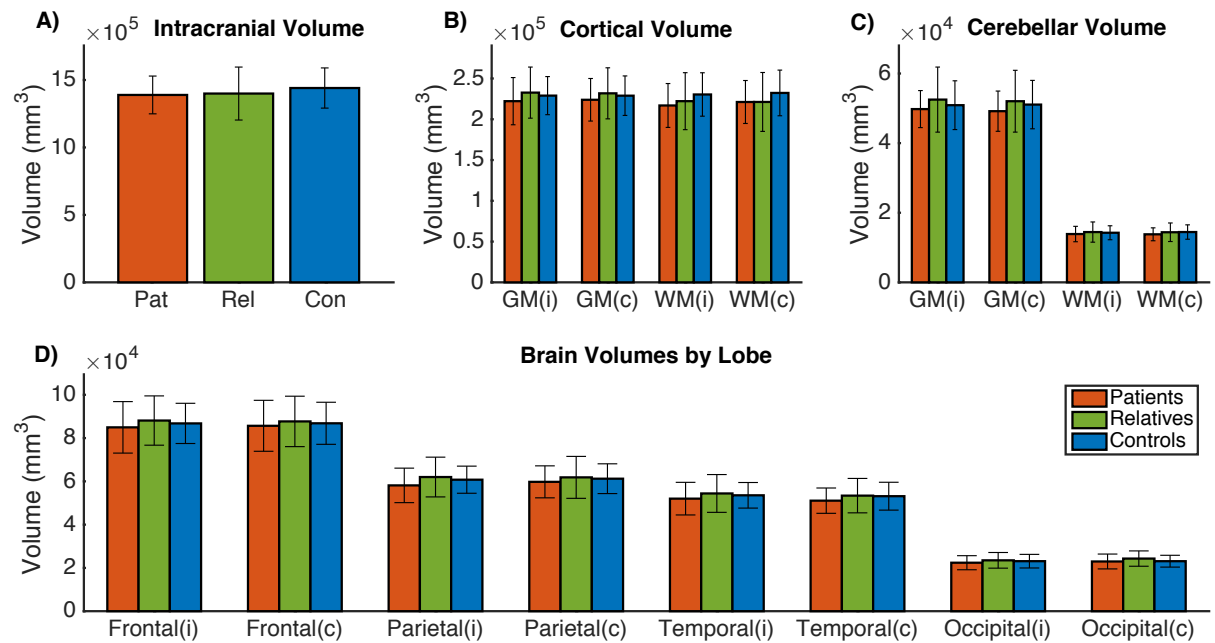


**Figure 6.2** Bar graphs of average cortical brain volumes. Plots show A) intracranial volumes, B) cortical grey and white matter volumes, C) cerebellar grey and white matter volumes, and D) cortical volumes by lobe. In plots B-C, (i) indicates ipsilateral side and (c) indicates contralateral side. Error bars shown are  $\pm 1$  standard deviation.

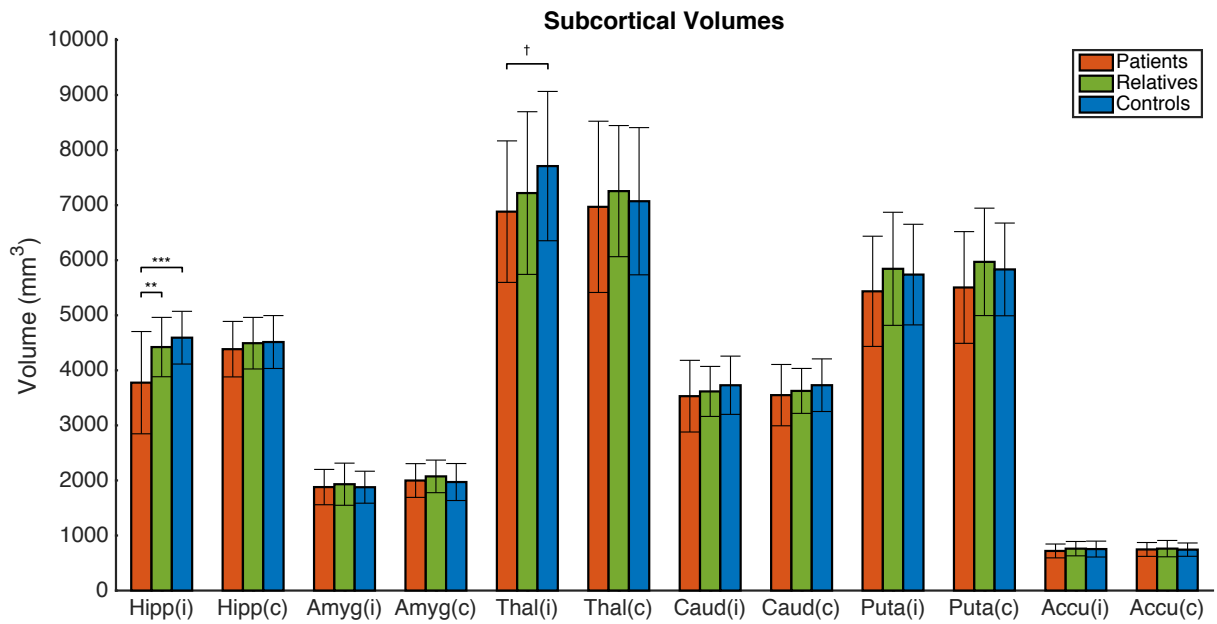


**Figure 6.3** Bar graphs of average subcortical volumes by group. Hipp = hippocampus, Amyg = amygdala, Thal = thalamus, Caud = caudate, Puta = putamen, Accu = accumbens. (i) indicates ipsilateral side and (c) indicates contralateral side. Error bars shown are  $\pm 1$  standard deviation. Group differences significant after Bonferroni correction at  $p < 0.01$  are denoted by \*\*,  $p < 0.001$  by \*\*\* and trends are denoted by †. A significant linear trend was found in the ipsilateral hippocampus.

#### 6.4.1.2 Morphometric analysis: metric distortion

In the vertex-wise analysis of metric distortion, MTLE patients had greater metric distortion in the ipsilateral temporal lobe compared to healthy controls. In top row of Figure 6.4, the cluster labelled “a” shows the significant cluster with a cluster-wise p-value of 0.0001 and cluster size of 2314.80mm<sup>2</sup>. The cluster extends over the parahippocampal gyrus, entorhinal cortex, fusiform gyrus, temporal pole, inferior temporal gyrus, middle temporal gyrus and superior temporal gyrus. There were no regions that showed significantly greater metric distortion in healthy controls compared to MTLE patients, and no significant differences in metric distortion between the two groups in the contralateral hemisphere.

When unaffected relatives were compared to healthy controls, relatives had increased metric distortion in the ipsilateral temporal lobe compared to the healthy control group. This cluster had high overlap but a smaller extent when compared with the cluster found in the MTLE patients and healthy controls contrast. The middle row in Figure 6.4 shows the significant cluster, cluster “b”, with a cluster-wise p-value of 0.0023 and cluster size of 1540.29mm<sup>2</sup>. The

cluster extended over parts of the parahippocampal gyrus, entorhinal cortex, fusiform gyrus, temporal pole, inferior temporal gyrus, and middle temporal gyrus. Relatives also had a cluster with significantly reduced metric distortion in the rostral middle frontal gyrus (cluster “c” in Figure 6.4). This cluster had a p-value of 0.0139 and size of 1189.48mm<sup>2</sup>. In the contralateral hemisphere, relatives had an area of increased metric distortion in the lingual gyrus (cluster-wise p-value = 0.0153, cluster size = 1305.33mm<sup>2</sup>), with the cluster extending over parts of the lingual gyrus and pericalcarine sulcus (cluster “d” in Figure 6.4).

Relatives showed significantly greater metric distortion than MTLE patients in the ipsilateral superior parietal lobe. The bottom row in Figure 6.4 shows the significant cluster, cluster “e”, with a cluster-wise p-value of 0.0061 and cluster size of 1471.67mm<sup>2</sup>. There were no significant differences in the contralateral hemisphere.

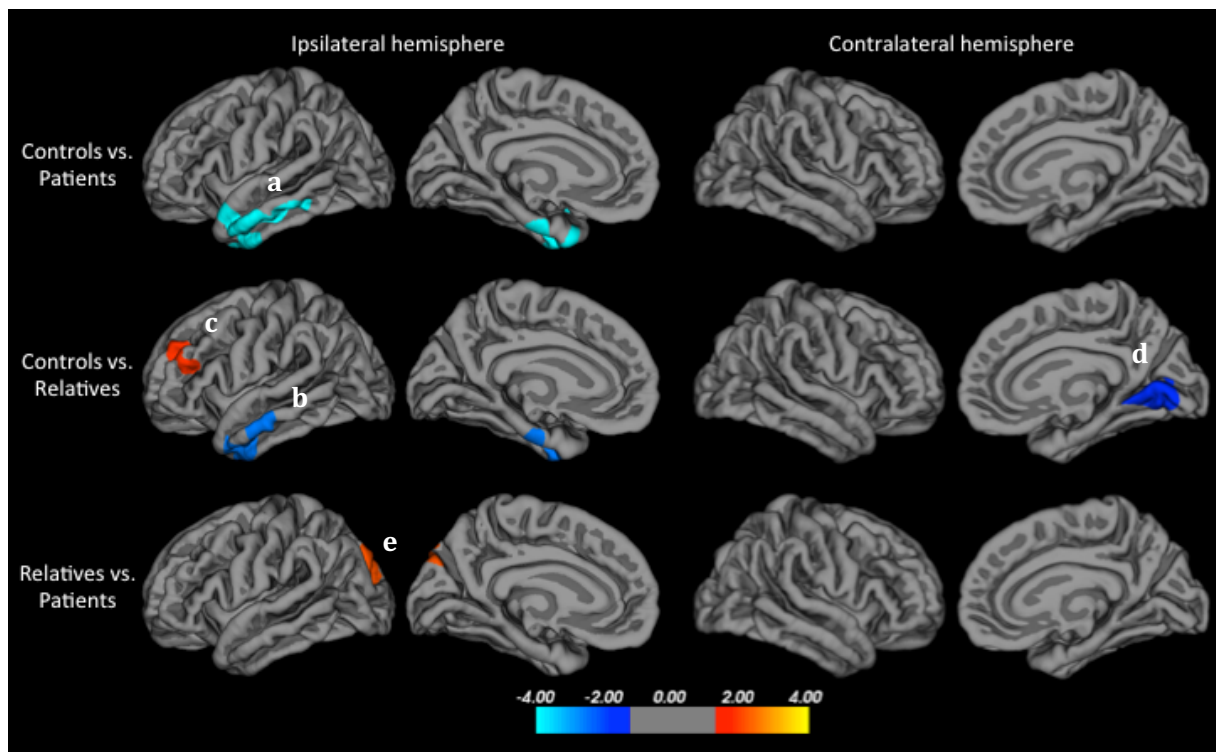


**Figure 6.4** Regional group differences in metric distortion. Top: Contrast showing healthy controls – MTLE patients. Middle: Contrast showing healthy controls – unaffected relatives. Bottom: Contrast showing unaffected relatives – MTLE patients. The colour bar corresponds to the Z-score statistic for each cluster after correction for multiple comparisons. Regions in red-yellow show increased metric distortion in the given contrasts while blue-light blue regions show reduced metric distortion. Clusters are labelled for easier reference in text.

#### 6.4.1.3 Morphometric analysis: surface area

MTLE patients had reduced surface area in the ipsilateral temporal lobe over a very similar cluster to that found in the metric distortion analysis. The top row in Figure 6.5 shows the significant cluster, labelled “a”, with a cluster-wise p-value of 0.0001 and cluster size of 2515.07mm<sup>2</sup>. The cluster extends over the parahippocampal gyrus, entorhinal cortex, fusiform gyrus, temporal pole, inferior temporal gyrus, middle temporal gyrus and superior temporal gyrus. There were no regions that showed significantly greater surface area in MTLE patients than in healthy controls.

In the relatives group compared to healthy controls (middle row Figure 6.5), there was reduced surface area in the ipsilateral temporal lobe (cluster “b”, cluster-wise p-value = 0.0061, cluster size = 1582.81mm<sup>2</sup>), with the cluster extending over parts of the entorhinal cortex, fusiform gyrus, inferior temporal gyrus and middle temporal gyrus. The superior parietal gyrus also showed reduced surface area in the relatives group (cluster “c”, cluster-wise p-value = 0.0464, cluster size = 1138.94mm<sup>2</sup>). There was also a cluster with increased surface area in the relatives group compared to controls. This cluster was in the ipsilateral rostral middle frontal gyrus (cluster “d”, cluster-wise p-value = 0.0337, cluster size = 1211.49mm<sup>2</sup>). In the contralateral hemisphere, relatives had reduced surface area in the lingual gyrus (cluster “e”, cluster-wise p-value = 0.0014, cluster size = 1958.86mm<sup>2</sup>).

Relatives had reduced surface area in the ipsilateral superior parietal lobe when compared to patients. The cluster had a p-value of 0.0038, cluster size of 1698.45 and is shown in the bottom row of Figure 6.5, labelled “f”.

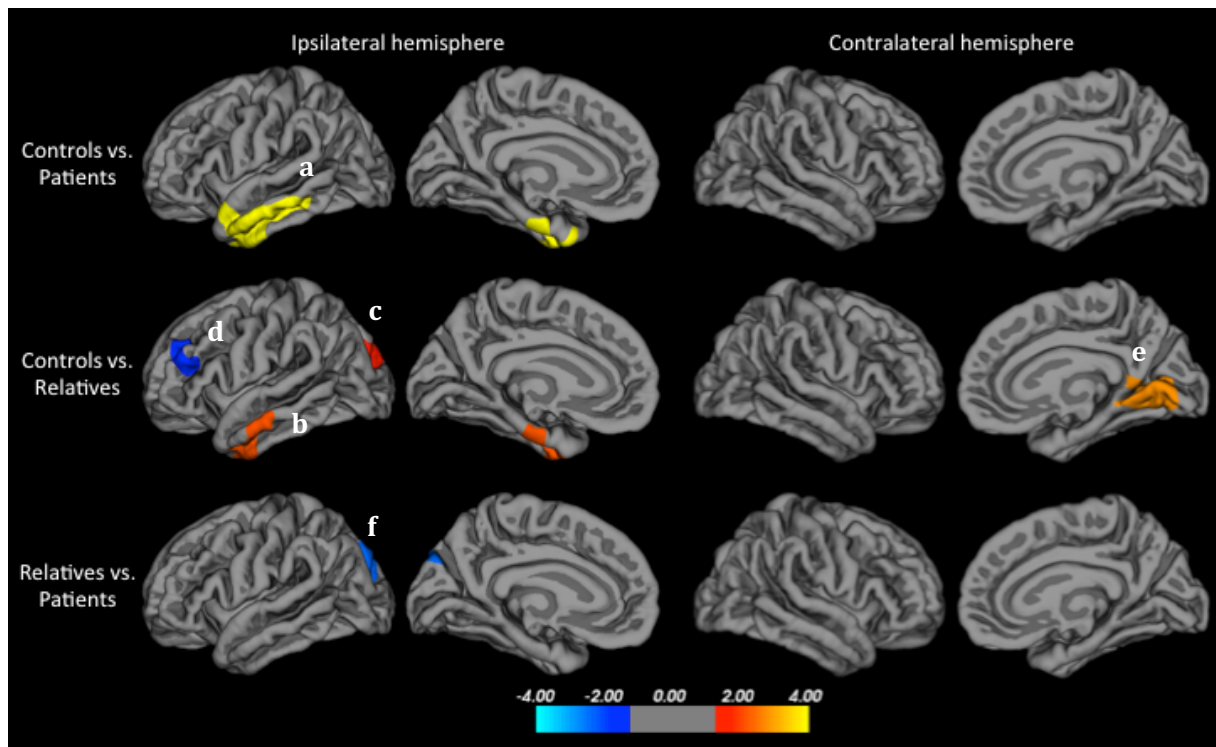


**Figure 6.5** Regional group differences in cortical surface area. Top: Contrast showing healthy controls – MTLE patients. Middle: Contrast showing healthy controls – relatives. Bottom: Contrast showing relatives – MTLE patients. The colour bar corresponds to the Z-score statistic for each cluster after correction for multiple comparisons. Regions in red-yellow show increased cortical surface area in the given contrasts while blue-light blue regions show reduced cortical surface area. Clusters are labelled for easier reference in text.

#### 6.4.1.4 Morphometric analysis: cortical thickness

Cortical thickness did not differ significantly between patients and controls in the ipsilateral hemisphere. In the contralateral hemisphere, the insula had greater cortical thickness in MTLE patients (cluster “a”, cluster-wise p-value = 0.004, cluster size 1230.59mm<sup>2</sup>; top row Figure 6.6).

Controls had greater cortical thickness in the ipsilateral temporal lobe when compared to relatives, with the cluster extending over parts of the middle temporal gyrus, superior temporal gyrus and inferior temporal gyrus (cluster “b”, cluster-wise p-value = 0.0194, cluster size = 936.67mm<sup>2</sup>). Parts of the inferior parietal lobe and temporal gyri showed greater thickness in relatives compared with controls (cluster “c”, cluster-wise p-value = 0.0001, cluster size = 1684.04mm<sup>2</sup>; middle row Figure 6.6). In the contralateral hemisphere, the insula (cluster “d”) had increased cortical thickness in the relatives, with a cluster-wise p-value of 0.0283 and cluster size 927.63mm<sup>2</sup>.

When relatives were compared with MTLE patients, lower cortical thickness was seen in patients in the lateral occipital cortex (cluster “e”, cluster-wise p-value = 0.0059, cluster size = 1090.00mm<sup>2</sup>), and greater cortical thickness in patients in the temporal lobe (cluster “f”, cluster-wise p-value = 0.0187, cluster size = 942.94mm<sup>2</sup>). There were no regions showing significantly different cortical thickness between relatives and patients in the contralateral hemisphere.

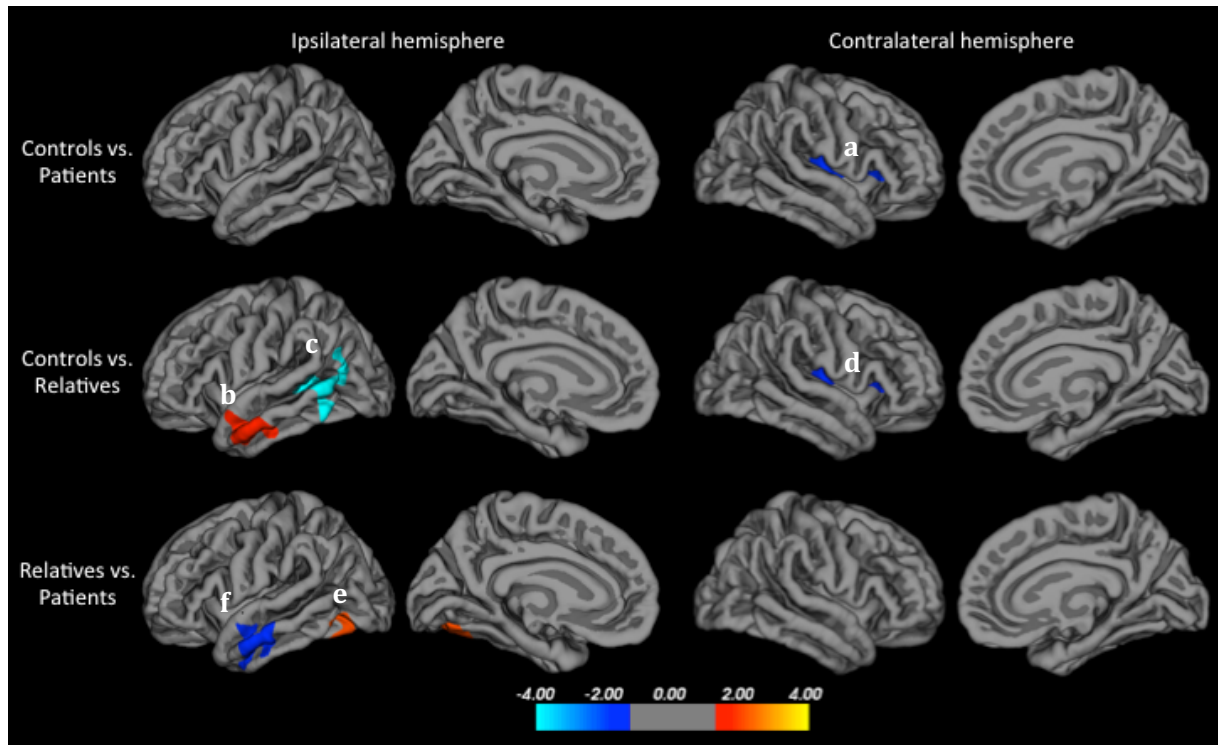


**Figure 6.6** Regional group differences in cortical thickness. Top: Contrast showing healthy controls – MTLE patients. Middle: Contrast showing healthy controls – relatives. Bottom: Contrast showing relatives – MTLE patients. The colour bar corresponds to the Z-score statistic for each cluster after correction for multiple comparisons. Regions in red-yellow show increased cortical thickness in the given contrasts while blue-light blue regions show reduced cortical thickness. Clusters are labelled for easier reference in text.

#### 6.4.2 FSL FIRST for subcortical shape analysis

Subcortical shape analysis revealed shape differences between MTLE patients and healthy controls in the ipsilateral hippocampus (**Error! Reference source not found.**; left), predominantly over the anterior hippocampus and body of the hippocampus. Using the significant region as a mask and comparing the average vertex shift across the mask in each subject revealed a predominant surface contraction across the vertices in the MTLE patient group in the ipsilateral hippocampus.

Comparing MTLE patients with relatives revealed significantly different shapes in the ipsilateral hippocampus across a similar set of vertices, but to a smaller extent than the differences seen in the comparison of MTLE patients with healthy controls (**Error! Reference source not found.**; middle). The differences were spread over the body of the hippocampus and did not extend much over the anterior hippocampus. This shape difference represented a contraction in the surface of the ipsilateral hippocampus.

Although there were no significant differences seen between unaffected relatives and healthy controls, an investigation of sub-threshold shape differences between the two groups at an uncorrected significance level of  $p < 0.01$  revealed ipsilateral hippocampal shape contraction in unaffected relatives, shown on the right panel of **Error! Reference source not found.** The less extensive hippocampal shape difference between MTLE patients and unaffected relatives, combined with sub-threshold shape differences between unaffected relatives and healthy controls suggests that there are subtle abnormalities in the shape of the ipsilateral hippocampus of unaffected relatives.

There were no significant differences in bilateral thalamic shape between any of the groups.

#### 6.4.3 FreeSurfer for hippocampal subfield volumes analysis

**Error! Reference source not found.** shows the average hippocampal subfield volumes in each group. Using Pillai's trace, there was a significant effect of group on ipsilateral hippocampal subfield volumes,  $F(24,122) = 3.14$ ,  $p < 0.001$ ,  $\eta^2 = 0.38$ , indicating a large effect size.

Separate univariate ANCOVAs on subcortical volumes revealed a significant effect of group in the presubiculum, subiculum, CA1, CA2/CA3, CA4, GC-DG, fimbria, molecular layer and hippocampal tail subfields on the ipsilateral side, all with effect sizes  $> 0.14$ , indicating large effects, except in the subiculum, where  $\eta^2 = 0.13$ , indicating a medium to large effect. Follow-up tests showed

significantly smaller volumes in MTLE patients compared with both healthy controls and relatives in all the above subfields except the fimbria and hippocampal tail. Linear trends were also significant in all the above sub-fields. There were no significant differences between unaffected relatives and healthy controls in any of the subfields except the fimbria, where unaffected relatives had higher fimbria volumes than both MTLE patients and healthy controls. Table 6.2 summarises the group differences in ipsilateral hippocampal subfields with the direction of the significant group differences from the follow-up tests given in the last column.

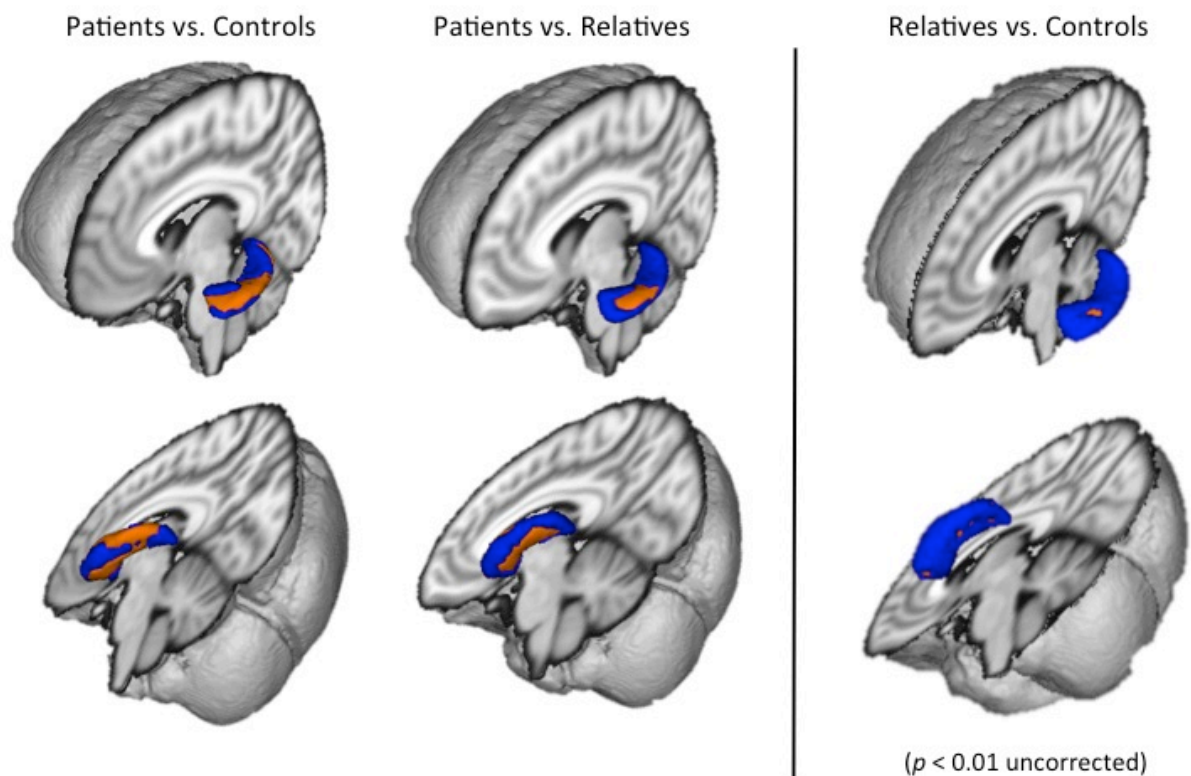


**Figure 6.7** Group differences in shape differences in the ipsilateral hippocampus. Shape differences between MTLE patients and healthy controls are shown on the left, and shape differences between MTLE patients and unaffected relatives are shown in the middle. On the right, sub-threshold shape differences between unaffected relatives and healthy controls are shown at  $p < 0.01$  uncorrected. The blue region indicates the hippocampus mask and the orange overlay indicates vertices that show a shape difference between groups.

In the contralateral hippocampus, using Pillai's Trace, the MANCOVA was not statistically significant, although there was a trend for a group effect on hippocampal subfields, with a large effect size:  $F(24,122) = 1.52$ ,  $p = 0.073$ ,  $\eta^2 = 0.23$ . Univariate ANCOVAs revealed the group difference was in the hippocampal fissure with a medium to large effect size:  $F(2,71) = 5.30$ ,  $p = 0.007$ ,  $\eta^2 = 0.13$ . There was also a trend toward a group difference in the hippocampal tail with a

medium effect size:  $F(2,71) = 2.42$ ,  $p = 0.096$ ,  $\eta^2 = 0.06$ . The linear trend was significant in the hippocampal fissure ( $p = 0.014$ ) and there was also a linear trend in the hippocampal tail ( $p = 0.077$ ) that was not statistically significant. MTLE patients showed a larger hippocampal fissure volume than unaffected relatives and healthy controls. In the hippocampal tail, the trend was toward a smaller volume in both MTLE patients and unaffected relatives compared to healthy controls.

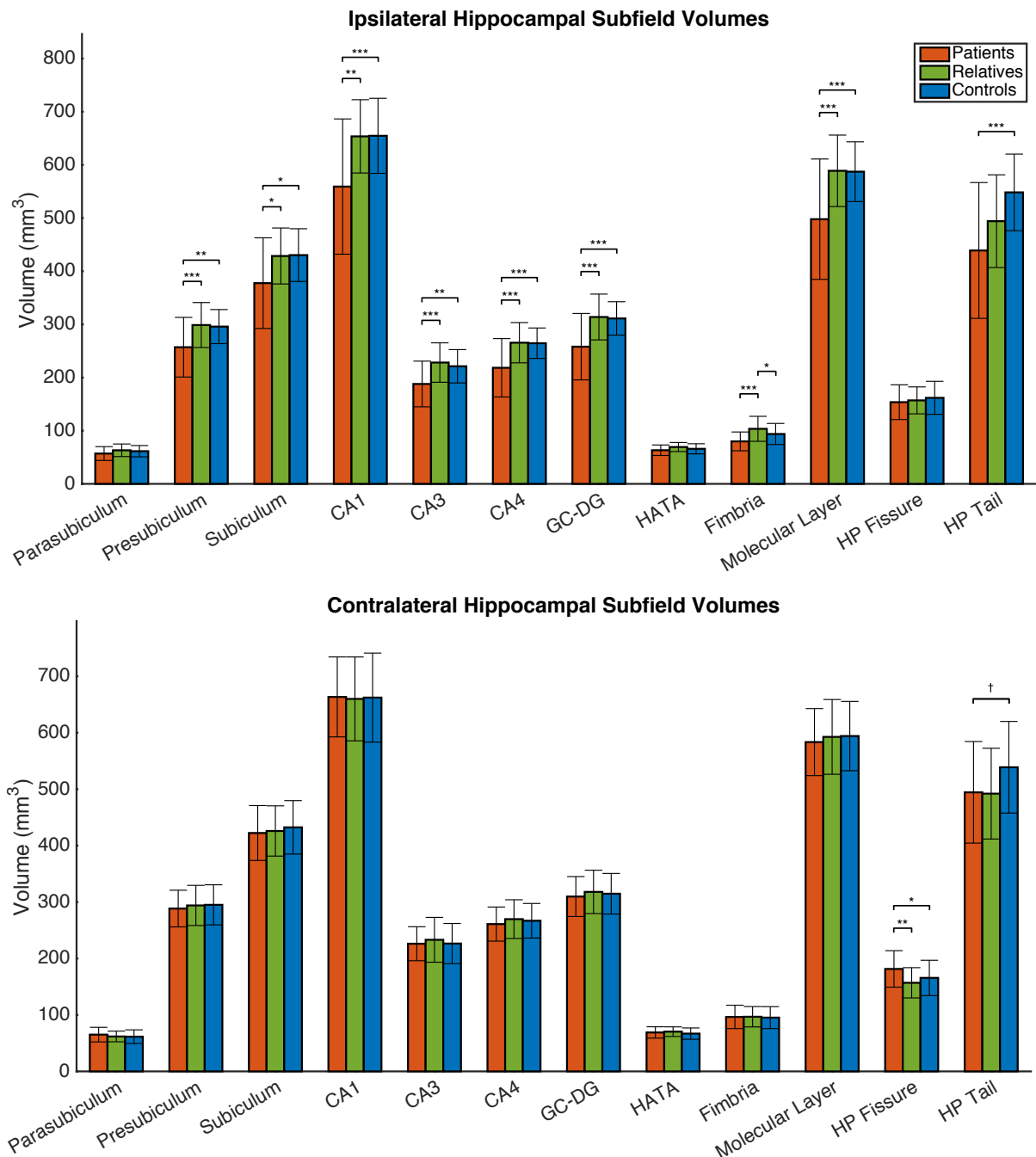


**Figure 6.8** Bar graph of average hippocampal subfield volumes in each group in the ipsilateral (top) and the contralateral (bottom) hippocampus. Error bars shown are  $\pm 1$  standard deviation. Significant differences after Bonferroni correction are denoted by: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

**Table 6.2** Group differences in ipsilateral hippocampal subfields

| Subfield            | <i>F</i> (2,71) | <i>p</i> -value | <sup>1</sup> Partial $\eta^2$ | <sup>2</sup> Group differences ( <i>p</i> -value)                                |
|---------------------|-----------------|-----------------|-------------------------------|----------------------------------------------------------------------------------|
| Parasubiculum       | 1.46            | 0.239           | 0.04                          | -                                                                                |
| Presubiculum        | 8.10            | 0.001           | 0.19                          | MTLE < Controls ( <i>p</i> = 0.005)<br>MTLE < Relatives ( <i>p</i> = 0.001)      |
| Subiculum           | 5.15            | 0.008           | 0.13                          | MTLE < Controls ( <i>p</i> = 0.019)<br>MTLE < Relatives ( <i>p</i> = 0.020)      |
| CA1                 | 8.41            | 0.001           | 0.19                          | MTLE < Controls ( <i>p</i> = 0.001)<br>MTLE < Relatives ( <i>p</i> = 0.002)      |
| CA2/CA3             | 8.39            | 0.001           | 0.19                          | MTLE < Controls ( <i>p</i> = 0.008)<br>MTLE < Relatives ( <i>p</i> = 0.001)      |
| CA4                 | 10.84           | < 0.001         | 0.23                          | MTLE < Controls ( <i>p</i> < 0.001)<br>MTLE < Relatives ( <i>p</i> < 0.001)      |
| GC-DG               | 12.10           | < 0.001         | 0.25                          | MTLE < Controls ( <i>p</i> < 0.001)<br>MTLE < Relatives ( <i>p</i> < 0.001)      |
| HATA                | 3.00            | 0.056           | 0.08                          | -                                                                                |
| Fimbria             | 10.50           | < 0.001         | 0.23                          | MTLE < Relatives ( <i>p</i> < 0.001)<br>Controls < Relatives ( <i>p</i> = 0.030) |
| Molecular Layer     | 10.74           | < 0.001         | 0.23                          | MTLE < Controls ( <i>p</i> < 0.001)<br>MTLE < Relatives ( <i>p</i> < 0.001)      |
| Hippocampal Fissure | 0.40            | 0.669           | 0.01                          | -                                                                                |
| Hippocampal Tail    | 8.25            | 0.001           | 0.19                          | MTLE < Controls ( <i>p</i> < 0.001)<br>Relatives < Controls ( <i>p</i> = 0.134)  |

<sup>1</sup> Effect size calculated using partial eta squared. <sup>2</sup> Corrected for multiple comparisons using Bonferroni.

## **6.5 Discussion**

This chapter aimed to characterise known patterns of structural differences in MTLE patients as compared to healthy controls and to investigate whether these differences also exist in unaffected relatives of MTLE patients. There were two main findings, the first was that there was ipsilateral hippocampal atrophy as a whole and across multiple subfields, as evidenced by reduced volumes and surface contractions in MTLE patients. There were also some subtle abnormalities in hippocampal volume and morphometry in unaffected relatives. The second finding was that both MTLE patients and unaffected relatives had ipsilateral mesial temporal, lateral temporal and temporal pole surface morphology alterations compared to healthy controls. Both the cortical and sub-cortical differences are well-established structural abnormalities in MTLE patients that have been previously reported in literature. The more subtle but similar patterns of altered temporal lobe cortical morphology seen in unaffected relatives has been reported in one other study, and the findings in this chapter provide additional evidence to suggest that structural abnormalities related to disease mechanisms may be heritable and could point to possible structural imaging endophenotypes for MTLE.

### **6.5.1 Ipsilateral hippocampal atrophy**

Across all analyses, the most consistent finding in the MTLE patients was the abnormality of the ipsilateral hippocampus. When compared with healthy controls, MTLE patients had significantly reduced overall ipsilateral hippocampal volumes, reduced volumes in 8 of the 12 ipsilateral hippocampal subfields, and a contraction of the ipsilateral hippocampal surface. This is an expected finding as hippocampal sclerosis is the most well-established pathology underlying MTLE and is associated with neuronal cell loss and gliosis, presenting as atrophy of the hippocampus on MRI.

In unaffected relatives, the fimbria subfield showed increased volume when compared to both MTLE patients and healthy controls. There has been no previous evidence for this, and in fact no

increases in hippocampal volumes of relatives of MTLE patients have been reported previously. Since the fimbria is one of the smallest subfield structures, accurate automatic segmentation for this subfield is difficult (Van Leemput et al., 2009), and thus this finding could be an artefact of the segmentation method. While overall hippocampal volumes and subfield volumes were not significantly reduced when compared to healthy controls, there was some evidence for subtle alterations in hippocampal shape in unaffected relatives, in the form of sub-threshold shape differences between relatives and controls, and a smaller extent of shape differences found when MTLE patients were compared to unaffected relatives (vs. when patients compared to healthy controls). These alterations are probably too subtle to be detected with the present sample size.

Evidence for altered hippocampi has been found in relatives of FMTLE, but in sporadic MTLE, the evidence has been inconclusive. Only one study has found altered hippocampal volumes and hippocampal asymmetry in relatives of MTLE patients, some of whom had a family history of epilepsy (although it wasn't clear whether these were familial MTLE cases) (Tsai et al., 2013). Two case reports looking at identical twin pairs discordant for MTLE found no abnormalities in the unaffected twin (Jackson et al., 1998; Karatas et al., 2012). In a preliminary study and a larger study using automated FreeSurfer segmentation, there were no significantly different hippocampal volumes found in unaffected relatives compared to healthy controls (Alhusaini et al., 2013; Scanlon, Ronan, et al., 2013).

The present investigations provide some evidence for altered hippocampi in relatives of sporadic MTLE cases. These trends toward altered hippocampal volume and morphometry may be because automated segmentation methods are not sensitive enough to detect subtle differences in the hippocampus in relatives.

### **6.5.2 Abnormalities in ipsilateral temporal lobe morphology**

The second main finding was that of increased metric distortion in the ipsilateral temporal lobe in MTLE patients that appear to be driven by reductions in surface area rather than cortical thickness. A very similar pattern of increased metric distortion was found in relatives of MTLE patients, albeit to a smaller extent. In relatives however, these differences in metric distortion appear to be driven by a combination of reduced surface area and cortical thinning.

Extra-hippocampal abnormalities are often reported alongside hippocampal alterations in MTLE patients, including atrophy in other ipsilateral temporal lobe structures (Bonilha et al., 2003; Richardson, 2010). Cortical volume reductions may have contributions from both surface area reductions and cortical thinning, which are thought to have separate genetic influences and it has been suggested that using volume alone in a study of imaging endophenotypes may be insufficient (Panizzon et al., 2009).

A study that characterised the different contributions of surface area and cortical thickness to the overall cortical surface distortion found that surface area contractions, rather than cortical thinning contributed to ipsilateral temporal lobe atrophy in MTLE patients (Alhusaini et al., 2012). They went on to investigate these morphological changes in unaffected relatives of MTLE patients and found a similar pattern of changes in relatives that appeared to be driven primarily by cortical surface area contractions in patients and relatives, but in relatives there was a non-significant trend for cortical thinning in the same antero-medial temporal lobe regions (Alhusaini, Whelan, Doherty, et al., 2016). The present study was able to reproduce these findings in relatives of MTLE patients, providing further evidence that such morphology changes in the temporal lobe may have a heritable component that is related to disease mechanisms.

The finding of altered cortical morphology extending to the ipsilateral temporal pole is interesting as this region has been implicated in pathology associated with MTLE (Kahane et al., 2002). It has been shown to play a critical role in temporal lobe seizures and may contribute to

failure of amygdalohippocampectomy in MTLE patients (Chabardès et al., 2005). In a post-mortem study, the temporal and frontal poles have been reported to show gliosis and microgliosis in cases of “classical” hippocampal sclerosis (Blanc et al., 2011). In the study presented here, cortical morphology alterations were seen in MTLE patients in the temporal pole, but not in unaffected relatives, suggesting alterations in this region may be specific to the patient group.

Mechanisms of structural changes in epilepsy remain poorly understood and it is uncertain whether the more widespread alterations outside of the ipsilateral hippocampus represent structural damage due to repeated seizures or whether they are due to genetic factors. From the evidence in unaffected relatives presented here, perhaps these subtle structural alterations represent a pre-disposition to epilepsy, but are not in themselves sufficient for epilepsy, suggesting a combination of genetic and environmental factors in epilepsy.

### **6.5.3 Lack of extra-temporal atrophy**

There were no extra-temporal abnormalities found in the present sample of MTLE patients compared to healthy controls, although abnormalities such as bilateral thalamic atrophy are frequently reported in other MTLE cohorts (Keller & Roberts, 2008). There was a linear trend toward smaller ipsilateral thalamus volumes in MTLE patients, although this was not statistically significant. This inconsistency could be due to several limitations of this study, including a relatively small sample size, combining left and right MTLE patients, and heterogeneity of the sample for hippocampal sclerosis. It is suggested that extra-hippocampal alterations are predominantly found in chronic epilepsy patients and relate to cognitive and memory deficits (Seidenberg et al., 2008; Stewart et al., 2009), but the study sample has a wide variation in disease duration and cognition and memory were not assessed.

#### **6.5.4 Implications for structural imaging endophenotypes in MTLE**

This chapter has demonstrated that ipsilateral temporal cortex morphology and to an extent hippocampal volumes may be suitable endophenotypes for MTLE and deserve further investigation. These structural alterations meet all 4 criteria for endophenotypes that were outlined in Chapter 1. They are well-characterised structural abnormalities in MTLE patients. These alterations are probably more trait- than state-dependent, since morphological alterations that are prominent enough to be captured using these analysis methods probably take longer to be established and are assumed to be present both ictally and interictally. They are presumed to be heritable. Heritability of the candidate endophenotypes could not be directly calculated as the present study did not have matching pairs of patients and relatives. A correlation between the candidate endophenotype values in each patient/relative pair to quantify “heritability” of the measure, could thus not be calculated. However, we do know that brain volumes and consequently cortical surface area and thickness are highly heritable (Panizzon et al., 2009; Peper et al., 2007; Wen et al., 2016) and age-related (Raznahan et al., 2011). Finally, they appear to have a higher occurrence in unaffected relatives than in the general healthy control population.

There were some morphological differences between healthy controls and unaffected relatives that were not found when controls were compared to MTLE patients. Considering morphological alterations in the context of endophenotypes, these are not as interesting as these alterations are not present in the patient population and thus do not meet the endophenotype criteria of being related to the disease trait or co-segregating within families.

#### **6.5.5 Limitations and further work**

Automated segmentation methods, though less time consuming, are prone to errors and manual checking of the segmented regions should be performed, which then introduces human errors due to errors of judgement and expertise. Although segmentations were visually checked before analysis, no manual intervention was used to correct any segmentations. A recent study also

found that manual intervention may improve segmentation in some cases, but does not affect overall mean volume, surface area and cortical thickness values, suggesting that manual intervention provides little extra benefit over the automated segmentation techniques (McCarthy et al., 2015).

The method for segmenting hippocampal subfields used in this chapter were originally developed for much higher imaging resolution than the 3T available in the present study. There is also no way of assessing whether the segmentations are of high enough quality since the boundaries of the different subfields may not be visible or may be at a smaller resolution than available with 3T. Differences found are likely to be due to an overall difference in hippocampal volumes rather than individual subfields, and the results from this analysis have to be interpreted with caution.

The study presented here provides additional evidence for subtle volumetric and morphometric alterations in the hippocampus and temporal lobe of unaffected relatives of MTLE patients. These findings suggest that alterations in these structures may be suitable candidates for structural imaging endophenotypes for MTLE. Replications of this work in larger cohorts is required to establish whether the subtle hippocampal volume alterations remain.

## Chapter 7 Alterations in fornix microstructure in MTLE

### 7.1 Introduction

In the previous chapter, hippocampal atrophy and widespread temporal lobe morphology alterations were observed in the current cohort of MTLE patients with subtle alterations in hippocampal volume and temporal lobe morphology also found in first-degree unaffected relatives. In addition to volumetric and morphometric grey matter structural changes, white matter (WM) microstructure alterations are also a well-established trait in MTLE patients, with widespread bilateral abnormalities reported in many studies of patients with unilateral MTLE (Gross, 2011; Rodríguez-Cruces & Concha, 2015; Yogarajah & Duncan, 2008). The most frequently reported alterations are reduced fractional anisotropy (FA) and increased mean diffusivity (MD)<sup>11</sup>. These alterations are most prominent around the affected hippocampus and in ipsilateral tracts with connections to the temporal lobe, and are less pronounced with increasing distance from the affected hippocampus (Otte et al., 2012).

WM organisation is highly heritable, as evidenced by data in healthy controls where genetic factors account for as much as 46% of inter-individual variance in FA and diffusivity values (Jahanshad et al., 2013; Kochunov et al., 2010). The well-established WM tract microstructure alterations in MTLE patients, together with evidence for the high heritability of WM organisation, lends credence to the idea that WM tract alterations in MTLE may be suitable endophenotypes for the disorder and deserves further investigation. This was the basis of a recently published study that found specific localised tract alterations in non-lesional MTLE patients and their unaffected siblings (Whelan et al., 2015). The authors of that study found alterations in localised regions of the ipsilateral superior longitudinal fasciculus and cortico-spinal tract of both patients and their unaffected siblings, that the MD in the tracts of siblings

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<sup>11</sup> FA and MD are DTI measures of white matter microstructure. MD is an index of the amount of water mobility in the tissue, with lower diffusivity values often seen in white matter, while FA is a measure of how much diffusivity is preferred in a particular direction. These measures are indirectly related to a number of tissue features including myelination of fibre bundles.

were increased but not the FA, and that the MD values were highly correlated between sibling and patient pairs.

In this chapter, I investigate whether white matter microstructure alterations could be considered candidate endophenotypes for MTLE in a group of predominantly lesional MTLE patients and their first-degree unaffected relatives. To my knowledge, this study represents the first investigation of white matter abnormalities in unaffected relatives of a group of predominantly lesional MTLE patients. Three white matter tracts with direct connections to and from the hippocampus and temporal lobes were manually defined in MTLE patients, unaffected relatives and healthy controls. I focussed on the fornix as it is the main fibre bundle connecting the hippocampus to limbic regions and has been shown to have reduced FA and increased MD bilaterally in unilateral MTLE patients (Concha, Beaulieu, et al., 2005). I also correlated hippocampal volumes with tract measures in each group to identify disease specific variations of WM microstructure. In addition, in a subset of data, I also manually defined two other tracts of interest in MTLE, the uncinate fasciculus and parahippocampal cingulum, both of which have also shown altered white matter microstructure in MTLE patients (Otte et al., 2012).

## **7.2 Aims and hypotheses**

This chapter aims to characterise neuroimaging measures related to microstructural characteristics of white matter tracts starting and terminating in the hippocampus and limbic regions in patients with MTLE and first-degree unaffected relatives compared to healthy control participants, and to assess the suitability of such measures as endophenotypes for MTLE. This was done by the virtual dissection of diffusion weighted images to identify tracts within the fornix in each subject and comparing several tract measures between groups. These measures are the FA, MD and the three eigenvalues representing diffusivity along the fibre tract ( $\lambda_1$ ), and perpendicular to the tract ( $\lambda_2$  and  $\lambda_3$ ). To relate WM tract integrity to the main disease trait of reduced hippocampal volume, I also correlated each of the tract values from the fornix to hippocampal volumes in each group. As a pilot analysis, the uncinate fasciculus (UNC) and

parahippocampal cingulum (PHC) were also virtually dissected in a smaller subset of subjects and tract measures were compared between groups.

The main hypotheses are that:

- 1) MTLE patients will show predominantly unilateral decreases in FA, increases in MD and increases in diffusivity in all 3 directions of the tracts compared to healthy controls.
- 2) Relatives of MTLE patients will show subtle differences in WM tracts with values intermediate between MTLE patients and healthy controls.
- 3) There will be a strong correlation between ipsilateral hippocampal volumes and tract measures and that the correlations would be in the same direction in both MTLE patients and relatives.

## **7.3 Method**

The general study protocol and image acquisition have been described in Chapter 5. The methods described below are specific to the analyses performed in this chapter.

### **7.3.1 Participants**

Data from 23 patients with MTLE, 21 relatives of patients with MTLE and 30 healthy controls were used in this analysis. As previously described, all data were flipped so that the pathological hippocampus is on the left for all participants and any differences on the left in the following analysis are considered ipsilateral to the affected hippocampus. Details of participants whose data have been left-to-right flipped are given in Appendix C.

Of the 23 MTLE patients, 14 had left-lateralised MTLE and 9 had right-lateralised MTLE. The sample characteristics are summarised in Table 7.1, and further information about which patients contributed to this analysis and any exclusions are given in Appendix C. Age was compared between groups using an ANOVA and gender was entered into a  $\chi^2$  test to assess for

group differences. A subset of 4 randomly chosen participants from each of the 3 groups were chosen for the pilot analysis of the UNC and PHC tracts.

**Table 7.1** Sample characteristics

|                                          | MTLE Patients | Relatives   | Controls   |
|------------------------------------------|---------------|-------------|------------|
| Number                                   | 23            | 21          | 30         |
| Age in years: mean (SD)                  | 38.1 (11.8)   | 33.2 (13.4) | 34.9 (9.9) |
| Gender: Female (%)                       | 10 (43.5%)    | 12 (57.1%)  | 16 (53.3%) |
| <b>Clinical Characteristics</b>          |               |             |            |
| Age of onset in years: mean (SD)         | 22.5 (8.5)    | -           | -          |
| Duration of epilepsy in years: mean (SD) | 16.3 (13.5)   | -           | -          |

### 7.3.2 Image processing

Diffusion weighted MR images were first corrected for field inhomogeneity distortions using FSL's *topup* software (Andersson et al., 2003) before being processed in ExploreDTI (v 4.8.5, <http://www.exploredti.com>, Leemans, Jeurissen, Sijbers, & Jones, 2009). All images were converted to ExploreDTI format and checked for obvious errors in conversion by checking that the colours in the DTI images represented the appropriate orientations for landmark regions like the corpus callosum (red, or left-right orientation), cingulum (green, or anterior-posterior orientation), and corticospinal tracts (blue, or superior-inferior orientation). Data were then corrected for participant motion and eddy current-induced distortions using inbuilt tools in ExploreDTI.

As tracts in the fornix are particularly susceptible to signal contamination due to the presence of free water in the nearby cerebrospinal fluid (Concha, Gross, et al., 2005), a free water correction algorithm was applied to reduce partial volume artefacts using custom Matlab scripts (Pasternak

et al., 2009). These scripts model the diffusion properties of brain tissues separately from the surrounding free water, producing a corrected DTI image as well as an image of the free water volume fraction.

Whole-brain tractography was performed on all pre-processed data in native diffusion space using a deterministic tractography algorithm based on constrained spherical deconvolution in ExploreDTI. A step size of 0.5mm, angle threshold of 45°, minimum fibre length of 50mm and maximum fibre length of 500mm were used.

### **7.3.3 Virtual tract delineation**

Three tracts were manually reconstructed following whole-brain tractography. There are well documented and highly reproducible methods for the manual virtual dissection of tracts (Catani & Thiebaut de Schotten, 2008; Catani et al., 2002) and the following protocol for manual tract delineation was adopted from Metzler-Baddeley and colleagues (Metzler-Baddeley et al., 2011). Tracts were defined based on the placement of SEED, AND and NOT regions of interest (ROIs), where SEED ROIs are the starting seed points of tracts, AND ROIs denote regions where tracts from the SEED must pass through, and NOT ROIs denote regions where there should not be any fibres passing through. Given the operator dependent nature of the methods, a number of the tracts were independently reconstructed by two observers (Dr Paul Wright and me) to allow intra- and inter-rater reliability for each tract to be tested.

#### ***Fornix***

The bilateral fornix was delineated by placing a SEED ROI at the curve of the fornix on a coronal slice of the image, and an AND ROI around the crus of the fornix bilaterally on an axial slice. Two NOT ROIs were drawn on a coronal section a few slices anterior to the SEED ROI and a few slices posterior to the crus of the fornix to exclude fibres extending anteriorly from the curve of the fornix and posteriorly into the occipital lobes. Another NOT ROI on the axial plane was also drawn through the largest part of the corpus callosum to exclude streamlines belonging to it.

Additional NOT ROIs were drawn on a subject specific basis to exclude any obvious tracts that do not belong to the fornix based on known anatomy. To split the fornix into left and right fibres, a NOT ROI was placed on the sagittal plane along the body of the fornix on the right and left sides respectively. An example of ROI placement for the fornix tract is given in Figure 7.1A.

### ***Uncinate Fasciculus (UNC)***

The left and right uncinate fasciculi were delineated separately. For each UNC, ROIs were placed in the corresponding hemisphere. A SEED ROI was placed on the first coronal slice anterior to the genu of the corpus callosum encompassing the anterior-posterior fibres of the UNC entering the frontal lobe. An AND ROI was placed in the axial plane where the UNC curves vertically, approximately at a level one slice superior to the upper pons. A NOT ROI encompassing the whole coronal cross-section in line with the posterior edge of the AND ROI was drawn to exclude fibres extending posteriorly. Additional NOT ROIs were placed on a subject specific basis to exclude frontal fibres veering superiorly, medially or laterally to remove obvious spurious fibres. An example of ROI placement for the UNC tract is given in Figure 7.1B.

### ***Parahippocampal Cingulum (PHC)***

The left and right PHC were delineated separately. For each tract bundle, ROIs were placed on the corresponding side. A SEED ROI was placed in the axial plane around the green anterior-posterior bundle of the PHC, approximately level with the most superior slice of the pons. No AND ROIs were used for this tract. A NOT ROI was placed across the whole sagittal midline plane to exclude cross-hemisphere fibres. Additional NOT ROIs were placed on a subject specific basis to exclude fibres from the dorsal cingulum, fibres extending into the occipital lobes, fibres extending posteriorly and inferiorly from the main body of the PHC, fibres extending medially or laterally from the main body of the PHC and fibres extending inferiorly from the anterior temporal end of the PHC. An example of ROI placement for the PHC tract is given in Figure 7.1C.

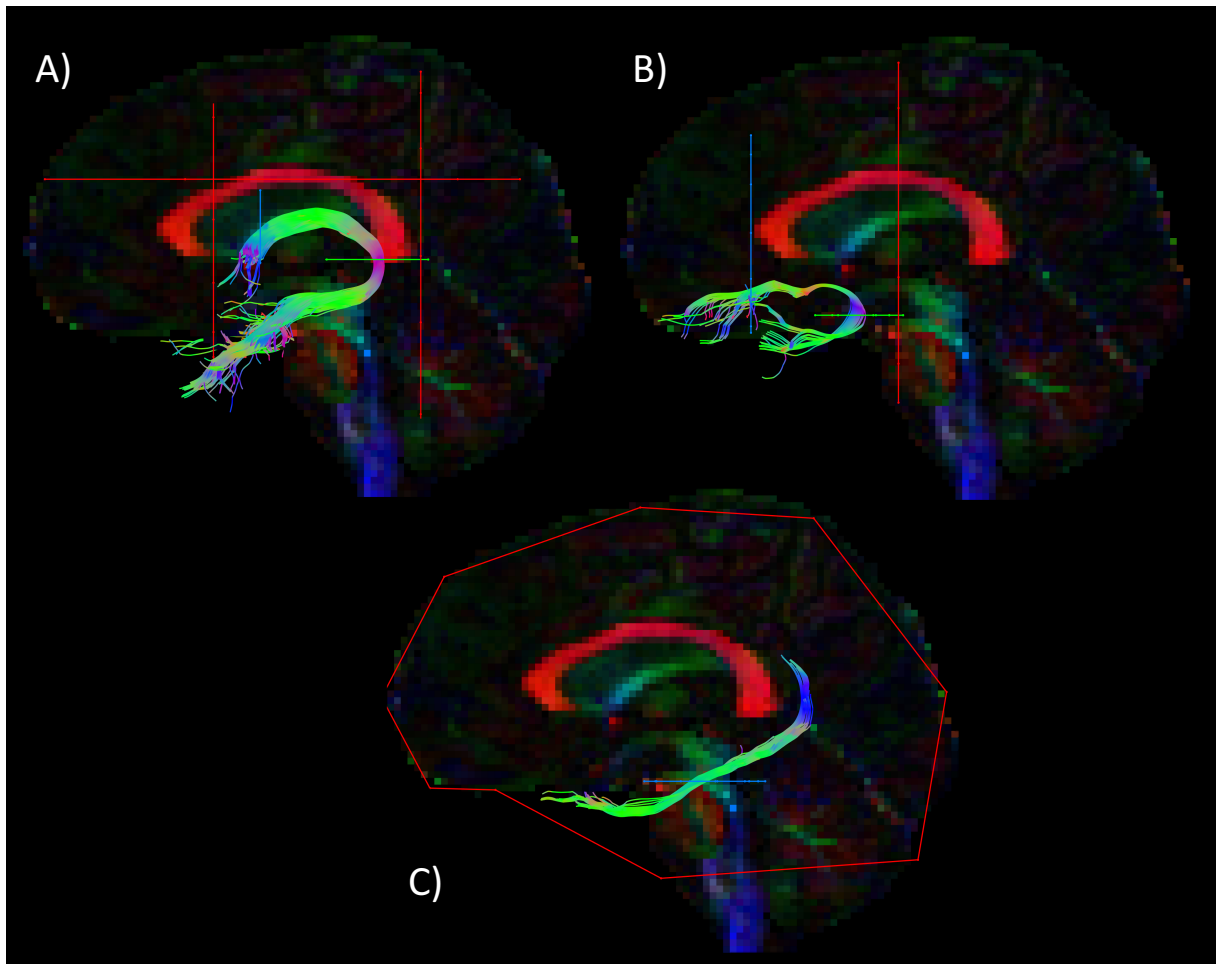


**Figure 7.1** Placement of ROIs to delineate the tracts of the (A) fornix, (B) uncinate fasciculus on the left side and (C) parahippocampal cingulum on the left side in an example control participant. Cross-sectional ROIs in the coronal and axial planes appear as “lines” in the sagittal view. The SEED ROIs are shown in blue, the AND ROIs are shown in green and the main NOT ROIs are shown in red. Additional NOT ROIs are subject-specific depending on the presence of extraneous tracts and not shown here.

### 7.3.4 Statistical analysis

Mean FA, MD and the three eigenvalues were extracted from each of the tract reconstructions in ExploreDTI giving ipsilateral and contralateral tract measures for each tract.

For the fornix, an initial MANCOVA was conducted in SPSS to assess for between-group differences in both ipsilateral and contralateral fornix tract measures with participant age as a covariate. Group effects were assessed for significance using Pillai’s trace and further univariate ANCOVAs were performed on significant effects. Follow-up t-tests were performed on significant univariate ANCOVAs and adjustment for multiple comparisons was done using Bonferroni correction. Planned linear contrasts were also performed for each measure to test the

hypothesis that volumes in unaffected relatives lie in between MTLE patients and healthy controls. The partial eta squared ( $\eta^2$ ) effect size was computed for the MANCOVA and each linear contrast. A rough guideline for small, medium and large effect sizes using  $\eta^2$  are 0.01, 0.06 and 0.14 respectively (Cohen, 1988).

Planned contrasts were specified, with both MTLE patients and relatives hypothesised to have reduced FA and increased MD compared to healthy controls. The MANCOVA was assessed for significance using Pillai's trace and custom contrasts assessed for significance at  $p < 0.008$  after adjustment for 6 multiple comparisons (3 groups, 2 measures) using Bonferroni's method.

The eigenvalues were assessed separately (and only if a significant effect was found with mean diffusivity) because both the FA and MD are derived from the eigenvalues and should not be assessed within the same analysis (Concha, Beaulieu, et al., 2005). Hence, if a significant effect of group was found with MD, a separate MANCOVA was conducted to determine group differences in the individual components which might underlie the MD change.

Pearson's correlation coefficients were computed to assess the relationships between ipsilateral hippocampal volumes (see Chapter 6) and these tract measures in each group. A one-tailed  $p$ -value for each correlation coefficient was computed and tested for significance at a  $p$ -value equivalent to  $p < 0.05$  after correction for multiple comparisons using Bonferroni's method.

For the UNC and PHC tracts, only the mean FA and mean MD were compared to identify summary differences in tract measures, as both of these values are derived from the three eigenvalues. A MANCOVA was conducted in SPSS with participant age as a covariate. Group effects were assessed using Pillai's trace and further univariate ANCOVAs were performed. Follow-up t-tests were performed on univariate ANCOVAs. Planned linear contrasts were also performed for each measure to test the hypothesis that volumes in unaffected relatives lie in between MTLE patients and healthy controls. No multiple corrections for multiple comparisons

were made as this was an exploratory pilot analysis on a small subset of data. The partial eta squared ( $\eta^2$ ) effect size was computed for the MANOVA and each univariate ANCOVA.

## 7.4 Results

The participant groups did not differ significantly in age or gender when assessed using a one-way ANOVA and  $\chi^2$  test.

Intra-rater reliability was determined to be high for all tracts as measured by the average intra-class correlation coefficients (ICC) of the FA and MD values from the bilateral tracts: fornix ICC = 0.99; UNC ICC = 0.95; PHC ICC = 0.91. Inter-rater reliability was also considered high: fornix ICC = 0.97; UNC ICC = 0.81; PHC ICC = 0.85.

### 7.4.1 Fornix

There was a significant effect of group on mean FA and MD values in the fornix tracts,  $F(8,136) = 2.65$ ,  $p = 0.010$ ,  $\eta^2 = 0.14$ , suggesting a large effect size. Univariate ANCOVAs revealed group differences were in the ipsilateral FA,  $F(2,70) = 6.78$ ,  $p = 0.002$ ,  $\eta^2 = 0.16$ , and ipsilateral MD,  $F(2,70) = 7.00$ ,  $p = 0.002$ ,  $\eta^2 = 0.17$ . Follow-up t-tests revealed MTLE patients had significantly lower ipsilateral FA when compared to unaffected relatives ( $p = 0.018$ ) and healthy controls ( $p = 0.002$ ). MTLE patients also had significantly higher ipsilateral MD when compared to unaffected relatives ( $p = 0.006$ ) and healthy controls ( $p = 0.004$ ). Although there were no significant group differences between unaffected relatives and healthy controls, planned linear contrasts were significant for both ipsilateral FA ( $p = 0.001$ ) and ipsilateral MD ( $p = 0.001$ ). There were no significant group differences in the contralateral measures. Bar graphs of FA and MD are shown in Figure 7.2 (top row).

Since there was a significant effect of group on ipsilateral MD values, the ipsilateral eigenvalues were compared in another MANCOVA and a significant effect of group on ipsilateral eigenvalues was also found:  $F(6,138) = 2.64$ ,  $p = 0.019$ ,  $\eta^2 = 0.10$ , indicating a medium to large effect size.

Univariate ANCOVAs revealed the group differences were in all three eigenvalues, with large effect sizes seen for  $\lambda_2$  and  $\lambda_3$  and a medium to large effect size in  $\lambda_1$  ( $\lambda_1$ :  $F(2,70) = 4.33$ ,  $p = 0.017$ ,  $\eta^2 = 0.11$ ;  $\lambda_2$ :  $F(2,70) = 7.55$ ,  $p = 0.001$ ,  $\eta^2 = 0.18$ ;  $\lambda_3$ :  $F(2,70) = 7.78$ ,  $p = 0.001$ ,  $\eta^2 = 0.18$ ). Planned linear contrasts were significant for all three measures ( $p = 0.020$ ,  $p = 0.001$ , and  $p = 0.001$ , respectively for  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$ ). Follow-up t-tests showed MTLE patients had higher  $\lambda_1$  values than unaffected relatives ( $p = 0.025$ ) and a trend toward higher values than healthy controls ( $p = 0.059$ ). MTLE patients also had higher  $\lambda_2$  and  $\lambda_3$  values than unaffected relatives ( $p = 0.006$  and  $p = 0.004$  for  $\lambda_2$  and  $\lambda_3$  respectively), and healthy controls ( $p = 0.002$  and  $p = 0.002$  for  $\lambda_2$  and  $\lambda_3$  respectively). There were no significant differences between unaffected relatives and healthy controls in any of the eigenvalues. Bar graphs of mean eigenvalues in each group are shown in Figure 7.2 (bottom row).

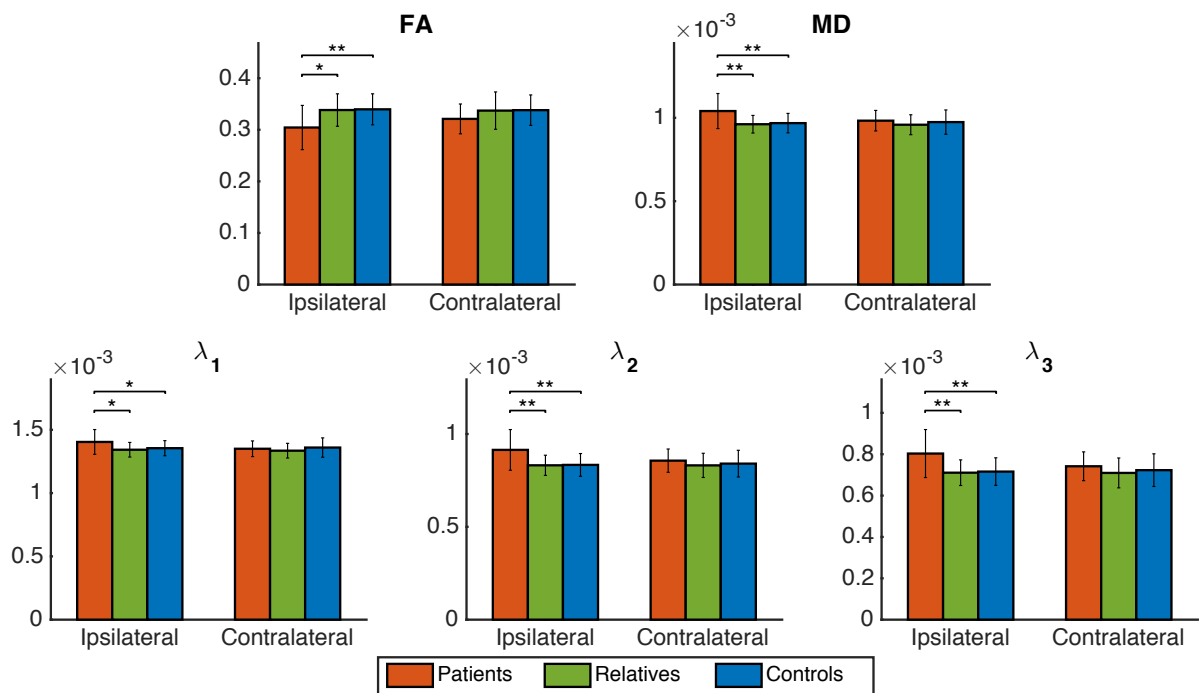


**Figure 7.2** Mean FA and diffusivity measures of the ipsilateral and contralateral fornix tract in each group. Error bars shown are  $\pm 1$  standard deviation. Significant differences after Bonferroni correction are denoted by \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

#### 7.4.2 Correlation of hippocampal volumes with fornix FA and MD measures

Ipsilateral FA and MD values were correlated with ipsilateral hippocampal volumes within each group. Corrections for 6 multiple comparisons were made using Bonferroni's method and  $p$ -

values were considered significant at  $p < 0.008$ . There was a significant positive correlation between FA values of the ipsilateral fornix and ipsilateral hippocampal volumes in MTLE patients ( $r = 0.75, p < 0.001$ ). A significant negative correlation was found between MD values of the ipsilateral fornix and ipsilateral hippocampal volumes in MTLE patients ( $r = -0.64, p = 0.001$ ). In unaffected relatives, there was no significant correlation between ipsilateral hippocampal volumes and ipsilateral fornix FA values, but there was a trend toward a negative correlation with ipsilateral MD values ( $r = -0.47, p = 0.016$ ). Ipsilateral hippocampal volumes did not correlate significantly with either FA or MD values in healthy controls. Correlations between ipsilateral hippocampal volumes and FA and MD in each group are shown in Figure 7.3.

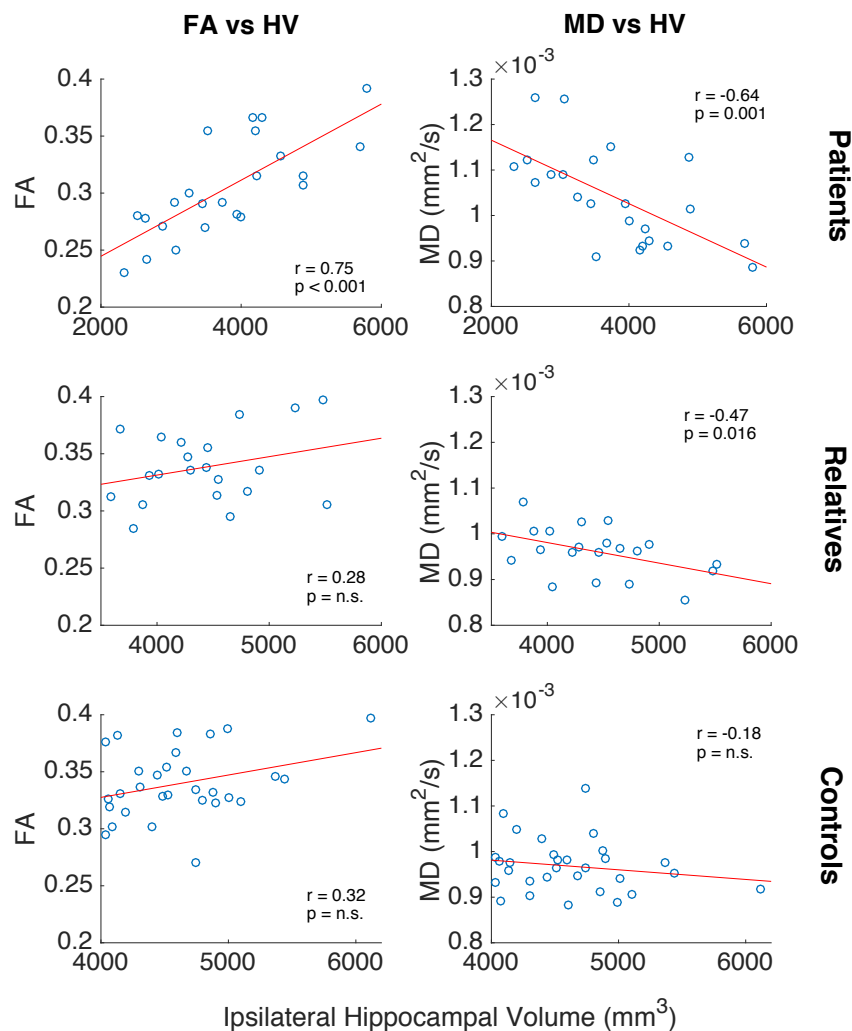


**Figure 7.3** Correlations between the ipsilateral hippocampal volumes (HV) and mean FA (left column) and MD (right column) of the ipsilateral fornix in MTLE patients (top row), unaffected relatives (middle row) and healthy controls (bottom row).

### 7.4.3 Uncinate fasciculus and parahippocampal cingulum pilot analysis

For the UNC tract, the MANCOVA was not significant (using Pillai's Trace), but a large effect size was observed:  $F(8,12) = 1.09$ ,  $p = 0.432$ ,  $\eta^2 = 0.42$ . Univariate ANCOVAs revealed large effect sizes for ipsilateral ( $\eta^2 = 0.45$ ) and contralateral ( $\eta^2 = 0.66$ ) FA values, and the linear trend was also significant for these measures ( $p = 0.04$  and  $p = 0.005$  respectively). Follow-up t-tests revealed that patients had lower ipsilateral and contralateral FA values in the UNC tract compared to healthy controls ( $p = 0.040$  and  $p = 0.005$  respectively) in the UNC. Patients also had lower contralateral FA values compared to relatives ( $p = 0.021$ ). There were no group differences in either MD or FA values between relatives and controls in the UNC.

In the PHC tracts, the MANCOVA was not significant (using Pillai's Trace), but a large effect size was observed:  $F(8,12) = 1.77$ ,  $p = 0.179$ ,  $\eta^2 = 0.54$ . Univariate ANCOVAs revealed a group effect in the ipsilateral FA values with a large effect size:  $F(2,8) = 8.09$ ,  $p = 0.012$ ,  $\eta^2 = 0.67$ , and the linear contrast was also significant ( $p = 0.006$ ). Follow-up t-tests showed MTLE patients had lower ipsilateral FA compared to healthy controls ( $p = 0.006$ ), and unaffected relatives ( $p = 0.012$ ). The ANCOVAs also showed large effect sizes for ipsilateral MD ( $\eta^2 = 0.21$ ) and contralateral FA ( $\eta^2 = 0.17$ ). Follow-up t-tests were not significant for these ANCOVAs, but there appeared to be a trend toward lower contralateral FA and higher ipsilateral MD in patients compared to healthy controls.

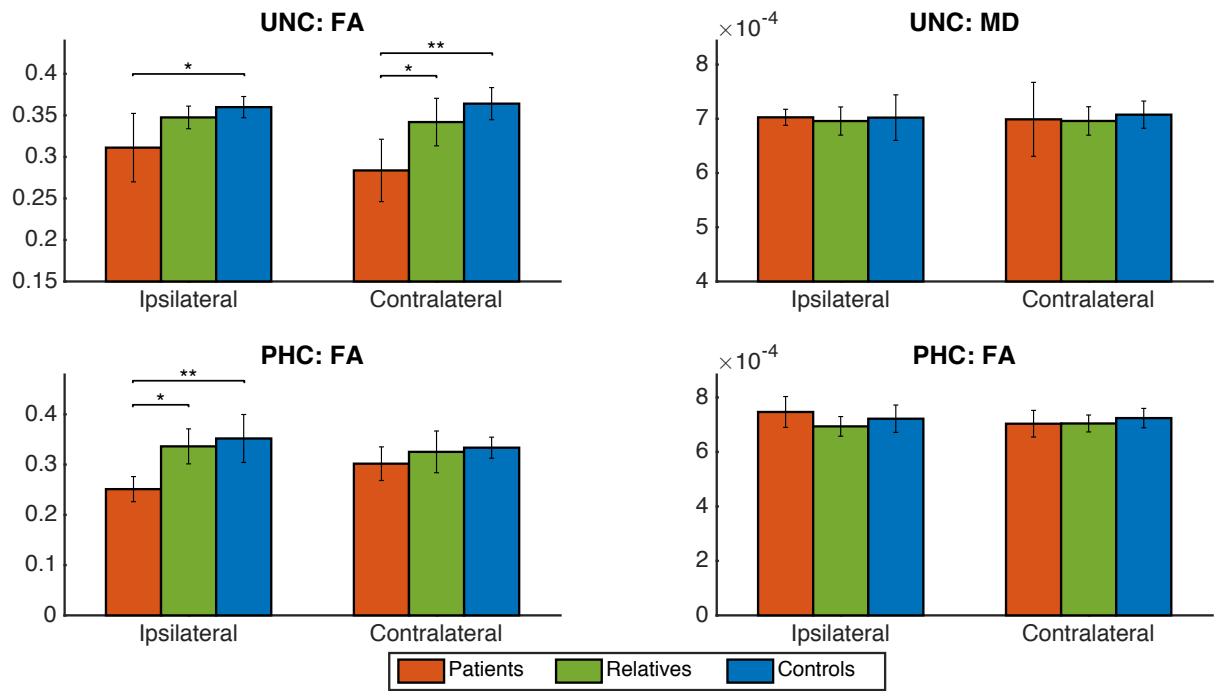


**Figure 7.4** Mean FA and MD measures of the ipsilateral and contralateral UNC and PHC tracts in each group. Error bars shown are  $\pm 1$  standard deviation. Significant differences (uncorrected) are denoted by \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

## 7.5 Discussion

In this chapter, the fornix was manually delineated and white matter tract measures were characterised in MTLE patients, unaffected relatives and healthy controls to identify alterations in tract microstructure in MTLE patients and determine whether similar alterations are also found in unaffected relatives. The main finding was that FA was lower and MD was higher in MTLE patients compared to healthy controls in the ipsilateral fornix, largely driven by diffusivity appearing higher in directions perpendicular ( $\lambda_2$  and  $\lambda_3$ ) to the tracts, although there was also a slight increase in diffusivity along the tract ( $\lambda_1$ ), which is consistent with previous reports of white matter alterations in MTLE. Both FA and MD of the ipsilateral fornix were also strongly correlated with ipsilateral hippocampal volumes in MTLE patients. Although there were no significant differences in fornix tract measures between unaffected relatives and healthy controls, there appeared to be a trend toward a correlation between MD of the ipsilateral fornix and ipsilateral hippocampal volumes in unaffected relatives. These findings suggest that there may be a disease-specific mechanism involved in alterations of both GM hippocampal volumes

and WM microstructure in MTLE patients and that unaffected relatives show subtle evidence for a similar mechanism governing grey and white matter variation in hippocampal regions.

In a pilot analysis on a subset of subjects for 2 other tracts, the UNC and PHC, 4 subjects from each group were compared and FA was found to be significantly reduced bilaterally in the UNC in MTLE patients, while reductions in FA were only found ipsilaterally in the PHC. No significant group differences were observed in these two tracts between unaffected relatives and healthy controls, but the linear trends indicated these FA measures increased linearly from MTLE patients to healthy controls, suggesting unaffected relatives had intermediate values.

### **7.5.1 Alterations in white matter microstructure of the fornix in MTLE patients**

Widespread alterations in white matter microstructure have been reported in MTLE patients in many previous studies. While the white matter alterations themselves are fairly well-established in MTLE, the mechanisms behind these alterations are less so. A possible theory for the mechanism of the spread of white matter changes in MTLE is axonal Wallerian degeneration. Wallerian degeneration is a process that occurs after a neuronal lesion or injury, and has been previously been characterised using DTI primarily in stroke and multiple sclerosis patients, but has also been characterised in both lesional and non-lesional MTLE patients (Concha, Beaulieu, et al., 2005; Shon et al., 2010). The characteristics of Wallerian degeneration observed using DTI are reduced tract fractional anisotropy and increased or normal tract mean diffusivity, with reduced diffusivity seen parallel to ( $\lambda_1$ ) and increased diffusivity perpendicular to the tract ( $\lambda_2$  and  $\lambda_3$ ) (Pierpaoli et al., 2001; Werring et al., 2000).

A recent meta-analysis of WM alterations in MTLE found more severe alterations of WM tracts directly connected to the ipsilateral hippocampus or epileptogenic temporal lobe, with the severity of WM alterations decreasing with increasing distance from the affected hippocampus (Otte et al., 2012). This provides some indirect evidence for the mechanism of Wallerian degeneration being at play in the spread of WM alterations in MTLE. The fornix is the ideal tract

for observing this type propagation of WM degeneration as it has direct connections to the hippocampus, which is known to be lesional in MTLE. The results from the study reported here, that of reduced FA, increased MD and increased perpendicular diffusivity in the ipsilateral fornix in MTLE patients, are broadly consistent with what is usually observed in Wallerian degeneration using DTI measures, suggesting that it may have a role in the propagation of WM changes in MTLE.

### **7.5.2 Correlations of ipsilateral hippocampal volumes with ipsilateral tract measures in MTLE patients and unaffected relatives**

A strong correlation was found between FA and MD in the ipsilateral fornix and ipsilateral hippocampal volumes in MTLE patients. This finding suggests that the severity of atrophy in the affected hippocampus has a strong relationship to white matter changes downstream in tracts directly connected to the hippocampus. This mechanism may be heritable (and thus not directly due to lesional tissue per se), as a correlation between MD and ipsilateral hippocampal volumes, although not reaching statistical significance after correction for multiple comparisons, was also found in unaffected relatives. The lack of correlation found between FA and hippocampal volumes in unaffected relatives however may imply different underlying mechanisms governing FA and MD changes in downstream tracts. In a study of sporadic non-lesional MTLE patients and their asymptomatic siblings, MD increases, but not FA changes, were found in specific locations of the ipsilateral corticospinal tract and ipsilateral superior longitudinal fasciculus (Whelan et al., 2015). Although these changes were not found in the tracts directly connected to the ipsilateral hippocampus, this does support the possibility that the underlying mechanisms of changes of MD and FA in tracts may be different.

### **7.5.3 Implications for DTI endophenotypes in MTLE**

WM alterations as measured by FA and MD in tracts in the vicinity of the affected hippocampus are a well-characterised alteration in MTLE patients and are highly heritable and should be good candidates for endophenotypes of MTLE if they have a higher occurrence in unaffected relatives

than in the general healthy population. The study reported here has demonstrated no significant differences between unaffected relatives and healthy controls, indicating that DTI measures of the fornix cannot directly be used as suitable endophenotypes for MTLE. However, a trend toward a correlation between ipsilateral hippocampal volumes and ipsilateral MD measures of the fornix was observed in unaffected relatives.

#### **7.5.4 Limitations and further work**

While the above study has shown some promise for the use of DTI measures as an endophenotype for MTLE, further work in larger cohorts is of course required to reproduce the results. Further limitations of this work are that left and right MTLE were combined in the study and the sample was heterogeneous for hippocampal sclerosis.

The preliminary findings from the pilot analysis on UNC and PHC tracts show reduced ipsilateral FA in MTLE patients and the linear trends suggest there may be some differences in unaffected relatives. This suggests it would be prudent to investigate tract alterations in other WM tracts connected to the temporal lobe, as WM alterations are known to be more widespread in MTLE. Furthermore, the only other study of unaffected relatives of sporadic MTLE patients found differences in relatives in two WM tracts distant from the affected hippocampus (Whelan et al., 2015), further supporting the need to investigate brain-wide tracts. One of the methods that has proven useful for this is an automatic tract segmentation method, TRActs Constrained by UnderLying Anatomy (TRACULA), that has been applied in patients with TLE (Kreilkamp et al., 2017). Automatic methods are advantageous as they do not require experienced personnel to dissect tracts, would not be affected by inter- and intra-rater reliability and would enable us to investigate a larger number of tracts in less time.

## **Chapter 8 EEG-fMRI: Alterations in networks related to occipital alpha rhythm**

### **8.1 Introduction**

Quantitative EEG has gained wide utility in epilepsy research, with many EEG studies in epilepsy focussed on studying epileptiform activity both during the ictal and interictal states. While equally important in characterising interictal brain activity at rest, background EEG rhythms have not been as widely studied in epilepsy and less is known about whether this activity is aberrant in epilepsy and what its influence may be on ictogenesis.

Alpha activity is one of the most prominent background rhythms observed in healthy adults and has been extensively studied since it was first reported by Hans Berger in 1929 (Adrian & Matthews, 1934; Karbowski, 2002). Alpha activity is a rhythmic activity typically in the 8 to 13Hz frequency range in healthy adults that can be observed on scalp EEG, most prominently in posterior electrodes with eyes closed. It characterises a state of relaxed wakefulness and is generally attenuated by both visual and non-visual stimuli, and mental tasks (Markand, 1990). There are three components of the alpha activity in humans: the classical occipital alpha, observable with scalp EEG; the Rolandic mu rhythm, not as reliably observed on scalp EEG; and the mid-temporal “third rhythm”, not detectable on scalp EEG unless there is a local bone defect (Niedermeyer, 1997).

Changes in the occipital alpha rhythm in patients with epilepsy, such as shifts toward lower frequencies or amplitude asymmetries, are rarely ever reported or described and are usually attributed to effects of anti-epileptic drugs (AEDs) (Larsson & Kostov, 2005). While it is known that the alpha rhythm is influenced by, among other things, AEDs (Clemens et al., 2006; Salinsky et al., 2002), changes in alpha rhythm have been reported in both unmedicated and medicated patients and have been related to disease severity. In a study using scalp EEG from 18 patients with epilepsy and 10 patients without epilepsy, who were referred to have an EEG due to a

range of other neurological symptoms (including headache and dizziness), patients with epilepsy had a decreased alpha frequency variation and decreased peak alpha frequency (PAF; defined as the frequency at which peak power occurs within the alpha frequency range) values over occipital and temporal electrodes, with patients on carbamazepine mono- or poly-therapy showing the largest shifts in PAF (Larsson & Kostov, 2005). In unpublished data from our group, alpha power was shown to be able to distinguish between patients with TLE, idiopathic generalised epilepsy (IGE), and healthy controls, and also to vary with the level of seizure control in patients after taking into account AED loads (Pawley et al., in preparation). Another study in post-ictal EEGs of TLE patients showed that EEG power shifts toward lower frequencies were related to increased severity and type of seizures, with seizures that impair consciousness being associated with more power at lower frequencies than seizures that do not impair consciousness (Yang et al., 2012). These studies show that PAF reductions are not fully explained by AEDs, and may be specific to epilepsy and be related to seizure liability.

The occipital alpha rhythm has been shown to be highly reproducible within individuals (Fernandez et al., 1993; Feshchenko et al., 2001), and there exists a large amount of inter-individual variation related to age, memory and cognition, and neurological conditions (Aurlien et al., 2004; Klimesch, 1997; McEvoy et al., 2001; Moretti et al., 2004; Posthuma et al., 2001). The alpha rhythm is also thought to be highly heritable (Davis & Davis, 1936), with many twin studies showing high genetic contributions to individual alpha frequency and alpha power (for reviews see Vogel, 1970; van Beijsterveldt and Boomsma, 1994). There is also evidence to suggest that developmental plasticity and individual experience have little effect on individual variance in alpha rhythm, with the bulk of variance being explained by genetic factors (Smit et al., 2006).

The generators of the alpha rhythm are thought to be thalamo-cortical in origin, based on several animal models where the alpha rhythm in the visual cortex occurs in synchrony with activity in specific nuclei of the thalamus, particularly in the lateral geniculate nucleus (Chatila et

al., 1993; Hughes, 2005; Hughes et al., 2004; Lopes da Silva et al., 1973, 1980). In support of this, non-invasive neuroimaging studies in humans have also shown positive correlations of alpha fluctuations with neuronal activity (e.g. blood oxygenation related in BOLD fMRI or glucose metabolism in PET) in the thalamus and negative correlations in the occipital cortex (Danos et al., 2001; Goldman et al., 2002; Liu, de Zwart, et al., 2012; Moosmann et al., 2003; Omata et al., 2013). The finding of positive correlations in the thalamus is sometimes inconsistent, however, and is highly variable between subjects and appears to depend on the experimental and analysis methods (Gonçalves et al., 2006; Larson et al., 1998; Laufs et al., 2003; Moosmann et al., 2003; Murta et al., 2015).

There is some evidence that EEG network topology measures derived from the “low alpha” frequency band (defined in the methods section below) constitute suitable endophenotypes for IGE, whereby both IGE patients and their unaffected relatives demonstrated greater degree distribution variance and clustering coefficients than healthy controls (Chowdhury et al., 2014).

To my knowledge, there have not been any studies that have evaluated the suitability of using measures of alpha rhythm as endophenotypes in MTLE, even though it appears from the abovementioned studies that PAF is reduced in patients with MTLE and is highly heritable. It is also unclear how this difference in alpha rhythm in patients is related to ictogenicity, and how the underlying generators of the alpha rhythm contribute to this. In this chapter, the PAF is characterised in a cohort of MTLE patients and their first-degree unaffected relatives as compared to healthy controls. PAF is then used as a covariate in a structural MRI VBM analysis to identify brain regions where local grey matter volume co-varies with PAF within each group. The alpha power fluctuations of each individual’s alpha frequency are also correlated with BOLD fMRI to investigate group differences in whole-brain functional networks associated with the occipital alpha rhythm. Finally, based on previous evidence for endophenotypes derived from brain networks in the “low alpha” range in IGE patients, I compared the topology and ictogenicity of these networks in each group.

## 8.2 Aims and hypotheses

The present chapter aims to investigate structural and functional brain regions and networks that are associated with differences in alpha rhythm in patients with MTLE and their first-degree unaffected relatives, and to assess the suitability of PAF as an endophenotype for MTLE. Based on previous evidence in IGE patients, the topology and ictogenicity of brain networks from the low alpha frequency band were also investigated for possible endophenotypes.

This will be achieved through four analyses applied to MTLE patients and their first-degree unaffected relatives and compared to a group of healthy controls:

- 1) Characterising PAF in EEG recorded outside the scanner and comparing this between groups.
- 2) Conducting a VBM analysis, using PAF as a covariate, to identify brain regions associated with PAF within groups.
- 3) Comparing whole-brain networks derived from BOLD fMRI correlates of alpha power fluctuations, using simultaneous EEG-fMRI.
- 4) Investigating network topology and ictogenicity, assessed using the critical coupling parameter<sup>12</sup>, in brain networks derived from the low alpha band in EEG.

The hypotheses were that:

- 1) MTLE patients will show a reduction in PAF as compared to healthy controls with reductions in PAF in first-degree unaffected relatives being intermediate to the two other groups.
- 2) These reductions in PAF in MTLE patients and unaffected relatives will be related to brain regions involved in alpha rhythm generation (e.g. the thalamus and occipital cortex), as evidenced by structural VBM and functional alterations related to alpha rhythm.

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<sup>12</sup> The critical coupling parameter is described in Chapter 5.

- 3) The EEG network topology (as described by mean degree, mean degree variance and clustering coefficient) and ictogenicity (computed from the critical coupling parameter) from the low alpha frequency band will be altered in MTLE patients and unaffected relatives compared to healthy controls. Patients were hypothesised to have higher mean degree, mean degree variance and clustering coefficient than controls, with relatives hypothesised to be intermediate. Critical coupling parameters were hypothesised to be lowest in patients, followed by relatives and highest in controls.

## 8.3 Method

The general study protocol and acquisition methods have been described in Chapter 5. The methods described below are specific to the analyses performed in this chapter.

### 8.3.1 Data acquisition

Participants had a 20-minute EEG recording outside the scanner, a high-resolution structural T1-weighted MRI and two 10-minute simultaneous EEG and fMRI recordings in the MRI scanner. Data were acquired from participants in an awake rest state with eyes closed (parameters and details have been described in Section 5.4.1). Not all participants completed the study protocol and as a result sample sizes for each of the three analyses presented here are different, as data were used where available<sup>13</sup>. For the PAF analysis, 10 min of EEG recorded outside the scanner was used, for the VBM analysis, the structural MRI scan and EEG recorded outside the scanner was used, and for the simultaneous EEG-fMRI analysis, and one simultaneous run was used for each subject as not all subjects completed two runs.

As previously described, all subjects' imaging data (sMRI and fMRI) were left-to-right flipped so that the left side is considered ipsilateral to the pathological hippocampus for all subjects (see Appendix C for details). As alpha power was averaged over bilateral occipital electrodes (see below), there was no need to flip EEG data.

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<sup>13</sup> A list of complete data from each participant is given in Appendix C.

### 8.3.2 Participants

Each analysis had different sample sizes, depending on the data available (see Appendix C for a complete list of available data for each subject). EEG recordings from 24 patients with MTLE, 24 relatives and 32 healthy controls were used in the PAF analysis. EEG recordings and structural MRI data from 23 patients with MTLE, 20 relatives and 31 healthy controls were used in the VBM analysis. Simultaneous EEG-fMRI data from 22 patients with MTLE, 18 relatives and 25 healthy controls were used in the EEG-fMRI analysis. The sample characteristics are shown in Table 8.1. Age was compared between groups using an ANOVA and gender was entered into a  $\chi^2$  test to assess for group differences.

**Table 8.1** Sample characteristics

|                                             | MTLE Patients | Relatives   | Controls    |
|---------------------------------------------|---------------|-------------|-------------|
| <b><u>PAF Analysis</u></b>                  |               |             |             |
| Number                                      | 24            | 24          | 32          |
| Age in years: mean (SD)                     | 39.8 (11.8)   | 37.0 (13.4) | 36.3 (10.8) |
| Gender: Female (%)                          | 11 (45.8%)    | 10 (58.3%)  | 12 (50.0%)  |
| <b>Clinical Characteristics</b>             |               |             |             |
| Age of onset in years: mean (SD)            | 22.8 (10.9)   | -           | -           |
| Duration of epilepsy in years: mean (SD)    | 17.0 (14.1)   | -           | -           |
| <b><u>VBM Analysis</u></b>                  |               |             |             |
| Number                                      | 23            | 20          | 31          |
| Age in years: mean (SD)                     | 39.5 (12.0)   | 34.6 (13.2) | 36.2 (10.9) |
| Gender: Female (%)                          | 11 (47.8%)    | 12 (60.0%)  | 15 (51.6%)  |
| <b>Clinical Characteristics<sup>1</sup></b> |               |             |             |

|                                          |             |             |            |
|------------------------------------------|-------------|-------------|------------|
| Age of onset in years: mean (SD)         | 23.7 (10.1) | -           | -          |
| Duration of epilepsy in years: mean (SD) | 15.9 (13.3) | -           | -          |
| <b><u>EEG-fMRI Analysis</u></b>          |             |             |            |
| Number                                   | 22          | 18          | 25         |
| Age in years: mean (SD)                  | 38.8 (12.7) | 35.3 (13.4) | 34.3 (7.9) |
| Gender: Female (%)                       | 11 (50.0%)  | 10 (55.6%)  | 12 (48.0%) |
| <b>Clinical Characteristics</b>          |             |             |            |
| Age of onset in years: mean (SD)         | 23.7 (10.1) | -           | -          |
| Duration of epilepsy in years: mean (SD) | 15.9 (13.3) | -           | -          |

<sup>1</sup> Age of onset and duration of epilepsy data was unavailable for 1 patient.

### 8.3.3 Data pre-processing and analysis

#### 8.3.3.1 EEG data: pre-processing and power spectrum analysis

EEG data acquired outside the scanner were used for power spectrum analysis. Data were processed in MATLAB (R2015b, The MathWorks, Inc., Natick, MA, 2015) using custom tools from the FieldTrip EEG software toolbox (Oostenveld et al., 2011) to exclude segments with noise related to participant motion, muscle and ocular artefacts. Data were first down-sampled to 250Hz, then divided into 20s segments, with the length of segments chosen based on qualitatively inspecting the data and maximising the number of samples while minimising the number of artefacts. In practice, the choice of segment length is a trade-off between stationarity of the data and frequency resolution with studies suggesting data of length between 2-10 seconds sampled at 500Hz are optimal (Jobert et al., 2013).

Data within each segment were re-referenced to the average of all channels except the Fp1, Fp2 and ECG electrodes (due to ocular artefacts generally present in Fp1 and Fp2 electrode

recordings even with eyes closed) and then de-trended, baseline corrected. A notch filter was applied to remove 50Hz line noise. Segments with artefacts were excluded using an algorithm locally developed by a colleague, Dr Isabella Premoli, designed to detect artefactual intensity spikes, muscle activity and electro-ocular artefacts. Artefactual intensity spikes were assessed across all channels with data points exceeding a sufficiently high Z-threshold of 30 identified as spikes. Muscle activity was assessed using the T7 and T8 electrodes, which are closest to the muscles of mastication that contribute to the bulk of muscle artefacts on EEG data. Data points exceeding a Z-threshold of 4 with high frequency (between 110 and 140Hz) were identified as muscle artefacts. Electro-ocular artefacts were identified from the Fp1 and Fp2 electrodes and data points exceeding a Z-threshold of 4 and between 1 and 15Hz were identified as ocular artefacts. Any 20s data segments with identified artefacts were rejected and the remaining 20s segments were visually inspected for remaining artefacts and the presence of interictal discharges (IEDs) in patients. One artefact-free 20s segment was chosen for each subject based on the presence of the alpha rhythm to ensure that all power spectrum comparisons were made on subjects in the awake eyes-closed rest state. A 20s segment was found to be sufficient to assess the occipital alpha rhythm from a single subject and did not vary between segments chosen from the start or end of a recording (Pawley et al., in preparation). Choosing a relatively short segment ensured stationarity of the data while maintaining a high data quality with minimal noise and representative of the eyes-closed rest state across all subjects.

Data were bandpass filtered between 0.5 and 70Hz (frequency range that encompasses all traditional frequency bands from theta to gamma) and the power spectral density (PSD) for each participant's 20s segment was estimated using Welch's method (Welch, 1967) with window length of 4s and 50% overlap, giving a frequency resolution of 0.25Hz. PSD data in the alpha frequency range between 6 and 13Hz were considered for this analysis. The lower boundary was modified to match the "low alpha" band (Shackman et al., 2010), in order to be able to detect shifts toward lower alpha frequencies. Only the occipital channels (O1, O2 and Oz) were considered for this analysis, as the posterior alpha rhythm has highest power over the occipital

lobes. The relative power within the alpha band was computed as the power at each frequency point (i.e. 0.25Hz band) divided by the total power between 0.5 to 70Hz. Values were averaged over the three occipital channels. The PAF, defined as the frequency at which the highest power occurs within the alpha frequency band, was then computed for each subject.

An ANCOVA was conducted in SPSS to identify main effects of group on the PAF and to test the hypothesis that patients had the lowest PAF values, followed by relatives, and then controls, a linear group contrast was specified (i.e. mean PAF values linearly increasing from patients to relatives to controls). Age was included as a covariate of non-interest, since the occipital alpha rhythm is known to vary with age. Three follow-up one-sided t-tests were also performed between 1) patients and controls, 2) patients and relatives, and 3) relatives and controls, with the first group hypothesised to have a lower PAF than the latter group in each comparison. Multiple comparisons corrections for 3 group comparisons were done using Bonferroni correction with the threshold for significance set at  $p = \frac{0.05}{3} = 0.017$ . Effect size of the ANCOVA was assessed using partial  $\eta^2$  (the default for ANCOVAs in SPSS), while effect sizes for each of the t-tests was assessed using Cohen's  $d$  (for t-tests). A rough guideline for small, medium and large effect sizes using  $\eta^2$  are 0.01, 0.06 and 0.14 respectively (Cohen, 1988). Using Cohen's  $d$ , small, medium and large effect sizes are 0.2, 0.5 and 0.8 respectively (Cohen, 1988).

#### **8.3.3.2 Structural MRI: VBM processing and analysis**

Structural MRI data were processed using the VBM software in SPM12 (<http://www.fil.ion.ucl.ac.uk/spm>). Images were first checked to be in the correct orientation using SPM's *Check Reg* and *Display* tools and the origin was manually adjusted where necessary. Tissue segmentation into grey matter, white matter and cerebrospinal fluid was then performed on each subject's image using "New Segment". The Dartel tool was used to iteratively estimate deformations required to align each subject's grey and white matter data to a custom average template of all subjects (Ashburner, 2007). The final custom template was then normalised to MNI space with an affine transform, allowing each subject's grey matter image, which from the

previous step had been aligned to the template, to also be aligned to MNI space. Grey matter images were then modulated by the Jacobian determinant from the deformation matrix obtained from the Dartel step in order to preserve the total volume of grey matter. Spatially normalised grey matter images were then spatially smoothed with an 8mm FWHM Gaussian, in order to meet the requirements for GLM statistics and to compensate for slight registration inaccuracies. The size of the smoothing kernel is typically recommended at between 8 to 12mm, and 8mm was used here so as to be able to detect small focal differences. Smoothed images were used for the voxel-wise analysis of grey matter changes related to PAF variations. Total intracranial volume for each subject was calculated from the sum of total grey matter, white matter and cerebrospinal fluid volumes from the earlier segmentation. A separate GLM was set up for each group to test for within group effects of PAF variations on voxel-wise grey matter volume differences after accounting for inter-individual differences in total intracranial volume by proportional scaling. This allowed for the comparison of relative volume changes at each voxel. Each GLM was set up as a multiple regression with PAF as the covariate of interest and age and gender as covariates of non-interest.

#### **8.3.3.3 Simultaneous EEG-fMRI data pre-processing and analysis**

EEG data recorded in the scanner were pre-processed using BrainVision Analyzer 2 (Brain Products GmbH, Gilching, Germany) to remove MR gradient and ballistocardiogram artefacts from the simultaneous EEG-fMRI runs. A sliding average template of MRI scanner artefacts using the average of 21 TR intervals identified by the gradient onset markers was subtracted from the EEG signal to correct for MR gradient artefacts. Data were then downsampled to 250Hz, which was the recommended down-sampling rate due to limits of the amplifier when used within the scanner. Within BrainVision Analyzer, the peaks of the R-waves were identified from the ECG signal in a semi-automated manner using a template pulse wave and subsequently manually checked and where necessary adjusted by eye. Ballistocardiogram artefacts were then corrected by subtracting a sliding average template for the R-waves from the EEG signal.

Pre-processed EEG data were then further processed using custom scripts in MATLAB, using MATLAB time series analysis tools and tools from the FieldTrip toolbox. Data were bandpass filtered between 6 and 13Hz to obtain data in the alpha band, including the “low alpha” range as above. Similar to the EEG data processed outside the scanner, only data from the occipital channels (O1, O2 and Oz) were considered for this analysis to increase the probability of capturing variations in the alpha rhythm. EEG data were averaged over the three occipital electrodes and a short-time Fourier transform was applied to compute the spectrogram using windows equal to the fMRI data sampling TR of 2.16s with no overlap. From this the alpha power time series regressor was computed for each subject. To account for intra-subject variation of peak alpha frequency, the alpha power regressor was computed as the mean over a narrow band of  $\pm 2$ Hz around the peak alpha frequency for each subject (i.e. frequency between 6 to 13Hz at which peak power occurs), chosen because the alpha rhythm is not monorhythmic and varies over about 1Hz within individuals (Aminoff, 2012). Outliers in the alpha power regressor were identified as data points where the amplitude exceeded the standard boxplot outlier definition,  $Q3 + 1.5 \times IQR$ <sup>14</sup>, and replaced by linearly interpolated values. Each alpha power time series was then truncated to exclude the first 5 and last 5 TR of data to exclude end effects from average template correction. The alpha power regressor was then normalised so that the amplitude range of each regressor was between 0 and 1 for each subject. Each alpha power regressor was then convolved with the canonical double-gamma hemodynamic response function.

fMRI data pre-processing steps were described in Chapter 5.

For each subject, a voxel-wise whole-brain GLM analysis of brain regions correlated to alpha power fluctuations was implemented in FEAT using with the alpha power time series as the EV of interest and the nuisance regressors as EVs of non-interest. The alpha power time series was

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<sup>14</sup> This is the standard outlier definition for box plots, where  $Q3$  denotes the third quartile of the data and  $IQR$  is the inter-quartile range.

convolved with the canonical double-gamma hemodynamic response function to take into account the delay associated with the BOLD response. A group-level mixed effects GLM analysis was performed within FEAT's higher-level analysis toolbox with age and gender as covariates of non-interest and corrections for multiple comparisons using family-wise error (FWE) cluster-correction at  $p < 0.05$ .

#### **8.3.3.4 EEG data: network analysis and critical coupling parameter**

EEG network analysis was implemented in MATLAB using custom scripts from a collaborator, Dr Helmut Schmidt (Department of Mathematics, University of Exeter). EEG data were bandpass filtered in the low alpha frequency band (6-9Hz) and Hilbert transformed to obtain instantaneous measures of phase and amplitudes at each timepoint. For each pair of channels, the phase-locking factor (PLF), a measure between 0 and 1 reflecting the level of synchronous activity between each pair of electrodes, and phase lag, a value between  $-\pi$  to  $\pi$  reflecting the angle of phase difference between each pair of electrodes and thus the "lag", were computed for each pair of channels. This gave a 63-by-63 channel PLF and lag matrix for each subject in the alpha band. The PLF matrix was multiplied by the positive lag matrix to give a directed and weighted matrix and spurious connections were removed up to the second order by removing weaker links where a stronger, more direct link between two nodes exists. The resulting sparse matrices were compared between groups.

Network measures for each PLF matrix (mean degree, degree variance and clustering) were computed. These network measures were chosen based on measures found to be possible endophenotypes in IGE data (Chowdhury et al., 2014). The Kuramoto oscillator model was applied to each subject's matrix to calculate the critical coupling parameter (CritC) for each network (Schmidt et al., 2014). A MANOVA was conducted in SPSS to identify any significant effects of group on the above measures. To test the hypothesis that patients have higher mean degree, mean degree variance and clustering coefficient than controls, with relatives intermediate, three follow-up one-sided t-tests were also performed between 1) patients and

controls, 2) patients and relatives, and 3) relatives and controls for each measure calculated. One-sided follow-up t-tests were also performed on CritC values to test the hypothesis that MTLE patients had the lowest CritC, followed by relatives and then controls. Multiple comparisons corrections for 12 comparisons (3 group comparisons and 4 measures) were performed using Bonferroni correction with the threshold for significance set at  $p = \frac{0.05}{12} = 0.004$ .

## 8.4 Results

The participant groups did not differ significantly in age or gender when assessed using a one-way ANOVA and  $\chi^2$  test.

### 8.4.1 EEG data: power spectrum analysis

EEG alpha peak frequency was shifted toward lower frequencies in TLE patients compared to controls, with relatives lying in between. Figure 8.1A shows the average PSD in each group where the peaks in the alpha power band of patients and relatives appear to be shifted toward lower frequencies. Patients had the lowest mean PAF value of 8.77Hz with a standard deviation of 1.13Hz, relatives had a mean PAF of  $9.51 \pm 1.14$  Hz, and controls had the highest PAF mean of  $9.96 \pm 1.25$  Hz.

The ANCOVA revealed a significant effect of group with a large effect size:  $F(2, 76) = 6.42, p = 0.003, \eta^2 = 0.15$ . The linear contrast was also significant ( $p = 0.001$ ), indicating that PAF values linearly increased from MTLE patients to healthy controls, with unaffected relatives intermediate. In pairwise one-tailed follow-up t-tests, there was a significant difference between MTLE patients had significantly lower PAF compared to healthy controls with a large effect size:  $t(54) = -3.89, p < 0.001, d = 1.06$ . MTLE patients showed a trend toward reduced PAF compared to relatives:  $t(46) = -2.03, p = 0.024, d = 0.59$ . Relatives also showed a trend toward reduced PAF compared to healthy controls:  $t(54) = -1.55, p = 0.063, d = 0.42$ . Although the differences

between patients and relatives and between relatives and controls were not statistically significant after Bonferroni adjustments of the p-value threshold for multiple comparisons, the effect size for each comparison suggests a medium effect of group on PAF (Cohen, 1988). Figure 8.1B shows the comparison between the peak frequency in the alpha band in each group.

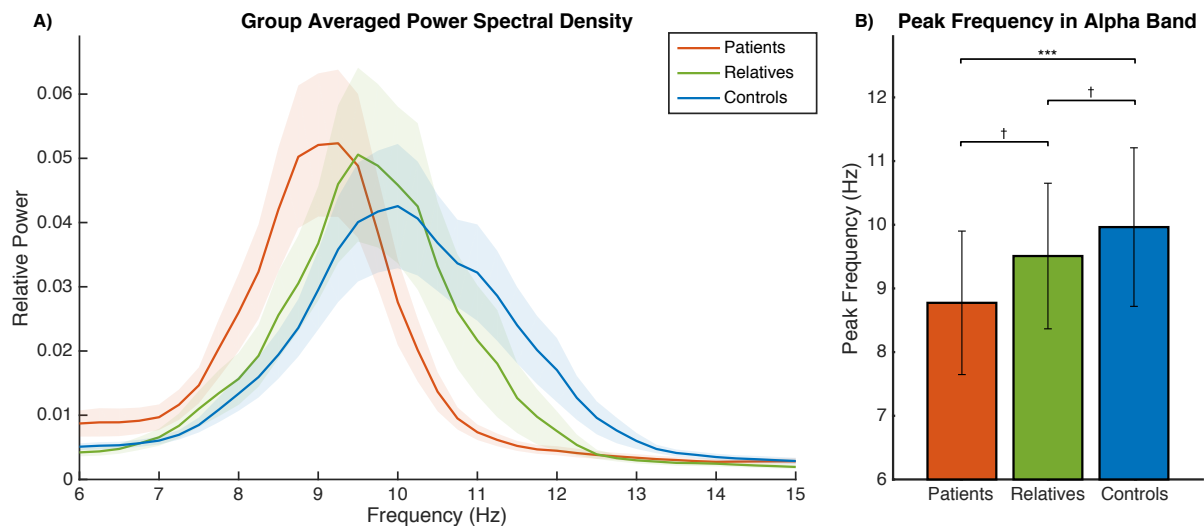


**Figure 8.1** A) Average power spectral density plots for each group. The shaded region around each plot represents the standard error of the mean at each frequency resolution point. B) Peak frequency plots for each group showing the frequency at which peak power occurs. Error bars shown are  $\pm 1$  standard deviation. \*\*\* indicates  $p < 0.001$  (Bonferroni corrected) and † indicates a trend.

#### 8.4.2 VBM analysis: Structural correlates of PAF

The VBM analysis showed a positive relationship between PAF values in the patient group and grey matter volume in a small number of voxels in the left frontal operculum/anterior insular cortex ( $p=0.033$ , cluster size=2 and  $p=0.039$ , cluster size=1) and 1 voxel in the left middle frontal gyrus ( $p=0.039$ ) that survived voxel-wise FWE correction at  $p<0.05$ . Taking an exploratory approach and looking at clusters showing PAF correlations at an initial voxel-wise threshold of  $p<0.001$  (uncorrected) with a cluster threshold of 1468 (cluster-correction threshold chosen based on smallest cluster surviving a cluster-wise FWE correction at  $p<0.05$ ), clusters that showed grey matter correlations with PAF were found in the left frontal operculum extending into the left anterior insular cortex, left middle frontal gyrus, right frontal operculum extending into the right insular cortex, right inferior frontal gyrus and left precuneus cortex extending into

the lingual gyrus (Figure 8.2A). Table 8.2 lists the cluster locations and their corresponding p-values.

**Table 8.2** Clusters where grey matter varies with PAF in the patient group

| Cluster location                                | Peak MNI Coordinates |      |      | Cluster p-value* | Cluster size |
|-------------------------------------------------|----------------------|------|------|------------------|--------------|
|                                                 | x                    | y    | z    |                  |              |
|                                                 | (mm)                 | (mm) | (mm) |                  |              |
| Left frontal operculum/anterior insular cortex  | -33                  | 24   | 14   | <0.001           | 4766         |
| Left middle frontal gyrus                       | -28                  | 20   | 54   | <0.001           | 6636         |
| Right frontal operculum/anterior insular cortex | 24                   | 21   | -8   | 0.001            | 1468         |
| Right inferior frontal gyrus                    | 51                   | 30   | -6   | <0.001           | 2402         |
| Left precuneus cortex/lingual gyrus             | -6                   | -72  | 30   | <0.001           | 1958         |

\* p-values are shown as cluster-wise FWE-corrected at  $p < 0.05$  after an initial uncorrected threshold of  $p < 0.001$

In the relative group, there were no clusters showing covariation between grey matter volume and PAF values that survived voxel-wise FWE correction, and while lowering the threshold to  $p < 0.001$  (uncorrected) with a cluster threshold of 10 revealed a cluster in the left insular cortex and another cluster in the left anterior frontal pole, these regions lay entirely within significant regions in the patients and did not survive corrections for multiple comparisons (Figure 8.2B). There were no clusters where local grey matter volume covaried with PAF values at either the FWE voxel-wise corrected threshold or the uncorrected level in the control group.

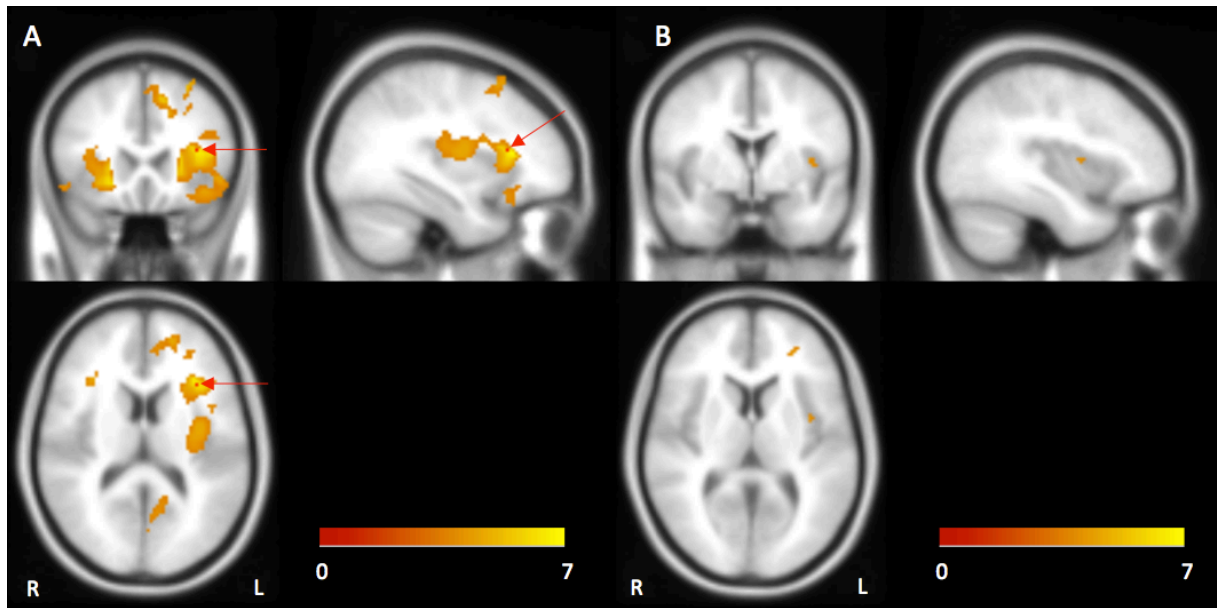


**Figure 8.2** (A) Clusters showing grey matter volume correlations with PAF in the patient group. Voxels in red (indicated by the red arrows) show results voxel-wise corrected at  $p < 0.05$ . Clusters in orange-yellow show regions at  $p < 0.001$ , with cluster-correction threshold of 1468. (B) Clusters showing grey matter changes correlated to PAF in relatives group, uncorrected at  $p < 0.001$ .

#### 8.4.3 Simultaneous EEG-fMRI data: BOLD correlates of alpha power analysis

An example alpha power timeseries and the corresponding single-subject map of regions where BOLD fMRI signal was significantly correlated with alpha power fluctuations is shown in Figure 8.3.

The mean alpha correlation map across all subjects is shown in Figure 8.4 at  $p < 0.001$  uncorrected to show the overall pattern of BOLD fMRI signal related to alpha power fluctuations. Regions that show positive correlation to alpha power include the bilateral thalami, the brainstem, and bilateral planum polare. Regions that showed negative correlations with alpha power fluctuations included the bilateral inferior frontal gyri and bilateral superior parietal lobes.

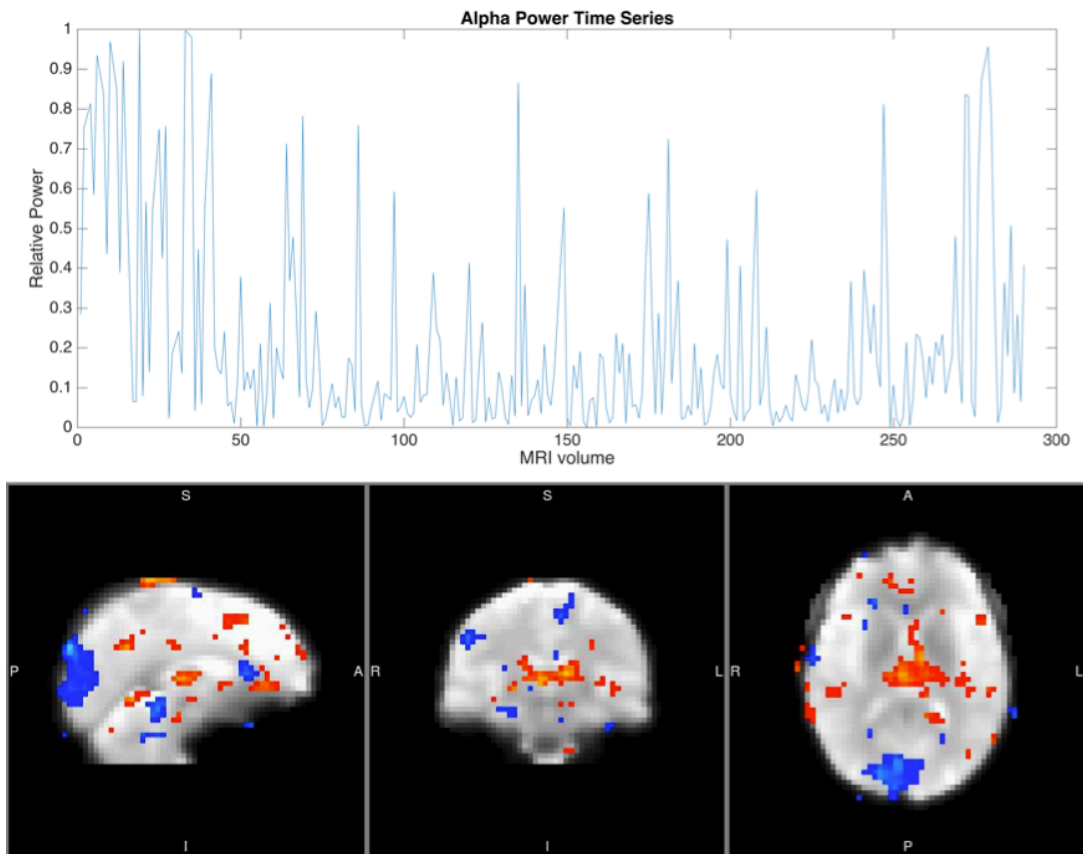


**Figure 8.3** Alpha power time series from a patient (TLE/P/014, top) and the corresponding correlation map of BOLD with alpha power fluctuations (bottom). The bottom image is a z-statistic image thresholded at  $p < 0.05$  uncorrected, with positive correlations shown in red-yellow and negative correlations shown in blue-light blue.

In group comparisons, patients had higher correlations between alpha rhythm and BOLD signal than healthy controls in the bilateral insular cortex, extending into the frontal operculum on both sides and the central operculum on the left, the cingulate gyrus extending into the supplementary motor area, and bilateral precentral gyri at a FWE cluster-corrected threshold of  $p < 0.05$ . The bilateral thalami also showed a trend toward higher correlations between alpha and BOLD signal in patients than in controls, but this was not significant after FWE correction and is shown in Figure 8.5A at  $p < 0.001$  uncorrected.

When patients were compared to relatives, only the left insular cortex extending into the central operculum showed increased correlation between alpha and BOLD signal in the patients at the FWE cluster-corrected threshold of  $p < 0.05$ , with some right insular cortex seen at  $p < 0.001$  uncorrected (Figure 8.5B). There were no significant differences in other group comparisons.

There were some similarities in the regions showing local volume changes related to PAF in the VBM analysis and the regions showing altered connectivity with alpha rhythm in patients the EEG-fMRI analysis. Figure 8.6 shows the regions that overlap between the two analyses, with the most overlap occurring in the bilateral insular regions and cingulate and precentral gyri.

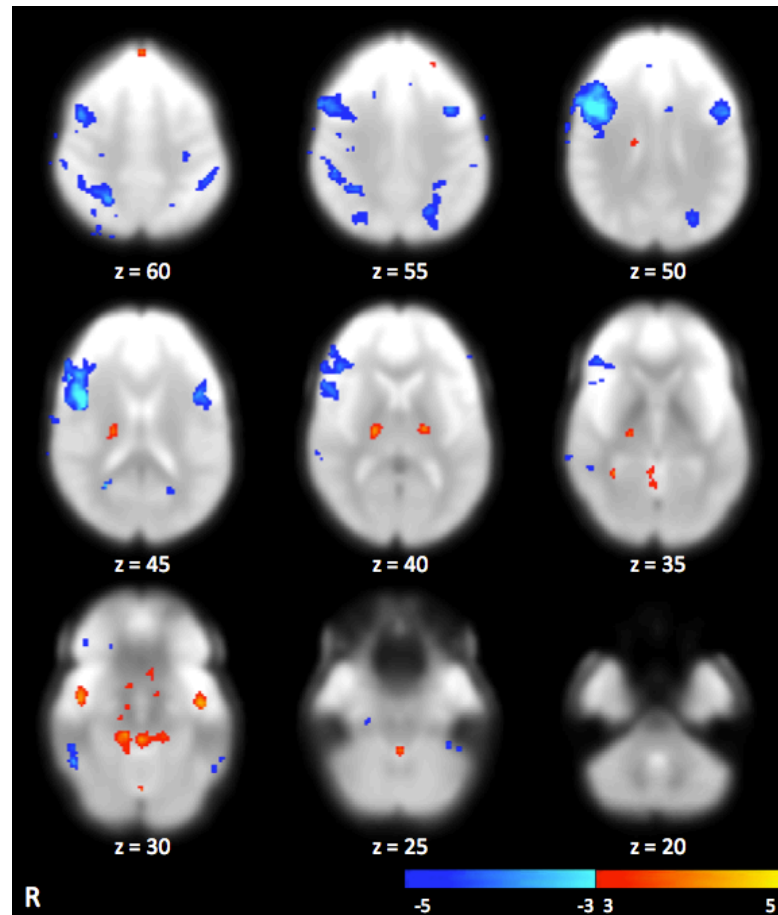


**Figure 8.4** Correlations between the BOLD signal and alpha power fluctuations across all subjects overlaid on the averaged subject fMRI in MNI standard space. Positive correlations are shown in red-yellow and negative correlations in blue-light blue. Correlations are shown as z-statistics at  $p < 0.001$  (uncorrected) to show the overall pattern of correlation.

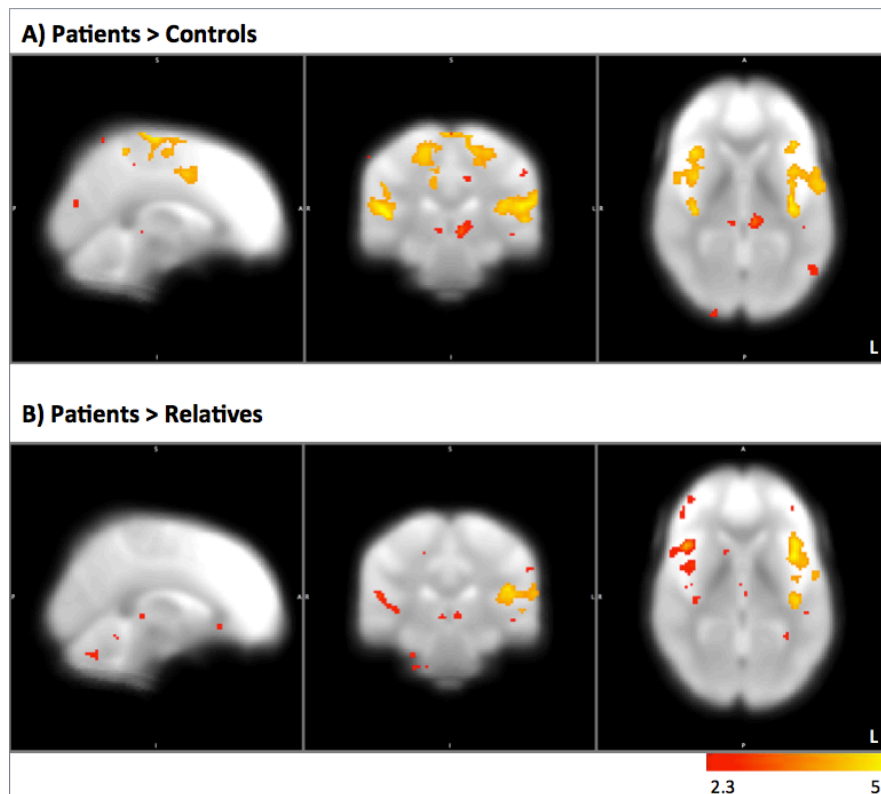


**Figure 8.5** Group comparisons showing greater positive correlations between alpha power fluctuations and BOLD signal in patients than in controls (A) and in patients than in relatives (B). Regions in orange-yellow are FWE cluster-corrected at  $p < 0.05$  and regions shown in red are  $p < 0.001$  uncorrected.

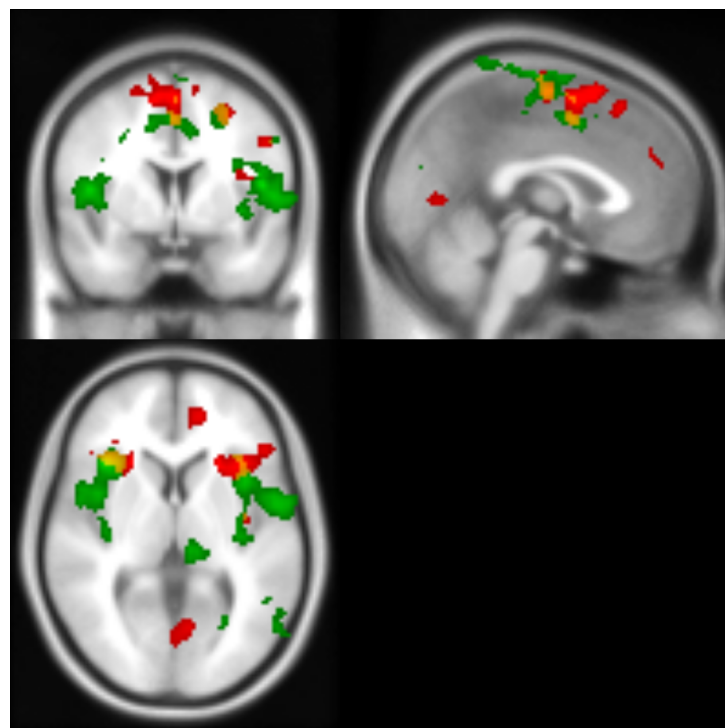


**Figure 8.6** Regions showing correlation between local volume changes and PAF in patients (in red), regions showing altered correlation with alpha rhythm in patients compared to healthy controls (in green), and regions showing an overlap between the two analyses (in orange). Left on image is right on the brain.

#### 8.4.4 EEG network analysis and critical coupling parameter

The averaged sparse PLF matrix of each group is shown in Figure 8.7A. Using Pillai's Trace, there was a significant main effect of group on network measures,  $F(8,150) = 2.18$ ,  $p = 0.032$ ,  $\eta^2 = 0.10$ . Univariate ANOVAs revealed a significant effect of group only on the mean degree,  $F(2,77) = 5.84$ ,  $p = 0.004$ ,  $\eta^2 = 0.132$ . A trend toward group effect was found on the critical coupling parameter,  $F(2,77) = 3.03$ ,  $p = 0.054$ ,  $\eta^2 = 0.073$ . No significant effects of group were found on the mean degree variance or clustering coefficients.

Follow-up t-tests revealed patients had significantly greater mean degree than relatives,  $t(46) = 3.19$ ,  $p = 0.001$ ,  $d = 0.92$ , and a trend toward greater mean degree than controls,  $t(54) = 1.82$ ,  $p = 0.038$ ,  $d = 0.49$ . There was also a trend toward lower critical coupling in patients than relatives,  $t(46) = -2.28$ ,  $p = 0.027$ ,  $d = 0.66$ . Effect sizes for these comparisons were all medium to large.

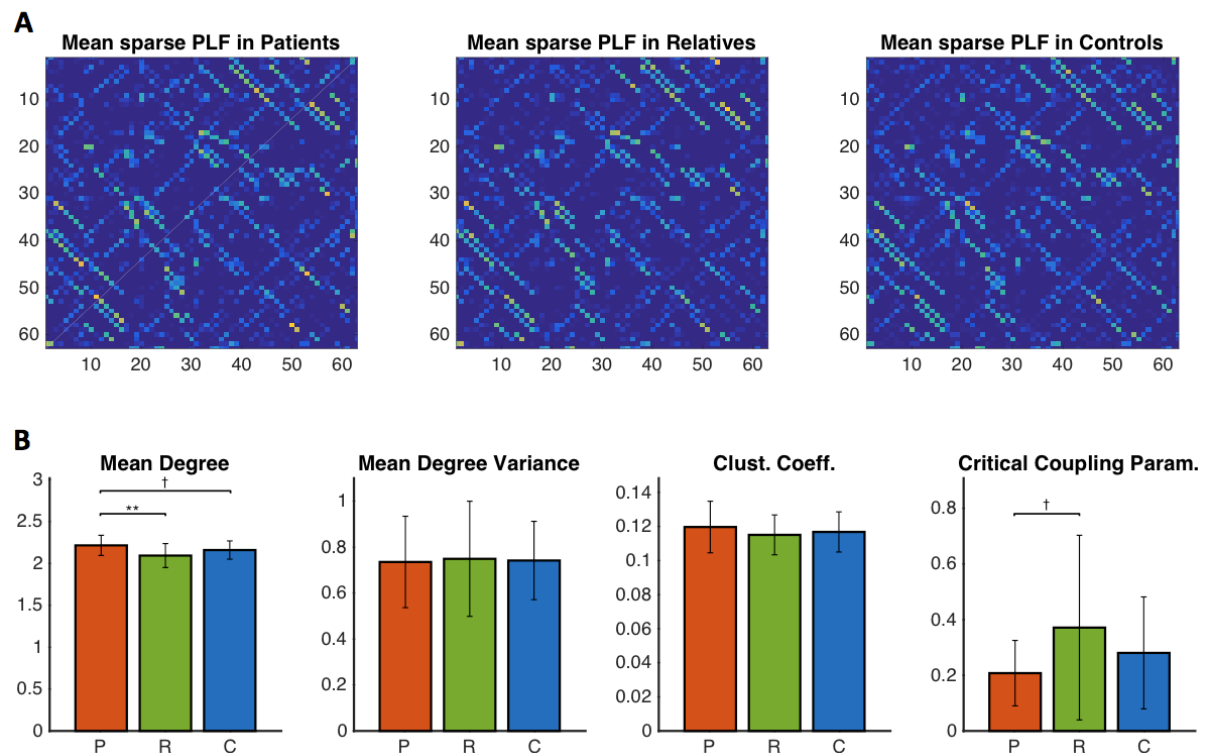


**Figure 8.7** Group averaged sparse PLF matrices. The numbers 1-63 correspond to the electrodes in the order given in Chapter 5, Figure 5.1.

## 8.5 Discussion

This chapter aimed to investigate brain network differences in MTLE patients and their relatives in relation to the occipital alpha rhythm using EEG, sMRI and fMRI data. The main findings are:

- 1) PAF is reduced in patients with MTLE as compared to healthy controls and there is a significant linear trend of PAF reduction with controls having the highest PAF values, followed by relatives and then patients.
- 2) PAF variation in patients is related to changes in brain volume in the left insular and frontal opercular cortices, and a non-significant trend was found to a lesser extent in a similar set of regions in relatives.
- 3) Alpha rhythm fluctuations appear to be more highly correlated to BOLD signal in a network of regions involving the left insular, frontal opercular, precentral and cingulate gyri in MTLE patients. There was some overlap between these regions and the regions showing grey matter loss in relation to PAF.
- 4) Finally, EEG networks in the low alpha frequency band in MTLE patients have a higher mean degree, suggesting a more strongly coupled network.

These findings suggest a possibility for using PAF as an endophenotype in MTLE. The findings also associate a network involving insular and frontal opercular regions with alpha rhythm and reduced PAF in MTLE patients. There is no strong evidence for using network measures of the low alpha frequency band in EEG as endophenotypes for MTLE.

### 8.5.1 Lower PAF in patients with MTLE

The main finding reported here was the reduction in PAF in patients with MTLE and a significant linear trend of reduced PAF in relatives of patients with MTLE when compared to healthy controls. The finding of reduced PAF in epilepsy has been reported previously (Larsson & Kostov, 2005), and has also been reported in several other neurological and psychiatric disorders including depression and Alzheimer's disease (Hughes, 2005; Niedermeyer, 1997). This is, to my knowledge, the first report of a significant linear trend of reduced PAF in first-

degree unaffected relatives of MTLE patients, suggesting PAF could be an inherited endophenotype in MTLE.

Focal slowing is sometimes seen in patients with underlying lesions, but this activity is most prominent over the region of the underlying lesion and usually in the delta frequency range (Blume et al., 1993; Cascino et al., 1996). In MTLE, focal slowing is usually seen over the temporal lobes on the side of seizure onset (Javidan, 2012). The shift of the alpha rhythm toward lower frequencies described in this chapter, however, reflects the slowing of the classic alpha rhythm over the posterior regions, rather than a focal slowing over the site of the hippocampal lesion. The mean of the PAF in patients ( $8.77 \pm 1.13$  Hz) is also within the limits of the alpha frequency band and not in the delta frequency range, further supporting the case that this is indeed a slowing of the classical posterior alpha rhythm.

The posterior alpha rhythm is thought to be thalamo-cortical in origin and a shift of the alpha rhythm toward lower frequencies has been referred to as “thalamic dysrhythmia”, as it is thought that the slowing results from dysfunction at the thalamic level (Llinás et al., 1999). It is believed that a hyperpolarization of thalamo-cortical neurons due to excess inhibition (possibly due to dysfunction of certain neurotransmitters) can lead to a slowing of alpha waves through the slowing of bursting in thalamic cells (Hughes, 2005), which might explain why thalamic dysrhythmia is observed in certain neurological and psychiatric conditions.

#### **8.5.2 PAF and alpha rhythm fluctuations related to structural and functional changes in a network of regions including the insular cortex and frontal operculum**

In the VBM analysis, local volumetric reductions in the anterior insular cortex, frontal operculum and middle frontal gyrus ipsilateral to the seizure onset side were found to be related to reduced PAF within the MTLE patient group, with relatives showing a trend toward a similar finding in the insular cortex. Whole-brain activity related to alpha power fluctuations was assessed with

simultaneous EEG-fMRI, and a similar set of regions was found to have increased activity related to fluctuations in alpha power in the patient group compared to healthy controls.

To my knowledge, there have been no other investigations of inter-individual peak alpha frequency variations and their relationship to morphometric changes in the brain.

Simultaneous neuroimaging and EEG studies have allowed for spatial mapping of alpha power fluctuations in the whole brain, but results have been inconsistent and mixed. Perhaps the most consistent finding is the negative correlation of posterior EEG alpha fluctuations with BOLD signal in occipital cortex (Goldman et al., 2002; Gonçalves et al., 2006; Liu, de Zwart, et al., 2012; Moosmann et al., 2003; Omata et al., 2013). Negative correlations with the temporal lobes and the fronto-parietal attention system have also been found (Laufs & Duncan, 2007; Mantini et al., 2007; Murta et al., 2015) suggesting alpha activity is a state of cognitive rest or reduced vigilance and attention. Predominantly positive correlations are also found in the thalamus (Goldman et al., 2002; Liu, de Zwart, et al., 2012; Omata et al., 2013; Sadato et al., 1998) although some studies have shown the opposite (Larson et al., 1998; Lindgren et al., 1999), this nevertheless supports the view that the thalamus plays a part in the generation of the alpha rhythm. Positive correlations with the insula were reported in two studies (Goldman et al., 2002; Sadato et al., 1998). There have been suggestions that the relationship between insular activity and the alpha rhythm is a physiological response to drowsiness, but the Sadato study (Sadato et al., 1998) (performed using PET) was conducted with eyes open, suggesting that the insular correlation cannot be fully explained by drowsiness and that it too may have a role in generating the alpha rhythm.

The insula is thought to underpin a variety of cognitive, affective and regulatory functions including attention and awareness. In particular, the anterior insular cortex has been postulated to be involved in the detection of salient events and the switching between other large-scale brain networks involved in attention and working memory in order to process or act on a salient

event. Strong connections between the insular cortex and anterior cingulate also allows for quick access to the motor system for action (Menon & Uddin, 2010). Since the alpha rhythm is associated with reduced attention, this modulation of attention networks may represent a link between the insular cortex and alpha rhythms, although the underlying mechanisms are still unclear.

Another link between the insular cortex and alpha rhythm is thought to be through the cholinergic basal forebrain. This is involved in modulating wakefulness and attention, and has a role in both memory and learning (Baxter & Chiba, 1999; Fibiger, 1991). The anterior insula is one of the few regions with direct connections to the cholinergic basal forebrain and it has been suggested that the link between the alpha rhythm and cholinergic basal forebrain activity is modulated or even controlled by the insula (Vakalopoulos, 2014). Decreased subcortical cholinergic arousal has also been suggested as a possible mechanism for loss of consciousness in focal TLE seizures (Motelow et al., 2015).

The insular cortex has also been implicated in MTLE (Bouilleret et al., 2002; Isnard et al., 2000). Similarities between temporal lobe seizure symptoms and responses from insular cortex stimulation have been described (Ostrowsky et al., 2000; Penfield & Faulk, 1955). A study has also suggested that temporal plus epilepsy is a leading cause of surgical failure in MTLE, as the seizure generating regions often extend outside of the resected mesial temporal lobe area into the anterior insular cortex (Barba et al., 2016). Taken together, these studies suggest that the insula may be part of a seizure generating network in MTLE and is a possible explanation for the alterations in these regions in relation to the altered alpha rhythm in MTLE patients reported in this chapter.

### **8.5.3 EEG network properties**

Since epilepsy is a disorder characterised by hyper-synchronous activity, the prevailing theory is that the brain networks during the interictal state would show a higher propensity to enter this

hyper-synchronous state, manifesting as increased functional connectivity in such interictal brain networks (Kramer & Cash, 2012). There has been some evidence to support the theory that specific network topologies can support seizure generation. A study simulating EEG networks found that the propensity for the networks to give rise to seizure-like activity was influenced not just by the presence of a pathological node, but also by the underlying network structure (Terry et al., 2012).

In this chapter, EEG brain networks in MTLE appear to show higher mean degree, suggesting increased connectivity, and a lower critical coupling parameter, suggesting a higher propensity to generate seizures.

#### **8.5.4 Limitations**

There is known to be large inter-individual variability in the alpha rhythm even within healthy controls, and some healthy adults don't show any occipital alpha rhythm at all (Niedermeyer, 1997), which may suggest the unsuitability of the alpha rhythm as a reliable measure. However, the alpha rhythm is fairly reproducible within individuals; the standard deviations shown here were equal across groups (as assessed by a Levene's test of equality of variance in SPSS). The clear and significant linear trend in reduced PAF seen here in the groups cannot completely be attributed to inter-individual differences as all the 20s EEG segments had been carefully chosen to have the canonical alpha rhythm while being blinded to group to reduce bias.

Slowing of alpha rhythms is also an indication of drowsiness, but the EEG data used to assess PAF differences was recorded outside the scanner and I was present for the duration of the recording to wake participants if they started to fall asleep. In the scanner however, I did not qualitatively or quantitatively assess differences in arousal. This could have been done using either sleep staging of the EEG in scanner or a post-scan interview to obtain a rating of drowsiness. Another explanation for this effect is that patients have undergone EEG and MRI

investigations before and are likely to be more relaxed during the procedures. However, this does not explain the linear trend seen in reduced PAF.

In this chapter, the correlation of EEG alpha fluctuations with BOLD signal in some subjects did not show the expected thalamic positive correlation and posterior negative correlation and in fact did not show any strong correlations at all with any brain regions. It has been suggested that the lack of fMRI correlations with EEG alpha rhythm could be due to low variance of alpha activity within the subject (Moosmann et al., 2003), however this has not been a consistent finding (Goldman et al., 2002) and in the present dataset, there were no significant differences in alpha power variation between any of the groups. It is unclear then in this dataset why BOLD-fMRI and alpha correlations are less prominent in some subjects.

### **8.5.5 Implications for EEG endophenotypes in MTLE**

The present chapter has demonstrated that PAF is reduced in patients and relatives compared to healthy controls and that this measure of EEG alpha rhythm may be a suitable endophenotype for MTLE and thus deserves further investigation. The PAF meets several criteria for endophenotypes as outlined in Chapter 1, including being a well-characterised abnormality in the patient group, being highly heritable as evidenced by twin studies described in the introduction of this chapter, being state independent and occurring in unaffected relatives, and appearing to have a higher occurrence in unaffected relatives than in the general healthy control population.

### **8.5.6 Conclusions**

There was evidence for altered occipital alpha rhythm in MTLE patients related to structural and functional alterations in a network including the ipsilateral insular cortex, frontal opercular cortex, and precentral and cingulate gyri. There was also a trend toward reduced alpha power in unaffected relatives of MTLE patients, suggesting a possibility for an endophenotype, but further work is required on larger datasets to identify whether these effects remain.

## Chapter 9 Large-scale DWI and fMRI connectivity networks in MTLE

### 9.1 Introduction

The concept of MTLE being a network disorder rather than a disorder of a single epileptogenic region is gaining traction, and efforts have been focussed into identifying and understanding this network of brain regions that contribute to epileptogenesis (see Chapter 3 for an introduction to this and Richardson, 2010, Laufs, 2012 and Kramer & Cash, 2012 for reviews). The ILAE has also recently revised its characterisation of focal seizures, including seizures in MTLE, as seizures that “are conceptualised as originating within networks limited to one hemisphere”; as opposed to a single epileptic focus (Berg et al., 2010).

One way to study brain networks from imaging data is to simplify the data into a set of nodes (or brain regions) and edges (the connections between brain regions) and infer network properties using the graph theoretical framework (see Chapter 5 for a brief description of Graph Theory). There have been a handful of studies of interictal networks, or connectomes, in MTLE using this approach in both functional and structural MRI data.

Using sMRI cortical thickness correlation networks, an increased path length and decreased clustering have been found in MTLE patients, with a reorganisation of cortical network hubs to temporo-limbic regions (Bernhardt et al., 2011). Structural connectivity networks derived from DWI data on the other hand have shown increased path length and decreased clustering, which correlated with increased cognitive impairments in MTLE patients (Vaessen et al., 2012). Structural connectomes have also been shown to have reduced long-range connectivity, but increased local efficiency in MTLE patients (Desalvo et al., 2014). Within the temporo-limbic network, often associated with seizure activity in MTLE, there is also evidence for network reorganisation. Increased node degree, but decreased clustering has been found in the hippocampus; however increased clustering, nodal efficiency and degree were observed in the surrounding temporo-limbic network (Bonilha et al., 2012). In another study of DWI networks,

MTLE patients showed decreases of global and local efficiency in ipsilateral temporal and bilateral frontal and parietal regions (Liu et al., 2014).

Networks derived from fMRI data have shown that while networks retained small-world features in MTLE patients, there was widespread decreased connectivity (decreased clustering and path length) within and between the occipital, temporal, parietal and frontal lobes, but increased connectivity within the temporal lobe (Liao et al., 2010). Another study using graph theory on limbic regions only also found reduced bilateral limbic connectivity in both left and right MTLE patients (Chiang et al., 2014). In a study of only right MTLE patients, a widespread increase in correlation was reported in functional networks, higher search information in structural networks, and a higher correlation between structural and functional connectivity of right MTLE patients was found, suggesting a less functional flexibility in the networks of MTLE patients (Wirsich et al., 2016).

A meta-analysis of studies of whole-brain network analysis in focal epilepsy across all modalities found increased path length and clustering in brain networks of epilepsy patients (van Diessen et al., 2014), suggesting an overall decreased integration and increased segregation in networks of focal epilepsies. While there are still some discrepancies in the findings, which may be due to different patient populations or methodological differences, what the studies above do demonstrate is that there is large-scale structural and functional network reorganisation in MTLE patients involving the epileptogenic network and its connections to extra-temporal regions (Bernhardt et al., 2013).

This network reorganisation has also been associated with the disease mechanism in MTLE. A study of pre-surgical structural connectomes of MTLE patients found that patients who were not seizure-free had higher connectivity between ipsilateral medial and temporal lobe regions, ipsilateral medial temporal and parietal regions and, contralateral temporal pole and parietal regions. The sub-network of the ipsilateral temporal lobe was also more highly connected within

itself but more disconnected from the rest of the brain (Bonilha et al., 2013). Another study investigating functional connectome changes following surgical resection in MTLE patients found that network topologies of seizure-free patients were more resilient to attack than patients who were not seizure-free (Liao et al., 2016).

Both brain network connectivity (Glahn et al., 2010) and network topology have been shown to be heritable, making them suitable candidates for endophenotypes. Moderate heritability has been reported for clustering, path lengths and small-worldness in EEG networks of twins (Smit et al., 2008). 60% of inter-individual variance in network cost-efficiency has also been attributed to genetic factors (Fornito et al., 2011). In epilepsy, there has been some evidence for altered degree distribution variance and clustering coefficient in EEG networks of both IGE patients and their unaffected relatives (Chowdhury et al., 2014). To my knowledge, there have been no studies investigating brain network endophenotypes in MTLE.

In Chapter 8, I identified some alterations in a specific EEG network associated with the low alpha rhythm MTLE patients, suggesting abnormal connectivity of a sub-network in MTLE patients. In this chapter, I seek to characterise whole-brain functional and structural network features in this cohort of MTLE patients and their first-degree unaffected relatives using fMRI and DWI data. I also investigate possible abnormalities in sub-networks of the left hippocampus and the set of hub nodes for each subject. Finally, I investigate the relationship between functional and structural brain networks in the subjects. Alterations in network features found in MTLE patients and similarly in unaffected relatives may indicate candidate network-based endophenotypes for MTLE.

## **9.2 Aims and hypotheses**

This chapter aims to characterise structural and functional network abnormalities in MTLE patients and their unaffected relatives compared to healthy controls, and to investigate whether there may be candidate network-based endophenotypes for MTLE.

This will be achieved through four analyses applied to brain networks derived from fMRI and DWI data of MTLE patients and their unaffected relatives, compared to a group of healthy controls:

- 1) Characterising whole-brain network properties across a range of network density thresholds.
- 2) Characterising network properties in a sub-network involving connections of the ipsilateral hippocampus.
- 3) Characterising network properties in a sub-network of highly connected hub regions.
- 4) Investigating structure-function network relationships and comparing them between groups.

The hypotheses were that:

- 1) MTLE patients will show altered connectivity in both whole-brain networks and sub-networks compared to healthy controls, with alterations in unaffected relatives being intermediate.
- 2) There will be an increase in structure-functional correlation in MTLE patients compared to healthy controls, with relatives intermediate.

## **9.3 Method**

### **9.3.1 Participants**

Twenty-four patients with MTLE, 22 first-degree relatives and 30 healthy controls were included in the study presented here. One patient with MTLE was excluded from the DWI analysis as they did not complete a full DWI scan. The sample characteristics are shown in Table 9.1. Age was compared between groups using an ANOVA and gender was entered into a  $\chi^2$  test to assess for group differences.

**Table 9.1** Participant demographics for DWI & fMRI connectome analysis

|                                          | MTLE Patients | Relatives   | Controls   |
|------------------------------------------|---------------|-------------|------------|
| <b><u>PAF Analysis</u></b>               |               |             |            |
| Number                                   | 24            | 22          | 30         |
| Age in years: mean (SD)                  | 39.0 (12.3)   | 33.5 (13.2) | 34.9 (9.9) |
| Gender: Female (%)                       | 11 (45.8%)    | 13 (59.1%)  | 16 (53.3%) |
| <b>Clinical Characteristics</b>          |               |             |            |
| Age of onset in years: mean (SD)         | 22.5 (8.5)    | -           | -          |
| Duration of epilepsy in years: mean (SD) | 16.3 (13.5)   | -           | -          |

### 9.3.2 Data acquisition

Participants had a structural T1 weighted MRI, two 10-minute long simultaneous EEG and fMRI recordings, and a full diffusion weighted scan with a shorter reverse phase-encoded scan. As explained in Chapter 5, imaging data were side-flipped where appropriate and all differences in the subsequent analyses will be considered as ipsilateral or contralateral to the pathological hippocampus. The full study protocol and acquisition methods have been described in Chapter 5. The methods described below are specific to the analyses performed in this chapter.

### 9.3.3 Choice of node parcellation for network construction

Many previous studies have used well-known anatomically-defined atlases to parcellate the brain, including the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) and the FreeSurfer cortical parcellations (Desikan et al., 2006; Fischl et al., 2004). As these atlases use anatomical landmarks to divide the brain, they often produce very large parcels and cannot accurately capture the functional heterogeneity within each parcel. Other studies have used functionally-defined atlases based on published task-based data (Power et al., 2011; Yeo et

al., 2011), or based on study-specific fMRI data using various clustering algorithms (Craddock et al., 2012; Maggioni et al., 2014; Shen et al., 2013), but these methods depend on the clustering algorithm used and the quality of the data they are applied on, which is a matter of debate in itself (Eickhoff et al., 2015).

A study that compared parcellation schemes for within parcel variance using fMRI data has shown that most of the parcellation schemes compared, including the AAL atlas, are able to delineate brain regions with above random accuracy when compared to a random parcellation method of the same order. It also found that in general, the larger the parcels, the higher the variance and heterogeneity of voxels within the parcel, and sub-dividing large regions increases within-parcel homogeneity, although this does ultimately depend on the smoothness of the data (Gorgolewski et al., 2016).

For this thesis, I have tried to find a balance between having a meaningful anatomically defined atlas, while maximising the homogeneity of fMRI connectivity within each parcellated region. For these reasons, I have chosen a broad, anatomically-defined atlas, the AAL atlas (Tzourio-Mazoyer et al., 2002; Figure 9.1A; hereafter referred to as the AAL90 atlas), and a sub-division of the atlas where there are 512 cortical and sub-cortical regions of approximately equal sizes (Zalesky, Fornito, Harding, et al., 2010; Figure 9.1B; hereafter referred to as the AAL512 atlas). Both parcellation schemes have anatomically defined boundaries, avoiding the confounds which arise from study-specific fMRI approaches.

The AAL90 atlas is widely used and the AAL512 atlas has been previously used to study both DWI and fMRI network connectome studies (Perry et al., 2015; Wirsich et al., 2016). Evaluating networks at two parcellation scales (AAL90 and AAL512) will also allow for a comparison of how scale might bias network topology and measures. The centre of gravity and anatomical label of each region in the AAL90 atlas is given in Appendix Table E1, while Appendix Table E2 lists

the centre of gravity of each region in the AAL512 parcellation and the corresponding broad anatomical label based on the AAL90 atlas.

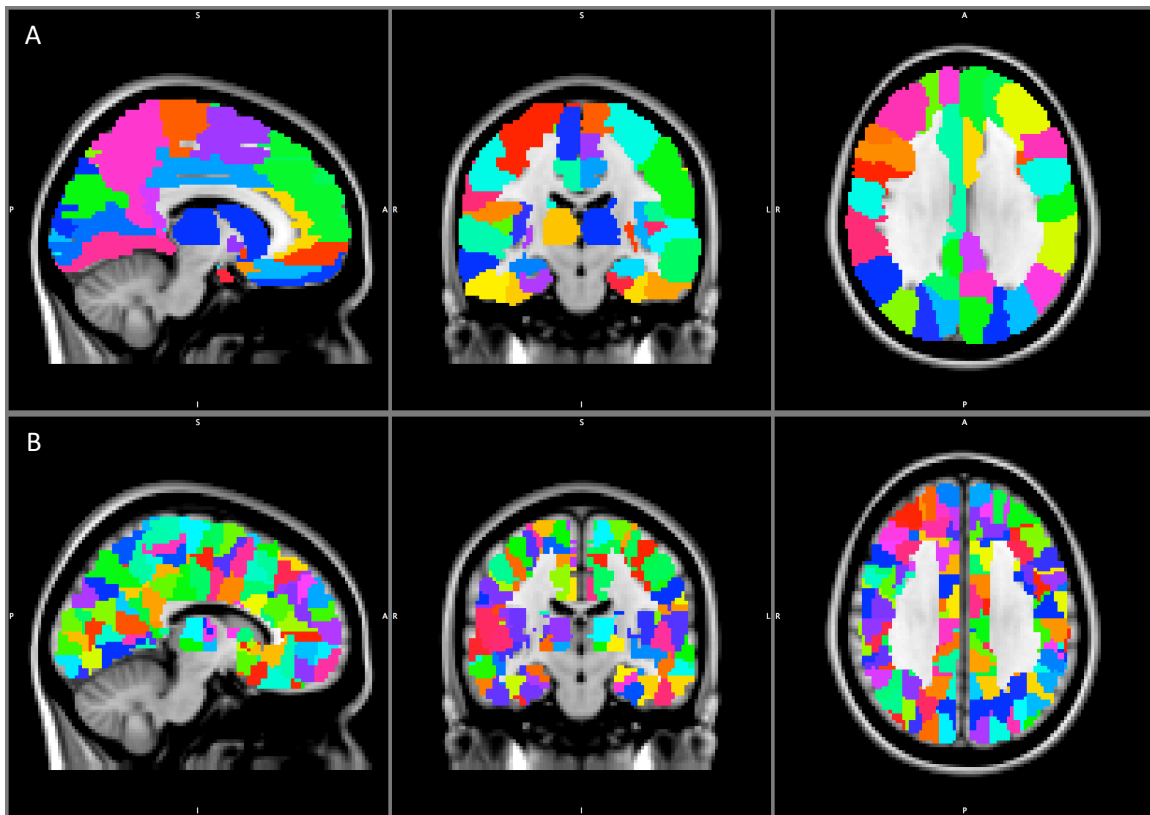


**Figure 9.1** Sagittal, coronal and axial view of original AAL parcellation with 90 regions (A) and AAL parcellation after sub-division into 512 regions of approximately uniform size (B). Each colour represents one sub-unit of the parcellation and colours are assigned at random and may be repeated.

### 9.3.4 Data pre-processing and connectome generation

#### 9.3.4.1 fMRI data

fMRI pre-processing and co-registration to standard MNI space were described in Chapter 5. Any subject with a maximum relative RMS displacement of >3mm (or 1 voxel) was excluded from the analysis and motion was compared between groups using an ANOVA to assess for any significant differences in fMRI motion between groups. For the present analysis, the nuisance regressors from the initial pre-processing were entered into a GLM for each subject, producing a residuals image of pre-processed and denoised data, which was used to construct the functional connectivity adjacency matrices.

Each AAL atlas, originally in MNI space, was transformed into each subject's native fMRI space by means of the previously computed non-linear warp and linear transforms, and using nearest-neighbour interpolation to preserve node boundaries and labels. Each subject's pre-processed and denoised fMRI images were then divided into 512 nodes using the co-registered atlas. The average time-series for each node was computed by taking the average of all voxel time-series in the node. A  $512 \times 512$  weighted functional connectivity matrix (denoted as  $FC_n$  for each subject  $n$ ) was created with each edge  $w_{ij}$  representing the Pearson's correlation coefficient between time-series in node  $i$  and node  $j$ . Correlation coefficients in each connectivity matrix were then Fisher-z transformed to variance normalise them; absolute values were taken so that both positive and negative correlations are treated as connections, and values were demeaned and normalised by the range so that all values lie between 0 and 1.

#### 9.3.4.2 DWI data

DWI data were processed using the MRtrix software package (J-D Tournier, Brain Research Institute, Melbourne, Australia, <https://github.com/MRtrix3/mrtrix3>; (Tournier et al., 2012) and tools from the FSL package. DWI data were first denoised using the *dwidenoise* tool from MRtrix that reduces effects of thermal noise. Next, the original and reverse phase-encoded scans with no diffusion weighting (*b0* scans) were used in FSL's *topup* to correct for field inhomogeneity distortion and FSL's *eddy* was used for eddy current-induced distortion correction and motion correction. The data were then bias field corrected, with the correction field estimated from the *b0* images and applied to all diffusion weighted volumes to correct for intensity non-uniformities caused by magnetic field inhomogeneities. Global intensity normalisation was performed across all subjects so that all subjects have the same median white matter value on their *b0* images. A group averaged response function was then estimated by averaging response functions across the group. This response function is the DWI signal obtained from a "single-fibre" bundle, where all fibres are oriented in the same direction, and is used as the kernel for the Constrained Spherical Deconvolution (CSD) step. CSD is then used to compute a fibre orientation distribution (FOD) image for each subject.

Each subject's T1 structural image was co-registered to its native DWI space by using a linear registration in FSL. To be able to make use of Anatomically Constrained Tractography (ACT) (Smith, Tournier, et al., 2012), the T1 image was first segmented into MRtrix's 5 tissue types (cortical grey matter, sub-cortical grey matter, white matter, cerebrospinal fluid and pathological tissue) using the *5ttgen* script (which interfaces with FSL's BET, FAST and FIRST tools). ACT allows for streamline propagation and termination at the grey-white matter boundaries. A probabilistic streamline algorithm (iFOD2) was used to generate 100 million fibre tracks sampled randomly across the whole brain and constrained by ACT. The default parameters were used: step size = 1mm, maximum angle = 45°, minimum track length = 4mm, maximum track length = 200mm, FOD termination threshold = 0.1. Spherical-deconvolution Informed Filtering of Tractograms (SIFT) was then performed to filter each set of 100 million tracks to produce streamline densities proportional to fibre densities estimated by spherical deconvolution across whole-brain white matter, giving an estimate of the number of streamlines between two regions as a proportion of the cross-sectional area of the fibres connecting them (Smith et al., 2013), resulting in 10 million tracks.

Each AAL atlas (in MNI space) was transformed into each subject's native diffusion space through a non-linear transformation to the T1 structural space and then a linear transform to diffusion space. A  $512 \times 512$  weighted structural connectivity matrix (denoted as  $SC_n$  for each subject  $n$ ) was created where each edge in the connectome  $w_{ij}$  represents the total streamline density between node  $i$  and node  $j$ . As suggested in a previous study, each edge in a weighted  $SC_n$  should be adjusted by the fibre length (Hagmann et al., 2007). Each streamline's contribution to the connectome edge was thus divided by its length in order to account for the effect of distance between nodes on the number of probabilistic streamlines likely to be found between the two nodes. As the tractography algorithm gives a streamline density at each voxel, no additional correction for node size was made. The values in each matrix were demeaned and normalised by the range so that all values lie between 0 and 1.

#### **9.3.4.3 Threshold selection for FC and SC matrices**

It is necessary to exclude spurious connections when studying connectomes derived from fMRI and DWI data by applying a threshold, so as to only include the most likely connections in the analysis, usually determined by strength of connection. Thresholds are also necessary to be able to compare networks of equal connection density across subjects so that any differences are not attributed to simply a difference in the overall density of the networks; a common technique for this is to select a range of thresholds within certain boundaries to ensure that any results are not biased by the matrix connection density (van Wijk et al., 2010).

However, there is no universally accepted method for thresholding FC and SC matrices and this is still a matter of debate (Bullmore & Bassett, 2011; Sporns, 2013). Several fMRI studies have justified their choice of threshold by selecting thresholds where all subjects' connectomes are fully-connected and exhibit the small-world network features known to exist in the human brain (Achard & Bullmore, 2007; Achard et al., 2006) and in general a threshold of around 10% is applied in most studies (Sporns, 2013). Global graph measures have also been found to be most reliable, with the highest within subject reliability across runs, at a range of 20-35% network density (Termenon et al., 2016).

For the whole-brain network analysis presented in this chapter, I examined networks across a range of network density thresholds from 5% to 45% densities. A range of thresholds was chosen in order to explore how network properties change across a range of network densities and to ensure that any group differences in measures are not biased by threshold selection. The sub-network analyses were not conducted across a range of thresholds and this is further explained below.

#### **9.3.5 Whole-brain network analysis**

Group differences in global network topology were characterised using graph network analysis implemented in Matlab, using scripts from the Brain Connectivity Toolbox (Rubinov and Sporns,

2010; <http://www.brain-connectivity-toolbox.net>). Descriptions and mathematical formulae of the network measures are given in Chapter 5 and Appendix D.

After thresholding at the network density determined in the above section, six global network measures that characterise the overall network structure, and which have been found to be different in previous studies of epilepsy patients, were calculated for each subject's FC and SC networks. These were the mean degree centrality ( $Deg_{mean}$ ), mean degree centrality variance ( $Deg_{var}$ ), mean clustering coefficient ( $CC_{mean}$ ), characteristic path length ( $PL_{char}$ ), global efficiency ( $Eff_{glob}$ ), and small-world index ( $\alpha$ ). These measures are commonly used in other studies of brain networks and global measures of brain organisation, in particular global efficiency and path length, have been shown in previous studies to be abnormal in TLE patients (Chiang & Haneef, 2014).

### 9.3.6 Hippocampal sub-network

To investigate the properties of the brain network associated with the ictogenic hippocampus, a sub-network was formed from the left hippocampus node and its first-degree connections. An initial threshold was used to exclude spurious connections.

For networks derived from fMRI data, each edge is a correlation coefficient with a corresponding  $p$ -value. Thus, fMRI networks were initially thresholded using  $p$ -values from the correlations, after adjustment for multiple correlations at the Bonferroni level (i.e.  $p < \frac{0.05}{N \times N - N}$  where  $N$  is the number of nodes).

Each subject's SC network was first thresholded at the subject's corresponding FC density, and then a threshold was chosen based on the density of the structural network containing connections in at least 90% of subjects from the control group. This provided a principled method to identify the density of connections most likely to be present in the structural core of the healthy brain. Each subject's network was then thresholded at this network density.

Different thresholds were chosen for FC and SC networks as they are likely to be related but are not necessarily equal, with FC networks typically having a greater variation and density, but constrained by the underlying SC networks (Honey et al., 2009; Sporns, 2013; van den Heuvel & Sporns, 2013a).

Global network properties of the ipsilateral hippocampal FC and SC sub-networks were characterised in all subjects using the same set of measures as described for the whole-brain analysis above.

### **9.3.7 Network hubs**

To investigate the properties of the core set of nodes within the FC or SC networks, hub nodes were determined for each group. Hub nodes within each subject's FC and SC networks were selected based on a composite index across three local node centrality measures known to describe different aspects the node's influence on the overall network (Betzel et al., 2014; Perry et al., 2015). These metrics were the node degree, node local efficiency and node betweenness centrality. The composite index was averaged across each group and the top 15% of nodes were considered network hubs.

A hub sub-network was formed from only the group-averaged hub nodes and global network properties were characterised in all subjects using the same set of measures as described above.

### **9.3.8 Statistical comparisons for network measures**

To identify group differences in global network measures across multiple thresholds, the area under the curve (AUC; across threshold) for each measure was first computed and group differences assessed using a MANOVA implemented in SPSS for 6 measures and 3 groups, with follow-up univariate ANOVAs and follow-up Tukey tests to determine the direction of significant effects. Planned linear contrasts were also performed for each measure to test the hypothesis that network measures in unaffected relatives lie in between MTLE patients and healthy

controls. The partial eta squared ( $\eta^2$ ) effect size was computed for the MANOVA and each linear contrast. A rough guideline for small, medium and large effect sizes using  $\eta^2$  are 0.01, 0.06 and 0.14 respectively (Cohen, 1988).

Network measures for each sub-network analysis were also assessed using a MANOVA and follow-up and planned contrasts as described above.

### **9.3.9 Structure-function relationships of networks**

To investigate the structure-function relationship of networks and whether it differs between groups, the Pearson's correlation coefficient was calculated between the FC and SC matrices of each subject and these values were compared between groups using an ANOVA and follow-up Tukey tests.

## **9.4 Results**

The participant groups did not differ in age or gender when assessed using a one-way ANOVA and chi-square test. The groups also did not differ significantly in maximum relative RMS motion in fMRI scans and no participants were excluded based on motion.

### **9.4.1 Whole-brain network analysis**

#### **9.4.1.1 Functional connectivity networks**

There were no significant group differences in the AUC of any of the network measures across the range of FC network thresholds for either the AAL90 or AAL512 parcellations (Figure 9.2). Networks across all three groups appeared to show small world properties ( $\sigma > 1$ ) at lower network densities.

At the AAL90 scale, linear contrast analysis showed that MTLE patients had consistently lower mean degrees and mean clustering coefficients, and longer path lengths than healthy controls,

with unaffected relatives intermediate, across all network densities. Although these differences were not statistically significant, the effect sizes were small to medium for group linear trends in mean degree and characteristic path length, and medium for mean clustering coefficient (Table 9.2).

**Table 9.2** p-values and effect sizes of linear trends in the AUC of network measures across multiple thresholds, at the AAL90 parcellation scales.

| Measure                            | AAL90           |                  |
|------------------------------------|-----------------|------------------|
|                                    | <i>p</i> -value | partial $\eta^2$ |
| <b>Mean degree</b>                 | 0.090           | 0.04             |
| <b>Mean degree variance</b>        | 0.267           | 0.02             |
| <b>Mean clustering coefficient</b> | 0.092           | <b>0.06</b>      |
| <b>Characteristic path length</b>  | 0.096           | 0.04             |
| <b>Global efficiency</b>           | 0.105           | 0.01             |
| <b>Small-world index</b>           | 0.656           | <0.01            |

#### 9.4.1.2 Structural connectivity networks

There were no significant group differences in any of the network measures across the range of SC network thresholds for either the AAL90 or AAL512 parcellations (Figure 9.3). As with the FC networks above, networks across all three groups appear to show small world properties ( $\sigma > 1$ ) at lower network densities. Path length and global efficiency for SC networks appeared to sharply plateau at a network density of 10% at the AAL90 parcellation scale, and was remarkably equal across all network densities at the AAL512 scale. This might suggest that structural networks should be assessed at very low network densities as measures tend to plateau after around 10-20% density.

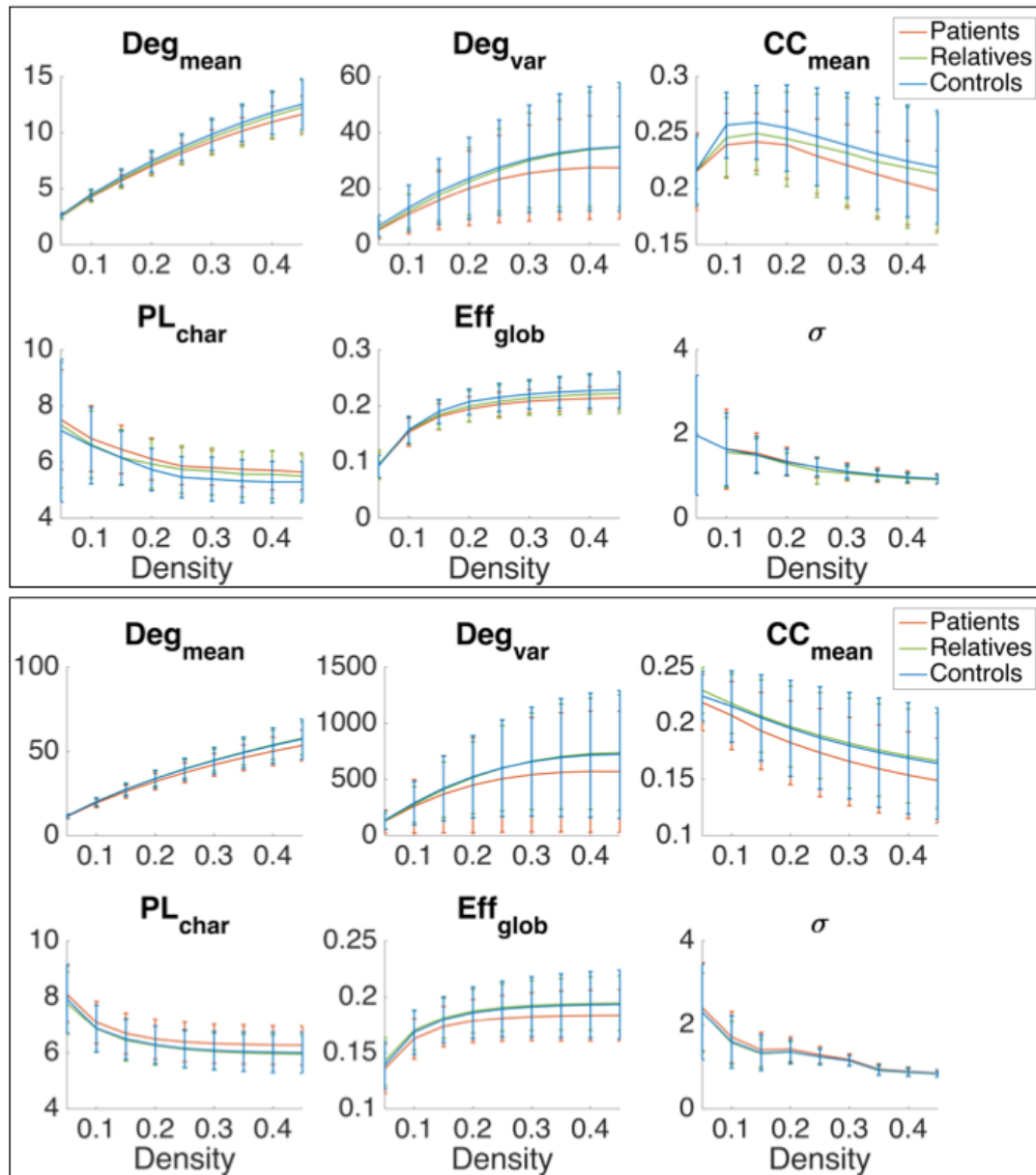


**Figure 9.2** Network measures for FC matrices from the AAL90 (top) and AAL512 (bottom) parcellation schemes across network densities ranging from 5% to 45%. Error bars shown are  $\pm 1$  standard deviation.

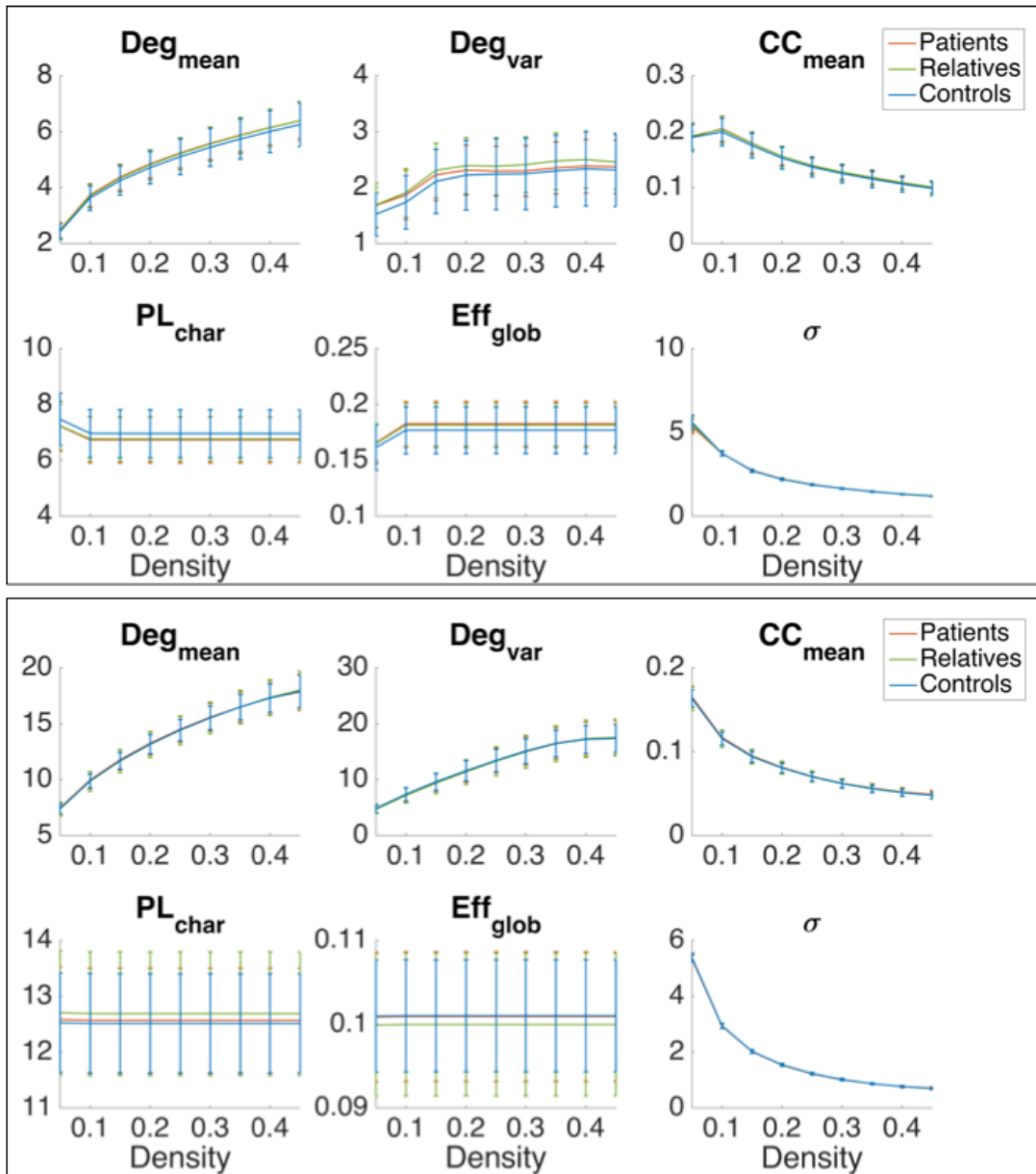


**Figure 9.3** Network measures for SC matrices from the AAL90 (top) and AAL512 (bottom) parcellation schemes across network densities ranging from 5% to 45%. Error bars shown are  $\pm 1$  standard deviation.

## 9.4.2 Hippocampal sub-network analysis

### 9.4.2.1 Initial thresholds

For FC networks, the network density of the matrices after Bonferroni correction was applied on the  $p$ -values from the correlation coefficients is shown in Figure 9.4. There were no significant group differences in network density. The network densities at the AAL90 scale were slightly higher than at the AAL512 scale, likely due to a lower  $p$ -value required to account for Bonferroni correction for more edges in the latter.

For SC networks, after the initial threshold based on the FC, the network density of the matrix for which each connection is present in at least 90% of healthy control participants was 24% for the AAL90 parcellation and 19% for the AAL512 parcellation.

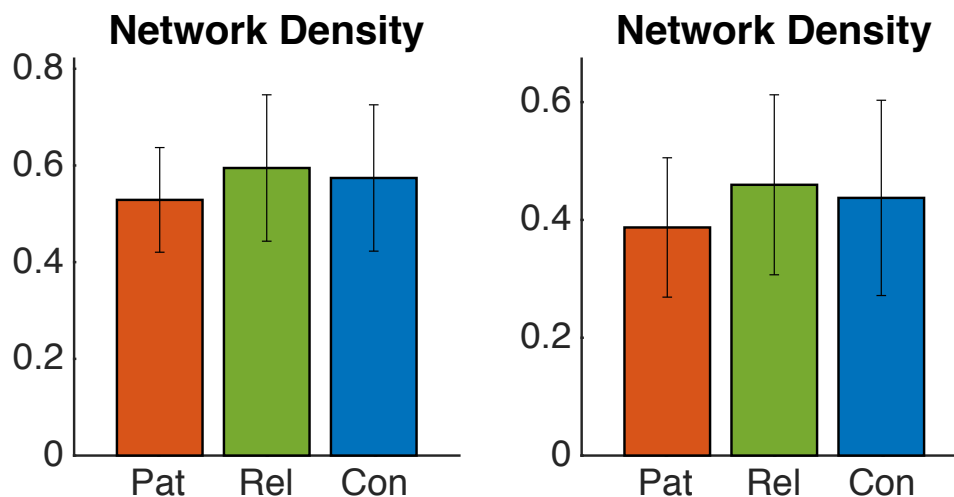


**Figure 9.4** Group comparisons of network density for FC networks after Bonferroni-correction of p-values of correlation coefficients. Left: AAL90 atlas, Right: AAL512 atlas.

#### 9.4.2.2 Hippocampal functional connectivity networks

The ipsilateral hippocampal FC sub-network was determined by considering only nodes with first-degree connections to the ipsilateral hippocampus node(s); i.e. node 19 in the AAL90 atlas, and nodes 90, 175 and 232 in the AAL512 atlas.

The ipsilateral hippocampal sub-network in each group is displayed in Figure 9.5. Each sphere represents the centre-of-gravity of a node. The sphere sizes and colours in the figure represent the proportion of subjects in each group that have that node as a part of their hippocampal sub-network. This could be thought of as the consistency of the node within the hippocampal sub-network of that group, with more consistent nodes being larger and in red, and less consistent nodes being smaller and in blue.

Across both parcellation schemes, there appear to be fewer nodes within the hippocampal sub-network of MTLE patients than healthy controls and unaffected relatives. The nodes appear

more consistent in hippocampal regions (larger and darker red spheres), however the spread of nodes within temporal regions and extra-temporal regions appear smaller in MTLE patients (fewer orange, yellow and green nodes in temporal regions, especially in contralateral temporal lobe of MTLE patients). These differences are more apparent at the AAL512 parcellation scale.

Network measures were computed based on individual subject hippocampal sub-networks. For the AAL90 atlas, the MANOVA revealed no significant group effect. However, planned contrasts revealed a linear trend in mean degree:  $F(2,73) = 3.75, p = 0.057, \eta^2 = 0.05$ ; and degree variance:  $F(2,73) = 3.72, p = 0.058, \eta^2 = 0.06$ . Patients had lower mean degree and degree variance than healthy controls. Although these linear trends did not meet the criteria for statistical significance, they had moderate effect sizes (Figure 9.6; top panel).

For the AAL512 atlas, using Pillai's trace, the MANOVA revealed a significant effect of group:  $F(12,138) = 2.12, p = 0.019, \eta^2 = 0.16$ . Univariate ANOVAs showed that the significant group effect was for mean degree variance:  $F(2,73) = 3.26, p = 0.044, \eta^2 = 0.08$ . Follow-up t-tests showed unaffected relatives had higher mean degree variance than MTLE patients ( $p = 0.035$ ), and there were no other significant group differences. The linear trend analysis was also not statistically significant (Figure 9.6; bottom panel).

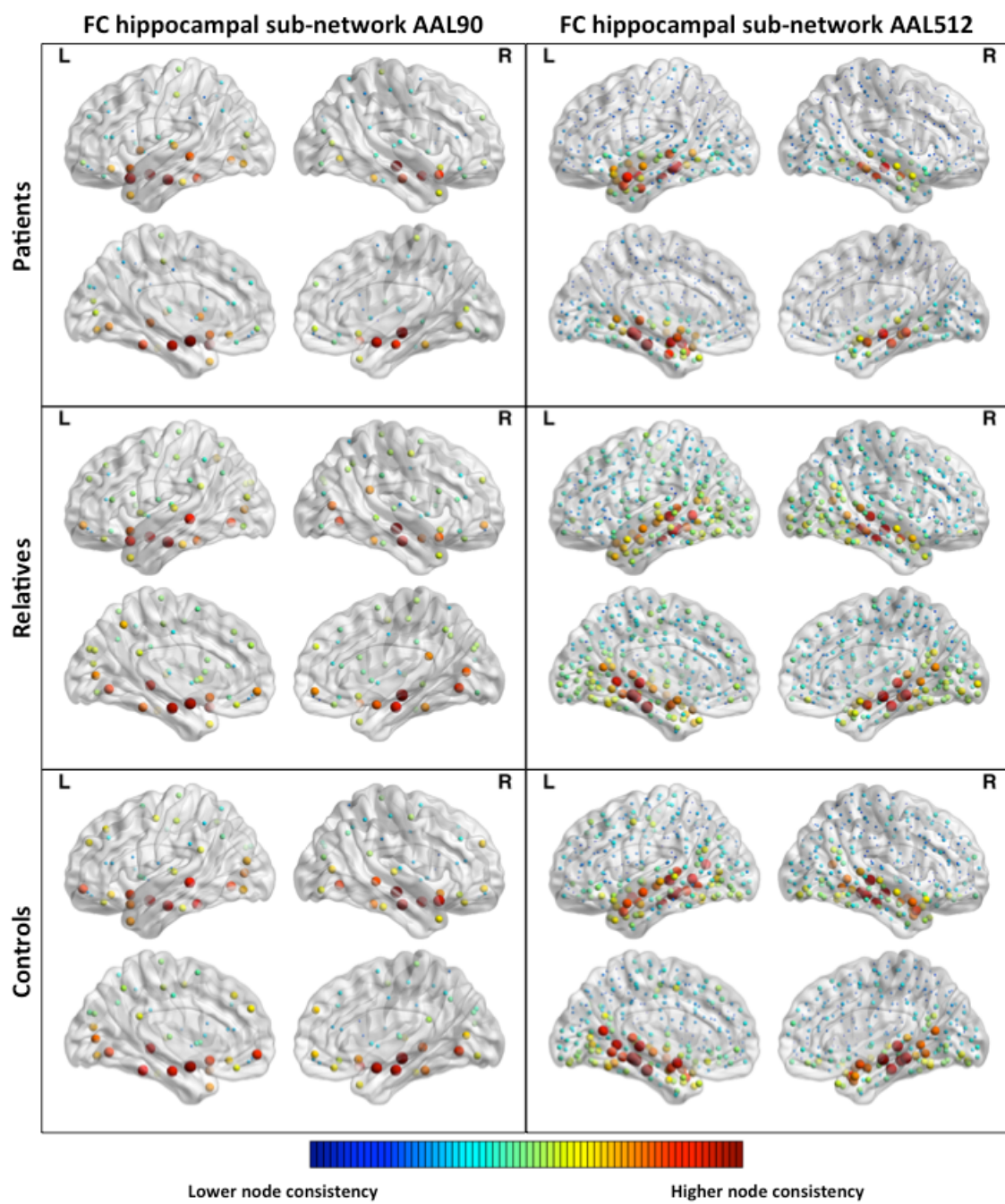


**Figure 9.5** Nodes within the FC hippocampal sub-networks across groups and parcellation scheme.

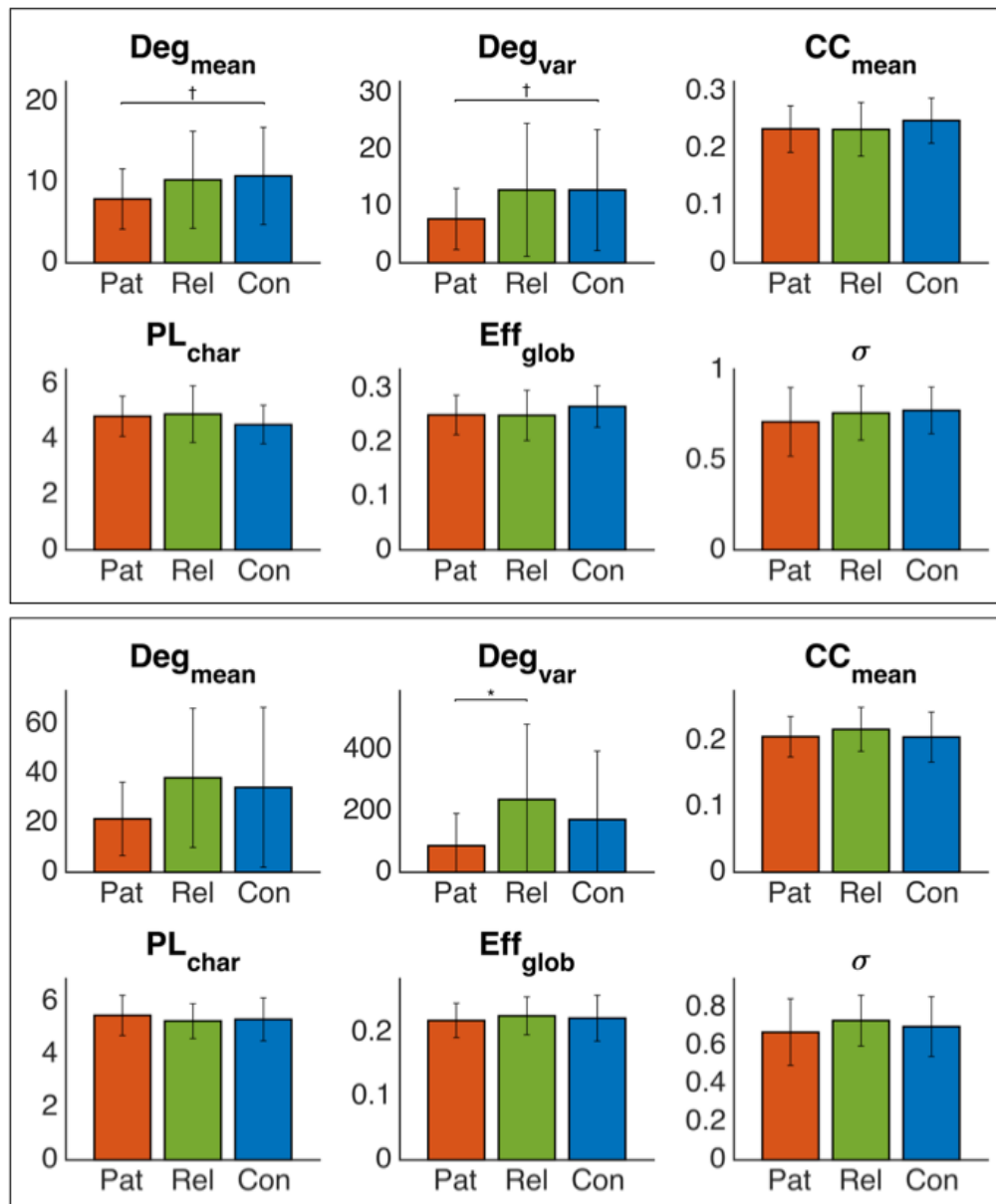


**Figure 9.6** Network measures in FC hippocampal sub-networks for the AAL90 atlas (top) and the AAL512 atlas (bottom). Error bars shown are  $\pm 1$  standard deviation. Group differences significant (using post-hoc Tukey tests) at  $p < 0.05$  are denoted by \* and trends are denoted by †.

#### 9.4.2.3 Hippocampal structural connectivity networks

The ipsilateral hippocampal SC sub-network in each group is displayed in Figure 9.7. As above, the colour and size of each sphere denotes the consistency of the node within the hippocampal sub-network of the group. At both parcellation scales, nodes within the SC hippocampal sub-networks of all three groups appear very similar with a spread mostly around temporal lobe and limbic regions and the posterior cingulum, and in the contralateral hippocampus at AAL512.

Network measures were computed based on individual subjects' hippocampal sub-networks. For the AAL90 atlas, using Pillai's trace, the group effect assessed using the MANOVA was not statistically significant, but had a large effect size:  $F(12,136) = 1.63$ ,  $p = 0.092$ ,  $\eta^2 = 0.13$ . Univariate ANOVAs showed that the trends for group effects were in the characteristic path length:  $F(2,72) = 4.18$ ,  $p = 0.019$ ,  $\eta^2 = 0.10$ ; and global efficiency:  $F(2,72) = 3.33$ ,  $p = 0.041$ ,  $\eta^2 = 0.09$ . Both trends also had moderate to large effect sizes. The linear contrast analysis showed a group linear trend in mean clustering coefficient, characteristic path length and global efficiency. MTLE patients and unaffected relatives had lower characteristic path length, higher clustering coefficient and higher global efficiency than healthy controls. Linear contrast test statistics for clustering coefficient:  $F(2,72) = 4.11$ ,  $p = 0.046$ ,  $\eta^2 = 0.05$ ; path length:  $F(2,72) = 6.61$ ,  $p = 0.012$ ,  $\eta^2 = 0.10$ ; and global efficiency:  $F(2,72) = 4.85$ ,  $p = 0.031$ ,  $\eta^2 = 0.08$ . The linear trends had medium effect sizes (Figure 9.8; top panel).

For the AAL512 atlas, the MANOVA revealed no significant group effects and planned linear contrasts did not yield any significant differences between groups (Figure 9.8; bottom panel).

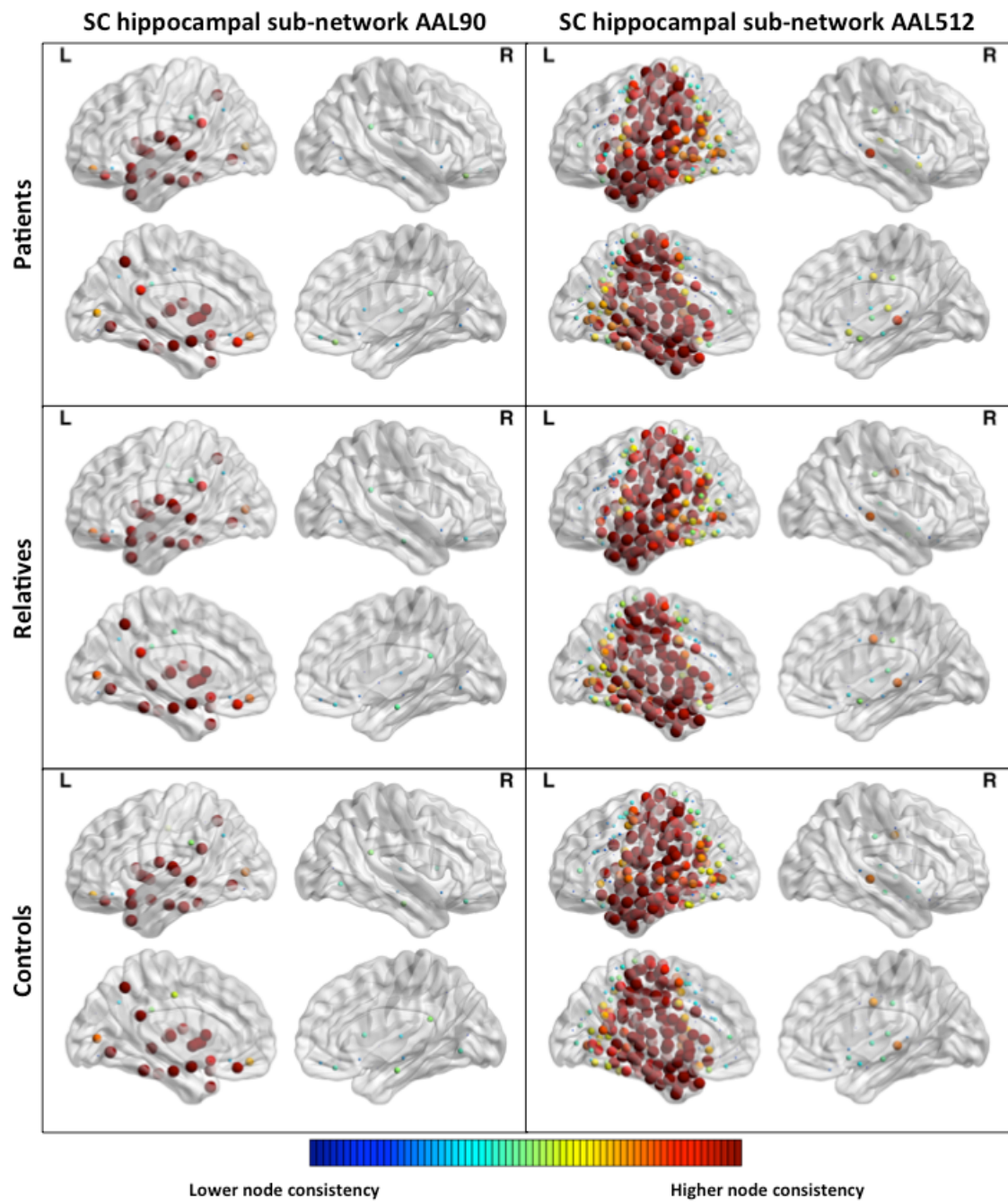


**Figure 9.7** Nodes within the SC hippocampal sub-networks across groups and parcellation scheme.

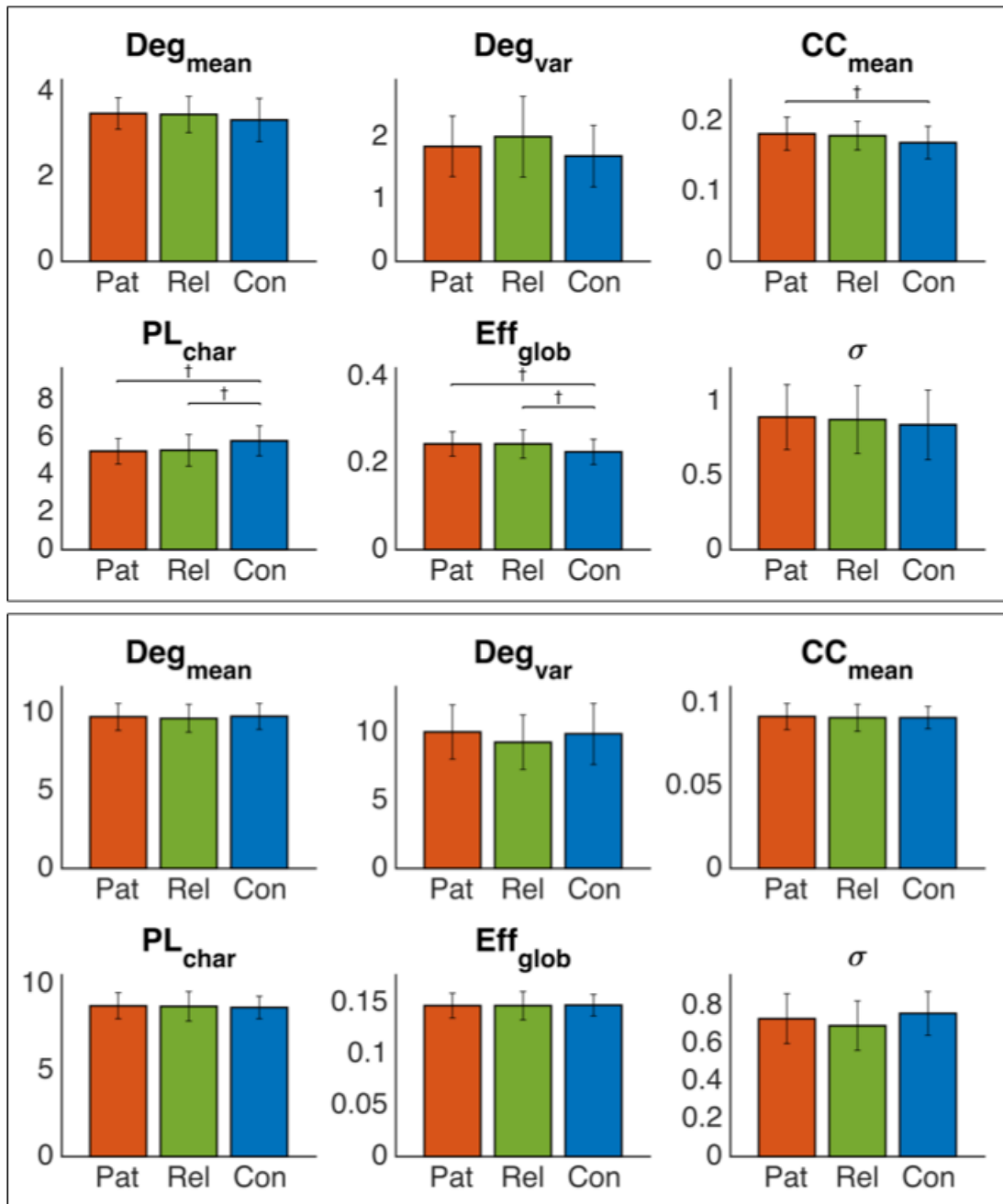


**Figure 9.8** Network measures in SC hippocampal sub-networks for the AAL90 atlas (top) and the AAL512 atlas (bottom). Error bars shown are  $\pm 1$  standard deviation. A trend toward a group difference is denoted by †.

### 9.4.3 Hub sub-network analysis

#### 9.4.3.1 Hub functional connectivity networks

For each participant group, an average hub sub-network was determined using a composite index comprised of the average of three local node measures for each subject. The composite index was averaged across group and then ranked. For each group and for each parcellation

scale, the top 15% of nodes were considered as hubs. The hub nodes in each group are shown in Figure 9.9. In the figure, each sphere represents a hub node and its size represents the number of subjects for which that node appears in the top 15% of nodes (for that particular subject). The connections between nodes are also shown as grey lines between nodes.

Network measures are shown in Figure 9.10. MANOVAs revealed no significant group differences and no significant linear trends at both parcellation scales.

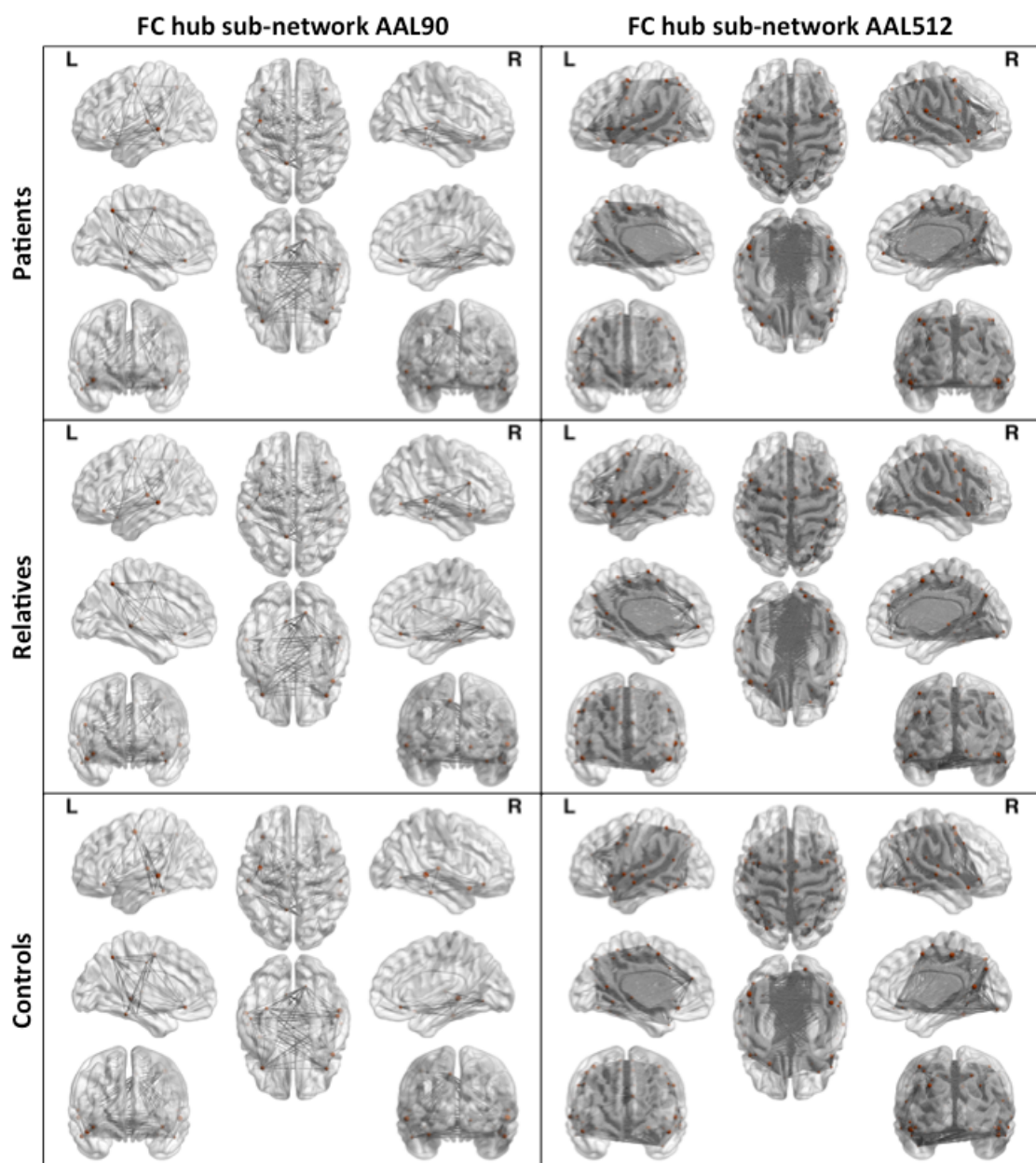


**Figure 9.9** FC network hub nodes in each group at the AAL90 and AAL512 parcellation scales.

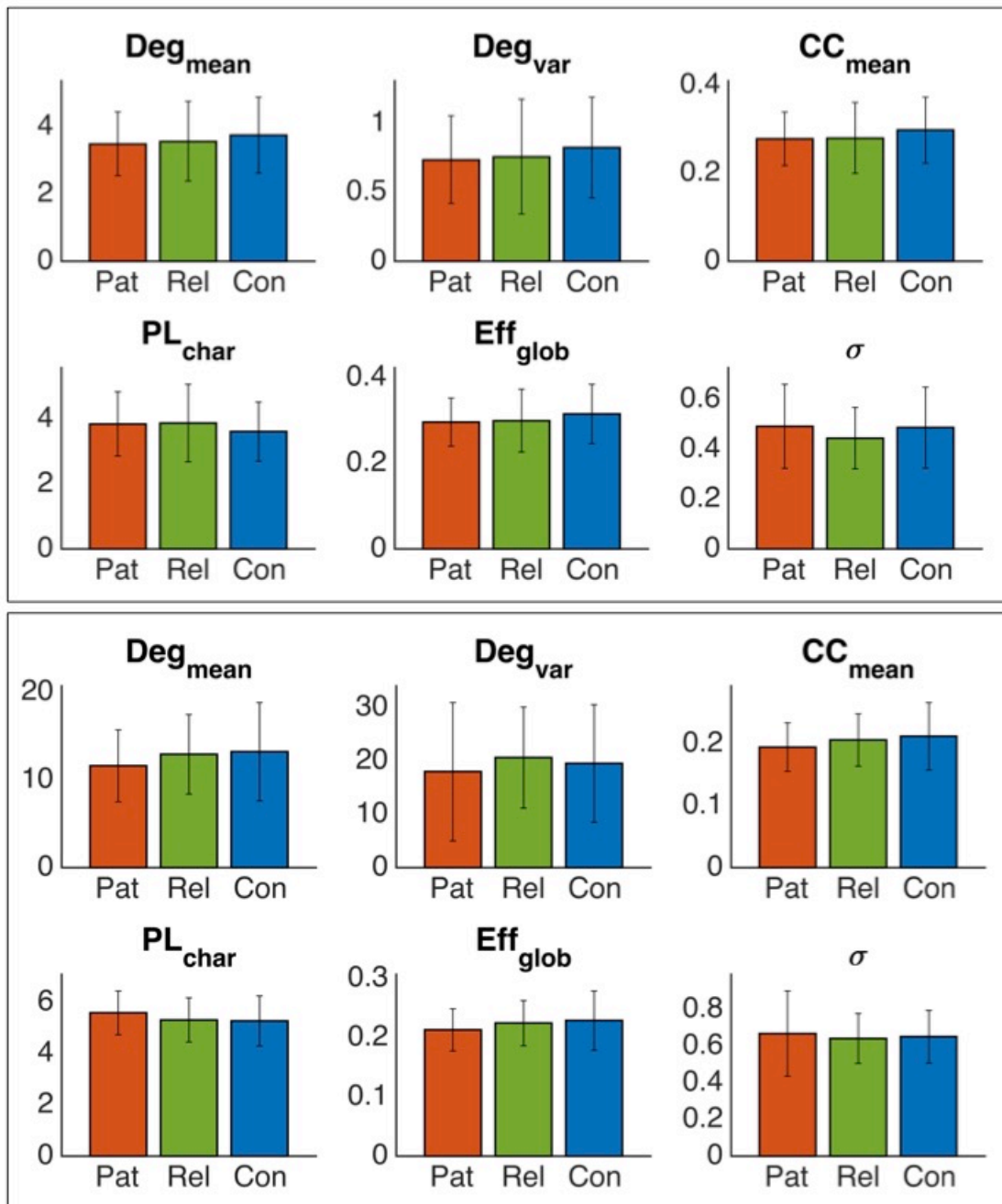


**Figure 9.10** Network measures in FC hub networks for the AAL90 atlas (top) and the AAL512 atlas (bottom). Error bars shown are  $\pm 1$  standard deviation.

#### 9.4.3.2 Hub structural connectivity networks

Hub networks were defined for SC in the same way as for FC. The hub nodes for SC networks in each group are shown in Figure 9.11 at the AAL90 and AAL512 scales.

There were no significant effects of group or linear trends at the AAL90 parcellation scale (Figure 9.12; top panel).

At the AAL512 parcellation scale, using Pillai's trace, the MANOVA revealed a significant effect of group:  $F(12,136) = 4.92, p < 0.001, \eta^2 = 0.30$ , indicating a large effect size. Univariate ANOVAs revealed a significant effect of group only on the mean degree:  $F(2,72) = 4.52, p = 0.014, \eta^2 = 0.11$ . Follow-up Tukey tests showed patients had significantly higher mean degree than controls ( $p = 0.024$ ), and a trend toward a higher mean degree in unaffected relatives than in healthy controls ( $p = 0.052$ ). Planned linear contrasts showed linear trends for mean degree:  $F(2,72) = 7.20, p = 0.009, \eta^2 = 0.11$ ; and degree variance:  $F(2,72) = 5.25, p = 0.025, \eta^2 = 0.07$ . The linear trends had moderate to large effect sizes.

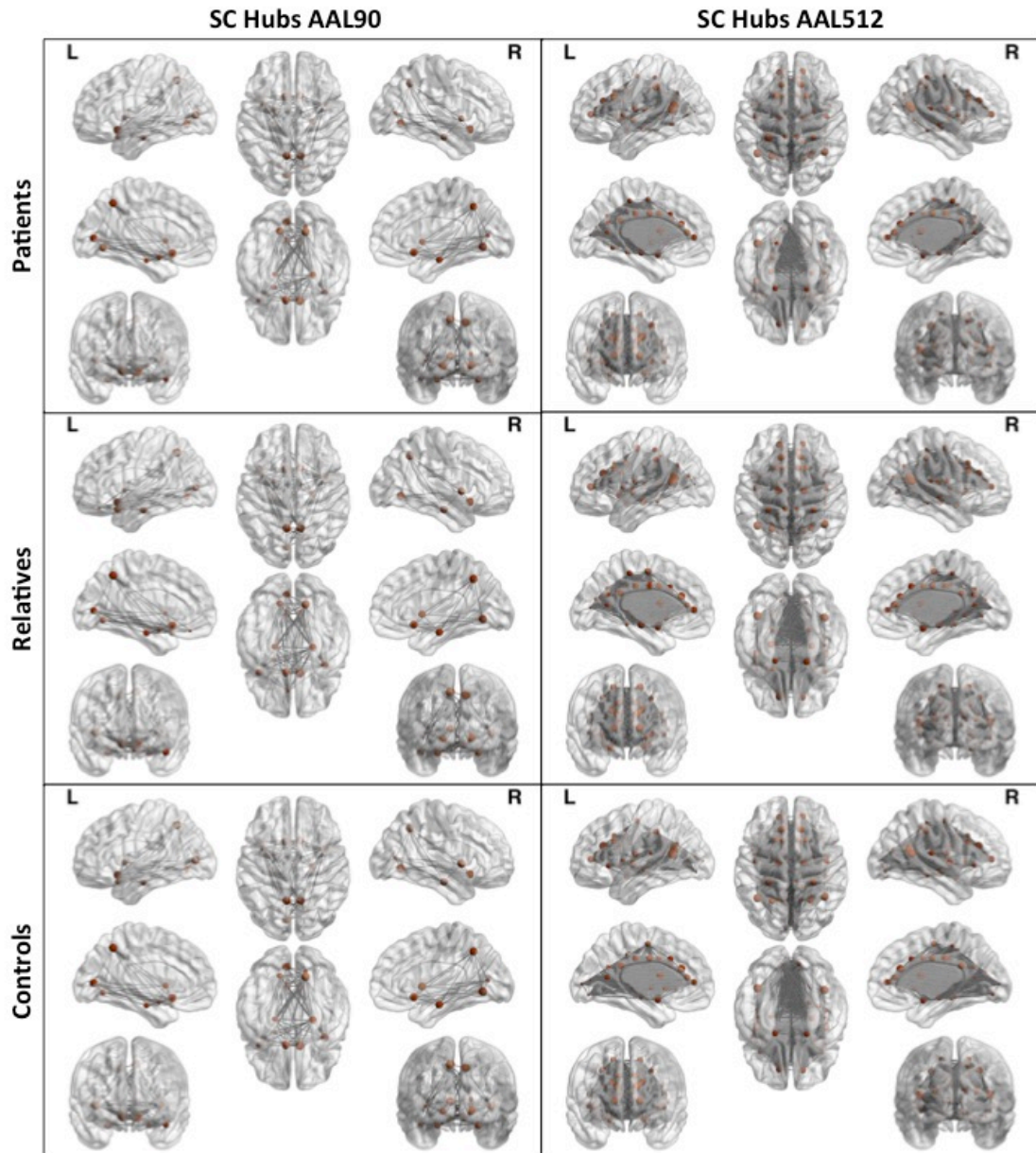


**Figure 9.11** SC network hub nodes in each group at the AAL90 and AAL512 parcellation scales.

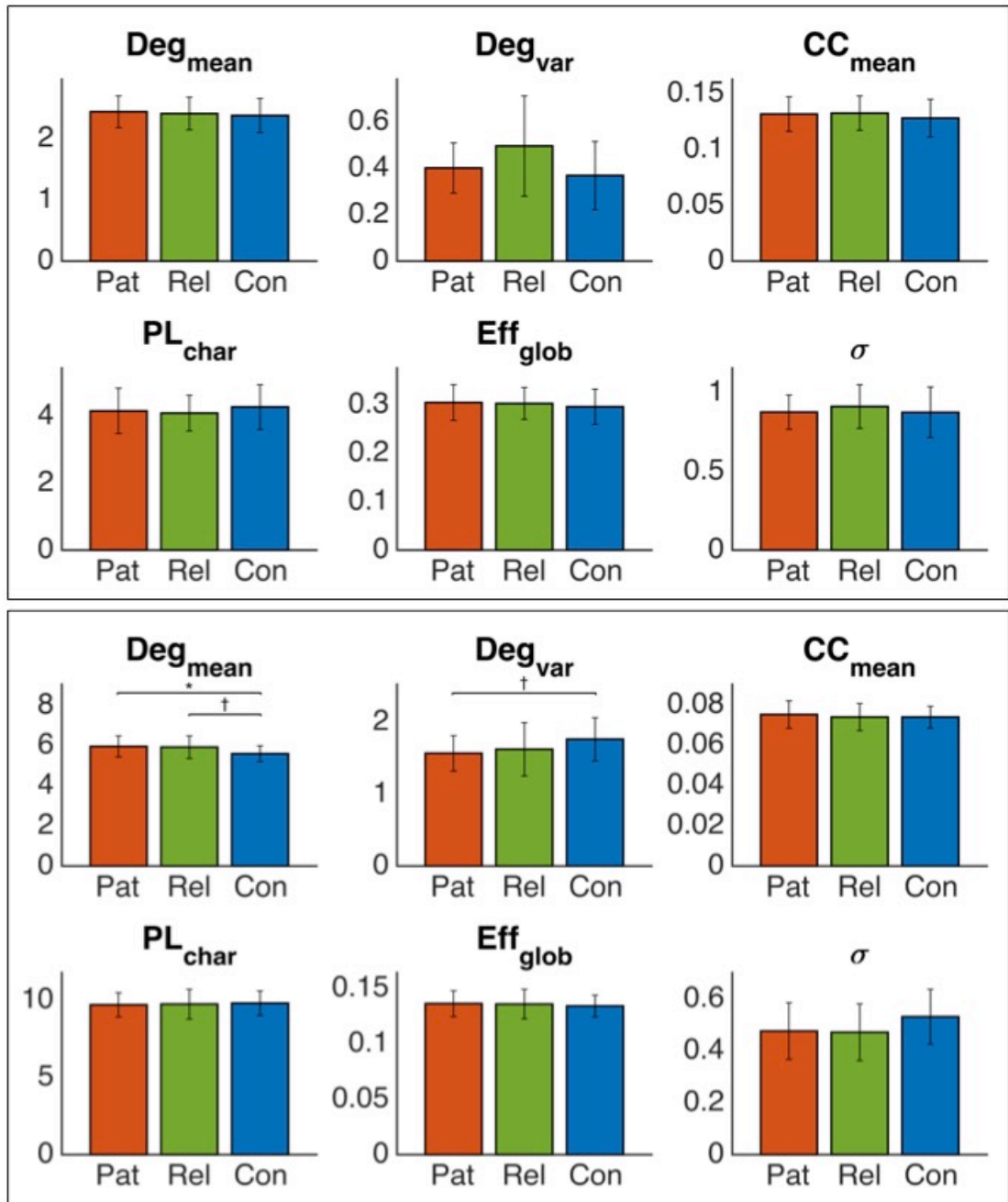


**Figure 9.12** Network measures in SC hub networks for the AAL90 atlas (top) and the AAL512 atlas (bottom). Error bars shown are  $\pm 1$  standard deviation. Group differences significant (using post-hoc Tukey tests) at  $p < 0.05$  are denoted by \* and trends are denoted by †.

#### 9.4.3.3 Node consistency within hubs

From Figure 9.9 and Figure 9.11, it can be observed that node sizes in SC networks are generally much larger than in FC networks, indicating a higher consistency of nodes within SC hub networks in all three groups.

#### 9.4.4 Structure-function relationships of networks

There were no significant differences in structural-functional network correlations between groups, although there appeared to be a trend toward higher SC-FC correlation values in MTLE patients than healthy controls and unaffected relatives (Figure 9.13).

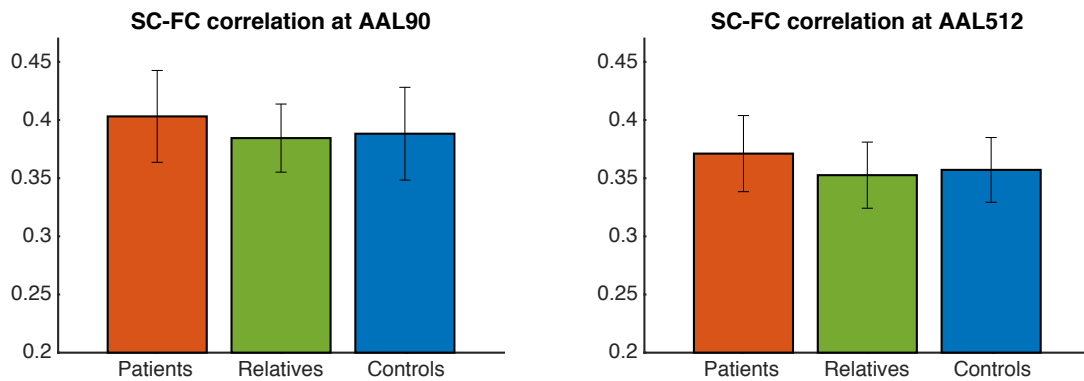


**Figure 9.13** Bar graphs of correlation values between structural connectivity and functional connectivity networks in each group at the AAL90 scale (left) and the AAL512 scale (right). Error bars shown are  $\pm 1$  standard deviation.

### 9.5 Discussion

This chapter aimed to investigate structural and functional whole-brain and sub-network alterations in MTLE patients and their unaffected relatives compared to healthy controls. The main findings are that:

- 1) At the whole-brain level of FC networks, there were medium effect sizes for linear trends of longer path lengths and lower mean degrees and clustering coefficients in MTLE patients than in healthy controls, with unaffected relatives intermediate. This suggests that whole-brain networks in MTLE patients are more segregated (longer path lengths) and less connected (lower mean degree and clustering coefficient) than networks in healthy controls, and that unaffected relatives' networks show similar alterations to a certain extent.
- 2) In hippocampal FC networks, there were medium effect sizes for linear trends of lower mean degree and mean degree variance in MTLE patients than in healthy controls, with unaffected relatives intermediate. This suggests that nodes within the hippocampal FC

networks may have more consistent but weaker intra-network connections in MTLE patients than in healthy controls.

- 3) In hippocampal SC networks, medium effect sizes were observed for linear trends of higher clustering coefficients and global efficiencies, and shorter characteristic path lengths in MTLE patients than in healthy controls, with unaffected relatives intermediate. These linear trends suggest a more highly integrated SC hippocampal sub-network in MTLE patients.
- 4) In the hub sub-network, there were no group differences in FC hubs, but SC hubs showed higher mean degree and lower mean degree variance in MTLE patients than in healthy controls, with relatives intermediate. This suggests a more strongly inter-connected SC hub sub-network in MTLE patients than in healthy controls.
- 5) Finally, there were no statistically significant group differences in the structural-functional network correlation.

These findings suggest functional and structural network reorganisation in MTLE patients. This was observed as a loss of functional connectivity at the whole-brain level, and stronger structural connectivity within sub-networks of the epileptogenic focus and highly influential hub nodes in MTLE patients compared to healthy controls, with relatives broadly showing network properties intermediate to the two other groups. While most of these differences were not statistically significant, they did have medium to large effect sizes suggesting that the comparisons may lack the power to detect significant differences at the current group sizes.

#### **9.5.1 Reduced whole-brain functional network connectivity**

The linear trends observed for longer path lengths and smaller mean degrees and clustering coefficients in MTLE patients than in healthy controls suggest that there is reduced whole-brain FC in MTLE patients than in healthy controls. The linear trends also suggest that unaffected relatives have network properties intermediate to MTLE patients and healthy controls.

In studies of MTLE patients, reduced FC has often been reported across the whole brain, in particular between epileptogenic regions and the rest of the brain. In a study using seed-based functional connectivity, decreased connectivity with the ipsilateral hippocampus was found in MTLE patients compared to healthy controls, with the reduction being more severe in left than in right MTLE patients (Pereira et al., 2010). The default mode network (DMN), a network of regions found to be highly connected in the brain at rest, has also been found to be less functionally connected in MTLE patients, particularly in the dorsal mesial prefrontal, mesial temporal and inferior temporal regions (Zhang et al., 2010). Functional connectivity between the DMN and epileptogenic regions such as the hippocampus and amygdala has also been found to be reduced in MTLE patients (Pittau et al., 2012). This study selected fMRI data without EEG spikes, suggesting that the decreased functional connectivity is not dependent on interictal spike activity. Decreased regional homogeneity (ReHo) was also found in DMN in patients with MTLE, but increased ReHo in ipsilateral regions thought to be associated with seizure generation and propagation (Zeng et al. 2013).

This widespread decrease in functional connectivity in MTLE patients is thought to underpin the various cognitive and neuropsychiatric disorders often found in MTLE. Among the networks shown to have reduced functional connectivity in MTLE patients are the language network in left MTLE (Waites et al., 2006), the auditory and sensorimotor networks (Zhang, Lu, Zhong, Tan, Liao, et al., 2009) and the dorsal attention network (Zhang, Lu, Zhong, Tan, Yang, et al., 2009). The decrease in functional connectivity in the dorsal attention network was found to be negatively correlated with scores on an attention task, suggesting that this functional connectivity alteration is indeed related to attention impairments in MTLE.

The mechanisms underlying the reduced functional connectivity are unknown, but it has been postulated that it may be a result of structural damage from recurrent seizures. Using fMRI and DTI data, functional and structural connectivity between the same regions of the DMN, the posterior cingulate/precuneus node, have been found to be decreased in MTLE patients (Liao et

al., 2011), indicating some relationship between reduced structural and functional connectivity. Further to this, studies have shown that the reduced functional connectivity is correlated with both duration and severity of seizures (Liao et al., 2010; Voets et al., 2012), suggesting there may be some credence to the theory.

Although I did not find any structural connectivity alterations in MTLE patients, the trends toward reduced overall functional network connectivity are in keeping with previous literature. The linear trend toward lower clustering in MTLE patients and unaffected relatives compared to healthy controls, although not statistically significant, has a medium effect size and suggests that some aspects of global connectivity in unaffected relatives may be altered and may represent candidate endophenotypes for further investigation.

### **9.5.2 Increased connectivity within the epileptogenic regions**

Hippocampal sub-networks derived from FC showed a trend toward more consistent but weaker intra-network connections, while the sub-networks derived from SC showed a trend toward a more highly connected hippocampal sub-network.

Evidence for both increased and reduced connectivity in the epileptogenic network of MTLE patients has been found. Decreased fMRI functional connectivity was found between epileptogenic regions including the amygdala, entorhinal cortex, and anterior and posterior hippocampi, with a contralateral, and possibly compensatory, increase in connectivity (Bettus et al., 2009). Using depth-EEG however, increased connectivity was found between mesial temporal lobe (MTL) structures including the hippocampus, entorhinal cortex and amygdala across all frequency bands, and was not explained by interictal spikes (Bettus et al., 2008). More evidence from depth-EEG recordings showed an overall increase in connectivity within MTL regions but also a more regular network arrangement, with increases in both clustering and path length (Bartolomei et al., 2013).

With fMRI data, another group found increased regional homogeneity within posterior cingulate and temporal lobe regions (Mankinen et al., 2011). Using graph theory on fMRI data, increased connectivity was also found within MTL structures, although decreased connectivity was found elsewhere in the brain (Liao et al., 2010).

In a study using DTI, although a general reduction of fibre density was found in MTLE patients, an increase in degree, clustering and efficiency was found in the limbic network and specific nodes including the ipsilateral insula, superior temporal lobes and thalamus (Bonilha et al., 2012). The same group also found that although there was a decrease in connectivity between ipsilateral thalamo-cortical regions, there was an increase in connectivity in the ipsilateral MTL, insular and frontal lobes (Bonilha et al., 2013).

While there has been evidence for both increases and decreases in connectivity within the ipsilateral epileptogenic network, what is clear from all the above studies and the analyses presented in this chapter is that there is network reorganisation within epileptogenic regions in MTLE patients. The differences in the reports could be due to the fact that many of the studies differed in terms of the spread of regions included in the “epileptogenic network”, with networks ranging from the entire ipsilateral temporal lobe, to specific regions like the entorhinal cortex and amygdala only. Different aspects of network reorganisation may also be captured using different modalities and methods, as evidenced by different frequency bands showing different patterns of altered connectivity (Quraan et al., 2013), and functional networks being influenced by but not necessarily similar to structural networks (Honey et al., 2009; Ponten et al., 2010).

A methodological reason for the difference between functional and structural networks could be the way hippocampal sub-networks were defined in the present study. In the above analysis, the hippocampal sub-network consisted of all nodes that have a first-degree connection with the ipsilateral hippocampus. From Figure 9.5 it can be observed that there appear to be fewer, less consistent nodes in the MTLE patients’ FC hippocampal sub-network compared to unaffected

relatives and healthy controls. While the core nodes within the network appear to be highly consistent and connected, taking the average across these and less consistent nodes could explain why it appears that the mean degree is lower in MTLE patients' sub-networks. SC networks were initially thresholded at a lower network density value, probably resulting in fewer spurious tracts. This might explain why structural hippocampal sub-networks appear more similar across groups in terms of the spread of nodes (Figure 9.7), but show global network properties that are different between groups.

### **9.5.3 Network hubs in MTLE**

Brain networks are complex networks with non-random small-world topologies that rely on "hubs" as the central core of the network (Bullmore & Sporns, 2009). These high centrality hubs have been found to be highly connected and are thought to be important in efficient information transfer in the brain. However, hubs tend to have higher metabolic demands than other brain regions making them more vulnerable to dysfunction and thus disorders of brain network dysfunction often involve alterations in network hubs (van den Heuvel & Sporns, 2013b). In fact, a study found that the hubs of the brain network often coincide with a higher probability of anatomical alterations in Alzheimer's disease and schizophrenia (Crossley et al., 2014). In MTLE, both fMRI and DTI data have shown a reorganisation of hub nodes (Liao et al., 2010; Liu et al., 2014), and a study using cortical thickness networks has shown that hubs in MTLE patients were less distributed across the whole brain and more clustered within the limbic and temporal association regions (Yasuda et al., 2015).

In the study presented here, the SC hub sub-networks in MTLE patients had higher streamline density connecting them compared to healthy controls, with unaffected relatives showing intermediate alterations. This suggests abnormally dense connections exist between hub regions in MTLE patients, and to an extent in unaffected relatives. These changes were only detected at the AAL512 parcellation scale and could perhaps be because such subtle differences cannot be observed when streamline densities are counted across larger regions as in the AAL90 atlas. The

majority of hub regions were association regions including the superior frontal cortex, precuneus, parietal lobes and several temporal association cortex nodes. Although there was no strong evidence for a reorganisation toward more hub nodes in MTLE patients, the hub nodes do have a high proportion of temporal lobe regions in general. From the previous analysis presented in Chapter 6, large parts of the temporal lobe showed altered morphology in MTLE patients, with subtle alterations in unaffected relatives. This may suggest that these structural alterations have an impact on whole-brain network architecture through altered network hub connections.

#### **9.5.4 Structure-function correlations**

There were no significant group differences in structure-function correlations, although there were some differences in structural and functional network properties between groups both at the global scale and at the sub-network level. Changes in FC are thought to be compensatory responses and secondary to changes in SC (Bernhardt et al., 2013). Alterations in FC have been shown to be related to SC alterations (Voets et al., 2012), and structure-function correlations have been reported to be reduced in MTLE patients, suggesting less “flexibility” in the MTLE brain (Wirsich et al., 2016).

#### **9.5.5 Methodological considerations**

The difference between functional and structural network findings may also be a result of how the networks are defined. FC network edges were defined based on an average time course across all voxels of each node, while SC network edges were a summation of streamline density at each voxel in the node. Ideally, nodes should represent voxels that are fairly “homogeneous” in function (and anatomical location), however, the parcellation schemes are not perfect. For example, the “hippocampal” nodes for the AAL512 atlas consisted mainly of voxels within the hippocampus, but also consisted of some nodes in the thalamus. This might explain why the SC hippocampal sub-network at the AAL512 scale encompassed a lot of nodes not traditionally thought to have connections with the hippocampus (Figure 9.7). FC hippocampal sub-networks

at the AAL512 scale appeared more typical of the spread of regions thought to be connected to the hippocampus because the time courses were averaged across the region where there is a greater number of voxels within the hippocampus than the thalamus. This problem is not observed with the AAL90 atlas because it respected traditional structural boundaries. Perhaps the results presented at the AAL512 scale should be treated with some caution.

In fMRI, 10 minutes of resting-state data has been shown to be a sufficiently long period to reliably estimate functional connectivity (Birn et al., 2013), and indeed the study presented here was able to reproduce some of the findings of altered FC in MTLE patients and uncover some subtle alterations in FC networks of unaffected relatives. However, brain activity is not static and networks and connections between networks are known to be dynamic and time-varying (Chang & Glover, 2010; Hutchison et al., 2013). In MTLE, greater variance in the dynamic FC of the hippocampus was suggested to be related to seizure semiology (Laufs et al., 2014). Averaging of functional connectivity across 10 minutes of data oversimplifies the complexity of the non-stationary dynamics in brain networks and further work is required to investigate these dynamic changes in FC and their relation to SC in MTLE patients.

The analysis software used for the DWI analysis is relatively new and employs some state of the art methods (including CSD and SIFT) to estimate the structural connectome. Many previous studies have used other software packages and it would be interesting to investigate the effect of the particular packages and algorithms used on the final results, but that this was beyond the scope of my thesis.

### **9.5.6 Conclusions**

The study presented here is one of the few multi-modal studies of network connectivity in MTLE patients and the first investigation of network alterations in unaffected relatives of MTLE patients. It showed some trends toward differences in both structural and functional connectivity in MTLE patients that suggest an increased connectivity within epileptogenic

networks and a decreased connectivity in widespread distal brain networks. Unaffected relatives also show subtler, but similar alterations in their brain networks. The effect sizes suggest there are some real alterations here but perhaps larger group sizes are required to identify statistically significant group differences. The field of investigating network alterations in MTLE patients is still new and more work is definitely required to understand the complex mechanisms of network alterations in MTLE patients before we can consider using these network features as endophenotypes.

## **Chapter 10 General discussion, conclusions and further work**

This thesis aimed to explore the suitability of several imaging and electrophysiological measures as endophenotypes for MTLE. Four experimental studies were undertaken to investigate candidate endophenotypes based on: structural volumetric and morphometric alterations (Chapter 6), structural connections based on white matter tracts (Chapter 7), a specific network related to electrophysiological alpha rhythms (Chapter 8), and large-scale whole-brain networks derived from functional and diffusion-weighted MRI (Chapter 9).

As there is already a wealth of knowledge and previous literature on both local and large-scale network alterations in MTLE, the strategy was to focus on characterising known alterations in an independent cohort of MTLE patients and investigating whether similar alterations are found in their first-degree unaffected relatives as compared to healthy controls. To my knowledge, the studies presented here represent one of the first few investigations of structural grey and white matter endophenotypes and the first investigations of functional MRI and EEG alterations in predominantly lesional sporadic MTLE patients and their unaffected relatives.

In this final chapter, I will summarise the key findings from the analyses described in Chapters 6 to 9 in relation to their respective study hypotheses. I will discuss their implications and contributions to the current field of work on endophenotypes for MTLE. I will also discuss the limitations of the current study and possible avenues for future research that could help to elucidate the complex genetic determinants of MTLE.

### **10.1 Summary of key findings**

Table 10.1 summarises the key findings from this thesis where the most promising findings for candidate endophenotypes for MTLE were found. Table 10.2 summarises the trends and non-significant findings for endophenotypes that could be investigated in larger, follow-up studies.

**Table 10.1 Summary of key findings**

| Candidate Endophenotype                           | Description                                                                                                                                                                                   |
|---------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reduced surface area in ipsilateral temporal lobe | Using vertex-wise structural MRI morphometry, both MTLE patients and unaffected relatives showed altered morphology the ipsilateral anterior temporal lobe driven by surface area reductions. |
| Reduced peak alpha frequency                      | Using power spectrum analysis of resting EEGs, there was a significant linear trend found for reduced PAF in both MTLE patients and unaffected relatives.                                     |

**Table 10.2 Summary of findings that show trends toward endophenotypes for MTLE**

| Candidate Endophenotype                                             | Description                                                                                                                                                                                                                                                                                                            |
|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ipsilateral hippocampal shape contraction                           | Using vertex-wise sub-cortical shape analysis, there was a trend for a shape contraction of the ipsilateral hippocampus in unaffected relatives.                                                                                                                                                                       |
| Ipsilateral hippocampal volumes correlate with MD of fornix         | Using manual tract dissection, the MD of the ipsilateral fornix was found to correlate with ipsilateral hippocampal volumes in unaffected relatives.                                                                                                                                                                   |
| PAF changes related to volume reduction on VBM                      | Using VBM, there was a trend for PAF correlation with reduced grey matter in a network involving ipsilateral insular and frontal opercular cortices in unaffected relatives.                                                                                                                                           |
| Altered network metrics in DTI-derived hippocampal and hub networks | Using graph theory on DTI-derived structural hippocampal networks, both MTLE patients and unaffected relatives showed a trend toward increased mean clustering coefficients and global efficiencies and decreased characteristic path lengths. In hub networks, relatives showed a trend toward increased mean degree. |

### **10.1.1 Volumetric and morphometric alterations of the ipsilateral hippocampus and temporal lobe**

Hippocampal atrophy is the best-established structural abnormality in MTLE patients. Many studies have also reported extra-hippocampal alterations in the ipsilateral temporal lobe, limbic regions and bilateral thalami (Keller & Roberts, 2008). In Chapter 6, structural MRI alterations in the present cohort of MTLE patients were characterised using whole-brain volumetric and morphometric approaches on FreeSurfer segmented images, sub-cortical shape analysis implemented in FSL FIRST, and hippocampal subfield volume analysis using FreeSurfer. It was hypothesised that MTLE patients would demonstrate reduced volumes in ipsilateral hippocampus and thalamus and show cortical shrinkage in the ipsilateral temporal lobe compared to healthy controls. Unaffected relatives were hypothesised to show a similar, but more subtle set of differences compared to healthy controls.

The primary finding was that ipsilateral temporal lobe morphology was altered in both MTLE patients and unaffected relatives compared to healthy controls. These alterations were spread across the ipsilateral medial and lateral temporal lobe and temporal pole in both groups. Compared to healthy controls, MTLE patients showed a larger extent of alterations than unaffected relatives. These cortical morphology alterations appeared to be driven only by surface area reductions in MTLE patients, but a combination of both surface area reduction and cortical thinning in unaffected relatives.

In the ipsilateral hippocampus, subfield volumetric analysis revealed reduced volumes in 8 of the 12 subfields in MTLE patients. Using vertex-wise sub-cortical shape analysis, contractions (i.e. atrophy) of the ipsilateral anterior hippocampus and body of the hippocampus were also found in MTLE patients compared to healthy controls. Although there were no significant volumetric or shape differences detected between unaffected relatives and healthy controls, unaffected relatives showed a sub-threshold atrophy compared to healthy controls. A reduced extent of shape differences was also found when MTLE patients were compared to unaffected

relatives as opposed to healthy controls, suggesting that subtle shape contractions may be present in unaffected relatives.

The above findings are in line with the hypothesis that MTLE patients would show alterations in the ipsilateral hippocampus and temporal lobe and that relatives would show subtle abnormalities in similar regions. These findings provide additional evidence for the use of cortical morphology of the ipsilateral temporal lobe as an endophenotype.

### **10.1.2 Tract reconstructions of the fornix**

Another well-established trait of MTLE patients is white matter microstructural alterations predominantly in tracts around the ipsilateral hippocampus and temporal lobe (Otte et al., 2012). In Chapter 7, I investigated the fornix, the main white matter fibre bundle connecting the hippocampus to limbic regions. The fibres of the fornix were reconstructed in each subject from diffusion-weighted data and tract averaged measures of fractional anisotropy and mean diffusivity were computed and compared between groups. It was hypothesised that ipsilateral fornix anisotropy would be reduced and diffusivity increased in MTLE patients compared to healthy controls, and that relatives would show subtle alterations of fornix tract measures similar to patients. I also hypothesised that the severity of fornix alterations would correlate with the severity of ipsilateral hippocampal volume reduction in both MTLE patients and unaffected relatives.

When compared to healthy controls, MTLE patients showed a clear pattern of reduced FA and increased MD in the ipsilateral fornix, but no significant differences or trends were detected in the ipsilateral fornix of unaffected relatives. Both ipsilateral fornix FA and MD correlated significantly with ipsilateral hippocampal volumes in MTLE patients, but only MD showed a trend toward a correlation with hippocampal volumes in unaffected relatives. There were also large effect sizes for reduced FA in the ipsilateral UNC and PHC tracts in MTLE patients, with linear trends showing reduced values in unaffected relatives as well.

The findings in the fornix tract did not align with the initial hypothesis that tract measures would be subtly different in unaffected relatives. However, there was some evidence for a correlation between severity of tract damage and hippocampal volume loss in unaffected relatives. This suggests that the unaffected relatives are a more heterogeneous group than healthy controls, with some more severely affected than others. Further work is required in the full dataset to further investigate differences in the UNC and PHC tracts.

### **10.1.3 Peak alpha frequency and alterations in a network related to the occipital alpha rhythm**

Peak alpha frequency (PAF) has been shown to be reduced in epilepsy patients (Larsson & Kostov, 2005) and alpha activity is a highly heritable trait (van Beijsterveldt & Boomsma, 1994). In Chapter 8, EEG measures of PAF were compared between groups and whole-brain voxel-wise patterns associated with alpha power and alpha fluctuations were investigated with voxel-based morphometry of structural MRI images and simultaneous EEG-fMRI. I hypothesised that both MTLE patients and unaffected relatives would show reductions in PAF compared to healthy controls, and that these reductions would be related to local structural grey matter volume alterations in regions involved in the generation of the alpha rhythm. I also hypothesised that the fMRI network associated with alpha power fluctuations would be altered in MTLE patients and unaffected relatives compared to healthy controls.

There was a significant linear trend of decreasing PAF, with healthy controls having the highest PAF values, unaffected relatives having PAF values in between and MTLE patients having the lowest PAF values. PAF was found to be related to changes in local volume in the ipsilateral insular and frontal opercular cortices in MTLE patients, with a non-significant trend in unaffected relatives. There was also higher correlation between BOLD signal and fluctuations in alpha power (in a similar set of brain regions, including the bilateral insular and frontal opercular cortices) in MTLE patients compared to healthy controls. There were no significant

differences when unaffected relatives were compared to healthy controls. When compared to MTLE patients however, the set of brain regions showing higher correlation with alpha rhythms in patients was reduced in extent and appeared more unilateral than bilateral, suggesting some abnormality in unaffected relatives too.

Only the PAF finding concurred with the hypothesis, while the two other findings appeared to show some non-significant trends. The findings suggest that PAF may be a suitable endophenotype for MTLE. It was rather unexpected to find that indices of the occipital alpha rhythm in MTLE patients correlated with both local volume changes and functional MRI activity in the same set of regions, as these regions are not usually those expected to be involved in alpha rhythm generation. Nevertheless, these were robust findings in two different imaging modalities.

#### **10.1.4 Large-scale functional and structural networks**

There has been increasing evidence for alterations in a widespread network involving regions outside of the affected hippocampus in MTLE (Richardson, 2010) and the first three analysis chapters have provided some more evidence of this. In Chapter 9, large-scale brain networks in MTLE patients and their unaffected relatives were characterised and compared to healthy controls using graph theory methods to model brain networks from fMRI and DTI data. Network measures across the whole-brain and in two sub-networks, a hippocampal sub-network and a “hub” sub-network, were compared between the groups.

Although the findings were not significant at the whole-brain level, there was some evidence (with a medium effect size) for more segregated and less connected whole-brain networks in MTLE patients compared to healthy controls, with unaffected relatives showing alterations intermediate to the two other groups. In the hippocampal sub-networks, there were again no significant effects, but there was medium effect size for a linear trend toward weaker connections in FC networks and more integrated SC networks in MTLE patients. In the SC hub

sub-networks, there was a significantly higher mean degree in MTLE patients, suggesting stronger connections between the hubs. No significant differences were found for FC hub sub-networks or in SC-FC network correlations.

Although mostly not statistically significant, some of the findings were in line with the hypothesis that network alterations would be present in MTLE patients and relatives would show similar alterations to a lesser extent. The significant finding of higher mean degree in MTLE patients in SC hub networks could be further investigated, because unaffected relatives also showed a similar (but not statistically significant) alteration.

## **10.2 Novel findings from this study**

The analysis in Chapter 6 represents the first replication, in an independent dataset, of a previously reported finding of altered temporal cortex morphology in unaffected relatives of sporadic MTLE patients with HS (Alhusaini, Whelan, Doherty, et al., 2016). There were some key differences in the study methods and analysis, which were:

- 1) left and right MTLE patients were combined in the study reported here due to the smaller sample size, but analysed separately in the study by Alhusaini and colleagues;
- 2) unaffected relatives included any first-degree relative of MTLE patients (i.e. all sharing 50% of their genes with the patients), while the Alhusaini study was a case-control study where only same sex siblings of MTLE patients were included;
- 3) the study reported here was performed at a higher MRI scanner strength (3T here vs. 1.5T in the Alhusaini study).

Despite these differences, the current study demonstrates that alterations of structural morphology in the ipsilateral anterior and medial temporal lobe represent a highly heritable trait related to MTLE and provides additional evidence for, and expands the scope of, the suggestion that this structural imaging trait may be a suitable endophenotype for MTLE that deserves further investigation.

Chapters 7 to 9 are the first studies to investigate white matter tracts directly connected to the hippocampus, EEG features, and functional and structural network features in unaffected relatives of a group of MTLE patients predominantly with MTS. Although Chapter 7 demonstrated that the tract measures themselves are unlikely to be suitable endophenotypes, the findings do suggest that tract MD is governed by a similar mechanism related to hippocampal integrity in both MTLE patients and unaffected relatives. Chapter 8 is the first demonstration of EEG PAF reduction in both MTLE patients and (to a smaller extent) unaffected relatives suggesting that this may be a suitable endophenotype for MTLE. Lastly, Chapter 9 demonstrated a more connected hub sub-network in MTLE patients, with unaffected relatives showing a similar (but not statistically significant) effect, suggesting a possibility for this measure to be used as an endophenotype.

### **10.3 Implications of findings on endophenotypes for MTLE**

Despite MTLE being the most common adult focal epilepsy, its genetic determinants remain largely a mystery. They are thought to be very complex with many genome-wide association studies failing to identify common genetic variants related to the disorder, possibly due to a strong environmental component (although there may be genetically determined risk factors), and heterogeneity of the samples in the studies, hence reduced power (Kasperaviciūte et al., 2010). Quantitative endophenotypes have been suggested to be a useful intermediary to aid in the identification of genetic variants associated with genetically complex disorders (Glahn et al., 2007; Pal & Strug, 2014).

The four analyses described here indicate the presence of structural and functional alterations in MTLE patients and demonstrate mixed evidence for similar changes in unaffected relatives. Two traits, morphology alterations in the temporal lobe and EEG peak alpha power, demonstrated the most promise for further evaluation as endophenotypes for MTLE and may have potential to be used in future genetic studies. In the following paragraphs I will discuss the suitability of these traits as endophenotypes according to the four criteria that these traits should: 1) have

high heritability, 2) be related to the phenotype or disorder, 3) be state independent, and 4) be present in unaffected relatives at a higher rate than in healthy controls (Glahn et al., 2007; Gottesman & Gould, 2003).

### 10.3.1 Heritability

Commonalties in brain structure and function within families are thought to be either due to genetic factors or a result of a shared environment. The analyses reported here have all investigated traits that have broadly been shown to be highly heritable in controls, however, further investigations are required to confirm the heritability of these specific traits in epilepsy. One way to estimate trait heritability, or what proportion of the variance of the trait is due to genetic factors, is from the correlation of offspring and parental phenotypic values (Wray & Visscher, 2008). Loosely applying this concept to the study reported here, I investigated correlations between the candidate endophenotype values of each patient and relative pair. However, in this study, there were only 7 matched samples of patient and relative pairs. Correlations of PAF values on the subset of the 7 patient and relative pairs (Figure 10.1) showed a moderate correlation of  $r = 0.47$  that was not statistically significant. Clearly not much can be concluded from this extremely modest sample, and further work is required to determine the heritability of these measures.

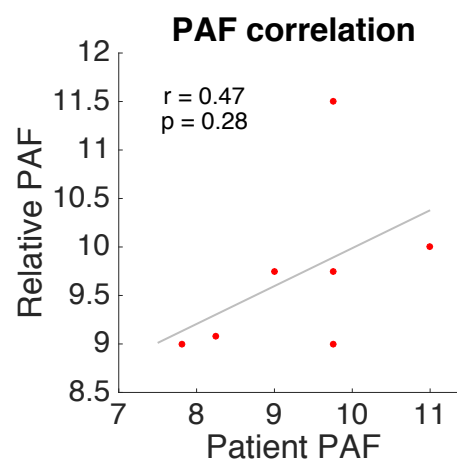


**Figure 10.1** Correlation of PAF values between patient and relative pairs.

### **10.3.2 Association with MTLE**

All the traits investigated as endophenotypes have been reported to be altered in MTLE patients compared to healthy controls from previously published studies, as described within each analysis chapter. In the study presented here, both candidate endophenotypes showed a higher degree of alteration in MTLE patients compared to unaffected relatives and healthy controls. These alterations could represent initial traits present in both unaffected relatives and MTLE patients related to disease predisposition. Further work is required to investigate associations with disease severity (e.g. epilepsy duration and severity or duration of seizures).

### **10.3.3 State independence**

It is important for endophenotypes to be trait- rather than state-dependent as this would mean they are more related to the genetic mechanisms of the disorder than the clinical state of the disorder. As such, endophenotypes should be present in both patients and unaffected relatives who do not exhibit the illness state. The MTLE patients in the present study were a heterogeneous mix of patients in terms of disease onset, medication and seizure frequency and severity. This suggests that the abnormalities found here are present in both chronic and recent-onset patients, are independent of medication and seizure status, and are likely to predate the onset of seizures, representing a trait rather than state dependence.

State independence of the endophenotypes can also be demonstrated if they are present in unaffected relatives of MTLE patients. Relatives in this study are considered “unaffected” if they do not have a diagnosis of epilepsy or have not had previous events compatible with seizures. However, this “normal” state can be difficult to define as evidenced by “EEG affected” but clinically asymptomatic relatives of juvenile myoclonic epilepsy patients showing a high rate of IEDs and EEG abnormalities similar to the patient group (Delgado-Escueta et al., 1989). In this study however, re-assessment of the EEG of relatives by a neurologist (Dr E. Abela) revealed no IEDs or aberrant activity found in the resting EEGs of relatives of MTLE patients in this study. The structural abnormalities found in unaffected relatives in Chapter 6 were also not obvious on

visual re-assessment of the original MR images. These further suggest that the endophenotype findings presented in this thesis state independent.

#### **10.3.4 Presence in unaffected relatives**

Endophenotypes should be present in unaffected relatives of probands at a higher rate than in the general population. They should also co-segregate within families, with individuals who are at higher genetic risk demonstrating a more severely affected trait. The two candidate endophenotypes presented here are both present in unaffected relatives at a higher rate than in healthy controls. The traits in unaffected relatives also appear to be less severely altered, suggesting that these traits do co-segregate within families, with MTLE patients, who are at higher genetic risk, demonstrating more severe alterations in the trait.

### **10.4 Implications of findings on underlying mechanisms of MTLE**

An ongoing debate about alterations in MTLE is whether these changes predate seizure onset or are a result of repeated seizures (Cendes et al., 1993; Thom et al., 2005). The finding of temporal lobe morphology changes in unaffected relatives could lend some insight into this. Since these alterations are present in unaffected and unmedicated relatives, they suggest traits that represent genetic susceptibility and could help disentangle the effects of recurring seizures and medication, as they are alterations that appear to be independent from the occurrence of seizures.

There were also some findings that have not been previously reported in MTLE patients, such as the involvement of the insular and frontal opercular cortex in relation to an altered alpha rhythm in MTLE patients. While I could speculate that this reflects an alteration in the salience network of MTLE patients, or reflects the role of the insula cortex in temporal lobe seizures, as discussed in Chapter 8, further work is required to determine why these patterns are observed and what their implications might be.

Structural hub networks were found to be more strongly inter-connected in MTLE patients compared to healthy controls. This finding represents network reorganisation in MTLE patients in the hubs of the network. Most studies of hubs in MTLE have shown a reorganisation of hub nodes toward hippocampal and limbic regions (Yasuda et al., 2015) and altered connectivity of hub nodes (Liao et al., 2011; Liu et al., 2014). More work is required to determine the influence of hubs on network organisation in MTLE patients and how this may relate to disease mechanisms.

Finally, several trends were found for alterations in whole-brain and hippocampal sub-networks in MTLE patients and unaffected relatives. There have been a number of previous studies demonstrating network alterations in MTLE patients, although effect sizes are not generally reported and the literature has been very mixed due to a range of different methods used to define networks. The lack of significant findings here could be due to methodological and sample differences, or that effect sizes for these differences are generally small. Further work is needed to better characterise the large-scale network differences in MTLE patients and investigate whether these indices may be suitable endophenotypes for MTLE.

## **10.5 Methodological considerations**

### **10.5.1 Study strengths**

The study presented here represents the first multi-modal investigation of both structural and functional imaging and electrophysiological changes in a group of MTLE patients and unaffected relatives of MTLE patients. Imaging investigations were conducted on a 3 Tesla scanner, and EEGs were investigated using a relatively high-density whole-brain coverage of 64 electrodes both with and without simultaneous fMRI. These methods provide a unique opportunity to fully characterise the spectrum of whole-brain structural and functional alterations within the same individuals.

This study is one of the first to investigate functional endophenotypes in unaffected relatives of MTLE patients in EEG and fMRI data. Previous investigations have concentrated on structural endophenotypes as these are the best-established alterations in MTLE patients, but there have also been many studies showing functional alterations in MTLE patients, indicating that these also deserve investigation as endophenotypes for MTLE.

This study also benefits from a relatively large sample size for an imaging study. The sample size of 76 in the last chapter is much larger than the median sample size of imaging studies of 28.5 in 2015 (Poldrack et al., 2017). The sample sizes in studies of MTLE patients range widely from hundreds of patients in structural imaging studies (Schoene-Bake et al., 2014) to under 10 patients in studies employing novel methods (Wirsich et al., 2016). Given the range of modalities investigated, the wide range of analysis methods, and the novel (at the time of study conception) group of unaffected relatives, in the study reported here, sample size calculations were challenging. To ensure the best chance of detecting both structural and functional alterations, sample size was calculated based on previous findings from both structural and functional studies of MTLE patients and the recruitment target was for an equal number of unaffected relatives.

The unaffected relatives recruited to this study were predominantly relatives of patients with MTS. The majority were relatives of patients with confirmed MTS, from clinical MRIs and evaluation of surgically removed tissue. Only 2 were relatives of patients with no MTS. As there has been evidence for genetic variation related to specific traits in MTLE patients, for example hippocampal sclerosis with a history of febrile seizures has been shown to be linked to genetic variation in SCN1A (Kasperavičiute et al., 2013), having sufficiently large samples of patients with a range of specific pathologies and their relatives potentially enables us to identify endophenotypes related to a specific pathology in MTLE (in this case, MTS).

The study presented here could be considered a cohort study, rather than a case control study, and did not include many directly matched pairs of unaffected relatives and MTLE patients. While case control studies are often preferred, in this case, this reduced the chance of shared environmental factors influencing the expression of endophenotypes in both the MTLE patient and unaffected relative groups, improving the chances of detecting endophenotypes with a shared genetic rather than environmental component. In fact, since 38% of the relatives were recruited from post-surgical MTLE patients with confirmed MTS, it was not possible to include the patients themselves in this study. Very few of the patients who were involved in the study had relatives that met the recruitment criteria or were willing to take part, resulting in a total of only 7 matched pairs of patients and relatives in the study.

### **10.5.2 Study limitations**

Although recruitment targets were exceeded in each of the 3 groups, data acquisition was incomplete for several unaffected relatives who were unable to complete the study protocol due to unforeseen circumstances (primarily claustrophobia and MRI contraindications not previously reported during study screening, see Appendix C for further details). Several findings reported here showed trends toward alterations in unaffected relatives, suggesting that the study may have been underpowered to detect changes in this population.

In the study presented here, to increase sample size and power, I side-flipped images so the ipsilateral side is on the left for all patients. This is common practice in the TLE literature when studies have small sample sizes that include both left- and right- onset patients (Coan et al., 2014; Zeng et al., 2013). This was assumed to be valid as it is thought that hippocampal sclerosis is usually a bilateral disease, and the mechanisms of seizure spread are thought to be similar. This is based on previous literature indicating post-mortem evidence of bilateral hippocampal sclerosis in around 31% of epilepsy patients previously thought to have unilateral HS (Margerison & Corsellis, 1966). However, neuroimaging literature has demonstrated mixed evidence for the homogeneity of left- and right-sided MTLE. Some studies have shown similar

alterations ipsilateral to the affected hippocampus in both left- and right-onset cases (Bernasconi et al., 2003, 2004; Bettus et al., 2009; Pittau et al., 2012). Other studies have shown more severe alterations in left MTLE (Ahmadi et al., 2009; Haneef et al., 2014, 2012; Pereira et al., 2010), while others have shown more severe alterations in right MTLE (Chiang et al., 2014; Dupont et al., 2002; Liu, Concha, et al., 2012). There is also some evidence for a difference in the general underlying mechanisms for left and right MTLE (de Campos et al., 2016; Fang et al., 2015). These studies show that it is unclear whether left- and right-onset MTLE can be considered the same disease. Such conflicting evidence may explain why no significant differences were found in the whole-brain network analysis. It would be useful to be able to investigate left- and right-onset MTLE patients separately in a larger sample.

Another sample limitation was the inclusion of patients both with and without MTS. The MTLE patient sample was predominantly lesional and there were only 4 patients with no detectable lesion on MRI, but with other abnormalities of the hippocampus and seizures and EEG strongly indicative of MTL onset. All 4 patients with no MTS did not have significant differences between left and right hippocampi from the volumetric analysis. There is a wide range of evidence for differences between the two patient groups, with lesional patients often showing more widespread and severe alterations (Liu, Concha, et al., 2012; Scanlon, Mueller, et al., 2013). The analysis could be repeated excluding those without MTS to investigate whether the results still hold.

I did not account for the effects of antiepileptic drugs (AEDs) in the patient group, which consisted of patients on a range of mono- and poly-therapies. As AEDs are known to affect EEGs (Clemens et al., 2006), the drug burden could have been calculated to account for this in the patient group. This can be by a previously derived formula in epilepsy patients that accounts for the dose and number of drugs, giving a single value representing the drug burden in patients which can then be used as a covariate in the analyses (Pawley et al., 2017). However, the relative group was not affected by this confound and still showed EEG differences in PAF.

Finally, on hindsight, it would have been prudent to have one run of eyes-open rest in addition to eyes-closed rest in the study fMRI protocol as the mechanisms underlying each condition are still not clear (Patriat et al., 2013) and the protocols differ within studies of resting-state functional connectivity in MTLE. Furthermore, it might have been easier to ensure participants stayed awake throughout the whole recording if they had their eyes open, as a study has found that a third of subjects fall asleep within 3 mins of a resting-state fMRI scan (Tagliazucchi & Laufs, 2014). However, we did have simultaneous EEG-fMRI and could visually identify whether subjects had fallen asleep during the fMRI scans.

## **10.6 Directions for future research**

### **10.6.1 Next steps for the present data**

The study could benefit from an increase in sample size in order to address several limitations outlined above. This would enable us to examine more homogeneous groups of patients, in terms of side of onset and the presence of a lesion. More matching pairs of patients and relatives could be included in the study so that preliminary estimates of trait heritability can be calculated for candidate endophenotypes.

In terms of analysis, the first steps for this dataset would be to investigate other frequency bands in the EEG as well as their structural and functional MRI correlates, as the theta band has also been implicated in MTLE (Bettus et al., 2008), and there has been pre-clinical evidence for alterations in networks of the delta frequency band in mice (Sheybani, 2016). Further work is also required to characterise alterations in other white matter tracts implicated in MTLE, e.g. the uncinate fasciculus and parahippocampal gyrus, and whole-brain automated methods could be used to achieve this. Along tract measures could also be calculated for these white matter tracts as alterations in unaffected relatives could be subtler than in MTLE patients and only confined to specific locations along the tract. Finally, as such a rich multi-modal dataset is available in a unique group of subjects, software such as The Virtual Brain (Jirsa et al., 2010; Ritter et al., 2013) could be applied to build models of brain activity through the integration of multi-modal data.

This could allow us to identify and predict specific network structures or dynamics that could be related to seizure liability and investigate whether these could be potential endophenotypes.

Collaborations with large imaging consortiums such as the ENIGMA consortium for epilepsy could further translate the present work into a much larger sample to confirm these findings and identify possible genetic contributions to the imaging and EEG endophenotypes in a larger group of unaffected relatives through genome-wide association studies.

### **10.6.2 Next steps for new data**

Further work on identifying endophenotypes could focus on particular contributors to MTLE risk, for example a previous history of febrile seizures, and also look into other non-imaging based endophenotypes. Neuropsychological measures have not been explored in MTLE, but may represent a less expensive approach to discovering endophenotypes that have been proven to be successful in investigations of other disorders (Gottesman and Gould, 2003; Glahn et al., 2004; Chowdhury et al., 2014). Executive function deficits have been demonstrated in patients with MTLE, and while there is some evidence that it may be a result of recurrent seizures and AEDs, this is still unclear (Bell et al., 2011; Zamarian et al., 2011). There is also evidence for a shared genetic susceptibility to migraine and epilepsy (Haan et al., 2008), in particular in familial MTLE (Gambardella et al., 2000). Further work on endophenotypes could investigate these in unaffected relatives of MTLE patients.

## **10.7 Conclusion**

In the thesis presented here, I have shown evidence for the presence of quantitative structural and functional traits in unaffected relatives of MTLE patients that may represent suitable endophenotypes for MTLE. The work presented here is part of a new field of research into neuroimaging and electrophysiological endophenotypes to identify risk factors, which holds promise for the further understanding of the etiology and pathogenesis of a genetically complex disorder such as MTLE.

## Appendices

## Appendix A. Study consent form

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### CONSENT FORM

(v2, 4.03.14)

**Title of project:** Brain Networks in Focal Epilepsy

**Name of Principal investigator:** Professor Mark Richardson

*Please initial box*

1. I confirm that I have read and understood the information sheet dated 15 October 2015 (version 3) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from King's College London or from regulatory authorities, or NHS Trust where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree that any relevant clinical information from my MRI and/or EEG tests can be used for future related research, which has been approved by a recognised Research Ethics Committee ☐
5. I agree to be contacted in the future by the BRC Bioresource team requesting my participation in similar studies or other related medical research studies. In some instances we may contact you for follow-up studies on the basis of your genetic/other biological information. ☐
6. I agree to take part in the above study. ☐

\_\_\_\_\_  
Name of Participant

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Name of Person taking consent

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

## Appendix B. Participant characteristics and clinical data

**Appendix Table B1:** MTLE patients – demographics and clinical characteristics

| No. | Participant ID | Age | Sex | MTLE laterality | Age of onset | MTS on MRI? | Other localisation features?                                                                                                                       | Seizure frequency (per month) <sup>1</sup> | Medication (daily dose in mg) <sup>2</sup> | History of FS? |
|-----|----------------|-----|-----|-----------------|--------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|--------------------------------------------|----------------|
| 1   | TLE/P/002      | 41  | F   | Right           | 24           | Yes         | Not documented                                                                                                                                     | 9-10                                       | LMT (550); LEV (1500); PER (4); CLB (60)   | *              |
| 2   | TLE/P/003      | 35  | M   | Left            | 15           | Yes         | *                                                                                                                                                  | 2                                          | LMT (400); VPA (900); CLB (10)             | *              |
| 3   | TLE/P/004      | 43  | F   | Left            | 20           | Yes         | Bitemporal onset on intra-cranial EEG.                                                                                                             | 3-7                                        | CAR (1400); LMT (200)                      | No             |
| 4   | TLE/P/005      | 57  | F   | Left            | 5            | Yes         | Left temporal discharges on EEG.                                                                                                                   | 6-7                                        | LEV (875); CIT (30)                        | No             |
| 5   | TLE/P/010      | 22  | M   | Left            | 16           | Yes         | Not documented                                                                                                                                     | 20-24                                      | CAR (1200)                                 | No             |
| 6   | TLE/P/011      | 34  | M   | Right           | 11           | Yes         | Right anterior temporal spike & wave epileptiform discharges on EEG.                                                                               | 2-3                                        | PHB (60); VPA(1200); OLA (7.5); CIT (*)    | No             |
| 7   | TLE/P/014      | 52  | F   | Left            | 15           | No          | 1) Loss of digitation in the left hippocampal head on MRI.<br>2) Left temporal hypo-metabolism on FDG PET.<br>3) Left temporal sharp waves on EEG. | 3-5                                        | LAC (150); CIT (50); LOR (2)               | No             |
| 8   | TLE/P/016      | 46  | M   | Right           | *            | Yes         | Focal area of established damage within the right frontal lobe on MRI.                                                                             | Unclear, cluster seizures                  | LEV (1600); PHE (350)                      | Yes            |
| 9   | TLE/P/021      | 51  | F   | Left            | 31           | Yes         | Not documented                                                                                                                                     | Well controlled                            | LAC (400)                                  | *              |
| 10  | TLE/P/022      | 31  | M   | Right           | 25           | Yes         | Not documented                                                                                                                                     | 16-20                                      | CAR (800); LEV (200); CLB (10)             | *              |

| No. | Participant ID | Age | Sex | MTLE laterality | Age of onset | MTS on MRI? | Other localisation features?                                                                                                                                                                                                                                                        | Seizure frequency (per month) <sup>1</sup> | Medication (daily dose in mg) <sup>2</sup> | History of FS? |
|-----|----------------|-----|-----|-----------------|--------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|--------------------------------------------|----------------|
| 11  | TLE/P/027      | 48  | M   | Right           | 33           | Yes         | Not documented                                                                                                                                                                                                                                                                      | Well controlled                            | LEV (3000); TOP (100)                      | Yes            |
| 12  | TLE/P/030      | 31  | M   | Right           | 21           | Yes         | 1) Area of gliosis at medial temporal pole on MRI.<br>2) Clear right temporal focus on EEG.<br>3) Right temporal hypo-metabolism on FDG PET.                                                                                                                                        | 3-4                                        | LEV (3000); ZON (200); CLN (2)             | No             |
| 13  | TLE/P/033      | 58  | F   | Left            | late         | Yes         | Not documented                                                                                                                                                                                                                                                                      | 3-4                                        | TOP (200); CLB (10)                        | *              |
| 14  | TLE/P/036      | 47  | M   | Right           | 40           | Yes         | Not documented                                                                                                                                                                                                                                                                      | Well controlled                            | LEV (400)                                  | *              |
| 15  | TLE/P/DVH1     | 49  | F   | Right           | *            | Yes         | EEG predominantly right-sided discharges                                                                                                                                                                                                                                            | *                                          | LMT (225); LAC (100); CLB (20)             | *              |
| 16  | TLE/P/041      | 24  | M   | Right           | 22           | No          | 1) Ectopic grey matter related to temporal horn of right lateral ventricle immediately lateral to body of right hippocampus on MRI.<br>2) EEG showed susceptibility to focal temporal lobe seizures with bursts of sharp transients over right temporal lobe during over breathing. | 3-4                                        | CAR (400)                                  | Uncertain      |
| 17  | TLE/P/029      | 25  | M   | Left            | 23           | No          | 1) EEG suggestive of left TLE.<br>2) Seizure semiology is predominantly nocturnal SGTCS with head turning to the right.                                                                                                                                                             | 3-4                                        | VPA (800); TOP (300)                       | No             |
| 18  | TLE/P/051      | 43  | F   | Left            | 40           | Yes         | Not documented                                                                                                                                                                                                                                                                      | 4                                          | CAR (800)                                  | No             |
| 19  | TLE/P/053      | 23  | M   | Left            | 22           | Yes         | Not documented                                                                                                                                                                                                                                                                      | Well controlled                            | ZON (200)                                  | Yes            |

| No. | Participant ID | Age | Sex | MTLE laterality | Age of onset | MTS on MRI? | Other localisation features?                                                                                                                                                           | Seizure frequency (per month) <sup>1</sup> | Medication (daily dose in mg) <sup>2</sup> | History of FS? |
|-----|----------------|-----|-----|-----------------|--------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|--------------------------------------------|----------------|
| 20  | TLE/P/043      | 47  | M   | Left            | 15           | No          | 1) Left temporal lobe epileptogenic focus on EEG.<br>2) Seizure semiology: brief & frequent episodes of flickering left eyes, tonic contraction of right hand and difficulty speaking. | 1-2                                        | CAR (600)                                  | No             |
| 21  | TLE/R/DVH2     | 24  | F   | Left            | *            | Yes         | *                                                                                                                                                                                      | *                                          | *                                          | *              |
| 22  | TLE/P/064      | 57  | M   | Right           | 20-30        | Yes         | Not documented                                                                                                                                                                         | Well controlled                            | LMT (300)                                  | *              |
| 23  | TLE/P/068      | 37  | F   | Left            | 27           | Yes         | Not documented                                                                                                                                                                         | 12                                         | CAR (400)                                  | Yes            |
| 24  | TLE/P/069      | 31  | F   | Left            | 22           | Yes         | Not documented                                                                                                                                                                         | 3                                          | LEV (3000)                                 | No             |
| 25  | TLE/P/071      | 44  | F   | Left            | 1            | Yes         | Seizure semiology is predominantly complex partial with stiffening of arms, dysphasia and incontinence.                                                                                | 4                                          | CAR (1200), CLB (10)                       | Uncertain      |
| 26  | TLE/P/077      | 22  | F   | Left            | 18           | Yes         | Seizure semiology: posturing of right hand                                                                                                                                             | 1-2                                        | Not started yet                            | No             |
| 27  | TLE/P/085      | 52  | M   | Right           | 20-30        | Yes         | Not documented                                                                                                                                                                         | 15                                         | LMT (750); PER (8)                         | *              |

\* indicates missing information

<sup>1</sup> In this cohort, well controlled = less than 2 per year.

<sup>2</sup> Drug abbreviations: CAR = Carbamazepine, CIT = Citalopram, CLB = Clobazam, CLN = Clonazepam, LAC = Lacosamide, LEV = Levetiracetam, LMT = Lamotrigine, LOR = Lorazepam, OLA = Olanzapine, PER = Perampanel, PHB = Phenobarbitone, PHE = Phenytoin, TOP = Topiramate, VPA = Sodium Valproate, ZON = Zonisamide.

**Appendix Table B2: Unaffected relatives – demographics and characteristics**

| No. | Participant ID | Age | Sex | Relative of patient in this study? | Relationship to patient with MTLE | MTLE laterality of related patient | Does related patient have MTS on MRI? |
|-----|----------------|-----|-----|------------------------------------|-----------------------------------|------------------------------------|---------------------------------------|
| 1   | TLE/R/007      | 49  | M   | No                                 | Son                               | Left                               | Yes                                   |
| 2   | TLE/R/012      | 24  | F   | Yes                                | Daughter                          | Left                               | No                                    |
| 3   | TLE/R/015      | 34  | M   | Yes                                | Twin brother                      | Right                              | Yes                                   |
| 4   | TLE/R/020      | 37  | F   | Yes                                | Daughter                          | Left                               | Yes                                   |
| 5   | TLE/R/023      | 49  | M   | No                                 | Brother                           | Left                               | Yes                                   |
| 6   | TLE/R/024      | 47  | M   | No                                 | Son                               | Left                               | Yes                                   |
| 7   | TLE/R/031      | 60  | F   | Yes                                | Mother                            | Right                              | Yes                                   |
| 8   | TLE/R/045      | 31  | F   | No                                 | Daughter                          | Right                              | Yes                                   |
| 9   | TLE/R/048      | 17  | M   | No                                 | Son                               | Left                               | Yes                                   |
| 10  | TLE/R/042      | 25  | M   | No                                 | Brother                           | Right                              | Yes                                   |
| 11  | TLE/R/028      | 30  | M   | No                                 | Son                               | Right                              | Yes                                   |
| 12  | TLE/R/050      | 44  | M   | No                                 | Brother                           | Right                              | Yes                                   |
| 13  | TLE/R/046      | 44  | F   | No                                 | Daughter                          | Right                              | Yes                                   |

| No. | Participant ID | Age | Sex | Relative of patient in this study? | Relationship to patient with MTLE | MTLE laterality of related patient | Does related patient have MTS on MRI? |
|-----|----------------|-----|-----|------------------------------------|-----------------------------------|------------------------------------|---------------------------------------|
| 14  | TLE/R/058      | 25  | M   | Yes                                | Brother                           | Left                               | Yes                                   |
| 15  | TLE/R/DVH3     | 54  | F   | Yes                                | Mother                            | Left                               | Yes                                   |
| 16  | TLE/R/063      | 24  | F   | No                                 | Daughter                          | Right                              | Yes                                   |
| 17  | TLE/R/052      | 18  | F   | Yes                                | Sister                            | Left                               | No                                    |
| 18  | TLE/R/066      | 40  | F   | No                                 | Sister                            | Bilateral                          | Yes                                   |
| 19  | TLE/R/067      | 40  | F   | No                                 | Sister                            | Bilateral                          | Yes                                   |
| 20  | TLE/R/080      | 20  | F   | No                                 | Daughter                          | Left                               | Yes                                   |
| 21  | TLE/R/073      | 60  | F   | No                                 | Mother                            | Left                               | Yes                                   |
| 22  | TLE/R/074      | 28  | M   | No                                 | Brother                           | Left                               | Yes                                   |
| 23  | TLE/R/075      | 57  | F   | No                                 | Sister                            | Left                               | Yes                                   |
| 24  | TLE/R/076      | 29  | F   | No                                 | Daughter                          | Right                              | Yes                                   |
| 25  | TLE/R/079      | 31  | F   | No                                 | Sister                            | Right                              | Yes                                   |
| 26  | TLE/R/084      | 17  | M   | No                                 | Son                               | Right                              | Yes                                   |

\* indicates missing information

**Appendix Table B3: Healthy controls – participant demographics**

| No. | Participant ID | Age | Sex |
|-----|----------------|-----|-----|
| 1   | TLE/C/001      | 61  | F   |
| 2   | TLE/C/006      | 41  | M   |
| 3   | TLE/C/008      | 45  | M   |
| 4   | TLE/C/009      | 21  | F   |
| 5   | TLE/C/013      | 32  | M   |
| 6   | TLE/C/017      | 32  | F   |
| 7   | TLE/C/018      | 42  | M   |
| 8   | TLE/C/019      | 29  | F   |
| 9   | TLE/C/025      | 30  | F   |
| 10  | TLE/C/026      | 34  | M   |
| 11  | TLE/C/032      | 31  | M   |
| 12  | TLE/C/035      | 34  | F   |
| 13  | TLE/C/037      | 34  | M   |

| No. | Participant ID | Age | Sex |
|-----|----------------|-----|-----|
| 14  | TLE/C/038      | 54  | F   |
| 15  | TLE/C/034      | 35  | M   |
| 16  | TLE/C/039      | 50  | M   |
| 17  | TLE/C/047      | 38  | F   |
| 18  | TLE/C/040      | 52  | M   |
| 19  | TLE/C/049      | 31  | F   |
| 20  | TLE/C/055      | 28  | F   |
| 21  | TLE/C/056      | 21  | F   |
| 22  | TLE/C/057      | 34  | M   |
| 23  | TLE/C/059      | 44  | F   |
| 24  | TLE/C/054      | 44  | M   |
| 25  | TLE/C/061      | 21  | F   |
| 26  | TLE/C/060      | 49  | M   |

| No. | Participant ID | Age | Sex |
|-----|----------------|-----|-----|
| 27  | TLE/C/065      | 22  | F   |
| 28  | TLE/C/062      | 55  | M   |
| 29  | TLE/C/072      | 36  | F   |
| 30  | TLE/C/080      | 21  | F   |
| 31  | TLE/C/081      | 32  | M   |
| 32  | TLE/C/082      | 30  | M   |
| 33  | TLE/C/086      | 35  | F   |

## Appendix C. List of complete study procedures for each participant

**Appendix Table C1:** List of completed study procedures for each participant and reasons for incomplete data or exclusions, and whether imaging data were left-right flipped

| No. | Participant ID | EEG<br>(outside<br>scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI | Reason for incomplete data/exclusion | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|-----------------------------|------|-------------------|-------------------|-----|--------------------------------------|----------------------------|-----------------------------|
| 1   | TLE/P/002      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 123                        | Yes                         |
| 2   | TLE/C/001      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 117                        | Yes                         |
| 3   | TLE/C/006      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 119                        | No                          |
| 4   | TLE/P/003      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 118                        | No                          |
| 5   | TLE/P/004      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 96                         | No                          |
| 6   | TLE/P/005      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 98                         | No                          |
| 7   | TLE/C/008      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 112                        | No                          |
| 8   | TLE/R/007      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 114                        | No                          |
| 9   | TLE/C/009      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 108                        | Yes                         |
| 10  | TLE/P/010      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 98                         | No                          |
| 11  | TLE/P/011      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                    | 97                         | Yes                         |

| No. | Participant ID | EEG<br>(outside scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI | Reason for incomplete data/exclusion                                                                              | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|--------------------------|------|-------------------|-------------------|-----|-------------------------------------------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 12  | TLE/R/012      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 102                        | No                          |
| 13  | TLE/C/013      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 123                        | No                          |
| 14  | TLE/P/014      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | *                          | No                          |
| 15  | TLE/R/015      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 100                        | Yes                         |
| 16  | TLE/P/016      | Yes                      | No   | No                | No                | No  | MRI contraindication: metal shrapnel injury in eye.                                                               | *                          | n.a.                        |
| 17  | TLE/C/017      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 112                        | No                          |
| 18  | TLE/C/018      | Yes                      | No   | No                | No                | No  | MRI contraindication: weight constraints.                                                                         | 120                        | n.a.                        |
| 19  | TLE/C/019      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 122                        | Yes                         |
| 20  | TLE/R/020      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 108                        | No                          |
| 21  | TLE/P/021      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | 112                        | No                          |
| 22  | TLE/P/022      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                                                                 | *                          | Yes                         |
| 23  | TLE/R/023      | Yes                      | No   | No                | No                | No  | MRI contraindication: surgical notes unavailable for previous hernia surgery; unable to ensure participant safety | 123                        | n.a.                        |
| 24  | TLE/R/024      | Yes                      | No   | No                | No                | No  | MRI contraindication: claustrophobia.                                                                             | 115                        | n.a.                        |

| No. | Participant ID | EEG<br>(outside<br>scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI                | Reason for incomplete data/exclusion                                                                                                           | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|-----------------------------|------|-------------------|-------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 25  | TLE/C/025      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | No                          |
| 26  | TLE/C/026      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | No                          |
| 27  | TLE/P/027      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | 112                        | Yes                         |
| 28  | TLE/C/032      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | Yes                         |
| 29  | TLE/R/031      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | Yes                         |
| 30  | TLE/P/030      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | Yes                         |
| 31  | TLE/P/033      | Yes                         | Yes  | Yes               | No                | Yes (no<br>top-up) | EEG-fMRI Run 2 aborted as participant was<br>uncomfortable and unable to continue with scan.<br>No top-up for DTI scan due to patient comfort. | *                          | No                          |
| 32  | TLE/C/035      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | No                          |
| 33  | TLE/P/036      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | Yes                         |
| 34  | TLE/C/037      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | *                          | No                          |
| 35  | TLE/C/038      | Yes                         | Yes  | No                | No                | No                 | MRI contraindication: claustrophobia;<br>participant only completed sMRI scan.                                                                 | *                          | Yes                         |
| 36  | TLE/C/034      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | 117                        | No                          |
| 37  | TLE/C/039      | Yes                         | Yes  | Yes               | Yes               | Yes                | -                                                                                                                                              | 111                        | No                          |

| No. | Participant ID | EEG<br>(outside<br>scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI | Reason for incomplete data/exclusion                                           | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|-----------------------------|------|-------------------|-------------------|-----|--------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 38  | TLE/C/047      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | Yes                         |
| 39  | TLE/P/DVH1     | Yes                         | Yes  | Yes               | Yes               | Yes | Excluded due to abnormal amount of atrophy.                                    | 112                        | n.a.                        |
| 40  | TLE/P/041      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 103                        | Yes                         |
| 41  | TLE/P/029      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 95                         | No                          |
| 42  | TLE/R/045      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 114                        | Yes                         |
| 43  | TLE/R/048      | Yes                         | Yes  | No EEG            | Yes               | Yes | EEG-fMRI Run 1: EEG equipment malfunction,<br>only fMRI acquired for this run. | 114                        | No                          |
| 44  | TLE/R/042      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 116                        | Yes                         |
| 45  | TLE/C/040      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 116                        | No                          |
| 46  | TLE/R/028      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 107                        | Yes                         |
| 47  | TLE/C/049      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 107                        | No                          |
| 48  | TLE/C/055      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 117                        | Yes                         |
| 49  | TLE/R/050      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 120                        | Yes                         |
| 50  | TLE/P/051      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | 104                        | No                          |

| No. | Participant ID | EEG<br>(outside scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI | Reason for incomplete data/exclusion                                           | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|--------------------------|------|-------------------|-------------------|-----|--------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 51  | TLE/C/056      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 112                        | No                          |
| 52  | TLE/C/057      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 112                        | No                          |
| 53  | TLE/C/059      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 119                        | Yes                         |
| 54  | TLE/C/054      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 123                        | No                          |
| 55  | TLE/R/046      | Yes                      | No   | No                | No                | No  | MRI contraindication: weight constraints.                                      | *                          | n.a.                        |
| 56  | TLE/P/053      | Yes                      | Yes  | No EEG            | Yes               | Yes | EEG-fMRI Run 1: EEG equipment malfunction,<br>only fMRI acquired for this run. | 119                        | No                          |
| 57  | TLE/P/043      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 124                        | No                          |
| 58  | TLE/R/058      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 116                        | No                          |
| 59  | TLE/C/061      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 123                        | No                          |
| 60  | TLE/C/060      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 120                        | Yes                         |
| 61  | TLE/R/DVH3     | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 119                        | No                          |
| 62  | TLE/R/DVH2     | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | No                          |
| 63  | TLE/R/063      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                              | 100                        | Yes                         |

| No. | Participant ID | EEG<br>(outside scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI | Reason for incomplete data/exclusion                                         | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|--------------------------|------|-------------------|-------------------|-----|------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 64  | TLE/P/064      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 102                        | Yes                         |
| 65  | TLE/C/065      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 115                        | No                          |
| 66  | TLE/C/062      | Yes                      | Yes  | No                | No                | No  | MRI contraindication: Metal injury to face that was not previously declared. | 106                        | No                          |
| 67  | TLE/C/072      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | *                          | Yes                         |
| 68  | TLE/R/052      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 90                         | No                          |
| 69  | TLE/P/068      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 107                        | No                          |
| 70  | TLE/P/069      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 115                        | No                          |
| 71  | TLE/R/066      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 111                        | No                          |
| 72  | TLE/R/067      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | 108                        | No                          |
| 73  | TLE/R/080      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | *                          | No                          |
| 74  | TLE/R/073      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | *                          | No                          |
| 75  | TLE/P/071      | Yes                      | Yes  | Yes               | Yes               | Yes | Excluded from MRI analyses due to unexplained T2 signal dropout.             | *                          | No                          |
| 76  | TLE/R/074      | Yes                      | Yes  | Yes               | Yes               | Yes | -                                                                            | *                          | No                          |

| No. | Participant ID | EEG<br>(outside<br>scanner) | sMRI | EEG-fMRI<br>Run 1 | EEG-fMRI<br>Run 2 | DTI | Reason for incomplete data/exclusion                                           | NART<br>score <sup>1</sup> | Imaging<br>data<br>flipped? |
|-----|----------------|-----------------------------|------|-------------------|-------------------|-----|--------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 77  | TLE/R/075      | Yes                         | No   | No                | No                | No  | MRI contraindication: claustrophobia.                                          | *                          | n.a.                        |
| 78  | TLE/C/080      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | No                          |
| 79  | TLE/C/081      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | No                          |
| 80  | TLE/C/082      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | Yes                         |
| 81  | TLE/P/077      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | No                          |
| 82  | TLE/R/076      | Yes                         | Yes  | No EEG            | Yes               | Yes | EEG-fMRI Run 1: EEG equipment malfunction,<br>only fMRI acquired for this run. | *                          | Yes                         |
| 83  | TLE/R/079      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | Yes                         |
| 84  | TLE/P/085      | Yes                         | Yes  | Yes               | Yes               | Yes | -                                                                              | *                          | Yes                         |
| 85  | TLE/R/084      | No                          | Yes  | No EEG            | No EEG            | Yes | Participant refused EEG.                                                       | *                          | Yes                         |
| 86  | TLE/C/086      | Yes                         | Yes  | No EEG            | Yes               | Yes | EEG-fMRI Run 1: EEG equipment malfunction,<br>only fMRI acquired for this run. | *                          | No                          |

<sup>1</sup> NART score is shown as the average of the three predicted IQ component scores (NART Full IQ, NART Verbal IQ and NART Performance IQ).

## Appendix D. Mathematical formulae for graph network measures

Network measures in this thesis were computed based on weighted graphs. All the formulae in the following table are based on a network  $G = (X, E)$  with  $X = \{1, \dots, n\}$  nodes and  $E = \{l_1, \dots, l_m\}$  edges. Networks are represented by the adjacency matrix  $A(G) = (a_{ij})$ , where  $a_{ij}$  represents the weighted edge between nodes  $i$  and  $j$  of the matrix.

**Appendix Table D1:** Mathematical formulae for graph network measures. Formulae adapted from (Newman, 2003).

| Network measure                                   | Mathematical formula                                                                                                                                                                                                                                                                                                            |
|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Node degree/<br>node degree strength<br>( $k_i$ ) | $k_i = \sum_{j=1}^n a_{ij}$                                                                                                                                                                                                                                                                                                     |
| Connection density<br>( $\Delta$ )                | $\Delta = \frac{2m}{n(n-1)}$                                                                                                                                                                                                                                                                                                    |
| Clustering coefficient<br>( $C_i$ )               | $C_i = \frac{2t_i}{k_i(k_i-1)}$ <p><math>t_i</math> is the number of triangles around node <math>i</math></p>                                                                                                                                                                                                                   |
| Characteristic path length<br>( $L$ )             | $L(G) = \frac{1}{n(n-1)} \sum_{j \neq k \in X} d_{jk}$                                                                                                                                                                                                                                                                          |
| Global efficiency<br>( $E$ )                      | $E(G) = \frac{1}{n(n-1)} \sum_{j \neq k \in X} \frac{1}{d_{jk}}$                                                                                                                                                                                                                                                                |
| Local efficiency<br>( $E_{loc}$ )                 | $E_{loc}(G) = \frac{1}{n} \sum_{i=1}^n E(G_i)$ <p><math>E(G_i)</math> is the efficiency of the sub-network <math>G_i</math> formed by the neighbours of node <math>i</math></p>                                                                                                                                                 |
| Betweenness centrality<br>( $b$ )                 | $b_i = \frac{1}{n(n-1)} \sum_{k, j \in X, i \neq j} \frac{n_{kj}(i)}{n_{kj}}$ <p><math>n_{kj}</math> is the number of different geodesics that join <math>k</math> and <math>j</math>,<br/>and <math>n_{kj}(i)</math> is the number of geodesics that join <math>k</math> and <math>j</math> passing through <math>i</math></p> |
| Eigenvector centrality<br>( $v$ )                 | $v_i \propto \sum_{j=1}^n a_{ji} v_j$                                                                                                                                                                                                                                                                                           |
| Small-world index<br>( $\sigma$ )                 | $\sigma = \frac{\gamma}{\lambda}; \text{ where } \gamma = \frac{C(G)}{C(G_{rand})} \text{ and } \lambda = \frac{P(G)}{P(G_{rand})}$ <p><math>G_{rand}</math> is a random network with the same node degree and degree distribution as <math>G</math></p>                                                                        |

## Appendix E. Atlas regions and centres of gravity

**Appendix Table E1:** Anatomical labels and centres of gravity for each parcellation region from the AAL90 atlas (Tzourio-Mazoyer et al., 2002).

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Label                |
|---------|------------|------------|------------|----------------------|
| 1       | -40        | -6         | 51         | Precentral L         |
| 2       | -19        | 35         | 42         | Frontal Sup L        |
| 3       | -18        | 47         | -13        | Frontal Sup Orb L    |
| 4       | -34        | 33         | 35         | Frontal Mid L        |
| 5       | -32        | 50         | -10        | Frontal Mid Orb L    |
| 6       | -49        | 13         | 19         | Frontal Inf Oper L   |
| 7       | -47        | 30         | 14         | Frontal Inf Tri L    |
| 8       | -37        | 31         | -12        | Frontal Inf Orb L    |
| 9       | -48        | -8         | 14         | Rolandic Oper L      |
| 10      | -6         | 5          | 61         | Supp Motor Area L    |
| 11      | -9         | 15         | -12        | Olfactory L          |
| 12      | -6         | 49         | 31         | Frontal Sup Medial L |
| 13      | -6         | 54         | -7         | Frontal Med Orb L    |
| 14      | -6         | 37         | -18        | Rectus L             |
| 15      | -36        | 7          | 3          | Insula L             |
| 16      | -5         | 35         | 14         | Cingulum Ant L       |
| 17      | -6         | -15        | 42         | Cingulum Mid L       |
| 18      | -6         | -43        | 25         | Cingulum Post L      |
| 19      | -26        | -21        | -10        | Hippocampus L        |
| 20      | -22        | -16        | -21        | ParaHippocampal L    |
| 21      | -24        | -1         | -17        | Amygdala L           |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Label                |
|---------|------------|------------|------------|----------------------|
| 22      | -8         | -79        | 6          | Calcarine L          |
| 23      | -7         | -80        | 27         | Cuneus L             |
| 24      | -16        | -68        | -5         | Lingual L            |
| 25      | -18        | -84        | 28         | Occipital Sup L      |
| 26      | -33        | -81        | 16         | Occipital Mid L      |
| 27      | -37        | -78        | -8         | Occipital Inf L      |
| 28      | -32        | -40        | -20        | Fusiform L           |
| 29      | -43        | -23        | 49         | Postcentral L        |
| 30      | -24        | -60        | 59         | Parietal Sup L       |
| 31      | -44        | -46        | 47         | Parietal Inf L       |
| 32      | -57        | -34        | 30         | SupraMarginal L      |
| 33      | -45        | -61        | 36         | Angular L            |
| 34      | -8         | -56        | 48         | Precuneus L          |
| 35      | -9         | -25        | 70         | Paracentral Lobule L |
| 36      | -43        | -19        | 10         | Heschl L             |
| 37      | -54        | -21        | 7          | Temporal Sup L       |
| 38      | -41        | 15         | -20        | Temporal Pole Sup L  |
| 39      | -57        | -34        | -2         | Temporal Mid L       |
| 40      | -37        | 15         | -34        | Temporal Pole Mid L  |
| 41      | -51        | -28        | -23        | Temporal Inf L       |
| 42      | -12        | 11         | 9          | Caudate L            |
| 43      | -25        | 4          | 2          | Putamen L            |
| 44      | -19        | 0          | 0          | Pallidum L           |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Label                |
|---------|------------|------------|------------|----------------------|
| 45      | -12        | -18        | 8          | Thalamus L           |
| 46      | 14         | 12         | 9          | Caudate R            |
| 47      | 27         | 5          | 2          | Putamen R            |
| 48      | 20         | 0          | 0          | Pallidum R           |
| 49      | 12         | -18        | 8          | Thalamus R           |
| 50      | 40         | -8         | 52         | Precentral R         |
| 51      | 20         | 31         | 44         | Frontal Sup R        |
| 52      | 17         | 48         | -14        | Frontal Sup Orb R    |
| 53      | 37         | 33         | 34         | Frontal Mid R        |
| 54      | 32         | 53         | -11        | Frontal Mid Orb R    |
| 55      | 49         | 15         | 21         | Frontal Inf Oper R   |
| 56      | 49         | 30         | 14         | Frontal Inf Tri R    |
| 57      | 40         | 32         | -12        | Frontal Inf Orb R    |
| 58      | 52         | -6         | 15         | Rolandic Oper R      |
| 59      | 8          | 0          | 62         | Supp Motor Area R    |
| 60      | 8          | 16         | -11        | Olfactory R          |
| 61      | 8          | 51         | 30         | Frontal Sup Medial R |
| 62      | 7          | 52         | -7         | Frontal Med Orb R    |
| 63      | 7          | 36         | -18        | Rectus R             |
| 64      | 38         | 6          | 2          | Insula R             |
| 65      | 7          | 37         | 16         | Cingulum Ant R       |
| 66      | 7          | -9         | 40         | Cingulum Mid R       |
| 67      | 6          | -42        | 22         | Cingulum Post R      |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Label                |
|---------|------------|------------|------------|----------------------|
| 68      | 28         | -20        | -10        | Hippocampus R        |
| 69      | 24         | -15        | -20        | ParaHippocampal R    |
| 70      | 26         | 1          | -18        | Amygdala R           |
| 71      | 15         | -73        | 9          | Calcarine R          |
| 72      | 13         | -79        | 28         | Cuneus R             |
| 73      | 15         | -67        | -4         | Lingual R            |
| 74      | 23         | -81        | 31         | Occipital Sup R      |
| 75      | 36         | -80        | 19         | Occipital Mid R      |
| 76      | 37         | -82        | -8         | Occipital Inf R      |
| 77      | 33         | -39        | -20        | Fusiform R           |
| 78      | 40         | -25        | 53         | Postcentral R        |
| 79      | 25         | -59        | 62         | Parietal Sup R       |
| 80      | 45         | -46        | 50         | Parietal Inf R       |
| 81      | 57         | -32        | 34         | SupraMarginal R      |
| 82      | 45         | -60        | 39         | Angular R            |
| 83      | 9          | -56        | 44         | Precuneus R          |
| 84      | 6          | -32        | 68         | Paracentral Lobule R |
| 85      | 45         | -17        | 10         | Heschl R             |
| 86      | 57         | -22        | 7          | Temporal Sup R       |
| 87      | 47         | 15         | -17        | Temporal Pole Sup R  |
| 88      | 56         | -37        | -1         | Temporal Mid R       |
| 89      | 43         | 15         | -32        | Temporal Pole Mid R  |
| 90      | 53         | -31        | -22        | Temporal Inf R       |

**Appendix Table E2: Anatomical labels and centres of gravity for each parcellation region of the AAL512 atlas (Zalesky et al., 2010; courtesy of Wirsich et al., 2016)**

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe            |
|---------|------------|------------|------------|------------------------|-----------------|
| 1       | -20        | -85        | 32         | Occipital Sup L.1      | Left Occipital  |
| 2       | -48        | -16        | 45         | Postcentral L.1        | Left Frontal    |
| 3       | -35        | -33        | 61         | Postcentral L.2        | Left Parietal   |
| 4       | -57        | -13        | 23         | Postcentral L.3        | Left Frontal    |
| 5       | -17        | 36         | 26         | Frontal Sup Medial L.1 | Left PreFrontal |
| 6       | -10        | -61        | 12         | Calcarine L.1          | Left Limbic     |
| 7       | -39        | -32        | 48         | Postcentral L.4        | Left Parietal   |
| 8       | -40        | 7          | 22         | Frontal Inf Oper L.1   | Left PreFrontal |
| 9       | -61        | -32        | -9         | Temporal Mid L.1       | Left Temporal   |
| 10      | -53        | 1          | 34         | Precentral L.1         | Left Frontal    |
| 11      | -56        | -24        | 27         | SupraMarginal L.1      | Left Parietal   |
| 12      | -49        | 9          | 13         | Frontal Inf Oper L.2   | Left PreFrontal |
| 13      | -8         | -81        | 9          | Calcarine L.2          | Left Occipital  |
| 14      | -29        | 53         | -8         | Frontal Mid Orb L.1    | Left PreFrontal |
| 15      | -50        | -56        | 27         | Angular L.1            | Left Parietal   |
| 16      | -47        | -31        | 7          | Temporal Sup L.1       | Left Temporal   |
| 17      | -27        | -88        | -13        | Occipital Inf L.1      | Left Occipital  |
| 18      | -55        | -9         | -10        | Temporal Mid L.2       | Left Temporal   |
| 19      | -37        | -80        | -5         | Occipital Mid L.1      | Left Occipital  |
| 20      | -21        | -64        | 52         | Parietal Sup L.1       | Left Parietal   |
| 21      | -49        | 16         | -18        | Temporal Pole Sup L.1  | Left Temporal   |
| 22      | -57        | 5          | 19         | Precentral L.2         | Left PreFrontal |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe            |
|---------|------------|------------|------------|------------------------|-----------------|
| 23      | -33        | 43         | 29         | Frontal Mid L.1        | Left PreFrontal |
| 24      | -45        | 18         | -2         | Frontal Inf Tri L.1    | Left PreFrontal |
| 25      | -36        | -79        | 16         | Occipital Mid L.2      | Left Temporal   |
| 26      | -21        | -8         | 0          | Putamen L.1            | Left Sub-Cortex |
| 27      | -34        | -74        | 26         | Occipital Mid L.3      | Left Parietal   |
| 28      | -17        | 35         | 47         | Frontal Sup L.1        | Left PreFrontal |
| 29      | -11        | -26        | 35         | Cingulum Mid L.1       | Left Limbic     |
| 30      | -10        | -74        | 19         | Cuneus L.1             | Left Occipital  |
| 31      | -17        | 24         | 53         | Frontal Sup L.2        | Left PreFrontal |
| 32      | -10        | -82        | -8         | Lingual L.1            | Left Occipital  |
| 33      | -26        | -7         | 9          | Insula L.1             | Left Sub-Cortex |
| 34      | -45        | -71        | 11         | Occipital Mid L.4      | Left Temporal   |
| 35      | -26        | -70        | 41         | Parietal Sup L.2       | Left Parietal   |
| 36      | -28        | -35        | 40         | Parietal Inf L.1       | Left Parietal   |
| 37      | -12        | -21        | 66         | Paracentral Lobule L.1 | Left Frontal    |
| 38      | -16        | -86        | 15         | Occipital Sup L.2      | Left Occipital  |
| 39      | -12        | -17        | 40         | Cingulum Mid L.2       | Left Limbic     |
| 40      | -35        | -13        | -32        | Temporal Inf L.1       | Left Temporal   |
| 41      | -24        | -12        | 67         | Precentral L.3         | Left Frontal    |
| 42      | -23        | -72        | 27         | Occipital Sup L.3      | Left Parietal   |
| 43      | -50        | 16         | 20         | Frontal Inf Tri L.2    | Left PreFrontal |
| 44      | -34        | 35         | -15        | Frontal Inf Orb L.1    | Left PreFrontal |
| 45      | -51        | -14        | 29         | Postcentral L.5        | Left Frontal    |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match | Lobe            |
|---------|------------|------------|------------|-------------------|-----------------|
| 46      | -38        | -80        | -13        | Occipital Inf L.2 | Left Occipital  |
| 47      | -11        | -85        | 27         | Cuneus L.2        | Left Occipital  |
| 48      | -55        | -6         | -2         | Temporal Sup L.2  | Left Temporal   |
| 49      | -43        | -66        | 0          | Occipital Mid L.5 | Left Temporal   |
| 50      | -37        | -21        | 56         | Precentral L.4    | Left Frontal    |
| 51      | -54        | -56        | 16         | Temporal Mid L.3  | Left Temporal   |
| 52      | -38        | -51        | 45         | Parietal Inf L.2  | Left Parietal   |
| 53      | -29        | -78        | 35         | Occipital Mid L.6 | Left Parietal   |
| 54      | -34        | 31         | 22         | Frontal Mid L.2   | Left PreFrontal |
| 55      | -37        | -1         | 56         | Precentral L.5    | Left Frontal    |
| 56      | -50        | -16        | -17        | Temporal Mid L.4  | Left Temporal   |
| 57      | -18        | -54        | -6         | Lingual L.2       | Left Limbic     |
| 58      | -11        | 1          | 35         | Cingulum Mid L.3  | Left Limbic     |
| 59      | -34        | -62        | 29         | Occipital Mid L.7 | Left Parietal   |
| 60      | -45        | 1          | -37        | Temporal Inf L.2  | Left Temporal   |
| 61      | -33        | -9         | -3         | Putamen L.2       | Left Sub-Cortex |
| 62      | -26        | 10         | 50         | Frontal Mid L.3   | Left Frontal    |
| 63      | -40        | -72        | 34         | Angular L.2       | Left Parietal   |
| 64      | -29        | 0          | -40        | Fusiform L.1      | Left Temporal   |
| 65      | -19        | -78        | 21         | Occipital Sup L.4 | Left Occipital  |
| 66      | -12        | -45        | 64         | Precuneus L.1     | Left Parietal   |
| 67      | -12        | -62        | 42         | Precuneus L.2     | Left Parietal   |
| 68      | -50        | -44        | 34         | Parietal Inf L.3  | Left Parietal   |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match   | Lobe            |
|---------|------------|------------|------------|---------------------|-----------------|
| 69      | -49        | -48        | -21        | Temporal Inf L.3    | Left Temporal   |
| 70      | -45        | -63        | 33         | Angular L.3         | Left Parietal   |
| 71      | -57        | -8         | 14         | Postcentral L.6     | Left Frontal    |
| 72      | -36        | -51        | -14        | Fusiform L.2        | Left Temporal   |
| 73      | -8         | 26         | -5         | Cingulum Ant L.1    | Left Limbic     |
| 74      | -31        | -68        | -14        | Fusiform L.3        | Left Occipital  |
| 75      | -44        | 22         | 23         | Frontal Inf Tri L.3 | Left PreFrontal |
| 76      | -42        | 40         | -10        | Frontal Inf Orb L.2 | Left PreFrontal |
| 77      | -17        | -60        | 32         | Precuneus L.3       | Left Parietal   |
| 78      | -23        | 49         | -11        | Frontal Mid Orb L.2 | Left PreFrontal |
| 79      | -21        | -57        | 61         | Parietal Sup L.3    | Left Parietal   |
| 80      | -15        | -40        | 44         | Cingulum Mid L.4    | Left Parietal   |
| 81      | -52        | -22        | -28        | Temporal Inf L.4    | Left Temporal   |
| 82      | -8         | -75        | 34         | Cuneus L.3          | Left Parietal   |
| 83      | -8         | -11        | 62         | Supp Motor Area L.1 | Left Frontal    |
| 84      | -15        | 18         | -18        | Rectus L.1          | Left PreFrontal |
| 85      | -15        | 10         | -10        | Putamen L.3         | Left Sub-Cortex |
| 86      | -11        | 35         | -9         | Rectus L.2          | Left PreFrontal |
| 87      | -18        | 16         | 27         | Cingulum Ant L.2    | Left Limbic     |
| 88      | -49        | -3         | -29        | Temporal Mid L.5    | Left Temporal   |
| 89      | -52        | 4          | -20        | Temporal Mid L.6    | Left Temporal   |
| 90      | -24        | -19        | -11        | Hippocampus L.1     | Left Limbic     |
| 91      | -9         | -53        | 46         | Precuneus L.4       | Left Parietal   |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                  |
|---------|------------|------------|------------|------------------------|-----------------------|
| 92      | -14        | -21        | 9          | Thalamus L.1           | Left Sub-Cortex       |
| 93      | -30        | 43         | 12         | Frontal Mid L.4        | Left PreFrontal       |
| 94      | -26        | -25        | -21        | ParaHippocampal L.1    | Left Limbic           |
| 95      | -39        | 4          | -9         | Insula L.2             | Left Frontal-Temporal |
| 96      | -26        | -6         | -29        | ParaHippocampal L.2    | Left Limbic           |
| 97      | -14        | 43         | 6          | Frontal Sup Medial L.2 | Left PreFrontal       |
| 98      | -30        | -15        | 59         | Precentral L.6         | Left Frontal          |
| 99      | -31        | 5          | 54         | Frontal Mid L.5        | Left Frontal          |
| 100     | -25        | -50        | 64         | Parietal Sup L.4       | Left Parietal         |
| 101     | -30        | -34        | 52         | Postcentral L.7        | Left Parietal         |
| 102     | -36        | -22        | 10         | Heschl L.1             | Left Frontal-Temporal |
| 103     | -8         | 49         | -4         | Frontal Med Orb L.1    | Left PreFrontal       |
| 104     | -11        | 1          | 63         | Supp Motor Area L.2    | Left Frontal          |
| 105     | -8         | 35         | 13         | Cingulum Ant L.3       | Left Limbic           |
| 106     | -29        | 10         | 0          | Putamen L.4            | Left Sub-Cortex       |
| 107     | -24        | 30         | 36         | Frontal Mid L.6        | Left PreFrontal       |
| 108     | -20        | 0          | -15        | Amygdala L.1           | Left Limbic           |
| 109     | -42        | -54        | 37         | Angular L.4            | Left Parietal         |
| 110     | -23        | 5          | -27        | Temporal Pole Sup L.2  | Left Limbic           |
| 111     | -39        | -20        | -6         | Temporal Sup L.3       | Left Frontal-Temporal |
| 112     | -11        | -96        | -1         | Calcarine L.3          | Left Occipital        |
| 113     | -8         | 1          | 52         | Supp Motor Area L.3    | Left Frontal          |
| 114     | -11        | 8          | 51         | Supp Motor Area L.4    | Left Frontal          |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                      |
|---------|------------|------------|------------|------------------------|---------------------------|
| 115     | -17        | -13        | 62         | Paracentral Lobule L.2 | Left Frontal              |
| 116     | -19        | -75        | 45         | Parietal Sup L.5       | Left Parietal             |
| 117     | -14        | 4          | 15         | Caudate L.1            | Left Sub-Cortex           |
| 118     | -57        | -34        | -17        | Temporal Mid L.7       | Left Temporal             |
| 119     | -55        | -50        | -4         | Temporal Mid L.8       | Left Temporal             |
| 120     | -17        | -88        | -14        | Lingual L.3            | Left Occipital            |
| 121     | -47        | 10         | 37         | Precentral L.7         | Left PreFrontal           |
| 122     | -42        | -7         | 0          | Insula L.3             | Left Frontal-<br>Temporal |
| 123     | -13        | -95        | -10        | Calcarine L.4          | Left Occipital            |
| 124     | -26        | 55         | 3          | Frontal Sup L.3        | Left PreFrontal           |
| 125     | -9         | -72        | 44         | Precuneus L.5          | Left Parietal             |
| 126     | -48        | -53        | 42         | Parietal Inf L.4       | Left Parietal             |
| 127     | -61        | -49        | -13        | Temporal Inf L.5       | Left Temporal             |
| 128     | -32        | -5         | -19        | Amygdala L.2           | Left Limbic               |
| 129     | -9         | 34         | 43         | Frontal Sup Medial L.3 | Left PreFrontal           |
| 130     | -57        | -45        | -22        | Temporal Inf L.6       | Left Temporal             |
| 131     | -8         | 42         | 23         | Frontal Sup Medial L.4 | Left PreFrontal           |
| 132     | -12        | 57         | -13        | Frontal Sup Orb L.1    | Left PreFrontal           |
| 133     | -15        | -38        | 59         | Precuneus L.6          | Left Frontal              |
| 134     | -41        | 14         | -34        | Temporal Pole Mid L.1  | Left Temporal             |
| 135     | -51        | 24         | 5          | Frontal Inf Tri L.4    | Left PreFrontal           |
| 136     | -31        | 19         | -31        | Temporal Pole Sup L.3  | Left Temporal             |
| 137     | -47        | -30        | -23        | Temporal Inf L.7       | Left Temporal             |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match   | Lobe                  |
|---------|------------|------------|------------|---------------------|-----------------------|
| 138     | -9         | -44        | 26         | Cingulum Post L.1   | Left Limbic           |
| 139     | -52        | -33        | 23         | SupraMarginal L.2   | Left Parietal         |
| 140     | -47        | -25        | 42         | Postcentral L.8     | Left Parietal         |
| 141     | -42        | -10        | 12         | Rolandic Oper L.1   | Left Frontal-Temporal |
| 142     | -34        | 50         | 4          | Frontal Mid L.7     | Left PreFrontal       |
| 143     | -45        | -71        | -13        | Occipital Inf L.3   | Left Occipital        |
| 144     | -17        | 37         | 38         | Frontal Sup L.4     | Left PreFrontal       |
| 145     | -49        | -57        | -14        | Temporal Inf L.8    | Left Temporal         |
| 146     | -18        | 10         | 59         | Frontal Sup L.5     | Left Frontal          |
| 147     | -7         | -90        | 5          | Calcarine L.5       | Left Occipital        |
| 148     | -25        | -94        | 7          | Occipital Mid L.8   | Left Occipital        |
| 149     | -60        | -35        | 18         | Temporal Sup L.4    | Left Temporal         |
| 150     | -40        | -23        | -23        | Temporal Inf L.9    | Left Temporal         |
| 151     | -9         | 26         | -20        | Rectus L.3          | Left PreFrontal       |
| 152     | -24        | 40         | -17        | Frontal Mid Orb L.3 | Left PreFrontal       |
| 153     | -58        | -17        | -24        | Temporal Inf L.10   | Left Temporal         |
| 154     | -37        | 23         | -1         | Frontal Inf Tri L.5 | Left PreFrontal       |
| 155     | -43        | -76        | 22         | Occipital Mid L.9   | Left Temporal         |
| 156     | -21        | -64        | -4         | Lingual L.4         | Left Occipital        |
| 157     | -31        | -54        | 54         | Parietal Sup L.6    | Left Parietal         |
| 158     | -15        | -55        | 23         | Precuneus L.7       | Left Parietal         |
| 159     | -11        | 29         | 22         | Cingulum Ant L.4    | Left Limbic           |
| 160     | -15        | 55         | -5         | Frontal Med Orb L.2 | Left PreFrontal       |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                  |
|---------|------------|------------|------------|------------------------|-----------------------|
| 161     | -8         | 56         | 18         | Frontal Sup Medial L.5 | Left PreFrontal       |
| 162     | -20        | 48         | 25         | Frontal Sup L.6        | Left PreFrontal       |
| 163     | -12        | -72        | 6          | Calcarine L.6          | Left Occipital        |
| 164     | -55        | -37        | 37         | Parietal Inf L.5       | Left Parietal         |
| 165     | -49        | -10        | 36         | Postcentral L.9        | Left Frontal          |
| 166     | -45        | 34         | -2         | Frontal Inf Tri L.6    | Left PreFrontal       |
| 167     | -44        | 1          | 32         | Precentral L.8         | Left Frontal          |
| 168     | -34        | 6          | -31        | Temporal Pole Sup L.4  | Left Temporal         |
| 169     | -42        | 28         | 12         | Frontal Inf Tri L.7    | Left PreFrontal       |
| 170     | -31        | -83        | 2          | Occipital Mid L.10     | Left Occipital        |
| 171     | -32        | 20         | 46         | Frontal Mid L.8        | Left PreFrontal       |
| 172     | -57        | -21        | 8          | Temporal Sup L.5       | Left Temporal         |
| 173     | -30        | -21        | -27        | Fusiform L.4           | Left Limbic           |
| 174     | -27        | -59        | 44         | Parietal Sup L.7       | Left Parietal         |
| 175     | -33        | -33        | -12        | Hippocampus L.2        | Left Limbic           |
| 176     | -10        | 15         | 34         | Cingulum Mid L.5       | Left Limbic           |
| 177     | -22        | 24         | -22        | Frontal Inf Orb L.3    | Left PreFrontal       |
| 178     | -54        | 4          | -1         | Rolandic Oper L.2      | Left Frontal-Temporal |
| 179     | -9         | -37        | 36         | Cingulum Mid L.6       | Left Limbic           |
| 180     | -28        | -86        | 13         | Occipital Mid L.11     | Left Occipital        |
| 181     | -29        | -5         | 50         | Precentral L.9         | Left Frontal          |
| 182     | -43        | 8          | -26        | Temporal Pole Mid L.2  | Left Temporal         |
| 183     | -35        | -74        | -1         | Occipital Mid L.12     | Left Occipital        |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe            |
|---------|------------|------------|------------|------------------------|-----------------|
| 184     | -35        | 27         | 37         | Frontal Mid L.9        | Left PreFrontal |
| 185     | -14        | -94        | 13         | Occipital Sup L.5      | Left Occipital  |
| 186     | -16        | 14         | 8          | Caudate L.2            | Left Sub-Cortex |
| 187     | -24        | 18         | 50         | Frontal Mid L.10       | Left PreFrontal |
| 188     | -34        | -57        | -17        | Fusiform L.5           | Left Occipital  |
| 189     | -26        | 46         | 23         | Frontal Mid L.11       | Left PreFrontal |
| 190     | -12        | 60         | 9          | Frontal Sup Medial L.6 | Left PreFrontal |
| 191     | -30        | 13         | -22        | Temporal Pole Sup L.5  | Left Temporal   |
| 192     | -15        | -30        | 0          | Thalamus L.2           | Left Limbic     |
| 193     | -43        | -38        | 55         | Postcentral L.10       | Left Parietal   |
| 194     | -53        | -56        | 7          | Temporal Mid L.9       | Left Temporal   |
| 195     | -42        | -14        | 50         | Precentral L.10        | Left Frontal    |
| 196     | -22        | 11         | -5         | Putamen L.5            | Left Sub-Cortex |
| 197     | -15        | 45         | 34         | Frontal Sup L.7        | Left PreFrontal |
| 198     | -26        | 25         | -13        | Frontal Inf Orb L.4    | Left PreFrontal |
| 199     | -7         | -59        | 24         | Precuneus L.8          | Left Parietal   |
| 200     | -15        | -35        | 69         | Paracentral Lobule L.3 | Left Parietal   |
| 201     | -49        | 16         | 31         | Frontal Inf Oper L.3   | Left PreFrontal |
| 202     | -13        | -60        | 59         | Precuneus L.9          | Left Parietal   |
| 203     | -44        | 0          | 48         | Precentral L.11        | Left Frontal    |
| 204     | -35        | 51         | 15         | Frontal Mid L.12       | Left PreFrontal |
| 205     | -7         | -34        | 50         | Paracentral Lobule L.4 | Left Frontal    |
| 206     | -60        | -21        | -15        | Temporal Mid L.10      | Left Temporal   |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match     | Lobe                      |
|---------|------------|------------|------------|-----------------------|---------------------------|
| 207     | -20        | -49        | 0          | Lingual L.5           | Left Limbic               |
| 208     | -42        | 30         | 32         | Frontal Mid L.13      | Left PreFrontal           |
| 209     | -40        | -53        | 13         | Temporal Mid L.11     | Left Temporal             |
| 210     | -51        | -44        | 22         | SupraMarginal L.3     | Left Parietal             |
| 211     | -57        | -43        | 10         | Temporal Sup L.6      | Left Temporal             |
| 212     | -43        | 15         | -13        | Temporal Pole Sup L.6 | Left PreFrontal           |
| 213     | -49        | -3         | 12         | Rolandic Oper L.3     | Left Frontal              |
| 214     | -23        | -56        | 17         | Angular L.5           | Left Limbic               |
| 215     | -44        | 42         | 13         | Frontal Mid L.14      | Left PreFrontal           |
| 216     | -41        | -29        | 14         | Rolandic Oper L.4     | Left Frontal-<br>Temporal |
| 217     | -49        | -20        | 2          | Temporal Sup L.7      | Left Temporal             |
| 218     | -35        | -63        | 51         | Parietal Sup L.8      | Left Parietal             |
| 219     | -52        | -30        | 15         | Temporal Sup L.8      | Left Parietal             |
| 220     | -17        | -47        | 49         | Precuneus L.10        | Left Parietal             |
| 221     | -51        | -62        | -6         | Temporal Mid L.12     | Left Temporal             |
| 222     | -23        | -23        | 70         | Precentral L.12       | Left Frontal              |
| 223     | -27        | -4         | 21         | Precentral L.13       | Left Sub-Cortex           |
| 224     | -36        | 19         | 32         | Frontal Mid L.15      | Left PreFrontal           |
| 225     | -20        | -44        | 71         | Parietal Sup L.9      | Left Parietal             |
| 226     | -36        | 4          | 43         | Precentral L.14       | Left Frontal              |
| 227     | -22        | -30        | 62         | Postcentral L.11      | Left Frontal              |
| 228     | -46        | 4          | 5          | Frontal Inf Oper L.4  | Left Frontal-<br>Temporal |
| 229     | -18        | -69        | 0          | Lingual L.6           | Left Occipital            |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                      |
|---------|------------|------------|------------|------------------------|---------------------------|
| 230     | -10        | 23         | 49         | Supp Motor Area L.5    | Left PreFrontal           |
| 231     | -10        | 53         | 30         | Frontal Sup Medial L.7 | Left PreFrontal           |
| 232     | -21        | -36        | 7          | Hippocampus L.3        | Left Limbic               |
| 233     | -42        | -41        | -21        | Temporal Inf L.11      | Left Temporal             |
| 234     | -58        | -35        | 2          | Temporal Mid L.13      | Left Temporal             |
| 235     | -30        | -9         | 41         | Precentral L.15        | Left Frontal              |
| 236     | -40        | 11         | 3          | Insula L.4             | Left Frontal-<br>Temporal |
| 237     | -49        | -63        | 5          | Temporal Mid L.14      | Left Temporal             |
| 238     | -29        | 22         | 5          | Frontal Inf Tri L.8    | Left PreFrontal           |
| 239     | -44        | -30        | 35         | Parietal Inf L.6       | Left Parietal             |
| 240     | -16        | -12        | 52         | Supp Motor Area L.6    | Left Frontal              |
| 241     | -53        | -6         | -20        | Temporal Mid L.15      | Left Temporal             |
| 242     | -9         | 45         | -15        | Rectus L.4             | Left PreFrontal           |
| 243     | -49        | 29         | -6         | Frontal Inf Orb L.5    | Left PreFrontal           |
| 244     | -7         | -10        | 36         | Cingulum Mid L.7       | Left Limbic               |
| 245     | -31        | -92        | -3         | Occipital Mid L.13     | Left Occipital            |
| 246     | -23        | -37        | -10        | ParaHippocampal L.3    | Left Limbic               |
| 247     | -40        | -48        | 55         | Parietal Inf L.7       | Left Parietal             |
| 248     | -50        | -34        | 46         | Parietal Inf L.8       | Left Parietal             |
| 249     | -42        | 35         | 14         | Frontal Inf Tri L.9    | Left PreFrontal           |
| 250     | -28        | -45        | -9         | Fusiform L.6           | Left Limbic               |
| 251     | -42        | 44         | 1          | Frontal Mid L.16       | Left PreFrontal           |
| 252     | -63        | -23        | -2         | Temporal Mid L.16      | Left Temporal             |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match    | Lobe             |
|---------|------------|------------|------------|----------------------|------------------|
| 253     | -21        | 60         | 10         | Frontal Sup L.8      | Left PreFrontal  |
| 254     | -13        | 16         | 43         | Supp Motor Area L.7  | Left PreFrontal  |
| 255     | -12        | -70        | -4         | Lingual L.7          | Left Occipital   |
| 256     | -56        | -42        | -3         | Temporal Mid L.17    | Left Temporal    |
| 257     | 19         | -85        | 33         | Occipital Sup R.1    | Right Occipital  |
| 258     | 47         | -15        | 45         | Postcentral R.1      | Right Frontal    |
| 259     | 34         | -32        | 62         | Postcentral R.2      | Right Parietal   |
| 260     | 57         | -11        | 24         | Postcentral R.3      | Right Frontal    |
| 261     | 16         | 36         | 26         | Frontal Sup R.1      | Right PreFrontal |
| 262     | 10         | -61        | 13         | Calcarine R.1        | Right Limbic     |
| 263     | 37         | -32        | 48         | Postcentral R.4      | Right Parietal   |
| 264     | 39         | 7          | 22         | Frontal Inf Oper R.1 | Right PreFrontal |
| 265     | 60         | -30        | -8         | Temporal Mid R.1     | Right Temporal   |
| 266     | 53         | 2          | 35         | Precentral R.1       | Right Frontal    |
| 267     | 55         | -24        | 28         | SupraMarginal R.1    | Right Parietal   |
| 268     | 48         | 11         | 13         | Frontal Inf Oper R.2 | Right PreFrontal |
| 269     | 7          | -81        | 9          | Calcarine R.2        | Right Occipital  |
| 270     | 28         | 53         | -8         | Frontal Mid Orb R.1  | Right PreFrontal |
| 271     | 49         | -54        | 28         | Angular R.1          | Right Parietal   |
| 272     | 46         | -30        | 8          | Temporal Sup R.1     | Right Temporal   |
| 273     | 27         | -88        | -12        | Occipital Inf R.1    | Right Occipital  |
| 274     | 54         | -7         | -10        | Temporal Sup R.2     | Right Temporal   |
| 275     | 36         | -79        | -5         | Occipital Inf R.2    | Right Occipital  |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match     | Lobe             |
|---------|------------|------------|------------|-----------------------|------------------|
| 276     | 20         | -64        | 52         | Parietal Sup R.1      | Right Parietal   |
| 277     | 48         | 17         | -17        | Temporal Pole Sup R.1 | Right Temporal   |
| 278     | 56         | 7          | 19         | Precentral R.2        | Right PreFrontal |
| 279     | 31         | 43         | 29         | Frontal Mid R.1       | Right PreFrontal |
| 280     | 44         | 19         | -2         | Frontal Inf Orb R.1   | Right PreFrontal |
| 281     | 35         | -79        | 17         | Occipital Mid R.1     | Right Temporal   |
| 282     | 20         | -8         | 1          | Putamen R.1           | Right Sub-Cortex |
| 283     | 33         | -73        | 27         | Occipital Mid R.2     | Right Parietal   |
| 284     | 17         | 35         | 47         | Frontal Sup R.2       | Right PreFrontal |
| 285     | 10         | -26        | 35         | Cingulum Mid R.1      | Right Limbic     |
| 286     | 10         | -74        | 19         | Cuneus R.1            | Right Occipital  |
| 287     | 16         | 24         | 54         | Frontal Sup R.3       | Right PreFrontal |
| 288     | 10         | -82        | -8         | Lingual R.1           | Right Occipital  |
| 289     | 24         | -7         | 9          | Insula R.1            | Right Sub-Cortex |
| 290     | 44         | -70        | 12         | Temporal Mid R.2      | Right Temporal   |
| 291     | 25         | -69        | 41         | Occipital Sup R.2     | Right Parietal   |
| 292     | 26         | -34        | 40         | Postcentral R.5       | Right Parietal   |
| 293     | 11         | -21        | 66         | Precentral R.3        | Right Frontal    |
| 294     | 16         | -86        | 14         | Occipital Sup R.3     | Right Occipital  |
| 295     | 11         | -17        | 40         | Cingulum Mid R.2      | Right Limbic     |
| 296     | 35         | -12        | -33        | Fusiform R.1          | Right Temporal   |
| 297     | 23         | -11        | 67         | Precentral R.4        | Right Frontal    |
| 298     | 23         | -72        | 28         | Occipital Sup R.4     | Right Parietal   |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match   | Lobe             |
|---------|------------|------------|------------|---------------------|------------------|
| 299     | 49         | 16         | 20         | Frontal Inf Tri R.1 | Right PreFrontal |
| 300     | 34         | 36         | -14        | Frontal Inf Orb R.2 | Right PreFrontal |
| 301     | 51         | -14        | 30         | Postcentral R.6     | Right Frontal    |
| 302     | 38         | -79        | -12        | Occipital Inf R.3   | Right Occipital  |
| 303     | 11         | -85        | 28         | Cuneus R.2          | Right Occipital  |
| 304     | 55         | -5         | -2         | Temporal Sup R.3    | Right Temporal   |
| 305     | 42         | -65        | 0          | Temporal Mid R.3    | Right Temporal   |
| 306     | 37         | -20        | 57         | Precentral R.5      | Right Frontal    |
| 307     | 53         | -56        | 17         | Temporal Mid R.4    | Right Temporal   |
| 308     | 38         | -50        | 46         | Parietal Inf R.1    | Right Parietal   |
| 309     | 28         | -78        | 35         | Occipital Mid R.3   | Right Parietal   |
| 310     | 33         | 32         | 22         | Frontal Mid R.2     | Right PreFrontal |
| 311     | 36         | 0          | 56         | Frontal Mid R.3     | Right Frontal    |
| 312     | 49         | -15        | -16        | Temporal Mid R.5    | Right Temporal   |
| 313     | 17         | -53        | -6         | Lingual R.2         | Right Limbic     |
| 314     | 10         | 2          | 35         | Cingulum Mid R.3    | Right Limbic     |
| 315     | 33         | -61        | 30         | Angular R.2         | Right Parietal   |
| 316     | 44         | 2          | -36        | Temporal Inf R.1    | Right Temporal   |
| 317     | 32         | -7         | -1         | Putamen R.2         | Right Sub-Cortex |
| 318     | 25         | 11         | 50         | Frontal Mid R.4     | Right Frontal    |
| 319     | 40         | -70        | 35         | Angular R.3         | Right Parietal   |
| 320     | 28         | 1          | -40        | Fusiform R.2        | Right Temporal   |
| 321     | 18         | -78        | 21         | Occipital Sup R.5   | Right Occipital  |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match   | Lobe             |
|---------|------------|------------|------------|---------------------|------------------|
| 322     | 11         | -44        | 64         | Postcentral R.7     | Right Parietal   |
| 323     | 11         | -62        | 42         | Precuneus R.1       | Right Parietal   |
| 324     | 49         | -43        | 34         | SupraMarginal R.2   | Right Parietal   |
| 325     | 48         | -47        | -21        | Temporal Inf R.2    | Right Temporal   |
| 326     | 44         | -61        | 33         | Angular R.4         | Right Parietal   |
| 327     | 56         | -7         | 16         | Postcentral R.8     | Right Frontal    |
| 328     | 36         | -51        | -14        | Fusiform R.3        | Right Temporal   |
| 329     | 9          | 25         | -4         | Caudate R.1         | Right Limbic     |
| 330     | 31         | -67        | -14        | Fusiform R.4        | Right Occipital  |
| 331     | 43         | 24         | 25         | Frontal Inf Tri R.2 | Right PreFrontal |
| 332     | 42         | 41         | -9         | Frontal Inf Orb R.3 | Right PreFrontal |
| 333     | 16         | -60        | 32         | Precuneus R.2       | Right Parietal   |
| 334     | 22         | 49         | -10        | Frontal Mid Orb R.2 | Right PreFrontal |
| 335     | 20         | -57        | 61         | Parietal Sup R.2    | Right Parietal   |
| 336     | 15         | -40        | 44         | Precuneus R.3       | Right Parietal   |
| 337     | 51         | -21        | -27        | Temporal Inf R.3    | Right Temporal   |
| 338     | 8          | -74        | 34         | Cuneus R.3          | Right Parietal   |
| 339     | 7          | -11        | 62         | Supp Motor Area R.1 | Right Frontal    |
| 340     | 14         | 19         | -17        | Frontal Sup Orb R.1 | Right PreFrontal |
| 341     | 14         | 10         | -10        | Putamen R.3         | Right Sub-Cortex |
| 342     | 11         | 36         | -9         | Frontal Med Orb R.1 | Right PreFrontal |
| 343     | 16         | 16         | 27         | Cingulum Ant R.1    | Right Limbic     |
| 344     | 49         | -1         | -29        | Temporal Mid R.6    | Right Temporal   |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                   |
|---------|------------|------------|------------|------------------------|------------------------|
| 345     | 52         | 5          | -19        | Temporal Pole Mid R.1  | Right Temporal         |
| 346     | 24         | -18        | -12        | Hippocampus R.1        | Right Limbic           |
| 347     | 8          | -53        | 47         | Precuneus R.4          | Right Parietal         |
| 348     | 14         | -21        | 10         | Thalamus R.1           | Right Sub-Cortex       |
| 349     | 29         | 43         | 12         | Frontal Mid R.5        | Right PreFrontal       |
| 350     | 26         | -25        | -20        | ParaHippocampal R.1    | Right Limbic           |
| 351     | 39         | 4          | -8         | Insula R.2             | Right Frontal-Temporal |
| 352     | 26         | -6         | -28        | ParaHippocampal R.2    | Right Limbic           |
| 353     | 13         | 45         | 7          | Frontal Sup Medial R.1 | Right PreFrontal       |
| 354     | 29         | -14        | 59         | Precentral R.6         | Right Frontal          |
| 355     | 30         | 5          | 54         | Frontal Mid R.6        | Right Frontal          |
| 356     | 25         | -49        | 64         | Parietal Sup R.3       | Right Parietal         |
| 357     | 29         | -34        | 52         | Postcentral R.9        | Right Parietal         |
| 358     | 35         | -20        | 11         | Heschl R.1             | Right Frontal-Temporal |
| 359     | 8          | 49         | -3         | Frontal Med Orb R.2    | Right PreFrontal       |
| 360     | 10         | 1          | 63         | Supp Motor Area R.2    | Right Frontal          |
| 361     | 7          | 35         | 13         | Cingulum Ant R.2       | Right Limbic           |
| 362     | 29         | 11         | 0          | Putamen R.4            | Right Sub-Cortex       |
| 363     | 23         | 30         | 36         | Frontal Mid R.7        | Right PreFrontal       |
| 364     | 19         | 1          | -15        | ParaHippocampal R.3    | Right Limbic           |
| 365     | 41         | -53        | 38         | Angular R.5            | Right Parietal         |
| 366     | 23         | 5          | -25        | ParaHippocampal R.4    | Right Limbic           |
| 367     | 39         | -19        | -6         | Temporal Sup R.4       | Right Frontal-Temporal |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                   |
|---------|------------|------------|------------|------------------------|------------------------|
| 368     | 10         | -96        | -1         | Calcarine R.3          | Right Occipital        |
| 369     | 7          | 1          | 52         | Supp Motor Area R.3    | Right Frontal          |
| 370     | 10         | 8          | 51         | Supp Motor Area R.4    | Right Frontal          |
| 371     | 16         | -13        | 62         | Frontal Sup R.4        | Right Frontal          |
| 372     | 18         | -74        | 45         | Parietal Sup R.4       | Right Parietal         |
| 373     | 14         | 4          | 15         | Caudate R.2            | Right Sub-Cortex       |
| 374     | 57         | -33        | -16        | Temporal Inf R.4       | Right Temporal         |
| 375     | 55         | -48        | -4         | Temporal Mid R.7       | Right Temporal         |
| 376     | 17         | -88        | -14        | Lingual R.3            | Right Occipital        |
| 377     | 47         | 11         | 36         | Precentral R.7         | Right PreFrontal       |
| 378     | 41         | -6         | 1          | Insula R.3             | Right Frontal-Temporal |
| 379     | 13         | -95        | -10        | Lingual R.4            | Right Occipital        |
| 380     | 24         | 55         | 3          | Frontal Sup R.5        | Right PreFrontal       |
| 381     | 8          | -72        | 44         | Precuneus R.5          | Right Parietal         |
| 382     | 48         | -52        | 43         | Parietal Inf R.2       | Right Parietal         |
| 383     | 60         | -48        | -12        | Temporal Inf R.5       | Right Temporal         |
| 384     | 31         | -4         | -18        | Amygdala R.1           | Right Limbic           |
| 385     | 8          | 34         | 43         | Frontal Sup Medial R.2 | Right PreFrontal       |
| 386     | 57         | -43        | -21        | Temporal Inf R.6       | Right Temporal         |
| 387     | 7          | 42         | 23         | Cingulum Ant R.3       | Right PreFrontal       |
| 388     | 11         | 57         | -13        | Frontal Sup Orb R.2    | Right PreFrontal       |
| 389     | 14         | -38        | 59         | Postcentral R.10       | Right Frontal          |
| 390     | 41         | 15         | -33        | Temporal Pole Mid R.2  | Right Temporal         |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match     | Lobe                   |
|---------|------------|------------|------------|-----------------------|------------------------|
| 391     | 51         | 25         | 5          | Frontal Inf Tri R.3   | Right PreFrontal       |
| 392     | 30         | 19         | -31        | Temporal Pole Mid R.3 | Right Temporal         |
| 393     | 46         | -29        | -23        | Temporal Inf R.7      | Right Temporal         |
| 394     | 9          | -44        | 26         | Cingulum Post R.1     | Right Limbic           |
| 395     | 51         | -31        | 24         | SupraMarginal R.3     | Right Parietal         |
| 396     | 46         | -25        | 44         | Postcentral R.11      | Right Parietal         |
| 397     | 41         | -9         | 13         | Rolandic Oper R.1     | Right Frontal-Temporal |
| 398     | 33         | 51         | 4          | Frontal Mid R.8       | Right PreFrontal       |
| 399     | 44         | -70        | -12        | Occipital Inf R.4     | Right Occipital        |
| 400     | 15         | 37         | 38         | Frontal Sup R.6       | Right PreFrontal       |
| 401     | 48         | -56        | -14        | Temporal Inf R.8      | Right Temporal         |
| 402     | 17         | 10         | 59         | Frontal Sup R.7       | Right Frontal          |
| 403     | 6          | -90        | 5          | Calcarine R.4         | Right Occipital        |
| 404     | 24         | -94        | 8          | Occipital Mid R.4     | Right Occipital        |
| 405     | 60         | -34        | 19         | Temporal Sup R.5      | Right Temporal         |
| 406     | 39         | -22        | -22        | Fusiform R.5          | Right Temporal         |
| 407     | 9          | 26         | -20        | Rectus R.1            | Right PreFrontal       |
| 408     | 23         | 39         | -16        | Frontal Mid Orb R.3   | Right PreFrontal       |
| 409     | 57         | -16        | -25        | Temporal Inf R.9      | Right Temporal         |
| 410     | 37         | 24         | -1         | Insula R.4            | Right PreFrontal       |
| 411     | 42         | -75        | 23         | Occipital Mid R.5     | Right Temporal         |
| 412     | 21         | -63        | -4         | Lingual R.5           | Right Occipital        |
| 413     | 30         | -53        | 54         | Parietal Sup R.5      | Right Parietal         |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                   |
|---------|------------|------------|------------|------------------------|------------------------|
| 414     | 14         | -55        | 23         | Precuneus R.6          | Right Parietal         |
| 415     | 10         | 29         | 22         | Cingulum Ant R.4       | Right Limbic           |
| 416     | 14         | 55         | -5         | Frontal Sup Orb R.3    | Right PreFrontal       |
| 417     | 8          | 57         | 19         | Frontal Sup Medial R.3 | Right PreFrontal       |
| 418     | 19         | 49         | 25         | Frontal Mid R.9        | Right PreFrontal       |
| 419     | 12         | -72        | 7          | Calcarine R.5          | Right Occipital        |
| 420     | 54         | -36        | 38         | SupraMarginal R.4      | Right Parietal         |
| 421     | 47         | -9         | 36         | Postcentral R.12       | Right Frontal          |
| 422     | 45         | 35         | -1         | Frontal Inf Tri R.4    | Right PreFrontal       |
| 423     | 43         | 2          | 33         | Precentral R.8         | Right Frontal          |
| 424     | 33         | 6          | -30        | Temporal Pole Mid R.4  | Right Temporal         |
| 425     | 41         | 29         | 12         | Frontal Inf Tri R.5    | Right PreFrontal       |
| 426     | 30         | -82        | 2          | Occipital Mid R.6      | Right Occipital        |
| 427     | 31         | 21         | 46         | Frontal Mid R.10       | Right PreFrontal       |
| 428     | 56         | -19        | 9          | Temporal Sup R.6       | Right Temporal         |
| 429     | 30         | -20        | -27        | Fusiform R.6           | Right Limbic           |
| 430     | 26         | -58        | 45         | Angular R.6            | Right Parietal         |
| 431     | 32         | -33        | -12        | Fusiform R.7           | Right Limbic           |
| 432     | 9          | 15         | 35         | Cingulum Mid R.4       | Right Limbic           |
| 433     | 21         | 25         | -22        | Frontal Inf Orb R.4    | Right PreFrontal       |
| 434     | 54         | 4          | 0          | Temporal Pole Sup R.2  | Right Frontal-Temporal |
| 435     | 8          | -37        | 36         | Cingulum Mid R.5       | Right Limbic           |
| 436     | 27         | -85        | 12         | Occipital Mid R.7      | Right Occipital        |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe             |
|---------|------------|------------|------------|------------------------|------------------|
| 437     | 28         | -4         | 50         | Precentral R.9         | Right Frontal    |
| 438     | 43         | 9          | -26        | Temporal Pole Mid R.5  | Right Temporal   |
| 439     | 34         | -73        | 0          | Occipital Mid R.8      | Right Occipital  |
| 440     | 34         | 28         | 38         | Frontal Mid R.11       | Right PreFrontal |
| 441     | 13         | -94        | 13         | Occipital Sup R.6      | Right Occipital  |
| 442     | 16         | 14         | 9          | Caudate R.3            | Right Sub-Cortex |
| 443     | 23         | 19         | 51         | Frontal Mid R.12       | Right PreFrontal |
| 444     | 33         | -56        | -16        | Fusiform R.8           | Right Occipital  |
| 445     | 25         | 47         | 23         | Frontal Mid R.13       | Right PreFrontal |
| 446     | 11         | 60         | 9          | Frontal Sup Medial R.4 | Right PreFrontal |
| 447     | 30         | 14         | -23        | Temporal Pole Sup R.3  | Right Temporal   |
| 448     | 14         | -30        | 0          | Thalamus R.2           | Right Limbic     |
| 449     | 43         | -36        | 55         | Parietal Inf R.3       | Right Parietal   |
| 450     | 52         | -54        | 7          | Temporal Mid R.8       | Right Temporal   |
| 451     | 41         | -13        | 51         | Precentral R.10        | Right Frontal    |
| 452     | 21         | 11         | -4         | Putamen R.5            | Right Sub-Cortex |
| 453     | 15         | 46         | 35         | Frontal Sup R.8        | Right PreFrontal |
| 454     | 26         | 25         | -13        | Frontal Inf Orb R.5    | Right PreFrontal |
| 455     | 7          | -59        | 24         | Precuneus R.7          | Right Parietal   |
| 456     | 14         | -35        | 70         | Postcentral R.13       | Right Parietal   |
| 457     | 48         | 17         | 30         | Frontal Inf Oper R.3   | Right PreFrontal |
| 458     | 12         | -60        | 58         | Parietal Sup R.6       | Right Parietal   |
| 459     | 43         | 1          | 48         | Precentral R.11        | Right Frontal    |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                       |
|---------|------------|------------|------------|------------------------|----------------------------|
| 460     | 35         | 52         | 16         | Frontal Mid R.14       | Right PreFrontal           |
| 461     | 6          | -33        | 50         | Paracentral Lobule R.1 | Right Frontal              |
| 462     | 59         | -20        | -15        | Temporal Mid R.9       | Right Temporal             |
| 463     | 18         | -48        | 1          | Lingual R.6            | Right Limbic               |
| 464     | 41         | 31         | 33         | Frontal Mid R.15       | Right PreFrontal           |
| 465     | 40         | -52        | 13         | Temporal Mid R.10      | Right Temporal             |
| 466     | 51         | -44        | 23         | Temporal Sup R.7       | Right Parietal             |
| 467     | 56         | -41        | 10         | Temporal Mid R.11      | Right Temporal             |
| 468     | 42         | 16         | -13        | Temporal Pole Sup R.4  | Right PreFrontal           |
| 469     | 48         | -2         | 14         | Rolandic Oper R.2      | Right Frontal              |
| 470     | 23         | -56        | 18         | Precuneus R.8          | Right Limbic               |
| 471     | 43         | 43         | 14         | Frontal Mid R.16       | Right PreFrontal           |
| 472     | 40         | -28        | 14         | Temporal Sup R.8       | Right Frontal-<br>Temporal |
| 473     | 48         | -19        | 3          | Temporal Sup R.9       | Right Temporal             |
| 474     | 33         | -62        | 51         | Angular R.7            | Right Parietal             |
| 475     | 52         | -28        | 16         | Temporal Sup R.10      | Right Parietal             |
| 476     | 16         | -46        | 49         | Parietal Sup R.7       | Right Parietal             |
| 477     | 50         | -61        | -6         | Temporal Inf R.10      | Right Temporal             |
| 478     | 22         | -22        | 70         | Precentral R.12        | Right Frontal              |
| 479     | 25         | -4         | 21         | Precentral R.13        | Right Sub-Cortex           |
| 480     | 35         | 19         | 32         | Frontal Mid R.17       | Right PreFrontal           |
| 481     | 19         | -44        | 71         | Postcentral R.14       | Right Parietal             |
| 482     | 35         | 4          | 44         | Frontal Mid R.18       | Right Frontal              |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match      | Lobe                   |
|---------|------------|------------|------------|------------------------|------------------------|
| 483     | 21         | -29        | 63         | Postcentral R.15       | Right Frontal          |
| 484     | 45         | 4          | 6          | Rolandic Oper R.3      | Right Frontal-Temporal |
| 485     | 17         | -69        | 0          | Lingual R.7            | Right Occipital        |
| 486     | 9          | 23         | 49         | Supp Motor Area R.5    | Right PreFrontal       |
| 487     | 9          | 53         | 30         | Frontal Sup Medial R.5 | Right PreFrontal       |
| 488     | 20         | -37        | 7          | Calcarine R.6          | Right Limbic           |
| 489     | 42         | -40        | -21        | Temporal Inf R.11      | Right Temporal         |
| 490     | 57         | -34        | 3          | Temporal Mid R.12      | Right Temporal         |
| 491     | 29         | -9         | 41         | Precentral R.14        | Right Frontal          |
| 492     | 39         | 12         | 3          | Insula R.5             | Right Frontal-Temporal |
| 493     | 48         | -62        | 6          | Temporal Mid R.13      | Right Temporal         |
| 494     | 28         | 23         | 5          | Insula R.6             | Right PreFrontal       |
| 495     | 43         | -29        | 35         | SupraMarginal R.5      | Right Parietal         |
| 496     | 15         | -12        | 52         | Frontal Sup R.9        | Right Frontal          |
| 497     | 52         | -5         | -20        | Temporal Mid R.14      | Right Temporal         |
| 498     | 9          | 44         | -14        | Rectus R.2             | Right PreFrontal       |
| 499     | 48         | 30         | -6         | Frontal Inf Orb R.6    | Right PreFrontal       |
| 500     | 6          | -10        | 36         | Cingulum Mid R.6       | Right Limbic           |
| 501     | 31         | -91        | -3         | Occipital Inf R.5      | Right Occipital        |
| 502     | 22         | -36        | -10        | ParaHippocampal R.5    | Right Limbic           |
| 503     | 38         | -46        | 56         | Parietal Sup R.8       | Right Parietal         |
| 504     | 50         | -33        | 46         | Parietal Inf R.4       | Right Parietal         |
| 505     | 41         | 36         | 16         | Frontal Mid R.19       | Right PreFrontal       |

| Node ID | X-Centroid | Y-Centroid | Z-Centroid | Closest AAL match | Lobe             |
|---------|------------|------------|------------|-------------------|------------------|
| 506     | 28         | -45        | -9         | Fusiform R.9      | Right Limbic     |
| 507     | 41         | 45         | 2          | Frontal Mid R.20  | Right PreFrontal |
| 508     | 62         | -21        | -1         | Temporal Sup R.11 | Right Temporal   |
| 509     | 21         | 60         | 10         | Frontal Sup R.10  | Right PreFrontal |
| 510     | 12         | 16         | 43         | Frontal Sup R.11  | Right PreFrontal |
| 511     | 11         | -70        | -5         | Lingual R.8       | Right Occipital  |
| 512     | 55         | -41        | -2         | Temporal Mid R.15 | Right Temporal   |

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