EEG-fMRI in Epilepsy and Sleep

by

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Abstract

This thesis used simultaneous electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI) to investigate both epilepsy and sleep. Initially, EEG-fMRI was used in a cohort of patients with complex epilepsy referred from a tertiary epilepsy clinic for both pre-surgical evaluation and diagnostic reasons. The results suggest a limited utility of EEG-fMRI in the epilepsy clinic with a very complex patient group. Following on, investigation of early blood oxygen level dependent (BOLD) signal changes in a group of patients with focal epilepsy demonstrated potentially meaningful BOLD changes occurring six seconds prior to interictal epileptiform discharges, and modelling less than this six seconds can result in overlap of the haemodynamic response function used to model BOLD changes. The same analysis was used to model endogenously occurring sleep paroxysms; K-complexes (KCs), vertex sharp waves (VSWs) and sleep spindles (SSs), finding early BOLD signal changes with SSs in group data. Finally, KCs and VSWs were investigated in more detail in a group of participants under both sleep deprived and non-deprived conditions, demonstrating an increase in overall activation for both KCs and VSWs following sleep deprivation. Overall, we find early BOLD changes are not restricted to pathological events and sleep deprivation can enhance BOLD responses.

Dedication

This thesis is dedicated to my family. My beautiful wife Sarah, whose unconditional love and unyielding support over these years has brought me here. I am humbled in the presence of her belief in me, her beauty, her patience and strength of character, and my spirit is lifted beyond the firmament simply by that smile. My precious daughter Evie, who at 3 ½ years old has shown a level of patience and understanding that would be a credit to an adult. Her quiet words of support and encouragement, and her very being gave me constant sustenance to see this through. My son Hugo, who, on the day I write this dedication and complete this thesis, took his first steps. That cheeky grin, those cheeks, and gentle eyes make me sure that like his mother and sister, he too has inherited that smile. I could never thank my family enough, but I will spend the rest of my life trying.

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List of abbreviations

AAS	Average artefact subtraction
AASM	American Academy of Sleep Medicine
ACC	Anterior cingulate cortex
ACh	Acetylcholine
ALFF	Amplitude of low frequency fluctuation
AP	Action potential
ARAS	Ascending reticular activating system
BCG	Ballistocardiographic
BETS	Benign epileptiform transients of sleep
BOLD	Blood oxygenation level dependent
CAE	Childhood absence epilepsy
CBF	Cerebral blood flow
CBV	Cerebral blood volume
$CMRO_2$	Cerebral metabolic rate of oxygen
CNS	Central nervous system
CPS	Complex partial seizure
CSF	Cerebral spinal fluid
DC	Direct current
dHb	Deoxy-haemoglobin
DMH	Dorsomedial nucleus of the hypothalamus
DMN	Default mode network
DNET	Dysembryoplastic neuroepithelial tumour
DR	Dorsal raphe nucleus
DTI	Diffusion tensor imaging
ECG	Electrocardiogram
ECoG	Electrocorticography
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
EPI	Echo planar imaging
EPSP	Excitatory postsynaptic potential
ERP	Event related potential
EZ	Epileptogenic zone

FC	Functional connectivity
FCD	Focal cortical dysplasia
FID	Free induction decay
FLE	Frontal lobe epilepsy
FLS	Frontal lobe seizures
fMRI	Functional magnetic resonance imaging
FMS	Focal motor seizures
FNE	First night effect
FWHM	Full width at half maximum
GABA	*Gamma*-aminobutyric acid
GLM	General linear model
GLMM	Generalised linear mixed model
GRE	Gradient echo
GTCS	Generalised tonic-clonic seizure
Hb	Haemoglobin
HRF	Haemodynamic response function
HS	Hippocampal sclerosis
IBE	International Bureau for Epilepsy
ICA	Independent component analysis
ICE	International Classification of Epilepsies
icEEG	Intracranial EEG
IED	Interictal epileptiform discharge
IGE	Idiopathic generalised epilepsy
ILAE	International League Against Epilepsy
In	Insula
IPSP	inhibitory postsynaptic potential
ISI	Inter-stimulus interval
KC	K-complex
LC	Locus coeruleus
LDT	Laterodorsal tegmental nucleus
LFP	Local field potential
LG	Lingual gyrus
LPT	Lateral pontine tegmentum
MCC	Mid-cingulate cortex
MCD	Malformation of cortical development
MCH	Melanin-concentrating hormone
MFG	Middle frontal gyrus
MNI	Montreal Neurological Institute
MnPO	Median preoptic nucleus
MOG	Mid-orbital gyrus
MRI	Magnetic resonance imaging
MTG	Middle temporal gyrus

mTLE	Mesial temporal lobe epilepsy
ND	Non-deprived
NEAD	Non-epileptic attack disorder
NICE	National Institute for Health and Clinical Excellence
NMDA	*N*-Methyl-D-aspartic acid
NREM	Non-rapid eye movement
OBS	Optimal basis sets
ORX	Orexin
OSA	Obstructive sleep apnoea
PB	Parabrachial nucleus
PC	Precoeruleus area
PC	Precuneus
PCA	principal component analysis
PCC	Posterior cingulate cortex
PDS	Paroxysmal depolarising shift
PET	Positron emission tomography
PoCG	Post-central gyrus
PPT	Pedunculopontine nucleus
PrCG	Pre-central gyrus
PSG	Polysomnography
PTE	Post-traumatic epilepsy
PTS	Post-traumatic seizures
RAS	Reticular activating system
rCBF	Regional cerebral blood flow
RE	Thalamic reticular
REM	Rapid eye movement
RF	Radio frequency
ROI	Region of interest
RSN	Resting-state network
SCN	Suprachiasmatic nucleus
SD	Sleep deprived
SLD	Sublaterodorsal region
SO	Slow oscillation
SPECT	Single-photon emission computed tomography
SPM	Statistical parametric mapping
SPS	Simple partial seizures
SPZ	Subparaventricular zone
SS	Sleep spindle
STG	Superior temporal gyrus
SWC	Spike and slow wave complex
SWS	Slow wave sleep
TBI	Traumatic brain injury

TC	Thalamocortical
TE	Echo time
Th	Thalamus
TLE	Temporal lobe epilepsy
TLS	Temporal lobe seizures
TMN	Tuberomamillary nucleus
TMS	Transcranial magnetic stimulation
TPJ	Temporoparietal junction
TR	Repetition time
TSC	Tuberous sclerosis
VAN	Ventral attention network
vlPAG	Ventrolateral periaqueductal grey
VLPO	Ventrolateral preoptic nucleus
vPAG	Ventral periaqueductal grey matter
VSW	Vertex sharp wave

Chapter 1

INTRODUCTION

This chapter will introduce the main themes of epilepsy and sleep followed by the historical discussion of both the electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI) and their simultaneous acquisition in both epilepsy and sleep. The progression from epilepsy to sleep is one of a practical progression, as clarified in section 1.2.3, rather than an investigation of their relationship.

1.1 EPILEPSY

1.1.1 Overview: history and epidemiology of epilepsy

Epilepsy, a disease characterised by habitual seizures, is a complex disease of many aetiologies, involving an interaction between dysfunctional neurons and aberrant brain networks, and of all diseases has one of the richest and most stigmatised histories across many cultures. The very first accounts of epilepsy can be traced back over 4000 years ago, circa 2000BC; documented in the Akkadian language, here the affliction was attributed to the god of the Moon (Magiorkinis, Sidiropoulou & Diamantis, 2010). Around 1000 years later the Babylonians produced the *Sakikku*, a medical text with descriptions of a variety of seizure disorders, both focal and generalised, offering instructions for diagnosis and treatment (Eadie, 1995). Perhaps the most famous early account of epilepsy however, came 500 years later from Hippocrates, a renowned Greek physician who penned the treaties *On the Sacred Disease* (Eadie, 1995). In this text Hippocrates was the first to differentiate between epilepsy as a "sacred disease" and one of natural causes like any other, and alongside his colleagues were astute enough to note the semiology of seizures following head injury would start contralateral to the side of the injury. The religious or supernatural pretext continued to be attributed as the underlying cause of epilepsy, with gospel writings in The New Testament from Mark (9:16), ascribing the cause of epilepsy in a child to the possession of an evil spirit that Jesus exorcised (DeToledo & Lowe, 2003). The supernatural origin of epilepsy persisted up until the 18th and 19th centuries where it slowly became accepted that there was an underlying physiological cause for epilepsy; though many misunderstandings still surrounded the disease, such as contagion (Masia & Devinsky, 2000). To this day the idea of a religious or supernatural basis of epilepsy still endures. A recent study conducted by Obeid and colleagues (2012) in Saudi Arabia, canvased university-educated

school teachers and undergraduate students on their knowledge of epilepsy, finding up to 50 per cent of undergraduate students and 40% of teachers stating possession as a cause for epilepsy (Obeid, Abulaban, Al-Ghatani, *et al.*, 2012). There have also been published case reports of epilepsy as instigated by Voodoo spirit possession in Haitian patients (Cavanna, Cavanna & Cavanna, 2010). Despite these enduring ideas it is now widely accepted that epilepsy, as Hippocrates originally postulated, is the result of a physiological disease process rather than a supernatural one.

Epilepsy is one of the most common neurological diseases that can affect individuals irrespective of age and it is estimated that the number of people worldwide with epilepsy stands at around 50 million (Anon, 2010). In Europe the annual incidence rate of epilepsy per year is around 50-55 per 100,000, though this varies between 30 and 100 per 100,000 depending on the age; in children and adolescents the incidence rate per 100,000 is 70, between the ages of 20 and 64 the incidence rate is 30 and for the elderly, 65 years and older, the incidence rate is 100 per 100,000 (Forsgren, Beghi, Oun, *et al.*, 2005). The prevalence of active epilepsy in Europe is estimated at around 6 per 1000, of course this varies with age with a higher prevalence observed in the elderly (Forsgren, Beghi, Oun, *et al.*, 2005). In comparison with the United States, the prevalence of active epilepsy is similar with a value of 6.8 per 1000 (Hauser, Annegers & Rocca, 1996). Some of the highest prevalence figures have been reported in developing countries with one study conducted in Chile reporting a prevalence of 17.6 per 1000, this was a more anomalous figure however, and not representative of developing countries where most reported a prevalence of less than 10 (Banerjee, Filippi & Allen Hauser, 2009). In the UK one of the largest community-based studies of neurological diseases was conducted by MacDonald and colleagues (2000), who prospectively determined the incidence of neurological diseases in 100,230 patients over an 18-month period (MacDonald, Cockerell, Sander, *et al.*,

2000). They identified epilepsy as the 3rd most prevalent neurological disorder, behind strokes and transient ischaemic attacks, with an incidence of 46 per 100,000 and a lifetime prevalence of active epilepsy of 4 per 1000. One of the most recent studies in the UK of 37.7 million patients found just over 304,000 had epilepsy, with a prevalence of 8 per 1000; though this study was not age or sex adjusted and relied heavily on the accurate GP coding of epilepsy in the Quality and Outcomes Framework from which the data were acquired (Steer, Pickrell, Kerr, *et al.*, 2014). In this ecologic investigation they interestingly revealed a large geographical variation in prevalence of epilepsy; a prevalence of 4.3 per 1000 was found in areas such as Kensington and Chelsea with 11.6 per 1000 in Blackpool, highlighting a correlation with epilepsy various measures of socioeconomic deprivation. With the numbers of those with epilepsy being so high, epilepsy has the potential to represent a significant economic burden on health and social services and indeed it does.

The total annual cost of epilepsy in the UK, based on data published in 2011, is an estimated €1.6bn, around £1.2bn (Gustavsson, Svensson, Jacobi, *et al.*, 2011). In young adults (18-25 years old) health and social care outlays for epilepsy costs the budgets £715m per year with around £17,000 spent per individual (Beecham, Snell, Perkins, *et al.*, 2010). The high costs of epilepsy can be a reflection of long term pharmacological treatment and recurrent hospital admissions. Of all the patients with epilepsy approximately 1 in 8 will develop a drug-resistant epilepsy, defined by inadequate seizure control through anti-epileptic medication, though figures of over 30% have also been reported (Kwan & Brodie, 2000; Picot, Baldy-Moulinier, Daurs, *et al.*, 2008). The accurate prevalence is contentious owing to varying definitions of a drug-resistant epilepsy and study design (Abimbola, Martiniuk, Hackett, *et al.*, 2011; Téllez-Zenteno, Hernández-Ronquillo, Buckley, *et al.*, 2014). In these cases, where a drug-resistant epilepsy has become established, it may be necessary to explore alternative treatment options,

in particular epilepsy surgery. The most common operative procedure for epilepsy is resective surgery, though neuromodulatory approaches, such as vagal nerve stimulation, are becoming more common, however, are not considered a curative option (Cascino, 2004; Ogbonnaya & Kaliaperumal, 2013). The hypothesis underlying resective surgery is the removal of the epileptogenic zone, the presumed site in which seizures initiate (Cascino, 2004; Rosenow & Lüders, 2001). The two key investigations for identifying the target for resection is the electroencephalogram (EEG) and magnetic resonance imaging (MRI), though other imaging modalities are also utilised (Rosenow & Lüders, 2001). The use of medical imaging such as MRI has increased rapidly over the years and has seen increased use in the pre-surgical evaluation of epilepsy (Hinde, Soares, Burch, *et al.*, 2014). As diagnostically useful as the EEG is, it is limited in terms of spatial resolution and as such the MRI and EEG are viewed as complementary investigations; the primary purpose of the MRI is to identify a lesion that could potentially be epileptogenic and is concordant with the findings from scalp EEG. Unfortunately, not all epilepsies originate from a focal epileptogenic lesion and the odds of seizure freedom following resective surgery are up to two to three times greater if there is a lesion on the MRI than if no lesion is identifiable (Téllez-Zenteno, Ronquillo, Moien-Afshari, *et al.*, 2010). In the absence of an identifiable lesion there may be a requirement of chronically implanted intracranial electrodes (sub-dural or intracerebral) to better identify and demarcate the epileptogenic zone and target of resection (Rosenow & Lüders, 2001). The advancement of imaging techniques over the last two decades has brought about the opportunity to use novel methods of delineating the seizure onset zone without the use of such invasive methods. The combination of EEG and functional-MRI (fMRI), a technique measuring changes in blood oxygenation, has been increasingly used to identify the brain regions responsible for the generation of interictal epileptiform discharges as recorded on EEG, with the aim of providing

non-invasive method to inform on potential resective targets (Gotman, Kobayashi, Bagshaw, *et al.*, 2006; Zijlmans, Huiskamp, Hersevoort, *et al.*, 2007).

The initial work in this thesis applies similar methods, using EEG-fMRI in a cohort of complicated heterogeneous epilepsy patients to assess the utility of EEG-fMRI in a clinical setting, as if this were a technique available to the epileptologist.

1.1.2 Definition of epilepsy

Epilepsy is a difficult disorder to define, due in large part to the lack of a singular cause, but rather it is a reflection of brain dysfunction from many possible aetiologies. The International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) have formed task groups with the role of defining epilepsy and producing a comprehensive classification system. In 2005, such a task force was charged with developing conceptual definitions of both epilepsy and an epileptic seizure; defining epilepsy as *"... a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure*" and an epileptic seizure as "*... a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain*" (Fisher, van Emde Boas, Blume, *et al.*, 2005). When published, these definitions and their further elaboration within the article met with some criticism from the wider community of epilepsy clinicians who felt they lacked clarity (Beghi, Berg, Carpio, *et al.*, 2005). The criticism was primarily directed to the lack of a practical definition of epilepsy that could be applied readily in a clinical setting, with clear explanations of terms within the definition. Concern was expressed that diagnosing epilepsy by the presence of a single seizure could cause an erroneous increase in epilepsy incidence and misdiagnosis (Beghi, Berg, Carpio,

et al., 2005). One significant issue is that the definition does not state the requirement of an unprovoked seizure, thus a single acute symptomatic seizure (i.e. seizure following a stroke), under this new definition, could be classified as epilepsy; such scenarios could result in a number of patients being misdiagnosed. The previous definition outlined by the ILAE required at least two unprovoked seizures for a diagnosis of epilepsy (Commission on Epidemiology and Prognosis, International League Against Epilepsy, 1993); this definition has been more widely accepted by clinicians and epidemiologists (Berg, 2008; Hauser & Beghi, 2008). The risk of recurrence following a single unprovoked seizure has been studied in multicentre trials, both with and without follow-up treatment. For example, the MESS (Multicentre Epilepsy and Single Seizure study) trial involved 1847 patients, with the 1443 who consented randomised into immediate or deferred treatment groups (Marson, Jacoby, Johnson, *et al.*, 2005). They found a 50% risk of a further seizure within 5 years following the first seizure when treatment was differed. These findings are similar to an earlier trial from the First Seizure Trial Group, 1993, who found the risk was 51% of seizure recurrence after 2 years. The risk of recurrence following a single acute symptomatic seizure was actually found to be lower than an unprovoked seizure (Hesdorffer, Benn, Cascino, *et al.*, 2009). Based on these findings if one adhered to the definition laid out by Fisher et al., 2005, a number of patients who may never have a recurrent seizure could be placed on treatment and diagnosed as epileptic. More recently, a "practical clinical definition of epilepsy" has been proposed that epilepsy can be established after a single unprovoked seizure where additional factors exist that could precipitate a high risk of recurrence, although in the absence of this the previous definition of two unprovoked seizures temporally separated by more than 24 hours still stands (Fisher, Acevedo, Arzimanoglou, *et al.*, 2014). In reality it is apparent that following a single unprovoked seizure or even a single acute symptomatic seizure, there are those who will go on to have recurrent

seizures, and at present there is no clear way of knowing who will have a propensity for seizures in the absence of specific markers (Sirven, 2009).

1.1.3 Classification of epilepsy

After the diagnosis of epilepsy is made, the epilepsy subsequently needs to be classified in order to best treat the patient. Currently the most widely used classification system for epilepsy was produced by the ILAE in the 1980s (Commission on Classification and Terminology, 1989). In the context of an advancing field, it might seem unusual that the International Classification of Epilepsies and Epileptic syndromes (ICE) produced in the '80s still remains at the forefront. Attempts at revising and updating the classifications of epilepsy took place in 2001, 2006 and 2010 (Engel 2001, Engel 2006, Berg 2010). Despite the changes and alterations to the ICE offered in the latest revision, the wider community of epilepsy specialists found the changes insufficient and are forced in many cases to refer to the ICE laid out in the '80s (Ferrie, 2010; Lüders, Amina, Baumgartner, *et al.*, 2012; Panayiotopoulos, 2012, 2011; Wolf, 2010). Although the revised classification made in 2010 (Berg, Berkovic, Brodie, *et al.*, 2010) has been the subject of criticism, some feel it is at least a move in the right direction, certainly with the inclusion of epilepsy as a network based disorder (Fisher, 2010; Shinnar, 2010). Producing a comprehensive and widely accepted classification based on the latest academic, clinical and epidemiological findings in epilepsy, would help unify and advance the field. Although no particular discussion to classification of epilepsies is made in the experimental body of this thesis, the classifications used are generally based on those from the 1980's and in 2001; this is due to the epileptologists from whom patients were recruited. The classification of epilepsy of the recruited patients into this study are from the patient's neurologist/epileptologist, and as

such based on the 1980's and 2001 classification they referred to at the time of recruitment (see Table 1.1.1. for an example of classifications).

Although there are various investigations available to the clinician in the diagnosis of epilepsy, the most prominent of which being the electroencephalogram (EEG), the diagnosis of epilepsy still remains clinically driven. Since the days of Hippocrates there have been detailed accounts of differing seizure manifestations based on careful observation of the seizure, the clinical presentation of which is referred to as the semiology of the seizure. The classification of a seizure disorder is generally based on clinical and EEG findings, though seizure semiology is viewed by some to be so fundamental as to warrant a classification system based on semiology alone (Lüders, Acharya, Baumgartner, *et al.*, 1999, 1998). Relying solely on the semiology of a seizure, however, may lead to incorrect classification or misdiagnosis, between epileptic seizures, syncopal attacks and psychogenic seizures, and there is also the risk of hidden propagation, undetectable on non-invasive EEG recordings, that can lead to incorrect localisation and classification (Panayiotopoulos, 2005). Accurate seizure classification is better achieved when using the diagnostic techniques available to the clinician, and some believe the current classification system needs to be updated to include the advances made in diagnostics (Lüders, Amina, Baumgartner, *et al.*, 2012).

Group of syndromes	Specific syndromes with group
Idiopathic focal epilepsies of infancy and childhood	Benign infantile seizures (nonfamilial)
	Benign childhood epilepsy with centrotemporal spikes
	Early-onset benign childhood occipital epilepsy (Panayiotopoulos type)
	Late-onset childhood occipital epilepsy (Gastaut type)
Familial (autosomal dominant) focal epilepsies	Benign familial neonatal seizures
	Benign familial infantile seizures
	Autosomal dominant nocturnal frontal lobe epilepsy
	Familial temporal lobe epilepsy
	Familial focal epilepsy with variable foci*a*
Symptomatic (or probably symptomatic) focal epilepsies	Limbic epilepsies Mesial temporal lobe epilepsy with hippocampal sclerosis Mesial temporal lobe epilepsy defined by specific etiologies Other types defined by location and etiology
	Neocortical epilepsies Rasmussen syndrome Hemiconvulsion–hemiplegia syndrome Other types defined by location and etiology Migrating partial seizures of early infancy*a*
Idiopathic generalized epilepsies	Benign myoclonic epilepsy in infancy
	Epilepsy with myoclonic astatic seizures
	Childhood absence epilepsy
	Epilepsy with myoclonic absences
	Idiopathic generalized epilepsies with variable phenotypes
	Juvenile absence epilepsy
	Juvenile myoclonic epilepsy
	Epilepsy with generalized tonic–clonic seizures only
	Generalized epilepsies with febrile seizures plus*a*
Reflex epilepsies	Idiopathic photosensitive occipital lobe epilepsy
	Other visual sensitive epilepsies
	Primary reading epilepsy
	Startle epilepsy
Epileptic encephalopathies (in which the epileptiform abnormalities may contribute to progressive dysfunction)	Early myoclonic encephalopathy
	Ohtahara syndrome
	West syndrome
	Dravet syndrome (previously known as severe myoclonic epilepsy in infancy)
	Myoclonic status in nonprogressive encephalopathies*a*
	Lennox–Gastaut syndrome
	Landau–Kleffner syndrome
	Epilepsy with continuous spike–waves during slow-wave sleep
Seizures not necessarily requiring a diagnosis of epilepsy	Benign neonatal seizures
	Febrile seizures
	Reflex seizures
	Alcohol-withdrawal seizures
	Drug or other chemically induced seizures
	Immediate and early posttraumatic seizures
	Single seizures or isolated clusters of seizures
	Rarely repeated seizures (oligoepilepsy)

Table 1.1.1. Overview of the classification of epilepsy syndromes and their respective group. Adapted from the 2001 classification, Engel, J., 2001. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 42, 796–803.

1.1.4 Mechanisms in epilepsy

This section covers the most common and well described mechanisms in epileptogenesis, with particular focus on the pathophysiology of childhood absence epilepsy and temporal lobe epilepsy, owing to their prevalence and well described mechanisms.

Channelopathies

As previously mentioned, epilepsy is a complex disease process of many aetiologies and consequently there is no single unifying model for pathogenesis, but rather several described models/mechanisms that best fit certain epilepsy types. A group of epilepsies termed idiopathic generalised epilepsy (IGE), are often associated with a genetic basis, and in most cases are the result of channelopathies (Badawy, Harvey & Macdonell, 2009; Mulley, Scheffer, Petrou, *et al.*, 2003). A number of these idiopathic epilepsies, are inherited through Mendelian inheritance; these mutations in single genes in turn cause mutations of certain ion channels, resulting in said channelopathy (for review on genetics of channelopathies and a list of described channelopathies in epilepsy syndromes see George, 2004). Ion channels are a group of proteins found in the lipid bilayer of the cell membrane of all animals and are essential in coordinating activity in the central and peripheral nervous system. The cell membrane is relatively impermeable to ions, so to allow passage, these ion channels provide a door through which ions may pass into or flow out of the cell. These channels may be non-gated, allowing the free movement of ions in the presence of changing ion gradients, or gated, where upon an external signal is required, either from specific binding molecules (i.e. neurotransmitter), or from local changes to the membrane potential near the channel; these are known as ligand-gated and voltage-gated ion channels, respectively. Voltage gated channelopathies have been

described in a number of epilepsies, such as potassium gated channelopathies in benign familial neonatal epilepsy, epileptic encephalopathy and benign epilepsy (Abidi, Devaux, Molinari, *et al.*, 2015; Maljevic & Lerche, 2014) and calcium gated channelopathies, in childhood absence epilepsy (Chen, Parker & Wang, 2014). Gamma aminobutyric acid (GABA) mediated ligand gated channelopathies have also been implicated in childhood absence epilepsy (CAE) and autosomal dominant juvenile myoclonic epilepsy (Baulac, Huberfeld, Gourfinkel-An, *et al.*, 2001; Cossette, Liu, Brisebois, *et al.*, 2002). For review of channelopathies in epilepsy see (George, 2004; Noebels, Avoli, Rogawski, *et al.*, 2012).

Malformations of cortical development

During the intrauterine development of the central nervous system, the neocortex is organised into six layers (Budday, Steinmann & Kuhl, 2015). This complex arrangement initiates with the migration of progenitor cells from the ventricular layer, occurring around week 5 of gestation, forming the sixth layer first with subsequent progenitor cells migrating through the forming layers (Budday, Steinmann & Kuhl, 2015). Disorders occurring as a result of abnormal development of this process were initially referred to as migration disorders, though now are better known as malformations of cortical development (MCD) (Aronica, Becker & Spreafico, 2012). MCD are closely associated with epilepsy, as of those with MCD 75 per cent will develop epilepsy at some point in their lifetime (Leventer, Guerrini & Dobyns, 2008). There are numerous types of MCD including tuberous sclerosis complex (TSC), focal cortical dysplasia (FCD), heterotopias, lissencephaly, polymicrogyria, hemimegalencephaly, and schizencephaly (Aronica, Becker & Spreafico, 2012). In TSC, mutation of the TSC1 and TSC2 genes have been implicated in this autosomal dominant disorder (Kandt, Haines, Smith, *et al.*,

1992; van Slegtenhorst, de Hoogt, Hermans, *et al.*, 1997), and it is associated with malady of the CNS in 90% of patients with TSC (Curatolo, Moavero & de Vries, 2015). Just over 85% of patients with TSC will have seizures, often developing multiple seizure types and a refractory epilepsy (Chu-Shore, Major, Camposano, *et al.*, 2010). Like TSC, FCDs are also highly associated with epilepsy, as reported by Wyllie et al., 1998, who in a surgical series of paediatric epilepsy cases found 26% to have FCD (Wyllie 1998). It was in 1971 when Taylor et al., observed large dysmorphic neurons and enlarged balloon cells in 10 epilepsy patients who had undergone resective surgery and first defined FCD (Taylor, Falconer, Bruton, *et al.*, 1971). The exact pathogenesis of FCD is not fully understood though it has been primarily linked to abnormalities in intrauterine neuronal migration but also both peri- and post-natal injury (Aronica, Becker & Spreafico, 2012; Coras, de Boer, Armstrong, *et al.*, 2012; Rakic, 1988). The ILAE have developed an extensive classification system for FCDs which should help unite the field and further develop our understanding (Blümcke, Thom, Aronica, *et al.*, 2011; Coras, de Boer, Armstrong, *et al.*, 2012).

Post-traumatic epilepsy

Post traumatic epilepsy (PTE) is a complication of a traumatic brain injury (TBI), and it is estimated that in 5% of patients with epilepsy have PTE (Agrawal, Timothy, Pandit, *et al.*, 2006). Whether one develops PTE following a TBI is dependent on a number of factors, such as a depressed skull fracture, intracranial bleed, penetrating head injury or a Glasgow coma scale of $\leq$10, among others (Temkin, 2003) and the risk of seizures following a TBI is between approximately 2-5% in the non-military population (Asikainen, Kaste & Sarna, 1999). An important risk factor for developing PTE is when a post traumatic seizure (PTS) occurs in

relation to the TBI. Early PTSs, occur within one week of TBI, whereas late PTSs occur after that period, with the risk of developing PTE greater if an early PTS has occurred (Frey, 2003). However, the risk of developing PTE/recurrent seizures following the first late PTS is also quite high (Haltiner, Temkin & Dikmen, 1997). The exact pathophysiology underlying PTE is not fully understood and varies dependent on type and severity of head injury. In haemorrhagic injuries there can be resulting haemosiderin deposits and gliosis, as identified on MRI (Messori, Polonara, Carle, *et al.*, 2005), which are known contributors to epileptogenesis (Pekny, Wilhelmsson & Pekna, 2014; Ruan, Yu, Shrestha, *et al.*, 2015). Ischemic injuries or penetrating injuries, for example, are likely to have separate mechanisms to a haemorrhagic injury, highlighting the complexity of epileptogenesis in this patient group (see Agrawal, Timothy, Pandit, *et al.*, 2006 for review)

Temporal lobe epilepsy

Partial/focal seizure disorders account for about 68-75% of adult epilepsies, and given that around 75% of new cases of epilepsy are in adults, this makes partial seizures the prevailing seizure type (Forsgren, Beghi, Oun, *et al.*, 2005). Of the partial epilepsies mesial TLE (mTLE) is the most prevalent type of epilepsy (Engel, 2001). It is becoming understood that mTLE is a progressive disorder and is often preceded by a traumatic event such as febrile convulsions, a period of status epilepticus, traumatic brain injury, or even encephalitis, with a several year latency period before seizure onset (Sharma, Reams, Jordan, *et al.*, 2007; Yang, Zhou & Stefan, 2010). The hippocampus, among other structures such as the amygdala, form part of the mesial temporal structures, and changes in the hippocampus are the most common cause of mTLE (Engel, 2001). Hippocampal sclerosis (HS) is a common neuropathological finding in patients

with refractory mTLE (Blümcke, Beck, Lie, *et al.*, 1999; Jefferys, 1999; Wieser, 2004). Neuronal loss in CA1, CA3 and CA4 regions of the hippocampus are well described in HS, as well as neuronal loss in the amygdala and neighbouring entorhinal cortex (Bernhardt, Kim & Bernasconi, 2013; Blümcke, Thom, Aronica, *et al.*, 2013; Yilmazer-Hanke, Wolf, Schramm, *et al.*, 2000). Aside from neuronal loss, other features are observed in both animal models and resected human tissue including gliosis and aberrant mossy fibre sprouting (O'Dell, Das, Wallace, *et al.*, 2012; Yang, Zhou & Stefan, 2010). Gliosis, a common feature of HS, occurs after damage or loss of neurons, a prominent feature of which is astrogliosis, a local increase and hypertrophy of astrocytes. It has been hypothesised that this astrogliosis, through the release of trophic factors (molecules that help maintain connections between neurons), can contribute to epileptogenesis through axonal sprouting, neurosynaptogenesis and increased glutamate release, all of which may in turn lead to chronic hyperexcitability (Crespel, Coubes, Rousset, *et al.*, 2002; Represa, Niquet, Pollard, *et al.*, 1995; Takahashi, Vargas & Wilcox, 2010). Gliosis has also been implicated in mossy fibre sprouting, a commonly observed feature across both animal models and human resected tissue (O'Dell, Das, Wallace, *et al.*, 2012; Sharma, Reams, Jordan, *et al.*, 2007; Thom, 2014). Mossy fibres, a form of axonal sprouting, originate from granule cells of the dentate gyrus and normally extend to pyramidal neurons (CA3 initially). However, as observed in HS, mossy fibres project inwards to the supra granular layer and inner molecular layer, possibly due to the loss of their original target neurons. These aberrant connections from mossy fibres potentially cause a form of short circuit, as, for example, they synapse with dendrites of other granule cells, facilitating recurrent excitatory loops, and are an integral part of the popular recurrent excitation hypothesis (Parent & Lowenstein, 1997; Pitkänen, Kharatishvili, Karhunen, *et al.*, 2007; Pitkänen & Sutula, 2002; Sharma, Reams, Jordan, *et al.*, 2007; Wuarin & Dudek, 1996). A contrasting hypothesis, the "dormant basket

cell hypothesis" revolves around the loss of hilar mossy cells, which are known to be vulnerable to excitotoxic damage. The hilar mossy cells usually provide input to inhibitory interneurons (basket cells) that inhibit granule cells. Loss of this inhibition essentially causes disinhibition of the dentate granule cells forming a dentate network that could be prone to seizures (Jinde, Zsiros & Nakazawa, 2013; Sloviter, 1991). This hypothesis is less commonly accepted due to various other experimental observations that have conflicting findings; including mossy hilar cells innervating granule cells rather than basket cells, and mossy hilar cell loss resulting in a reduction in granule cell excitability (for review see Sharma, Reams, Jordan, *et al*., 2007).

The mechanisms underlying mTLE, although becoming increasingly understood, have yet to be fully elucidated. A recent focus of research has been the observed progression of mTLE, underpinning it as a progressive disorder. As previously mentioned, mTLE usually occurs sometime following a traumatic event, indicating a possible hidden progression towards seizures. Patients with TLE often become pharmacoresistant over time and present with worsening cognitive symptoms which may be related to the progression of mTLE through possible inflammatory mechanisms (Das, Wallace, Holmes, *et al.*, 2012; Helmstaedter & Elger, 2009; Marques, Caboclo, da Silva, *et al.*, 2007; Yang, Zhou & Stefan, 2010). There are an increasing number of studies investigating the link between inflammation and progression of mTLE (for review see Yang, Zhou & Stefan, 2010), offering a potential new treatment pathway in halting inflammation to slow or stop disease progression and provide a better means for seizure control.

Childhood absence apilepsy

Unlike mTLE, childhood absence epilepsy (CAE) is classified as a generalised epilepsy rather than a focal one, manifesting primarily in a transient cessation of function and impairment of consciousness (Matricardi, Verrotti, Chiarelli, *et al.*, 2014). Accounting for about 10–12% of epilepsies in paediatrics and presenting in children primarily between the ages of four and eight, CAE is one of the most common presentations of generalised epilepsy in the paediatric population (Berg, Shinnar, Levy, *et al.*, 1999; Callenbach, Geerts, Arts, *et al.*, 1998; Hughes, 2009). The favoured mechanism in the generation of these seizures is believed to come from a thalamocortical network that is integral to transitioning sleep states and the generation of sleep paroxysms (Futatsugi & Riviello, 1998; Matricardi, Verrotti, Chiarelli, *et al.*, 2014; Steriade, 2005). This thalamocortical circuit comprises primarily of three groups of neurons; pyramidal neurons of the neocortex, thalamic relay neurons and reticular thalamic neurons. Reticular thalamic neurons provide projections, mediated by $GABA_B$ receptors, to thalamic relay neurons, which then project onto cortical neurons, and both cortical neurons and thalamic relay neurons provide excitatory input onto reticular thalamic neurons. This thalamocortical circuit is also modulated by neighbouring thalamic relay neurons projecting inwards via GABAergic pathways ($GABA_A$) and cortical interneurons (Chen, Parker & Wang, 2014; Steriade, 2006). Interaction between these groups of neurons enables activation of two firing modes of cortical pyramidal neurons; burst and tonic firing modes. The tonic mode is active during wakefulness and rapid eye movement (REM) sleep, and the burst mode is active during non-REM sleep (Domich, Oakson & Steriade, 1986; Fuentealba & Steriade, 2005; Steriade, 2005). The burst firing mode observed in non-REM sleep is a consequence of the T-type calcium channels that enable low threshold depolarisations and subsequently bursts of action potentials (Perez-Reyes, 2003). These bursts are the underlying basis for sleep spindles, a sleep paroxysm observed in

non-REM sleep, mediated by thalamic reticular neurons which are considered the pacemaker for sleep spindles (Contreras & Steriade, 1996; Steriade, 2003). Aberrations in this thalamocortical circuit, specifically in T-type calcium channels, are hypothesised to be the cause of absence seizures and the accompanying 3-4Hz spike and slow wave complexes (SWC) evident on the EEG during this ictal period (Chen, Parker & Wang, 2014; Pinault & O'Brien, 2005). These T-type calcium channels are located at all nodes of the circuit, i.e. thalamic reticular, thalamic relay and cortical pyramidal neurons, though where in the circuit absence seizures are generated is not yet fully elucidated. There is evidence for both a thalamic origin (Seidenbecher, Staak & Pape, 1998) and a cortical origin (Meeren, Pijn, Van Luijtelaar, *et al.*, 2002; Polack, Guillemain, Hu, *et al.*, 2007; van Luijtelaar, Hramov, Sitnikova, *et al.*, 2011) for absence seizures though recently it has been shown that both cortex and thalamus are intrinsic in the generation of SWC and absence seizures, with recent findings also implicating thalamic and cortical glial cells (van Luijtelaar & Zobeiri, 2014). It is apparent that exact mechanisms of absence seizures and the generation of SWC are still yet to be clearly elucidated.

Networks in epilepsy

Much of the understanding of the mechanisms underlying epilepsy come from studies at the neuronal level, but how this translates into wider brain networks has become an evolving area of interest. The advancement in this area has prompted revisions in the ILAE classification and terminology including brain networks in epilepsy as a core component in the new revisions (Berg, Berkovic, Brodie, *et al.*, 2010). An example of the importance of these networks lies in the thalamocortical networks as previously mentioned; absence seizures are often accompanied by characteristic 3-4Hz SWC, though SWCs are not seen exclusively with absence seizures or

at 3-4Hz, but variable in frequency, morphology, electrographic distribution and across different epilepsies, both focal and generalised. Differing pathological mechanisms and networks may give rise to SWCs as the ultimate result of this process (Blumenfeld, 2005). Neuroimaging studies using modalities such as positron emission tomography (PET), single-photon emission computed tomography (SPECT), MRI, diffusion tensor imaging (DTI) and fMRI allow for non-invasive investigation of brain regions, functional networks and structural networks that may be linked to ictogenesis (Gotman, 2008; Gotman, Kobayashi, Bagshaw, *et al.*, 2006; Liu, Concha, Lebel, *et al.*, 2012; Richardson, 2010; Scanlon, Mueller, Cheong, *et al.*, 2013). Theoretical work has demonstrated that seizure discharges can occur independently of a single abnormality in a brain region and occur from alterations in network structure (Terry, Benjamin & Richardson, 2012). Elaborations on this theoretical work, using graph theory, identify increased seizure susceptibility as a result of functional network structure (Schmidt, Petkov, Richardson, *et al.*, 2014). In patients with TLE, structural changes in white matter tracts, as identified by DTI, have been reported in tracts beyond the temporal lobe, indicating different dysfunctional networks may be present and that TLE is a systemic disorder (Gross, 2011; Liu, Chen, Beaulieu, *et al.*, 2014; Liu, Concha, Lebel, *et al.*, 2012). Functional studies using fMRI data have identified aberrations in well-defined functional brain networks, as well as functional connectivity changes in multiple brain regions with differing epilepsy types (Haneef, Lenartowicz, Yeh, *et al.*, 2014; Lopes, Moeller, Besson, *et al.*, 2014; Luo, Li, Lai, *et al.*, 2011; Maneshi, Moeller, Fahoum, *et al.*, 2012; Wei, An, Zeng, *et al.*, 2015a). Computational models of neuronal systems, structural and functional imaging of brain regions and networks have broadened our understanding of epilepsy and may help elucidate the genesis of seizures from neuron to network. This is further elaborated on in section 1.5.2.

1.2 SLEEP

Sleep, in one form or another, is ubiquitous across the animal kingdom and dominates a third of our lives. The function of sleep has long been the subject of debate and does not necessarily subserve the same functions, or is expressed in the same way, across species (Siegel, 2008). A popular theory on the function of sleep is the conservation of energy, a passive process unobtainable during waking states (Berger & Phillips, 1995). However, sleep is not a passive state, as demonstrated by the increased activity of the brain during rapid eye movement (REM) sleep (Braun, Balkin, Wesenten, *et al.*, 1997; Madsen & Vorstrup, 1991; Maquet, 2000). If not conservation of energy, another common theory is that of restoration (Benington & Heller, 1995), in which the brain can replenish glycogen stores (Dworak, McCarley, Kim, *et al.*, 2010; Petit, Burlet-Godinot, Magistretti, *et al.*, 2015) and, in-line with restoration, clear potentially neurotoxic interstitial waste products (Xie, Kang, Xu, *et al.*, 2013). There is also considerable evidence that sleep facilitates memory formation, consolidation and synaptic plasticity (Benington & Frank, 2003; Chauvette, Seigneur & Timofeev, 2012). Attempts have been made to create a unifying hypothesis of sleep, a hypothesis that neatly packages the functions of sleep; the energy allocation model, a model that focuses on an organisms need to optimally allocate limited energy resources (Schmidt, 2014) and the synaptic homeostasis hypothesis, which states "*sleep is the price the brain pays for plasticity*" (Tononi & Cirelli, 2014, 2006), are two such theories, though the latter has been challenged due to its poorly defined mechanisms and lack of empirical basis (Frank, 2013). Ultimately sleep serves many functions and it is difficult to package into a single answer or hypothesis that fits everyone. Given the diversity of sleep research, functions and mechanisms in sleep, the focus of this section on sleep will be to give a general overview of some basic mechanisms in sleep and of the chief sleep paroxysms that

occur during non-REM (NREM) periods of the NREM-REM sleep cycle, discussed in the next section.

1.2.1 Mechanisms of sleep

Sleep-Wake Networks

The transitions between sleep and wake states involve a number of brain regions and their complex interactions. It was Moruzzi and Magoun, in 1949, who described in detail the reticular activating system (RAS) as a mechanism of regulating wakefulness (Moruzzi & Magoun, 1949), though it was later shown that the reticular formation was not solely responsible for arousal but also a number of specific cells groups are involved (Saper, Chou & Scammell, 2001). The modern day idea of the ascending reticular activating system (ARAS) is a combination of these additional cell groups and the reticular formation (McGinty & Szymusiak, 2011). The ARAS can be divided into two major constituent branches, one that projects to the thalamus and the other to the cortex, via the lateral hypothalamus and basal forebrain, circumventing the thalamus (Jones, 2003; Saper, Chou & Scammell, 2001). Input to the thalamus comes from two cell groups containing cholinergic neurons, the pedunculopontine nucleus (PPT) and the laterodorsal tegmental nucleus (LDT), both producing acetylcholine (ACh), a neurotransmitter (Saper, Chou & Scammell, 2001). These neurons fire most rapidly during both a waking state and during REM sleep (Kajimura, Uchiyama, Takayama, *et al.*, 1999; Kayama, Ohta & Jodo, 1992) and, when activated during NREM sleep, can initiate REM sleep periods (Van Dort, Zachs, Kenny, *et al.*, 2015). The input of these neurons to the reticular thalamic nucleus decouples the synchronisation in the thalamocortical networks which produce sleep oscillations, thus allowing a state of cortical excitability necessary for wakefulness and

cognition (McCormick, 1989; Steriade, 1994). Although the PPT and LDT are the principle nuclei in wakefulness and arousal, other inputs to the thalamus also play a role in arousal; intralaminar and midline nuclei and parabrachial nucleus (see (Saper, Scammell & Lu, 2005) for review). The other branch that evades the thalamus, projecting primarily to the cortex, contains a number of cell groups, that, unlike the cholinergic neurons of the PPT and LDT, contain a number of different neurotransmitters (Jones, 2003; Kayama & Koyama, 2003). The noradrenergic neurons of the locus coeruleus (LC), the serotoninergic neurons of the raphe nuclei, the histaminergic neurons of the tuberomamillary nucleus (TMN), and the dopaminergic neurons of the ventral periaqueductal grey matter (vPAG) are the origin of the second branch providing input directly to the cortex, and comprising the monoaminergic system (Jones, 2003; Kayama & Koyama, 2003; Saper, Scammell & Lu, 2005). Additional input is provided by the basal forebrain, which contains both cholinergic and GABAergic neurons (Zaborszky & Duque, 2003) and also the lateral hypothalamus with neurons contains melanin concentrating hormone (Monti, Torterolo & Lagos, 2013) and orexin (Alexandre, Andermann & Scammell, 2013). Unlike the previous branch containing the cholinergic PPT and LDT, the monoaminergic neurons of this branch are not active during REM sleep, but again fire at their highest rates during wakefulness (Jones, 2003).

If there are systems that keep us awake, there also must be a system which facilitates sleep. The neurons of the ventrolateral preoptic nucleus (VLPO) send outputs to the hypothalamus, the TMN, LC, raphe nuclei, vPAG and the cholinergic cell bodies, essentially, the majority of the major components of the ARAS (Sherin, Elmquist, Torrealba, *et al.*, 1998; Sherin, Shiromani, McCarley, *et al.*, 1996). The galanin-positive neurons of the VLPO project in majority to the TMN but also to the LC and raphe nuclei, with extended regions of the VLPO, i.e. median preoptic nucleus (MnPO), providing GABAergic efferent projections to the VLPO, vPAG, LC

and dorsal raphe nuclei (Gaus, Strecker, Tate, *et al.*, 2002; Sherin, Elmquist, Torrealba, *et al.*, 1998; Uschakov, Gong, McGinty, *et al.*, 2007). These projections to the major components of the ARAS inhibit said system, however, afferents from the monoaminergic systems that project to the VLPO provide inhibitory input; the interaction between the two systems provides the basis for a model of switching between sleep states, the flip-flop switch model (Saper, Fuller, Pedersen, *et al.*, 2010). See figure 1.2.1.

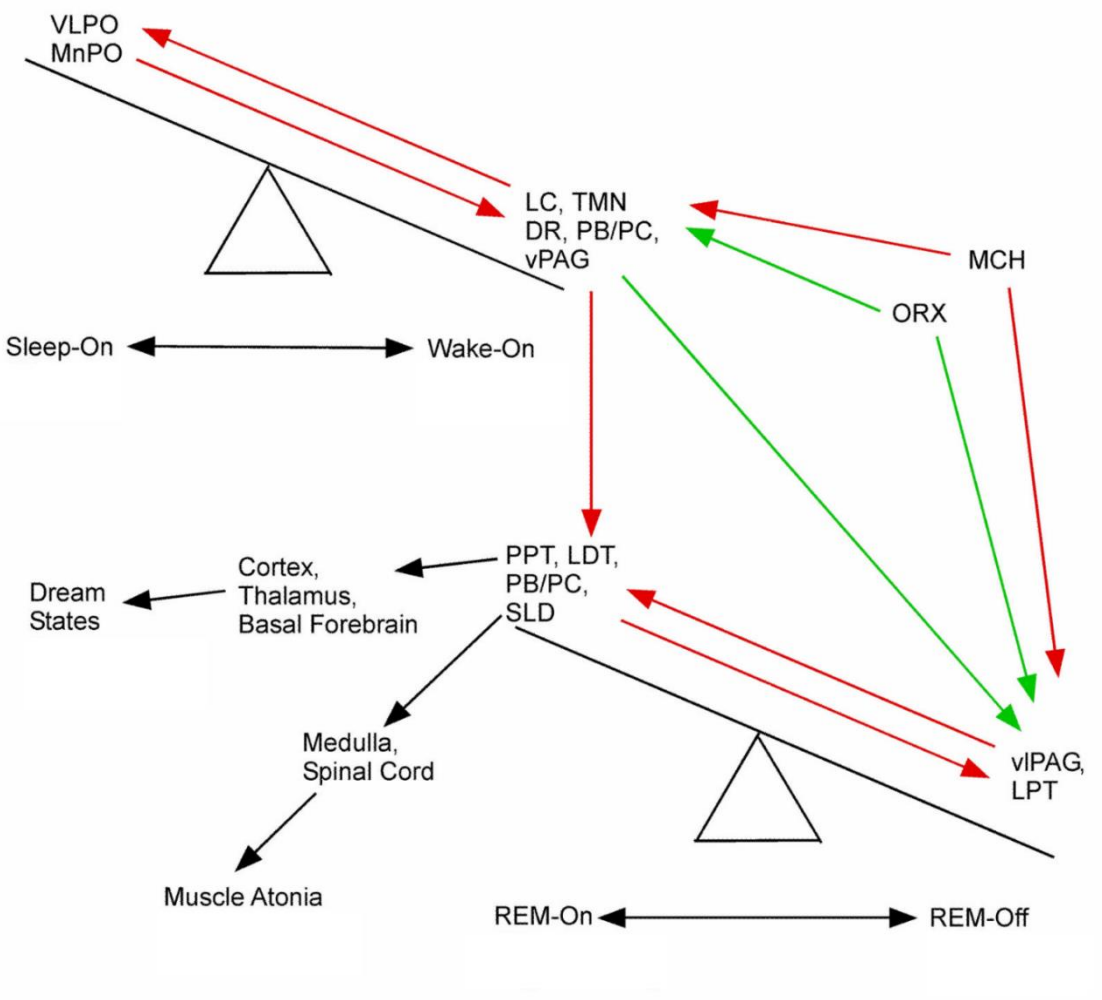


Figure 1.2.1. Summary of the wake and sleep promoting regions of the brain and their interactions with the flip-flop switch model. The red arrows represent inhibitory projections and the green excitatory. The same monoaminergic arousal systems that promotes wakefulness through inhibition of the VLPO, provides inhibitory input to the "REM-on" neurons and excitatory input to the "REM-off" neurons, thus preventing the rapid switch from a state of wakefulness into REM sleep when sleep occurs. This system is stabilised by orexin (ORX) neurons, as demonstrated in narcolepsy, where loss of ORX signalling causes instability on both switches and thus permitting rapid transitions to REM sleep. Melanin-concentrating hormone (MCH) neurons have the opposite profile of ORX neurons, they mainly fire during REM sleep, inhibit the same targets that ORX neurons excite.

VLPO = ventrolateral preoptic nucleus, MnPO = median preoptic nucleus, LC = locus coeruleus, TMN = tuberomamillary nucleus, DR = dorsal raphe nucleus, PB = parabrachial nucleus, PC = precoeruleus area, vPAG = ventral periaqueductal grey, PPT = pedunculopontine nucleus, LDT = laterodorsal tegmental nucleus, SLD = sublaterodorsal region, vlPAG = ventrolateral periaqueductal grey, LPT = lateral pontine tegmentum

Figure taken and modified slightly from Saper et al., 2010.

Switching between wake-sleep states

The model of the flip-flop switch (Figure 1.2.1) is described in great detail in an excellent review by Saper, Fuller, Pedersen, *et al.*, 2010. This mechanism of switching between sleep states enables a rapid transition from wake to sleep and vice-versa. The monoaminergic neurons of the LC, TMN and raphe nucleus, supported by orexin neurons, inhibit the VLPO, thus promoting a waking state. The sleep transition occurs when the VLPO inhibits the same monoaminergic cell groups it receives inhibition from, and consequently inhibits the orexin neurons, inhibiting their support of the monoaminergic cell groups; it is hypothesised that it is orexin neurons from the lateral hypothalamus that are the key to stabilising the switch (Mochizuki, Crocker, McCormack, *et al.*, 2004; Peyron, Tighe, van den Pol, *et al.*, 1998; Saper, Fuller, Pedersen, *et al.*, 2010), a suggestion which comes from the observation that patients with narcolepsy, who experience uncontrollable state transitions, lack orexin neurons (De la Herrán-Arita, Guerra-Crespo & Drucker-Colín, 2011; Hara, Beuckmann, Nambu, *et al.*, 2001).

Homeostatic and circadian regulation of sleep

The most prominent model of sleep-wake regulation comes from the interaction between a homeostatic process, a circadian process, and an ultradian process (Borbély & Achermann, 1992). The homeostatic process of sleep, termed process S by Borbély and colleagues (Achermann & Borbély, 2011), presumes some form of trigger for sleep that accumulates during wakefulness to enable the switch from wake to sleep states, i.e. allowing the VLPO to exert its influence and flip the switch, as in the flip-flop switch model (Saper, Scammell & Lu, 2005). The exact process for this remains unclear, however an accumulation of the neuromodulator adenosine, particularly in the basal forebrain, occurs during wakefulness

(Boonstra, Stins, Daffertshofer, *et al.*, 2007; Strecker, Morairty, Thakkar, *et al.*, 2000) and has been shown to exert an influence on reducing inhibitory GABAergic inputs to the VLPO and the posterior/anterior hypothalamus (Chamberlin, Arrigoni, Chou, *et al.*, 2003; Strecker, Morairty, Thakkar, *et al.*, 2000) which may flip the switch towards sleep.

The circadian process of sleep, termed "process C" (Achermann & Borbély, 2011), is separate from the homeostatic regulation of sleep and is influenced by the suprachiasmatic nucleus (SCN) of the hypothalamus, also referred to as the "master clock" of the brain (Reppert & Weaver, 2002). The SCN receives input from a direct retinohypothalamic tract and in turn modulates the timings in the cortex and in peripheral organs (LeSauter, Silver, Cloues, *et al.*, 2011; Liu & Reppert, 2000). The SCN projects to the VLPO, though the majority of its projections extend to the dorsomedial nucleus of the hypothalamus (DMH) and the subparaventricular zone (SPZ), while the SPZ extends projections to the DMH, and the DMH provides a significant input to the VLPO (Aston-Jones, Chen, Zhu, *et al.*, 2001; Chou, Scammell, Gooley, *et al.*, 2003). This circuit enables the transfer of light information to the VLPO. The two process model proposes an interaction of process S and process C, where ultimately both coincide to promote sleep at specific phases of the light-dark cycle (Borbély & Achermann, 1992).

An ultradian rhythm refers to a rhythm that is less than 24 hours, and in the case of sleep specifically refers to the NREM-REM cycle observed in adult human sleep (Achermann & Borbély, 2011; Hobson, McCarley & Wyzinski, 1975). A model for this ultradian rhythm is the reciprocal-interaction model (Hobson, McCarley & Wyzinski, 1975), which proposed that the changes between NREM and REM sleep are facilitated by an interaction of aminergic and cholinergic cell groups at the mesopontine junction. Evidence supports cholinergic facilitation of REM sleep from the PPT, LDT and brainstem reticular formation, and aminergic inhibition

of REM sleep from raphe nuclei and the LC (Hobson & Pace-Schott, 2002; Pace-Schott & Hobson, 2002). The ultradian NREM-REM rhythm demonstrates an 80-120-minute periodicity (Figure 1.2.2) with a gradual increase in REM duration as sleep progresses (Achermann & Borbély, 2011; Blume, del Giudice, Wislowska, *et al.*, 2015; Carskadon & Dement, 2011). The simplest graphical representation of the NREM-REM ultradian rhythm is the hypnogram (Figure 1.2.2), usually derived from the polysomnography (PSG), a specialised sleep recording (see section 1.3.4). The PSG, in short, stages sleep into, wake, 3 stages of NREM sleep (N1, N2, N3) and REM sleep, based on scoring criteria (Iber, Ancoli-Israel, Chesson, *et al.*, 2007; Rechtschaffen & Kales, 1968). In the scoring criteria for the PSG, sleep paroxysms, K-complexes (KCs), sleep spindles (SSs) and vertex sharp waves (VSWs) feature heavily, with the KC and SS forming the basis of the transition between stage N1 and N2 and VSWs, a solid indicator of N1 sleep.

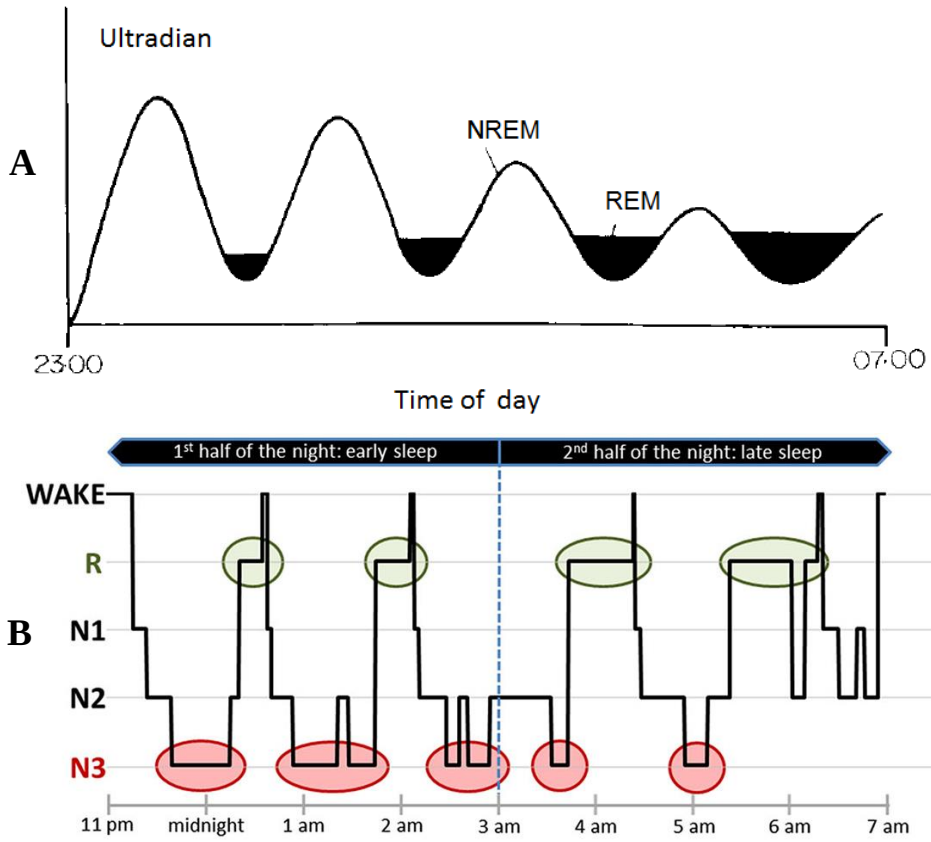


Figure 1.2.2. Ultradian rhythm of NREM-REM sleep and a normal hypnogram. A) Ultradian rhythm over a typical night, NREM decreases over the night and REM increases, this correlates with B) a normal hypnogram which demonstrates a gradual reduction in slow wave sleep and an increase in REM.

A) modified from Borbély and Achermann, 1992, and B) modified from Blume, del Giudice, Wislowska, *et al*., 2015.

Sleep Deprivation

Sleep deprivation, the acute or chronic loss of sleep, has a profound negative effect on cognitive function and our physical and psychological wellbeing, and from a scientific point of view offers a unique means of studying the function of sleep (Killgore, 2010). Although variable between individuals the average sleep length is between 7 – 8.5 hours per day (Alhola & Polo-Kantola, 2007) and, ideally, adults require a minimum of 7 hours sleep per night to maintain

optimum health (Watson, Badr, Belenky, *et al.*, 2015). Restriction of sleep, i.e. less than 7 hours per night, can have profound negative consequences on health, including obesity, diabetes, hypertension, depression, and increased mortality (Watson, Badr, Belenky, *et al.*, 2015). Sleep deprivation is also associated with impaired performance and cognitive function, with chronic sleep restriction of 6 hours or less demonstrating performance deficits in attention/alertness comparable to two nights of total sleep deprivation (Van Dongen, Maislin, Mullington, *et al.*, 2003). Additionally, sleep deprivation has also been shown to have a negative effect on the processes of learning and memory (Goel, Rao, Durmer, *et al.*, 2009). In the United States, the National Commission on Sleep Disorders Research was tasked with investigating the economic impact of sleep deprivation and reported an impact of $43 to $56 billion (Leger, 1994). A large contributor to this economic impact is motor vehicle accidents, which are particularly prevalent in working populations associated with sleep deprivation, such as shift workers, airline pilots, and junior doctors (Goel, Rao, Durmer, *et al.*, 2009). This is further corroborated by neuroimaging studies, one of the first, using PET, demonstrated reduced metabolic activity in brain regions associated with attention (Thomas, Sing, Belenky, *et al.*, 2000). Additionally, fMRI studies have demonstrated reduced activation in frontal cortical controls systems and parietal attention regions following sleep deprivation (Chee & Chuah, 2008; Chee, Tan, Zheng, *et al.*, 2008; Chee & Tan, 2010). Given the profound and diverse negative effect sleep deprivation has on our physical and mental wellbeing and its detrimental consequences on cognition (see Killgore, 2010 for review), it is clear how sufficient sleep is necessary in our daily lives, and the experimental investigation of the effects of sleep deprivation can help elucidate the many functions of sleep.

1.2.2 Sleep paroxysms

The Slow Oscillation

Although not defined as a sleep paroxysm, the slow oscillation (SO) is intimately linked to them and warrants discourse before moving onto discussing the specific sleep paroxysms themselves. The SO was first described by Mercia Steriade and colleagues in 1993, from intracellular recordings from cats and EEG recordings in humans. They demonstrated that delta waves had a periodicity of between 0.4 to 0.9 Hz and defined a <1Hz oscillation (Achermann & Borbely, 1997; Steriade, Nunez & Amzica, 1993). Its cortical origin was established following studies in which the SO persisted in the cortex in the absence of a thalamus (Steriade, Nuñez & Amzica, 1993) and was abolished in the thalamus when the cortex was disconnected (Timofeev & Steriade, 1996). Almost all cortical neurons participate in the SO, and although cortical in origin they also entrain and synchronise thalamic cells (Contreras & Steriade, 1997, 1995). There are two phases to the SO, an up-state and a down-state. The up-state is a depolarising phase, made up of synaptic activity, brought about by NMDA-mediated EPSPs, enhanced by a DC hyperpolarizing current, which results from non-NMDA dependent EPSPs and fast IPSPs within a local cortical circuit (Steriade, Nunez & Amzica, 1993). The hyperpolarising down-state, defined by lack of activity in the network, occurs as the result of removal of synaptic input in intracortical and thalamocortical networks and depletion of extracellular calcium ions (Contreras, Timofeev & Steriade, 1996; Steriade, 2006). As sleep deepens the SO increases in frequency, brought about by the progressive hyperpolarisation of thalamocortical neurons and decrease in the depolarising phase duration, correlating with transition to slow waves observed during slow wave sleep (Amzica & Steriade, 2002, 1998). One of the key functions of the SO is its importance in grouping other brain rhythms, some of which are discussed in the next

section, during NREM sleep such as delta activity, sleep spindles, K-complexes and faster frequencies, i.e. beta and gamma (for review see Steriade, 2006).

K-Complexes

The K-complex (KC), was first defined by Alfred Lee Loomis in 1938, as a large positive-negative-positive waveform (Loomis, Harvey & Hobart, 1938). The years following its discovery led to numerous investigations by means of evoked potentials in sleep, primarily eliciting KCs using auditory stimuli, but also inspiratory occlusion, leading to the definition of the N550 evoked response in sleep, intimately associated with the KC (Bastien, Crowley & Colrain, 2002; Colrain, Webster & Hirst, 1999; Colrain & Campbell, 2007; Halász, 2005). The KC is a biphasic waveform, consisting of an initial surface positive deflection followed by a large surface negative component (Amzica & Steriade, 1997). The underlying mechanisms for these two components are linked to the previously described SO. The initial surface positive potential reflects the depolarising phase of the SO and the large surface negative potential reflects the hyperpolarisation of cortical neurons (Amzica & Steriade, 2002). The KC occurs with the SO and is postulated to actually reflect a SO (Amzica & Steriade, 2002), supported by the fact that there is an increase in the occurrence of KCs in proximity to slow wave sleep as KCs give way to delta waves (De Gennaro, Ferrara & Bertini, 2000); which in themselves are likely in part to constitute KCs (Amzica & Steriade, 2002, 1997). One of the functional roles KCs play is in the grouping of other sleep rhythms, such as sleep spindles or delta oscillations, and they do this by providing a synchronous input onto the reticular nucleus of the thalamus (Contreras & Steriade, 1995). The KC is also theorised to provide a sleep protective mechanism; initially thought of as a mechanism of arousal (Ehrhart, Ehrhart, Muzet, *et al.*, 1981), it was

later discovered that the KC protects the continuity of sleep (De Gennaro, Ferrara & Bertini, 2000; Halász, 2005) and may allow for brief windows of information processing (Jahnke, von Wegner, Morzelewski, *et al.*, 2012), though this has yet to be validated with behavioural data.

Vertex sharp waves

Observed on scalp EEG across the vertex, or regions contiguous to it, the vertex sharp wave (VSW) is a large surface negative potential that occurs during the early stages of sleep (Niedermeyer & Silva, 2005; Yasoshima, Hayashi, Iijima, *et al.*, 1984). It can be seen to occur spontaneously in the EEG but can also be elicited by stimulation (Colrain, Webster, Hirst, *et al.*, 2000) and contributes to the N350 evoked response in evoked potential studies of sleep (Bastien, Crowley & Colrain, 2002; Gora, Colrain & Trinder, 2001). The VSW occurs at around the same time the SO is seen, and at the cellular level is believed to be underpinned by similar mechanisms as the KC, and may also represent a similar phenomenon as the KC but at an earlier stage of development (Amzica & Steriade, 1998). VSWs are discussed further in Chapter 5.

Sleep spindles

As with KCs, sleep spindles (SSs) were also first termed by Loomis and colleagues (Loomis, Harvey & Hobart, 1935), and appear in the second stage of sleep (Iber, Ancoli-Israel, Chesson, *et al.*, 2007). Previous mention has been made to the relationship between the KC in grouping SSs, though SSs can occur both with and without an associated KC (De Gennaro & Ferrara, 2003). SSs can occur with a frequency of between 7 and 15 Hz (De Gennaro & Ferrara, 2003; Niedermeyer & Silva, 2005; Steriade, McCormick & Sejnowski, 1993) and have been divided into two categories, fast- (>13Hz) and slow- (<13Hz) spindles (Anderer, Klösch, Gruber, *et*

al., 2001; Werth, Achermann, Dijk, *et al.*, 1997). Their origin is located in the thalamus, as demonstrated by their absence in athalamic cats (Steriade, 1995). They are generated through the synchronous cortical input exerted by the SO onto GABAergic cells of the thalamic reticular nucleus (RE), which is transferred back to the cortex via glutamatergic thalamocortical neurons, which in turn also project back onto the RE producing a recurrent inhibitory circuit that shapes SSs (Amzica & Steriade, 2000; Contreras & Steriade, 1995). Fast and slow SSs are topographically distinct, with the former being primarily located over centro-parietal regions and the latter more frontally predominant (Anderer, Klösch, Gruber, *et al.*, 2001; Werth, Achermann, Dijk, *et al.*, 1997) and they occur at different phases of the SO, with fast spindles being linked with the upstate of the SO and slow spindles with transition between the up to down state of the SO (Klinzing, Mölle, Weber, *et al.*, 2016; Mölle, Bergmann, Marshall, *et al.*, 2011). Investigation between these two spindle types has led to some controversy of the classically accepted mechanism of spindle generation, as previously described, as it may not necessarily underpin both spindle types, and, although it may be applicable to fast spindles (Ayoub, Aumann, Hörschelmann, *et al.*, 2013; Timofeev & Chauvette, 2013), slow spindles may be generated in non-specific thalamic nuclei or even through intracortical mechanisms (Timofeev & Chauvette, 2013).

The function of sleep spindles has been the topic of great interest over the last couple of decades with a focus on their role in learning and plasticity (Diekelmann & Born, 2010; Lindemann, Ahlbeck, Bitzenhofer, *et al.*, 2016). A recent study has even demonstrated the temporal relationship between the SO, spindles and hippocampal ripples demonstrating the nesting of ripples in the troughs of spindles, presumably facilitating information transfer from hippocampus to neocortical structures (Staresina, Bergmann, Bonnefond, *et al.*, 2015). It has also been postulated that SSs provide a sleep protective mechanism, originally the evidence for

this was not compelling (De Gennaro & Ferrara, 2003), though recent imaging studies have demonstrated that processing of sound is constrained by the presence of spindles (Dang-Vu, Bonjean, Schabus, *et al.*, 2011; Dang-Vu, McKinney, Buxton, *et al.*, 2010; Schabus, Dang-Vu, Heib, *et al.*, 2012) which may serve to protect sleep.

1.2.3 Epilepsy and sleep: the link

The link between epilepsy and sleep has been well established throughout history. Since circa 300BC the association between epilepsy and sleep has been documented, by both Aristotle and Hippocrates (Temkin, 1994). Deprivation of sleep is a well-documented precipitant of seizures in epilepsy (Méndez & Radtke, 2001) and is widely used in a clinical setting to provoke interictal epileptiform activity and improve sensitivity of the EEG in epilepsy diagnostics (Molaie & Cruz, 1988; Roupakiotis, Gatzonis, Triantafyllou, *et al.*, 2000; Veldhuizen, Binnie & Beintema, 1983). Patients who suffer from both epilepsy and obstructive sleep apnoea (OSA), and are chronically sleep deprived as a result of the OSA, demonstrate an improvement in seizure control following OSA treatment (Vaughn, D'Cruz, Beach, *et al.*, 1996). Additionally, the propensity for seizures to occur during sleep have been well described (Dinner, 2002; Shouse, da Silva & Sammaritano, 1996), especially with frontal lobe epilepsy (O'Muircheartaigh & Richardson, 2012), and continuous epileptiform discharges can occur during sleep, as in electrical status epilepticus of sleep (Nickels & Wirrell, 2008). Despite the clear intimate relationship between epilepsy and sleep the link presented in this thesis is not a direct investigation of their relationship but rather a practical progression from epilepsy to sleep, with development of a genuine interest in paroxysmal sleep activity and sleep in general. The link comes between chapter 3 and chapter 4, where the same experimental methodology is

applied to both pathological paroxysmal EEG events, i.e. IEDs (chapter 3), and non-pathological endogenous paroxysms as observed in sleep (chapter 4), to ascertain if the results observed in chapter 3 are unique to pathological epileptiform paroxysms or if they are also a feature of normal paroxysmal activity observed in sleep. Owing in part to the constraints in recruiting patients with epilepsy and growing interest in the nature of these sleep paroxysms, chapter 5 is solely focused on sleep, specifically, the effect sleep deprivation has on KCs and VSWs.

1.3 ELECTROENCEPHALOGRAPHY

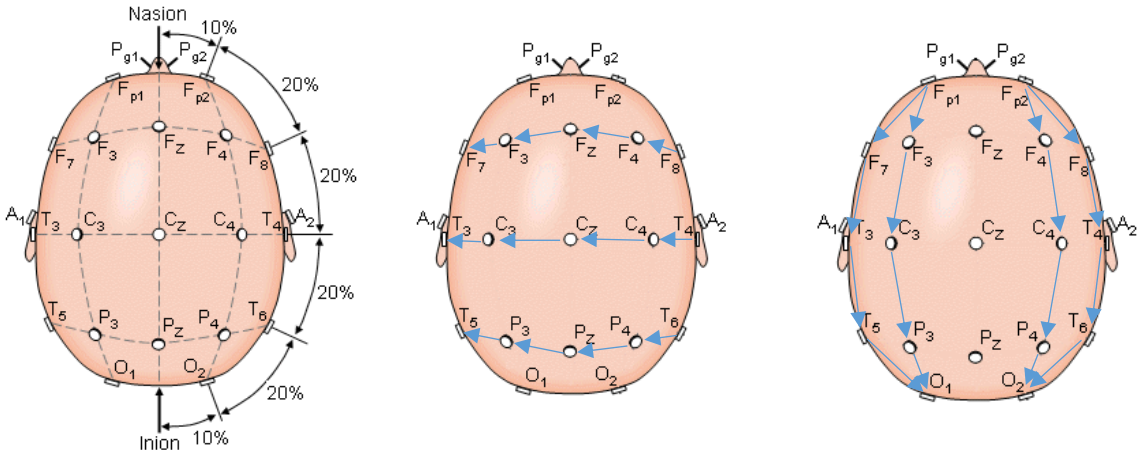
In the mid 1870's, a physician in Liverpool, Richard Caton, presented experimental findings of what were to be the first electroencephalographic recordings in animals, though later he lost interest in this line of research eventually moving on to become the mayor of Liverpool (Brazier, 1963). Some years later, in 1929, Hans Berger, a German neuropsychiatrist of little renown at the time, published the first report of EEG recordings in humans; a single channel bipolar recording from a Siemens galvanometer (Gloor, 1969; La Vaque, 1999). However, it wasn't until 1935 that EEG would find its true calling in the clinical context of epilepsy, when, in America, Frederic Gibbs and colleagues recorded the EEG of a small cohort of 12 children with epilepsy, publishing the first accounts of 3 per second spike and wave activity (Gibbs, Davis & Lennox, 1968; Gibbs, Gibbs & Lennox, 1937). At the same time, in 1937, Alfred Lee Loomis and colleagues were using EEG from over 200 recordings to investigate sleep and were the first to identify the principal paroxysms of sleep (VSWs, KCs and SSs), and divided sleep into 5 stages (Loomis, Harvey & Hobart, 1937). Since the late 1930's, the EEG has become a mainstay feature in both tertiary epilepsy centres, sleep clinics and general hospitals across the world, providing the gold standard in epilepsy diagnostics and a pivotal role in sleep diagnostics. The EEG is used in many different guises in the field of neurocognition, epilepsy and sleep, from dense array EEG employing hundreds of scalp electrodes to single unit recordings in animals and humans (Feng, Hu, Pan, *et al.*, 2016; Miller, Neufang, Solway, *et al.*, 2013; Niedermeyer & Silva, 2005; Pisarenco, Caporro, Prosperetti, *et al.*, 2014; Truccolo, Donoghue, Hochberg, *et al.*, 2011). The following in this section will introduce EEG in more detail covering the technical aspects of scalp EEG and the origin of EEG signals; primarily in the context of epilepsy and sleep.

1.3.1 Technical aspects of EEG

The bioelectric signals that constitute the EEG are usually recorded from the scalp via electrodes and a conductive medium (conductive gel or paste). In its simplest form only three electrodes are required to record these signals, an active electrode, a reference electrode and a ground electrode. The electrical activity originating from the brain has to traverse many layers of different tissue types, i.e. cerebral spinal fluid (CSF), dura, skin and bone, attenuating and blurring the electrical signal until all that remains is in the order of microvolts (μV). Given the scale of these signals, amplifiers are needed to bring the signals into a range where they can be converted into digital signals using an analog-to-digital converter. Two of the most important considerations for scalp EEG recordings are impedance and direct current (DC) offset voltage (Bronzino, 1999; Niedermeyer & Silva, 2005). A steady DC potential is generated between the site of the electrode and the connecting electrolyte junction, comprising the skin and conductive media; a formation of an electrical bilayer brought about by the flow of ions from the metal of the electrode to the conductive media and vice versa. Depending on the materials used i.e. properties of the electrode and of the conductive media, the DC offset voltage can be many times greater than the electrical activity derived from the brain. EEG amplifiers are designed to account for a DC offset voltage but if the materials used are unsuitable and generate a high DC offset voltage, brain signals may be abolished. The second issue is impedance, this is dependent on both the properties of the electrode used and the electrical resistance of the skin at the site of juncture between scalp and electrode via conductive media. Silver (Ag) electrodes with a silver chloride coating (AgCl) are usually used to minimise both the DC offset voltage and impedance at the electrode/conductive media junction, and skin, which has a naturally high resistance to electricity, needs to be prepared appropriately to lower impedance (Almasi & Schmitt, 1970; Kiloh, McComas & Osselton, 2013).

Placement of electrodes are important and most centres internationally use the international 10-20 measurement system (Klem, Lüders, Jasper, *et al.*, 1999; Sharbrough, Chatrian, Lesser, *et al.*, 1991). This means electrodes are placed in standardised location based upon percentage measurements derived from measurements taken between anatomical landmarks (see Figure 1.3.1). Theoretically as children grow and if EEGs are done elsewhere in the world, using this system means electrodes are generally placed over consistent anatomical locations.

Electrodes carry the signals to amplifiers which amplify the potential difference between two electrodes, usually the referential electrode and the active electrode. The signal is digitised and stored electronically, enabling post hoc analysis and manipulation of the data. The resolution of the digitally converted signal is dependent on the sampling frequency and the quality is dependent on a number of factors, prominently organic and nonorganic artefacts (unwanted electrical information in the EEG data). EEG data can be viewed in either referential montages or in bipolar montages. Figure 1.3.1 gives an overview of the basic EEG montages, placement and potential artefacts.



Physiological Artefacts

- Ocular artefacts – movements of the positively charged cornea create artefacts in surrounding electrodes.
- Myogenic artefacts – electrical activity from surrounding activated muscles.
- ECG artefact – electrodes pick up potentials generated by the heart, seen more commonly in obese patients with cardiomegaly or when there are large distances between electrodes.
- Electrodermal artefacts – caused by patient perspiration creating slow potentials.

Pulse artefacts – waveforms temporally coupled with the ECG caused by scalp arteries pulsating under/near an electrode

Non-Physiological Artefacts

- Mains interference – 50 or 60 Hz interference depending on country, generated by power lines in close proximity. More prominent with high impedance/mismatched impedances.
- Implanted devices – devices such as pacemakers or deep brain stimulators may produce artefacts depending on parameters of device.
- External devices – oscillating ventilators, infusion pumps, other nearby medical devices.
- Electrode artefacts – movements of electrodes, poor construction, material construction (stainless steel electrodes more likely to generate artefact than Ag/AgCl electrodes)

Figure 1.3.1. Diagram of electrode placement according to the international 10-20 system and examples of common montage configurations. Head in the top left shows 10-20 electrode placements, middle head shows and example of a transverse bipolar montage and head on the right an example of an anterior-posterior bipolar montage. Although not displayed, referential montages are all electrode referred to a single reference electrode, and average-reference montages uses an average of all scalp electrodes (apart from frontals due to ocular artefacts) as a reference. Below head diagrams are two tables of the more commonly encountered physiological and non-physiological artefacts.

Head diagram modified from Sharbrough et al., 1991.

1.3.2 Origin of EEG signals

The basic understanding of the origin of EEG signals is essential to anybody using EEG in either a clinical or academic setting. Understanding some of the underlying mechanisms helps us to interpret and understand the EEG data we acquire. Signals recorded from scalp EEG are, in its simplest form, the summation of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). Postsynaptic potentials originate from an action potential (AP) which is generated at the cell body of a neuron. The AP was first defined by Hodgkin and Huxley in 1952, based on observation of ionic currents of a giant squid nerve axon (Hodgkin & Huxley, 1952). All cells have a resting membrane potential, though the AP only occurs at the cell membrane of cells containing the appropriate ion channels and ion pumps, meaning not all cells are capable of generating an AP. An AP occurs when the resting membrane potential, usually around -70mV, receives sufficient stimulus to reach a threshold potential; if the threshold is reached the membrane depolarises, becoming less negative, caused by the influx of sodium ions (Na^+) into the cell. At the peak of the action potential all Na^+ channels are open and this raised voltage triggers the closure and inactivation of said channels. The membrane potential falls back towards its resting potential as potassium ion (K^+) channels open allowing the efflux of K^+ ions. Once the membrane resting potential is reached K^+ ion channels do not close immediately, with the continued efflux resulting in an afterhyperpolarisation period. During this period of hyperpolarisation the membrane is inactivated, incapable of producing another AP until resting potential end equilibrium is reached (Hammond, 2015). The AP propagates down the axon of the neuron until it reaches a synapse where the AP triggers a release of a neurotransmitter across the synaptic cleft, from pre to post synapse. Whether the resulting synaptic potential is inhibitory or excitatory depends on the neurotransmitter. APs are very short lived, usually with a duration of less than a millisecond, whereas a postsynaptic

potential is longer in duration, several milliseconds and sometime much longer. Although the amplitude of an AP is larger, the relatively short duration means they do not summate as well as postsynaptic potentials and thus contribute little, except under certain circumstances, to the electric potentials which are paramount and form the basis of EEG recordings. Electric potentials are often referred to as local field potentials (LFPs), and are recorded from within the cortex, whereas electric fields are recorded from scalp EEG, or the surface of the cortex, and are the difference between the electric potential and a reference point (Buzsáki, Anastassiou & Koch, 2012; Petsche, Pockberger & Rappelsberger, 1984) Depending on whether the AP reaches an excitatory or inhibitory synapse dictates whether an EPSP or a hyperpolarising IPSP occurs (Niedermeyer & Silva, 2005). Although PSPs provide the most ubiquitous contribution to electric fields, and ultimately the EEG, there are other mechanisms that also contribute to these fields, including; neuron-glia interactions, providing indirect contribution through enhancing neuronal synchrony; ephaptic effects, referring to the conducting properties of the extracellular space; calcium spikes, a non-synaptic contribution as calcium spikes can propagate within a cell, are large (10-50mV) and long lasting (10-100ms) and in some circumstances contribute significantly to extracellular fields; and spike after-hyperpolarisations, which can be similar in size and duration to a synaptic potential (Buzsáki, Anastassiou & Koch, 2012).

Another important consideration is neuronal geometry. The majority of EEG signals that are recorded result from pyramidal cells, as not only are they the most abundant cell type, their long apical dendrites are aligned parallel to one another, generating an open field; an ideal condition for summation and synchrony of dipoles. The gyration of the cortex means the orientation of pyramidal cells and their dendrites may be radial, projecting the maximum voltage of the dipole towards the scalp, or tangential, where the polarities of the dipole fall on either side of the

recording site and the voltage recorded is comparatively low. In terms of dipoles and scalp potentials, when a negative wave (upward deflection on scalp EEG) is recorded at the scalp this can arise from either a superficial excitatory input or a deep inhibitory input; the deep inhibitory input will be smaller in size due to its distance from the scalp. The opposite is true for downward deflections, or positive scalp potentials, as they arise from either a superficial inhibitory input or a superficial excitatory one (Kirschstein & Köhling, 2009). However, much of the information on neuronal activity and local field potentials are lost to scalp recordings, given it takes electrical synchrony from at least several square centimetres of cortex for anything to be visible on scalp EEG (Ebersole, Husain & Nordli (Jr.), 2014).

The normal human EEG comprises of five primary frequency bands; delta (below 3.5Hz), theta (4-7.5Hz), alpha (8-13Hz), beta (14-30Hz) and, of less clinical importance, gamma (above 30Hz). Other high frequency and low frequency bands also exist, as do paroxysmal variations within a band, though in clinical EEG, paroxysmal activity excluded, the EEG is broken down into the four bandwidths of alpha, beta, theta and delta (see figure 1.3.2).

Alpha activity is primarily recorded from the posterior derivations of EEG, maximal over the occipital regions, occurring during relaxed wakefulness, most prominently with eye closure. The cellular mechanisms that generate alpha activity are yet to be fully elucidated, it is a complex rhythm that is suppressed when the eyes are open but enhanced during certain tasks, such as anticipation of a visual stimulus when the stimulus presentation is controlled by the participant (Stenner, Bauer, Haggard, *et al.*, 2014), and the individual cycles of the alpha oscillation have been linked to discrete windows of perceptual processing (Palva & Palva, 2007). It has been shown however that both intracortical propagation across the neocortex and modulation by thalamic nuclei and thalamocortical high threshold bursting cells play a role in alpha generation (Hughes & Crunelli, 2005; Lopes da Silva, 1991).

The faster frequency range observed in the EEG is beta activity. Beta activity is present in almost every adult in one form or another and can be observed with an anterior, central, or posterior predominance, or indeed diffusely across both hemispheres. There has been considerable focus on beta oscillations in the somatomotor cortex, with links to tasks of fine motor control and attention (Brown, 2000; Engel & Fries, 2010; Kristeva-Feige, Fritsch, Timmer, *et al.*, 2002). The origin of beta oscillations is not entirely clear, nor is the extent to which these oscillations in the somatomotor cortex relate to thalamic/subcortical activity (Lopes da Silva, 2009). There is evidence of beta oscillations originating in layer V of the somatosensory cortex from intrinsically bursting neurons, possibly the result of expectation of movement in response to sensory information (Roopun, Middleton, Cunningham, *et al.*, 2006).

Activity in the theta bandwidth can be divided into two broad categories; cortical theta activity (Niedermeyer & Silva, 2005) and theta oscillations in the hippocampus (Buzsáki, 2002; Colgin, 2016). Cortical theta activity is frequently observed in the matured adult EEG in very small amounts, though it is seen abundantly during sleep and in the maturing brain. Differing underlying pathologies can also give rise to theta activity, focal structural lesions, non-specific general cerebral dysfunction (e.g. in an encephalitis, encephalopathy, anoxic brain injury), and epilepsy; as such the mechanisms of generation can differ with pathology. Theta oscillations in the hippocampus are better described; generated through GABAergic cells of the medial septum, they play a pivotal role in learning and memory (Colgin, 2016).

Delta waves, like theta, can have different underlying mechanisms dependent on underlying pathology, such as encephalopathies, lesions etc., though the mechanisms underlying delta oscillations are better described. As with theta activity there is a degree of distinction between waves and oscillations, with waves appearing in a more singular fashion and oscillations a repeated pattern of waves. Delta oscillations can be thalamic, generated by intrinsic currents of

thalamocortical cells, or cortical, from pyramidal neurons (the slow oscillation is discussed in more detail in Section 1.2.2)

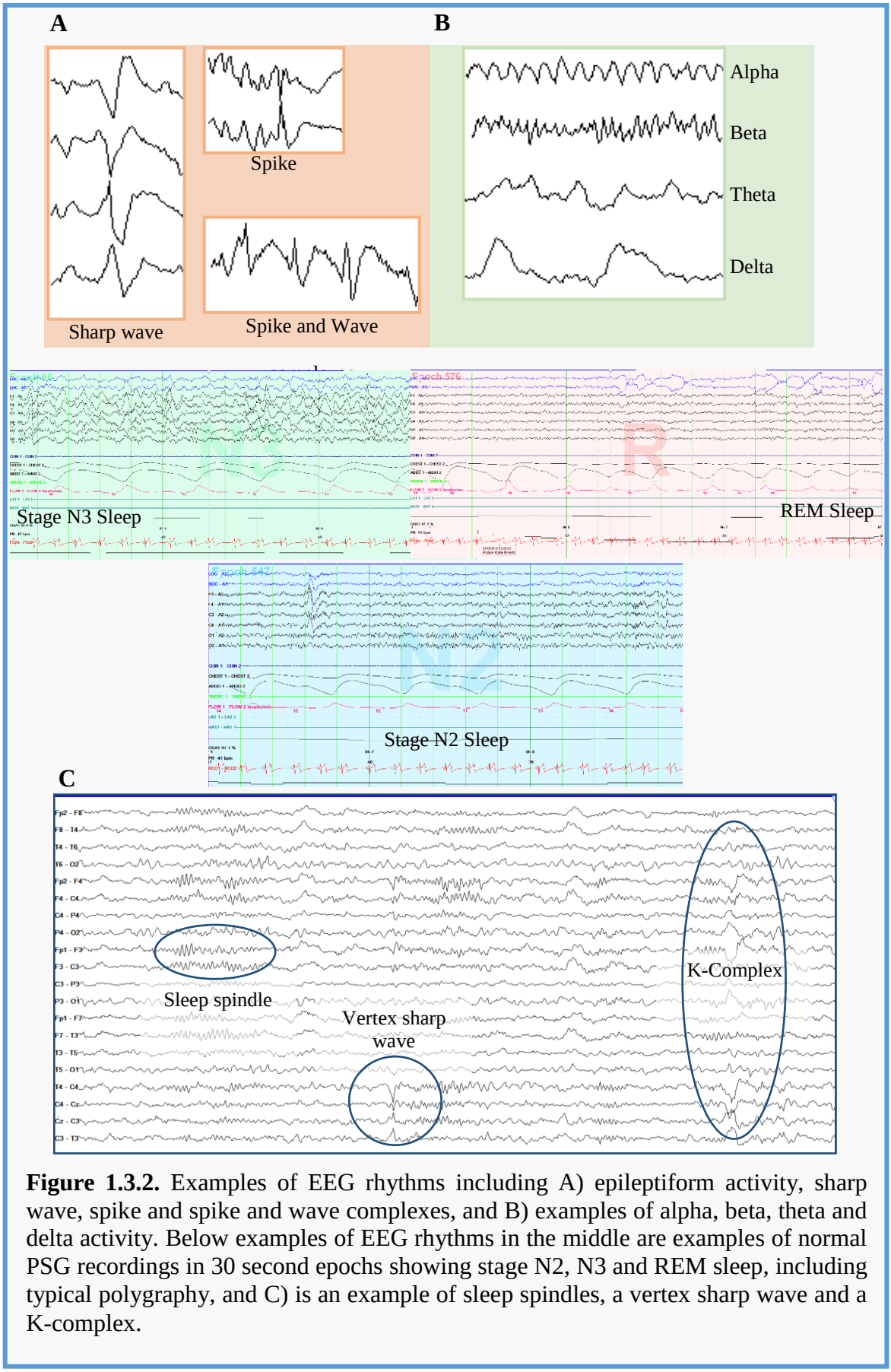


Figure 1.3.2. Examples of EEG rhythms including A) epileptiform activity, sharp wave, spike and spike and wave complexes, and B) examples of alpha, beta, theta and delta activity. Below examples of EEG rhythms in the middle are examples of normal PSG recordings in 30 second epochs showing stage N2, N3 and REM sleep, including typical polygraphy, and C) is an example of sleep spindles, a vertex sharp wave and a K-complex.

1.3.3 EEG in epilepsy

The diagnosis of epilepsy is best made as an electro-clinical diagnosis, and as such EEG plays a pivotal role in epilepsy management (Berg, Berkovic, Brodie, *et al.*, 2010; Smith, 2005). The primary aim of the EEG in epilepsy diagnostics is the identification and localisation of interictal activity, the period between seizures, and/or ictal activity, the discrete period relating to a seizure. In the adult EEG, interictal epileptiform discharges (IEDs) synonymous with epilepsy (see figure 1.3.2) can be divided roughly into spikes, sharp waves and spike and slow wave complexes (Noachtar, Binnie, Ebersole, *et al.*, 1999; Noachtar & Rémi, 2009). Focal IEDs, such as focal spikes and/or sharp waves can help localise an epilepsy to a lobe of the brain, e.g. temporal lobe epilepsy, and help both diagnostically and in pre-surgical evaluation, though focal ictal recordings can contribute significantly to a pre-surgical workup for epilepsy (Ebersole, Husain & Nordli (Jr.), 2014; Raghavendra, Nooraine & Mirsattari, 2012). Generalised IEDs can also contribute significantly to epilepsy diagnostics and classification, such as the characteristic pattern of 3 per second spike and wave activity synonymous with childhood absence epilepsy (Hughes, 2009). A strict criterion for defining an epileptic spike or sharp wave is lacking, and clinically is based on subjective opinion often dependent on the electroencephalographer's experience and expertise; as such, in reviewing EEGs, there can be generally low inter-observer reliability (Abend, Gutierrez-Colina, Zhao, *et al.*, 2011; Azuma, Hori, Nakanishi, *et al.*, 2003; Williams, Lüders, Brickner, *et al.*, 1985) and even the most capable have a 21% chance of getting it wrong (Grant, Abdel-Baki, Weedon, *et al.*, 2014). However, there are some defining characteristics of an epileptiform spike or sharp wave; a spike or sharp wave is generally observed on scalp EEG with a prominent surface negative polarity (Noachtar, Binnie, Ebersole, *et al.*, 1999) and a duration of 70 – 200 ms for a sharp wave and 20 - <70 ms for a spike (International Federation of Societies for Clinical Neurophysiology,

1974). The lack of a strict criterion is down to the variable nature of EEG and also the presence of normal electrographic paroxysms with a sharp morphology; some of these normal variants, such as wicket spikes and benign epileptiform transients of sleep (BETS), could be interpreted incorrectly as IEDs (Noachtar & Rémi, 2009).

Correct identification of IEDs aside, one must capture these paroxysmal discharges on the EEG first. In most clinical settings a routine outpatient EEG is recorded for around 20 minutes and the general yield for recording an IED in a 20-minute period is around 37-45% (Badry, 2013; Narayanan, Labar & Schaul, 2008), thus even in patients with epilepsy many can have a normal EEG, with repeated EEGs increasing diagnostic yield (Noachtar & Rémi, 2009). In order to increase the yield of the EEG, provocation techniques, such as hyperventilation, photic stimulation and sleep deprivation, are used in most clinical settings (Mendez & Brenner, 2006). The lack of abundant IEDs in clinical EEG may not be at all surprising given the volume of brain needed to produce a scalp spike. Intracranial-EEG recordings reveal IEDs that are not visible on scalp EEG, or are indistinguishable from the background EEG, and it is estimated that it requires 10-20 cm^2 of synchronous/temporally overlapping gyral cortex to produce a scalp spike (Tao, Ray, Hawes-Ebersole, *et al.*, 2005). The underlying mechanism in generating scalp spikes, termed the paroxysmal depolarising shift (PDS), is characterised by rapid bursting of action potentials superimposed upon a slow depolarising potential (de Curtis & Avanzini, 2001). The localisation of interictal spikes or sharp waves can be informative, providing not only valuable diagnostic information but also providing a possible targeting strategy for intracranial recordings in pre-surgical evaluation.

Spike and slow wave complexes (SWCs) are much easier to identify on the scalp EEG as compared to interictal spikes or sharp waves. Characterised electrographically by a surface negative spike followed by a surface negative slow wave, the SWC is maximal in frontal regions

though is generalised across both hemispheres. The negative spike has been associated with EPSPs of apical dendrites and the slow wave with IPSPs located at the soma of pyramidal cells (Niedermeyer & Silva, 2005). There are generally two hypotheses based around the generation of SWCs, both involving interactions between thalamus and cortex, and founded on animal experiments primarily investigating models of absence epilepsy; an epileptic syndrome synonymous with SWCs, notably the 3 per second SWC. According to the cortico-reticular theory (Kostopoulos, 2000), SWCs may be generated within the same circuits responsible for the generation of sleep spindles (see Section 1.2.2); initiated in the thalamus, thalamocortical volleys to cortical neurons pace spindles, increased output from cortex engages inhibitory feedback which transforms the sleep spindle to a SWC. This theory has been challenged by the more recent "cortical" theory, which proposes a cortical origin to SWCs with the thalamus playing a secondary role (Meeren, Pijn, Van Luijtelaar, *et al.*, 2002). Despite the primary focus of SWCs around models of absence epilepsy, SWCs can be observed on the EEG in focal seizure disorders, not just generalised seizure disorders (Westmoreland, 1998). The frequency at which the SWCs occur is also important, given that 6 per second SWCs can be considered benign or at least of uncertain significance (Ebersole, Husain & Nordli (Jr.), 2014; Tatum, Husain, Benbadis, *et al.*, 2006).

As previously mentioned, intracranial recordings are done in the pre-surgical work up for epilepsy surgery, and can be necessary for delineation of the epileptogenic zone (EZ). The EZ is defined in surgical terms as "*the minimum amount of cortex that must be resected (inactivated or completely disconnected) to produce seizure freedom*" and must be considered in the context of other zones (Lüders, Najm, Nair, *et al.*, 2006). A number of other zones are also defined; the irritative zone, "*an area of cortex which generates interictal spikes*"; the seizure onset zone, "*area of cortex that initiates clinical seizures*"; the symptomatogenic zone, "*area of cortex*

which, when activated, produces the initial symptoms or signs", the functional deficit zone is the "*area of cortex that is not functioning normally in the interictal period*", and finally, if present, the epileptogenic lesion (for more information see (Lüders, Najm, Nair, *et al.*, 2006) from where these definitions originate). Determining the extent of these zones ultimately helps the epileptologist and the neurosurgeon in defining the resective target for epilepsy surgery. The contribution from EEG and intracranial EEG (icEEG) is to help determine the extent of both the irritative zone and the seizure-onset zone which in turn provides vital information in defining the EZ. In the absence of a lesion on MR imaging icEEG can provide vital information in order to progress with surgery, though even with an identifiable lesion icEEG may be used to prove, or disprove, the lesion as the EZ. Electrode can be placed on the surface of the cortex as a subdural grid following a craniotomy, or stereotactic placed depth electrodes which penetrate the cortex, and a number of implantation strategies have been described (Cardinale, Cossu, Castana, *et al.*, 2013; Lachaux, Rudrauf & Kahane, 2003). The recorded voltages from icEEG compared to scalp EEG are quite large due to signals not having to pass through bone and scalp etc. (see figure 1.3.3).

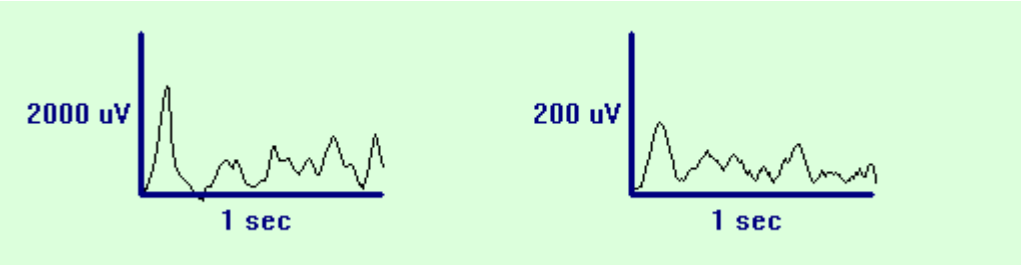


Figure 1.3.3 Visual representation of the difference in magnitude between a sharp wave recorded at the scalp level and intracranially. On the left is a sharp wave recorded from the hippocampus using a depth electrode, and on the right, is a sharp wave recorded from a scalp electrode, note these are not the same electrographic event. This is taken from the EEG data of patient #13, discussed in more detail in chapter 2.

1.3.4 EEG in sleep

As with epilepsy the EEG has also become an integral part of sleep medicine and research, though generally utilised clinically in a slightly different manner (see below). The progression into and through sleep is accompanied by stark changes on the EEG, so much so that the EEG can be broken down into 30 second epochs and classified into one of five stages; wake, stage N1, N2, N3 and rapid eye movement (REM) sleep, with the "N" denoting a non-REM sleep stage (Iber, Ancoli-Israel, Chesson, *et al.*, 2007). Previously, stage N3 sleep was divided into two separate stages, 3, and 4 (Hori, Sugita, Koga, *et al.*, 2001; Rechtschaffen & Kales, 1968), though later consolidated into the one stage. Since its introduction in 2007, the American Academy of Sleep Medicine (AASM) scoring manual (Iber, Ancoli-Israel, Chesson, *et al.*, 2007), has become widely used and accepted, and continues to adapt to the needs of the sleep community (Grigg-Damberger, 2012). As such scoring guidelines used within this thesis will be limited to the AASM guidelines rather than the earlier Rechtschaffen and Kale, 1968 guidelines.

During the early stage of sleep, N1, the most notable change in the background rhythms of the scalp EEG is usually the dropout of alpha activity; the record can become quite low voltage before the more regular appearance of theta activity. It is in this light stage of sleep that vertex sharp waves (VSWs) make an appearance, usually following deeper progression into this stage (see section 1.2.2 for more on the VSW, KC, and SS). The background slowly starts to become more dominated by theta and delta activity with faster frequencies in the beta bandwidth commonly observed. However, the EEG is not recognised as stage N2 until the occurrence of a K-complex (KC) in the absence of any associated arousal (a paroxysmal shift to higher frequencies lasting longer than 3 seconds), or a train of sleep spindles (SSs). Stage N3, also

denominated as slow wave sleep (SWS), is defined as having at least 20 per cent of the 30 second epoch being slow waves exceeding 75μV with a frequency of 0.5 – 2Hz. During REM sleep the EEG becomes desynchronised and moderately low amplitude with accompanying excessive ocular movements.

Given the fairly clear distinctions made between each stage of sleep, visual scoring of sleep studies is easily performed. The investigation synonymous with sleep studies is the polysomnogram (PSG), an overnight investigation that utilises EEG recording, and a range of polygraphy, including electromyography (EMG), electrooculography (EOG) and sensors to monitor breathing and respiration (Iber, Ancoli-Israel, Chesson, *et al.*, 2007). The EEG recordings are limited to frontal, central and occipital regions as not as many electrodes are required to stage sleep EEG as compared to localising IEDs in epilepsy. One principle end product of an overnight sleep study/PSG is the hypnogram, a graph that plots sleep stage as a function of time (see figure 1.2.2). The hypnogram can be used to evaluate quality of sleep and aid in diagnosis of sleep disorders, such as obstructive sleep apnoea that can demonstrate frequent transitions from sleep to wake on the hypnogram (Bianchi & Thomas, 2013; Swihart, Caffo, Bandeen-Roche, *et al.*, 2008). As with the identification of IEDs on an EEG, sleep staging is also somewhat subjective, despite scoring guidelines, and can be staged incorrectly if the individual performing the staging is not experienced enough (Collop, 2002); with AASM scoring standards the overall interrater reliability is around 82% (Danker-Hopfe, Anderer, Zeitlhofer, *et al.*, 2009). Despite this, when it comes to sleep diagnostics the PSG is still regarded as the gold standard (Kushida, Chang, Gadkary, *et al.*, 2001). The PSG has also been indicated in the use of differentiating between nocturnal frontal lobe epilepsies and parasomnias (disorders of sleep that can manifest similar clinical presentations to frontal lobe seizures), by

using combined video PSG and EEG (for review see (Foldvary-Schaefer & Alsheikhtaha, 2013).

1.4 MRI AND FUNCTIONAL MRI

Magnetic resonance imaging (MRI) was pioneered by Peter Mansfield and Paul Lauterbur, mostly in the 1970's, and they were subsequently awarded, in 2003, the Nobel prize in medicine for their discoveries (Mayor, 2003). Since its development MRI has become integral to both medical diagnostics and research and continues to play a pivotal role in both fields.

1.4.1 Principles of MRI

Almost all atoms/isotopes of atoms possess a nuclear spin, i.e. the nuclei of an atom/isotope will rotate aligned with a perpendicular axis to direction of rotation. Nuclei that possess spin are able to absorb and emit radio frequency (RF) energy when placed in a magnetic field. When wanting to image the human body, the most obvious choice of atom to use is hydrogen, or more specifically its isotope protium (^{1}H) due to its high natural abundance. Tissues in the body primarily contain water and fat, and hydrogen is present in both. When a positively charged nucleus, as in ^{1}H, spins, it creates a localised magnetic field, known as a magnetic moment. The orientation of hydrogen protons in a given body of tissue will be distributed randomly, i.e. spin vectors will be randomly orientated. This results in no net magnetisation of said tissue. Placed into a magnetic field (B_0), the spin vectors from a proportion of the nuclei will precess parallel to B_0 oriented along the z-axis. The frequency of this precession is proportional to B_0 strength and referred to as the Larmor frequency. Whilst the z-component of the spin is now aligned to B_0, the x and y components are still randomly distributed with no net magnetisation, but for the z-component we observe a Zeeman interaction, where the protium nucleus will either lie parallel to B_0, in a low energy state, also referred to as spin-up, or antiparallel in a high energy state, referred to as spin-down. More protons will possess a spin-up orientation thus producing

an overall net magnetisation (M_0) when in the magnetic field, thus whatever tissue (or otherwise) is in the field is said to be magnetised. The key to obtaining MR images is based on M_0, and involves presentation of a radiofrequency (RF) energy pulse. The protons will absorb RF energy at the Larmor frequency, where if aligned parallel, in B_0, in a low energy state will be excited to the higher energy state and those of high energy state will release energy and drop to a low energy state. As mentioned before, initially there are more protons in a low energy state, so the overall effect is that of energy absorption. Following the RF pulse, the direction of the of magnetisation can be rotated perpendicular to B_0 and regarded as an additional field (B_1), with rotation dependent on the RF pulses flip angle, i.e. a flip of 90º will rotate to the transverse plane, whereas a flip and of 180º will rotate in the opposite direction to the static field.

After the RF pulse the energy is released and the protons return to previous energy states and equilibrium. The resulting free induction decay (FID) signal is dependent on M_0. Relaxations times, based on the FID, refer to the time taken for the energy to be released, and are referred to as T1 and T2. T1 relaxation time is also referred to as the longitudinal magnetisation relaxation time, and, following an RF pulse, is the time required for the original magnetisation to return to 63% of its value prior to the RF pulse. Tissues have different relaxation times and thus their own T1 value (Akber, 1996), some have long T1 times, such as cerebrospinal fluid (CSF) and take a long time to relax, others have shorter T1 times such as cerebral grey matter, thus a contrast is formed between the two tissues. In the case of a clinical T1 MRI scan, CSF appears dark as it only returned to a relatively small percentage of its original magnetisation whereas grey matter will appear brighter, and white matter even brighter still as it has an even shorter T1 relaxation time than grey matter.

During MRI acquisition an RF pulse is repeatedly administered with a fixed interval between pulses, this is called the repetition time (TR). The TR is generally always shorter than the

relaxation time of the tissue thus the second RF pulse will return less magnetisation, as will the third, though this effect plateaus after the first few pulses thus the returning magnetisation becomes stable. To accommodate this the first few RF pulses are not included. T1 weighted scans tend to have a short TR so as to maximise contrast.

T2 relaxation time refers to the transverse magnetisation, and the time it takes for it to decay to 37% of its initial value immediately following an RF pulse. Following a 90º RF pulse the net magnetisation (M_0) is in the transverse plane, but loses coherence as protons realign to B_0, producing FID, eventually losing all transverse magnetisation. A primary feature in the loss of transverse magnetisation is spin-spin relaxation, in which one proton transfers its energy to another. Efficient energy transfer within a tissue equates to a shorter T2. Other factors effect loss of coherence such as inhomogeneities in the magnetic field or imaging gradients. Again different tissues have different T2 relaxation times, for example in a 3 Telsa (3T) field (thus B_0 = 3T), the T2 relaxation time of grey matter is ~99ms, and ~69ms for white matter (Stanisz, Odrobina, Pun, *et al.*, 2005), hence white matter appears darker on T2 weighted scans due to the shorter T2 relaxation time. The point in time that the data is acquired, as the RF energy is released, is dependent on the echo time (TE), in the case of T2 scans a long TE is required to get the best contrast between tissues.

The spatial localisation of proton frequencies is determined by way of gradient fields, an applied linear distortion of B_0, orientated around the isocenter of the scanners magnet. Three gradients x, y and z are used in order to put the data into 3-dimensional space and display the image, which is made up of numerous voxels, each containing volume intensity data from the protons within said voxel.

For a more detailed review of MRI physics and applications see (Dale, Brown & Semelka, 2015).

1.4.2 Functional MRI

The basis for fMRI is the T2* relaxation decay of transverse magnetisation which is observed when using gradient echo (GRE) imaging. As previously mentioned, local inhomogeneities contribute to dephasing of transverse magnetisation, this can be eliminated by using a spin-echo sequence, a 180° RF pulse, which results in a "true" T2 relaxation time (Chavhan, Babyn, Thomas, *et al.*, 2009). In GRE imaging, the T2* relaxation time is a combination of local inhomogeneities and this true T2 relaxation time. T2* relaxation times are shorter than the T2 relaxation time given dephasing of transverse magnetisation is more rapid. This is fundamental to fMRI as the primary measure is based on blood oxygenation, i.e. haemoglobin (Hb) and whether the Hb is oxygenated and diamagnetic, or deoxygenated (dHb) and paramagnetic. Due to the unbound haem group in dHb a charge is present and thus a small magnetic field, which introduces local inhomogeneities and thus increases dephasing (a shorter T2* relaxation time) of surrounding water and tissue (Ogawa, Lee, Nayak, *et al.*, 1990). For EEG fMRI studies, scanning parameters can vary from study to study, the TR could be 2-4 seconds, TE 30-50ms, 30-50 slices, 80-90º flip angle, voxels from 2 x 2 x 2 mm to 5 x 5 x 5 mm, with the number of volumes captured depending on desired length of scan.

It was the work of Ogawa and colleagues in 1990, who demonstrated an oxygen-dependent contrast in rodents under a high strength magnetic field (Ogawa, Lee, Kay, *et al.*, 1990; Ogawa, Lee, Nayak, *et al.*, 1990; Ogawa & Lee, 1990). In this work they defined the blood oxygenation level-dependent (BOLD) contrast in which the dHb is the contrast agent. The BOLD signal is

influenced by the level of blood oxygenation, cerebral blood flow (CBF), cerebral blood volume (CBV) and the cerebral metabolic rate of oxygen ($CMRO_2$) (Huettel, Song & McCarthy, 2014). The haemodynamic response that encompasses these measures results from neurovascular coupling associated with increases in neuronal activity (Arthurs & Boniface, 2002; Logothetis & Wandell, 2004). The BOLD signal is believed to reflect local field potentials rather than action potentials, occurring at synapses rather than soma of neurons and is an indirect measure of neuronal activity (Lauritzen, 2005; Logothetis, Pauls, Augath, *et al.*, 2001).

Modelling the BOLD responses for analysis is generally done using a haemodynamic response function (HRF). The HRF is a model of the expected changes in the BOLD response to an evoked or endogenous neuronal event (see figure 1.4.1). There are different HRF models, such as the Glover HRF derived from sensorimotor and auditory BOLD responses (Glover, 1999), the statistical parametric mapping (SPM) canonical HRF implemented in SPM (Friston, Ashburner, Kiebel, *et al.*, 2007) or even subject specific HRFs (Storti, Formaggio, Bertoldo, *et al.*, 2013). Temporally the haemodynamic response is sluggish, with an initial dip or delay of 1-2s, peaking at ~6s, and with post-stimulus undershoots that can last for 30s or more, dependent on stimulus duration (Buxton, Uludağ, Dubowitz, *et al.*, 2004). Estimated parameters of the HRF, i.e. response height, peak latency and full width at half maximum (FWHM), may yield useful information, when accurately modelled, regarding the underlying neuronal activity (Lindquist & Wager, 2007). Accurate modelling of the haemodynamic response is important to minimise false positive and negative results; a poor fitting model will result in a loss of statistical power and reduce validity of results (Handwerker, Ollinger & D'Esposito, 2004; Lindquist & Wager, 2007).

Most EEG-fMRI studies in epilepsy employ an event related design, where the timing of the IED is used and modelled in a general linear model (GLM) as a stick (zero-duration) or box-

car (with a given duration) function with the HRF; in SPM the canonical HRF comprises of two gamma functions, one to model the peak and the other the undershoot of the response. Further basis functions can be added to the canonical HRF to account for variations in peak latency and duration of peak response; the temporal derivative and dispersion derivative, respectively. The addition of the temporal and dispersion derivatives helps to account for any deviation from the predicted response (Friston, Fletcher, Josephs, *et al.*, 1998). In SPM the model is constructed via a design matrix which will include the timing of the IED, possibly it's derivatives, and any regressors of no interest, such as motion correction parameters, cardiac events, other paroxysmal EEG events of no interest etc. (see figure 1.4.2). The time course for each voxel is then analysed for fit of the predicted response (model) i.e. is there increased signal that occurs following an IED the is explained by the predicted response (HRF). A response that occurs outside the prediction will not be picked up, hence the reason for accurate modelling, this is especially pertinent in epilepsy due to the variable nature of BOLD responses to IEDs (for reviews see (Gotman, Kobayashi, Bagshaw, *et al.*, 2006; Gotman & Pittau, 2011; Murta, Leite, Carmichael, *et al.*, 2015b; van Graan, Lemieux & Chaudhary, 2015).

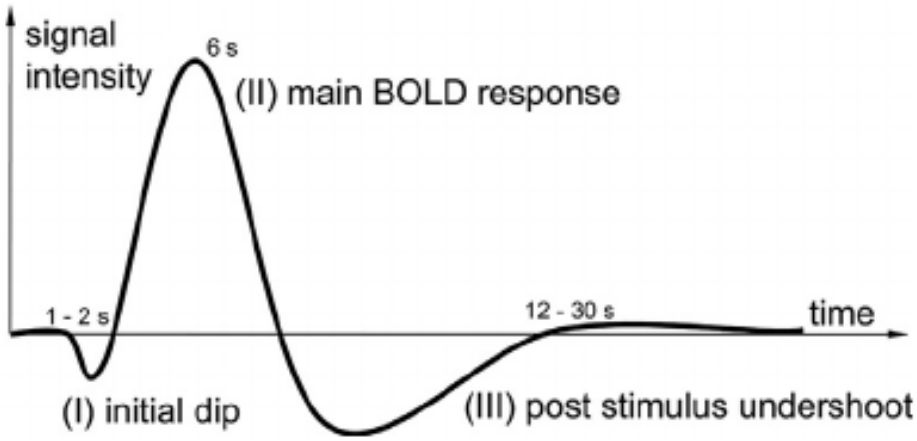


Figure 1.4.1. Example of a haemodynamic response.
Modified from Siero et al., 2013.

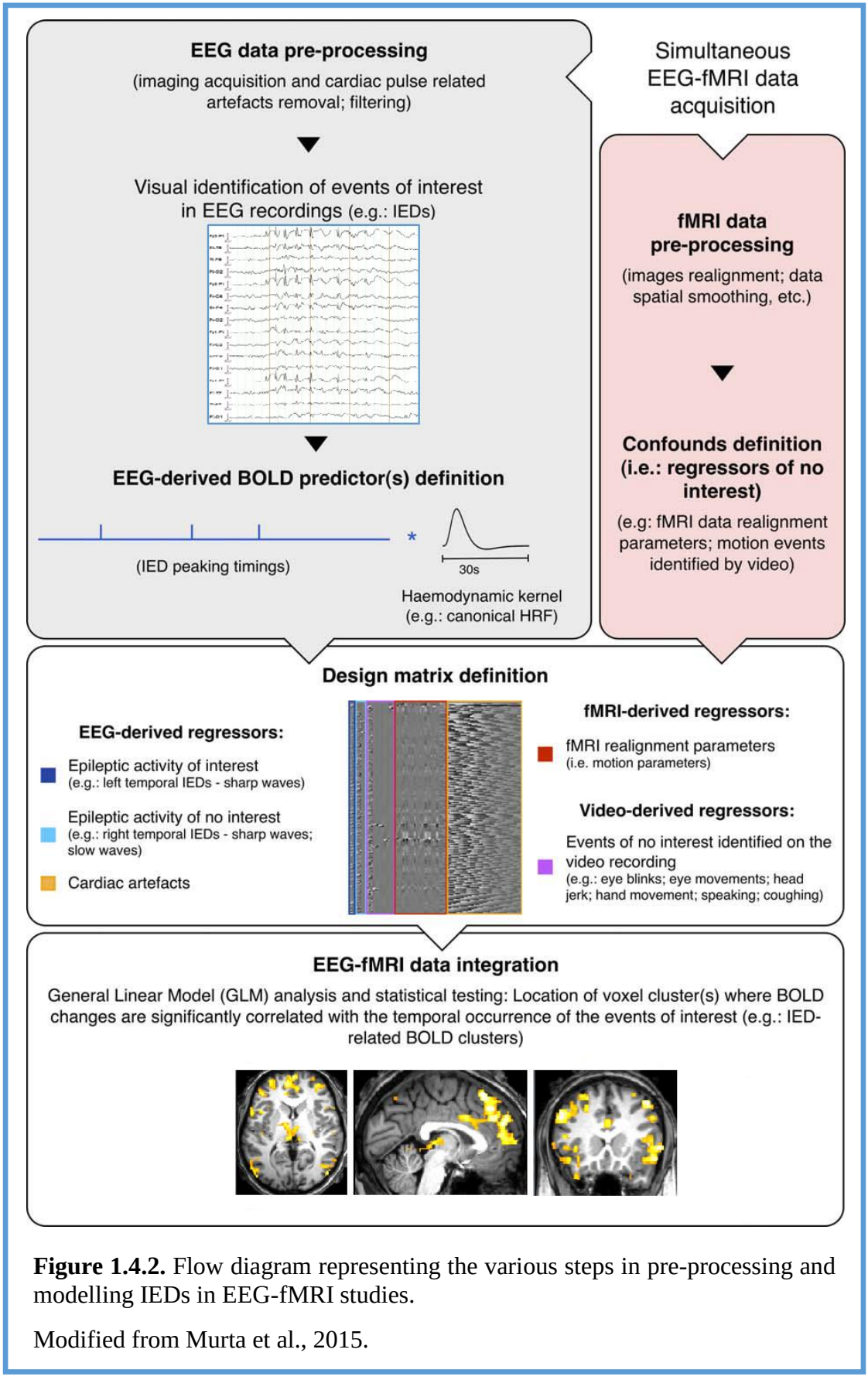


Figure 1.4.2. Flow diagram representing the various steps in pre-processing and modelling IEDs in EEG-fMRI studies.

Modified from Murta et al., 2015.

1.4.3 Pre-processing of fMRI data

The pre-processing of fMRI data is necessary for further analysis and largely consists of four steps; realignment or motion correction, slice timing correction, spatial normalisation and spatial smoothing. These processes allow correction for any displacements that occur during data acquisition and allow for spatially improved comparisons between subjects or for group analysis (for detailed reviews of these steps see (Huettel, Song & McCarthy, 2014; Aguirre, 2006)).

Realignment/Motion Correction

During scanning, no matter how cooperative a participant is or how well their head is padded within the head coil, there will be some movements. Movement correction attempts to account for this by comparing each subsequent image of the brain to the first one acquired. The primary method for this uses a rigid-body correction with six motion parameters (three translations and three rotation). Although this realignment to the first image accounts for movements it does not remove for signal artefacts caused by said movements. Studies usually include the motion parameters as a covariate of no interest to account for the effects of these artefacts on the BOLD signal.

Slice timing correction

Slice-timing correction is done to account for the slight delays between slices obtained for a given volume of fMRI data. Multiple 2D slices are obtained in order to build a 3D volume, e.g. 20 slices; as a slight delay is incurred for each slice the temporal shift is accumulated in the 3D

volume and thus in the haemodynamic response. As analysis of fMRI data is based on the fMRI time course, an event-related design, especially, would be compromised as the time of event would not match the haemodynamic response. Slice-timing correction is a necessary pre-processing step, especially in event-related designs (Sladky, Friston, Tröstl, *et al.*, 2011).

Spatial normalisation

Spatial normalisation is done to warp a subject's anatomical structure to a template. Putting a subject's brain into standard space allows for testing and comparison of brain regions across subjects. This is particularly important for analysis of group data and even combining imaging modalities. The Montreal Neurological Institute (MNI) defined a standard brain space using a large number of MRI scans from normal controls, and this MNI brain space is used in SPM software packages.

Spatial smoothing

Finally, spatial smoothing reduces the statistical burden in fMRI analysis associated with the multiple statistical tests per voxel. The greater the number of statistical tests per voxel, the higher the false positive rate and thus the higher the statistical threshold needs to be. Spatial smoothing reduces the number of tests required. Too much smoothing however will reduce sensitivity for small areas of activation and too little will reduce for large areas. A FWHM Gaussian filter of at least 8mm or more is recommended for group data (Mikl, Marecek, Hlustík, *et al.*, 2008). Though generally a smoothing of 2 times the maximum voxel size is used.

1.5 COMBINED EEG AND FMRI

The first fMRI study in humans was performed in 1992 by Kenneth Kwong and colleagues who demonstrated increases in signal intensity in the visual cortex and motor cortex following flash stimulation and a hand squeezing task, respectively (Kwong, Belliveau, Chesler, *et al.*, 1992). It was a year later in 1993 when John Ives and colleagues demonstrated it was possible to acquire EEG data in such a hostile environment. From these first steps into fMRI and combined recording of EEG and fMRI in humans, this has led to a plethora of studies investigating normal and pathological brain function.

1.5.1 EEG data acquisition during fMRI

The MRI scanner presents a hostile environment for recording the tiny voltages of scalp EEG, but also the materials used can have a detrimental impact on fMRI causing artefacts during scanning. Selecting the right equipment is essential for EEG-fMRI studies, for example Ag/AgCl electrodes produce more artefact on fMRI scans than carbon electrodes, whereas carbon composition resistors attached to electrodes produce much more artefact than cermet film resistors, thus poorly optimised equipment will lead to artefacts intruding into cortex (Krakow, Allen, Symms, *et al.*, 2000). Rather than individually placed electrodes, the use of specialised MR-compatible EEG caps are also recommended (Bonmassar, Hadjikhani, Ives, *et al.*, 2001). Due to the rise in popularity of EEG-fMRI, companies exist specialising in optimised equipment specifically designed for EEG-fMRI studies. Optimised equipment will minimise artefacts in the fMRI data but the EEG data will still be rendered uninterpretable by two significant contributors of artefacts; gradient artefacts and ballistocardiographic (BCG) artefacts.

Gradient artefacts are caused by the rapid switching of magnetic field gradients that occurs during scanning to give spatial information (see section 1.4.1). This high frequency artefact completely obscures the EEG data and can saturate amplifiers with insufficient dynamic range, but over the years several methods have been described in order to remove the artefact; the average artefact subtraction (AAS) method (Allen, Josephs & Turner, 2000); a variation of the AAS method, using principal component analysis (PCA) to define optimal basis sets (OBS) for artefact removal (Niazy, Beckmann, Iannetti, *et al.*, 2005); the optimised moving-average filtering method (Ferreira, Wu, Besseling, *et al.*, 2016); and the inclusion of any movements that occur during scanning into the artefact subtraction method (Freyer, Becker, Anami, *et al.*, 2009; Sun & Hinrichs, 2009). It has also been demonstrated that optimising the subject's axial position can attenuate gradient artefacts (Mullinger, Yan & Bowtell, 2011). For most methods, to obtain optimal gradient artefact removal other additional conditions need to be satisfied; firstly, the synchronisation between MRI scanner and EEG acquisition clocks (Mandelkow, Halder, Boesiger, *et al.*, 2006; Mullinger, Morgan & Bowtell, 2008), secondly, reducing the magnitude of the gradient artefact using a low-pass hardware filter, typically set to 250Hz (Mullinger, Castellone & Bowtell, 2013) and finally a sufficiently high sampling rate, ≥5KHz (Allen, Josephs & Turner, 2000).

BCG artefacts are temporally coupled with the QRS complex of the ECG, occurring on the EEG with a slight delay, and are a combination of small movements of the electrodes as the scalp expands and contracts with the cardiac cycle (i.e. two phases, systolic and diastolic); small movements of the body and head related to cardiac cycle; and variation of the Hall voltage caused by changes in speed of the blood passing through arteries (Sijbers, Van Audekerke, Verhoye, *et al.*, 2000). As with gradient artefact removal the same methods previously can be used to create a template to subtract BCG artefacts, i.e. AAS method (Allen, Josephs & Turner,

2000), or the OBS method (Niazy, Beckmann, Iannetti, *et al.*, 2005). Field strength is an important consideration with regards to BCG artefact removal; as BCG artefact is a variable physiological artefact, when the magnetic field strength increases so does the amplitude and spatial variability of the artefact, making it increasingly difficult to remove (Debener, Mullinger, Niazy, *et al.*, 2008).

For a detailed overview of the practicalities of EEG-fMRI acquisition see Mullinger, Castellone & Bowtell, 2013

1.5.2 EEG-fMRI in epilepsy

The first EEG-fMRI experiments investigating epilepsy did not use fully simultaneous recording of EEG and fMRI, rather the EEG was used to trigger fMRI acquisition, with up to a few seconds delay in fMRI acquisition following IEDs (Krakow, Woermann, Symms, *et al.*, 1999; Seeck, Lazeyras, Michel, *et al.*, 1998; Warach, Ives, Schlaug, *et al.*, 1996). These studies successfully demonstrated regional increases in signal intensity associated with scalp IEDs. With the development of recording techniques and methods of artefact subtraction, as previously described, early studies using combined EEG-fMRI in epilepsy showed it was possible to implement this technique, again demonstrating regional increases in the BOLD signal associated with IEDs (Bénar, Gross, Wang, *et al.*, 2002; Lemieux, Salek-Haddadi, Josephs, *et al.*, 2001). From these initial studies, numerous have followed investigating focal epilepsies (Aghakhani, Kobayashi, Bagshaw, *et al.*, 2006; Kobayashi, Bagshaw, Bénar, *et al.*, 2006; Lengler, Kafadar, Neubauer, *et al.*, 2007; Liu, Yang, Yang, *et al.*, 2008; Manganotti, Formaggio, Gasparini, *et al.*, 2008), generalised epilepsies (Aghakhani, 2004; Benuzzi, Ballotta, Mirandola, *et al.*, 2015; Hamandi, Laufs, Nöth, *et al.*, 2008) and even recording ictal

events during EEG-fMRI (Chaudhary, Duncan & Lemieux, 2013; Di Bonaventura, Vaudano, Carnì, *et al.*, 2006; Liu, Yang, Yang, *et al.*, 2008; Salek-Haddadi, 2002; Sierra-Marcos, Maestro, Falcón, *et al.*, 2013). The potential of EEG-fMRI to localise epileptic foci has led to its proposed primary clinical application in the work-up to epilepsy surgery (van Graan, Lemieux & Chaudhary, 2015; Zijlmans, Huiskamp, Hersevoort, *et al.*, 2007), and where this places in the surgical pathway can be seen in figure 1.5.1 (Chapter 2 covers this in more detail).

Since the early days of EEG-fMRI in epilepsy it was discovered that not all IEDs demonstrate a BOLD response that conforms to a canonical HRF shape (Bénar, Gross, Wang, *et al.*, 2002; Lemieux, Laufs, Carmichael, *et al.*, 2007; Masterton, Harvey, Archer, *et al.*, 2010), and not all IEDs seem to be associated with BOLD changes (Aghakhani, Kobayashi, Bagshaw, *et al.*, 2006; Salek-Haddadi, Diehl, Hamandi, *et al.*, 2006). Two important factors may play a part in this, the modelling techniques used to model IEDs (discussed in section 2.5), and the neurovascular coupling that underpins the BOLD signal is potentially different in pathological neuronal events, such as an IED (Schwartz, 2007), as opposed to BOLD signal changes in response to a task in a normal brain, e.g. astrocytes that contribute significantly to CBF changes (Buxton, 2012; Murta, Leite, Carmichael, *et al.*, 2015a) are known mediators of epileptiform activity (Devinsky, Vezzani, Najjar, *et al.*, 2013), with recent computational models demonstrating nonlinear contributions of astrocytes to CBF magnitudes in interictal events (Blanchard, Saillet, Ivanov, *et al.*, 2016). As iterated in a review by Buxton, 2012, understanding of the physiological mechanisms behind the BOLD signal still requires further research (Buxton, 2012), this is especially true of pathological brain events.

The conceptual development of epilepsy as a condition involving large-scale brain networks, often spanning hemispheres and involving multiple brain lobes, has evolved over the last couple of decades (Halász, 2010a, 2010b), to the extent of being recognised in recent standards of

classification (Berg, Berkovic, Brodie, *et al.*, 2010). The network basis of epilepsy has also been investigated with EEG-fMRI studies, using group analysis of patients with epilepsy to identify common regions across subjects (An, Dubeau & Gotman, 2015; Fahoum, Lopes, Pittau, *et al.*, 2012; Kobayashi, Grova, Tyvaert, *et al.*, 2009; Laufs, Richardson, Salek-Haddadi, *et al.*, 2011). From these studies, a common region of activation associated with IEDs, in a group with focal epilepsy, was identified in the ipsilateral frontal piriform cortex (Laufs, Richardson, Salek-Haddadi, *et al.*, 2011); in a more homogenous group, of TLE patients, activation was reported in ipsilateral mesial temporal and bilateral neocortical-temporal structures and basal ganglia (Kobayashi, Grova, Tyvaert, *et al.*, 2009); and a study by Fahoum et al., 2012, identified multiple brain regions associated with three distinct groups of frontal lobe epilepsy (FLE), TLE and posterior quadrant epilepsy. Group deactivations were also reported in regions of the default mode network (DMN) (Fahoum, Lopes, Pittau, *et al.*, 2012; Kobayashi, Grova, Tyvaert, *et al.*, 2009), a resting state network of the brain (Raichle, MacLeod, Snyder, *et al.*, 2001).

The advent of fMRI, specifically the development of resting-state fMRI, has contributed significantly to the characterisation of numerous resting-state networks (RSNs) composed of often distant regions functionally correlated (i.e., demonstrating correlation of their time series) to form specific networks common between subjects (Damoiseaux, Rombouts, Barkhof, *et al.*, 2006; Fox, Corbetta, Snyder, *et al.*, 2006; Raichle, MacLeod, Snyder, *et al.*, 2001; Raichle & Snyder, 2007; Seeley, Menon, Schatzberg, *et al.*, 2007). Alterations in the DMN (Gotman, Grova, Bagshaw, *et al.*, 2005a; Haneef, Lenartowicz, Yeh, *et al.*, 2012; Luo, Li, Lai, *et al.*, 2011), attention networks (Chen, Huang, Chen, *et al.*, 2015; Zhang, Lu, Zhong, *et al.*, 2009; Zheng, Qin, Dang, *et al.*, 2012), fronto-parietal control network (Wei, An, Zeng, *et al.*, 2015b), and executive control network (Vlooswijk, Jansen, Jeukens, *et al.*, 2011) have been reported in various epilepsy syndromes. As well as disruption of well-defined RSNs caused by an

underlying epilepsy syndrome, recent research has also begun to utilise similar methods to investigate epilepsy specific fluctuations in resting state fMRI, i.e. regions implicated in the epilepsy. Changes in functional connectivity (FC), specifically a reduction, between affected amygdala/hippocampus and the DMN/contralateral homologous structures has been demonstrated in unilateral mesial TLE (Pittau, Grova, Moeller, *et al.*, 2012). Additionally, amplitude of low frequency fluctuation (ALFF) measurements, which measures low frequency activity (0.01-0.08Hz) of BOLD signal data, presumably associated with the brain's spontaneous neuronal activity during rest (Zang, He, Zhu, *et al.*, 2007; Zou, Zhu, Yang, *et al.*, 2008), has demonstrated regional increases in mesial TLE with moderate sensitivity and high specificity in lateralisation (Zhang, Lu, Zhong, *et al.*, 2010), and may even aid in classification of TLE subtypes (Reyes, Thesen, Wang, *et al.*, 2016). Such techniques, group analysis of homogenous epilepsies, investigating alterations in RSNs as a function of epilepsy, and changes in FC and ALFF, again allow for potentially clinically relevant information and may help in pre-surgical evaluation of epilepsy. Indeed, studies have begun to use resting state EEG-fMRI and FC measures as an assessment tool in pre-surgical workup for epilepsy. In an EEG-fMRI study by Negishi et al., 2011, computed FC maps, seeded from the concordant region of activation derived from spike related EEG-fMRI, demonstrated less lateralised FC in groups of patients with poor outcome following resective surgery, i.e. seizure recurrence, indicating this technique may potentially be used as a predictor of outcome (Negishi, Martuzzi, Novotny, *et al.*, 2011). Seizure freedom following surgery is one outcome, another outcome to consider is potential neuro-deficits following surgery. Recent studies have also shown that resting state fMRI may help predict neurocognitive outcomes following surgery (Bonelli, Powell, Yogarajah, *et al.*, 2010; Doucet, Rider, Taylor, *et al.*, 2015). (Pittau & Vulliemoz, 2015).

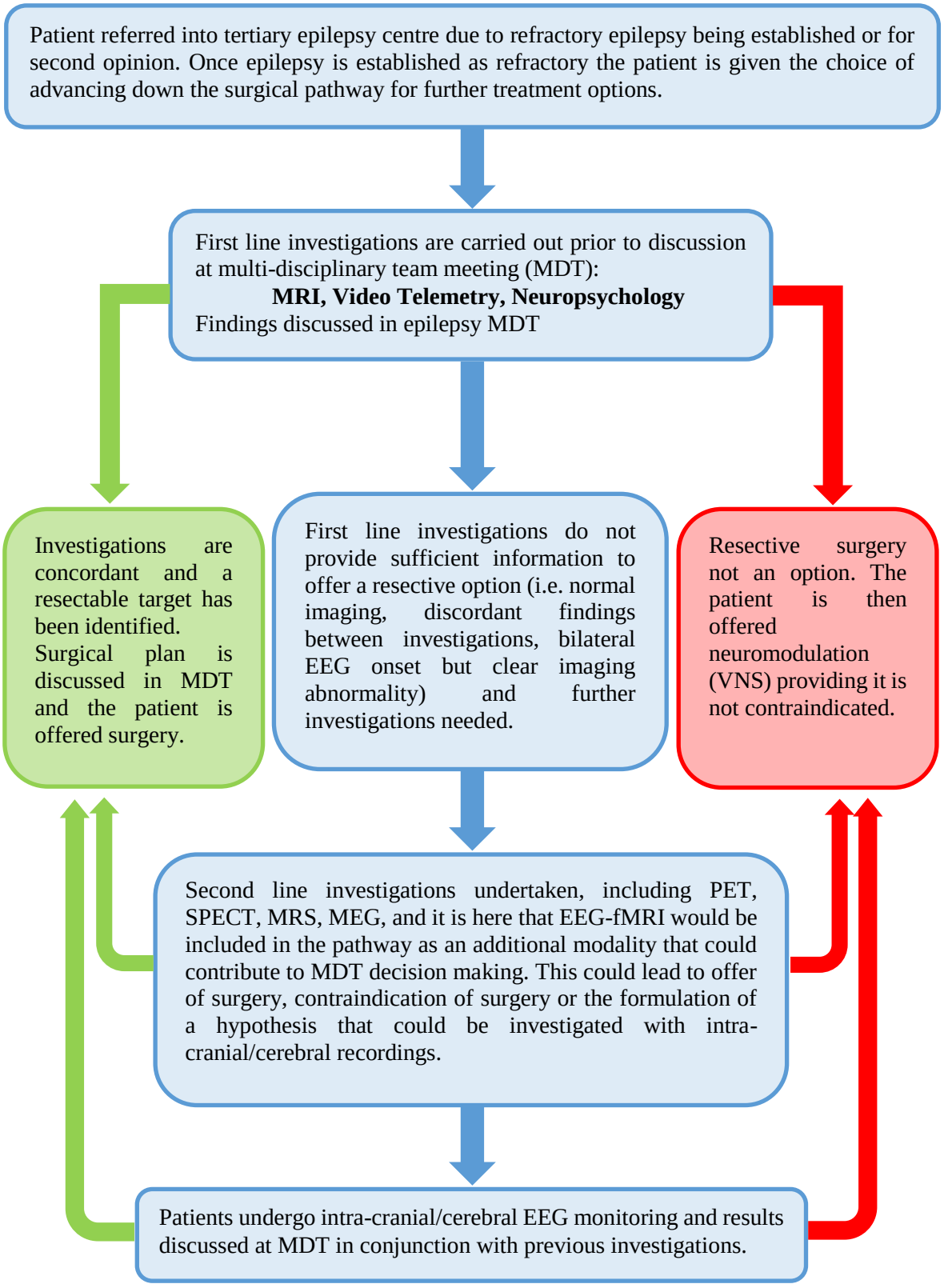


Figure 1.5.1. Epilepsy surgery MDT pathway. A summary of the surgical pathway at the Queen Elizabeth Hospital Birmingham, Birmingham, UK, indicating where EEG-fMRI might fit in.
VNS = vagal nerve stimulator, PET = positron emissions tomography, MRS = magnetic resonance spectroscopy, SPECT = single positron emission tomography, MEG = magnetoencephalogram

1.5.3 EEG-fMRI in sleep

Studies investigating cognitive processing of sensory stimuli during sleep have primarily focused on using EEG and the subsequent investigation of event related potentials (ERPs) to various acoustic paradigms (Bastuji, García-Larrea, Franc, *et al.*, 1995; Colrain & Campbell, 2007; Cote & Campbell, 1999). Acoustic stimuli are used in ERP studies preferentially, given, as an experimental variable, it is easier to manipulate, deliver and measure, as opposed to respiratory occlusion for example (for review of ERPs in sleep see Campbell, 2010). One of the earliest studies to use EEG-fMRI during sleep sought to do something similar, and presented auditory stimuli during NREM sleep, identifying increased activity in left amygdala and prefrontal cortex to novel stimuli, and concluding that the brain can process meaningful stimuli during sleep (Portas, Krakow, Allen, *et al.*, 2000). A further EEG-fMRI study by Czisch et al., 2002, using complex auditory stimulation of a recording of a Mark Twain story, demonstrated a reduction in activation in the auditory cortex and a deactivation in the visual cortex accompanying NREM sleep (Czisch, Wetter, Kaufmann, *et al.*, 2002). This deactivation in the visual cortex was also corroborated by an fMRI and PET study (Born, Law, Lund, *et al.*, 2002), and was interpreted as a mechanism of the brain to prevent arousal from external stimuli (Czisch, Wetter, Kaufmann, *et al.*, 2002). Czisch et al., 2004, later expanded on this work mapping the correlation between the fMRI response and the EEG changes associated with acoustic stimuli, i.e. delta power and KCs, demonstrating BOLD signal decreases that correlated with an increase in delta power and KCs and that were absent during SWS, indicating a stage-dependent sleep-protective mechanism (Czisch, Wehrle, Kaufmann, *et al.*, 2004). It has also been shown, in a very recent study, that there is reduced auditory responses to stimuli with a hierarchy of language processing during sleep, with activation in regions related to higher order language processing absent during NREM (Wilf, Ramot, Furman-Haran, *et al.*, 2016).

The processing of incoming auditory stimuli has also been studied in relation to sleep paroxysms, SSs (Dang-Vu, Bonjean, Schabus, *et al.*, 2011; Dang-Vu, McKinney, Buxton, *et al.*, 2010; Schabus, Dang-Vu, Heib, *et al.*, 2012) and KCs (Dang-Vu, Bonjean, Schabus, *et al.*, 2011). Behavioural studies have demonstrated that responses to auditory stimulus in the presence of a SS are diminished and more variable as compared to responses outside the presence of a SS, indicating a disruption in the transmission of external stimuli to the cortex (Dang-Vu, Bonjean, Schabus, *et al.*, 2011; Dang-Vu, McKinney, Buxton, *et al.*, 2010). Auditory responses followed by a KC were enhanced as compared to auditory responses in the absence of a KC, also producing frontal activations similar to those seen with a SO (Dang-Vu, Bonjean, Schabus, *et al.*, 2011). Similar findings were also demonstrated by Czisch et al., 2009, who observed stronger auditory responses associated with KCs than not (Czisch, Wehrle, Stiegler, *et al.*, 2009).

Aside from their interaction with incoming stimuli, SSs and KCs have also been a subject of study in their own right (Caporro, Haneef, Yeh, *et al.*, 2012; Jahnke, von Wegner, Morzelewski, *et al.*, 2012; Schabus, Dang-Vu, Albouy, *et al.*, 2007) as have VSWs (Stern, Caporro, Haneef, *et al.*, 2011), as investigation of these paroxysms using EEG-fMRI may yield information regarding function or networks involved in their generation. Activation associated with sleep spindles has been reported in the thalamus, anterior and posterior cingulate cortices, insula cortex, temporal lobes and paracentral cortex (Caporro, Haneef, Yeh, *et al.*, 2012; Schabus, Dang-Vu, Albouy, *et al.*, 2007), whilst activation associated with KCs has been reported in the thalamus, superior temporal lobes, paracentral gyri, occipital, parietal and frontal lobes (Caporro, Haneef, Yeh, *et al.*, 2012), as well the brainstem and primary sensory regions (Jahnke, von Wegner, Morzelewski, *et al.*, 2012). EEG-fMRI of VSWs demonstrated signal increases predominating in primary sensory and motor regions (Stern, Caporro, Haneef, *et al.*,

2011). Interestingly only one of these studies report negative BOLD changes, Jahnke et al., 2012, in the anterior insula, this was interpreted as part of a mechanism to prevent higher order processing of any basic sensory information that may be processed during a KC. They also used dynamic causal modelling to support a cortical origin of the KC (Jahnke, von Wegner, Morzelewski, *et al.*, 2012). In addition to these sleep paroxysms, the cerebral correlates of slow waves and delta waves have also been investigated linking several brain regions, including frontal cortical regions, posterior cingulate cortex and the precuneus, synchronised to the slow oscillation (Dang-Vu, Schabus, Desseilles, *et al.*, 2008).

The effect of sleep on established brain networks has also begun to unfurl with particular focus on the DMN (Horovitz, Braun, Carr, *et al.*, 2009; Koike, Kan, Misaki, *et al.*, 2011; Sämann, Wehrle, Hoehn, *et al.*, 2011). The deepening of sleep has demonstrated the decoupling of the frontal cortical components of the DMN (Horovitz, Braun, Carr, *et al.*, 2009), and a general reduction in corticocortical functional connectivity in the DMN, notably between the anterior and posterior node, and between the DMN and its anti-correlated network (Sämann, Wehrle, Hoehn, *et al.*, 2011). The effects of sleep deprivation on the DMN has also been investigated with just fMRI, demonstrating reduced functional connectivity within the DMN with sleep deprivation (De Havas, Parimal, Soon, *et al.*, 2012). For a review on the utility of EEG-fMRI in sleep and the relationship between sleep and brain networks, see Duyn, 2012, and Picchioni, Duyn & Horovitz, 2013.

1.6 THESIS OVERVIEW

The first experiment in this thesis involved the recruitment of patients with a complex epilepsy from a tertiary epilepsy centre. The aim here was to assess the utility of EEG-fMRI in a clinical setting as if this investigation were freely available to the epileptologist or epilepsy neurosurgeon. The patients "referred" in for this theoretical service were primarily those who were on an epilepsy surgical pathway, though some were non-surgical complex diagnostic cases. Following on from this the second experiment expanded on previous work investigating BOLD signal changes preceding IEDs, this time applying a pre- and postspike analysis to both a subset of patients with focal epilepsy and a visual experiment in normal participants. In the next experiment this same analysis was then applied to sleep paroxysms (VSWs, KCs and SSs) from a group of sleep deprived participants who managed to sleep in the MRI scanner. The purpose of this experiment was to determine if early BOLD signal changes are unique to pathological paroxysmal activity or also observable in non-pathological paroxysmal activity. The initial hypothesis was that "prespike" changes might be absent and thus validate the findings of prespike changes in epilepsy. This experiment became quite orientated towards sleep and ultimately marked the point at which the thesis began to deviate towards the investigation of sleep. The final experiment focused solely on the investigation of sleep paroxysms, explicitly VSWs and KCs, this time re-recruiting previous sleep deprived participants who slept in the scanner, to achieve data reflective of "normal" rather than recovery sleep, and examine the effect of sleep deprivation on these transients.

Chapter 2

UTILITY OF EEG-fMRI IN PATIENTS REFERRED FROM THE TERTIARY EPILEPSY CLINIC

2.1 ABSTRACT

Epilepsy is one of the most common neurological disorders and onset can occur at any age. It is a complex disease of many aetiologies and can be challenging in its diagnosis, classification and management. In approximately 30 per cent of cases the epilepsy is not adequately controlled with pharmacological treatment and a subset of these patient may progress to epilepsy surgery as a potential curative option, an option that often requires a considerable work-up. The aim of this study was to assess the potential use of EEG-fMRI as a clinical tool if it were freely available to the epileptologists/neurosurgeon in the setting of a tertiary centre with a complex epilepsy service. In this study, 26 patients were recruited from epileptologists who "referred" in complex epilepsy patients. The primary aim for most patients was that of localisation for pre-surgical work-up but in some cases it was a diagnostic question that predominated. Of the 26 recruited only 13 demonstrated any interictal epileptiform discharges on the EEG obtained within the scanner. Of those 13, two were primarily "referred" for more diagnostic reasons and the others for further localisation. In 3 (12%) of cases overall, the results from the EEG-fMRI were judged by the epileptologist to have been contributory to patient management or aid progression down a surgical pathway. These results suggest, deployed in its simpler form, EEG-fMRI has some limited use in the tertiary epilepsy clinic.

2.2 INTRODUCTION

Epilepsy is a relatively common neurological disease affecting an estimated 50 million people worldwide, across all age groups (Anon, 2010). Of these patients approximately 20 - 30 per cent will develop a refractory epilepsy, one that is not amenable to pharmacological intervention (Arroyo, Brodie, Avanzini, *et al.*, 2002; Sander, 1993). According to guidelines laid down by the National Institute for Health and Clinical Excellence (NICE), refractory epilepsy is established after 2 years of unsuccessful pharmacological treatment and after trial of at least two medications. Following this, patients are recommended to be referred to a tertiary centre for further management and possible entry on to an epilepsy surgery program (National Clinical Guideline Centre (UK), 2012). For patients with refractory epilepsy who enter the surgical program, there are two surgical options; cranial surgery, usually resective, and neuromodulation, with resective surgery being the only potentially curative option (Fauser & Zentner, 2012). Given seizure freedom rates of between 50 – 80 percent following resective surgery (de Tisi, Bell, Peacock, *et al.*, 2011; Elwes, Dunn, Binnie, *et al.*, 1991; Polkey, 2004) pursuing this option is indeed worthwhile for many. The decision on surgical treatments is only made following a battery of diagnostic tests; most commonly video-EEG, MRI, and neuropsychological testing (Bernstein, Prather & Rey-Casserly, 1995; Uijl, Leijten, Parra, *et al.*, 2005; Urbach, Mast, Egger, *et al.*, 2015). Lack of concordance between diagnostic modalities, though not necessarily discordance, can often hamper progression to resective surgery and this is most notable in the presence of normal MR imaging (Alarcon, 2006; Scott, Fish, Smith, *et al.*, 1999). In the absence of an MRI abnormality the use of more novel imaging techniques has increased in recent years with the particular aim of formulating a hypothesis on localising the epileptogenic zone (EZ), which may be amenable to further

investigation with intracranial electrodes (Nowell, Rodionov, Zombori, *et al.*, 2015; Rodionov, Vollmar, Nowell, *et al.*, 2013). The use of intracranial recordings has been shown to be effective in both MRI-negative and lesional epilepsies in equal measures, though formulating an implant strategy is still dependent on the information gained from non-invasive techniques (McGonigal, Bartolomei, Regis, *et al.*, 2007). Given around 30 – 50 per cent of adult patients with focal epilepsy have no identifiable lesion on MR imaging (Nguyen, Mbacfou, Nguyen, *et al.*, 2013; Toledo, Sarria-Estrada, Quintana, *et al.*, 2013), further information regarding localisation is invaluable, and utilisation of multimodal imaging techniques can significantly alter decisions made regarding epilepsy surgery (Nowell, Rodionov, Zombori, *et al.*, 2015).

Combined EEG-fMRI is one such non-invasive imaging technique that has been developing over the last two decades and gaining more support for its potential role in epilepsy surgical work-up (Dorfer, Widjaja, Ochi, *et al.*, 2015; Ramli, Rahmat, Lim, *et al.*, 2015; van Graan, Lemieux & Chaudhary, 2015). Given the difficulties in formulating a hypothesis on resective targets for epilepsy surgery, any further information that could be provided by non-invasive techniques is welcome. Initially EEG-fMRI studies tended to report on case studies of a small group of, often heterogeneous, patients with epilepsy (Bénar, Gross, Wang, *et al.*, 2002; Lemieux, Salek-Haddadi, Josephs, *et al.*, 2001; Salek-Haddadi, Merschhemke, Lemieux, *et al.*, 2002), only later focussing on more homogenous epilepsy forms, such as focal TLE (Kobayashi, Bagshaw, Bénar, *et al.*, 2006; Morgan, Gore & Abou-Khalil, 2007), or generalised epilepsies (Aghakhani, 2004; Benuzzi, Ballotta, Mirandola, *et al.*, 2015; Hamandi, Laufs, Nöth, *et al.*, 2008; Hamandi, Salek-Haddadi, Laufs, *et al.*, 2006). The utilisation of EEG-fMRI in the work-up for epilepsy surgery has already gained momentum, and is proving to be a useful addition in the battery of non-invasive

investigations available to clinicians and surgeons (Coan, Chaudhary, Grouiller, *et al.*, 2016; Moeller, Tyvaert, Nguyen, *et al.*, 2009; Thornton, Laufs, Rodionov, *et al.*, 2010; Zijlmans, Huiskamp, Hersevoort, *et al.*, 2007). The vast majority of these outcome studies focus on patients already entered onto an epilepsy surgery program rather than refractory patients who might be difficult to manage. How clinicians, who may not have any vested interest or working knowledge of EEG-fMRI, would utilise this test were it freely available to them is not fully reported. Would it be used in difficult to manage patients or exclusively for pre-surgical work-up where localisation is difficult with just basic modalities available?

The aim of this study was to investigate the potential utility of EEG-fMRI in a group of heterogeneous patients with epilepsy, freely referred from the complex epilepsy service of a tertiary NHS Trust, and follow them up over a period of at least 3 years to see if EEG-fMRI made any contribution to patient management, surgical or otherwise.

2.3 METHODS

Patients

Twenty-six patients (13 females, 37±19 years) with a diagnosis of epilepsy were recruited from Consultant Neurologists with a special interest in Epilepsy, from the University Hospital Birmingham NHSF Trust and The Barbary BSMH Trust in accordance with National Health Service ethical approval (REC reference 06/Q2702/69). Patients were referred in by Epileptologists with the expressed intent of gaining more information on clinically difficult to manage patients, not necessarily on any surgical program. Patients were accepted on the basis of frequency of IED

(estimated rate more than 10 IEDs per hour based on routine outpatient EEG) and a seizure frequency of less than one per week to minimise the risk of a seizure occurring in the MRI scanner. Written and informed consent was obtained, and the patients were screened to ensure suitability for scanning. One subject was scanned twice on two separate occasions; the first dataset for this patient is included. Following scanning and presenting the results to the epileptologists who referred the patients for EEG-fMRI, they were asked whether they felt the additional information provided by the results was contributory to patient management. The neurologists/epileptologists were fully aware that EEG-fMRI, at the time, was not a clinically validated investigative technique and any results were treated as such.

EEG-fMRI data acquisition

EEG data were acquired at 5 kHz continuously during scanning using an MR compatible 64-channel EEG cap (BrainProducts, Munich, Germany) and two 32 channel MR compatible amplifiers (BrainAmp MR Plus, Brain Products, Munich, Germany). The EEG recording cap consisted of 62 scalp electrodes distributed in accordance with the international 10-20 system, with a further 2 electrodes to record ECG and ocular movements. Electrode impedances were kept below 10kΩ.

A 3T Phillips Achieva MR scanner (Philips, Netherlands), with an 8-channel SENSE head coil, was used for the acquisition of echo-planar imaging (EPI) data (patients 1 – 8, 2.5mm isotropic voxel, TR 3000ms, TE 35ms, 50 slices, flip angel 80°, 120 volumes/run; patients 9 – 25, 3x3x4mm voxels, TR 2000ms, TE 35ms, 32 slices, flip angel 80°, 180 volumes/run) and a T1-weighted scan

(1mm isotropic voxels) for anatomical reference of fMRI data. Each run of fMRI lasted approximately 6 minutes, with the number of runs acquired dependent on patient cooperation and comfort.

EEG processing

Removal of MR gradient artefacts was performed offline using Brain Vision Analyser (Brain Products, Munich, Germany) using a template subtraction approach (Allen, Josephs & Turner, 2000). Ballistocardiogram artefacts were removed using the Optimal Basis Set (OBS) plug-in (Niazy, Beckmann, Iannetti, *et al.*, 2005) to EEGLAB (Delorme & Makeig, 2004). An experienced electroencephalographer marked the onset of any epileptiform electrographic discharges, categorising the events based on morphology, topography and duration. If a patient had more than one IED type, this was modelled within the same design matrix.

fMRI processing

EPI data were processed in SPM5 (Wellcome Department of Imaging Neuroscience, UCL, UK). Initially the images underwent realignment for motion correction, followed by slice timing correction, anatomical normalisation to MNI space and then finally the data were smoothed with a Gaussian kernel (6mm). Images were flipped so that the right side of the image represents the right side of the brain (i.e. images are presented according to neurological convention). The timings of the IEDs extracted from the EEG data were modelled in an event related design as a stick function using a canonical HRF and its temporal derivative (Friston, Fletcher, Josephs, *et al.*, 1998)

implemented in SPM5 (Friston, Ashburner, Kiebel, *et al.*, 2007). Six motion correction parameters were also included in the analysis as multiple regressors. Statistical t-maps were created with a liberal thresholding of $p<0.001$, uncorrected.

2.4 RESULTS

Of the 26 patients initially recruited into the study only 13 (8 females, 22±18 years) were included in the final analysis. One patient was excluded due to having a seizure in the scanner before EPI acquisition, one due to excessive myogenic artefact on the EEG recording obtained within the scanner and one due to not wanting to go inside the scanner due to the feeling that the scanner environment was too uncomfortable. The remaining 10 patients were excluded due to having no IEDs whilst in the scanner (Table 2.4.1. outlines brief details on each patient and reason for exclusion). One patient (Patient 17) demonstrated only 8 irregular theta paroxysms with a slightly sharpened morphology occurring in the left temporal region during scanning. The clinical EEG, 12 months prior to participation in this study, demonstrated well defined left temporal sharp waves; unfortunately, these were not evident during scanning. No significant activation was associated with the paroxysmal theta activity that was captured on the EEG during scanning.

For patients who went on to have surgery or where the epileptologists felt that the results of the EEG-fMRI were contributory in patient management, the results are presented as short case studies. In addition, a summary of 5 patients who were included, but where the results were non-contributory or did not progress to epilepsy surgery, can be found in Table 2.4.2. Coordinates and

anatomical locations highlighted in bold are the location of the maximum t-statistic in both the case studies and in Table 2.4.2.

Patient	Age/Sex	Seizure type	IEDs in scanner	Included/Excluded
1	40/M	L arm FMS	137 R T SW	Included
2	20/M	R TLS	Nil recorded	Excluded – no IEDs
3	18/M	GTCS	7 GSW	Included
4	35/F	R TLS	Nil recorded	Excluded – no IEDs
5	30/M	R TLS, SPS	14 R T SW	Included
6	36/M	L TLS, GTCS	Nil recorded	Excluded – no IEDs
7	29/M	GTCS	Myogenic artefact	Excluded - artefact
8	24/F	GTCS, SPS	15 R P Sp	Included
9	36/M	L TLS, GTCS	Refusal to be scanned	Excluded
10	18/F	L TLS	21 L T SW	Included
11	47/M	GTCS	Nil recorded	Excluded – no IEDs
12	25/F	L FLS	59 L F SW	Included
13	21/F	L TLS	45 L T Sp	Included
14	18/F	L TLS, GTCS	17 L T SW	Included
15	31/M	GTCS, drop attacks	Nil recorded	Excluded – no IEDs
16	49/F	R TLS	Nil recorded	Excluded – no IEDs
17	29/M	L TLS	8 L T theta	Included
18	56/F	L TLS, NEAD	Nil recorded	Excluded – no IEDs
19	19/F	L TLS, GTCS	19 L F-T SW	Included
20	55/M	R TLS	Nil recorded	Excluded – no IEDs
21	30/F	FLS	Nil recorded	Excluded – no IEDs
22	25/F	FLS (hyperkinetic)	Nil recorded	Excluded – no IEDs
23	33/F	R FS	7 R T SW	Included
24	30/M	L FMS	8 L F-C SW	Included
25	38/M	FLS (hyperkinetic)	Seizure in scanner	Excluded
26	37/F	L TLS	4 L T SW	Included

Table 2.4.1. Summary of patients recruited into study. Patients recruited into the final analysis are highlighted and reasons given if not included in study. Most common reason for exclusion is lack of IEDs recorded in the scanner.
Abbreviations: L = left, R = right, F = frontal, T = temporal, C = central, P = parietal, IED = interictal epileptiform discharge, FMS = focal motor seizures, TLS = temporal lobe seizures, GTCS = generalised tonic clonic seizures, SPS = simple partial seizures, FLS = frontal lobe seizures, SW = sharp waves, GSW = generalised spike and wave, Sp = spikes, NEAD = non-epileptic attack disorder.

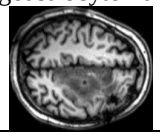
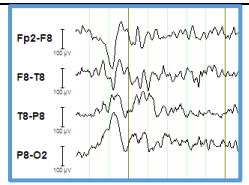
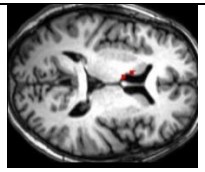
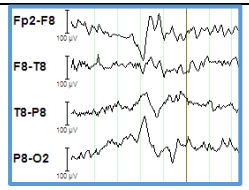
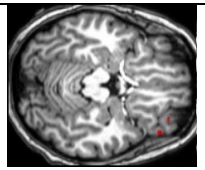
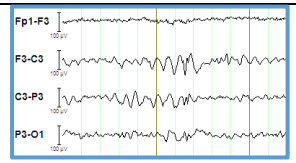
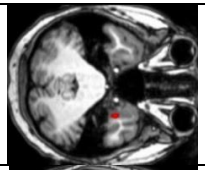
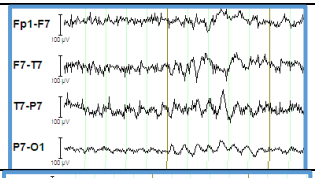
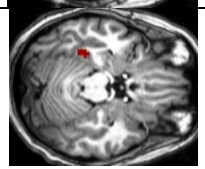
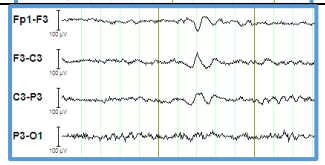
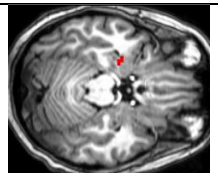
Patient	Clinical MRI	Seizure semiology	EEG	fMRI results	fMRI activation
1, 40/M	Extensive right oligoastrocytoma 	Focal motor seizures of the left arm lasting only several seconds. Retained awareness.		Small region of activation in **left caudate (-6, 0, 8).** Non-contributory by view of consultant.	
5, 30/M	No abnormality	Epigastric aura, lip smacking, loss of awareness, lasting up to 2 minutes.		**Right inferior frontal (46, 38, -18)**, very small cluster anterior insula (40, 14, 7). Non-contributory by view of consultant.	
8, 24/F	No abnormality	Paroxysmal episodes of parasthesia/dysthesia of right arm, with retained awareness. GTCS.		**Right perirhinal cortex (30, -6, -40),** right mid-cingulate region (18, -30, 43). Non-contributory by view of consultant.	
10, 18/F	No abnormality	Complex visual hallucinations with retained awareness. Episodes of loss of awareness, lip smacking, speech arrest.		**Left fusiform gyrus (-36, -39, -24)**, left temporal pole (-36, 18, -40), left hippocampus (-37, -14, -16). Non-contributory by view of consultant.	
12, 25/F	No abnormality	Loss of awareness, dystonic posturing of right arm, infrequent nocturnal events with hyperkinetic semiology.		**Left hippocampus (-27, -6, -20)**, left temporal pole (-27, 12, -28), left middle frontal gyrus (-30, 48, 4). Non-contributory by view of consultant	

Table 2.4.2. Summary of patients included in the study but who did not progress to surgery or where it was felt by medical team that the EEG-fMRI results were non-contributory to patient management.

CASE 1. PATIENT 3

History. Right handed, 18-year-old male with onset of seizures at the age of 13 years. Typical seizure burden of GTCS, though possibly hyperkinetic, about once every 2-4 weeks. Witness description of daytime seizures compatible with GTCS though possible thrashing around at night. A mixture of nocturnal and daytime seizures. Provisional diagnosis of idiopathic generalised epilepsy (IGE).

Medicated with Phenytoin and Lamotrigine.

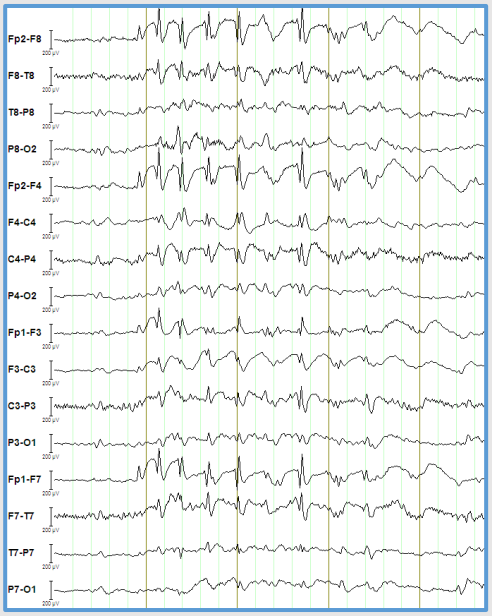
Clinical diagnostics. Routine outpatient clinical EEG demonstrated bursts of SWC activity with a right sided emphasis.

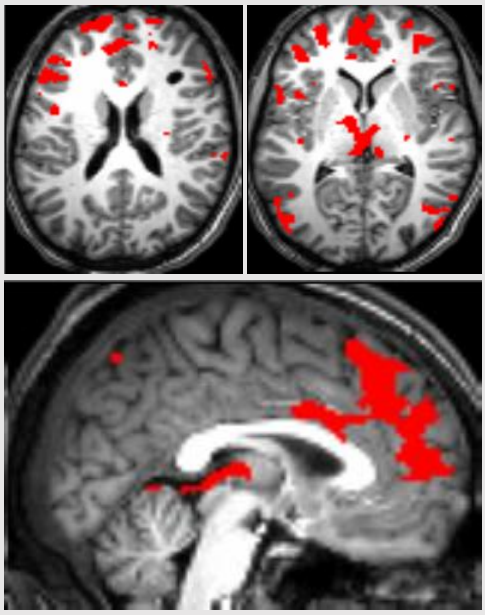
Clinical MR imaging revealed a right frontal cyst.

EEG-fMRI. Patient recruited into the study via epileptologist who expressed a specific interest on whether the right frontal cyst could be epileptogenic or not, i.e. was the patient's epilepsy truly an IGE or of focal origin.

EEG recording during fMRI scanning demonstrated seven bursts of SWC activity, maximal in amplitude over the right anterior quadrant.

fMRI results showed widespread activation in the frontal lobe, bilaterally in posterior middle temporal gyrus, precuneus, bilateral thalamus, anterior/mid-cingulate cortices. No activation was observed in the region of the cyst.





Outcome. Epileptologist satisfied that both clinical history, EEG and EEG-fMRI sufficient to rule out cyst as cause of epilepsy and felt that the EEG-fMRI results were contributory to this decision. Epilepsy went into remission following changes in pharmacological therapy.

CASE 2. PATIENT 13

History. Right handed, 21-year-old female with onset of seizures at the age of 13 years. Typical seizure burden of one complex partial seizure (CPS) a week; loss of awareness, aphasic, occasional lip smacking and fear. Patient had previous excision of left temporal oligoastrocytoma for oncological reasons. Seizure free for 6 months following surgery but seizures started again.

Medicated with Levetiracetam and Lamotrigine.

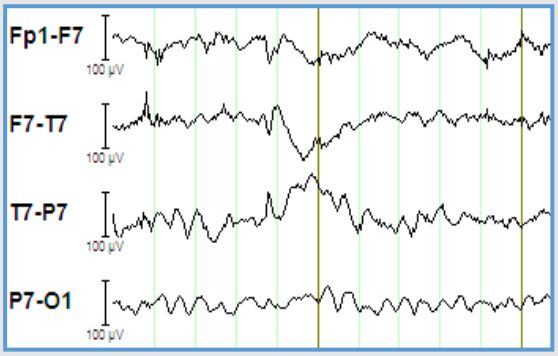
Clinical diagnostics. Routine outpatient clinical EEG demonstrated fairly frequent left temporal sharp waves and occasional independent right temporal sharp waves. Video-EEG captured a single seizure arising from the left hemisphere.

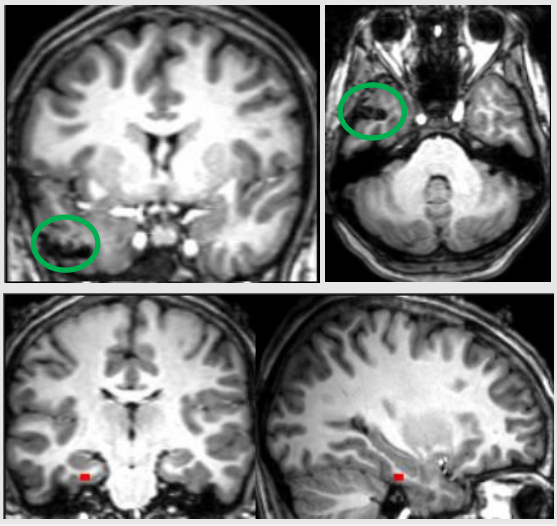
Clinical MRI revealed site of previous excision of left temporal oligoastrocytoma (highlighted with green circles). No primary/secondary features of hippocampal sclerosis.

EEG-fMRI. Patient recruited into the study via epileptologist who expressed a specific interest in whether the hippocampus that remained following tumour based surgery could be epileptogenic.

EEG recording during fMRI scanning demonstrated 45 left temporal spikes.

fMRI results showed a single small cluster of activation in the **left parahippocampal gyrus (-30, -21, -24).**





Outcome. Despite small region of activation, the epileptologist and neurosurgeon felt justified in offering a period of intracerebral video-EEG which revealed seizure onset from left hippocampus. Plan was for second surgery, this time an anterior temporal lobe resection and hippocampectomy, however patient's epilepsy went into remission so any surgical interventions were suspended. The patient is now >2 years' seizure free.

CASE 3. PATIENT 14

History. Right handed, 18-year-old female with onset of seizures at the age of 15 years. Seizure semiology described as episodes of loss of awareness (described by carer as absences) and GTCS; "absences" occurring once a week and GTCS every month.

Medicated with Lamotrigine.

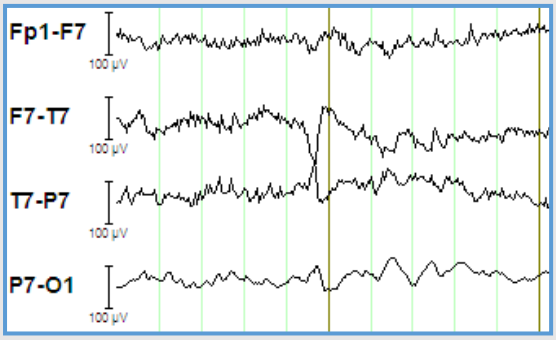
Clinical diagnostics. Routine outpatient clinical EEG demonstrated both left and right temporal sharp waves, with a greater preponderance in the left. Video-EEG following aggressive medication withdrawal demonstrated generalised EEG changes with GTCS.

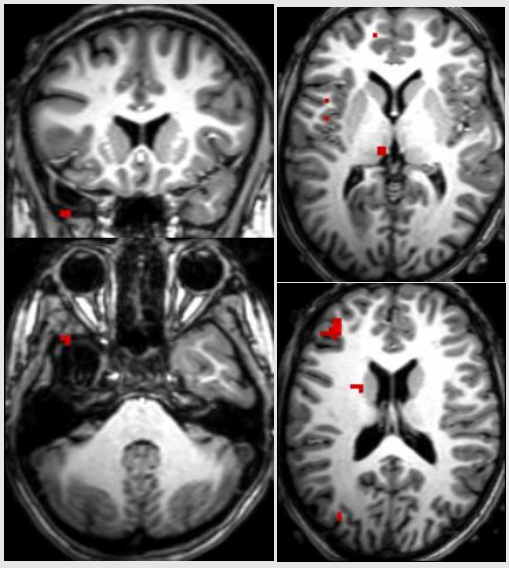
Clinical MR Imaging showed possible left temporal dysembryoplastic neuroepithelial tumour (DNET).

EEG-fMRI. Patient recruited into the study via epileptologist given discordance between ictal EEG and potentially highly DNET.

EEG recording during fMRI scanning demonstrated 17 left temporal sharp waves.

fMRI results showed clusters of activation in the left temporal pole (-42, 18, -36), **left middle frontal gyrus (-36, 45, 20),** left thalamus (-7, -20, 3), posterior middle temporal gyrus (-43, -61, -1).





Outcome. Results of EEG-fMRI (left sided activations) prompted epileptologist to redo video-EEG without aggressive medication withdrawal. Patient had two ictal events during second video-EEG which revealed a left temporal onset. Patient had left temporal lobe resective surgery though did not become seizure free.

CASE 4. PATIENT 19

History. Right handed, 19-year-old female with onset of seizures at the age of 4 years. Seizure semiology described as right sided sensory symptoms, loss of awareness, right hand grasping the left, dysphasia.

Medicated with Lamotrigine and Oxcarbazepine

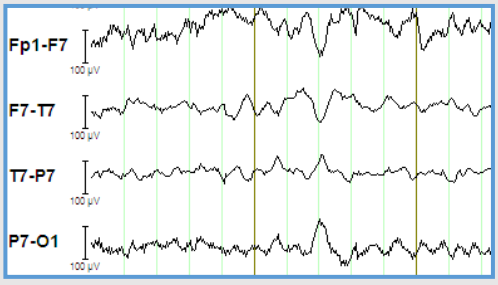
Clinical diagnostics. Routine outpatient clinical EEG demonstrated left temporal sharp waves, occasionally independent right temporal sharp waves. Video-EEG demonstrated 8 seizures with a left temporal onset.

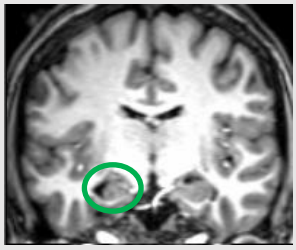
Clinical MR Imaging showed left hippocampal sclerosis (circled in green).

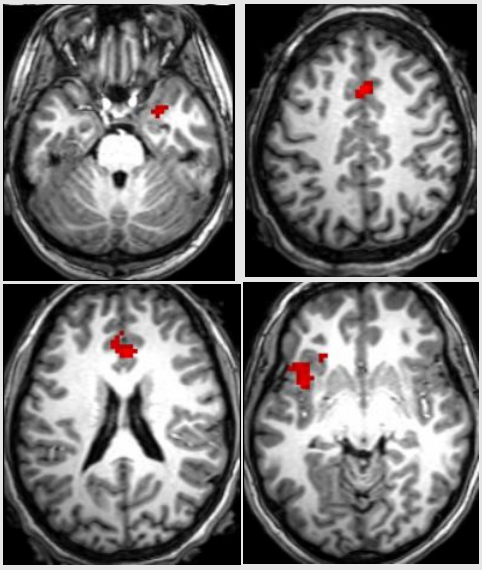
EEG-fMRI. Patient recruited into the study via epileptologist as a further investigation for pre-surgical work up.

EEG recording during fMRI scanning demonstrated 19 left temporal sharp waves.

fMRI results showed activation in **left insula (-36, 15, -4),** left mid cingulate (0, 15, 44), left anterior cingulate (3, 30, 20), left inferior temporal gyrus (-42, -12, -20), right amygdala (30, 6, -25).







Outcome. Results of EEG-fMRI (left sided activations) viewed, by epileptologist, as neither concordant or discordant and in this case viewed as non-contributory as other routine diagnostics were concordant (i.e. video-EEG, MRI and neuropsychometry). Patient had left temporal lobe resective surgery and became seizure free (>2 years).

CASE 5. PATIENT 23

History. Right handed, 33-year-old female with onset of seizures at the age of 22 years. Two main seizure types; out of body experiences lasting a few seconds and focal motor seizures of mainly the right foot, occasionally progressing to GTCS.

Medicated with Lamotrigine.

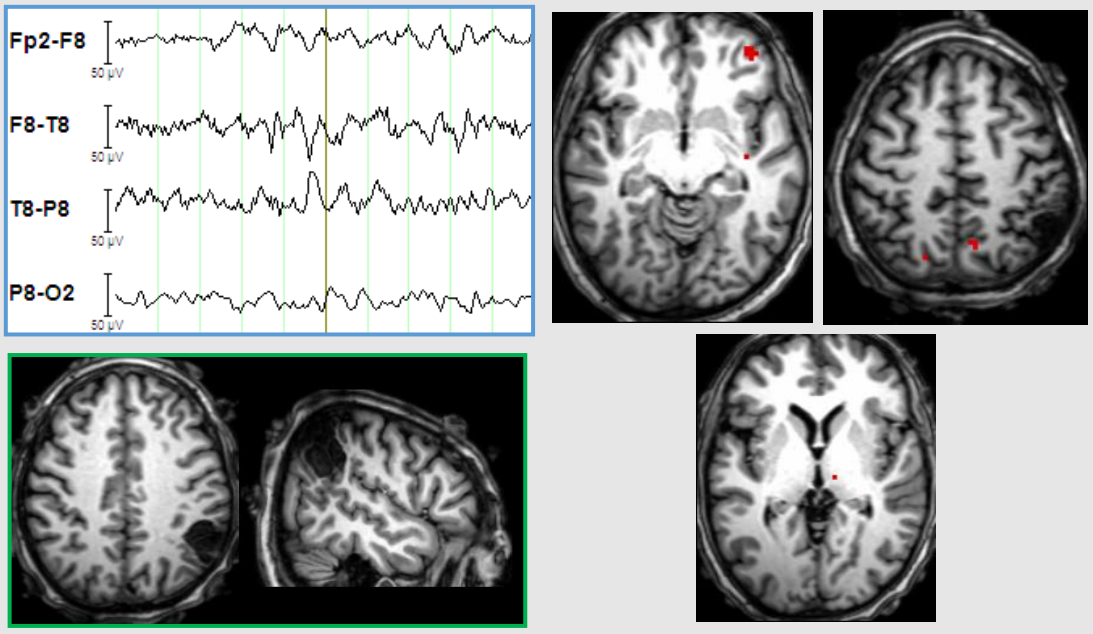
Clinical diagnostics. Routine outpatient clinical EEG demonstrated right sided paroxysmal theta activity. Video-EEG captured 3 seizures with bilateral slowing at onset, more prominent on the right.

Clinical MR imaging revealed a right parietal astrocytoma (WHO 1).

EEG-fMRI. Patient recruited into the study via epileptologist given the seemingly bilateral onset of seizures recorded on the video-EEG

EEG recording during fMRI scanning demonstrated seven right temporal sharp waves.

fMRI results showed activation mainly in the **right inferior frontal gyrus (pars orbitalis) (42, 48, -12),** right precuneus (12, -63, 56) and a small cluster in the right



Outcome. Decided at multi-disciplinary meeting to remove tumour for mainly oncological reasons but using electrocorticography (ECoG) to ascertain epileptogenicity of tumour. Acute ECoG revealed frequent spiking around tumour; following resection spikes remained and margin of resection increased slightly. Following this no spikes were evident. Unfortunately, the patient was not seizure free

CASE 6. PATIENT 24

History. Right handed, 30-year-old male with onset of seizures at the age of 21 years. Seizures were frequent focal motor seizures involving the right arm occasionally hyperkinetic seizures in sleep.

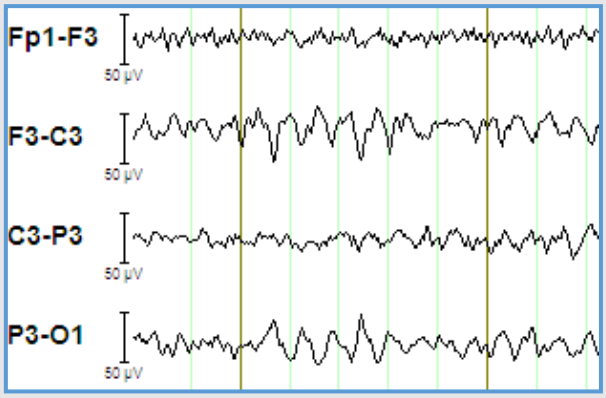
Medicated with Lacosamide, Levetiracetam and Carbamazepine.

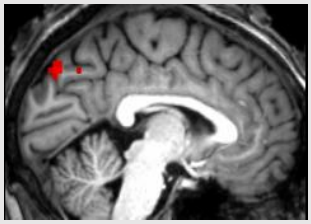
Clinical diagnostics. Routine outpatient clinical EEG demonstrated left central/fronto-central sharp waves. Video-EEG captured 4 focal motor seizures a left sided onset, most prominent in the left fronto-central region.

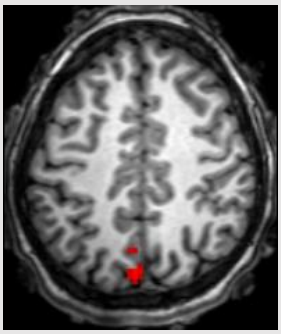
Clinical MR imaging revealed a left focal cortical dysplasia next to the motor strip.

EEG-fMRI. Patient recruited into the study via epileptologist given the left hemispheric onset of seizures to see if more localisation in relation to the dysplastic region could be obtained. EEG recording during fMRI scanning demonstrated eight left centro-parietal sharp waves.

fMRI results showed activation in the **left precuneus (-3, -78, 44).**







T1 scan was corrupted. Activation map is superimposed over the normalised T1 scan of a patient in the study with no MRI abnormality.

Outcome. Results from the fMRI deemed by epileptologist and neurosurgeon to be non-contributory. Patient went for ECoG guided resective surgery. Motor strip was mapped intraoperatively using cortical stimulation and ECoG. Dysplastic region demonstrated frequent spiking. Following resection, the patient became seizure free (>2 years).

CASE 7. PATIENT 26

History. Right handed, 37-year-old female with onset of seizures at the age 19 years. Patient retains some degree of awareness during typical seizure, flexor posturing of right arm, ictal retching; seizures last about ½ minute with rapid recovery.

Medicated with Carbamazepine and Lamotrigine.

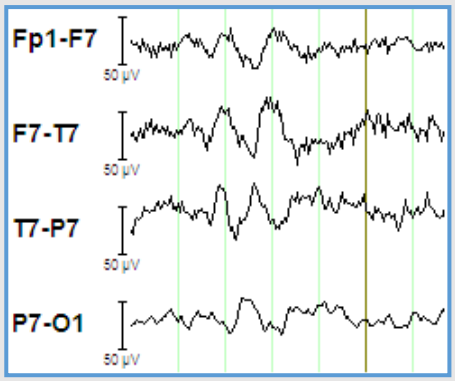
Clinical diagnostics. Routine outpatient clinical EEG demonstrated left temporal sharp waves. Video-EEG captured 5 stereotypes seizures as described above. One event showed a clear left temporal onset, three were obscured by myogenic artefact and one demonstrated possible bilateral changes at onset though this was uncertain due to myogenic artefacts. No independent IEDs were found in the right hemisphere.

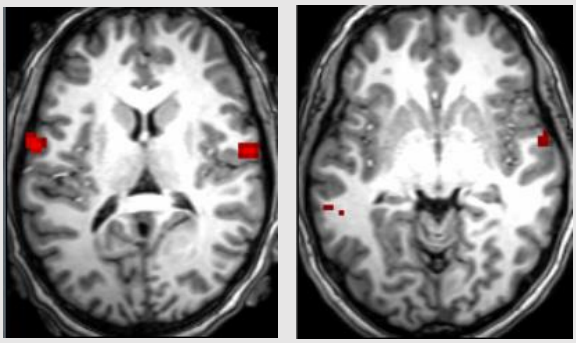
Clinical MR imaging revealed no clear abnormality. PET scan showed hypo-metabolism in left temporal lobe, particularly mesial temporal lobe.

EEG-fMRI. Patient recruited into the study via epileptologist given the ambiguity of the video-EEG results and lack of clear MRI abnormality. Further localisation was required.

EEG recording during fMRI scanning demonstrated only 4 left temporal sharp waves.

fMRI results showed activation in the **left rolandic operculum (-63, -3, 12),** left superior temporal gyrus (-45, -3, -8) and the right rolandic operculum/superior temporal gyrus (63, -6, 8)





Outcome. With or without the EEG-fMRI results the consensus from the epileptologist and neurosurgeon was to proceed with intracranial-EEG recordings. It was felt however that the EEG-fMRI results were concordant with this decision. Intracranial-EEG with bilateral mesial temporal strip electrodes found seizures originating from the left. Patient went on to have surgery for left temporal lobe epilepsy and is now seizure free (>2 years). Histology of resected hippocampus revealed histological evidence of hippocampal sclerosis.

2.5 DISCUSSION

In this study 26 patients were recruited from local tertiary epilepsy services via epileptologists who specifically expressed an interest in utilising EEG-fMRI as an investigation that may provide further useful information to guide patient management. It was down to the opinion of the patient's primary care team (mainly neurologist but on a few occasions also their neurosurgeon) to determine if EEG-fMRI had contributed at all to patient management. This is a very subjective measure in which to report results, but also a very real one. Epilepsy, as with many other medical conditions, is managed and diagnosed primarily though the expertise of the patient's primary care team, most notably their neurologist, with less attributed to diagnostic interventions (Engel Jr, 2005; Nunes, Sawyer, Neilson, *et al.*, 2012; Smith, 2005). As such, it makes sense to base the clinical "usefulness" of EEG-fMRI on clinical opinion. In this present study of the 26 patients recruited, only 13 (50%) were recruited into the final analysis, and of the 26 initially recruited, EEG-fMRI was deemed to be contributory to patient management in 3 (12%) of the patients. Of the 13 patients in the final analysis only 2 had an underlying diagnostic question, patient #1 and patient #3, the others were related to progression down the surgical pathway for epilepsy.

The results for patient #1, which were deemed to have not contributed, demonstrated a very small region in the left caudate. The query from the "referring" epileptologist was related to discrepancy between ictal semiology and interictal EEG findings. Clinical EEG findings demonstrated clear temporal lobe spikes/sharp waves but the patient had focal motor seizures, rarely with any loss of awareness. The epileptologists sought to clarify any temporal origin of seizures or a secondary focus. The results ultimately did not help answer this question. The remaining 4 patients where results were deemed non-contributory were in the context of possible surgical consideration. The

results for these patients did not aid entry or progression down the surgical pathway and thus did not aid in any way in patient management.

The first patient in the individual cases reported in the current study (Case 1, Patient #3) was recruited from their epileptologist who had a genuine diagnostic question as to whether this patient had an IGE or a focal frontal lobe epilepsy. Clinical presentation was of seizures both nocturnal and during the day with a description fitting GTCS during the day but with an unclear description of the nocturnal events; possibly even hyperkinetic. The term IGE covers a number of electro-clinical epilepsy syndromes, often including GTCS as a primary seizure type, and was originally classified in the absence of any focal features (Commission on Classification and Terminology, 1989). This was decided as too rigid criteria and later amended (Berg, Berkovic, Brodie, *et al.*, 2010); indeed IGE has been shown to demonstrate some features akin to focal seizure disorders which can potentially lead to a misdiagnosis of IGE as a focal disorder (Seneviratne, Cook & D'Souza, 2014). Frontal lobe seizures can present with a variety of different semiology including GTCS and hyperkinetic seizures, and it is often difficult to localise a focal onset based on semiology alone (O'Muircheartaigh & Richardson, 2012). Even though frontal lobe epilepsy is a focal epilepsy it can also present with generalised spike and wave activity (Niedermeyer, 1998; Westmoreland, 1998), which is commonly observed in an IGE (Commission on Classification and Terminology, 1989), and was seen in this patient's case. The patient's MRI demonstrated a right frontal cyst which raised some diagnostic doubt over the diagnosis of IGE. The EEG-fMRI results demonstrated activation in the frontal regions as well as the thalamus, a typical pattern observed in spike and wave (Aghakhani, 2004; Gotman, Grova, Bagshaw, *et al.*, 2005b), with these findings tending to be homogenous in patients with IGE (Gotman, Grova, Bagshaw, *et al.*, 2005b). No

activation was observed near the cyst; this, together with the pattern of activation observed from the fMRI results, helped the epileptologist decided to remain with the original IGE diagnosis. Pharmacological therapy was optimised and the patient's epilepsy went into remission. This also helped to abate the patient's and family's concerns over the involvement of the cyst and also whether resective surgery of the cyst would be an option in the future. The epileptologist decided that the EEG-fMRI in this case was contributory to the patient's management, not from the point of view of a pre-surgical evaluation, but in the context of a clear diagnostic question.

In many EEG-fMRI studies of epilepsy the focus has been on pre-surgical evaluation, given the localising potential of EEG-fMRI, and the primary benchmark which has been used to validate findings has been comparison with surgical outcome (An, Fahoum, Hall, *et al.*, 2013; Coan, Chaudhary, Grouiller, *et al.*, 2016; Thornton, Laufs, Rodionov, *et al.*, 2010; van Houdt, de Munck, Leijten, *et al.*, 2013; Zijlmans, Huiskamp, Hersevoort, *et al.*, 2007). In the present study, of the 26 patients initially recruited five patients went on to have resective surgery and one had an offer of surgery before becoming seizure free on pharmacological therapy. Of those five who underwent surgery three became seizure free for a period of at least 2 years.

In the six patients considered for surgery the EEG-fMRI results were deemed contributory by the patient's epileptologist/neurosurgeon in two (Case 2 and Case 3). Where Case 2 did not ultimately progress to surgery, Case 3 did and this resulted in a failure to gain seizure freedom. In Case 4, the request was made for EEG-fMRI as part of the pre-surgical work-up despite the epileptologists/neurosurgeon having a hypothesis of resectable left mTLE with HS, aside from IEDs also originating from the contralateral temporal region, which can be a common feature of unilateral TLE (Schulz, Lüders, Hoppe, *et al.*, 2000; Steinhoff, So, Lim, *et al.*, 1995), other non-

invasive diagnostics were concordant with left mTLE with HS. In this case the results from the EEG-fMRI, although demonstrating the most significant and preponderant activation in the left hemisphere, there was a small region of activation in the right amygdala. The consensus from the epileptologist and neurosurgeon was this did not dissuade them from offering surgery and the left predominant activation, was not deemed localised enough to the hypothesised EZ, to be relevant, thus EEG-fMRI in this case was deemed to have not contributed to this particular case. Surgical intervention in Case 5 was prompted mainly due to oncological reasons with epilepsy surgery a secondary objective. Bilateral-onset seizures on video-EEG prompted referral for EEG-fMRI, as hypothesised EZ was the lesion and possibly surrounding tissue. The predominant cluster from the EEG-fMRI results was located in the right inferior frontal region, distant to the presumed EZ. ECoG during surgery revealed the epileptogenic nature of the tumour, and a full resection, including the margin of the tumour was done, achieving abolition of epileptic activity identified on ECoG. Unfortunately, the patient was not seizure free following surgery. Despite the confirmed epileptogenicity of the lesion it could be that the patient developed secondary epileptogenesis in an ipsilaterally connected region. Although the exact mechanisms are disputed, development of an epileptogenic foci, secondary to a primary focal lesion has been well documented (see Cibula & Gilmore, 1997; Morrell, 1989 for review). Whether the activation on EEG-fMRI could reflect an area of secondary epileptogenesis is purely speculative at best, though ultimately the EEG-fMRI results were regarded by the epileptologist and neurosurgeon to have not contributed to this patient's work-up, primarily due to the lack of activation around the tumour, or a very clear and convincing region that could have prompted the generation of a hypothesised region outside the lesional zone. In Case 6, the EEG-fMRI results were deemed by the epileptologist and neurosurgeon to have not contributed to the final decision, primarily as the strongly favoured

hypothesis of the dysplastic region near the motor strip as being the EZ, was distant to the activation which was located in the left precuneus. The desire from the surgeon was a clear area of activation to outline the EZ to help demarcate the potential area of resection from the motor strip, which was perhaps an unrealistic expectation of the EEG-fMRI study. The final case, Case 7, was requested in the context of normal MR imaging and slightly ambiguous results from video-EEG, namely a possible bilateral onset seizure. An implantation strategy for intracranial-EEG was formulated based on video-EEG results, seizure semiology, and neuropsychometry, at this point EEG-fMRI was also decided upon. The consensus from the epileptologist and neurosurgeon was the results from the EEG-fMRI, maximal activation in the left rolandic operculum/superior temporal gyrus but similar in the right, reflected the ambiguous video-EEG results and supported the implant strategy.

The contributions made by EEG-fMRI in the two patients (Case 2 and 3) were in the context of progression down the surgical pathway where otherwise progression may have been difficult. In Case 2 the only activation present was in the parahippocampal gyri, so focal to encourage the progression onto intracranial-EEG which demonstrated the hippocampus to be the seizure onset zone. It is impossible to say whether resection would have resulted in seizure freedom but it did certainly result in an offer of surgery. In Case 3 the contribution was subtler, with the results prompting a second video-EEG which ultimately encouraged progression onto surgery.

Is there any information in the fMRI results that may indicate why some patients gained seizure freedom and some did not? A recent study by An et al., 2013, investigated 35 patients who had undergone EEG-fMRI, had significant activation and progressed to surgery (An, Fahoum, Hall, *et al.*, 2013). They formed four groups depending on how localised the cluster with the maximum

statistical response was to the site of resection, and found, in those where the maximum cluster was within the area of resection, a good outcome was much more likely than in those where the cluster was distant from the area of resection. In the present study, in the two patients who failed to become seizure free following surgery the cluster with the maximal statistical value was distant to the site of resection. Though this was also observed in the patients who became seizure free. From the observations in the current study, prediction of surgical outcomes based on EEG-fMRI is not at all straight forward and should be viewed with caution.

In the first study to utilise EEG-fMRI as a clinical tool in a cohort of complex adult surgical cases, Zijlmans et al., 2007, analysed the BOLD responses from 29 patients who had entered a surgical pathway and were rejected for various reasons (Zijlmans, Huiskamp, Hersevoort, *et al.*, 2007). From these patients, four had new surgical prospects open to them as a result of the added investigation. These results somewhat mirror the findings here, with EEG-fMRI opening an otherwise closed door for surgery to a few patients. As an imaging modality it is one that is not yet utilised readily in the UK (Mouthaan, Rados, Barsi, *et al.*, 2016). Other imaging studies, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) are more readily utilised in pre-surgical assessment to aid in localisation (Mouthaan, Rados, Barsi, *et al.*, 2016), with a very recent study quoting a sensitivity of ~63% and ~49% respectively (Lascano, Perneger, Vulliemoz, *et al.*, 2016). In cases where more readily available imaging such as PET and SPECT are unable to contribute EEG-fMRI may help progress surgery in a small number of cases.

One of the most significant issues that is highlighted in the results of this study is the lack of IEDs recorded during scanning in a large proportion of patients (50%). Despite the most recent clinical

EEG being used to determine the frequency of IEDs per hour, the EEG may well be out of date, and IED rates high enough for EEG-fMRI are relatively uncommon. Given the underlying ethos of treating the patient and not the EEG, following diagnosis, repeat EEGs are not often indicated (Smith, 2005). In a clinical setting EEG-fMRI should ideally follow a recent EEG or video-EEG to ascertain a current IED load. The lack of IEDs is not an issue restricted to just this study and comparable figures of 40% have been reported before of no IEDs being recorded in the scanner (Salek-Haddadi, Diehl, Hamandi, *et al.*, 2006). In order to improve IED detection and increase IED yield, studies have used novel means of analysis, including, independent component analysis (ICA) and wavelet analysis of the EEG recorded in the scanner to improve IED identification (Formaggio, Storti, Bertoldo, *et al.*, 2011); using voltage maps derived from epilepsy specific averaged IEDs obtained from prolonged EEG recordings outside the scanner and applying them to data obtained during scanning (Grouiller, Thornton, Groening, *et al.*, 2011); and using ICA in the decomposition of fMRI data to identify epileptic independent components related to the seizure onset zone that are not dependant on scalp IEDs, thus removing dependence on identification of scalp IEDs (Maziero, Sturzbecher, Velasco, *et al.*, 2015; Rodionov, De Martino, Laufs, *et al.*, 2007; van Houdt, Ossenblok, Colon, *et al.*, 2015). If they are able to overcome the limitation of the number of IEDs, and potentially remove the reliance on IEDs altogether, these type of approaches could considerably improve the clinical utility of EEG-fMRI.

Lack of IEDs recorded in the scanner is one problem, another is lack of significant BOLD responses even in the presence of IEDs. In the present study one patient (Patient #17) did not demonstrate any significant activation linked to sharpened paroxysmal theta discharges recorded during scanning. This is not an uncommon finding in epilepsy EEG-fMRI studies where IEDs are

identified on scalp EEG during scanning but fail to show any significant BOLD responses (Aghakhani, Kobayashi, Bagshaw, *et al.*, 2006; Salek-Haddadi, Diehl, Hamandi, *et al.*, 2006). Additionally, some results reported in the present study, although demonstrating significant responses, only showed very small regions of activation (e.g. patient #1) which could easily not be present with less liberal thresholding. In order to improve the sensitivity of EEG-fMRI, several studies have advocated the inclusion of various physiological artefacts and measurements into the design matrix, incorporating; eye blinks, swallowing, chewing, and motion artefacts identified by video (Chaudhary, Rodionov, Carmichael, *et al.*, 2012; Ruggieri, Vaudano, Benuzzi, *et al.*, 2015); any sleep paroxysms recorded during scanning (Moehring, Coropceanu, Galka, *et al.*, 2011); cardiac related noise (Liston, Lund, Salek-Haddadi, *et al.*, 2006); and respiration related noise as variations in pulse height derived from photoplethysmogram (van Houdt, Ossenblok, Boon, *et al.*, 2010). Aside from improving sensitivity by inclusion of various sources of noise into the design matrix, IEDs have been shown not to conform to the haemodynamic response function (HRF) commonly used to model BOLD signal changes related to IEDs (Bénar, Gross, Wang, *et al.*, 2002; Lemieux, Laufs, Carmichael, *et al.*, 2007) and improved sensitivity can be achieved by using a patient specific HRF (Kang, Bénar, Al-Asmi, *et al.*, 2003; Storti, Formaggio, Bertoldo, *et al.*, 2013), voxel-specific HRFs (Lu, Grova, Kobayashi, *et al.*, 2007; Lu, Bagshaw, Grova, *et al.*, 2006) or by using alternate peak times of the HRF (Bagshaw, Aghakhani, Bénar, *et al.*, 2004; Jacobs, Levan, Moeller, *et al.*, 2009; Rollings, Assecondi, Ostwald, *et al.*, 2015). One of the most recent studies, using topographical maps derived from data outside of the scanner, demonstrated a sensitivity of 81% in identifying patients (with TLE) with good surgical outcome where the BOLD response overlapped with the area of resection (Coan, Chaudhary, Grouiller, *et al.*, 2016).

2.6 CONCLUSION

In the present study, EEG-fMRI was deployed in a heterogeneous and complex group of epilepsy patients. The patients were referred in by epileptologists who felt further diagnostic tests were required; in most cases, for surgical evaluation, in others for answering more diagnostic related questions. This group of patients was very representative of the patients one would likely see referred for EEG-fMRI if the investigation was freely available. The aim of this study was just that, to ascertain how useful, from a clinical point of view, EEG-fMRI could be to a referring clinician. In 12% of patients included in the study, EEG-fMRI was deemed by the epileptologists to be contributory in patient management. One of the most significant issues hampering the study was the lack of IEDs in 50% of patients. If IEDs were recorded in all patients this figure could well be higher; though this is the reality of EEG-fMRI in epilepsy. In this study EEG-fMRI was delivered in its simplest form as this would represent the minimum needed for a centre to deploy the test. Application of more advanced methods, some of which have been discussed, would no doubt improve clinical relevance. However, the more complex and time consuming an investigation the less likely it is to make it to a clinical setting, primarily due to cost. Some studies have already begun to address this by combining multiple imaging modalities which can potentially minimise time and cost of complex imaging investigations (Grouiller, Delattre, Pittau, *et al.*, 2015; Shin, Jewells, Sheikh, *et al.*, 2015). Developing these techniques into a package that could be implemented in a time efficient and cost effective way is key to transition from academic settings to a clinical one. Implemented here in its simplest form, the results presented here show EEG-fMRI has some limited use in a clinical setting, though this is in the context of a very difficult to manage patient group.

Chapter 3

EARLY HAEMODYNAMIC CHANGES OBSERVED IN PATIENTS WITH EPILEPSY AND IN A VISUAL EXPERIMENT

The work in this chapter has contributed to

Rollings DT, Assecondi S, Ostwald D, Porcaro C, McCorry D, Bagary M, Soryal I, Bagshaw AP. Early haemodynamic changes observed in patients with epilepsy, in a visual experiment and in simulations. Clin Neurophysiol 127(1) 245 – 253 (2016)

3.1 ABSTRACT

The objective of this study was to investigate whether previously reported early blood oxygen level dependent (BOLD) changes in epilepsy could occur as a result of the modelling techniques rather than physiological changes. EEG-fMRI data were analysed from seven patients with focal epilepsy and six control subjects undergoing a visual experiment. In six separate analyses the event timing was shifted by either -9,-6,-3,+3,+6 or +9 seconds relative to the onset of the interictal epileptiform discharge (IED) or visual stimulus. The visual dataset demonstrated an overlap between modelled haemodynamic response function (HRF) at event onset and at +/-3s relative to onset, which diminished at +/-6s. In the epilepsy dataset, pre-spike analysis at -6s improved concordance with the assumed IED generating lobe relative to the standard HRF in 43% of patients. The visual dataset findings indicate a form of "temporal bleeding", an overlap between the modelled HRF at time 0 and at +/-3s which is attenuated at +/-6s, suggesting that this form of analysis should be performed at 6 seconds prior to onset of IED to minimise the temporal bleeding effect. In the epilepsy dataset, the results also support the presence of relevant BOLD responses occurring prior to IEDs and prespike analysis may improve concordance.

3.2 INTRODUCTION

Simultaneous acquisition of electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) is a non-invasive technique (EEG-fMRI) that allows the indirect investigation of epileptiform neuronal activity in the human brain (for a review see Gotman et al., 2006). This method exploits the haemodynamic changes associated with interictal epileptiform discharges (IED) that are identified on the EEG. The concurrent blood oxygenation level dependent (BOLD) signal changes that accompany this activity are typically modelled with a haemodynamic response function (HRF) that peaks 5 – 6 seconds after the electrographic discharge (Friston, Fletcher, Josephs, *et al.*, 1998; Glover, 1999). This HRF, which for example may be derived from auditory responses in healthy volunteers (Glover, 1999), has previously been shown to be suitable for modelling BOLD changes related to IEDs (Bénar, Gross, Wang, *et al.*, 2002). However, studies have also demonstrated that the haemodynamic response in epilepsy is subject to variability in both peak timing and shape (Kang, Bénar, Al-Asmi, *et al.*, 2003; Lemieux, Laufs, Carmichael, *et al.*, 2007; Lu, Bagshaw, Grova, *et al.*, 2006), and that the sensitivity of EEG-fMRI to detect regions of activation can be improved by shifting the timing of the HRF relative to the IED (Bagshaw, Aghakhani, Bénar, *et al.*, 2004; Hawco, Bagshaw, Lu, *et al.*, 2007; Jacobs, Kobayashi, Boor, *et al.*, 2007).

In a study by Hawco et al., 2007, they shifted the HRF to peak at 3 and 1 seconds prior to the onset of the IEDs, and successfully demonstrated BOLD responses with a similar degree of concordance to later peaking HRFs (Hawco, Bagshaw, Lu, *et al.*, 2007). These findings suggested the presence of metabolically-demanding neuronal activity, not visible on the EEG, but preceding the electrographically evident spike. The notion that haemodynamic responses might occur prior to electrographic epileptic activity was previously suggested by observations during the transition from the interictal to the ictal state by Federico, 2005, who observed BOLD

changes several minutes prior to ictal onset (Federico, 2005). Makiranta et al., 2005, were also able to demonstrate BOLD changes preceding IEDs in an animal model following penicillin injection (Mäkiranta, Ruohonen, Suominen, *et al.*, 2005). They observed BOLD changes occurring several seconds prior to onset of the IED. For a review of other studies which have observed pre-ictal or pre-interictal haemodynamic changes, see Schwartz, Hong, Bagshaw, *et al.*, 2011. It has been suggested that these early BOLD responses are potentially more representative of the epileptogenic zone, resulting in improved localisation, since they may represent activity prior to propagation. For example, Jacobs et al., 2009, found that pre spike BOLD changes that occurred in nine of thirteen patients yielded more focal results than later responses with a strong degree of concordance with the electrographic spike field (Jacobs, Levan, Moeller, *et al.*, 2009).

Given the clinical potential of EEG-fMRI recordings in patients with epilepsy, and the frequent difficulty in interpreting the results, any improvement in methodology and in the basic understanding of the technique would be welcome. However, the analysis strategy whereby HRFs are shifted to investigate haemodynamic activity prior to electrographic activity, while being relatively common in epilepsy studies, has not previously been applied to EEG-fMRI data from control subjects in task conditions. This leaves open the possibility that the observations made in patients with epilepsy regarding early peaking HRFs could be an artefact of the methodology. In order to address this issue, and to determine whether these observations are exclusive to spontaneously occurring, endogenous epileptiform events, early and late peaking HRFs were investigated in a control group participating in a visual task, as well as a group of patients with focal epilepsy. Since it is reasonable to assume that no such early BOLD changes would occur to random exogenous stimuli, their absence in an analysis with shifted

HRFs would add validity to the pre-interictal BOLD responses that have been previously reported.

3.3 METHODS

Subjects

Visual experiment data

Six healthy volunteers (3 females, 3 males, mean age 21.8 years) were randomly selected from a group of fourteen subjects who had taken part in a previously reported experiment (Ostwald, Porcaro & Bagshaw, 2010; Porcaro, Ostwald & Bagshaw, 2010). All participants had normal or corrected to normal visual acuity. Informed and written consent was obtained from the volunteers, and they were paid for their participation. The study was approved by the research ethics committee of the University of Birmingham.

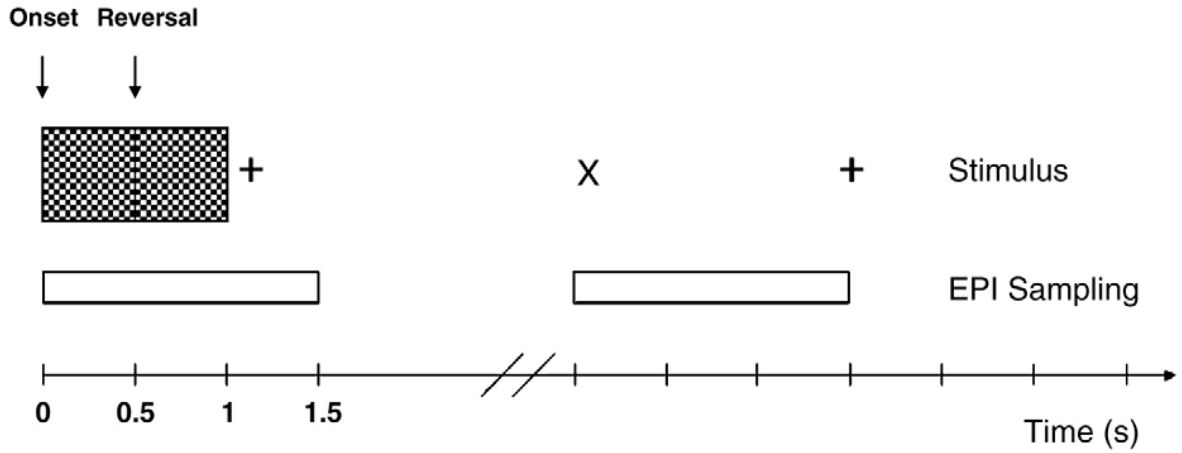


Figure 3.4.1. Experimental design of the visual experiment. Onset of checkerboard presentation at time 0 with reversal occurring at 500 ms, with offset of checkboard after a further 500ms. A subset of trials presented an × in place of the fixation cross to which participants were asked to press a button to indicate. (Modified from Ostwald et al., 2010).

The experiment, in brief, consisted of a sparsely presented 2Hz reversing checkerboard pattern in the left hemi-field (ISI 16.5–21s, discretised to 1.5s, spatial frequency 2 cycles per degree of visual angle, two contrast levels, high (CMichelson=1) and low (CMichelson=0.25)). Stimuli were presented with a central fixation cross for 1s (i.e. one checkerboard reversal), contrasts were randomized, and in total 85 trials of each contrast were presented (paradigm summarised in figure 3.4.1). For full details see (Ostwald, Porcaro & Bagshaw, 2010; Porcaro, Ostwald & Bagshaw, 2010).

Epilepsy patient data

Fifteen patients with a diagnosis of focal epilepsy were recruited from Consultant Neurologists with a special interest in Epilepsy, from the University Hospital Birmingham NHSF trust and The Barbary BSMH trust in accordance with National Health Service ethical approval (REC reference 06/Q2702/69). Patients were selected on the basis of frequency of IED (more than 10 IEDs per hour) and a seizure frequency of less than one per week. Written and informed consent was obtained, and the patients were screened to ensure suitability for scanning. Five subjects were excluded due to lack of IEDs during the scanning session, and three were excluded due to excessive movement in the scanner resulting in poor data quality. The seven patients included in the study had an age range of 18 – 40 years (mean 25.3 years, 5 females).

EEG-fMRI data acquisition

The same EEG acquisition protocol and equipment were used for the control subjects and the epilepsy patients. Continuous 64-channel EEG data were acquired at 5 kHz during scanning using MR-compatible EEG equipment (BrainAmp MR Plus, Brain Products, Munich,

Germany). The EEG recording cap (Brain Products, Munich, Germany) consisted of 62 scalp electrodes distributed in accordance with the international 10-20 system, with a further 2 electrodes to record ECG and ocular movements. Electrode impedances were kept below 10kΩ.

A 3T Philips Achieva MR Scanner (Philips, Netherlands) with an 8-channel SENSE head coil was used for the acquisition of echo-planar imaging (EPI) data, and a T1-weighted scan (1mm isotropic voxels) used for anatomical localisation of superimposed statistical parametric maps. For the visual experiment, EPI data were acquired from 20 slices covering occipital cortex in five runs of approximately 11 minutes (2.5x2.5x3mm voxels, TR 1500ms, TE 35ms, flip angle 80°, 441 volumes/run). For the data acquired from epilepsy patients, multiple scans of 6 minutes were acquired dependent on patient cooperation and comfort (patients 1 – 3 2.5mm isotropic voxels, TR 3000ms, TE 35ms, 50 slices, flip angle 85°, 120 volumes/run; patients 4 – 7, 3x3x4mm voxels, TR 2000ms, TE 35ms, 32 slices, flip angle 80°, 180 volumes/run).

EEG processing

Removal of MR gradient artefacts was performed offline using Brain Vision Analyser (Brain Products, Munich, Germany) using a template subtraction approach (Allen, Josephs & Turner, 2000). Ballistocardiogram artefacts were removed using the Optimal Basis Set (OBS) plug-in (Niazy, Beckmann, Iannetti, *et al.*, 2005) to EEGLAB (Delorme & Makeig, 2004). For the epilepsy data, an experienced electroencephalographer marked the onset of any epileptiform electrographic discharges, categorising the events based on morphology, topography and duration. If a patient had more than one IED type, this was modelled within the same design matrix. Interictal events occurring within 15 seconds of each other were excluded from the

analysis. No EEG processing was necessary for the visual dataset as only the stimulus event markers were needed.

Given the aim of this experiment was not to report solely on the localising potential of EEG-fMRI, but rather to ascertain the validity of the early BOLD changes, concordance between EEG and fMRI was based on localisation to the same brain lobe as identified from the surface EEG recording. If activation was seen in the concordant lobe in the pre-event maps but absent in the standard HRF map then this was considered as an improvement in concordance.

fMRI processing

EPI data were processed in SPM5 (Wellcome Department of Imaging Neuroscience, UCL, UK). Initially the images underwent realignment for motion correction, followed by slice timing correction, anatomical normalisation to MNI space and then finally the data were smoothed with a Gaussian kernel (5mm for the visual data, 6mm for the epilepsy data). Images were flipped so that the right side of the image represents the right side of the brain (i.e. images are presented according to neurological convention).

The experimental data were modelled using a general linear model (GLM) in an event related design convolved with a canonical HRF implemented in SPM5 (Friston, Ashburner, Kiebel, *et al.*, 2007). The canonical HRF was modelled with time derivatives, and six motion correction parameters were also included in the analysis as multiple regressors. To change the peak of the HRF, the timing of the events in both the epilepsy and visual datasets were shifted by -3, -6 and -9 seconds for pre-event analysis and +3, +6 and +9 for post-event analysis. For both the visual and epilepsy datasets a threshold of $p<0.001$ uncorrected for multiple voxels was used but Bonferroni corrected to take account of the use of multiple HRFs.

ROI analysis

For each GLM analysis, performed on all data sets, a 5mm radius spherical region of interest (ROI) was defined around the activated voxel with the maximal t-statistic. The time course for each event was extracted over a 40/42 second time window, depending on the TR of the EPI acquisition, with 20/21 seconds pre and post event. These single event HRFs were then averaged for each analysis of a data set.

3.4 RESULTS

3.4.1 Visual dataset

GLM results

In all subjects, as expected given the stimulation paradigm, maximal activation was seen in the right primary visual cortex using the canonical HRF. Significant activation was also seen in the same region across all pre- and post-stimulus HRFs at the relatively liberal threshold of $p<0.001$ that is often used in interictal epilepsy studies. The maximum t statistics were associated with the standard peaking canonical HRF and the lowest with HRF-6 (Figure 3.4.2). Shifting the peak time of the HRF by 3 seconds either pre- or post-stimulus resulted in moderately robust activation, with the areas of maximal activation mostly confined to the visual cortex (Figure 3.4.3). As would be expected, when the peak of the HRF was shifted further from the stimulus event, less activation was noted and the T-statistic was reduced. The only exception was when the peak was shifted 9 seconds pre-stimulus, which resulted in a marginal increase in the average T-statistic and the number of activated clusters. This is likely to be a result of the

timings of the stimulus presentation. This analysis suggests that a considerable amount of residual, artefactual activation is able to survive the statistical threshold often used in epilepsy studies (i.e., uncorrected $p<0.001$), but that this activity is minimised when shifting the event times by 6s.

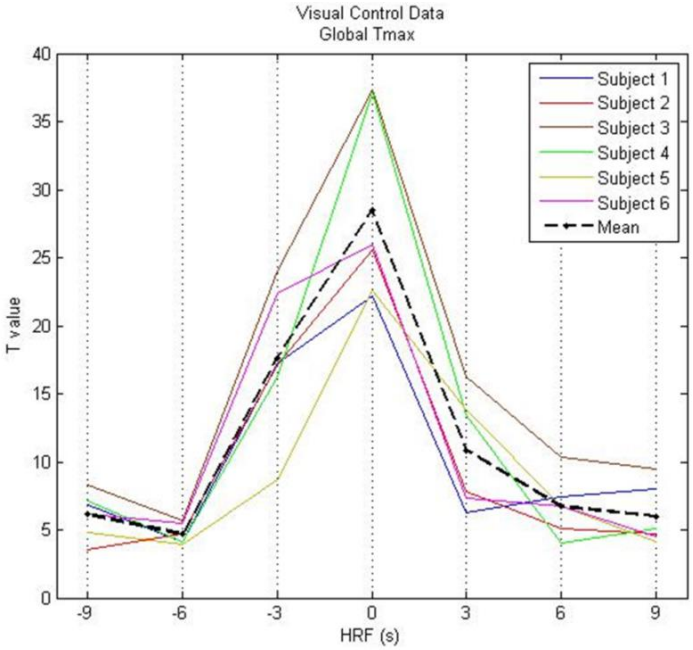


Figure 3.4.2. Maximum t statistic across all HRF analysis for the 6 visual datasets. Greatest dip in T value occurs 6 seconds pre/post stimulus.

ROI analysis

As expected, the extracted time course showed a very consistent canonical response for each trial when modelled with the standard HRF, with the maximal signal change peaking at about 5 seconds post stimulus (Figure 3.4.3). A canonical response could also be seen at HRF-3 and HRF+3, although as expected the peak signal change fell at around 7 seconds and 4 seconds respectively, in line with the idea that these analyses were sampling the actual response to the stimuli presented at time zero. There was no convincing canonical shaped response seen in any of the other pre- or post-event analyses.

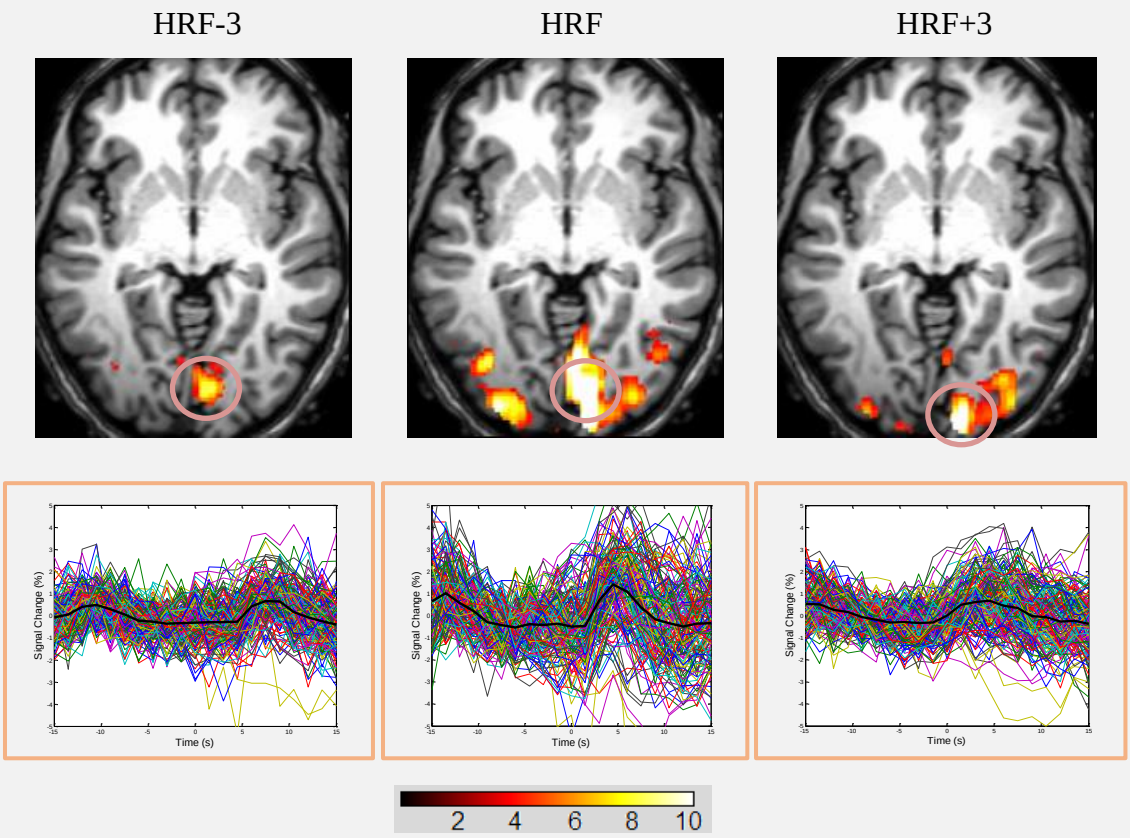


Figure 3.4.3. Visual dataset. Example from a randomly selected participant. Statistical t map (t>3.6, Bonferroni corrected for analysis with multiple HRFs, with no cluster threshold) for HRF and HRF-3,+3 superimposed over subject specific T1 anatomical scan normalised to MNI space. Axial slice through HRF focused on maximally activated voxel. No clusters of activation were observed in the visual cortex at HRF-6,-9,+6,+9 with activated clusters here being random and distant. Extracted time course from ROI shows a plot for each event and mean (black line).

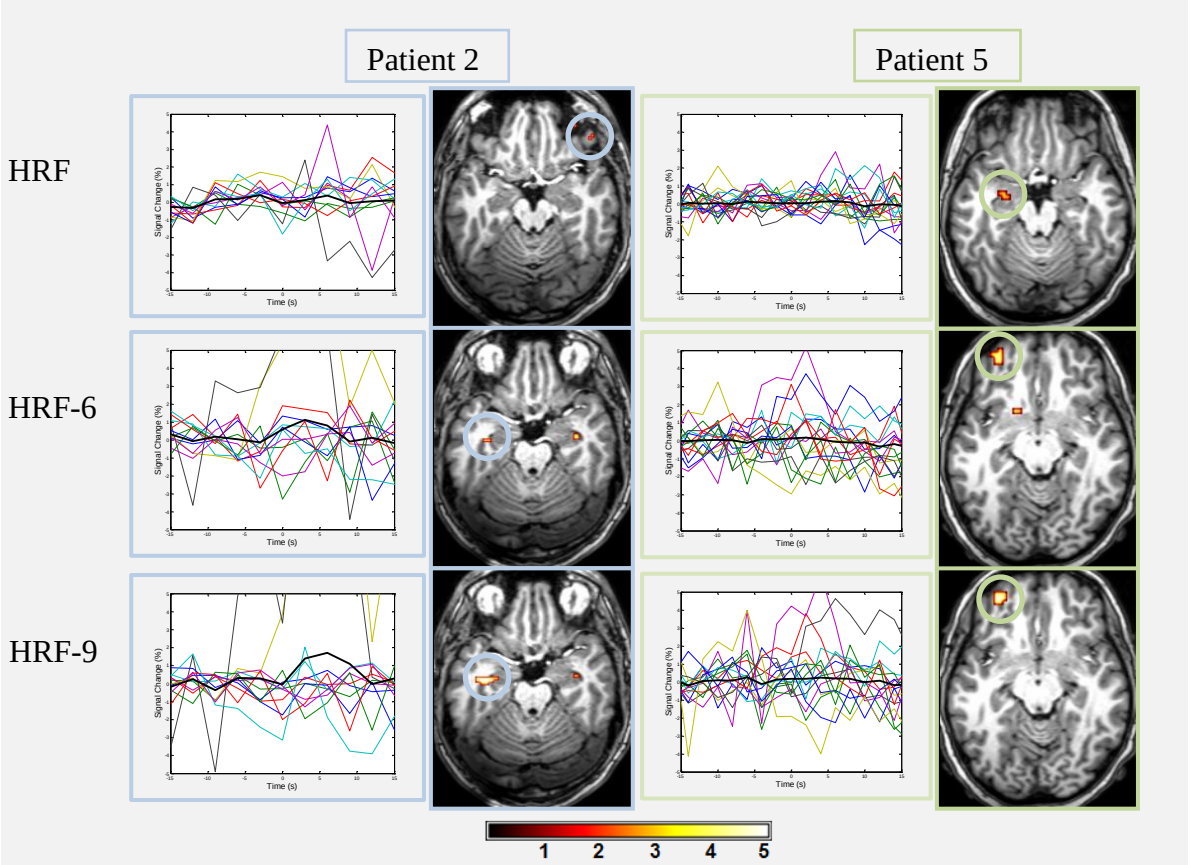


Figure 3.4.4. Epilepsy dataset. Example from two patients showing maximally activated clusters (t-contrast, t>3.6 Bonferroni corrected threshold, no cluster extent threshold) for standard HRF and pre event maps; HRF-6,-9. Bi- inferior temporal activation seen in the pre event maps of patient 2 at HRF-6 and HRF-9 to sharp waves in the right temporal region. Left frontal sharp waves of patient 5 demonstrated clear activation evident in the left inferior frontal gyrus at HRF-6 and HRF -9. Extracted time course from ROI shows a plot for each event and mean (black line). Note, right side of MRI image represents the right side of the brain (neurological convention).

3.4.2 Epilepsy dataset

Patients

The demographics and clinical details of the seven patients included in the study are summarised in Table 3.4.1. Patient 4 had two distinct IED types, although the right temporal sharp waves recorded from this patient failed to demonstrate any significant activation. The IEDs occurred randomly as isolated events in the EEG in all patients, with four IEDs excluded from analysis in patient 1 due to close proximity of the discharges.

Patient	Age/Sex	Epilepsy/Seizure type	EEG	MRI	IED (#)
1	40/M	TLE/CPS	R T SW	R Oligoastrocytoma	137
2	30/M	TLE/CPS	R T SW	Normal	14
3	25/F	PLE/SPS,CPS & GTCS	R P Sp	Normal	15
4	18/F	TLE/CPS	L T SW	Normal	56
5	25/F	FLE/CPS	L F SW	Normal	59
6	21/F	TLE/CPS	L T Sp	Previous excision of L T Oligoastrocytoma	45
7	18/F	TLE/CPS	L T SW	L T DNET	17

Table 3.4.1. Summary of patients. Abbreviations: FE = focal epilepsy, TLE = temporal lobe epilepsy, FLE = frontal lobe epilepsy, PLE = parietal lobe epilepsy, SPS = simple partial seizures, CPS = complex partial seizures, GTCS = generalised tonic clonic seizures, R = right, L = left, F = frontal, T = temporal, P = parietal, SW = sharp wave, Sp = spike.

PATIENT	HRF	HRF-3	HRF-6	HRF-9	HRF+3	HRF+6	HRF+9
1	n/a	**R Sup Med Gyrus (3.86),** L Hippocampus, L Sup Med Gyrus	**R Inf T gyrus (3.87)**, R rolandic operculum	**L caudate (4.08)**, L fusiform gyrus.	**L caudate (4.16)**	**L Fusiform gyrus (3.97)**, R Fusiform Gyrus	**R Inf T Gyrus (4.20)**, R Sup T Gyrus, L Fusiform Gyrus,
2	**R Inf F Gyrus (4.00)**, R Sup F Gyrus.	**R Rectal Gyrus (3.69)**	**R Hippocampus (4.64),** L Fusiform Gyrus.	**L Fusiform Gyrus (6.03),** R Parahippocampal Gyrus.	**R Mid F Gyrus (4.52)**, R Rolandic Operculum	**R Mid F Gyrus (5.16)**, R Insula, R Precentral Gyrus	**R Insula (4.64)**, R Inf F Gyrus, R Putamen
3	**R Fusiform Gyrus (4.85)**	n/a	n/a	n/a	**R Hippocampus (6.21)**	**L Lingual Gyrus (3.83)**	**L Inf T Gyrus (6.09)**, R Cingulate, R Inf F Gyrus.
4	**L Hippocampus (4.44),** L Med T, L Mid F Gyrus	**R Sup F Gyrus (4.31),** L Parahippocampal Gyrus, L Amygdala	**R Inf T Gyrus (4.17)**, R Sup P	**R Inf P (4.47),** R Inf T Gyrus, L Mid F Gyrus	**R Fusiform Gyrus (3.77)**	**L Insula (3.75)**	**R Sup F Gyrus (3.97)**, L T Pole, L Sup Orbital Gyrus.
5	**L Hippocampus (3.63)**, L T Pole, L Mid F Gyrus	**L Mid F Gyrus (4.02)**	**L Inf F Gyrus (4.46),** L Sup F Gyrus, L Rectal Gyrus.	**L Mid Orbital Gyrus (4.41)**, L Inf F Gyrus, R Sup Orbital Gyrus	**L Mid Orbital Gyrus (4.22),** L Mid F Gyrus, L Sup Orbital Gyrus	**L Mid F Gyrus (4.61),** L Inf F Gyrus	**L Sup F Gyrus (4.11)**, L Inf F Gyrus
6	n/a	n/a	**R Thalamus (3.99),** L Fusiform Gyrus	**R Paracentral (3.70)**	n/a	**L Mid T Gyrus (3.82)**	**R Thalamus (4.39)**, L Med T Pole
7	**L Inf F Gyrus (4.08)**, L Mid T Gyrus, L Mid F Gyrus	**L Caudate (3.94)**	n/a	**Genu of Corpus Callosum (3.65)**	**L Sup Med Gyrus (3.93)**, R Mid F gyrus, L Mid F Gyrus	**R Sup Med Gyrus (4.90)**, L Mid F Gyrus	**L Inf O Gyrus (4.26)**, L Inf F Gyrus, L Mid T Gyrus

Table 3.4.2. Anatomical locations of activations across all HRF analyses. Regions highlighted in bold represent cluster with maximum t statistic for each analysis and the t value in parentheses.

Abbreviations: "n/a" = no activation survived threshold, R = right, L = left, F = frontal, T = temporal, P = parietal, O = occipital, Inf = inferior, Sup = superior, Med = medial, Mid = middle

GLM results: HRF

Using a threshold of $p<0.001$ uncorrected for multiple voxels but Bonferroni corrected to take account of the use of multiple HRFs (height threshold $t>3.6$), patients 1 and 6 did not show any significant activation associated with the standard peaking HRF (Table 3.4.2). Concordance with the electrographic spike field and the maximally activated region was seen in patient 4 (left temporal sharp waves and activation evident in left hippocampus and left medial temporal gyrus) with the standard peaking HRF. Concordant activation was also seen in patient 5 (left frontal sharp waves with activation in left middle frontal gyrus) and patient 7 (left temporal spikes with activation in the left middle temporal gyrus); however, these were not the clusters with the greatest T statistic. With the standard HRF, areas of deactivation were noted in 6 of the 7 patients but they were not concordant with the spike field in any of the patients.

GLM results: early HRF

In line with the results from the visual data set, analysis focused on events shifted by 6s prior to the real IEDs (i.e., HRF-6). For patient 1 at HRF-6, there was an increase in BOLD signal in the inferior aspect of the superior temporal gyrus and the right rolandic operculum, showing a degree of concordance with the spike field (right temporal sharp waves). Right and left inferior temporal activation was seen in patient 2 when the right temporal IEDs were modelled with HRF-6. In patient 3 no prespike activation survived statistical threshold. Patient 4 demonstrated an increase in BOLD signal in the right inferior temporal gyrus into the supramarginal gyrus at HRF-6; this was contralateral to the electrographic IEDs. Data from patient 5 yielded similar results between the

three pre-spike analyses, with more robust activation seen in the left inferior frontal region with HRF-6, concomitant with the spike field of left frontal sharp waves. No activation was noted when using HRF or HRF-3 models for patient 6, however at HRF-6, activation was noted in the electrographically concordant lobe, although this was not the maximally activated cluster.

A small area of deactivation in the left hippocampus, concordant with the spike field, was evident in patient 3 at HRF-6. The only other dataset to demonstrate deactivation near the region of the spike field was patient 5 with changes in the left rectal/midorbital gyrus at HRF-3,-6,-9 with additional deactivation seen in the left hippocampus at HRF-9.

From this group of patients, concordance was evident in 4/7 (57%) when shifting the HRF to peak 6 seconds earlier, including patients 1, 2 and 6 where no concordant activation was evident in the standard HRF analysis.

GLM results: late HRF

Post event analyses for all patients demonstrated significant activation at HRF+6, however only patient 5 demonstrated activation concordant with the electrographic spike field. This activation was observed in the most rostral area of the left superior frontal gyrus at HRF+6, with this activation moving more left lateral at HRF+9. Deactivations were observed in five patients at HRF+6, with left inferior frontal and middle orbital deactivation seen in patient 5 at HRF+6, which was concordant with the electrographic spike field of this patient.

ROI analysis

There was considerable variability in the haemodynamic responses to individual IEDs for each patient, and this was common across all HRF analyses (Figure 3.4.4). This variability resulted in a mean time course which did not show a clear canonical shaped response in the majority of the data, with only a few exceptions. In the original HRF analysis the mean time course from patient 3 was the only one to show maximum signal change at around 5-6 seconds, an observation which was not evident in the other patients. The prespike analysis for patient 2 demonstrated a clear maximum signal change at 3 seconds and 6 seconds for HRF-6 and HRF-9 respectively, and had a resemblance of a canonical shape. This was not the case in any of the other prespike analysis. The mean time course for patient 2 (Figure 3.4.4) showed peak signal change at 3 seconds when modelled with HRF -6 and peaked at 6 seconds when modelled with HRF-9.

3.5 DISCUSSION

The primary aim of this study was to ascertain if the event related GLM design used in most EEG-fMRI studies of patients with epilepsy is appropriate in modelling pre-spike BOLD changes. To this end the same pre- and post-spike analysis was applied to a control group who participated in a visual experimental paradigm. Pre and post-spike activation was observed in the visual dataset despite the exogenous stimuli where one would not expect any pre event signal changes. The most notable changes occurred at HRF-3 and HRF+3, where significant activation was noted in the visual cortex in all datasets. The area of activation was similar to that activated when modelled with the standard canonical HRF. These findings are explained in terms of a simple overlap of the

HRFs used to model these responses. The HRF shifted by +/-3s overlaps with the increase signal in the fMRI time course associated with the event, resulting in a pattern of activation similar to the HRF at time 0, as in essence the same event is being modelled. This effect has been termed "temporal bleeding" as the rise and fall in the fMRI time course "bleed" into HRFs modelled at -3s and +3s respectively (Rollings, Assecondi, Ostwald, *et al.*, 2015). The results from the time series extraction from the visual dataset highlight signal changes in the time series that overlap with the modelled canonical HRF. The study by Rollings et al., 2015, to which this chapter contributes in majority, gives further corroboration for this interpretation from simulated data showing a gradual rather than an abrupt drop in T-value from event onset, thus allowing some overlap in pre and post event analyses; demonstrating from a simulation of a physiological scenario, only slight variations in timing when using different TRs, indicating differing TRs do not substantially alter the results. Based on these findings, when using this method of analysis, shifting event timings by seconds, rather than number of TRs, would be a valid approach.

In the present study, pre-spike analysis at HRF-6 demonstrated activation in 5 of the 7 (71%) epilepsy patients, with activation occurring in the spike field in 4 of the 7 (57%) datasets. However not all the concordant clusters contained the maximum T value. In these findings, 3 (43%) of the 7 patients showed activation clusters concordant with the spike field in addition to containing the maximally activated voxel in prespike analysis. In a study by Jacobs et al., 2009, prespike BOLD responses were observed in 11 of the 13 (85%) studies, comparable to the present study (Jacobs, Levan, Moeller, *et al.*, 2009). They demonstrated focal activations concordant with the spike field in 4 studies, with the HRF peaking -9 to -5 seconds, and in 7 of the studies when the HRF peak was -3 to +1 seconds; this increased concordance observed closer to the actual event time may

reflect the overlap in the models as previously discussed. One of the first studies to investigate early BOLD changes was conducted by Hawco et al., 2007, who demonstrated BOLD signal changes prior to scalp discharge (Hawco, Bagshaw, Lu, *et al.*, 2007). The degree of concordance between these early activations and the spike field was comparable to the present study and that of Jacobs et al., 2009, with 5 of the 7 patients showing concordance in those that had early HRFs. From the present study, one patient (#2) demonstrated clear signal changes in the time course peaking at 6 seconds when modelled with HRF-9 and at 3 seconds when modelled at HRF-6 without a clear change in signal with the standard HRF. From the observations made in the visual dataset shifting the event time forwards caused the peak signal change in the time course to be shifted backwards, i.e. from 6 seconds to 3 seconds. This would indicate that the response from patient 2 at HRF-9 could be the genuine BOLD response and the signal changes seen at HRF-6 would result from this temporal bleeding effect. Given these findings it is possible that the BOLD signal changes observed 9 seconds prior to the IEDs could represent genuine pre spike activation. While electrophysiologically this is a considerable amount of time, it is consistent with previous work and suggestive of metabolically-demanding processes occurring considerably before a scalp discharge.

A clear distinction between the time courses observed for the visual and epilepsy datasets is the absence of consistent responses on an event-by-event basis from the epilepsy data. Figure 3.4.3 shows obvious and robust responses to brief visual stimuli, whereas the responses to IEDs are much more variable. Non-canonical HRFs have been commented upon previously (Lemieux, Laufs, Carmichael, *et al.*, 2007; Lu, Bagshaw, Grova, *et al.*, 2006), and data in this study also highlights that not all IEDs, despite being morphologically similar in the EEG, are associated with BOLD

signal increases consistent with a canonical HRF. This variability in the BOLD signal between individual IEDs within the same subject may explain why EEG-fMRI studies in focal epilepsy are often plagued by low statistical values. It is not clear why IEDs of the same type within a subject vary in their BOLD response so much, but this may suggest variability in metabolic demand or in the mechanisms of generation.

The activity driving the early BOLD response does not appear to result in an identifiable electrographic discharge (Jacobs, Levan, Moeller, *et al.*, 2009), but given the substantial area of synchronized cortex required to generate an electrographic discharge visible on scalp EEG (Ray, Tao, Hawes-Ebersole, *et al.*, 2007; Tao, Baldwin, Hawes-Ebersole, *et al.*, 2007), this is hardly surprising. This prespike activity has been shown to be more focal than later modelled responses (Jacobs, Levan, Moeller, *et al.*, 2009) and thus prespike activations could potentially be more representative of the epileptogenic zone. The mechanism that drives these early BOLD changes has been the subject of considerable debate since they were first observed. In order to investigate the mechanisms underlying this phenomenon, Pittau et al., 2011, conducted an EEG-fMRI study comparing early observed BOLD activations to intracerebral EEG recordings. In their study of 4 patients they found electrical discharges in the prespike period which corresponded to the early BOLD response in only one patient (Pittau, Levan, Moeller, *et al.*, 2011). They concluded that these commonly observed BOLD responses that precede epileptiform discharges are due to metabolic events starting prior to scalp spikes resulting from both neuronal and non-neuronal mechanisms. These findings demonstrating little electrical activity preceding scalp spikes aid in the explanation of the absence of prespike EEG power changes noted by Jacobs et al., 2009. Overall, these early BOLD responses are evidently a consistent phenomenon that is reproducible

across studies in a variety of epilepsy subtypes in both adults and children (Schwartz, Hong, Bagshaw, *et al.*, 2011). Despite these observations it is not currently possible to reliably predict which IED or epilepsy type will demonstrate early BOLD changes. In this study of heterogeneous patients, no reliable and consistent pattern was evident.

Deactivations in epilepsy have been reported for both generalised and focal seizure disorders (Aghakhani, 2004; Bagshaw, Aghakhani, Bénar, *et al.*, 2004; Hamandi, Salek-Haddadi, Laufs, *et al.*, 2006; Hawco, Bagshaw, Lu, *et al.*, 2007; Jacobs, Levan, Moeller, *et al.*, 2009; Kobayashi, Bagshaw, Grova, *et al.*, 2006; Moeller, Siebner, Wolff, *et al.*, 2008; Rathakrishnan, Moeller, Levan, *et al.*, 2010; Stefanovic, Warnking, Kobayashi, *et al.*, 2005). The present study observed pre-spike and post-spike deactivations, with deactivations notably less common than activations. Deactivations concordant with the spike field were evident in only two patients across all analyses (patients 3 and 5). Deactivations observed using the standard canonical HRF were often distant from the electrographic spike field and inconsistent given the heterogeneity of the group. A study by Rathakrishnan et al., 2010, noted a partial overlap between areas of deactivation with preceding activation (Rathakrishnan, Moeller, Levan, *et al.*, 2010). This observation was not evident in the findings here apart from one patient (#5) who demonstrated deactivation concordant with both spike field, pre-spike activations and at +3, +6 and +9 seconds post-spike. The findings reported in the present study are also less consistent than those found by Jacobs et al., 2009, who found concordant deactivation precipitated by pre-spike activation in three of their subjects. The observations in the present study could be due to the heterogeneity of the patient group and consequently suggest that IED type plays a pivotal role in the patterns of deactivation observed, both pre- and post-spike.

The primary limitations of this study are both subject numbers and the heterogeneity of the patients recruited into the study. A greater number of patients and more homogenous groups (i.e. unilateral TLE) would have enabled a more thorough investigation and any early BOLD responses.

3.6 CONCLUSION

In summary, reliability of pre-spike changes observed in IEDs is highly variable and likely varies between subjects and IED types, and thus any improved concordance observed from this type of analysis should be viewed with caution. From the visual data the results of this study suggest that any pre/post-event analyses need to be restricted to approximately 6 seconds either side of event onset. Although unlikely to eliminate the effects completely, this will serve to minimise the likelihood of temporal bleeding between models. Rollings et al., 2015, report a relatively small influence of TR and suggest that restricting analyses to 6 seconds rather than a specified number of TRs (i.e., four to five TRs if TR=1.5s, two TRs if TR=3s) would be sufficient. More extensive studies with more homogenous groups of patients showing single IED types may yield more information about the factors which contribute to the observation and reliability of pre-spike fMRI.

Chapter 4

EARLY BOLD SIGNAL CHANGES IN SLEEP PAROXYSMS

4.1 ABSTRACT

Sleep paroxysms such as vertex sharp waves (VSWs), K-complexes (KCs) and sleep spindles (SSs), are spontaneously occurring endogenous graphoelements of sleep and are entirely non-pathological. Interictal epileptiform discharges (IEDs) are also paroxysmal, spontaneously occurring endogenous EEG events however they are pathological. Studies have previously reported early BOLD signal changes preceding IEDs that may be meaningful, though the same analysis has not been previously applied to sleep paroxysms. In this study, the aim was to investigate if early BOLD signal changes are unique to pathological IEDs or if they can also be observed in non-pathological paroxysmal activity. Nineteen participants underwent a period of EEG-fMRI following a night of sleep deprivation. Thirteen managed to achieve sleep in the scanner. A group analysis of VSWs, KCs and SSs were done using the onset times of the evens marked in the EEG during scanning and a second group analysis using the event times -6 seconds. No early BOLD responses were observed in VSWs or KCs, however early BOLD changes were observed for SSs, in the bilateral superior temporal gyri and Heschl's gyri, anterior cingulate cortex, supplementary motor cortex, cuneus, and posterior insula. This is the first account of early BOLD changes occurring in SSs, or any non-pathological, paroxysmal sleep activity, though the significance of these changes are unclear.

4.2 INTRODUCTION

Interictal epileptiform discharges (IEDs) are spontaneously occurring abnormal paroxysmal events, recorded on intracranial or scalp electroencephalography (EEG), requiring a large area of synchronised cortical activity of up to 20 cm^2 of cortex to be visible on the scalp (Tao, Baldwin, Hawes-Ebersole, *et al.*, 2007). This interictal activity is one of the hallmarks of epilepsy and used in the diagnosis and/or the classification of an epileptic disorder (Fisher, Scharfman & deCurtis, 2014; Smith, 2005). The morphological features of these IEDs enable a broad classification of IED type, i.e. a spike, sharp wave or a spike and wave complex (International Federation of Societies for Clinical Neurophysiology, 1974). These transient epileptiform events are not unique in terms of paroxysmal electrographic discharges in the brain, as during the early stages of sleep, and its progression, several paroxysmal events can be observed; most consistently, vertex sharp waves, sleep spindles and K-complexes (Bastien, Crowley & Colrain, 2002; Halász, 2005; Jankel & Niedermeyer, 1985). Consistently observed during sleep in the normal brain, these paroxysms are distinct from the background EEG, just like IEDs, and vary in their morphology. The sleep spindle (SS) is a brief train of rhythmical 12 – 15 Hz activity lasting between 0.5 and 3 seconds originating in the thalamus and transferred to the cortex via thalamocortical and corticothalamic circuits (Bonjean, Baker, Lemieux, *et al.*, 2011; Contreras & Steriade, 1996; De Gennaro & Ferrara, 2003; Jankel & Niedermeyer, 1985). A K-complex (KC) is a large, frontally predominant, biphasic slow wave complex originating from a highly synchronised cortex, mediated by corticothalamic and thalamocortical circuits, that can occur spontaneously or be evoked (Amzica & Steriade, 2002, 1998; Halász, 2005). Although morphologically dissimilar to IEDs both SSs and KCs are spontaneously occurring endogenous paroxysms, like IEDs, and provide a reliable basis for

investigating paroxysmal events in the human brain. The vertex sharp wave (VSW) or the vertex sharp transient (Yasoshima, Hayashi, Iijima, *et al.*, 1984), is again an entirely normal feature of early sleep yet, unlike the SS or KC, its morphological appearance is strikingly similar to an epileptiform sharp wave. The duration of an epileptic sharp wave is briefer than a VSW, defined as between 70ms and 200ms (Noachtar, Binnie, Ebersole, *et al.*, 1999), whereas a VSW is a broader complex often with a duration of around 200ms (Yasoshima, Hayashi, Iijima, *et al.*, 1984). Additionally, the VSW occurs across the vertex of the head and/or regions contiguous to it, whereas an IED can occur anywhere on scalp-EEG dependent on the location of the epileptogenic zone. Spontaneously occurring VSWs coincide with the onset of the cortical slow oscillation during sleep, and are likely to reflect the emerging synchronization of cortical networks that occurs during sleep progression (Amzica & Steriade, 1998). Unlike the VSW, KC and SS which are generated through an arrangement of thalamocortical, corticocortical and corticothalamic circuits. the mechanisms proposed to underlie the epileptiform spike or sharp wave result from an intrinsic feature of the epileptic neuron, a paroxysmal depolarisation shift (PDS). This is an excessive depolarisation that allows for a burst of action potentials (Gorji & Speckmann, 2009; McCormick & Contreras, 2001).

For about two decades combined EEG-fMRI has been used to investigate the blood oxygenation level dependent (BOLD) signal changes in response to spontaneous IEDs as recorded on scalp EEG (Warach, Ives, Schlaug, *et al.*, 1996). The vast majority of these studies used an event related design, taking the onset of the IED as the event time, and a canonical haemodynamic response function (HRF) to model BOLD changes (Lemieux, Salek-Haddadi, Hoffmann, *et al.*, 2002; Gotman, Kobayashi, Bagshaw, *et al.*, 2006; Gotman, 2008). The results from such studies have

demonstrated the BOLD correlates of focal IEDs, such as spikes and sharp waves, are quite variable between subjects and even demonstrate non-canonical responses (Grouiller, Vercueil, Krainik, *et al.*, 2010; Lemieux, Laufs, Carmichael, *et al.*, 2007; Storti, Formaggio, Bertoldo, *et al.*, 2013). To improve the sensitivity of this method some epilepsy EEG-fMRI studies have shifted the time used in this analysis to precede the actual timing of IED to investigate the presence of prespike activity (Bagshaw, Aghakhani, Bénar, *et al.*, 2004; Hawco, Bagshaw, Lu, *et al.*, 2007; Jacobs, Kobayashi, Boor, *et al.*, 2007; Pittau, Levan, Moeller, *et al.*, 2011; Rollings, Assecondi, Ostwald, *et al.*, 2015). Evidence from these studies suggests that there are meaningful BOLD signal changes that precede the electrographic scalp IED. However, the origin of these changes is not clear. Presumably they are the result of metabolically demanding activity that is not visible on scalp EEG, which has some support (Pittau, Levan, Moeller, *et al.*, 2011) and is consistent with the observation of pre-ictal haemodynamic phenomena observed with a variety of methods (Schwartz, Hong, Bagshaw, *et al.*, 2011). The question of whether pre-event BOLD responses occur as a result of pathological, epileptic neurophysiological events, or simply because IEDs are internally generated, paroxysmal discharges, remains to be answered.

These sleep paroxysms, KCs, VSWs and SSs, are the only reliably occurring paroxysmal discharges that are present on the normal EEG, and as such represent an opportunity to understand more about how BOLD responses are coupled with paroxysmal discharges in the absence of pathology. Imaging studies using EEG-fMRI have also been conducted to investigate the BOLD signal correlates of VSWs, KCs and SS (Caporro, Haneef, Yeh, *et al.*, 2012; Jahnke, von Wegner, Morzelewski, *et al.*, 2012; Schabus, Dang-Vu, Albouy, *et al.*, 2007; Stern, Caporro, Haneef, *et al.*,

2011), but no previous imaging studies have used the method of shifting the peak timing of the HRF when modelling these sleep paroxysms.

The aim of this study was to investigate any potential early BOLD signal changes related to sleep paroxysms, or whether these early signal changes are unique to IEDs.

4.3 METHODS

Subjects & Procedure

Nineteen participants (10 females, 29±9 years) were recruited into this study. Subjects were without known abnormal neurology and screened for excessive daytime sleepiness which may have indicated possible sleep disorders using the Epworth Sleepiness Scale (Johns, 1991). Subjects were asked to wear an Actiwatch (Philips Respironics) and keep a sleep diary for 1 week prior to scanning, to ensure no obviously disordered sleep was evident. They were requested to refrain from alcohol and caffeine consumption 24 hours prior to scanning.

Subjects underwent a session of EEG-fMRI under fully sleep deprived conditions in which they were asked to remain awake for 24 hours prior to scanning. They attended for the scanning session at approximately 07:00hrs. During the scanning session, subjects were given earplugs and headphones to minimise the noise from MRI acquisition. The study was approved by the University of Birmingham Research Ethics Committee. Subjects gave written informed consent and were paid for their participation.

Data acquisition

EEG data were acquired continuously with a sampling rate of 5 KHz using a 64-channel EEG cap (Brain Products, Munich, Germany), connected to two 32-channel MR-compatible amplifiers (BrainAmp MR Plus, Brain Products, Munich, Germany). Placement of single electrodes on the left shoulder and sub-mandibular suprahyoid muscles ensured recording of the ECG and proximal myogenic activity. Electrode impedances were kept below 10kΩ.

A 3T Phillips Achieva MR Scanner (Phillips, Netherlands) with an 8-channel SENSE head coil was used for the acquisition of EPI data (3x3x4mm voxels, TR 2000ms, TE 35ms, 32 slices, flip angle 80°, 450 dynamics per scan), and a T1-weighted scan (1mm isotropic voxels) used for anatomical localisation. Following the T1-weighted scan, subjects were instructed to remain still with eyes closed. Each EPI scan was 15 minutes in duration, with a 1-minute period between each run of EPI acquisition to allow time for the subject to terminate scanning if required. Subjects remained in the scanner between 45 and 90 minutes.

Data pre-processing & analysis

EEG data.

Removal of MR gradient artefacts was performed offline using Brain Vision Analyzer software (version 1, Brain Products, Munich, Germany) implementing a template subtraction approach as described by (Allen, Josephs & Turner, 2000). The data were then downsampled to 200Hz and ballistocardiogram artefacts were removed using the Optimal Basis Set (OBS) plug-in (Niazy, Beckmann, Iannetti, *et al.*, 2005) to EEGLAB (Delorme and Makeig, 2004). All events (VSW, KC and SS), were marked by an experienced electroencephalographer in accordance with guidelines laid out by the American Association of Sleep Medicine (Iber, Ancoli-Israel, Chesson, *et al.*, 2007).

To allow unambiguous identification of the activation specifically associated with VSW, KC or SS, any events overlapped by 10 seconds either side of the event (e.g. a VSW occurring within 10 seconds of a KC or a SS occurring within 10 seconds of a KC etc.), or linked, i.e. KC associated with a SS, were excluded from the analysis.

fMRI data.

EPI data were processed in SPM5 (Wellcome Department of Imaging Neuroscience, UCL, UK). Initially the images underwent realignment for motion correction, followed by slice timing correction, anatomical normalisation to MNI space and smoothing with a Gaussian kernel (6mm, FWHM). All marked events were convolved with a canonical HRF and its first temporal derivative using a general linear model (GLM) in an event related design. To account for noise contribution from cardiac rhythms and associated motion artefacts, six motion parameters and the timings for the Q-peaks, derived from the single lead ECG, were included as regressors in the design matrix.

An additional GLM analysis was performed to investigate any potential BOLD changes that preceded the onset of the sleep paroxysms. In this case the peak of the HRF was changed by deducting 6 seconds from the timing of the onset of the sleep paroxysms used in the original GLM, in accordance with a previous investigation of early peaking HRFs in epilepsy (Rollings, Assecondi, Ostwald, *et al.*, 2015).

Group analyses including each paroxysm (VSW, KC and SS) were performed in SPM5. A Bonferroni corrected threshold of $p<0.0005$ ($P<0.001/2$) was used to take account of the use of two different peaking HRFs used.

4.4 RESULTS

4.4.1 EEG results

Of the 19 participants recruited into the study, 13 (7 males, 30 years ± 8 years) were able to achieve sleep in the scanner and demonstrate recordable sleep paroxysms (i.e. VSWs, KCs and/or SS), and thus were included in further analysis. Not all participants exhibited all paroxysms on the EEG during the scanning session; all 13 demonstrated VSWs, with KCs and SSs only observed in 7 of the participants. A total of 112 VSWs, 53 KCs, 34 SSs were recorded across all included subjects and scanning sessions. Previous studies have identified two distinct spindle types, fast- and slow-SSs (Anderer, Klösch, Gruber, *et al.*, 2001; Schabus, Dang-Vu, Albouy, *et al.*, 2007; Werth, Achermann, Dijk, *et al.*, 1997), however in this study it was decided to focus on investigating common generators/regions between both spindle types. Additionally, since the total number of SSs recorded was relatively low, separating them out would have reduced the numbers even further with 21 fast-SSs and only 13 slow-SSs. Topographical distribution of the sleep paroxysms on the EEG was as expected with VSWs maximal across the vertex, KCs showed a fairly widespread distribution across scalp electrodes but maximal in the frontal derivations, and SSs either occurred in frontal regions predominantly or in the central/parietal region depending on whether it was slow or fast spindles respectively.

4.4.2 fMRI results

Only positive t values were considered further as no negative responses where observed at the single or group level. With the standard HRF, group fMRI data for VSWs (n=13) demonstrated

widespread activation across several cortical and subcortical structures (Figure 4.4.1.a.). Activation was observed across anterior, middle and posterior cingulate cortices, pre- and post-central gyri, superior temporal gyri, inferior, middle and superior frontal gyri, and in primary visual cortices. Subcortical activation was also noted in the thalamus bilaterally and in the brainstem. The group data for the shifted HRF occurring 6 seconds prior to VSW onset did not demonstrate any significant activation.

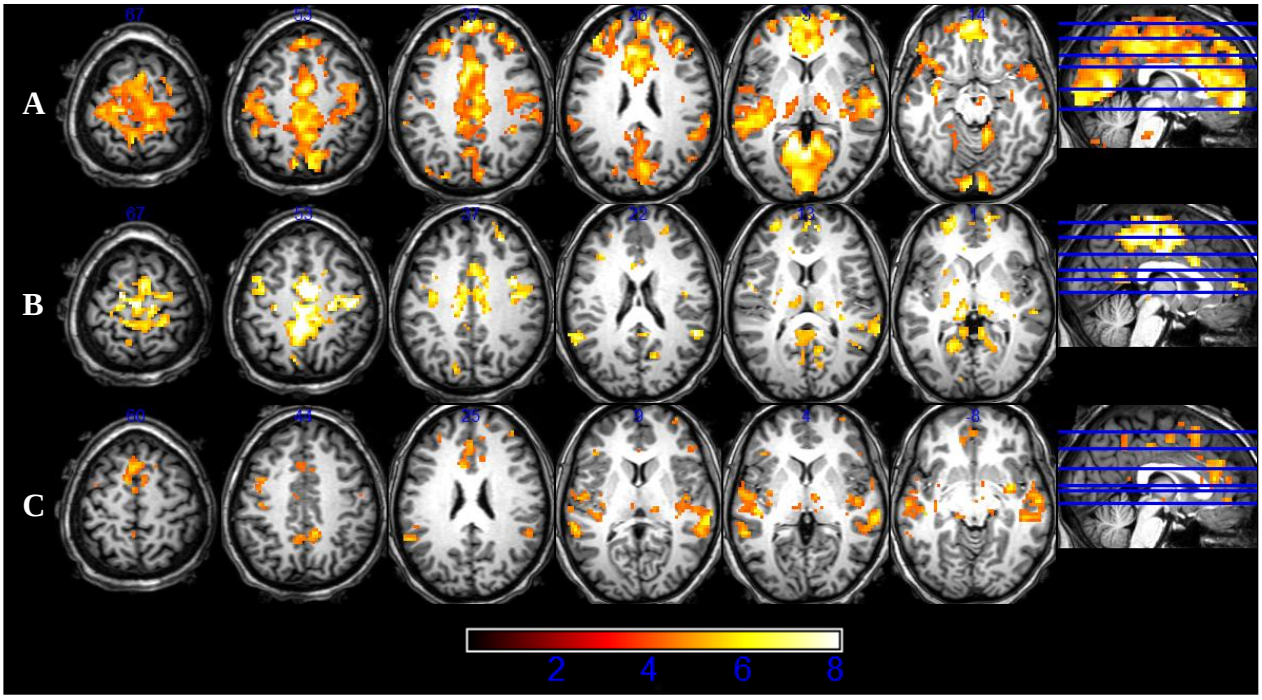


Figure 4.4.1. Fixed effects group responses for the total A) 112 VSWs (n=13), B) 53 KCs (n=7), and C) 34 SSs (n=7). Activation maps (p<0.0005, uncorrected, no cluster threshold) superimposed on to a normalised T1 (MNI space) from one of the participants.

With actual event times, the group data for KCs (n=7) demonstrated several regions of cortical activation as well as subcortical activation (Figure 4.4.1.b.). A large cluster of activation was observed in the middle cingulate cortex and into primary motor and sensory cortices. Activation was present bilaterally in the superior temporal gyrus, calcarine cortex, precuneus, some in the

posterior cingulate cortex and also in the prefrontal cortex. Subcortical activation was evident in bilaterally in the thalamus, some in the rostral brainstem and also the left putamen/globus pallidus. Again, shifting the HRF to peak 6 seconds prior to the onset of the KC did not demonstrate any significant activation.

In the group data for SSs (n=7) using the actual event times, activation was observed in both cortical and subcortical structures (Figure 4.4.1.c.). Cortical regions involved included bilateral superior and middle temporal gyri, bilateral posterior insula, precuneus, anterior cingulate cortex (ACC), middle frontal gyrus, and supplementary motor area. Bilateral thalamic activation was also noted. The group map for the shifted HRF demonstrated activation in a number of cortical regions (Figure 4.4.2.): bilateral superior temporal gyri and Heschl's gyri, anterior cingulate cortex, supplementary motor cortex, cuneus, and posterior insula; most notably the right insula.

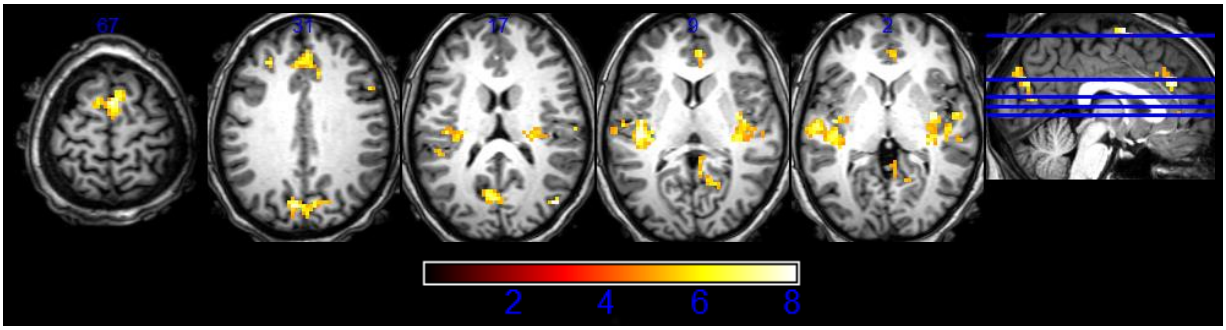


Figure 4.4.2. Group results for early BOLD responses for SSs (n=7). Activation maps ($p<0.0005$, uncorrected, no cluster threshold). Regions include posterior insula, anterior cingulate, Heschl's gyrus, and superior temporal gyrus.

4.5 DISCUSSION

The primary aim of this study was to ascertain if early BOLD signal changes that have been observed in EEG-fMRI studies in epilepsy are also observed in spontaneously occurring sleep paroxysms. Early BOLD changes in epilepsy have been observed in both focal and generalised epilepsy (Jacobs, Kobayashi, Boor, *et al.*, 2007; Moeller, Siebner, Wolff, *et al.*, 2008; Rollings, Assecondi, Ostwald, *et al.*, 2015). It has been postulated they result from neurons or glial structures involved in the generation of focal IEDs that are yet unsynchronised (Pittau, Levan, Moeller, *et al.*, 2011). Given this, it would be unlikely to observe any early BOLD responses in spontaneously occurring sleep paroxysms; KCs, VSWs and SSs.

The areas of activation observed in the group data for VSWs and KCs were fairly widespread and consistent with previous studies (Caporro, Haneef, Yeh, *et al.*, 2012; Jahnke, von Wegner, Morzelewski, *et al.*, 2012; Stern, Caporro, Haneef, *et al.*, 2011). For both KCs and VSWs, no significant activation was observed in the analysis of early BOLD signals, at the group or single subject level. Given the potential pitfall of "temporal bleeding", or an overlap in the HRF models (Rollings, Assecondi, Ostwald, *et al.*, 2015), using this type of analysis to investigate early BOLD signals, it is reassuring to find early BOLD responses are not observed in VSWs and KCs despite having such extensive activation; especially as seen with VSWs. If early responses were a result of an overlap between the HRF models, then one would certainly expect to see such overlap when the responses are so significant and widespread. Thus the early BOLD changes observed in epilepsy studies become even more convincing, and less likely to occur as a result of modelling techniques.

At the group level using the canonical HRF SSs demonstrated regions of activation including bilateral superior temporal gyri, posterior insula, precuneus, anterior cingulate cortex, middle frontal gyrus, supplementary motor area and thalamus. These regions are similar to previous EEG-fMRI studies of SSs (Andrade, Spoormaker, Dresler, *et al.*, 2011; Schabus, Dang-Vu, Albouy, *et al.*, 2007). Of particular note, significant early BOLD signal changes were observed in SSs. No previous study has investigated early BOLD changes in sleep paroxysms and as such this is the first report of early activation preceding the scalp SS. The regions that demonstrate early increases in BOLD signal include bilateral superior temporal gyri and Heschl's gyri, anterior cingulate cortex, supplementary motor cortex, cuneus, and posterior insula; most notably the right insula. Although similar regions of activation are observed between both the standard and early HRF models for SSs, there are clear distinctions between the two maps (Figure 4.4.2.), suggesting that temporal bleeding is an unlikely explanation. Given that no early responses were observed in either VSWs or KCs, and the BOLD responses with the canonical HRF were greater and more extensive, it is unlikely that the early responses observed in SSs are a result of the modelling techniques used, but rather they are genuine BOLD signal increases that precede the SS. The origin of these early activations and their significance are the palpable questions that arise from such results.

Spindles originate in the thalamus as thalamic reticular (RE) neurons enact rhythmic inhibitory post synaptic potentials (IPSPs) upon thalamocortical (TC) cells which can lead to rebound spike bursts transferring to the cortex, resulting in spindles through rhythmical interaction between RE and TC neurons (Contreras & Steriade, 1996; Steriade, 1995). The thalamic origin of SSs is supported by *in vivo* recordings in decorticated cats (Morison & Bassett, 1945; Steriade, 1995) and synchronisation though the cortex is potentiated by the neocortex and reciprocal interactions

between TC and RE neurons (Contreras & Steriade, 1996; Destexhe, Contreras & Steriade, 1998). Given that the evidence indicates clearly a thalamic origin of spindles, it appears counter intuitive that early BOLD changes would not involve the thalamus and rather purely cortical structures. Recent research, using computational models and *in vivo* recordings from both the thalamus and cortex, indicate that initiation of spindle sequences may occur via pyramidal neurons through recruiting bursts in RE neurons (Bonjean, Baker, Lemieux, *et al.*, 2011). This suggests that spindles arise through a complex interaction between the thalamus and cortex and are heavily influenced by corticothalamic involvement. Recent studies of slow SSs and fast SSs suggest that they are generated through different mechanisms, and slow SSs may have an intracortical origin (Ayoub, Aumann, Hörschelmann, *et al.*, 2013; Timofeev & Chauvette, 2013). In this present study both slow and fast SSs were included in the same analysis, and as such it is unclear as to what the contributions from each spindle type to the overall results are. These findings in part may lend explanation as to why early BOLD changes in SSs, observed in this present study, are purely cortical. Why these responses occur in the first place remains elusive and a topic for further investigation. It is feasible that preceding changes in cortical activity may indicate the development of a favourable substrate for spindle generation when initiated by the thalamus; one such substrate is the slow oscillation.

The slow oscillation (SO) originates in the cortex (Timofeev, Grenier, Bazhenov, *et al.*, 2000), oscillates between ~0.3 – 1 Hz (Steriade, McCormick & Sejnowski, 1993). It reflects the highly synchronous activity of nearly all cortical neurons between a depolarising upstate, consisting of inhibitory and excitatory postsynaptic potentials, and a hyperpolarising downstate, in which neuronal firing is silenced (Steriade, McCormick & Sejnowski, 1993; Volgushev, Chauvette,

Mukovski, *et al.*, 2006; Amzica & Steriade, 2000). SSs are temporally linked to the phase of the SO; specifically fast spindles are associated with the upstate and slow spindles in the transition from upstate to downstate (Klinzing, Mölle, Weber, *et al.*, 2016; Mölle, Marshall, Gais, *et al.*, 2002). Imaging studies using positron emission tomography (PET) have demonstrated, among other regions, anterior cingulate and insular cortices involvement in the SO (Dang-Vu, Desseilles, Laureys, *et al.*, 2005; Maquet, 2000). Similar findings have been reported in an EEG-fMRI study by Kaufmann et al., 2006, who demonstrated decreased brain activity in several regions correlated with deepening of sleep, most notably the involvement of the cingulate and insular cortices (Kaufmann, Wehrle, Wetter, *et al.*, 2006). In PET studies, regional cerebral blood flow (rCBF) is found to be lowest in regions most involved in slow sleep oscillations, likely associated with synchronised hyperpolarisation of the neurons in these regions (Maquet, 2000). This interpretation is also applied to decreases in BOLD signal and increases in delta power observed with deepening sleep (Czisch, Wehrle, Kaufmann, *et al.*, 2004; Kaufmann, Wehrle, Wetter, *et al.*, 2006). A study using high density-EEG reported that spontaneous slow waves during sleep occurred in numerous distributed cortical sites, but preferentially in the insula and cingulate cortex (Murphy, Riedner, Huber, *et al.*, 2009). The involvement of the insula and ACC in the generation of slow activity, which is inextricably linked to the SO, is therefore evident across different imaging modalities, and is consistent with the early BOLD changes observed in relation to SSs in the current study. However, despite the activation of similar regions and the temporal relationship between the SO and SSs, it is perplexing as to why early BOLD responses 6 seconds before the SS would occur. This explanation therefore remains speculative.

In addition to the early BOLD signal changes observed in the insula and ACC, the results in this study demonstrated early activation in superior temporal gyri and Heschl's gyri. A possible explanation for this lies in the role of SSs. Spindles have been described as having a sleep protective role (Dang-Vu, McKinney, Buxton, *et al.*, 2010; De Gennaro & Ferrara, 2003) which can inhibit responses to incoming stimuli (Elton, Winter, Heslenfeld, *et al.*, 1997; Schabus, Dang-Vu, Heib, *et al.*, 2012; Steriade, McCormick & Sejnowski, 1993) and may even represent the inhibition of information processing by the thalamus (Cote, De Lugt, Langley, *et al.*, 1999). Auditory stimuli during NREM sleep can elicit fMRI activation at any phase of the SO in the auditory cortex but activation in the STG is phase dependent; i.e. the negative to positive slope of the SO (Schabus, Dang-Vu, Heib, *et al.*, 2012). Given that spindles are temporally related to the phase of the SO, and auditory responses can occur during any phase of the SO, one possible explanation is that the early activation observed in this present study is related to auditory stimulation resulting from scanner noise. This is probably unlikely however given the response to the noise would essentially be occurring with a delay of six seconds.

Given the relationship between the SO and SSs and the reported contribution of both the insula and ACC, and the early BOLD response reported here in this present study, it is plausible that the insula and ACC may play a meaningful role in spindle generation. The involvement of auditory cortex may be related to incoming stimuli from scanner noise, though this is not possible to establish in this study. This could be investigated by use of tone stimulation during scanning to ascertain if these early BOLD responses are most evident during stimulus delivery. In this study SSs were coalesced and not analysed separately as fast and slow spindles. It is thus unknown whether one spindle type or another is the main contributor to the early BOLD responses observed here, or

whether this is a feature of both fast and slow SSs. A larger cohort and greater numbers of SSs would allow for separation of the two spindle types to investigate this further.

4.6 CONCLUSION

The aim of this study was to ascertain if early BOLD signal changes observed in epilepsy studies were also evident in sleep paroxysms; notably VSWs, KCs and SSs. The absence of early BOLD responses in VSWs and KCs gives supports that the findings of prespike activity observed in IEDs is a genuine phenomenon rather than an effect of the modelling used (i.e. shifting HRF peak time). This is the first study to report early BOLD signal responses in SSs, and indeed in relation to any normal paroxysmal brain activity. As this has not been previously investigated or reported their significance and meaning remain unclear and warrant further investigation. These changes could represent an emerging network involved in the generation and propagation of sleep spindles. Further studies are needed with the specific aim of investigating these responses to elucidate their validity and significance.

Chapter 5

THE EFFECT OF SLEEP DEPRIVATION ON VERTEX SHARP WAVES AND K-COMPLEXES

5.1 ABSTRACT

The K-complex (KC) and vertex sharp wave (VSW) are two major graphoelements that are unique to sleep. Their relationship has been established in event related potential (ERP) studies during sleep though their characterised roles have been both described as similar and divergent at one time or another. At present both are postulated to provide a sleep maintenance role. The aim of this EEG-fMRI experiment was to further elucidate the relationship between the two paroxysms and to further elucidate the functional role by manipulating homeostatic sleep pressure through sleep deprivation. Group data from six subjects who were scanned under bother sleep deprived (SD) and non-deprived (ND) conditions demonstrated widespread regions of cortical activation in both KCs and VSWs. Regions of interest (ROI) were defined from this data and beta values extracted. This data were used in a generalised linear mixed model (GLMM). From the GLMM there was a significant overall increase in the BOLD response when under SD conditions, indicating sleep deprivation increases the BOLD response. In KCs specific regions (bilateral middle frontal and middle temporal gyri) demonstrated a decrease, rather than increase following SD; these regions associated with attentional networks may represent reduced attentional activation when SD. Widespread activation was present in primary sensory regions more so with VSWs than KCs, indicating the VSW may be priming the cortex for a rapid transition from a sleep to wake state should the need arise. Overall these results suggest the KC has a more sleep protective role than the VSW though the two may indeed be related with the VSW perhaps a precursor of the KC. Additionally, sleep deprivation has been shown to increase the BOLD response and this should be taken into account when using sleep deprivation to study sleep using EEG-fMRI.

5.2 INTRODUCTION

The progression through the stages of human sleep is heralded by stark electrographic features unique to each brain state. The vertex sharp wave (VSW), observed late in drowsiness into stage 1 sleep, and the K-complex (KC), first evident during the transition from stage 1 to stage 2 sleep, are two such graphoelements. While both VSW and KC have been observed for several decades, and are included in standard protocols for sleep staging (Iber, Ancoli-Israel, Chesson, *et al.*, 2007) the brain regions that generate them and their functional significance are yet to be fully elucidated.

The VSW is topographically observed most prominently over the vertex (Yasoshima, Hayashi, Iijima, *et al.*, 1984) comprising of a large surface negative potential preceded by a small positive wave and is often associated with the N350 component of event related potentials (ERP) in sleep studies (Colrain, Webster, Hirst, *et al.*, 2000; Harsh, Voss, Hull, *et al.*, 1994). First described in 1938 by Loomis and colleagues, the KC is a fundamental landmark of stage 2 sleep, occurring spontaneously or evoked by exogenous/endogenous stimuli (Colrain, 2005; Loomis, Harvey & Hobart, 1938). The KC is predominantly associated with a prominent surface negative component, in ERP studies occurring at 550 ms (N550), with an initial preceding positive deflection. A negative peak at 300 – 350 ms can often be observed associated with the KC though is not considered to be a primary component of that KC but rather attributed to the VSW (Bastien & Campbell, 1992; Bastien, Crowley & Colrain, 2002; Colrain, Webster, Hirst, *et al.*, 2000; Halász, 2005).

Due largely to the work of Amzica and Steriade, the electrophysiological basis of KCs is well understood (Amzica & Steriade, 2002, 1998, 1997). The two phases of the biphasic KC, the initial positive deflection, representing synchronous neuronal excitation, and the more electrographically prominent negative components resulting from a period of hyperpolarisation, cause widespread

cortical synchronisation. Subsequent triggering of sleep spindles or delta oscillations has been proposed as a consequence of the synchronous input into thalamic neurons (Amzica & Steriade, 2002, 1998). This is corroborated by the frequently observed presence of sleep spindles following KCs (Halász, 2005). While similar mechanisms are thought to subserve the generation of VSWs, their electrophysiology has received less attention (See section 1.2 for more details on sleep paroxysms).

Initial research suggested that the functional significance of KCs was limited to an arousal mechanism (Ehrhart, Ehrhart, Muzet, *et al.*, 1981; Roth, Shaw & Green, 1956). However, more recent indications favour a sleep protective mechanism rather than that of a simple arousal response (Bastien, Ladouceur & Campbell, 2000; Colrain, 2005; Forget, Morin & Bastien, 2011; Peszka & Harsh, 2002). This theory has been further substantiated by recent studies using functional imaging methods to identify brain regions and networks associated with KCs (Caporro, Haneef, Yeh, *et al.*, 2012; Jahnke, von Wegner, Morzelewski, *et al.*, 2012). Both Copporo et al., 2012, and Jahnke et al., 2012, identified activation in primary sensory regions in addition to other cortical and subcortical regions. From this it has been inferred that there may be a degree of information processing, allowing the sleeper to monitor environmental changes potentially requiring action, but also to maintain uninterrupted sleep should no arousing response to the environmental stimuli be required.

The sensory regions identified in these imaging studies are remarkably similar to those seen in response to VSW (Stern, Caporro, Haneef, *et al.*, 2011). The VSW has received far less attention than the KC despite its stark morphology and consistently observed appearance during the early stages of sleep. The primary component of the VSW, the large surface negative potential, is observed maximal in amplitude across the midline and can be elicited by both auditory and

inspiratory occlusion stimuli (Colrain, Webster, Hirst, *et al.*, 2000; Bastien, Crowley & Colrain, 2002). Despite differing topographical distribution of the primary components of the VSW and the KC, it has been proposed that the VSW may also contribute a sleep protective mechanism, possibly sharing similar neural generators with the KC (Amzica & Steriade, 1998; Colrain & Campbell, 2007). A relationship between the VSW and KC is possible given the presence of the N350 across both sleep paroxysms in studies investigating ERPs in sleep. It was initially suggested that the N350 may act as a trigger for the N550 once a threshold had been reached, though subsequently it was demonstrated that there was no significant difference in amplitudes between the N350 occurring independently or in conjunction with the N550 (Bastien & Campbell, 1992; Gora, Colrain & Trinder, 2001). A functional relationship between VSWs and KCs remains probable, but at present the evidence is ambiguous.

The VSW and KC have both been implicated in providing a sleep protective role, possibly via gating of external stimuli in order to maintain sleep. Under sleep deprived conditions, which increases sleep drive, Nicholas et al., 2002, identified a significant increase in both spontaneous and evoked KCs compared with normal sleep (Nicholas, Trinder & Colrain, 2002). They concluded a sleep maintenance role for KCs, though no change in density was noted for VSW. Subsequent studies have failed to show significant changes in KC density following sleep deprivation (Curcio, Ferrara, Pellicciari, *et al.*, 2003; Sforza, Chapotot, Pigeau, *et al.*, 2004) and the effect of sleep deprivation on VSW has been neglected.

In this study we used EEG-fMRI to investigate the brain regions involved in the generation of VSWs and KCs under both sleep deprived and non-deprived conditions. The study had two primary aims: 1) to investigate the relationship between the two sleep transients in normal and recovery sleep and 2) to use manipulation of homeostatic sleep drive via sleep deprivation to observe any

potential effect on VSWs and KCs and whether this may help to further elucidate the function of these sleep transients.

5.3 METHODS

Subjects & Procedure

Eight participants (5 males, mean age 32±6 years) were recruited into this study. Subjects were without known abnormal neurology and screened for excessive daytime sleepiness which may have indicated possible sleep disorders using the Epworth Sleepiness Scale (Johns, 1991). Subjects were asked to wear an Actiwatch (Philips Respironics) and keep a sleep diary for 1 week prior to scanning, and to keep a regular sleep onset time. They were requested to refrain from alcohol and caffeine consumption 24 hours prior to scanning.

Subjects initially underwent a session of EEG-fMRI under fully sleep deprived conditions in which they were asked to remain awake for 24 hours prior to scanning. They attended for the scanning session at approximately 07:00hrs (sleep deprived (SD) condition). Subjects were asked to return for a second scanning session, separated by at least 2 weeks, this time under non-deprived conditions (start of scanning session approximately 23.00hrs depending on subjects' normal bedtime, non-deprived (ND) condition)).

During both scanning sessions, subjects were given earplugs and headphones to minimise the noise from MRI acquisition. The study was approved by the University of Birmingham Research Ethics Committee. Subjects gave written informed consent and were paid for their participation.

Data acquisition

EEG data were acquired continuously with a sampling rate of 5 KHz using a 64-channel EEG cap (Brain Products, Munich, Germany), connected to two 32-channel MR-compatible amplifiers (BrainAmp MR Plus, Brain Products, Munich, Germany). Placement of single electrodes on the left shoulder and sub-mandibular suprahyoid muscles ensured recording of the ECG and proximal myogenic activity. Electrode impedances were kept below 10kΩ.

A 3T Phillips Achieva MR Scanner (Phillips, Netherlands) with an 8-channel SENSE head coil was used for the acquisition of EPI data (3x3x4mm voxels, TR 2000ms, TE 35ms, 32 slices, flip angle 80°, 450 dynamics per scan), and a T1-weighted scan (1mm isotropic voxels) used for anatomical localisation. Following the T1-weighted scan, subjects were instructed to remain still with eyes closed and not to resist sleep. Each EPI scan was 15 minutes in duration, with a 1-minute period between each run of EPI acquisition to allow time for the subject to terminate scanning if required. Subjects remained in the scanner between 45 and 90 minutes.

Data preprocessing & analysis

EEG data. Removal of MR gradient artefacts was performed offline using Brain Vision Analyzer software (version 1, Brain Products, Munich, Germany) implementing a template subtraction approach as described by Allen et al., 2000 (Allen, Josephs & Turner, 2000). The data were then downsampled to 200Hz and ballistocardiogram artefacts were removed using the Optimal Basis Set (OBS) plug-in (Niazy, Beckmann, Iannetti, *et al.*, 2005) EEGLAB (Delorme & Makeig, 2004). All electrographic transients of sleep (VSW, KC and sleep spindles), were marked by an experienced electroencephalographer in accordance with guidelines laid out by the American Association of Sleep Medicine (Iber, Ancoli-Israel, Chesson, *et al.*, 2007). To allow unambiguous

identification of the activation specifically associated with VSW or KC, VSW associated with a sleep spindle or KC were excluded from analysis as were KCs associated with sleep spindles or delta waves. As this occurred very infrequently these few events were not included as regressors. KCs closely preceded by a VSW were also excluded to avoid any potential overlap in activation or contamination between the two paroxysms.

fMRI data

EPI data were processed in SPM5 (Wellcome Department of Imaging Neuroscience, UCL, UK). Initially the images underwent realignment for motion correction, followed by slice timing correction, anatomical normalisation to MNI space and smoothing with a Gaussian kernel (6mm, FWHM). All marked events were convolved with a canonical HRF and its first temporal derivative using a general linear model (GLM) in an event related design. To account for noise contribution from cardiac rhythms and associated motion artefacts, six motion parameters and the timings for the Q-peaks were included as regressors in the design matrix. Sleep spindles were also included in the design as a regressor of no interest, though sleep spindles and KCs occurring concurrently were excluded.

A group analysis including each condition (ND and SD) and paroxysm (VSW and KC) was performed in SPM5. An F-contrast was created in SPM including both conditions and paroxysms and the resulting statistical map (FWE corrected, $p < 0.05$) was used to formulate regions of interest (ROI) for later analyses (see Figure 5.4.1.). From the largest observed clusters, the voxel with the maximum t-value was selected and a 10 mm spherical ROI was defined around this coordinate in the MarsBaR plugin (Brett, Anton, Valabregue, *et al.*, 2002) to SPM (Table 5.3.1). The beta values for each subject were extracted for each paroxysm and condition giving a total of four beta values

per subject for each ROI. The beta values were then used in a generalised linear mixed-model (GLMM) implemented in SPSS (IBM SPSS Statistics, version 22, Chicago, IL) modelling the fixed effects of sleep type, sleep transient, ROI and also the two-way (sleep-transient, sleep-ROI, transient-ROI) and three-way (sleep-transient-ROI) interactions. The model also included the random-effects of subject.

ROI	Anatomical Abbreviation	Anatomical location	MNI coordinates
1	R MFG	Right middle frontal gyrus	27, 57, 0
2	L MFG	Left middle frontal gyrus	-33, 54, 8
3	MOG	Mid-Orbital gyrus	-3, 54, -4
4	R In	Right insula	36, -18, 12
5	L In	Left insula	-33, -18, 8
6	ACC	Anterior cingulate cortex	6, 24, 24
7	MCC	Mid-cingulate cortex	0, -21, 44
8	PCC	Posterior cingulate cortex	3, -49, 30
9	PC	Precuneus	6, -66, 52
10	L PrCG	Left pre-central gyrus	-39, -24, 58
11	R PrCG	Right pre-central gyrus	39, -24, 64
12	L PoCG	Left post-central gyrus	-33, -39, 64
13	R PoCG	Right post-central gyrus	36, -36, 64
14	R STG	Right superior temporal gyrus	57, -33, 8
15	L STG	Left superior temporal gyrus	-60, -39, 16
16	L MTG	Left middle temporal gyrus	-48, -60, 8
17	R MTG	Right middle temporal gyrus	51, -60, 8
18	L LG	Left lingual gyrus	-6, -69, 8
19	R LG	Right lingual gyrus	18, -63, 0
20	L Th	Left thalamus	-9, -21, 4
21	R Th	Right thalamus	9, -21, 4
22	Pons	Brainstem (Pons region)	-3, -30, -40

Table 5.3.1.. Anatomical location and MNI coordinates of each defined 10mm spherical ROI with identifier.

5.4 RESULTS

5.4.1 EEG results

All but one subject reached at least stage N2 sleep in both sleep deprived (SD) and non-deprived (ND) scanning sessions. The subject who failed to reach stage N2 sleep in both conditions was excluded. A further subject was excluded due to corrupted EPI data, despite reaching stage N2 sleep. From the 6 subjects (4 males, 29 years ± 4 years) included in the analysis, the combined scanning time for EPI acquisition of the ND group was 435 minutes, and 420 minutes for SD group. A total of 60 VSWs were recorded during the ND session and 67 during the SD session. In the ND group 40 KCs were recorded and 49 from the SD group. Although slightly more of the sleep paroxysms were recorded during the SD session, paired t-tests showed there was no significant difference between the two groups in the number of VSW ($p = 0.64$) or KC ($p = 0.18$) and no difference between the mean amplitudes between the two conditions for VSW ($p = 0.09$) and KC ($p = 0.23$). The electrode site demonstrating maximal amplitude varied between subjects but was consistent within subjects. The maximal amplitude for VSWs was located at the vertex or contiguous to it, with the maximal amplitude for the KCs located more anteriorly.

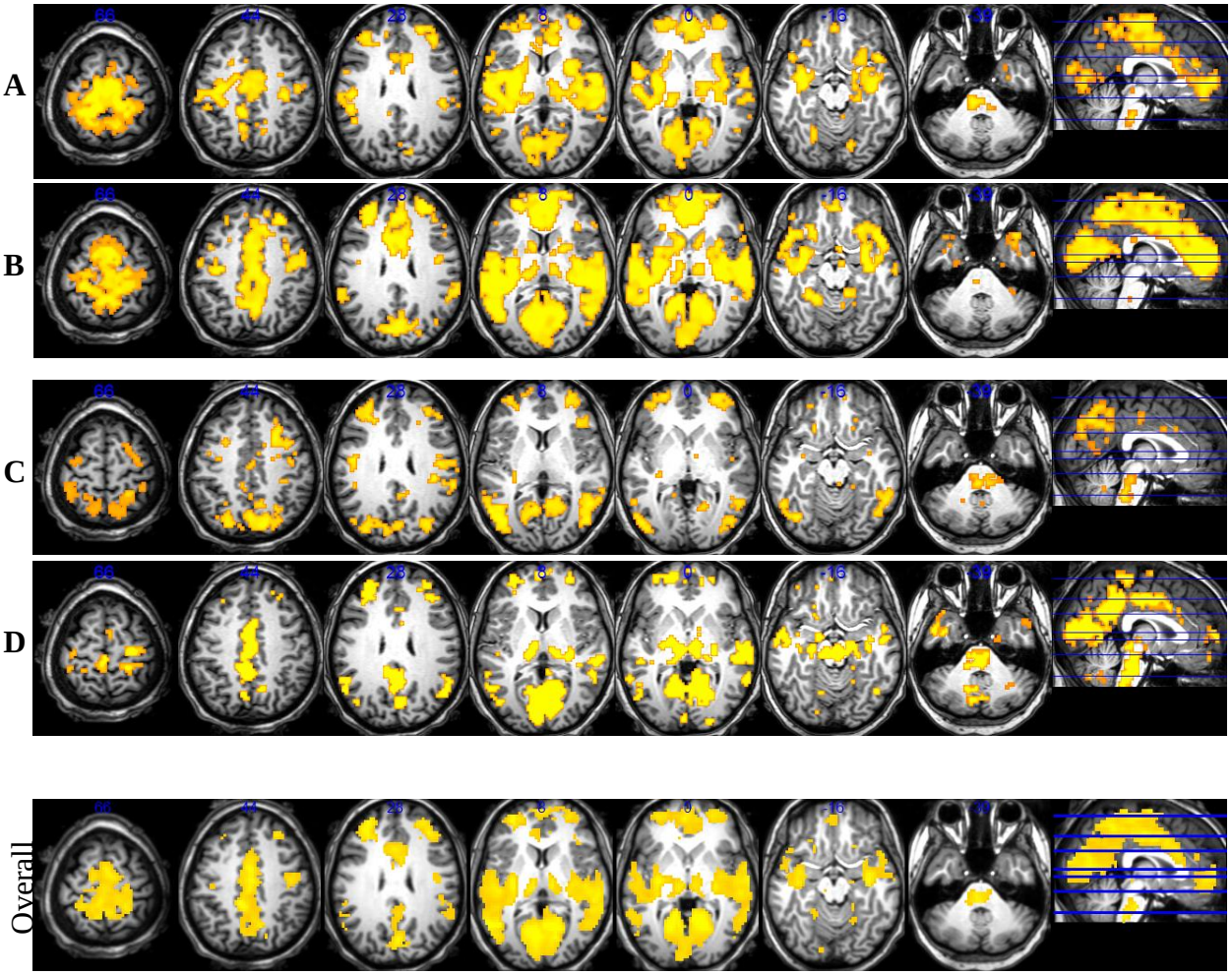


Figure. 5.4.1. Group results showing regions of activation in relation to (A) VSW under non-sleep deprived conditions, (B) VSW under sleep deprived conditions, (C) KCs under non-sleep deprived conditions and (D) KCs under sleep deprived conditions. Activations maps (displayed at $p < 0.001$, uncorrected) superimposed on normalised anatomical T1 scan from one subject. The final "Overall" map at the bottom is an F-contrast including both VSW and KC under both ND and SD conditions ($p < 0.05$, FWE corrected).

5.4.2 fMRI results

For both conditions and sleep paroxysms, no deactivations at group or single subject level were observed, so only positive BOLD responses are discussed.

Group analysis for VSW under ND conditions (n=6) demonstrated BOLD signal increases in widespread regions (Figure 5.4.1.). Large clusters of activation were observed in anterior cingulate cortex, middle frontal and superior frontal gyri bilaterally, insula cortices, cuneus and bilaterally in superior and middle temporal gyri. Most of the primary sensory and motor regions also demonstrated an increase in BOLD signal. Sub cortical activation was also noted bilaterally in the thalamus and in the brainstem at the level of the pons. Under SD conditions similar regions of activation were observed though more abundant activation was seen across the cortex as compared to the ND group. Posterior temporal regions bilaterally also demonstrated BOLD signal increases that were not evident in the ND group. Subcortically, bilateral thalamic activation was noted as was bilateral caudate.

For the KC group under ND conditions, increased BOLD signal changes were observed bilaterally in the inferior frontal gyrus, middle frontal gyrus, middle temporal gyrus and inferior parietal cortices (Figure 5.4.1.). Additionally, signal increases were seen in the posterior cingulate cortex, precuneus and cuneus. Primary sensory (auditory, visual, somatosensory) and motor regions were also involved as were bilateral thalamus and medial brainstem at the level of the pons. Under SD conditions, KC activation patterns were similar to those observed under ND conditions with more activation in the posterior cingulate cortex, cuneus, thalamus and brainstem. Increases in BOLD signal compared to the ND condition were observed in the prefrontal cortex and to a lesser extent the posterior temporal region.

The F-contrast including both paroxysm types across both ND and SD conditions, performed to investigate the overall effects of sleep paroxysm and sleep type and on which the ROI analysis was based, on can be seen in Figure 5.4.1. (D).

	VSW				KC			
Region	**Std**	**t**	**df**	**Sig.**	**Std**	**t**	**df**	**Sig.**
R MFG	0.603	0.329	19	0.746	0.603	2.584	19	0.018
L MFG	0.611	-0.349	19	0.731	0.611	2.800	19	0.011
MOG	0.617	-2.746	20	0.013	0.617	-3.197	20	0.005
R In	0.581	-2.187	19	0.041	0.581	0.000	19	1.000
L In	0.409	-3.268	19	0.004	0.409	0.000	19	1.000
ACC	0.730	-2.924	20	0.008	0.703	-2.806	20	0.011
MCC	0.779	1.764	19	0.093	0.779	-2.707	19	0.014
PCC	0.836	-2.715	20	0.013	0.836	-4.067	20	0.001
PC	0.753	-1.733	19	0.099	0.753	1.859	19	0.078
L PrCG	0.663	-0.420	20	0.679	0.663	0.035	20	0.972
R PrCG	0.369	-0.190	18	0.852	0.369	0.000	18	1.000
L PoCG	0.547	-0.615	19	0.546	0.547	1.340	19	0.196
R PoCG	0.541	0.897	20	0.381	0.541	1.587	20	0.129
R STG	0.769	-3.242	20	0.004	0.769	-0.241	20	0.812
L STG	1.183	-2.927	20	0.008	1.183	-4.556	20	0.000
L MTG	0.435	-6.160	19	0.000	0.435	3.114	19	0.006
R MTG	0.565	-5.211	19	0.000	0.565	2.970	19	0.008
L LG	1.635	-1.613	20	0.123	1.635	-2.547	20	0.019
R LG	1.688	-2.161	20	0.043	1.688	-2.165	20	0.043
L Th	0.849	-2.151	19	0.044	0.849	-5.396	19	0.000
R Th	0.872	-2.290	20	0.033	0.872	-5.372	20	0.000
Pons	0.522	0.000	19	1.000	0.522	-3.669	19	0.002

Table 5.4.1. Results of the three-way effects within the GLMM. Rows highlighted indicated regions that are significant across both sleep transients, where as individual highlighted p-values are regions significant for that particular sleep transient.

5.4.3 ROI results

The GLMM demonstrated that the main effect of sleep type was significant ($F(1,161 = 20.637$, $p < 0.001$), with greater beta values observed in the SD group when compared to the ND group. The main effect of transient type was also significant ($F(1,161) = 39.713$, $p < 0.001$) with higher beta values observed in VSW compared to KCs, and similarly the main effect of region was significant ($F(8,34) = 6.594$, $p< 0.001$). There was a significant interaction between sleep type and region ($F(21,39) = 7.118$, $p < 0.001$), and between transient type and region ($F(21,39) = 27.383$, $p < 0.001$); a breakdown of the individual regions can be seen in Figure 5.4.2. Sleep and transient type did not show a significant interaction ($F(1,206) = 1.103$, $p = 0.295$), initially indicating sleep type, ND or SD, did not have a significant effect on sleep transient. A three-way fixed effect interaction, however, between sleep type, transient type and region was significant ($F(21,39) = 5.500$, $p < 0.001$) indicating that the interaction between sleep type and transient that was region dependent (Figure 5.4.3).

In Table 5.4.1 several regions are highlighted as showing significant increases or decreases in beta value as an effect of sleep type; some were common to both VSWs and KCs, and others exclusive to each transient. Regions that were commonly activated and showed a significant effect of sleep type included pre-frontal cortex, anterior and posterior cingulate cortex, left superior temporal gyrus, bilateral posterior middle temporal gyri and bilateral thalamus.

Under SD conditions VSWs demonstrated increases in beta values across all significant regions when compared to ND conditions. Although the majority of significant regions for KCs showed an increase in beta value in SD, there were some regions that showed a decrease: bilateral middle frontal gyri and bilateral posterior middle temporal gyri.

There were some instances where regions had no activation in the defined ROI but there was activation present in the general region. This was most notable in the brainstem (ROI 22), where activation was observed more caudally in the brainstem for VSW compared to the defined ROI.

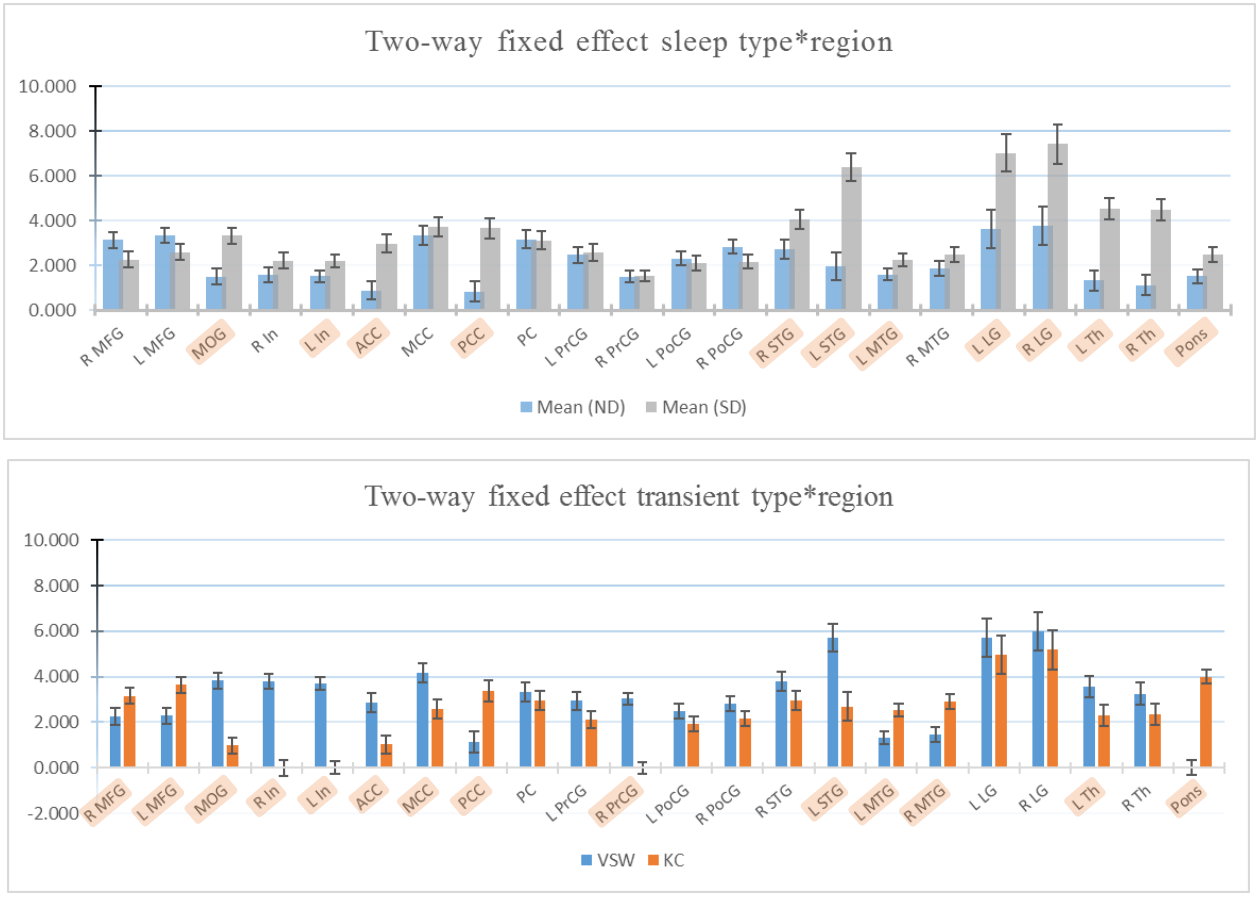


Figure. 5.4.2. Results from the GLMM showing the two-way fixed effects between sleep type and region and transient type and region. Highlighted ROIs on the graph are where the effect is significant ($p < 0.05$).

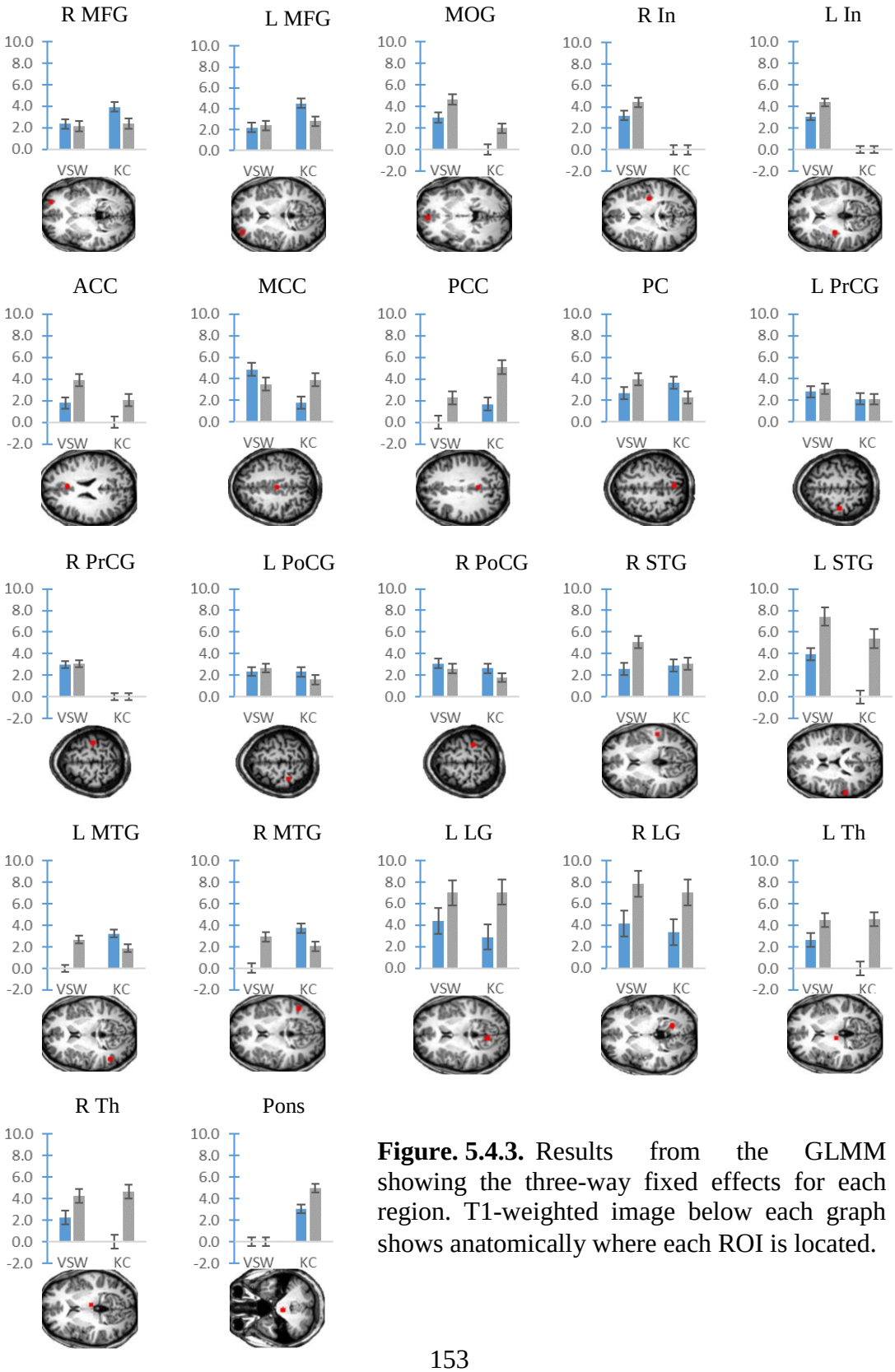


Figure. 5.4.3. Results from the GLMM showing the three-way fixed effects for each region. T1-weighted image below each graph shows anatomically where each ROI is located.

5.5 DISCUSSION

This study investigated the BOLD signal correlates of VSWs and KCs in healthy subjects under both ND and SD conditions. Positive BOLD signal changes were observed in widespread regions for both VSWs and KCs, including primary sensory and motor regions, cingulate cortices, precuneus and prefrontal cortex. Increased BOLD signal changes were also found in subcortical structures (thalamus, basal ganglia and brainstem). No significant BOLD deactivations at the single subject or group level were observed.

5.5.1 The relationship between VSWs and KCs

In the present study the maximal amplitude of the VSW was located at the vertex or regions contiguous to the vertex in surface EEG recordings, as demonstrated in other studies (Bastien, Crowley & Colrain, 2002; Colrain, Webster, Hirst, *et al.*, 2000). Despite the electrographic distribution, the BOLD signal increases associated with VSWs demonstrate widespread involvement of cortical and subcortical regions. The regions of activation noted in the present study are similar to those presented by Stern et al., 2011, who analysed two hundred VSWs from seven partially sleep deprived subjects (Stern, Caporro, Haneef, *et al.*, 2011). From a group analysis they identified several regions of increased signal, explicitly in the medial and lateral central regions, cuneus and lingual gyrus, superior temporal regions, cingulate and precuneus. Contrary to the current study, the authors noted that there was no signal increase in certain subcortical regions; thalamus and superior brainstem. Despite some differences, the regions of increased signal are comparable between studies. It is noteworthy that a number of the cortical regions involved are

also components of well-established resting state networks (RSN); all nodes of the sensorimotor network (Biswal, Yetkin, Haughton, *et al.*, 1995; van de Ven, Formisano, Prvulovic, *et al.*, 2004), involving primary somatosensory and motor cortex and premotor cortex; the auditory cortex network (Biswal, Van Kylen & Hyde, 1997), involving primary auditory cortex bilaterally; the primary visual network (van den Heuvel & Hulshoff Pol, 2010), involving primary and secondary visual cortex; the frontal attention network, involving the insula and middle frontal gyri (Corbetta, Patel & Shulman, 2008; Corbetta & Shulman, 2002); and nodes of the default mode network (Raichle, MacLeod, Snyder, *et al.*, 2001), involving prefrontal cortex, posterior cingulate cortex and precuneus (for an overview of RSNs see (Damoiseaux, Rombouts, Barkhof, *et al.*, 2006), are encompassed in the pattern of activation associated with VSWs. The elevation of activity observed in all these network regions (notably the primary motor and sensory cortices) certainly suggest the VSW may act as a priming mechanism, perhaps readying the sleeping individual to wake and orientate quickly should the need arise.

The KC, although a larger electrographic paroxysm, demonstrated less widespread activation than the VSW. The pattern of activation of KCs, although exhibiting a number of similarities, differed conspicuously from the overall pattern of VSWs. Less widespread activation as observed in the frontal regions, auditory, sensory and motor cortices, and no activation in the insula cortex, whilst there was more widespread activation observed in the posterior cingulate cortex and brainstem. The regions of activation observed in KCs are similar to those reported in other studies. Caporro et al., 2012 investigated the BOLD signal correlates of KCs in seven partially sleep deprived participants (Caporro, Haneef, Yeh, *et al.*, 2012). They found BOLD signal increases in several cortical regions; paracentral gyri, temporal regions, posterior cingulate cortex, precuneus, insula

and, although not specifically stated, the cuneus/occipital regions. In the same year, Jahnke et al. demonstrated BOLD signal changes in primary sensory regions (auditory, visual, paracentral and postcentral gyri, supplementary motor area), prefrontal cortex, cingulate cortices (anterior and mid), precuneus and inferior parietal regions, amongst others (Jahnke, von Wegner, Morzelewski, *et al.*, 2012). They also noted signal increases subcortically in the thalamus and brainstem. Jahnke et al., 2012, interpreted their results as the KC having two roles, protecting sleep whilst also monitoring the environment and allowing basic information processing. The KC has been well established as serving a sleep protective mechanism (Amzica & Steriade, 2002; Halász, 2005) and has long been associated with the VSW (Bastien, Crowley & Colrain, 2002). Despite the link between the KC and VSW the widespread involvement of the cortex, primary sensory regions in particular, suggests that the VSW provides a more favourable substrate for vigilance during early sleep, creating a window of opportunity in which sensory information can be processed and if needed acted upon. This theoretically would facilitate the transition from light sleep to wake allowing one to orientate to the environment quickly and act upon any relevant environmental change. This is particularly pertinent given that the appearance of VSWs has been indicated as the herald of the transition from wake to sleep (Tanaka, Hayashi & Hori, 2000). Perhaps, as sleep progresses and deepens, priming of sensory regions in environmental monitoring is reduced, and less activation of primary sensory regions observed with KCs reflects the deepening of sleep and gradual reduction in responsiveness to environmental stimuli.

5.5.2 ROI analysis and the effects of sleep deprivation

In this study significantly greater BOLD responses were observed under SD conditions when compared to ND conditions. When observing the individual group maps (Figure 5.4.1.) this is visually apparent with more widespread activation noted under SD conditions, especially for VSWs. One of the first questions that arises from this is, why is there more activation present under SD conditions? The augmentation of activation observed under sleep deprivation may be a result of the progressive hyperpolarisation of thalamocortical cells that occurs at sleep onset (Amzica & Steriade, 2002; Steriade, McCormick & Sejnowski, 1993) producing a favourable substrate for the generation of low frequency activity, such as VSWs and KCs, as well as widespread synchronisation. Following a period of prolonged wakefulness there is a progressive accumulation of extracellular adenosine, a neuromodulatory transmitter with an inhibitory effect on cholinergic neurons (Boonstra, Stins, Daffertshofer, *et al.*, 2007; Strecker, Morairty, Thakkar, *et al.*, 2000). This inhibitory effect, that also induces a reduction in cortical levels of acetylcholine, has been shown to increase slow wave activity (Benington & Heller, 1995; Boonstra, Stins, Daffertshofer, *et al.*, 2007) Studies using EEG have demonstrated slow wave activity during sleep is altered as a function of increased homeostatic sleep pressure, with increases in slow wave activity accompanying greater sleep pressure which reduces during the course of sleep (Bersagliere & Achermann, 2010; Borbély, Baumann, Brandeis, *et al.*, 1981). The slow oscillation (SO), a cortical event occurring at <1 Hz, is characterised by depolarizing upstates and hyperpolarising downstates (Mölle, Marshall, Gais, *et al.*, 2002; Steriade, McCormick & Sejnowski, 1993; Timofeev, Grenier, Bazhenov, *et al.*, 2000), and has also been shown to be modified as a function of sleep deprivation (Bersagliere & Achermann, 2010; Perrault, Carrier, Desautels, *et al.*, 2013). Increases in the density of the SO (Perrault, Carrier, Desautels, *et al.*, 2013), and its frequency and amplitude (Bersagliere

& Achermann, 2010), have been reported with increased sleep pressure. This correlates with animal studies that demonstrate more synchronous and stable entry of neurons into up- and downstates with sustained wakefulness (Vyazovskiy, Olcese, Lazimy, *et al.*, 2009). Additionally, the slow oscillation is implicated in the grouping and temporal arrangement of sleep paroxysms such as sleep spindles, KCs and possibly VSWs (Amzica & Steriade, 1998; Mölle, Marshall, Gais, *et al.*, 2002). Greater synchronisation across the cortex implicates increased recruitment of neurons which may contribute to the increased BOLD signal observed in the present study following sleep deprivation. Exactly how this increased cortical synchronisation that occurs following increased homeostatic sleep pressure effects the BOLD signal is speculative, and requires further investigation.

In addition to increased cortical synchronisation during sleep following sleep deprivation, animal studies have demonstrated increased cortical excitability with wakefulness and increasing with extended period of wakefulness, both in vitro (Liu, Faraguna, Cirelli, *et al.*, 2010) and in vivo (Vyazovskiy, Olcese, Lazimy, *et al.*, 2009). In the study by Lui et al., 2010, the amplitude and frequency of miniature excitatory post-synaptic currents increased following wakefulness in cortical slices from SD rats. In a study of live rats, Vyazovskiy et al., 2009, found that cortical firing rates increased following sustained wakefulness, and continued to increase, to a point, during sleep deprivation. This increase in cortical excitability has also been demonstrated in humans using combined transcranial magnetic stimulation (TMS) and EEG (Huber, Maki, Rosanova, *et al.*, 2013). In this TMS-EEG study the slope and amplitude of the EEG response to TMS was progressively increased with prolonged wake time, concluding that ongoing wakefulness is concomitant with increased cortical excitability (Huber, Maki, Rosanova, *et al.*, 2013). This

increase in cortical excitability following sustained wakefulness, reported in both animals and humans, could well expound the overall increase in activation observed following sleep deprivation in this present study. In fact, there is recent evidence demonstrating that sleep deprivation causes an increase in the whole brain signal in fMRI data (Yeo, Tandi & Chee, 2015). The increase in BOLD signal and the increased involvement of more cortical areas observed in both VSWs and KCs in the present study (see Figure 5.4.1.) may be explained by both or a combination of these processes, i.e. increased cortical excitability following prolonged wakefulness and increased cortical synchronisation during recovery sleep.

The underlying changes in neuronal substrate brought about by sleep deprivation may be responsible for the overall increase in activation reported in this study, however this does not explain why some regions in KCs show a decrease; namely, middle frontal gyrus (MFG) and middle temporal gyrus (MTG) bilaterally. The changes in these four regions could purport to a specific feature of KCs, alternatively these regions may be differentially affected by increasing sleep pressure/prolonged wakefulness. Given that these regions, together with all other regions, show an increase following SD conditions when investigating VSWs, it is unlikely that these regions are affected differently following sleep deprivation. It is therefore more plausible that the decreases in these four regions, rather than an increase, indicate these regions are specifically affected by KCs. The question then remains as to why these four regions behave differently compared to all the others? The reason behind this may be postulated when considering the function of the brain regions involved and/or their relation to brain networks. The MFG and MTG have both been associated with attentional brain networks, notably the ventral attention network (VAN) (Corbetta, Patel & Shulman, 2008; Corbetta & Shulman, 2002; Japee, Holiday, Satyshur, *et al.*,

2015; Shulman, Astafiev, Franke, *et al.*, 2009), and it has been demonstrated that there is activation in regions associated with the VAN when presented with an unexpected and potentially meaningful stimulus (Corbetta, Patel & Shulman, 2008; Downar, Crawley, Mikulis, *et al.*, 2000). The temporoparietal junction (TPJ) and the ventral frontal cortex (which involves parts of the MFG) form primary nodes of the VAN (Corbetta, Patel & Shulman, 2008). The exact anatomical correlates of the TPJ and its function are still subject of ongoing investigation, though there is evidence that the TPJ can be subdivided based on functional and structural connectivity (Mars, Sallet, Schuffelgen, *et al.*, 2012). In the study by Mars et al., 2011, the TPJ was subdivided into anterior and posterior components and it was demonstrated that the posterior aspect of the TPJ was functionally connected with the MTG and MFG, among other areas. It is therefore possible that the regions identified in the present study that show a decrease in activation may fall within the regions associated with the VAN. In this case these findings may suggest that there is a decreased attentional response to stimuli when homeostatic sleep pressure is increased, in line with the notion that the KC acts in part as a sleep protective mechanism, perhaps exhibiting a dampened response to external stimuli in favour of sleep preservation. These hypotheses remain to be tested with explicit stimulation during sleep, although this is technically challenging in the MRI scanner.

5.5.3 Limitations

There are a number of considerations to be taken into account. Firstly, the number of subjects included in the final analysis is low, and thus the number of paroxysms included in the group analysis. The findings reported here would benefit from validation by a similar study using a larger cohort. Secondly, the MRI scanner does not produce a favourable environment for sleep, both in

terms of comfort and noise. It is unknown how much scanner noise and discomfort would contribute to the results given both VSWs and KCs can be evoked by various stimuli. Apart from the technical limitations of the scanner and low participant numbers, there are also physiological considerations. Sleeping in the scanner is an unfamiliar and foreign environment and likely subject to the first night effect (FNE). The FNE is characterised by poorer sleep in the first night when sleeping in a novel environment and can increase sleep latency, decrease the time asleep and increase arousals (Curcio, Ferrara, Piergianni, *et al.*, 2004; Tamaki, Bang, Watanabe, *et al.*, 2016; Tamaki, Nittono, Hayashi, *et al.*, 2005). The FNE is also associated with a more vigilant brain (Tamaki, Bang, Watanabe, *et al.*, 2016). It is unknown as to what influence the FNE would have on these results and would need to be investigated further, though a scanner environment is unlikely to be an environment one could habituate to. A further potential confound is the times in which the scanning commenced. Scanning at 07:00hrs (SD session) in the morning and 23:00hrs (ND session) at night falls at two very different phases of the circadian rhythm. In the morning at around 07:00hrs would be the trough of the circadian phase, with the lowest sleep pressure and at 23:00hrs would be towards the peak of the circadian phase with highest sleep pressure (Schmidt, Collette, Cajochen, *et al.*, 2007). Circadian phase has been shown to alter the BOLD response in task related fMRI studies (Schmidt, Peigneux, Leclercq, *et al.*, 2012) and also shows alterations in resting state fMRI (Shannon, Dosenbach, Su, *et al.*, 2013). Phase advanced sleep (sleeping earlier than usual) and phase delayed sleep (sleeping later than usual) has been shown to have an impact on sleep architecture, with an advance associated with decreased REM and SWS, and an delay increasing REM and decreasing stage 2 (Gonnissen, Mazuy, Rutters, *et al.*, 2013). How this impacts on this study is unclear, and in any future studies it is advisable to scan at the same time to avoid any potential influence of circadian misalignment.

5.6 CONCLUSION

This study is the first to compare VSWs and KCs between SD and ND conditions. The findings indicate that although sharing similarities, VSWs and KCs are separate entities likely sharing analogous and deviant functions. Whilst both could be attributed to having a sleep protective mechanism, the findings in this study suggest that the VSW provides a favourable substrate to waking should it be necessary.

The involvement of several nodes of different RSNs indicate that VSWs and, to a lesser degree, KCs activate multiple brain networks. The significance of this would need to be explored in the future, with this specific purpose in mind.

The effect of sleep deprivation on both VSWs and KCs reported in this present study may well be explicated by the progressive excitability of the cortex caused by prolonged wakefulness, the increased synchronisation of the cortex during sleep following sleep deprivation, or a combination of both. Studies investigating sleep using fMRI should pay careful attention to the effects of sleep deprivation on the underlying neuronal substrate and how this may alter the BOLD signal. Further comparative studies between SD and ND conditions are needed to fully understand this effect.

Chapter 6

THESIS SUMMARY AND CONCLUDING REMARKS

6.1 THESIS SUMMARY

6.1.1 EEG-fMRI in epilepsy

The initial aim of this thesis was to investigate the use of EEG-fMRI as a clinical tool in epilepsy. With the objective of recruiting homogenous groups of patients with particular epilepsy syndromes, e.g. groups with IGE, TLE, FLE, the plan was to explore the value of EEG-fMRI in these groups. However, recruitment was difficult and it soon emerged that recruitment was biased towards patients of a complex nature in whom further investigations were needed to assist in management. This shifted the aim of the initial investigation to assess how useful EEG-fMRI could be from a clinical point of view in the very group of patients who would most likely be referred; thus recruitment became more akin to referrals into this theoretical EEG-fMRI service. The principal reason for referral was related to pre-surgical evaluation though in some cases the question was more diagnostic. Overall EEG-fMRI was found to be clinically contributory in 12% of cases. This demonstrated a quite a limited value of EEG-fMRI in this clinical setting, with complex epilepsy patients where other more standard diagnostic modalities may have been inadequate, though the investigation was implemented in its simplest form, one that, with the right equipment, could be deployed in a clinical setting with relative ease (discussed in more detail in chapter 2).

Single-photon emission computed tomography (SPECT) and positron emission tomography (PET) are specialist imaging investigations that are generally used in epilepsy tertiary centres, predominately in pre-surgical evaluation (la Fougère, Rominger, Förster, *et al.*, 2009; Mouthaan, Rados, Barsi, *et al.*, 2016). The sensitivity of PET, specifically F-18 fluorodeoxy-glucose (^{18}FDG) PET, in identifying the EZ depends on the epilepsy type, i.e. ^{18}FDG-PET is quite sensitive in TLE,

at around 71-89% (Burneo, Poon, Kellett, *et al.*, 2015), which drops in extratemporal epilepsies, such as FLE to 55% (Kim, Lee, Lee, *et al.*, 2002). The sensitivity of interictal SPECT is on the lower side, around 50% (Spanaki, Spencer, Corsi, *et al.*, 1999), which increases to 70-90% for ictal SPECT, depending on epilepsy type (Ergün, Saygi, Yalnizoglu, *et al.*, 2016). A recent EEG-fMRI study demonstrated a sensitivity of 81% of concordant BOLD responses, to area of resection in a group of TLE patients who underwent surgery, and good surgical outcome (Coan, Chaudhary, Grouiller, *et al.*, 2016). In terms of utility, the results reported in chapter 2 suggest a more limited use of EEG-fMRI, though the results reported in chapter 3 suggest this could potentially have been improved by modelling earlier HRFs. Increasing the complexity of data modelling or introducing multiples of the same model but at different peak times (as described in chapter 3) decrease the likelihood of EEG-fMRI making it into a clinical setting from an academic on unless packaged in software that could be used with ease. Given the high sensitivity reported by Coan et al., 2016, one would wonder why this technique not readily implemented in the tertiary epilepsy centre, and complexity of modelling may be one such hurdle. Coan et al., 2016, used similar methods described by Grouiller et al, 2011, who created topographical EEG maps from interictal data obtained from long term monitoring outside the scanner, and used these maps to investigate associated haemodynamic changes, consequently significantly increasing the yield of interictal activity recorded in the scanner (Grouiller, Thornton, Groening, *et al.*, 2011). The methods applied in chapter 2 where simpler and unable to model interictal activity in its observable absence. Again, as to why EEG-fMRI isn't implemented more readily there may be a number of contributing factors. The more complex a method becomes the harder it becomes to implement in a clinical setting; the required expertise, extra specialised equipment, time taken to perform an investigation and to analyse the data, these all impart a cost. In the face of NHS over spends (Triggle, 2015) and

a reduction in the capital budget to repair and replace equipment (Broomfield, 2016), it is unlikely that an expensive imaging investigation is going to become a mainstay in epilepsy tertiary centres. One of the largest areas in growth in activity for diagnostics is for MRI scans with an average monthly growth of 1.1% and a yearly increase of 0.7%, from 2015 to 2016, of patients waiting over 6 weeks (NHS England, 2016). In addition to this, The Royal College of Radiologists report a shortage in staff numbers and warn about the overuse of MRI scanners in delivering a 24/7 service (The Royal College of Radiologists, 2016). This demand on imaging services does not lend itself to the delivery of a service (EEG-fMRI) that requires possibly 2 hours of scanning time, especially as there has been an emphasis on optimising MRI protocols to reduce scan times (Hollingsworth, 2015; Mekle, Wu, Meckel, *et al.*, 2006). This is not to say fMRI cannot become a principal tool in the diagnostic arsenal of the epileptologist/neurosurgeon. Indeed, fMRI has seen an increasing presence in the work-up for epilepsy surgery but in lateralising language and memory (Duncan, 2009) rather than identifying the EZ, and has a definite role in replacing the invasive Wada test for lateralising language (Dym, Burns, Freeman, *et al.*, 2011; Kashida, Otsubo, Hanaya, *et al.*, 2016). With the continued advancement of resting state fMRI (see Pittau & Vulliemoz, 2015 for review), and as tertiary epilepsy centres will already have an MRI scanner available, it may only be a matter of time before this modality becomes more widely implemented. This will be dependent to a degree on whether analysis can be automated and neatly packaged into software that does not require a heavy research background to operate. Although there was an incidence reported in chapter 2 of EEG-fMRI being diagnostically useful, the principal use of EEG-fMRI is in pre-surgical evaluation. It is unlikely that this method will see clinical use outside this setting. Given many of the larger NHS Trusts who house a tertiary epilepsy centre are affiliated with Universities with an academic based imaging centre, many of the hurdles regarding the use of EEG-fMRI in an epilepsy

setting may be addressed. This would largely depend on the mutual cooperation and interest between the two establishments, which is not always easy to achieve (Cooksey, 2006). If available EEG-fMRI should be used and has the potential to provide the necessary information that could ultimately result in a patient with refractory epilepsy becoming seizure free. It is unlikely that EEG-fMRI will move outside of a cooperative academic-clinical setting into a purely clinical one in the near future, but in tertiary epilepsy centres with access to academic imaging centres and the necessary expertise there-in, every effort should be made to produce a mutually beneficial arrangement in both the advancement of science and best treatment and care for the patient.

6.1.2 EEG-fMRI in sleep

The jump from epilepsy to sleep in this thesis came about for two main reasons, firstly, the difficulty in recruiting patients with epilepsy and secondly the interest behind sleep paroxysms themselves, in which there was a dearth of EEG-fMRI studies. The initial logic was to apply the analysis in chapter 3 to sleep paroxysms; VSWs, KCs and SSs, with the hypothesis that little would be evident in terms of early BOLD signal changes. This is not the first time sleep paroxysms have been a focus in EEG-fMRI studies in epilepsy, Moehring et al., 2011, specifically included VSWs, KCs, and SSs in their model to improve sensitivity to IED associated BOLD signal changes (Moehring, Coropceanu, Galka, *et al.*, 2011). In chapter 4, the first study of early BOLD signal changes in sleep paroxysms, it was originally thought that early BOLD changes observed with IEDs were more likely pathological rather than a feature of paroxysmal activity. We discovered early BOLD signal changes occurring in SSs, which suggest early BOLD changes are not

specifically related to pathological phenomenon but now also observable in both pathological and non-pathological paroxysmal activity. Understanding the early responses associated normal paroxysmal activity may help further our understanding of the prespike activity observed with IEDs and may provide a better understanding between the link between epilepsy and sleep; especially given sleep paroxysms have an intimate relationship with epilepsy, such as the more recently challenged hypothesis that SWCs are a transformation of SSs (Leresche, Lambert, Errington, *et al.*, 2012; Sitnikova, Hramov, Koronovsky, *et al.*, 2009), or the initiation of frontal lobe seizures by KCs (El Helou, Navarro, Depienne, *et al.*, 2008). The lack of early BOLD changes in VSWs and KCs were reassuring that modelling effects are actually minimised 6 seconds prior to onset, and lend some weight to the validity of the results observed in the epilepsy data (chapter 3). The real issue became how to interpret the early changes now observed with SSs. A thalamic origin of SSs has been well established (Steriade, 1995), so why would activation in cortical regions precede thalamically generated sleep spindles? As discussed in chapter 3 this could potentially be the result of the contribution of slow SSs to the group analysis which may yet have a cortical origin (Timofeev & Chauvette, 2013). These results need further investigation and may have an impact on the significant community with a particular interest in SSs.

In the final experimental chapter KCs ad VSWs were examined in more detail, due in part to their relationship as evoked responses during sleep (Bastien, Crowley & Colrain, 2002). Here, sleep-deprivation was used as a mechanism to increase sleep pressure in order to examine the effect this would have on these two sleep paroxysms. Overall there was a significant increase in the BOLD response following sleep deprivation. Discussed in detail in chapter 5, these results do have a wider implication on EEG-fMRI/fMRI studies into sleep, in that BOLD responses do alter as a function

of increased sleep pressure, and when using sleep deprivation simply as a mechanism to encourage sleeping in the scanner there may be an influence on the BOLD response. The patterns of activation noted for both VSWs and KCs demonstrated some overlap, but there was noticeably less involvement of primary sensory regions with KCs as compared to VSWs; this was interpreted as a gradual loss of response to external stimuli as sleep progresses, where sleep is favoured over a preparation to transition from sleep to wake. Additionally, although all regions included in the analysis demonstrated an increase in BOLD signal when sleep deprived as compared to non-deprived for VSWs; in the case of KCs four regions demonstrated a decrease in BOLD signal, i.e. a greater BOLD signal was observed in the non-deprived condition to the sleep deprived condition. These four regions, located in the bilateral middle frontal gyrus and bilateral middle temporal gyrus, fall within attention networks (Corbetta & Shulman, 2002) and may represent a reduced attentional response to stimuli, keeping in-line with the notion that KCs play a role in sleep maintenance (discussed further in chapter 5).

6.2 FUTURE DIRECTIONS

Our findings show EEG-fMRI implemented in its simplest form is limited when deployed in the setting of a complex epilepsy service, though this is very dependent on the nature of the patients referred; as previously mentioned the patients referred for EEG-fMRI were both very complex and lacked IEDs in the scanner. It is worth noting EEG, the primary diagnostic test in epilepsy, only has a sensitivity of 25-56% (Smith, 2005). There are many studies, one of the most recent being Coan et al, 2016, which have demonstrated the potential of EEG-fMRI as being of high value in

localising the EZ. The need for specialised MR-compatible equipment to record EEG in the scanner, scanner time required, and cost pressure within the NHS are likely reasons why EEG-fMRI hasn't become more prevalent in tertiary epilepsy centres, despite the growing body of evidence of its potential. Streamlining analysis into easy to implement software packages or identifying IEDs from the fMRI data alone (Hamandi, Salek Haddadi, Liston, *et al.*, 2005), thus removing the need for extra specialised equipment, are possible means to improve the availability/accessibility of EEG-fMRI/fMRI in the epilepsy clinic in the UK.

The early BOLD signal changes observed in epilepsy (chapter 3), have yet to be validated by surgical outcome in a sufficient cohort of epilepsy surgery patients. The improved concordance described in chapter 3 and elsewhere (Jacobs, Kobayashi, Boor, *et al.*, 2007), may improve localisation of the EZ. Previous studies have correlated EEG-fMRI with surgical outcome (Coan, Chaudhary, Grouiller, *et al.*, 2016; Thornton, Laufs, Rodionov, *et al.*, 2010; van Houdt, de Munck, Leijten, *et al.*, 2013) or used EEG-fMRI to predict surgical outcome (An, Fahoum, Hall, *et al.*, 2013), and have shown a high sensitivity and predictive value, but none have done the same with early haemodynamic changes in epilepsy. Inclusion of prespike investigation in such studies may further improve the sensitivity and specificity of EEG-fMRI but also extend our understanding of these prespike changes, as to whether they are more localising of the EZ or not.

The early BOLD responses observed in SSs certainly warrants further investigation. Similar analysis applied to a greater number of participants with separation of slow- and fast-SSs will elucidate as to whether the contributor to these early BOLD signal changes are slow or fast-SSs, or whether they are both contributors. There are limited studies investigating functional connectivity (FC) in relation to sleep spindles. In a study of FC in the human hippocampus during

sleep, increased FC between the hippocampal formation and neocortical regions was observed in the presence of SSs (Andrade, Spoormaker, Dresler, *et al.*, 2011). A more recent study, specifically investigating functional connectivity in relation to SSs, used the magnetoencephalogram (MEG) to identify specific patterns of connectivity related to both fast and slow spindles, indicating strong intrahemispheric connectivity during fast spindles with slow spindles more associated with a stronger connectivity between long range and interhemispheric connections (Zerouali, Lina, Sekerovic, *et al.*, 2014). A seed based correlation analysis FC approach could be used to further investigate SSs, perhaps seeding from regions identified in the early BOLD signal activation maps which may uncover a specific network relating to SSs. The same could be said for VSWs and KCs, as there have been no studies to investigate the FC of these paroxysms. Given the widespread activation observed with these paroxysms, it may be possible they recruit multiple brain networks. Many previous studies have used auditory stimulus to study these sleep paroxysms (Schabus, Dang-Vu, Heib, *et al.*, 2012; Colrain, 2005; Bastien, Crowley & Colrain, 2002), future studies could also use these methods during EEG-fMRI to investigate VSWs and KCs in more detail; for example differences in evoked and spontaneous paroxysms in the fMRI, and if this influences patterns of activation or FC, and perhaps including more accurate characterisation of EEG features (e.g. amplitude, latency, topography) and how these relate to the BOLD response. Further studies may help understand their function beyond what we already know.

6.3 CONCLUSION

In this thesis of two halves we first demonstrated a limited use of EEG-fMRI as a diagnostic/presurgical investigation when deployed in its simplest form in a group of complex epilepsy patients, rather than more straightforward homogenous group of patients, TLE for example. Patient selection is important for EEG-fMRI studies, as lack of any IEDs can hamper these studies, and development of software packages to automate a lot of the analysis would be needed for the transition to a clinical setting. Though the greatest hurdle is potential cost, given the most cost effective strategies include video-EEG, MRI, PET, and icVT and not other modalities such as SPECT, MEG, DTI, which often need individual funding requests, it is unlikely EEG-fMRI will become available in all tertiary epilepsy centres (Burch, Hinde, Palmer, *et al.*, 2012). Fostering the relationship between academic imaging centres and epilepsy tertiary centres is likely the best way to make this investigation available to patients who could benefit greatly from it. The early BOLD signal changes we report are best studied at 6 seconds prior to IEDs when using a GLM based approach, to avoid overlap in models, and this can improve concordance.

In the second half of this thesis we demonstrate for the first time haemodynamic changes that precede SSs, which may open an avenue for further investigation and understanding of this sleep transient. We also find some evidence to support the sleep protective role of KCs and show an enhanced BOLD response following sleep deprivation which may have an impact on other studies using sleep deprivation as a method to study sleep.

References

Abend, N.S., Gutierrez-Colina, A., Zhao, H., Guo, R., et al. (2011) Interobserver reproducibility of electroencephalogram interpretation in critically ill children. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society*. [Online] 28 (1), 15–19. Available from: doi:10.1097/WNP.0b013e3182051123.

Abidi, A., Devaux, J.J., Molinari, F., Alcaraz, G., et al. (2015) A recurrent KCNQ2 pore mutation causing early onset epileptic encephalopathy has a moderate effect on M current but alters subcellular localization of Kv7 channels. *Neurobiology of Disease*. [Online] 8080–92. Available from: doi:10.1016/j.nbd.2015.04.017.

Abimbola, S., Martiniuk, A.L.C., Hackett, M.L. & Anderson, C.S. (2011) The Influence of Design and Definition on the Proportion of General Epilepsy Cohorts with Remission and Intractability. *Neuroepidemiology*. [Online] 36 (3), 204–212. Available from: doi:10.1159/000327497.

Achermann, P. & Borbély, A.A. (2011) Chapter 37 - Sleep Homeostasis and Models of Sleep Regulation. In: Meir H. Kryger, Thomas Roth, & William C. Dement (eds.). *Principles and Practice of Sleep Medicine*. 5th edition. [Online]. Philadelphia, W.B. Saunders. pp. 431–444. Available from: http://www.sciencedirect.com/science/article/pii/B9781416066453000372 [Accessed: 28 August 2016].

Achermann, P. & Borbely, A.A. (1997) Low-frequency (< 1Hz) oscillations in the human sleep electroencephalogram. *Neuroscience*. 81 (1), 213–222.

Aghakhani, Y. (2004) fMRI activation during spike and wave discharges in idiopathic generalized epilepsy. *Brain*. [Online] 127 (5), 1127–1144. Available from: doi:10.1093/brain/awh136.

Aghakhani, Y., Kobayashi, E., Bagshaw, A., Hawco, C., et al. (2006) Cortical and thalamic fMRI responses in partial epilepsy with focal and bilateral synchronous spikes. *Clinical Neurophysiology*. [Online] 117 (1), 177–191. Available from: doi:10.1016/j.clinph.2005.08.028.

Agrawal, A., Timothy, J., Pandit, L. & Manju, M. (2006) Post-traumatic epilepsy: An overview. *Clinical Neurology and Neurosurgery*. [Online] 108 (5), 433–439. Available from: doi:10.1016/j.clineuro.2005.09.001.

Aguirre, G.K. (2006) Experimental Design and Data Analysis for fMRI. In: Scott H. Faro & Feroze B. Mohamed (eds.). *Functional MRI: Basic Principles and Clinical Applications*. Springer. pp. 58–74.

Akber, S.F. (1996) NMR relaxation data of water proton in normal tissues. *Physiological chemistry and physics and medical NMR*. 28 (4), 205–238.

Alarcon, G. (2006) Is it worth pursuing surgery for epilepsy in patients with normal neuroimaging? *Journal of Neurology, Neurosurgery & Psychiatry*. [Online] 77 (4), 474–480. Available from: doi:10.1136/jnnp.2005.077289.

Alexandre, C., Andermann, M.L. & Scammell, T.E. (2013) Control of arousal by the orexin neurons. *Current opinion in neurobiology*. [Online] 23 (5), 752–759. Available from: doi:10.1016/j.conb.2013.04.008.

Alhola, P. & Polo-Kantola, P. (2007) Sleep deprivation: Impact on cognitive performance. *Neuropsychiatric Disease and Treatment*. 3 (5), 553–567.

Allen, P.J., Josephs, O. & Turner, R. (2000) A method for removing imaging artifact from continuous EEG recorded during functional MRI. *NeuroImage*. [Online] 12 (2), 230–239. Available from: doi:10.1006/nimg.2000.0599.

Almasi, J.J. & Schmitt, O.H. (1970) Systemic and Random Variations of ECG Electrode System Impedance. *Annals of the New York Academy of Sciences*. [Online] 170 (2), 509–519. Available from: doi:10.1111/j.1749-6632.1970.tb17718.x.

Amzica, F. & Steriade, M. (1998) Cellular substrates and laminar profile of sleep K-complex. *Neuroscience*. 82 (3), 671–686.

Amzica, F. & Steriade, M. (2000) Integration of low-frequency sleep oscillations in corticothalamic networks. *Acta neurobiologiae experimentalis*. 60 (2), 229–246.

Amzica, F. & Steriade, M. (2002) The functional significance of K-complexes. *Sleep Medicine Reviews*. [Online] 6 (2), 139–149. Available from: doi:10.1053/smrv.2001.0181.

Amzica, F. & Steriade, M. (1997) The K-complex: its slow (<1-Hz) rhythmicity and relation to delta waves. *Neurology*. 49 (4), 952–959.

An, D., Dubeau, F. & Gotman, J. (2015) BOLD responses related to focal spikes and widespread bilateral synchronous discharges generated in the frontal lobe. *Epilepsia*. [Online] 56 (3), 366–374. Available from: doi:10.1111/epi.12909.

An, D., Fahoum, F., Hall, J., Olivier, A., et al. (2013) Electroencephalography/functional magnetic resonance imaging responses help predict surgical outcome in focal epilepsy. *Epilepsia*. [Online] 54 (12), 2184–2194. Available from: doi:10.1111/epi.12434.

Anderer, P., Klösch, G., Gruber, G., Trenker, E., et al. (2001) Low-resolution brain electromagnetic tomography revealed simultaneously active frontal and parietal sleep spindle sources in the human cortex. *Neuroscience*. 103 (3), 581–592.

Andrade, K.C., Spoormaker, V.I., Dresler, M., Wehrle, R., et al. (2011) Sleep Spindles and Hippocampal Functional Connectivity in Human NREM Sleep. *Journal of Neuroscience*. [Online] 31 (28), 10331–10339. Available from: doi:10.1523/JNEUROSCI.5660-10.2011.

Aronica, E., Becker, A.J. & Spreafico, R. (2012) Malformations of Cortical Development: Malformations. *Brain Pathology*. [Online] 22 (3), 380–401. Available from: doi:10.1111/j.1750-3639.2012.00581.x.

Arroyo, S., Brodie, M.J., Avanzini, G., Baumgartner, C., et al. (2002) Is refractory epilepsy preventable? *Epilepsia*. 43 (4), 437–444.

Arthurs, O.J. & Boniface, S. (2002) How well do we understand the neural origins of the fMRI BOLD signal? *Trends in neurosciences*. 25 (1), 27–31.

Asikainen, I., Kaste, M. & Sarna, S. (1999) Early and late posttraumatic seizures in traumatic brain injury rehabilitation patients: brain injury factors causing late seizures and influence of seizures on long-term outcome. *Epilepsia*. 40 (5), 584–589.

Aston-Jones, G., Chen, S., Zhu, Y. & Oshinsky, M.L. (2001) A neural circuit for circadian regulation of arousal. *Nature Neuroscience*. [Online] 4 (7), 732–738. Available from: doi:10.1038/89522.

Ayoub, A., Aumann, D., Hörschelmann, A., Kouchekmanesch, A., et al. (2013) Differential Effects on Fast and Slow Spindle Activity, and the Sleep Slow Oscillation in Humans with Carbamazepine and Flunarizine to Antagonize Voltage-Dependent Na+ and Ca2+ Channel Activity. *Sleep*. [Online] 36 (6), 905–911. Available from: doi:10.5665/sleep.2722.

Azuma, H., Hori, S., Nakanishi, M., Fujimoto, S., et al. (2003) An intervention to improve the interrater reliability of clinical EEG interpretations. *Psychiatry and Clinical Neurosciences*. [Online] 57 (5), 485–489. Available from: doi:10.1046/j.1440-1819.2003.01152.x.

Badawy, R.A.B., Harvey, A.S. & Macdonell, R.A.L. (2009) Cortical hyperexcitability and epileptogenesis: Understanding the mechanisms of epilepsy – Part 1. *Journal of Clinical Neuroscience*. [Online] 16 (3), 355–365. Available from: doi:10.1016/j.jocn.2008.08.026.

Badry, R. (2013) Latency to the first epileptiform activity in the EEG of epileptic patients. *The International Journal of Neuroscience*. [Online] 123 (9), 646–649. Available from: doi:10.3109/00207454.2013.785543.

Bagshaw, A.P., Aghakhani, Y., Bénar, C.-G., Kobayashi, E., et al. (2004) EEG-fMRI of focal epileptic spikes: Analysis with multiple haemodynamic functions and comparison with gadolinium-enhanced MR angiograms. *Human Brain Mapping*. [Online] 22 (3), 179–192. Available from: doi:10.1002/hbm.20024.

Banerjee, P.N., Filippi, D. & Allen Hauser, W. (2009) The descriptive epidemiology of epilepsy—A review. *Epilepsy Research*. [Online] 85 (1), 31–45. Available from: doi:10.1016/j.eplepsyres.2009.03.003.

Bastien, C. & Campbell, K. (1992) The evoked K-complex: all-or-none phenomenon? *Sleep*. 15 (3), 236–245.

Bastien, C.H., Crowley, K.E. & Colrain, I.M. (2002) Evoked potential components unique to non-REM sleep: relationship to evoked K-complexes and vertex sharp waves. *International journal of psychophysiology*. 46 (3), 257–274.

Bastien, C.H., Ladouceur, C. & Campbell, K.B. (2000) EEG characteristics prior to and following the evoked K-Complex. *Canadian Journal of Experimental Psychology = Revue Canadienne De Psychologie Expérimentale*. 54 (4), 255–265.

Bastuji, H., García-Larrea, L., Franc, C. & Mauguière, F. (1995) Brain processing of stimulus deviance during slow-wave and paradoxical sleep: a study of human auditory evoked responses using the oddball paradigm. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 12 (2), 155–167.

Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., et al. (2001) First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nature Genetics*. [Online] 28 (1), 46–48. Available from: doi:10.1038/88254.

Beecham, J., Snell, T., Perkins, M. & Knapp, M. (2010) Health and social care costs for young adults with epilepsy in the UK: Support for young adults with epilepsy. *Health & Social Care in the Community*. [Online] 18 (5), 465–473. Available from: doi:10.1111/j.1365-2524.2010.00919.x.

Beghi, E., Berg, A., Carpio, A., Forsgren, L., et al. (2005) Comment on epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*. 46 (10), 1698–1699.

Bénar, C.-G., Gross, D.W., Wang, Y., Petre, V., et al. (2002) The BOLD Response to Interictal Epileptiform Discharges. *NeuroImage*. [Online] 17 (3), 1182–1192. Available from: doi:10.1006/nimg.2002.1164.

Benington, J.H. & Frank, M.G. (2003) Cellular and molecular connections between sleep and synaptic plasticity. *Progress in Neurobiology*. 69 (2), 71–101.

Benington, J.H. & Heller, H.C. (1995) Restoration of brain energy metabolism as the function of sleep. *Progress in Neurobiology*. 45 (4), 347–360.

Benuzzi, F., Ballotta, D., Mirandola, L., Ruggieri, A., et al. (2015) An EEG-fMRI Study on the Termination of Generalized Spike-And-Wave Discharges in Absence Epilepsy. *PLoS ONE*. [Online] 10 (7). Available from: doi:10.1371/journal.pone.0130943 [Accessed: 31 July 2016].

Berg, A.T. (2008) Risk of recurrence after a first unprovoked seizure: Risk of Recurrence. *Epilepsia*. [Online] 4913–18. Available from: doi:10.1111/j.1528-1167.2008.01444.x.

Berg, A.T., Berkovic, S.F., Brodie, M.J., Buchhalter, J., et al. (2010) Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia*. [Online] 51 (4), 676–685. Available from: doi:10.1111/j.1528-1167.2010.02522.x.

Berg, A.T., Shinnar, S., Levy, S.R. & Testa, F.M. (1999) Newly diagnosed epilepsy in children: presentation at diagnosis. *Epilepsia*. 40 (4), 445–452.

Berger, R.J. & Phillips, N.H. (1995) Energy conservation and sleep. *Behavioural Brain Research*. [Online] 69 (1–2), 65–73. Available from: doi:10.1016/0166-4328(95)00002-B.

Bernhardt, B.C., Kim, H. & Bernasconi, N. (2013) Patterns of subregional mesiotemporal disease progression in temporal lobe epilepsy. *Neurology*. [Online] 81 (21), 1840–1847. Available from: doi:10.1212/01.wnl.0000436069.20513.92.

Bernstein, J.H., Prather, P.A. & Rey-Casserly, C. (1995) Neuropsychological assessment in preoperative and postoperative evaluation. *Neurosurgery Clinics of North America*. 6 (3), 443–454.

Bersagliere, A. & Achermann, P. (2010) Slow oscillations in human non-rapid eye movement sleep electroencephalogram: effects of increased sleep pressure. *Journal of Sleep Research*. [Online] 19 (1p2), 228–237. Available from: doi:10.1111/j.1365-2869.2009.00775.x.

Bianchi, M.T. & Thomas, R.J. (2013) Technical advances in the characterization of the complexity of sleep and sleep disorders. *Progress in neuro-psychopharmacology & biological psychiatry*. [Online] 45277–286. Available from: doi:10.1016/j.pnpbp.2012.09.017.

Biswal, B., Yetkin, F.Z., Haughton, V.M. & Hyde, J.S. (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine*. 34 (4), 537–541.

Biswal, B.B., Van Kylen, J. & Hyde, J.S. (1997) Simultaneous assessment of flow and BOLD signals in resting-state functional connectivity maps. *NMR in biomedicine*. 10 (4–5), 165–170.

Blanchard, S., Saillet, S., Ivanov, A., Benquet, P., et al. (2016) A New Computational Model for Neuro-Glio-Vascular Coupling: Astrocyte Activation Can Explain Cerebral Blood Flow Nonlinear Response to Interictal Events. *PLoS ONE*. [Online] 11 (2). Available from: doi:10.1371/journal.pone.0147292.

Blümcke, I., Beck, H., Lie, A.A. & Wiestler, O.D. (1999) Molecular neuropathology of human mesial temporal lobe epilepsy. *Epilepsy Research*. 36 (2–3), 205–223.

Blümcke, I., Thom, M., Aronica, E., Armstrong, D.D., et al. (2013) International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: A Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia*. [Online] 54 (7), 1315–1329. Available from: doi:10.1111/epi.12220.

Blümcke, I., Thom, M., Aronica, E., Armstrong, D.D., et al. (2011) The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia*. [Online] 52 (1), 158–174. Available from: doi:10.1111/j.1528-1167.2010.02777.x.

Blume, C., del Giudice, R., Wislowska, M., Lechinger, J., et al. (2015) Across the consciousness continuum—from unresponsive wakefulness to sleep. *Frontiers in Human Neuroscience*. [Online] 9105. Available from: doi:10.3389/fnhum.2015.00105.

Blumenfeld, H. (2005) Cellular and Network Mechanisms of Spike-Wave Seizures. *Epilepsia*. 46 (s9), 21–33.

Bonelli, S.B., Powell, R.H.W., Yogarajah, M., Samson, R.S., et al. (2010) Imaging memory in temporal lobe epilepsy: predicting the effects of temporal lobe resection. *Brain: A Journal of Neurology*. [Online] 133 (Pt 4), 1186–1199. Available from: doi:10.1093/brain/awq006.

Bonjean, M., Baker, T., Lemieux, M., Timofeev, I., et al. (2011) Corticothalamic Feedback Controls Sleep Spindle Duration In Vivo. *Journal of Neuroscience*. [Online] 31 (25), 9124–9134. Available from: doi:10.1523/JNEUROSCI.0077-11.2011.

Bonmassar, G., Hadjikhani, N., Ives, J.R., Hinton, D., et al. (2001) Influence of EEG electrodes on the BOLD fMRI signal. *Human brain mapping*. 14 (2), 108–115.

Boonstra, T.W., Stins, J.F., Daffertshofer, A. & Beek, P.J. (2007) Effects of sleep deprivation on neural functioning: an integrative review. *Cellular and molecular life sciences: CMLS*. [Online] 64 (7–8), 934–946. Available from: doi:10.1007/s00018-007-6457-8.

Borbély, A.A. & Achermann, P. (1992) Concepts and models of sleep regulation: an overview. *Journal of sleep research*. 1 (2), 63–79.

Borbély, A.A., Baumann, F., Brandeis, D., Strauch, I., et al. (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalography and Clinical Neurophysiology*. 51 (5), 483–495.

Born, A.P., Law, I., Lund, T.E., Rostrup, E., et al. (2002) Cortical Deactivation Induced by Visual Stimulation in Human Slow-Wave Sleep. *NeuroImage*. [Online] 17 (3), 1325–1335. Available from: doi:10.1006/nimg.2002.1249.

Braun, A.R., Balkin, T.J., Wesenten, N.J., Carson, R.E., et al. (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2 (15) O PET study. *Brain*. 120 (7), 1173–1197.

Brazier, M.A.B. (1963) THE HISTORY OF THE ELECTRICAL ACTIVITY OF THE BRAIN AS A METHOD FOR LOCALIZING SENSORY FUNCTION. *Medical History*. 7 (3), 199–211.

Brett, M., Anton, J.-L., Valabregue, R. & Poline, J.-B. (2002) *Region of interest analysis using an SPM toolbox [abstract] Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2-6, 2002, Sendai, Japan. Available on CD-ROM in NeuroImage, Vol 16, No 2.*

Bronzino, J.D. (1999) *The Biomedical Engineering Handbook*. 2nd edition. Springer Science & Business Media.

Broomfield, M. (2016) George Osborne cut £1.1bn from the NHS budget without anyone noticing. *The Independent*. [Online] 20 March. Available from: http://www.independent.co.uk/news/uk/politics/budget-2016-george-osborne-cuts-11bn-from-nhs-repairs-fund-a6942301.html [Accessed: 19 September 2016].

Brown, P. (2000) Cortical drives to human muscle: the Piper and related rhythms. *Progress in Neurobiology*. 60 (1), 97–108.

Budday, S., Steinmann, P. & Kuhl, E. (2015) Physical biology of human brain development. *Frontiers in Cellular Neuroscience*. [Online] 9. Available from: doi:10.3389/fncel.2015.00257 [Accessed: 31 July 2015].

Burch, J., Hinde, S., Palmer, S., Beyer, F., et al. (2012) The clinical effectiveness and cost-effectiveness of technologies used to visualise the seizure focus in people with refractory epilepsy being considered for surgery: a systematic review and decision-analytical model. *Health Technology Assessment (Winchester, England)*. [Online] 16 (34), 1–157, iii–iv. Available from: doi:10.3310/hta16340.

Burneo, J.G., Poon, R., Kellett, S. & Snead, O.C. (2015) The Utility of Positron Emission Tomography in Epilepsy. *The Canadian Journal of Neurological Sciences. Le Journal Canadien Des Sciences Neurologiques*. [Online] 42 (6), 360–371. Available from: doi:10.1017/cjn.2015.279.

Buxton, R.B. (2012) Dynamic Models of BOLD Contrast. *NeuroImage*. [Online] 62 (2), 953–961. Available from: doi:10.1016/j.neuroimage.2012.01.012.

Buxton, R.B., Uludağ, K., Dubowitz, D.J. & Liu, T.T. (2004) Modeling the hemodynamic response to brain activation. *NeuroImage*. [Online] 23, Supplement 1S220–S233. Available from: doi:10.1016/j.neuroimage.2004.07.013.

Buzsáki, G. (2002) Theta oscillations in the hippocampus. *Neuron*. 33 (3), 325–340.

Buzsáki, G., Anastassiou, C.A. & Koch, C. (2012) The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. *Nature Reviews Neuroscience*. [Online] 13 (6), 407–420. Available from: doi:10.1038/nrn3241.

Callenbach, P.M., Geerts, A.T., Arts, W.F., van Donselaar, C.A., et al. (1998) Familial occurrence of epilepsy in children with newly diagnosed multiple seizures: Dutch Study of Epilepsy in Childhood. *Epilepsia*. 39 (3), 331–336.

Campbell, K. (2010) Event-related potentials as a measure of sleep disturbance: a tutorial review. *Noise & Health*. [Online] 12 (47), 137–153. Available from: doi:10.4103/1463-1741.63216.

Caporro, M., Haneef, Z., Yeh, H.J., Lenartowicz, A., et al. (2012) Functional MRI of sleep spindles and K-complexes. *Clinical Neurophysiology*. [Online] 123 (2), 303–309. Available from: doi:10.1016/j.clinph.2011.06.018.

Cardinale, F., Cossu, M., Castana, L., Casaceli, G., et al. (2013) Stereoelectroencephalography: surgical methodology, safety, and stereotactic application accuracy in 500 procedures. *Neurosurgery*. [Online] 72 (3), 353–366; discussion 366. Available from: doi:10.1227/NEU.0b013e31827d1161.

Carskadon, M.A. & Dement, W.C. (2011) Normal Human Sleep: An Overview. In: Meir H. Kryger, Thomas Roth, & William C. Dement (eds.). *Principles and Practice of Sleep Medicine*. 5th edition. [Online]. Philadelphia, W.B. Saunders. pp. 16–26. Available from: http://www.sciencedirect.com/science/article/pii/B9781416066453000025.

Cascino, G.D. (2004) Surgical treatment for epilepsy. *Epilepsy Research*. [Online] 60 (2–3), 179–186. Available from: doi:10.1016/j.eplepsyres.2004.07.003.

Cavanna, A.E., Cavanna, S. & Cavanna, A. (2010) Epileptic seizures and spirit possession in Haitian culture: Report of four cases and review of the literature. *Epilepsy & Behavior*. [Online] 19 (1), 89–91. Available from: doi:10.1016/j.yebeh.2010.07.002.

Chamberlin, N.L., Arrigoni, E., Chou, T.C., Scammell, T.E., et al. (2003) Effects of adenosine on gabaergic synaptic inputs to identified ventrolateral preoptic neurons. *Neuroscience*. 119 (4), 913–918.

Chaudhary, U.J., Duncan, J.S. & Lemieux, L. (2013) Mapping hemodynamic correlates of seizures using fMRI: A review. *Human Brain Mapping*. [Online] 34 (2), 447–466. Available from: doi:10.1002/hbm.21448.

Chaudhary, U.J., Rodionov, R., Carmichael, D.W., Thornton, R.C., et al. (2012) Improving the sensitivity of EEG-fMRI studies of epileptic activity by modelling eye blinks, swallowing and other video-EEG detected physiological confounds. *NeuroImage*. [Online] 61 (4), 1383–1393. Available from: doi:10.1016/j.neuroimage.2012.03.028.

Chauvette, S., Seigneur, J. & Timofeev, I. (2012) Sleep oscillations in the thalamocortical system induce long-term neuronal plasticity. *Neuron*. [Online] 75 (6), 1105–1113. Available from: doi:10.1016/j.neuron.2012.08.034.

Chavhan, G.B., Babyn, P.S., Thomas, B., Shroff, M.M., et al. (2009) Principles, Techniques, and Applications of T2*-based MR Imaging and Its Special Applications. *Radiographics*. [Online] 29 (5), 1433–1449. Available from: doi:10.1148/rg.295095034.

Chee, M.W. & Chuah, L.Y. (2008) Functional neuroimaging insights into how sleep and sleep deprivation affect memory and cognition. *Current opinion in neurology*. 21 (4), 417–423.

Chee, M.W.L. & Tan, J.C. (2010) Lapsing when sleep deprived: neural activation characteristics of resistant and vulnerable individuals. *NeuroImage*. 51 (2), 835–843.

Chee, M.W.L., Tan, J.C., Zheng, H., Parimal, S., et al. (2008) Lapsing during sleep deprivation is associated with distributed changes in brain activation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 28 (21), 5519–5528.

Chen, X.-M., Huang, D.-H., Chen, Z.-R., Ye, W., et al. (2015) Temporal lobe epilepsy: decreased thalamic resting-state functional connectivity and their relationships with alertness performance. *Epilepsy & Behavior: E&B*. [Online] 4447–54. Available from: doi:10.1016/j.yebeh.2014.12.013.

Chen, Y., Parker, W.D. & Wang, K. (2014) The Role of T-Type Calcium Channel Genes in Absence Seizures. *Frontiers in Neurology*. [Online] 5. Available from: doi:10.3389/fneur.2014.00045 [Accessed: 19 July 2015].

Chou, T.C., Scammell, T.E., Gooley, J.J., Gaus, S.E., et al. (2003) Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 23 (33), 10691–10702.

Chu-Shore, C.J., Major, P., Camposano, S., Muzykewicz, D., et al. (2010) The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia*. [Online] 51 (7), 1236–1241. Available from: doi:10.1111/j.1528-1167.2009.02474.x.

Cibula, J.E. & Gilmore, R.L. (1997) Secondary epileptogenesis in humans. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 14 (2), 111–127.

Coan, A.C., Chaudhary, U.J., Grouiller, F., Campos, B.M., et al. (2016) EEG-fMRI in the presurgical evaluation of temporal lobe epilepsy. *Journal of Neurology, Neurosurgery, and Psychiatry*. [Online] 87 (6), 642–649. Available from: doi:10.1136/jnnp-2015-310401.

Colgin, L.L. (2016) Rhythms of the hippocampal network. *Nature Reviews. Neuroscience*. [Online] 17 (4), 239–249. Available from: doi:10.1038/nrn.2016.21.

Collop, N.A. (2002) Scoring variability between polysomnography technologists in different sleep laboratories. *Sleep Medicine*. [Online] 3 (1), 43–47. Available from: doi:10.1016/S1389-9457(01)00115-0.

Colrain, I.M. (2005) The K-complex: a 7-decade history. *Sleep*. 28 (2), 255–273.

Colrain, I.M. & Campbell, K.B. (2007) The use of evoked potentials in sleep research. *Sleep Medicine Reviews*. [Online] 11 (4), 277–293. Available from: doi:10.1016/j.smrv.2007.05.001.

Colrain, I.M., Webster, K.E. & Hirst, G. (1999) The N550 component of the evoked K-complex: A modality non-specific response? *Journal of sleep research*. 8 (4), 273–280.

Colrain, I.M., Webster, K.E., Hirst, G. & Campbell, K.B. (2000) The roles of vertex sharp waves and K-complexes in the generation of N300 in auditory and respiratory-related evoked potentials during early stage 2 NREM sleep. *Sleep*. 23 (1), 97–106.

Commission on Classification and Terminology (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia*. 30 (4), 389–399.

Commission on Epidemiology and Prognosis, International League Against Epilepsy (1993) Guidelines for epidemiologic studies on epilepsy. *Epilepsia*. 34 (4), 592–596.

Contreras, D. & Steriade, M. (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *The Journal of Neuroscience*. 15 (1), 604–622.

Contreras, D. & Steriade, M. (1996) Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. *The Journal of Physiology*. 490 (Pt 1)159–179.

Contreras, D. & Steriade, M. (1997) Synchronization of low-frequency rhythms in corticothalamic networks. *Neuroscience*. 76 (1), 11–24.

Contreras, D., Timofeev, I. & Steriade, M. (1996) Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *The Journal of Physiology*. 494 (Pt 1), 251–264.

Cooksey, D. (2006) *A review of UK health research funding*. [Online]. The Stationery Office. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/228984/0118404881.pdf.

Coras, R., de Boer, O.J., Armstrong, D., Becker, A., et al. (2012) Good interobserver and intraobserver agreement in the evaluation of the new ILAE classification of focal cortical dysplasias. *Epilepsia*. [Online] 53 (8), 1341–1348. Available from: doi:10.1111/j.1528-1167.2012.03508.x.

Corbetta, M., Patel, G. & Shulman, G.L. (2008) The Reorienting System of the Human Brain: From Environment to Theory of Mind. *Neuron*. [Online] 58 (3), 306–324. Available from: doi:10.1016/j.neuron.2008.04.017.

Corbetta, M. & Shulman, G.L. (2002) CONTROL OF GOAL-DIRECTED AND STIMULUS-DRIVEN ATTENTION IN THE BRAIN. *Nature Reviews Neuroscience*. [Online] 3 (3), 215–229. Available from: doi:10.1038/nrn755.

Cossette, P., Liu, L., Brisebois, K., Dong, H., et al. (2002) Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nature Genetics*. [Online] 31 (2), 184–189. Available from: doi:10.1038/ng885.

Cote, K.A. & Campbell, K.B. (1999) P300 to high intensity stimuli during REM sleep. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. 110 (8), 1345–1350.

Cote, K.A., De Lugt, D.R., Langley, S.D. & Campbell, K.B. (1999) Scalp topography of the auditory evoked K-complex in stage 2 and slow wave sleep. *Journal of sleep research*. 8 (4), 263–272.

Crespel, A., Coubes, P., Rousset, M.-C., Brana, C., et al. (2002) Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Research*. 952 (2), 159–169.

Curatolo, P., Moavero, R. & de Vries, P.J. (2015) Neurological and neuropsychiatric aspects of tuberous sclerosis complex. *The Lancet. Neurology*. [Online] 14 (7), 733–745. Available from: doi:10.1016/S1474-4422(15)00069-1.

Curcio, G., Ferrara, M., Pellicciari, M.C., Cristiani, R., et al. (2003) Effect of total sleep deprivation on the landmarks of stage 2 sleep. *Clinical Neurophysiology*. [Online] 114 (12), 2279–2285. Available from: doi:10.1016/S1388-2457(03)00276-1.

Curcio, G., Ferrara, M., Piergianni, A., Fratello, F., et al. (2004) Paradoxes of the first-night effect: a quantitative analysis of antero-posterior EEG topography. *Clinical Neurophysiology*. [Online] 115 (5), 1178–1188. Available from: doi:10.1016/j.clinph.2003.12.018.

de Curtis, M. & Avanzini, G. (2001) Interictal spikes in focal epileptogenesis. *Progress in neurobiology*. 63 (5), 541–567.

Czisch, M., Wehrle, R., Kaufmann, C., Wetter, T.C., et al. (2004) Functional MRI during sleep: BOLD signal decreases and their electrophysiological correlates. *European Journal of Neuroscience*. [Online] 20 (2), 566–574. Available from: doi:10.1111/j.1460-9568.2004.03518.x.

Czisch, M., Wehrle, R., Stiegler, A., Peters, H., et al. (2009) Acoustic Oddball during NREM Sleep: A Combined EEG/fMRI Study. *PLoS ONE*. [Online] 4 (8). Available from: doi:10.1371/journal.pone.0006749 [Accessed: 4 September 2016].

Czisch, M., Wetter, T.C., Kaufmann, C., Pollmächer, T., et al. (2002) Altered Processing of Acoustic Stimuli during Sleep: Reduced Auditory Activation and Visual Deactivation Detected by a Combined fMRI/EEG Study. *NeuroImage*. [Online] 16 (1), 251–258. Available from: doi:10.1006/nimg.2002.1071.

Dale, B.M., Brown, M.A. & Semelka, R.C. (2015) *MRI: Basic Principles and Applications*. 5th edition. John Wiley & Sons.

Damoiseaux, J.S., Rombouts, S.A.R.B., Barkhof, F., Scheltens, P., et al. (2006) Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences*. [Online] 103 (37), 13848–13853. Available from: doi:10.1073/pnas.0601417103.

Dang-Vu, T.T., Bonjean, M., Schabus, M., Boly, M., et al. (2011) Interplay between spontaneous and induced brain activity during human non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences*. [Online] 108 (37), 15438–15443. Available from: doi:10.1073/pnas.1112503108.

Dang-Vu, T.T., Desseilles, M., Laureys, S., Degueldre, C., et al. (2005) Cerebral correlates of delta waves during non-REM sleep revisited. *NeuroImage*. [Online] 28 (1), 14–21. Available from: doi:10.1016/j.neuroimage.2005.05.028.

Dang-Vu, T.T., McKinney, S.M., Buxton, O.M., Solet, J.M., et al. (2010) Spontaneous brain rhythms predict sleep stability in the face of noise. *Current biology: CB*. [Online] 20 (15), R626-627. Available from: doi:10.1016/j.cub.2010.06.032.

Dang-Vu, T.T., Schabus, M., Desseilles, M., Albouy, G., et al. (2008) Spontaneous neural activity during human slow wave sleep. *Proceedings of the National Academy of Sciences*. 105 (39), 15160–15165.

Danker-Hopfe, H., Anderer, P., Zeitlhofer, J., Boeck, M., et al. (2009) Interrater reliability for sleep scoring according to the Rechtschaffen & Kales and the new AASM standard. *Journal of Sleep Research*. [Online] 18 (1), 74–84. Available from: doi:10.1111/j.1365-2869.2008.00700.x.

Das, A., Wallace, G.C., Holmes, C., McDowell, M.L., et al. (2012) Hippocampal tissue of patients with refractory temporal lobe epilepsy is associated with astrocyte activation, inflammation, and altered expression of channels and receptors. *Neuroscience*. [Online] 220237–246. Available from: doi:10.1016/j.neuroscience.2012.06.002.

De Gennaro, L. & Ferrara, M. (2003) Sleep spindles: an overview. *Sleep Medicine Reviews*. 7 (5), 423–440.

De Gennaro, L., Ferrara, M. & Bertini, M. (2000) The spontaneous K-complex during stage 2 sleep: is it the 'forerunner' of delta waves? *Neuroscience Letters*. 291 (1), 41–43.

De Havas, J.A., Parimal, S., Soon, C.S. & Chee, M.W.L. (2012) Sleep deprivation reduces default mode network connectivity and anti-correlation during rest and task performance. *NeuroImage*. [Online] 59 (2), 1745–1751. Available from: doi:10.1016/j.neuroimage.2011.08.026.

De la Herrán-Arita, A.K., Guerra-Crespo, M. & Drucker-Colín, R. (2011) Narcolepsy and Orexins: An Example of Progress in Sleep Research. *Frontiers in Neurology*. [Online] 2. Available from: doi:10.3389/fneur.2011.00026 [Accessed: 7 September 2016].

Debener, S., Mullinger, K.J., Niazy, R.K. & Bowtell, R.W. (2008) Properties of the ballistocardiogram artefact as revealed by EEG recordings at 1.5, 3 and 7 T static magnetic field strength. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*. [Online] 67 (3), 189–199. Available from: doi:10.1016/j.ijpsycho.2007.05.015.

Delorme, A. & Makeig, S. (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*. [Online] 134 (1), 9–21. Available from: doi:10.1016/j.jneumeth.2003.10.009.

Destexhe, A., Contreras, D. & Steriade, M. (1998) Mechanisms Underlying the Synchronizing Action of Corticothalamic Feedback Through Inhibition of Thalamic Relay Cells. *Journal of Neurophysiology*. 79 (2), 999–1016.

DeToledo, J.C. & Lowe, M.R. (2003) Epilepsy, demonic possessions, and fasting: another look at translations of Mark 9:16. *Epilepsy & Behavior*. [Online] 4 (3), 338–339. Available from: doi:10.1016/S1525-5050(03)00038-6.

Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N.C., et al. (2013) Glia and epilepsy: excitability and inflammation. *Trends in Neurosciences*. [Online] 36 (3), 174–184. Available from: doi:10.1016/j.tins.2012.11.008.

Di Bonaventura, C., Vaudano, A.E., Carnì, M., Pantano, P., et al. (2006) EEG/fMRI study of ictal and interictal epileptic activity: methodological issues and future perspectives in clinical practice. *Epilepsia*. [Online] 47 Suppl 552–58. Available from: doi:10.1111/j.1528-1167.2006.00878.x.

Diekelmann, S. & Born, J. (2010) The memory function of sleep. *Nature Reviews. Neuroscience*. [Online] 11 (2), 114–126. Available from: doi:10.1038/nrn2762.

Dinner, D.S. (2002) Effect of sleep on epilepsy. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 19 (6), 504–513.

Domich, L., Oakson, G. & Steriade, M. (1986) Thalamic burst patterns in the naturally sleeping cat: a comparison between cortically projecting and reticularis neurones. *The Journal of Physiology*. 379429–449.

van Dongen, H.P.A., Maislin, G., Mullington, J.M. & Dinges, D.F. (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*. 26 (2), 117–126.

Dorfer, C., Widjaja, E., Ochi, A., Carter Snead Iii, O., et al. (2015) Epilepsy surgery: recent advances in brain mapping, neuroimaging and surgical procedures. *Journal of Neurosurgical Sciences*. 59 (2), 141–155.

Doucet, G.E., Rider, R., Taylor, N., Skidmore, C., et al. (2015) Presurgery resting-state local graph-theory measures predict neurocognitive outcomes after brain surgery in temporal lobe epilepsy. *Epilepsia*. [Online] 56 (4), 517–526. Available from: doi:10.1111/epi.12936.

Downar, J., Crawley, A.P., Mikulis, D.J. & Davis, K.D. (2000) A multimodal cortical network for the detection of changes in the sensory environment. *Nature neuroscience*. 3 (3), 277–283.

Duncan, J. (2009) The current status of neuroimaging for epilepsy. *Current Opinion in Neurology*. [Online] 22 (2), 179–184. Available from: doi:10.1097/WCO.0b013e328328f260.

Duyn, J.H. (2012) EEG-fMRI Methods for the Study of Brain Networks during Sleep. *Frontiers in Neurology*. [Online] 3. Available from: doi:10.3389/fneur.2012.00100 [Accessed: 4 September 2016].

Dworak, M., McCarley, R.W., Kim, T., Kalinchuk, A.V., et al. (2010) Sleep and Brain Energy Levels: ATP Changes during Sleep. *The Journal of Neuroscience*. [Online] 30 (26), 9007–9016. Available from: doi:10.1523/JNEUROSCI.1423-10.2010.

Dym, R.J., Burns, J., Freeman, K. & Lipton, M.L. (2011) Is functional MR imaging assessment of hemispheric language dominance as good as the Wada test?: a meta-analysis. *Radiology*. [Online] 261 (2), 446–455. Available from: doi:10.1148/radiol.11101344.

Eadie, M. (1995) Epilepsy—from the Sakikku to hughlings Jackson. *Journal of Clinical Neuroscience*. [Online] 2 (2), 156–162. Available from: doi:10.1016/0967-5868(95)90010-1.

Ebersole, J.S., Husain, A.M. & Nordli (Jr.), D.R. (2014) *Current Practice of Clinical Electroencephalography*. 4th edition.

Ehrhart, J., Ehrhart, M., Muzet, A., Schieber, J.P., et al. (1981) K-complexes and sleep spindles before transient activation during sleep. *Sleep*. 4 (4), 400–407.

El Helou, J., Navarro, V., Depienne, C., Fedirko, E., et al. (2008) K-complex-induced seizures in autosomal dominant nocturnal frontal lobe epilepsy. *Clinical Neurophysiology*. [Online] 119 (10), 2201–2204. Available from: doi:10.1016/j.clinph.2008.07.212.

Elton, M., Winter, O., Heslenfeld, D., Loewy, D., et al. (1997) Event-related potentials to tones in the absence and presence of sleep spindles. *Journal of Sleep Research*. 6 (2), 78–83.

Elwes, R.D., Dunn, G., Binnie, C.D. & Polkey, C.E. (1991) Outcome following resective surgery for temporal lobe epilepsy: a prospective follow up study of 102 consecutive cases. *Journal of Neurology, Neurosurgery, and Psychiatry*. 54 (11), 949–952.

Engel, A.K. & Fries, P. (2010) Beta-band oscillations--signalling the status quo? *Current Opinion in Neurobiology*. [Online] 20 (2), 156–165. Available from: doi:10.1016/j.conb.2010.02.015.

Engel, J. (2001) Mesial temporal lobe epilepsy: what have we learned? *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*. 7 (4), 340–352.

Engel Jr, J. (2005) *Epilepsy: Global Issues for the Practicing Neurologist*. Demos Medical Publishing.

Ergün, E.L., Saygi, S., Yalnizoglu, D., Oguz, K.K., et al. (2016) SPECT-PET in Epilepsy and Clinical Approach in Evaluation. *Seminars in Nuclear Medicine*. [Online] 46 (4), 294–307. Available from: doi:10.1053/j.semnuclmed.2016.01.003.

Fahoum, F., Lopes, R., Pittau, F., Dubeau, F., et al. (2012) Widespread epileptic networks in focal epilepsies: EEG-fMRI study. *Epilepsia*. [Online] 53 (9), 1618–1627. Available from: doi:10.1111/j.1528-1167.2012.03533.x.

Fauser, S. & Zentner, J. (2012) Critical review of palliative surgical techniques for intractable epilepsy. *Advances and Technical Standards in Neurosurgery*. [Online] 39165–194. Available from: doi:10.1007/978-3-7091-1360-8_7.

Federico, P. (2005) Functional MRI of the pre-ictal state. *Brain*. [Online] 128 (8), 1811–1817. Available from: doi:10.1093/brain/awh533.

Feng, R., Hu, J., Pan, L., Wu, J., et al. (2016) Application of 256-channel dense array electroencephalographic source imaging in presurgical workup of temporal lobe epilepsy. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. [Online] 127 (1), 108–116. Available from: doi:10.1016/j.clinph.2015.03.009.

Ferreira, J.L., Wu, Y., Besseling, R.M.H., Lamerichs, R., et al. (2016) Gradient Artefact Correction and Evaluation of the EEG Recorded Simultaneously with fMRI Data Using Optimised Moving-Average. *Journal of Medical Engineering*. [Online] 2016. Available from: doi:10.1155/2016/9614323 [Accessed: 3 September 2016].

Ferrie, C.D. (2010) Terminology and organization of seizures and epilepsies: radical changes not justified by new evidence. *Epilepsia*. 51 (4), 713–714.

Fisher, R.S. (2010) What is a classification essay? *Epilepsia*. 51 (4), 714–715.

Fisher, R.S., Acevedo, C., Arzimanoglou, A., Bogacz, A., et al. (2014) ILAE Official Report: A practical clinical definition of epilepsy. *Epilepsia*. [Online] 55 (4), 475–482. Available from: doi:10.1111/epi.12550.

Fisher, R.S., van Emde Boas, W., Blume, W., Elger, C., et al. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*. [Online] 46 (4), 470–472. Available from: doi:10.1111/j.0013-9580.2005.66104.x.

Fisher, R.S., Scharfman, H.E. & deCurtis, M. (2014) How Can We Identify Ictal and Interictal Abnormal Activity? In: Helen E. Scharfman & Paul S. Buckmaster (eds.). *Issues in Clinical Epileptology: A View from the Bench*. [Online]. Dordrecht, Springer Netherlands. pp. 3–23. Available from: http://link.springer.com/10.1007/978-94-017-8914-1_1 [Accessed: 6 July 2016].

Foldvary-Schaefer, N. & Alsheikhtaha, Z. (2013) Complex nocturnal behaviors: nocturnal seizures and parasomnias. *Continuum (Minneapolis, Minn.)*. [Online] 19 (1 Sleep Disorders), 104–131. Available from: doi:10.1212/01.CON.0000427210.98305.8f.

Forget, D., Morin, C.M. & Bastien, C.H. (2011) The Role of the Spontaneous and Evoked K-Complex in Good-Sleeper Controls and in Individuals with Insomnia. *SLEEP*. [Online] Available from: doi:10.5665/sleep.1250.

Formaggio, E., Storti, S.F., Bertoldo, A., Manganotti, P., et al. (2011) Integrating EEG and fMRI in epilepsy. *NeuroImage*. [Online] 54 (4), 2719–2731. Available from: doi:10.1016/j.neuroimage.2010.11.038.

Forsgren, L., Beghi, E., Oun, A. & Sillanpää, M. (2005) The epidemiology of epilepsy in Europe–a systematic review. *European Journal of Neurology*. 12 (4), 245–253.

la Fougère, C., Rominger, A., Förster, S., Geisler, J., et al. (2009) PET and SPECT in epilepsy: A critical review. *Epilepsy & Behavior*. [Online] 15 (1), 50–55. Available from: doi:10.1016/j.yebeh.2009.02.025.

Fox, M.D., Corbetta, M., Snyder, A.Z., Vincent, J.L., et al. (2006) Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proceedings of the National Academy of Sciences of the United States of America*. [Online] 103 (26), 10046–10051. Available from: doi:10.1073/pnas.0604187103.

Frank, M.G. (2013) Why I Am Not SHY: A Reply to Tononi and Cirelli. *Neural Plasticity*. [Online] 2013. Available from: doi:10.1155/2013/394946 [Accessed: 26 August 2016].

Frey, L.C. (2003) Epidemiology of posttraumatic epilepsy: a critical review. *Epilepsia*. 44 (s10), 11–17.

Freyer, F., Becker, R., Anami, K., Curio, G., et al. (2009) Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction. *NeuroImage*. [Online] 48 (1), 94–108. Available from: doi:10.1016/j.neuroimage.2009.06.022.

Friston, K.J., Ashburner, J.T., Kiebel, S.J., Nichols, T.E., et al. (2007) *Statistical Parametric Mapping: The Analysis of Functional Brain Images*. [Online]. Academic Press. Available from: http://www.sciencedirect.com/science/book/9780123725608.

Friston, K.J., Fletcher, P., Josephs, O., Holmes, A., et al. (1998) Event-related fMRI: characterizing differential responses. *NeuroImage*. [Online] 7 (1), 30–40. Available from: doi:10.1006/nimg.1997.0306.

Fuentealba, P. & Steriade, M. (2005) The reticular nucleus revisited: Intrinsic and network properties of a thalamic pacemaker. *Progress in Neurobiology*. [Online] 75 (2), 125–141. Available from: doi:10.1016/j.pneurobio.2005.01.002.

Futatsugi, Y. & Riviello, J.J. (1998) Mechanisms of generalized absence epilepsy. *Brain & Development*. 20 (2), 75–79.

Gaus, S.E., Strecker, R.E., Tate, B.A., Parker, R.A., et al. (2002) Ventrolateral preoptic nucleus contains sleep-active, galaninergic neurons in multiple mammalian species. *Neuroscience*. 115 (1), 285–294.

George, A.L. (2004) Inherited Channelopathies Associated with Epilepsy. *Epilepsy Currents*. [Online] 4 (2), 65–70. Available from: doi:10.1111/j.1535-7597.2004.42010.x.

Gibbs, F.A., Davis, H. & Lennox, W.G. (1968) The Electro Encephalogram in Epilepsy and in Conditions of Impaired Consciousness. *American Journal of EEG Technology*. [Online] 8 (2), 59–73. Available from: doi:10.1080/00029238.1968.11080707.

Gibbs, F.A., Gibbs, E.L. & Lennox, W.G. (1937) Epilepsy: A Paroxysmal Cerebral Dysrhythmia. *Brain*. [Online] 60 (4), 377–388. Available from: doi:10.1093/brain/60.4.377.

Gloor, P. (1969) Hans Berger on Electroencephalography. *American Journal of EEG Technology*. [Online] 9 (1), 1–8. Available from: doi:10.1080/00029238.1969.11080728.

Glover, G.H. (1999) Deconvolution of impulse response in event-related BOLD fMRI. *NeuroImage*. 9 (4), 416–429.

Goel, N., Rao, H., Durmer, J.S. & Dinges, D.F. (2009) Neurocognitive Consequences of Sleep Deprivation. *Seminars in neurology*. [Online] 29 (4), 320–339. Available from: doi:10.1055/s-0029-1237117.

Gonnissen, H.K.J., Mazuy, C., Rutters, F., Martens, E.A.P., et al. (2013) Sleep Architecture When Sleeping at an Unusual Circadian Time and Associations with Insulin Sensitivity. *PLoS ONE*. [Online] 8 (8). Available from: doi:10.1371/journal.pone.0072877.

Gora, J., Colrain, I.M. & Trinder, J. (2001) The investigation of K-complex and vertex sharp wave activity in response to mid-inspiratory occlusions and complete obstructions to breathing during NREM sleep. *Sleep*. 24 (1), 81–89.

Gorji, A. & Speckmann, E.-J. (2009) Epileptiform EEG spikes and their functional significance. *Clinical EEG and neuroscience*. 40 (4), 230–233.

Gotman, J. (2008) Epileptic networks studied with EEG-fMRI. *Epilepsia*. [Online] 49 (s3), 42–51. Available from: doi:10.1111/j.1528-1167.2008.01509.x.

Gotman, J., Grova, C., Bagshaw, A., Kobayashi, E., et al. (2005a) Generalized epileptic discharges show thalamocortical activation and suspension of the default state of the brain. *Proceedings of the National Academy of Sciences of the United States of America*. [Online] 102 (42), 15236–15240. Available from: doi:10.1073/pnas.0504935102.

Gotman, J., Grova, C., Bagshaw, A., Kobayashi, E., et al. (2005b) Generalized epileptic discharges show thalamocortical activation and suspension of the default state of the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 102 (42), 15236–15240.

Gotman, J., Kobayashi, E., Bagshaw, A.P., Bénar, C.-G., et al. (2006) Combining EEG and fMRI: A multimodal tool for epilepsy research. *Journal of Magnetic Resonance Imaging*. [Online] 23 (6), 906–920. Available from: doi:10.1002/jmri.20577.

Gotman, J. & Pittau, F. (2011) Combining EEG and fMRI in the study of epileptic discharges. *Epilepsia*. [Online] 52 (0 4), 38–42. Available from: doi:10.1111/j.1528-1167.2011.03151.x.

van Graan, L.A., Lemieux, L. & Chaudhary, U.J. (2015) Methods and utility of EEG-fMRI in epilepsy. *Quantitative Imaging in Medicine and Surgery*. [Online] 5 (2), 300–312. Available from: doi:10.3978/j.issn.2223-4292.2015.02.04.

Grant, A.C., Abdel-Baki, S.G., Weedon, J., Arnedo, V., et al. (2014) EEG Interpretation Reliability and Interpreter Confidence: A Large Single Center Study. *Epilepsy & behavior : E&B*. [Online] 32102–107. Available from: doi:10.1016/j.yebeh.2014.01.011.

Grigg-Damberger, M.M. (2012) The AASM Scoring Manual Four Years Later. *Journal of Clinical Sleep Medicine : JCSM : Official Publication of the American Academy of Sleep Medicine*. [Online] 8 (3), 323–332. Available from: doi:10.5664/jcsm.1928.

Gross, D.W. (2011) Diffusion tensor imaging in temporal lobe epilepsy. *Epilepsia*. [Online] 52 Suppl 432–34. Available from: doi:10.1111/j.1528-1167.2011.03149.x.

Grouiller, F., Delattre, B.M.A., Pittau, F., Heinzer, S., et al. (2015) All-in-one interictal presurgical imaging in patients with epilepsy: single-session EEG/PET/(f)MRI. *European Journal of Nuclear Medicine and Molecular Imaging*. [Online] 42 (7), 1133–1143. Available from: doi:10.1007/s00259-015-3045-2.

Grouiller, F., Thornton, R.C., Groening, K., Spinelli, L., et al. (2011) With or without spikes: localization of focal epileptic activity by simultaneous electroencephalography and functional magnetic resonance imaging. *Brain*. [Online] 134 (10), 2867–2886. Available from: doi:10.1093/brain/awr156.

Grouiller, F., Vercueil, L., Krainik, A., Segebarth, C., et al. (2010) Characterization of the hemodynamic modes associated with interictal epileptic activity using a deformable model-based analysis of combined EEG and functional MRI recordings. *Human Brain Mapping*. [Online] 31 (8), 1157–1173. Available from: doi:10.1002/hbm.20925.

Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., et al. (2011) Cost of disorders of the brain in Europe 2010. *European Neuropsychopharmacology*. [Online] 21 (10), 718–779. Available from: doi:10.1016/j.euroneuro.2011.08.008.

Halász, P. (2005) K-complex, a reactive EEG graphoelement of NREM sleep: an old chap in a new garment. *Sleep Medicine Reviews*. [Online] 9 (5), 391–412. Available from: doi:10.1016/j.smrv.2005.04.003.

Halász, P. (2010a) The concept of epileptic networks. Part 1. *Ideggyógyászati Szemle*. 63 (9–10), 293–303.

Halász, P. (2010b) The concept of epileptic networks. Part 2. *Ideggyógyászati Szemle*. 63 (11–12), 377–384.

Haltiner, A.M., Temkin, N.R. & Dikmen, S.S. (1997) Risk of seizure recurrence after the first late posttraumatic seizure. *Archives of Physical Medicine and Rehabilitation*. 78 (8), 835–840.

Hamandi, K., Laufs, H., Nöth, U., Carmichael, D.W., et al. (2008) BOLD and perfusion changes during epileptic generalised spike wave activity. *NeuroImage*. [Online] 39 (2), 608–618. Available from: doi:10.1016/j.neuroimage.2007.07.009.

Hamandi, K., Salek Haddadi, A., Liston, A., Laufs, H., et al. (2005) fMRI temporal clustering analysis in patients with frequent interictal epileptiform discharges: Comparison with EEG-driven analysis. *NeuroImage*. [Online] 26 (1), 309–316. Available from: doi:10.1016/j.neuroimage.2005.01.052.

Hamandi, K., Salek-Haddadi, A., Laufs, H., Liston, A., et al. (2006) EEG–fMRI of idiopathic and secondarily generalized epilepsies. *NeuroImage*. [Online] 31 (4), 1700–1710. Available from: doi:10.1016/j.neuroimage.2006.02.016.

Hammond, C. (2015) *Cellular and Molecular Neurophysiology*. 4th edition. Academic Press.

Handwerker, D.A., Ollinger, J.M. & D'Esposito, M. (2004) Variation of BOLD hemodynamic responses across subjects and brain regions and their effects on statistical analyses. *NeuroImage*. [Online] 21 (4), 1639–1651. Available from: doi:10.1016/j.neuroimage.2003.11.029.

Haneef, Z., Lenartowicz, A., Yeh, H.J., Engel, J., et al. (2012) Effect of lateralized temporal lobe epilepsy on the default mode network. *Epilepsy & behavior : E&B*. [Online] 25 (3), 350–357. Available from: doi:10.1016/j.yebeh.2012.07.019.

Haneef, Z., Lenartowicz, A., Yeh, H.J., Levin, H.S., et al. (2014) Functional Connectivity of Hippocampal Networks in Temporal Lobe Epilepsy. *Epilepsia*. [Online] 55 (1), 137–145. Available from: doi:10.1111/epi.12476.

Hara, J., Beuckmann, C.T., Nambu, T., Willie, J.T., et al. (2001) Genetic Ablation of Orexin Neurons in Mice Results in Narcolepsy, Hypophagia, and Obesity. *Neuron*. [Online] 30 (2), 345–354. Available from: doi:10.1016/S0896-6273(01)00293-8.

Harsh, J., Voss, U., Hull, J., Schrepfer, S., et al. (1994) ERP and behavioral changes during the wake/sleep transition. *Psychophysiology*. 31 (3), 244–252.

Hauser, W.A., Annegers, J.F. & Rocca, W.A. (1996) Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. *Mayo Clinic Proceedings*. [Online] 71 (6), 576–586. Available from: doi:10.1016/S0025-6196(11)64115-3.

Hauser, W.A. & Beghi, E. (2008) First seizure definitions and worldwide incidence and mortality: First Seizure Definitions, Incidence, Mortality. *Epilepsia*. [Online] 498–12. Available from: doi:10.1111/j.1528-1167.2008.01443.x.

Hawco, C.S., Bagshaw, A.P., Lu, Y., Dubeau, F., et al. (2007) BOLD changes occur prior to epileptic spikes seen on scalp EEG. *NeuroImage*. [Online] 35 (4), 1450–1458. Available from: doi:10.1016/j.neuroimage.2006.12.042.

Helmstaedter, C. & Elger, C.E. (2009) Chronic temporal lobe epilepsy: a neurodevelopmental or progressively dementing disease? *Brain: A Journal of Neurology*. [Online] 132 (Pt 10), 2822–2830. Available from: doi:10.1093/brain/awp182.

Hesdorffer, D.C., Benn, E.K.T., Cascino, G.D. & Hauser, W.A. (2009) Is a first acute symptomatic seizure epilepsy? Mortality and risk for recurrent seizure. *Epilepsia*. [Online] 50 (5), 1102–1108. Available from: doi:10.1111/j.1528-1167.2008.01945.x.

van den Heuvel, M.P. & Hulshoff Pol, H.E. (2010) Exploring the brain network: A review on resting-state fMRI functional connectivity. *European Neuropsychopharmacology*. [Online] 20 (8), 519–534. Available from: doi:10.1016/j.euroneuro.2010.03.008.

Hinde, S., Soares, M., Burch, J., Marson, A., et al. (2014) The added clinical and economic value of diagnostic testing for epilepsy surgery. *Epilepsy Research*. [Online] 108 (4), 775–781. Available from: doi:10.1016/j.eplepsyres.2014.02.002.

Hobson, J.A., McCarley, R.W. & Wyzinski, P.W. (1975) Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science*. [Online] 189 (4196), 55–58. Available from: doi:10.1126/science.1094539.

Hobson, J.A. & Pace-Schott, E.F. (2002) The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nature Reviews Neuroscience*. [Online] 3 (9), 679–693. Available from: doi:10.1038/nrn915.

Hodgkin, A.L. & Huxley, A.F. (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of Physiology*. 117 (4), 500–544.

Hollingsworth, K.G. (2015) Reducing acquisition time in clinical MRI by data undersampling and compressed sensing reconstruction. *Physics in Medicine and Biology*. [Online] 60 (21), R297-322. Available from: doi:10.1088/0031-9155/60/21/R297.

Hori, T., Sugita, Y., Koga, E., Shirakawa, S., et al. (2001) Proposed supplements and amendments to 'A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects', the Rechtschaffen & Kales (1968) standard. *Psychiatry and Clinical Neurosciences*. [Online] 55 (3), 305–310. Available from: doi:10.1046/j.1440-1819.2001.00810.x.

Horovitz, S.G., Braun, A.R., Carr, W.S., Picchioni, D., et al. (2009) Decoupling of the brain's default mode network during deep sleep. *Proceedings of the National Academy of Sciences*. 106 (27), 11376–11381.

van Houdt, P.J., de Munck, J.C., Leijten, F.S.S., Huiskamp, G.J.M., et al. (2013) EEG-fMRI correlation patterns in the presurgical evaluation of focal epilepsy: a comparison with electrocorticographic data and surgical outcome measures. *NeuroImage*. [Online] 75238–248. Available from: doi:10.1016/j.neuroimage.2013.02.033.

van Houdt, P.J., Ossenblok, P.P.W., Boon, P.A.J.M., Leijten, F.S.S., et al. (2010) Correction for pulse height variability reduces physiological noise in functional MRI when studying spontaneous brain activity. *Human Brain Mapping*. [Online] 31 (2), 311–325. Available from: doi:10.1002/hbm.20866.

van Houdt, P.J., Ossenblok, P.P.W., Colon, A.J., Hermans, K.H.M., et al. (2015) Are Epilepsy-Related fMRI Components Dependent on the Presence of Interictal Epileptic Discharges in Scalp EEG? *Brain Topography*. [Online] 28 (4), 606–618. Available from: doi:10.1007/s10548-014-0407-1.

Huber, R., Maki, H., Rosanova, M., Casarotto, S., et al. (2013) Human Cortical Excitability Increases with Time Awake. *Cerebral Cortex*. [Online] 23 (2), 1–7. Available from: doi:10.1093/cercor/bhs014.

Huettel, S.A., Song, A.W. & McCarthy, G. (2014) *Functional Magnetic Resonance Imaging*. 3rd edition. Sinauer Associates, Incorporated.

Hughes, J.R. (2009) Absence seizures: A review of recent reports with new concepts. *Epilepsy & Behavior*. [Online] 15 (4), 404–412. Available from: doi:10.1016/j.yebeh.2009.06.007.

Hughes, S.W. & Crunelli, V. (2005) Thalamic mechanisms of EEG alpha rhythms and their pathological implications. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*. [Online] 11 (4), 357–372. Available from: doi:10.1177/1073858405277450.

Iber, C., Ancoli-Israel, S., Chesson, A. & Quan, S. (2007) *for the American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. 1st ed.*

International Federation of Societies for Clinical Neurophysiology (1974) A glossary of terms most commonly used by clinical electroencephalographers. *Electroencephalography and Clinical Neurophysiology*. 37 (5), 538–548.

Jacobs, J., Kobayashi, E., Boor, R., Muhle, H., et al. (2007) Hemodynamic Responses to Interictal Epileptiform Discharges in Children with Symptomatic Epilepsy. *Epilepsia*. [Online] 48 (11), 2068–2078. Available from: doi:10.1111/j.1528-1167.2007.01192.x.

Jacobs, J., Levan, P., Moeller, F., Boor, R., et al. (2009) Hemodynamic changes preceding the interictal EEG spike in patients with focal epilepsy investigated using simultaneous EEG-fMRI. *NeuroImage*. [Online] 45 (4), 1220–1231. Available from: doi:10.1016/j.neuroimage.2009.01.014.

Jahnke, K., von Wegner, F., Morzelewski, A., Borisov, S., et al. (2012) To wake or not to wake? The two-sided nature of the human K-complex. *NeuroImage*. [Online] 59 (2), 1631–1638. Available from: doi:10.1016/j.neuroimage.2011.09.013.

Jankel, W.R. & Niedermeyer, E. (1985) Sleep spindles. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 2 (1), 1–35.

Japee, S., Holiday, K., Satyshur, M.D., Mukai, I., et al. (2015) A role of right middle frontal gyrus in reorienting of attention: a case study. *Frontiers in Systems Neuroscience*. [Online] 9. Available from: doi:10.3389/fnsys.2015.00023.

Jefferys, J.G. (1999) Hippocampal sclerosis and temporal lobe epilepsy: cause or consequence? *Brain*. 122 (6), 1007–1008.

Jinde, S., Zsiros, V. & Nakazawa, K. (2013) Hilar mossy cell circuitry controlling dentate granule cell excitability. *Frontiers in Neural Circuits*. [Online] 7. Available from: doi:10.3389/fncir.2013.00014.

Johns, M.W. (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*. 14 (6), 540–545.

Jones, B.E. (2003) Arousal systems. *Frontiers in Bioscience: A Journal and Virtual Library*. 8s438-451.

Kajimura, N., Uchiyama, M., Takayama, Y., Uchida, S., et al. (1999) Activity of midbrain reticular formation and neocortex during the progression of human non-rapid eye movement sleep. *The Journal of neuroscience*. 19 (22), 10065–10073.

Kandt, R.S., Haines, J.L., Smith, M., Northrup, H., et al. (1992) Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. *Nature Genetics*. [Online] 2 (1), 37–41. Available from: doi:10.1038/ng0992-37.

Kang, J.K., Bénar, C.-G., Al-Asmi, A., Khani, Y.A., et al. (2003) Using patient-specific hemodynamic response functions in combined EEG-fMRI studies in epilepsy. *NeuroImage*. [Online] 20 (2), 1162–1170. Available from: doi:10.1016/S1053-8119(03)00290-8.

Kashida, Y., Otsubo, T., Hanaya, R., Kodabashi, A., et al. (2016) Determination of hemispheric language dominance using functional magnetic resonance imaging and the Shiritori (Japanese word chain) task in patients with epilepsy: Comparison with the Wada test. *Epilepsy Research*. [Online] 12416–22. Available from: doi:10.1016/j.eplepsyres.2016.04.005.

Kaufmann, C., Wehrle, R., Wetter, T.C., Holsboer, F., et al. (2006) Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: an EEG/fMRI study. *Brain: A Journal of Neurology*. [Online] 129 (Pt 3), 655–667. Available from: doi:10.1093/brain/awh686.

Kayama, Y. & Koyama, Y. (2003) Control of sleep and wakefulness by brainstem monoaminergic and cholinergic neurons. *Acta Neurochirurgica. Supplement*. 873–6.

Kayama, Y., Ohta, M. & Jodo, E. (1992) Firing of 'possibly' cholinergic neurons in the rat laterodorsal tegmental nucleus during sleep and wakefulness. *Brain Research*. 569 (2), 210–220.

Killgore, W.D.S. (2010) Effects of sleep deprivation on cognition. *Progress in Brain Research*. [Online] 185105–129. Available from: doi:10.1016/B978-0-444-53702-7.00007-5.

Kiloh, L.G., McComas, A.J. & Osselton, J.W. (2013) *Clinical Electroencephalography*. Butterworth-Heinemann.

Kim, Y.K., Lee, D.S., Lee, S.K., Chung, C.K., et al. (2002) (18)F-FDG PET in localization of frontal lobe epilepsy: comparison of visual and SPM analysis. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*. 43 (9), 1167–1174.

Kirschstein, T. & Köhling, R. (2009) What is the Source of the EEG? *Clinical EEG and neuroscience*. 40 (3), 146–149.

Klem, G.H., Lüders, H.O., Jasper, H.H. & Elger, C. (1999) The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalography and Clinical Neurophysiology. Supplement*. 523–6.

Klinzing, J.G., Mölle, M., Weber, F., Supp, G., et al. (2016) Spindle activity phase-locked to sleep slow oscillations. *NeuroImage*. [Online] 134607–616. Available from: doi:10.1016/j.neuroimage.2016.04.031.

Kobayashi, E., Bagshaw, A.P., Bénar, C.-G., Aghakhani, Y., et al. (2006) Temporal and extratemporal BOLD responses to temporal lobe interictal spikes. *Epilepsia*. 47 (2), 343–354.

Kobayashi, E., Bagshaw, A.P., Grova, C., Dubeau, F., et al. (2006) Negative BOLD responses to epileptic spikes. *Human Brain Mapping*. [Online] 27 (6), 488–497. Available from: doi:10.1002/hbm.20193.

Kobayashi, E., Grova, C., Tyvaert, L., Dubeau, F., et al. (2009) Structures involved at the time of temporal lobe spikes revealed by interindividual group analysis of EEG/fMRI data. *Epilepsia*. [Online] 50 (12), 2549–2556. Available from: doi:10.1111/j.1528-1167.2009.02180.x.

Koike, T., Kan, S., Misaki, M. & Miyauchi, S. (2011) Connectivity pattern changes in default-mode network with deep non-REM and REM sleep. *Neuroscience Research*. [Online] 69 (4), 322–330. Available from: doi:10.1016/j.neures.2010.12.018.

Kostopoulos, G.K. (2000) Spike-and-wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. 111 Suppl 2S27-38.

Krakow, K., Allen, P.J., Symms, M.R., Lemieux, L., et al. (2000) EEG recording during fMRI experiments: image quality. *Human Brain Mapping*. 10 (1), 10–15.

Krakow, K., Woermann, F.G., Symms, M.R., Allen, P.J., et al. (1999) EEG-triggered functional MRI of interictal epileptiform activity in patients with partial seizures. *Brain: A Journal of Neurology*. 122 (Pt 9)1679–1688.

Kristeva-Feige, R., Fritsch, C., Timmer, J. & Lücking, C.-H. (2002) Effects of attention and precision of exerted force on beta range EEG-EMG synchronization during a maintained motor contraction task. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. 113 (1), 124–131.

Kushida, C.A., Chang, A., Gadkary, C., Guilleminault, C., et al. (2001) Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. *Sleep Medicine*. [Online] 2 (5), 389–396. Available from: doi:10.1016/S1389-9457(00)00098-8.

Kwan, P. & Brodie, M.J. (2000) Early Identification of Refractory Epilepsy. *New England Journal of Medicine*. [Online] 342 (5), 314–319. Available from: doi:10.1056/NEJM200002033420503.

Kwong, K.K., Belliveau, J.W., Chesler, D.A., Goldberg, I.E., et al. (1992) Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proceedings of the National Academy of Sciences of the United States of America*. 89 (12), 5675–5679.

La Vaque, T.J. (1999) The History of EEG Hans Berger. *Journal of Neurotherapy*. [Online] 3 (2), 1–9. Available from: doi:10.1300/J184v03n02_01.

Lachaux, J.P., Rudrauf, D. & Kahane, P. (2003) Intracranial EEG and human brain mapping. *Journal of Physiology-Paris*. [Online] 97 (4–6), 613–628. Available from: doi:10.1016/j.jphysparis.2004.01.018.

Lascano, A.M., Perneger, T., Vulliemoz, S., Spinelli, L., et al. (2016) Yield of MRI, high-density electric source imaging (HD-ESI), SPECT and PET in epilepsy surgery candidates. *Clinical Neurophysiology*. [Online] 127 (1), 150–155. Available from: doi:10.1016/j.clinph.2015.03.025.

Laufs, H., Richardson, M.P., Salek-Haddadi, A., Vollmar, C., et al. (2011) Converging PET and fMRI evidence for a common area involved in human focal epilepsies. *Neurology*. [Online] 77 (9), 904–910. Available from: doi:10.1212/WNL.0b013e31822c90f2.

Lauritzen, M. (2005) Reading vascular changes in brain imaging: is dendritic calcium the key? *Nature Reviews. Neuroscience*. [Online] 6 (1), 77–85. Available from: doi:10.1038/nrn1589.

Leger, D. (1994) The cost of sleep-related accidents: a report for the National Commission on Sleep Disorders Research. *Sleep*. 17 (1), 84–93.

Lemieux, L., Laufs, H., Carmichael, D., Paul, J.S., et al. (2007) Noncanonical spike-related BOLD responses in focal epilepsy. *Human Brain Mapping*. [Online] 29 (3), 329–345. Available from: doi:10.1002/hbm.20389.

Lemieux, L., Salek-Haddadi, A., Hoffmann, A., Gotman, J., et al. (2002) EEG-Correlated Functional MRI: Recent Methodologic Progress and Current Issues. *Epilepsia*. [Online] 4364–68. Available from: doi:10.1046/j.1528-1157.2002.043s1064.x.

Lemieux, L., Salek-Haddadi, A., Josephs, O., Allen, P., et al. (2001) Event-Related fMRI with Simultaneous and Continuous EEG: Description of the Method and Initial Case Report. *NeuroImage*. [Online] 14 (3), 780–787. Available from: doi:10.1006/nimg.2001.0853.

Lengler, U., Kafadar, I., Neubauer, B.A. & Krakow, K. (2007) fMRI correlates of interictal epileptic activity in patients with idiopathic benign focal epilepsy of childhood. *Epilepsy Research*. [Online] 75 (1), 29–38. Available from: doi:10.1016/j.eplepsyres.2007.03.016.

Leresche, N., Lambert, R.C., Errington, A.C. & Crunelli, V. (2012) From sleep spindles of natural sleep to spike and wave discharges of typical absence seizures: is the hypothesis still valid? *Pflügers Archiv: European Journal of Physiology*. [Online] 463 (1), 201–212. Available from: doi:10.1007/s00424-011-1009-3.

LeSauter, J., Silver, R., Cloues, R. & Witkovsky, P. (2011) Light exposure induces short- and long-term changes in the excitability of retinorecipient neurons in suprachiasmatic nucleus. *Journal of Neurophysiology*. [Online] 106 (2), 576–588. Available from: doi:10.1152/jn.00060.2011.

Leventer, R.J., Guerrini, R. & Dobyns, W.B. (2008) Malformations of cortical development and epilepsy. *Dialogues in Clinical Neuroscience*. 10 (1), 47–62.

Lindemann, C., Ahlbeck, J., Bitzenhofer, S.H. & Hanganu-Opatz, I.L. (2016) Spindle Activity Orchestrates Plasticity during Development and Sleep. *Neural Plasticity*. [Online] 2016. Available from: doi:10.1155/2016/5787423.

Lindquist, M.A. & Wager, T.D. (2007) Validity and Power in Hemodynamic Response Modeling: A Comparison Study and a New Approach. *Human Brain Mapping*. [Online] 28 (8), 764–784. Available from: doi:10.1002/hbm.20310.

Liston, A.D., Lund, T.E., Salek-Haddadi, A., Hamandi, K., et al. (2006) Modelling cardiac signal as a confound in EEG-fMRI and its application in focal epilepsy studies. *NeuroImage*. [Online] 30 (3), 827–834. Available from: doi:10.1016/j.neuroimage.2005.10.025.

Liu, C. & Reppert, S.M. (2000) GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron*. 25 (1), 123–128.

Liu, M., Chen, Z., Beaulieu, C. & Gross, D.W. (2014) Disrupted anatomic white matter network in left mesial temporal lobe epilepsy. *Epilepsia*. [Online] 55 (5), 674–682. Available from: doi:10.1111/epi.12581.

Liu, M., Concha, L., Lebel, C., Beaulieu, C., et al. (2012) Mesial temporal sclerosis is linked with more widespread white matter changes in temporal lobe epilepsy. *NeuroImage. Clinical*. [Online] 1 (1), 99–105. Available from: doi:10.1016/j.nicl.2012.09.010.

Liu, Y., Yang, T., Yang, X., Liu, I., et al. (2008) EEG-fMRI study of the interictal epileptic activity in patients with partial epilepsy. *Journal of the Neurological Sciences*. [Online] 268 (1–2), 117–123. Available from: doi:10.1016/j.jns.2007.11.019.

Liu, Z.-W., Faraguna, U., Cirelli, C., Tononi, G., et al. (2010) Direct Evidence for Wake-Related Increases and Sleep-Related Decreases in Synaptic Strength in Rodent Cortex. *Journal of Neuroscience*. [Online] 30 (25), 8671–8675. Available from: doi:10.1523/JNEUROSCI.1409-10.2010.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., et al. (2001) Neurophysiological investigation of the basis of the fMRI signal. *Nature*. 412 (6843), 150–157.

Logothetis, N.K. & Wandell, B.A. (2004) Interpreting the BOLD Signal. *Annual Review of Physiology*. [Online] 66 (1), 735–769. Available from: doi:10.1146/annurev.physiol.66.082602.092845.

Loomis, A.L., Harvey, E.N. & Hobart, G. (1937) Cerebral states during sleep, as studied by human brain potentials. *Journal of Experimental Psychology*. [Online] 21 (2), 127–144. Available from: doi:10.1037/h0057431.

Loomis, A.L., Harvey, E.N. & Hobart, G. (1935) POTENTIAL RHYTHMS OF THE CEREBRAL CORTEX DURING SLEEP. *Science (New York, N.Y.)*. [Online] 81 (2111), 597–598. Available from: doi:10.1126/science.81.2111.597.

Loomis, A.L., Harvey, E.N. & Hobart, G.A. (1938) Distribution of Disturbance-Patterns in the Human Electroencephalogram, with Special Reference to Sleep. *Journal of Neurophysiology*. 1 (5), 413–430.

Lopes, R., Moeller, F., Besson, P., Ogez, F., et al. (2014) Study on the Relationships between Intrinsic Functional Connectivity of the Default Mode Network and Transient Epileptic Activity. *Frontiers in Neurology*. [Online] 5. Available from: doi:10.3389/fneur.2014.00201.

Lopes da Silva, F. (2009) EEG: Origin and Measurement. In: Christoph Mulert & Louis Lemieux (eds.). *EEG - fMRI*. [Online]. Berlin, Heidelberg, Springer Berlin Heidelberg. pp. 19–38. Available from: http://link.springer.com/10.1007/978-3-540-87919-0_2.

Lopes da Silva, F. (1991) Neural mechanisms underlying brain waves: from neural membranes to networks. *Electroencephalography and Clinical Neurophysiology*. 79 (2), 81–93.

Lu, Y., Bagshaw, A.P., Grova, C., Kobayashi, E., et al. (2006) Using voxel-specific hemodynamic response function in EEG-fMRI data analysis. *NeuroImage*. [Online] 32 (1), 238–247. Available from: doi:10.1016/j.neuroimage.2005.11.040.

Lu, Y., Grova, C., Kobayashi, E., Dubeau, F., et al. (2007) Using voxel-specific hemodynamic response function in EEG-fMRI data analysis: An estimation and detection model. *NeuroImage*. [Online] 34 (1), 195–203. Available from: doi:10.1016/j.neuroimage.2006.08.023.

Lüders, H., Acharya, J., Baumgartner, C., Benbadis, S., et al. (1999) A new epileptic seizure classification based exclusively on ictal semiology. *Acta Neurologica Scandinavica*. 99 (3), 137–141.

Lüders, H., Acharya, J., Baumgartner, C., Benbadis, S., et al. (1998) Semiological seizure classification. *Epilepsia*. 39 (9), 1006–1013.

Lüders, H.O., Amina, S., Baumgartner, C., Benbadis, S., et al. (2012) Modern technology calls for a modern approach to classification of epileptic seizures and the epilepsies: Classification of Epilepsies. *Epilepsia*. [Online] 53 (3), 405–411. Available from: doi:10.1111/j.1528-1167.2011.03376.x.

Lüders, H.O., Najm, I., Nair, D., Widdess-Walsh, P., et al. (2006) The epileptogenic zone: general principles. *Epileptic disorders*. 8S1.

van Luijtelaar, G., Hramov, A., Sitnikova, E. & Koronovskii, A. (2011) Spike–wave discharges in WAG/Rij rats are preceded by delta and theta precursor activity in cortex and thalamus. *Clinical Neurophysiology*. [Online] 122 (4), 687–695. Available from: doi:10.1016/j.clinph.2010.10.038.

van Luijtelaar, G. & Zobeiri, M. (2014) Progress and outlooks in a genetic absence epilepsy model (WAG/Rij). *Current Medicinal Chemistry*. 21 (6), 704–721.

Luo, C., Li, Q., Lai, Y., Xia, Y., et al. (2011) Altered functional connectivity in default mode network in absence epilepsy: a resting-state fMRI study. *Human Brain Mapping*. [Online] 32 (3), 438–449. Available from: doi:10.1002/hbm.21034.

MacDonald, B.K., Cockerell, O.C., Sander, J.W. & Shorvon, S.D. (2000) The incidence and lifetime prevalence of neurological disorders in a prospective community-based study in the UK. *Brain: A Journal of Neurology*. 123 (Pt 4)665–676.

Madsen, P.L. & Vorstrup, S. (1991) Cerebral blood flow and metabolism during sleep. *Cerebrovascular and Brain Metabolism Reviews*. 3 (4), 281–296.

Magiorkinis, E., Sidiropoulou, K. & Diamantis, A. (2010) Hallmarks in the history of epilepsy: Epilepsy in antiquity. *Epilepsy & Behavior*. [Online] 17 (1), 103–108. Available from: doi:10.1016/j.yebeh.2009.10.023.

Mäkiranta, M., Ruohonen, J., Suominen, K., Niinimäki, J., et al. (2005) BOLD signal increase preceeds EEG spike activity—a dynamic penicillin induced focal epilepsy in deep anesthesia. *NeuroImage*. [Online] 27 (4), 715–724. Available from: doi:10.1016/j.neuroimage.2005.05.025.

Maljevic, S. & Lerche, H. (2014) Potassium channel genes and benign familial neonatal epilepsy. *Progress in Brain Research*. [Online] 21317–53. Available from: doi:10.1016/B978-0-444-63326-2.00002-8.

Mandelkow, H., Halder, P., Boesiger, P. & Brandeis, D. (2006) Synchronization facilitates removal of MRI artefacts from concurrent EEG recordings and increases usable bandwidth. *NeuroImage*. [Online] 32 (3), 1120–1126. Available from: doi:10.1016/j.neuroimage.2006.04.231.

Maneshi, M., Moeller, F., Fahoum, F., Gotman, J., et al. (2012) Resting-state connectivity of the sustained attention network correlates with disease duration in idiopathic generalized epilepsy. *PloS One*. [Online] 7 (12), e50359. Available from: doi:10.1371/journal.pone.0050359.

Manganotti, P., Formaggio, E., Gasparini, A., Cerini, R., et al. (2008) Continuous EEG–fMRI in patients with partial epilepsy and focal interictal slow-wave discharges on EEG. *Magnetic Resonance Imaging*. [Online] 26 (8), 1089–1100. Available from: doi:10.1016/j.mri.2008.02.023.

Maquet, P. (2000) Functional neuroimaging of normal human sleep by positron emission tomography. *Journal of Sleep Research*. 9 (3), 207–231.

Marques, C.M., Caboclo, L.O.S.F., da Silva, T.I., Noffs, M.H. da S., et al. (2007) Cognitive decline in temporal lobe epilepsy due to unilateral hippocampal sclerosis. *Epilepsy & Behavior: E&B*. [Online] 10 (3), 477–485. Available from: doi:10.1016/j.yebeh.2007.02.002.

Mars, R.B., Sallet, J., Schuffelgen, U., Jbabdi, S., et al. (2012) Connectivity-Based Subdivisions of the Human Right 'Temporoparietal Junction Area': Evidence for Different Areas Participating in Different Cortical Networks. *Cerebral Cortex*. [Online] 22 (8), 1894–1903. Available from: doi:10.1093/cercor/bhr268.

Marson, A., Jacoby, A., Johnson, A., Kim, L., et al. (2005) Immediate versus deferred antiepileptic drug treatment for early epilepsy and single seizures: a randomised controlled trial. *Lancet*

(London, England). [Online] 365 (9476), 2007–2013. Available from: doi:10.1016/S0140-6736(05)66694-9.

Masia, S.L. & Devinsky, O. (2000) Epilepsy and Behavior: A Brief History. *Epilepsy & Behavior*. [Online] 1 (1), 27–36. Available from: doi:10.1006/ebeh.1999.0021.

Masterton, R.A.J., Harvey, A.S., Archer, J.S., Lillywhite, L.M., et al. (2010) Focal epileptiform spikes do not show a canonical BOLD response in patients with benign rolandic epilepsy (BECTS). *NeuroImage*. [Online] 51 (1), 252–260. Available from: doi:10.1016/j.neuroimage.2010.01.109.

Matricardi, S., Verrotti, A., Chiarelli, F., Cerminara, C., et al. (2014) Current Advances in Childhood Absence Epilepsy. *Pediatric Neurology*. [Online] 50 (3), 205–212. Available from: doi:10.1016/j.pediatrneurol.2013.10.009.

Mayor, S. (2003) Nobel prize in medicine awarded to MRI pioneers. *BMJ : British Medical Journal*. 327 (7419), 827.

Maziero, D., Sturzbecher, M., Velasco, T.R., Rondinoni, C., et al. (2015) A Comparison of Independent Component Analysis (ICA) of fMRI and Electrical Source Imaging (ESI) in Focal Epilepsy Reveals Misclassification Using a Classifier. *Brain Topography*. [Online] 28 (6), 813–831. Available from: doi:10.1007/s10548-015-0436-4.

McCormick, D.A. (1989) Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends in Neurosciences*. 12 (6), 215–221.

McCormick, D.A. & Contreras, D. (2001) On the cellular and network bases of epileptic seizures. *Annual Review of Physiology*. 63 (1), 815–846.

McGinty, D. & Szymusiak, R. (2011) Neural Control of Sleep in Mammals. In: Meir H. Kryger, Thomas Roth, & William C. Dement (eds.). *Principles and Practice of Sleep Medicine*. 5th edition. [Online]. Philadelphia, W.B. Saunders. pp. 76–91. Available from: http://www.sciencedirect.com/science/article/pii/B9781416066453000074.

McGonigal, A., Bartolomei, F., Regis, J., Guye, M., et al. (2007) Stereoelectroencephalography in presurgical assessment of MRI-negative epilepsy. *Brain*. [Online] 130 (12), 3169–3183. Available from: doi:10.1093/brain/awm218.

Meeren, H.K.M., Pijn, J.P.M., Van Luijtelaar, E.L.J.M., Coenen, A.M.L., et al. (2002) Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 22 (4), 1480–1495.

Mekle, R., Wu, E.X., Meckel, S., Wetzel, S.G., et al. (2006) Combo acquisitions: balancing scan time reduction and image quality. *Magnetic Resonance in Medicine*. [Online] 55 (5), 1093–1105. Available from: doi:10.1002/mrm.20882.

Méndez, M. & Radtke, R.A. (2001) Interactions between sleep and epilepsy. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 18 (2), 106–127.

Mendez, O.E. & Brenner, R.P. (2006) Increasing the yield of EEG. *Journal of clinical neurophysiology*. 23 (4), 282–293.

Messori, A., Polonara, G., Carle, F., Gesuita, R., et al. (2005) Predicting posttraumatic epilepsy with MRI: prospective longitudinal morphologic study in adults. *Epilepsia*. [Online] 46 (9), 1472–1481. Available from: doi:10.1111/j.1528-1167.2005.34004.x.

Mikl, M., Marecek, R., Hlustík, P., Pavlicová, M., et al. (2008) Effects of spatial smoothing on fMRI group inferences. *Magnetic Resonance Imaging*. [Online] 26 (4), 490–503. Available from: doi:10.1016/j.mri.2007.08.006.

Miller, J.F., Neufang, M., Solway, A., Brandt, A., et al. (2013) Neural Activity in Human Hippocampal Formation Reveals the Spatial Context of Retrieved Memories. *Science (New York, N.Y.)*. [Online] 342 (6162), 1111–1114. Available from: doi:10.1126/science.1244056.

Mochizuki, T., Crocker, A., McCormack, S., Yanagisawa, M., et al. (2004) Behavioral state instability in orexin knock-out mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. [Online] 24 (28), 6291–6300. Available from: doi:10.1523/JNEUROSCI.0586-04.2004.

Moehring, J., Coropceanu, D., Galka, A., Moeller, F., et al. (2011) Improving sensitivity of EEG-fMRI studies in epilepsy: the role of sleep-specific activity. *Neuroscience Letters*. [Online] 505 (2), 211–215. Available from: doi:10.1016/j.neulet.2011.10.028.

Moeller, F., Siebner, H.R., Wolff, S., Muhle, H., et al. (2008) Changes in activity of striato-thalamo-cortical network precede generalized spike wave discharges. *NeuroImage*. [Online] 39 (4), 1839–1849. Available from: doi:10.1016/j.neuroimage.2007.10.058.

Moeller, F., Tyvaert, L., Nguyen, D.K., LeVan, P., et al. (2009) EEG-fMRI Adding to standard evaluations of patients with nonlesional frontal lobe epilepsy. *Neurology*. 73 (23), 2023–2030.

Molaie, M. & Cruz, A. (1988) The effect of sleep deprivation on the rate of focal interictal epileptiform discharges. *Electroencephalography and Clinical Neurophysiology*. [Online] 70 (4), 288–292. Available from: doi:10.1016/0013-4694(88)90047-8.

Mölle, M., Bergmann, T.O., Marshall, L. & Born, J. (2011) Fast and Slow Spindles during the Sleep Slow Oscillation: Disparate Coalescence and Engagement in Memory Processing. *Sleep*. [Online] 34 (10), 1411–1421. Available from: doi:10.5665/SLEEP.1290.

Mölle, M., Marshall, L., Gais, S. & Born, J. (2002) Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *The Journal of neuroscience*. 22 (24), 10941–10947.

Monti, J.M., Torterolo, P. & Lagos, P. (2013) Melanin-concentrating hormone control of sleep-wake behavior. *Sleep Medicine Reviews*. [Online] 17 (4), 293–298. Available from: doi:10.1016/j.smrv.2012.10.002.

Morgan, V.L., Gore, J.C. & Abou-Khalil, B. (2007) Cluster analysis detection of functional MRI activity in temporal lobe epilepsy. *Epilepsy research*. [Online] 76 (1), 22–33. Available from: doi:10.1016/j.eplepsyres.2007.06.008.

Morison, R.S. & Bassett, D.L. (1945) Electrical Activity of the Thalamus and Basal Ganglia in Decorticate Cats. *Journal of Neurophysiology*. 8 (5), 309–314.

Morrell, F. (1989) Varieties of human secondary epileptogenesis. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 6 (3), 227–275.

Moruzzi, G. & Magoun, H.W. (1949) Brain stem reticular formation and activation of the EEG. *Electroencephalography and Clinical Neurophysiology*. 1 (4), 455–473.

Mouthaan, B.E., Rados, M., Barsi, P., Boon, P., et al. (2016) Current use of imaging and electromagnetic source localization procedures in epilepsy surgery centers across Europe. *Epilepsia*. [Online] 57 (5), 770–776. Available from: doi:10.1111/epi.13347.

Mulley, J.C., Scheffer, I.E., Petrou, S. & Berkovic, S.F. (2003) Channelopathies as a genetic cause of epilepsy. *Current opinion in neurology*. 16 (2), 171–176.

Mullinger, K.J., Castellone, P. & Bowtell, R. (2013) Best Current Practice for Obtaining High Quality EEG Data During Simultaneous fMRI. *Journal of Visualized Experiments : JoVE*. [Online] (76). Available from: doi:10.3791/50283 [Accessed: 3 September 2016].

Mullinger, K.J., Morgan, P.S. & Bowtell, R.W. (2008) Improved artifact correction for combined electroencephalography/functional MRI by means of synchronization and use of vectorcardiogram recordings. *Journal of magnetic resonance imaging: JMRI*. [Online] 27 (3), 607–616. Available from: doi:10.1002/jmri.21277.

Mullinger, K.J., Yan, W.X. & Bowtell, R. (2011) Reducing the gradient artefact in simultaneous EEG-fMRI by adjusting the subject's axial position. *NeuroImage*. [Online] 54 (3), 1942–1950. Available from: doi:10.1016/j.neuroimage.2010.09.079.

Murphy, M., Riedner, B.A., Huber, R., Massimini, M., et al. (2009) Source modeling sleep slow waves. *Proceedings of the National Academy of Sciences*. [Online] 106 (5), 1608–1613. Available from: doi:10.1073/pnas.0807933106.

Murta, T., Leite, M., Carmichael, D.W., Figueiredo, P., et al. (2015a) Electrophysiological correlates of the BOLD signal for EEG-informed fMRI. *Human Brain Mapping*. [Online] 36 (1), 391–414. Available from: doi:10.1002/hbm.22623.

Murta, T., Leite, M., Carmichael, D.W., Figueiredo, P., et al. (2015b) Electrophysiological correlates of the BOLD signal for EEG-informed fMRI: Electrophysiological Correlates of the BOLD Signal. *Human Brain Mapping*. [Online] 36 (1), 391–414. Available from: doi:10.1002/hbm.22623.

Narayanan, J.T., Labar, D.R. & Schaul, N. (2008) Latency to first spike in the EEG of epilepsy patients. *Seizure*. [Online] 17 (1), 34–41. Available from: doi:10.1016/j.seizure.2007.06.003.

National Clinical Guideline Centre (UK) (2012) *The Epilepsies: The Diagnosis and Management of the Epilepsies in Adults and Children in Primary and Secondary Care: Pharmacological Update of Clinical Guideline 20*. National Institute for Health and Clinical Excellence: Guidance. [Online]. London, Royal College of Physicians (UK). Available from: http://www.ncbi.nlm.nih.gov/books/NBK247130/ [Accessed: 27 July 2016].

Negishi, M., Martuzzi, R., Novotny, E.J., Spencer, D.D., et al. (2011) Functional MRI connectivity as a predictor of the surgical outcome of epilepsy. *Epilepsia*. [Online] 52 (9), 1733–1740. Available from: doi:10.1111/j.1528-1167.2011.03191.x.

Nguyen, D.K., Mbacfou, M.T., Nguyen, D.B. & Lassonde, M. (2013) Prevalence of nonlesional focal epilepsy in an adult epilepsy clinic. *The Canadian Journal of Neurological Sciences. Le Journal Canadien Des Sciences Neurologiques*. 40 (2), 198–202.

NHS England (2016) *NHS Diagnostic Waiting Times and Activity Data - April 2016 Monthly Report*. [Online]. June 2016. Available from: https://www.england.nhs.uk/statistics/wp-content/uploads/sites/2/2016/06/DWTA-Report-April-2016.pdf [Accessed: 21 September 2016].

Niazy, R.K., Beckmann, C.F., Iannetti, G.D., Brady, J.M., et al. (2005) Removal of FMRI environment artifacts from EEG data using optimal basis sets. *NeuroImage*. [Online] 28 (3), 720–737. Available from: doi:10.1016/j.neuroimage.2005.06.067.

Nicholas, C.L., Trinder, J. & Colrain, I.M. (2002) Increased production of evoked and spontaneous K-complexes following a night of fragmented sleep. *Sleep*. 25 (8), 882–887.

Nickels, K. & Wirrell, E. (2008) Electrical status epilepticus in sleep. *Seminars in Pediatric Neurology*. [Online] 15 (2), 50–60. Available from: doi:10.1016/j.spen.2008.03.002.

Niedermeyer, E. (1998) Frontal lobe epilepsy: the next frontier. *Clinical EEG (electroencephalography)*. 29 (4), 163–169.

Niedermeyer, E. & Silva, F.H.L. da (2005) *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*. 5th edition. Lippincott Williams & Wilkins.

Noachtar, S., Binnie, C., Ebersole, J., Mauguière, F., et al. (1999) A glossary of terms most commonly used by clinical electroencephalographers and proposal for the report form for the EEG findings. The International Federation of Clinical Neurophysiology. *Electroencephalography and Clinical Neurophysiology. Supplement*. 5221–41.

Noachtar, S. & Rémi, J. (2009) The role of EEG in epilepsy: A critical review. *Epilepsy & Behavior*. [Online] 15 (1), 22–33. Available from: doi:10.1016/j.yebeh.2009.02.035.

Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W., et al. (2012) *Jasper's Basic Mechanisms of the Epilepsies*. 4th edition. Bethesda (MD), National Center for Biotechnology Information (US).

Nowell, M., Rodionov, R., Zombori, G., Sparks, R., et al. (2015) Utility of 3D multimodality imaging in the implantation of intracranial electrodes in epilepsy. *Epilepsia*. [Online] 56 (3), 403–413. Available from: doi:10.1111/epi.12924.

Nunes, V.D., Sawyer, L., Neilson, J., Sarri, G., et al. (2012) Diagnosis and management of the epilepsies in adults and children: summary of updated NICE guidance. *BMJ (Clinical research ed.)*. 344e281.

Obeid, T., Abulaban, A., Al-Ghatani, F., Al-Malki, A.R., et al. (2012) Possession by 'Jinn' as a cause of epilepsy (Saraa): A study from Saudi Arabia. *Seizure*. [Online] 21 (4), 245–249. Available from: doi:10.1016/j.seizure.2012.01.001.

O'Dell, C.M., Das, A., Wallace, G., Ray, S.K., et al. (2012) Understanding the basic mechanisms underlying seizures in mesial temporal lobe epilepsy and possible therapeutic targets: A review. *Journal of Neuroscience Research*. [Online] 90 (5), 913–924. Available from: doi:10.1002/jnr.22829.

Ogawa, S. & Lee, T.M. (1990) Magnetic resonance imaging of blood vessels at high fields: in vivo and in vitro measurements and image simulation. *Magnetic Resonance in Medicine*. 16 (1), 9–18.

Ogawa, S., Lee, T.M., Kay, A.R. & Tank, D.W. (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proceedings of the National Academy of Sciences of the United States of America*. 87 (24), 9868–9872.

Ogawa, S., Lee, T.M., Nayak, A.S. & Glynn, P. (1990) Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magnetic Resonance in Medicine*. 14 (1), 68–78.

Ogbonnaya, S. & Kaliaperumal, C. (2013) Vagal nerve stimulator: Evolving trends. *Journal of Natural Science, Biology and Medicine*. [Online] 4 (1), 8. Available from: doi:10.4103/0976-9668.107254.

O'Muircheartaigh, J. & Richardson, M.P. (2012) Epilepsy and the frontal lobes. *Cortex*. [Online] 48 (2), 144–155. Available from: doi:10.1016/j.cortex.2011.11.012.

Ostwald, D., Porcaro, C. & Bagshaw, A.P. (2010) An information theoretic approach to EEG-fMRI integration of visually evoked responses. *NeuroImage*. [Online] 49 (1), 498–516. Available from: doi:10.1016/j.neuroimage.2009.07.038.

Pace-Schott, E.F. & Hobson, J.A. (2002) The Neurobiology of Sleep: Genetics, cellular physiology and subcortical networks. *Nature Reviews Neuroscience*. [Online] 3 (8), 591–605. Available from: doi:10.1038/nrn895.

Palva, S. & Palva, J.M. (2007) New vistas for α-frequency band oscillations. *Trends in Neurosciences*. [Online] 30 (4), 150–158. Available from: doi:10.1016/j.tins.2007.02.001.

Panayiotopoulos, C.P. (2005) *The Epilepsies: Seizures, Syndromes and Management : Based on the ILAE Classifications and Practice Parameter Guidelines*. Bladon Medical Publishing.

Panayiotopoulos, C.P. (2011) The new ILAE report on terminology and concepts for organization of epileptic seizures: A clinician's critical view and contribution: *The ILAE Classification Report: Critical View*. *Epilepsia*. [Online] 52 (12), 2155–2160. Available from: doi:10.1111/j.1528-1167.2011.03288.x.

Panayiotopoulos, C.P. (2012) The new ILAE report on terminology and concepts for the organization of epilepsies: Critical review and contribution: Critical Review on Organization of Epilepsies. *Epilepsia*. [Online] 53 (3), 399–404. Available from: doi:10.1111/j.1528-1167.2011.03381.x.

Parent, J.M. & Lowenstein, D.H. (1997) Mossy fiber reorganization in the epileptic hippocampus. *Current Opinion in Neurology*. 10 (2), 103–109.

Pekny, M., Wilhelmsson, U. & Pekna, M. (2014) The dual role of astrocyte activation and reactive gliosis. *Neuroscience Letters*. [Online] 56530–38. Available from: doi:10.1016/j.neulet.2013.12.071.

Perez-Reyes, E. (2003) Molecular physiology of low-voltage-activated t-type calcium channels. *Physiological Reviews*. [Online] 83 (1), 117–161. Available from: doi:10.1152/physrev.00018.2002.

Perrault, R., Carrier, J., Desautels, A., Montplaisir, J., et al. (2013) Slow wave activity and slow oscillations in sleepwalkers and controls: effects of 38 h of sleep deprivation. *Journal of Sleep Research*. [Online] 22 (4), 430–433. Available from: doi:10.1111/jsr.12041.

Peszka, J. & Harsh, J. (2002) Effect of sleep deprivation on NREM sleep ERPs and related activity at sleep onset. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*. 46 (3), 275–286.

Petit, J.-M., Burlet-Godinot, S., Magistretti, P.J. & Allaman, I. (2015) Glycogen metabolism and the homeostatic regulation of sleep. *Metabolic Brain Disease*. [Online] 30 (1), 263–279. Available from: doi:10.1007/s11011-014-9629-x.

Petsche, H., Pockberger, H. & Rappelsberger, P. (1984) On the search for the sources of the electroencephalogram. *Neuroscience*. 11 (1), 1–27.

Peyron, C., Tighe, D.K., van den Pol, A.N., de Lecea, L., et al. (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 18 (23), 9996–10015.

Picchioni, D., Duyn, J.H. & Horovitz, S.G. (2013) Sleep and the Functional Connectome. *NeuroImage*. [Online] 80387–396. Available from: doi:10.1016/j.neuroimage.2013.05.067.

Picot, M.-C., Baldy-Moulinier, M., Daurs, J.-P., Dujols, P., et al. (2008) The prevalence of epilepsy and pharmacoresistant epilepsy in adults: A population-based study in a Western European country. *Epilepsia*. [Online] 49 (7), 1230–1238. Available from: doi:10.1111/j.1528-1167.2008.01579.x.

Pinault, D. & O'Brien, T.J. (2005) Cellular and network mechanisms of genetically-determined absence seizures. *Thalamus & Related Systems*. [Online] 3 (3), 181–203. Available from: doi:10.1017/S1472928807000209.

Pisarenco, I., Caporro, M., Prosperetti, C. & Manconi, M. (2014) High-density electroencephalography as an innovative tool to explore sleep physiology and sleep related disorders. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*. [Online] Available from: doi:10.1016/j.ijpsycho.2014.01.002.

Pitkänen, A., Kharatishvili, I., Karhunen, H., Lukasiuk, K., et al. (2007) Epileptogenesis in experimental models. *Epilepsia*. 48 Suppl 213–20.

Pitkänen, A. & Sutula, T.P. (2002) Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *The Lancet Neurology*. 1 (3), 173–181.

Pittau, F., Grova, C., Moeller, F., Dubeau, F., et al. (2012) Patterns of altered functional connectivity in mesial temporal lobe epilepsy. *Epilepsia*. [Online] 53 (6), 1013–1023. Available from: doi:10.1111/j.1528-1167.2012.03464.x.

Pittau, F., Levan, P., Moeller, F., Gholipour, T., et al. (2011) Changes preceding interictal epileptic EEG abnormalities: comparison between EEG/fMRI and intracerebral EEG. *Epilepsia*. [Online] 52 (6), 1120–1129. Available from: doi:10.1111/j.1528-1167.2011.03072.x.

Pittau, F. & Vulliemoz, S. (2015) Functional brain networks in epilepsy: recent advances in noninvasive mapping. *Current Opinion in Neurology*. [Online] 28 (4), 338–343. Available from: doi:10.1097/WCO.0000000000000221.

Polack, P.-O., Guillemain, I., Hu, E., Deransart, C., et al. (2007) Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. [Online] 27 (24), 6590–6599. Available from: doi:10.1523/JNEUROSCI.0753-07.2007.

Polkey, C.E. (2004) Clinical outcome of epilepsy surgery. *Current Opinion in Neurology*. 17 (2), 173–178.

Porcaro, C., Ostwald, D. & Bagshaw, A.P. (2010) Functional source separation improves the quality of single trial visual evoked potentials recorded during concurrent EEG-fMRI. *NeuroImage*. [Online] 50 (1), 112–123. Available from: doi:10.1016/j.neuroimage.2009.12.002.

Portas, C.M., Krakow, K., Allen, P., Josephs, O., et al. (2000) Auditory processing across the sleep-wake cycle: simultaneous EEG and fMRI monitoring in humans. *Neuron*. 28 (3), 991–999.

Raghavendra, S., Nooraine, J. & Mirsattari, S.M. (2012) Role of Electroencephalography in Presurgical Evaluation of Temporal Lobe Epilepsy. *Epilepsy Research and Treatment*. [Online] 2012. Available from: doi:10.1155/2012/204693 [Accessed: 8 September 2016].

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., et al. (2001) A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America*. [Online] 98 (2), 676–682. Available from: doi:10.1073/pnas.98.2.676.

Raichle, M.E. & Snyder, A.Z. (2007) A default mode of brain function: A brief history of an evolving idea. *NeuroImage*. [Online] 37 (4), 1083–1090. Available from: doi:10.1016/j.neuroimage.2007.02.041.

Rakic, P. (1988) Defects of neuronal migration and the pathogenesis of cortical malformations. *Progress in Brain Research*. 7315–37.

Ramli, N., Rahmat, K., Lim, K.S. & Tan, C.T. (2015) Neuroimaging in refractory epilepsy. Current practice and evolving trends. *European Journal of Radiology*. [Online] 84 (9), 1791–1800. Available from: doi:10.1016/j.ejrad.2015.03.024.

Rathakrishnan, R., Moeller, F., Levan, P., Dubeau, F., et al. (2010) BOLD signal changes preceding negative responses in EEG-fMRI in patients with focal epilepsy. *Epilepsia*. [Online] 51 (9), 1837–1845. Available from: doi:10.1111/j.1528-1167.2010.02643.x.

Ray, A., Tao, J.X., Hawes-Ebersole, S.M. & Ebersole, J.S. (2007) Localizing value of scalp EEG spikes: A simultaneous scalp and intracranial study. *Clinical Neurophysiology*. [Online] 118 (1), 69–79. Available from: doi:10.1016/j.clinph.2006.09.010.

Rechtschaffen, A. & Kales, A. (1968) *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects*. Brain Information Service/Brain Research Institute, University of California.

Reppert, S.M. & Weaver, D.R. (2002) Coordination of circadian timing in mammals. *Nature*. [Online] 418 (6901), 935–941. Available from: doi:10.1038/nature00965.

Represa, A., Niquet, J., Pollard, H. & Ben-Ari, Y. (1995) Cell death, gliosis, and synaptic remodeling in the hippocampus of epileptic rats. *Journal of Neurobiology*. [Online] 26 (3), 413–425. Available from: doi:10.1002/neu.480260313.

Reyes, A., Thesen, T., Wang, X., Hahn, D., et al. (2016) Resting-state functional MRI distinguishes temporal lobe epilepsy subtypes. *Epilepsia*. [Online] Available from: doi:10.1111/epi.13456.

Richardson, M. (2010) Current themes in neuroimaging of epilepsy: Brain networks, dynamic phenomena, and clinical relevance. *Clinical Neurophysiology*. [Online] 121 (8), 1153–1175. Available from: doi:10.1016/j.clinph.2010.01.004.

Rodionov, R., De Martino, F., Laufs, H., Carmichael, D.W., et al. (2007) Independent component analysis of interictal fMRI in focal epilepsy: Comparison with general linear model-based EEG-correlated fMRI. *NeuroImage*. [Online] 38 (3), 488–500. Available from: doi:10.1016/j.neuroimage.2007.08.003.

Rodionov, R., Vollmar, C., Nowell, M., Miserocchi, A., et al. (2013) Feasibility of multimodal 3D neuroimaging to guide implantation of intracranial EEG electrodes. *Epilepsy Research*. [Online] 107 (1–2), 91–100. Available from: doi:10.1016/j.eplepsyres.2013.08.002.

Rollings, D.T., Assecondi, S., Ostwald, D., Porcaro, C., et al. (2015) Early haemodynamic changes observed in patients with epilepsy, in a visual experiment and in simulations. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. [Online] Available from: doi:10.1016/j.clinph.2015.07.008.

Roopun, A.K., Middleton, S.J., Cunningham, M.O., LeBeau, F.E.N., et al. (2006) A beta2-frequency (20–30 Hz) oscillation in nonsynaptic networks of somatosensory cortex. *Proceedings of the National Academy of Sciences of the United States of America*. [Online] 103 (42), 15646–15650. Available from: doi:10.1073/pnas.0607443103.

Rosenow, F. & Lüders, H. (2001) Presurgical evaluation of epilepsy. *Brain: A Journal of Neurology*. 124 (Pt 9), 1683–1700.

Roth, M., Shaw, J. & Green, J. (1956) The form voltage distribution and physiological significance of the K-complex. *Electroencephalography and Clinical Neurophysiology*. 8 (3), 385–402.

Roupakiotis, S.C., Gatzonis, S.D., Triantafyllou, N., Mantouvalos, V., et al. (2000) The usefulness of sleep and sleep deprivation as activating methods in electroencephalographic recording: contribution to a long-standing discussion. *Seizure*. [Online] 9 (8), 580–584. Available from: doi:10.1053/seiz.2000.0462.

Ruan, D., Yu, X.-B., Shrestha, S., Wang, L., et al. (2015) The Role of Hemosiderin Excision in Seizure Outcome in Cerebral Cavernous Malformation Surgery: A Systematic Review and Meta-Analysis. *PloS One*. [Online] 10 (8), e0136619. Available from: doi:10.1371/journal.pone.0136619.

Ruggieri, A., Vaudano, A.E., Benuzzi, F., Serafini, M., et al. (2015) Mapping (and modeling) physiological movements during EEG-fMRI recordings: the added value of the video acquired simultaneously. *Journal of Neuroscience Methods*. [Online] 239223–237. Available from: doi:10.1016/j.jneumeth.2014.10.005.

Salek-Haddadi, A. (2002) Simultaneous EEG-Correlated Ictal fMRI. *NeuroImage*. [Online] 16 (1), 32–40. Available from: doi:10.1006/nimg.2002.1073.

Salek-Haddadi, A., Diehl, B., Hamandi, K., Merschhemke, M., et al. (2006) Hemodynamic correlates of epileptiform discharges: An EEG-fMRI study of 63 patients with focal epilepsy. *Brain Research*. [Online] 1088 (1), 148–166. Available from: doi:10.1016/j.brainres.2006.02.098.

Salek-Haddadi, A., Merschhemke, M., Lemieux, L. & Fish, D.R. (2002) Simultaneous EEG-Correlated Ictal fMRI. *NeuroImage*. [Online] 16 (1), 32–40. Available from: doi:10.1006/nimg.2002.1073.

Sämann, P.G., Wehrle, R., Hoehn, D., Spoormaker, V.I., et al. (2011) Development of the brain's default mode network from wakefulness to slow wave sleep. *Cerebral Cortex (New York, N.Y.: 1991)*. [Online] 21 (9), 2082–2093. Available from: doi:10.1093/cercor/bhq295.

Sander, J.W. (1993) Some aspects of prognosis in the epilepsies: a review. *Epilepsia*. 34 (6), 1007–1016.

Saper, C.B., Chou, T.C. & Scammell, T.E. (2001) The sleep switch: hypothalamic control of sleep and wakefulness. *Trends in Neurosciences*. 24 (12), 726–731.

Saper, C.B., Fuller, P.M., Pedersen, N.P., Lu, J., et al. (2010) Sleep State Switching. *Neuron*. [Online] 68 (6), 1023–1042. Available from: doi:10.1016/j.neuron.2010.11.032.

Saper, C.B., Scammell, T.E. & Lu, J. (2005) Hypothalamic regulation of sleep and circadian rhythms. *Nature*. [Online] 437 (7063), 1257–1263. Available from: doi:10.1038/nature04284.

Scanlon, C., Mueller, S.G., Cheong, I., Hartig, M., et al. (2013) Grey and white matter abnormalities in temporal lobe epilepsy with and without mesial temporal sclerosis. *Journal of Neurology*. [Online] 260 (9), 2320–2329. Available from: doi:10.1007/s00415-013-6974-3.

Schabus, M., Dang-Vu, T.T., Albouy, G., Balteau, E., et al. (2007) Hemodynamic cerebral correlates of sleep spindles during human non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences*. 104 (32), 13164–13169.

Schabus, M., Dang-Vu, T.T., Heib, D.P.J., Boly, M., et al. (2012) The Fate of Incoming Stimuli during NREM Sleep is Determined by Spindles and the Phase of the Slow Oscillation. *Frontiers in Neurology*. [Online] 3. Available from: doi:10.3389/fneur.2012.00040 [Accessed: 8 June 2016].

Schmidt, C., Collette, F., Cajochen, C. & Peigneux, P. (2007) A time to think: Circadian rhythms in human cognition. *Cognitive Neuropsychology*. [Online] 24 (7), 755–789. Available from: doi:10.1080/02643290701754158.

Schmidt, C., Peigneux, P., Leclercq, Y., Sterpenich, V., et al. (2012) Circadian Preference Modulates the Neural Substrate of Conflict Processing across the Day Shin Yamazaki (ed.). *PLoS ONE*. [Online] 7 (1), e29658. Available from: doi:10.1371/journal.pone.0029658.

Schmidt, H., Petkov, G., Richardson, M.P. & Terry, J.R. (2014) Dynamics on Networks: The Role of Local Dynamics and Global Networks on the Emergence of Hypersynchronous Neural Activity Jean Daunizeau (ed.). *PLoS Computational Biology*. [Online] 10 (11), e1003947. Available from: doi:10.1371/journal.pcbi.1003947.

Schmidt, M.H. (2014) The energy allocation function of sleep: A unifying theory of sleep, torpor, and continuous wakefulness. *Neuroscience & Biobehavioral Reviews*. [Online] 47122–153. Available from: doi:10.1016/j.neubiorev.2014.08.001.

Schulz, R., Lüders, H.O., Hoppe, M., Tuxhorn, I., et al. (2000) Interictal EEG and ictal scalp EEG propagation are highly predictive of surgical outcome in mesial temporal lobe epilepsy. *Epilepsia*. 41 (5), 564–570.

Schwartz, T.H. (2007) Neurovascular Coupling and Epilepsy: Hemodynamic Markers for Localizing and Predicting Seizure Onset. *Epilepsy Currents*. [Online] 7 (4), 91–94. Available from: doi:10.1111/j.1535-7511.2007.00183.x.

Schwartz, T.H., Hong, S.-B., Bagshaw, A.P., Chauvel, P., et al. (2011) Preictal changes in cerebral haemodynamics: review of findings and insights from intracerebral EEG. *Epilepsy Research*. [Online] 97 (3), 252–266. Available from: doi:10.1016/j.eplepsyres.2011.07.013.

Scott, C.A., Fish, D.R., Smith, S.J., Free, S.L., et al. (1999) Presurgical evaluation of patients with epilepsy and normal MRI: role of scalp video-EEG telemetry. *Journal of Neurology, Neurosurgery, and Psychiatry*. 66 (1), 69–71.

Seeck, M., Lazeyras, F., Michel, C.M., Blanke, O., et al. (1998) Non-invasive epileptic focus localization using EEG-triggered functional MRI and electromagnetic tomography. *Electroencephalography and clinical Neurophysiology*. 106 (6), 508–512.

Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., et al. (2007) Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. *Journal of Neuroscience*. [Online] 27 (9), 2349–2356. Available from: doi:10.1523/JNEUROSCI.5587-06.2007.

Seidenbecher, T., Staak, R. & Pape, H.C. (1998) Relations between cortical and thalamic cellular activities during absence seizures in rats. *The European Journal of Neuroscience*. 10 (3), 1103–1112.

Seneviratne, U., Cook, M. & D'Souza, W. (2014) Focal abnormalities in idiopathic generalized epilepsy: A critical review of the literature. *Epilepsia*. [Online] 55 (8), 1157–1169. Available from: doi:10.1111/epi.12688.

Sforza, E., Chapotot, F., Pigeau, R., Paul, P.N., et al. (2004) Effects of sleep deprivation on spontaneous arousals in humans. *Sleep*. 27 (6), 1068–1075.

Shannon, B.J., Dosenbach, R.A., Su, Y., Vlassenko, A.G., et al. (2013) Morning-evening variation in human brain metabolism and memory circuits. *Journal of Neurophysiology*. [Online] 109 (5), 1444–1456. Available from: doi:10.1152/jn.00651.2012.

Sharbrough, F., Chatrian, G.-E., Lesser, R.P., Lüders, H., et al. (1991) American Electroencephalographic Society guidelines for standard electrode position nomenclature. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 8 (2), 200–202.

Sharma, A.K., Reams, R.Y., Jordan, W.H., Miller, M.A., et al. (2007) Mesial Temporal Lobe Epilepsy: Pathogenesis, Induced Rodent Models and Lesions. *Toxicologic Pathology*. [Online] 35 (7), 984–999. Available from: doi:10.1080/01926230701748305.

Sherin, J.E., Elmquist, J.K., Torrealba, F. & Saper, C.B. (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 18 (12), 4705–4721.

Sherin, J.E., Shiromani, P.J., McCarley, R.W. & Saper, C.B. (1996) Activation of ventrolateral preoptic neurons during sleep. *Science (New York, N.Y.)*. 271 (5246), 216–219.

Shin, H.W., Jewells, V., Sheikh, A., Zhang, J., et al. (2015) Initial experience in hybrid PET-MRI for evaluation of refractory focal onset epilepsy. *Seizure*. [Online] 311–4. Available from: doi:10.1016/j.seizure.2015.06.010.

Shinnar, S. (2010) The new ILAE classification. *Epilepsia*. [Online] 51 (4), 715–717. Available from: doi:10.1111/j.1528-1167.2010.02542.x.

Shouse, M.N., da Silva, A.M. & Sammaritano, M. (1996) Circadian rhythm, sleep, and epilepsy. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. 13 (1), 32–50.

Shulman, G.L., Astafiev, S.V., Franke, D., Pope, D.L.W., et al. (2009) Interaction of Stimulus-Driven Reorienting and Expectation in Ventral and Dorsal Frontoparietal and Basal Ganglia-Cortical Networks. *Journal of Neuroscience*. [Online] 29 (14), 4392–4407. Available from: doi:10.1523/JNEUROSCI.5609-08.2009.

Siegel, J.M. (2008) Do all animals sleep? *Trends in Neurosciences*. [Online] 31 (4), 208–213. Available from: doi:10.1016/j.tins.2008.02.001.

Sierra-Marcos, A., Maestro, I., Falcón, C., Donaire, A., et al. (2013) Ictal EEG-fMRI in localization of epileptogenic area in patients with refractory neocortical focal epilepsy. *Epilepsia*. [Online] 54 (9), 1688–1698. Available from: doi:10.1111/epi.12329.

Sijbers, J., Van Audekerke, J., Verhoye, M., Van der Linden, A., et al. (2000) Reduction of ECG and gradient related artifacts in simultaneously recorded human EEG/MRI data. *Magnetic*

Resonance Imaging. [Online] 18 (7), 881–886. Available from: doi:10.1016/S0730-725X(00)00178-8.

Sirven, J.I. (2009) Not all first seizures are created equally. *Epilepsy Currents*. 9 (6), 164–165.

Sitnikova, E., Hramov, A.E., Koronovsky, A.A. & van Luijtelaar, G. (2009) Sleep spindles and spike-wave discharges in EEG: Their generic features, similarities and distinctions disclosed with Fourier transform and continuous wavelet analysis. *Journal of Neuroscience Methods*. [Online] 180 (2), 304–316. Available from: doi:10.1016/j.jneumeth.2009.04.006.

Sladky, R., Friston, K.J., Tröstl, J., Cunnington, R., et al. (2011) Slice-timing effects and their correction in functional MRI. *Neuroimage*. [Online] 58 (2–2), 588–594. Available from: doi:10.1016/j.neuroimage.2011.06.078.

van Slegtenhorst, M., de Hoogt, R., Hermans, C., Nellist, M., et al. (1997) Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science (New York, N.Y.)*. 277 (5327), 805–808.

Sloviter, R.S. (1991) Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the ‘dormant basket cell’ hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus*. [Online] 1 (1), 41–66. Available from: doi:10.1002/hipo.450010106.

Smith, S.J.M. (2005) EEG in the diagnosis, classification, and management of patients with epilepsy. *Journal of Neurology, Neurosurgery & Psychiatry*. [Online] 76 (suppl_2), ii2-ii7. Available from: doi:10.1136/jnnp.2005.069245.

Spanaki, M.V., Spencer, S.S., Corsi, M., MacMullan, J., et al. (1999) Sensitivity and specificity of quantitative difference SPECT analysis in seizure localization. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*. 40 (5), 730–736.

Stanisz, G.J., Odrobina, E.E., Pun, J., Escaravage, M., et al. (2005) T1, T2 relaxation and magnetization transfer in tissue at 3T. *Magnetic Resonance in Medicine*. [Online] 54 (3), 507–512. Available from: doi:10.1002/mrm.20605.

Staresina, B.P., Bergmann, T.O., Bonnefond, M., van der Meij, R., et al. (2015) Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature Neuroscience*. [Online] 18 (11), 1679–1686. Available from: doi:10.1038/nn.4119.

Steer, S., Pickrell, W.O., Kerr, M.P. & Thomas, R.H. (2014) Epilepsy prevalence and socioeconomic deprivation in England. *Epilepsia*. [Online] 55 (10), 1634–1641. Available from: doi:10.1111/epi.12763.

Stefanovic, B., Warnking, J.M., Kobayashi, E., Bagshaw, A.P., et al. (2005) Hemodynamic and metabolic responses to activation, deactivation and epileptic discharges. *NeuroImage*. [Online] 28 (1), 205–215. Available from: doi:10.1016/j.neuroimage.2005.05.038.

Steinhoff, B.J., So, N.K., Lim, S. & Lüders, H.O. (1995) Ictal scalp EEG in temporal lobe epilepsy with unitemporal versus bitemporal interictal epileptiform discharges. *Neurology*. 45 (5), 889–896.

Stenner, M.-P., Bauer, M., Haggard, P., Heinze, H.-J., et al. (2014) Enhanced Alpha-oscillations in Visual Cortex during Anticipation of Self-generated Visual Stimulation. *Journal of Cognitive Neuroscience*. [Online] 26 (11), 2540–2551. Available from: doi:10.1162/jocn_a_00658.

Steriade, M. (2006) Grouping of brain rhythms in corticothalamic systems. *Neuroscience*. [Online] 137 (4), 1087–1106. Available from: doi:10.1016/j.neuroscience.2005.10.029.

Steriade, M. (2005) Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends in Neurosciences*. [Online] 28 (6), 317–324. Available from: doi:10.1016/j.tins.2005.03.007.

Steriade, M. (1994) Sleep oscillations and their blockage by activating systems. *Journal of Psychiatry and Neuroscience*. 19 (5), 354–358.

Steriade, M. (1995) Thalamic origin of sleep spindles: Morison and Bassett (1945). *Journal of Neurophysiology*. 73 (3), 921–922.

Steriade, M. (2003) The corticothalamic system in sleep. *Frontiers in Bioscience: A Journal and Virtual Library*. 8d878-899.

Steriade, M., McCormick, D.A. & Sejnowski, T.J. (1993) Thalamocortical oscillations in the sleeping and aroused brain. *Science (New York, N.Y.)*. 262 (5134), 679–685.

Steriade, M., Nunez, A. & Amzica, F. (1993) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *The Journal of Neuroscience*. 13 (8), 3252–3265.

Steriade, M., Nuñez, A. & Amzica, F. (1993) Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 13 (8), 3266–3283.

Stern, J.M., Caporro, M., Haneef, Z., Yeh, H.J., et al. (2011) Functional imaging of sleep vertex sharp transients. *Clinical Neurophysiology*. [Online] 122 (7), 1382–1386. Available from: doi:10.1016/j.clinph.2010.12.049.

Storti, S.F., Formaggio, E., Bertoldo, A., Manganotti, P., et al. (2013) Modelling hemodynamic response function in epilepsy. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. [Online] 124 (11), 2108–2118. Available from: doi:10.1016/j.clinph.2013.05.024.

Strecker, R.E., Morairty, S., Thakkar, M.M., Porkka-Heiskanen, T., et al. (2000) Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behavioural Brain Research*. 115 (2), 183–204.

Sun, L. & Hinrichs, H. (2009) Simultaneously recorded EEG-fMRI: removal of gradient artifacts by subtraction of head movement related average artifact waveforms. *Human Brain Mapping*. [Online] 30 (10), 3361–3377. Available from: doi:10.1002/hbm.20758.

Swihart, B.J., Caffo, B., Bandeen-Roche, K. & Punjabi, N.M. (2008) Characterizing Sleep Structure Using the Hypnogram. *Journal of Clinical Sleep Medicine : JCSM : official publication of the American Academy of Sleep Medicine*. 4 (4), 349–355.

Takahashi, D.K., Vargas, J.R. & Wilcox, K.S. (2010) Increased coupling and altered glutamate transport currents in astrocytes following kainic-acid-induced status epilepticus. *Neurobiology of Disease*. [Online] 40 (3), 573–585. Available from: doi:10.1016/j.nbd.2010.07.018.

Tamaki, M., Bang, J.W., Watanabe, T. & Sasaki, Y. (2016) Night Watch in One Brain Hemisphere during Sleep Associated with the First-Night Effect in Humans. *Current Biology*. [Online] 26 (9), 1190–1194. Available from: doi:10.1016/j.cub.2016.02.063.

Tamaki, M., Nittono, H., Hayashi, M. & Hori, T. (2005) Examination of the first-night effect during the sleep-onset period. *Sleep*. 28 (2), 195–202.

Tanaka, H., Hayashi, M. & Hori, T. (2000) Topographical characteristics of slow wave activities during the transition from wakefulness to sleep. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*. 111 (3), 417–427.

Tao, J.X., Baldwin, M., Hawes-Ebersole, S. & Ebersole, J.S. (2007) Cortical substrates of scalp EEG epileptiform discharges. *Journal of Clinical Neurophysiology*. 24 (2), 96–100.

Tao, J.X., Ray, A., Hawes-Ebersole, S. & Ebersole, J.S. (2005) Intracranial EEG substrates of scalp EEG interictal spikes. *Epilepsia*. [Online] 46 (5), 669–676. Available from: doi:10.1111/j.1528-1167.2005.11404.x.

Tatum, W.O., Husain, A.M., Benbadis, S.R. & Kaplan, P.W. (2006) Normal adult EEG and patterns of uncertain significance. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*. [Online] 23 (3), 194–207. Available from: doi:10.1097/01.wnp.0000220110.92126.a6.

Taylor, D.C., Falconer, M.A., Bruton, C.J. & Corsellis, J.A. (1971) Focal dysplasia of the cerebral cortex in epilepsy. *Journal of Neurology, Neurosurgery, and Psychiatry*. 34 (4), 369–387.

Téllez-Zenteno, J.F., Hernández-Ronquillo, L., Buckley, S., Zahagun, R., et al. (2014) A validation of the new definition of drug-resistant epilepsy by the International League Against Epilepsy. *Epilepsia*. [Online] 55 (6), 829–834. Available from: doi:10.1111/epi.12633.

Téllez-Zenteno, J.F., Ronquillo, L.H., Moien-Afshari, F. & Wiebe, S. (2010) Surgical outcomes in lesional and non-lesional epilepsy: A systematic review and meta-analysis. *Epilepsy Research*. [Online] 89 (2–3), 310–318. Available from: doi:10.1016/j.eplepsyres.2010.02.007.

Temkin, N.R. (2003) Risk factors for posttraumatic seizures in adults. *Epilepsia*. 44 Suppl 1018–20.

Temkin, O. (1994) *The Falling Sickness: A History of Epilepsy from the Greeks to the Beginnings of Modern Neurology*. 2nd, revised edition. JHU Press.

Terry, J.R., Benjamin, O. & Richardson, M.P. (2012) Seizure generation: The role of nodes and networks: Networks and Seizure Generation. *Epilepsia*. [Online] 53 (9), e166–e169. Available from: doi:10.1111/j.1528-1167.2012.03560.x.

The Royal College of Radiologists (2016) *Information submitted to Health Education England workforce planning and education commissioning round - 2015/16*. [Online]. 2016. Available from: https://www.rcr.ac.uk/sites/default/files/documents/hee_evidence_2015-16_clinical_radiology.pdf [Accessed: 21 September 2016].

Thom, M. (2014) Review: Hippocampal sclerosis in epilepsy: a neuropathology review: Hippocampal sclerosis in epilepsy. *Neuropathology and Applied Neurobiology*. [Online] 40 (5), 520–543. Available from: doi:10.1111/nan.12150.

Thomas, M., Sing, H., Belenky, G., Holcomb, H., et al. (2000) Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *Journal of Sleep Research*. 9 (4), 335–352.

Thornton, R., Laufs, H., Rodionov, R., Cannadathu, S., et al. (2010) EEG correlated functional MRI and postoperative outcome in focal epilepsy. *Journal of Neurology, Neurosurgery, and Psychiatry*. [Online] 81 (8), 922–927. Available from: doi:10.1136/jnnp.2009.196253.

Timofeev, I. & Chauvette, S. (2013) The Spindles: Are They Still Thalamic? *Sleep*. [Online] 36 (6), 825–826. Available from: doi:10.5665/sleep.2702.

Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T.J., et al. (2000) Origin of slow cortical oscillations in deafferented cortical slabs. *Cerebral Cortex (New York, N.Y.: 1991)*. 10 (12), 1185–1199.

Timofeev, I. & Steriade, M. (1996) Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *Journal of Neurophysiology*. 76 (6), 4152–4168.

de Tisi, J., Bell, G.S., Peacock, J.L., McEvoy, A.W., et al. (2011) The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. *The Lancet*. 378 (9800), 1388–1395.

Toledo, M., Sarria-Estrada, S., Quintana, M., Auger, C., et al. (2013) 3 TESLA MR imaging in adults with focal onset epilepsy. *Clinical Neurology and Neurosurgery*. [Online] 115 (10), 2111–2116. Available from: doi:10.1016/j.clineuro.2013.07.039.

Tononi, G. & Cirelli, C. (2014) Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. *Neuron*. [Online] 81 (1), 12–34. Available from: doi:10.1016/j.neuron.2013.12.025.

Tononi, G. & Cirelli, C. (2006) Sleep function and synaptic homeostasis. *Sleep Medicine Reviews*. [Online] 10 (1), 49–62. Available from: doi:10.1016/j.smrv.2005.05.002.

Triggle, N. (2015) NHS trusts' overspend hits £1.6bn. *BBC News*. [Online] 20 November. Available from: http://www.bbc.co.uk/news/health-34879194 [Accessed: 19 September 2016].

Truccolo, W., Donoghue, J.A., Hochberg, L.R., Eskandar, E.N., et al. (2011) Single-neuron dynamics in human focal epilepsy. *Nature neuroscience*. [Online] 14 (5), 635–641. Available from: doi:10.1038/nn.2782.

Uijl, S.G., Leijten, F.S.S., Parra, J., Arends, J.B.A.M., et al. (2005) What is the current evidence on decision-making after referral for temporal lobe epilepsy surgery? *Seizure*. [Online] 14 (8), 534–540. Available from: doi:10.1016/j.seizure.2005.08.001.

Urbach, H., Mast, H., Egger, K. & Mader, I. (2015) Presurgical MR Imaging in Epilepsy. *Clinical Neuroradiology*. [Online] 25 Suppl 2151–155. Available from: doi:10.1007/s00062-015-0387-x.

Uschakov, A., Gong, H., McGinty, D. & Szymusiak, R. (2007) Efferent projections from the median preoptic nucleus to sleep- and arousal-regulatory nuclei in the rat brain. *Neuroscience*. [Online] 150 (1), 104–120. Available from: doi:10.1016/j.neuroscience.2007.05.055.

Van Dort, C.J., Zachs, D.P., Kenny, J.D., Zheng, S., et al. (2015) Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. *Proceedings of the National Academy of Sciences of the United States of America*. [Online] 112 (2), 584–589. Available from: doi:10.1073/pnas.1423136112.

Vaughn, B.V., D'Cruz, O.F., Beach, R. & Messenheimer, J.A. (1996) Improvement of epileptic seizure control with treatment of obstructive sleep apnoea. *Seizure*. 5 (1), 73–78.

Veldhuizen, R., Binnie, C.D. & Beintema, D.J. (1983) The effect of sleep deprivation on the EEG in epilepsy. *Electroencephalography and Clinical Neurophysiology*. [Online] 55 (5), 505–512. Available from: doi:10.1016/0013-4694(83)90161-X.

van de Ven, V.G., Formisano, E., Prvulovic, D., Roeder, C.H., et al. (2004) Functional connectivity as revealed by spatial independent component analysis of fMRI measurements during rest. *Human Brain Mapping*. [Online] 22 (3), 165–178. Available from: doi:10.1002/hbm.20022.

Vlooswijk, M.C.G., Jansen, J.F.A., Jeukens, C.R.L.P.N., Majoie, H.J.M., et al. (2011) Memory processes and prefrontal network dysfunction in cryptogenic epilepsy. *Epilepsia*. [Online] 52 (8), 1467–1475. Available from: doi:10.1111/j.1528-1167.2011.03108.x.

Volgushev, M., Chauvette, S., Mukovski, M. & Timofeev, I. (2006) Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations [corrected]. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. [Online] 26 (21), 5665–5672. Available from: doi:10.1523/JNEUROSCI.0279-06.2006.

Vyazovskiy, V.V., Olcese, U., Lazimy, Y.M., Faraguna, U., et al. (2009) Cortical Firing and Sleep Homeostasis. *Neuron*. [Online] 63 (6), 865–878. Available from: doi:10.1016/j.neuron.2009.08.024.

Warach, S., Ives, J.R., Schlaug, G., Patel, M.R., et al. (1996) EEG-triggered echo-planar functional MRI in epilepsy. *Neurology*. 47 (1), 89–93.

Watson, N.F., Badr, M.S., Belenky, G., Bliwise, D.L., et al. (2015) Recommended Amount of Sleep for a Healthy Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society. *Sleep*. 38 (6), 843–844.

Wei, H.L., An, J., Zeng, L.L., Shen, H., et al. (2015a) Altered functional connectivity among default, attention, and control networks in idiopathic generalized epilepsy. *Epilepsy & Behavior: E&B*. [Online] 46118–125. Available from: doi:10.1016/j.yebeh.2015.03.031.

Wei, H.L., An, J., Zeng, L.L., Shen, H., et al. (2015b) Altered functional connectivity among default, attention, and control networks in idiopathic generalized epilepsy. *Epilepsy & Behavior: E&B*. [Online] 46118–125. Available from: doi:10.1016/j.yebeh.2015.03.031.

Werth, E., Achermann, P., Dijk, D.J. & Borbély, A.A. (1997) Spindle frequency activity in the sleep EEG: individual differences and topographic distribution. *Electroencephalography and Clinical Neurophysiology*. 103 (5), 535–542.

Westmoreland, B.F. (1998) The EEG findings in extratemporal seizures. *Epilepsia*. 39 Suppl 4S1-8.

Wieser, H.-G. (2004) ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia*. [Online] 45 (6), 695–714. Available from: doi:10.1111/j.0013-9580.2004.09004.x.

Wilf, M., Ramot, M., Furman-Haran, E., Arzi, A., et al. (2016) Diminished Auditory Responses during NREM Sleep Correlate with the Hierarchy of Language Processing. *PloS One*. [Online] 11 (6), e0157143. Available from: doi:10.1371/journal.pone.0157143.

Williams, G.W., Lüders, H.O., Brickner, A., Goormastic, M., et al. (1985) Interobserver variability in EEG interpretation. *Neurology*. 35 (12), 1714–1719.

Wolf, P. (2010) Much ado about nothing? *Epilepsia*. 51 (4), 717–718.

World Health Organization (ed.) (2010) *Epilepsy in the WHO Eastern Mediterranean region: bridging the gap*. Cairo, Egypt, World Health Organization, Regional Office for the Eastern Mediterranean.

Wuarin, J.P. & Dudek, F.E. (1996) Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 16 (14), 4438–4448.

Xie, L., Kang, H., Xu, Q., Chen, M.J., et al. (2013) Sleep Drives Metabolite Clearance from the Adult Brain. *Science (New York, N.Y.)*. [Online] 342 (6156). Available from: doi:10.1126/science.1241224 [Accessed: 25 August 2016].

Yang, T., Zhou, D. & Stefan, H. (2010) Why mesial temporal lobe epilepsy with hippocampal sclerosis is progressive: Uncontrolled inflammation drives disease progression? *Journal of the Neurological Sciences*. [Online] 296 (1–2), 1–6. Available from: doi:10.1016/j.jns.2010.06.002.

Yasoshima, A., Hayashi, H., Iijima, S., Sugita, Y., et al. (1984) Potential distribution of vertex sharp wave and saw-toothed wave on the scalp. *Electroencephalography and Clinical Neurophysiology*. 58 (1), 73–76.

Yeo, B.T.T., Tandi, J. & Chee, M.W.L. (2015) Functional connectivity during rested wakefulness predicts vulnerability to sleep deprivation. *NeuroImage*. [Online] 111147–158. Available from: doi:10.1016/j.neuroimage.2015.02.018.

Yilmazer-Hanke, D.M., Wolf, H.K., Schramm, J., Elger, C.E., et al. (2000) Subregional pathology of the amygdala complex and entorhinal region in surgical specimens from patients with pharmacoresistant temporal lobe epilepsy. *Journal of Neuropathology and Experimental Neurology*. 59 (10), 907–920.

Zaborszky, L. & Duque, A. (2003) Sleep-wake mechanisms and basal forebrain circuitry. *Frontiers in Bioscience: A Journal and Virtual Library*. 8d1146-1169.

Zang, Y.-F., He, Y., Zhu, C.-Z., Cao, Q.-J., et al. (2007) Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain & Development*. [Online] 29 (2), 83–91. Available from: doi:10.1016/j.braindev.2006.07.002.

Zerouali, Y., Lina, J.-M., Sekerovic, Z., Godbout, J., et al. (2014) A time-frequency analysis of the dynamics of cortical networks of sleep spindles from MEG-EEG recordings. *Frontiers in Neuroscience*. [Online] 8. Available from: doi:10.3389/fnins.2014.00310 [Accessed: 8 June 2016].

Zhang, Z., Lu, G., Zhong, Y., Tan, Q., et al. (2010) fMRI study of mesial temporal lobe epilepsy using amplitude of low-frequency fluctuation analysis. *Human Brain Mapping*. [Online] 31 (12), 1851–1861. Available from: doi:10.1002/hbm.20982.

Zhang, Z., Lu, G., Zhong, Y., Tan, Q., et al. (2009) Impaired attention network in temporal lobe epilepsy: a resting FMRI study. *Neuroscience Letters*. [Online] 458 (3), 97–101. Available from: doi:10.1016/j.neulet.2009.04.040.

Zheng, J., Qin, B., Dang, C., Ye, W., et al. (2012) Alertness network in patients with temporal lobe epilepsy: a fMRI study. *Epilepsy Research*. [Online] 100 (1–2), 67–73. Available from: doi:10.1016/j.eplepsyres.2012.01.006.

Zijlmans, M., Huiskamp, G., Hersevoort, M., Seppenwoolde, J.-H., et al. (2007) EEG-fMRI in the preoperative work-up for epilepsy surgery. *Brain*. [Online] 130 (9), 2343–2353. Available from: doi:10.1093/brain/awm141.

Zou, Q.-H., Zhu, C.-Z., Yang, Y., Zuo, X.-N., et al. (2008) An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: Fractional ALFF. *Journal of neuroscience methods*. [Online] 172 (1), 137–141. Available from: doi:10.1016/j.jneumeth.2008.04.012.