A Prospective Advanced Magnetic Resonance Imaging Study of Newly Diagnosed Epilepsy

By

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Abstract

According to the World Health Organization (WHO), approximately 50 million people in the world have active epilepsy. Epilepsy is the most common neurological disorder after migraine, stroke and Alzheimer's disease. Epilepsy disorder affects men and women of all ages, races and social classes. There is an extensive neuroimaging literature describing patients with chronic epilepsy. However, few studies have investigated brain structural changes in patients with newly diagnosed epilepsy (NDE) using quantitative magnetic resonance imaging (MRI).

The main goal of this thesis was to determine the nature and extent of brain structural and functional differences in patients with NDE using different MRI techniques compared with healthy controls. The first study was to determine the morphometric changes in patients with NDE compared to healthy controls using quantitative MRI analysis. The second study was to identify functional connectivity differences (in the whole brain and regions of interest) in patients with NDE and healthy controls using resting state functional magnetic resonance imaging (RS-fMRI).

All study participants were recruited from the Walton Centre NHS Foundation Trust, Liverpool. All had been diagnosed with focal epilepsy by a consultant neurologist and recruited for MRI scanning within 12 months of diagnosis. Twenty-seven patients with NDE were recruited (14 male, 13 female, with mean age (M)=33.2) and 32 healthy matched controls (14 male, 18 female, M=33.07). Control and NDE study participants were matched for age, handedness and gender. All participants were scanned using a Siemens 3T Trio whole-body scanner (Siemens, Erlangen, Germany) with eight-channel radiofrequency (RF) head coil together with foam padding to comfortably restrict head motion at the Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool. Various MRI sequences were conducted including: 3D MPRAGE T1-weighted anatomical data, and RS-fMRI

In the first study, shape, surface based, and voxel based morphometry analysis were applied, and the results suggested differences to the morphology of the brain stem and both the right and left thalami in patients with NDE. The independent component analysis of RS-fMRI showed abnormal different functional connectivity in visual and attention networks in patients with NDE relative to healthy controls while ROI-ROI demonstrated increased functional connectivity between the subcallosal cortex and both thalami in NDE patients. This is the first extensive programme of research to employ various analysis techniques and advanced MRI sequences to study structural and functional differences in patients with NDE compared to healthy controls. The results of this thesis show that structural and functional differences occur in both thalami in patients with NDE. These findings suggest that the thalamus plays a very important role in epilepsy pathophysiology. The results of this thesis offer further understanding regarding the role of structural and functional differences in NDE. They highlight the need for future quantitative MRI analysis studies of NDE to help patients avoid the chronic stage of the disorder and improve their quality of life.

Declaration

I hereby certify that this thesis constitutes my own work. The material contained in this thesis has not been presented, nor is currently being presented, either wholly or part, for any other degree or qualification.

The research work was carried out at the Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool and the Walton Centre NHS Foundation Trust.

Signed,

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Abbreviations

- **AC** Anterior Commissure
- **AEDs** Antiepileptic Drugs
- **AFQ** automated fibre quantification
- **ALS** amyotrophic lateral sclerosis
- **B_0** External Magnetic field applied in MRI
- **BOLD** Blood Oxygenation Level Dependent
- **CBF** Regional Cerebral Blood Flow
- **CNS** Central nervous system
- **CSF** Cerebrospinal Fluid
- **EEG** Electroencephalography
- **EPI** Echo Planar Image
- **FC** Functional connectivity
- **FDR** False Discovery Rate
- **FMRI** Functional Magnetic Resonance Imaging
- **FMRIB** Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
- **FSL** FMRIB's software library
- **GLM** General Linear Model
- **GM** Grey matter
- **ICA** Independent component analysis
- **ICC** Intra-class correlation coefficient
- **IGE** Idiopathic generalised epilepsy
- **JME** Juvenile myoclonic epilepsy
- **MEG** Magnetoencephalography
- **MNI** Montreal Neurological Institute
- **MRI** Magnetic Resonance imaging
- **NDE** Newly Diagnosed Epilepsy
- **NMR** Nuclear Magnetic Resonance
- **NMV** Net Magnetization Vector
- **PACS** picture archiving and communication system
- **PET** Positron emission tomography

- **ROI** Region of interest
- **SD** Standard deviation
- **SNR** Signal To Noise Ratio
- **SPET** Single Photon Emission Tomography
- **T1** Longitudinal Relaxation Time
- **T2** Dephasing Relaxation Time
- **$T2^*$** Transverse Magnetization Time
- **TE** Echo Time
- **TR** Repetition Time
- **TRN** thalamic reticular nucleus
- **VBM** Voxel-Based Morphometric
- **WHO** World Health Organization
- **WM** White Matter

Chapter 1 General introduction

1.1 Background

According to the World Health Organization (WHO), approximately 50 million people in the world have active epilepsy. Of those patients with epilepsy, approximately 80% live in developing countries (WHO, 2016). Epilepsy is the most common neurological disorder after migraine, stroke and Alzheimer's disease (Hirtz et al., 2007). Epilepsy disorder affects men and women of all ages, races and social classes (Neligan and Sander, 2009). The physical health issues of epilepsy disorder have significant financial costs. In Europe the estimated cost of treating those with epilepsy disorder in 2004 was €15.5 billion (Pugliatti et al., 2007). According to the National Centre for Chronic Disease Prevention and Health Promotion in the USA, the estimated cost of epilepsy was around $15.5 billion in 2010 (England et al., 2012). However, compared with other neurological disorders (e.g., Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease and stroke), epilepsy has a low rate of research funding for the improvement of medical treatment (Meador et al., 2011).

At present, no medical treatment has had a significant effect on the morbidity associated with epilepsy. There is an urgent need to improve our understanding of epilepsy pathophysiology, to develop new approaches for medical treatment (Pohlmann-Eden, 2011). Epilepsy management, including medical and surgical treatment, can be helpful for many patients

with epilepsy; however, these management interventions offer no guarantee of seizure cessation and control. Response to antiepileptic drugs (AEDs) is most favourable in patients with newly diagnosed epilepsy (NDE) (Cendes, 2011). Nearly 60% of patients with NDE will respond to and have seizures controlled by AEDs after 12 months of treatment (Bonnett et al., 2012) and 30% of patients with NDE will become medically intractable.

Patients with epilepsy who do not respond to AEDs and have uncontrolled epilepsy may ultimately experience brain injury (Cascino, 2009). Around 30% of patients with epilepsy develop pharmacoresistance (Wiebe and Jette, 2012). Patients with epilepsy commonly develop resistance to AEDs after not responding to two or more AEDs (Wiebe and Jette, 2012). After surgical intervention, 65% of patients with pharmacoresistant focal epilepsy achieve continued seizure remission (Wiebe and Jette, 2012). Uncontrolled seizures in patients with epilepsy increases the risk of injury and sudden death (Tellez-Zenteno et al., 2005). For between 40% and 60% of patients who develop medically intractable epilepsy and are diagnosed as having focal seizures, surgery is an option that can be used to resect epileptogenic tissue and minimize seizures (de Tisi et al., 2011).

In patients with NDE, neuroimaging techniques are used in diagnosis, classification and evaluation. Magnetic resonance imaging (MRI) can identify gross lesions in approximately 30% of patients with NDE via clinical scans (Bonnett et al., 2012, Liu et al., 2002). These lesions in

epilepsy patients may represent damage caused by seizures that AEDs failed to control (Bonnett et al., 2012). At the time of diagnosis in patients with NDE, there are alterations in regions of the brain that cause seizures and cognitive impairment (Liu et al., 2002, Taylor et al., 2010). Despite the significance of these alterations, they may be not reported in most patients with NDE using routine MRI clinical sequences.

1.2 Main goal of thesis and research problem

The main goal of this thesis is to determine the nature and extent of brain structural and functional alterations in patients with NDE. It is hypothesized that sophisticated MRI techniques will show structural and functional alterations in the brains of patients with epilepsy.

While research has focused on determining differences between healthy controls and patients with chronic epilepsy, no study has examined the difference between healthy controls and patients with NDE. Furthermore, few studies have applied quantitative MRI to assess structural features in the brain of patients with NDE (Liu et al., 2001, Liu et al., 2002, Liu et al., 2003a, Schmidt and Pohlmann-Eden, 2011, Van Paesschen et al., 1998); research has concentrated on chronic epilepsy.

The majority of the MRI work in NDE has focused on the hippocampus (Briellmann et al., 2002, Salmenperä et al., 2005, Van Paesschen et al., 1998). There is an urgent need to understand the mechanisms in patients

with epilepsy to improve new medical treatments and prevent the progression of NDE to chronic stages of the disease (Pohlmann-Eden, 2011). There is a need for knowledge regarding the fundamental pathophysiologic mechanisms of epileptogenesis (Schmidt and Pohlmann-Eden, 2011) to develop treatment and management plans for patients with epilepsy. To my knowledge, there are no sophisticated analyses of advanced MRI sequences applied in patients with NDE (Cendes, 2011, Pohlmann-Eden, 2011, Schmidt and Pohlmann-Eden, 2011). Advanced and post-processing analyses of sophisticated MRI sequences, for example resting state functional MRI (ASL), and T1 structural sequences are extremely sensitive to structural and functional changes in patients with neurological disorders. Such advanced analyses are necessary to achieve a more detailed understanding of the aetiology, development and prognosis of epilepsy.

The research objectives are as follows:

1. To determine the morphometric change in patients with NDE compared to healthy controls using quantitative MRI analysis. (This objective is discussed in Chapter 7).

2. To identify functional connectivity differences (in the whole brain and regions of interest) in patients with NDE and healthy controls using novel resting state methods. (This objective is discussed in Chapter 8).

1.3 Organisation of the thesis

Chapter 1. Chapter 1 provides a general background about epilepsy, the main goal of thesis, the research problem, the research objectives and a brief summary for each chapter in this thesis.

Chapter 2: Brain anatomy. Chapter 2 provides a general background about the basic structural and functional components of brain. This chapter also provides a brief description of the nerve cells and lobes of the brain, including the frontal lobe, parietal lobe, occipital lobe, temporal lobe and insula. The other parts discussed in brain anatomy include the cerebellum, brainstem and other subcortical structures (i.e., the basal ganglia, hippocampus and thalamus). The last section in this chapter discusses the white matter tracts of the cerebral hemispheres.

Chapter 3: Introduction to epilepsy. Chapter 3 provides a background on the disorder of epilepsy, including the basic concepts of epilepsy, the epidemiology and classification of epileptic seizures and syndromes.

Chapter 4: Principles of magnetic resonance imaging. Chapter 4 provides a brief description of the basic principles of MRI, including the following: the basic physics of MRI (Lamor frequency, hydrogen, magnetic field, tesla and gyromagnetic ratio), MRI instrumentation, image parameters, pules sequence imaging techniques, resting state functional MRI and MRS.

Chapter 5: Magnetic resonance imaging in epilepsy. Chapter 5 discusses the MRI research that has been done in NDE and the literature on chronic epilepsy in different imaging analysis, including ;resting state functional MRI, fMRI, MRS and ASL.

Chapter 6: Subjects and methods. Chapter 6 provides full information regarding the subjects in the present study and brief information regarding common methods used in this thesis. The information on the subjects includes the study population, study ethics information, recruitment criteria, healthy controls, patients with NDE and Edinburgh Handedness Inventory. A brief explanation of MRI data acquisition (routine clinical sequences and research sequences) and medical imaging analysis software is also provided.

Chapter 7: Brain morphology in patients with newly diagnosed epilepsy. Chapter 7 provides an overview of quantitative MRI analysis methods used to study brain morphology and investigate the morphological differences in the whole brain between patients with NDE and healthy controls. The first aim in this chapter is vertex-based shape analyses applied to assess shape differences in subcortical structures of the brain in patients with NDE compared with healthy controls using FIRST FSL toolbox. The second aim in Chapter 7 involved using FreeSurfer software to perform subcortical volume analyses segmentation in patients with NDE and healthy controls. The last aim in this chapter describes the application of automated voxel-based morphometry on MRIs

used to study differences in the whole brain between patients with NDE and healthy controls. Chapter 7 provides a full technical description of the steps necessary for FIRST FSL, FreeSurfer, and VBM analysis methods.

Chapter 8: Functional MRI studies in patients with NDE. Three aims are discussed in this chapter. The first aim is to investigate the functional connectivity in the whole brain using independent component analysis (ICA) FSL in patients with NDE relative to healthy controls. The second aim is to use the CONN toolbox functional connectivity toolbox to investigate functional connectivity in regions of interest (ROI) of both thalami in patients with NDE and healthy controls based on structural changes in the shape and volume of both thalami from the previous chapter.

Chapter 9: Summary, strengths, limitations, future work and conclusions

This chapter reviews the aims of my thesis, describes the main results and discusses the strengths and limitations of the research.

Chapter 2 Brain Anatomy

2.1 The brain

The brain is the most complex organ in the human body. The mass of the human brain represents 2% of the total body mass, but it consumes 20% of the body's energy (Ellenbogen et al., 2012). The brain is often considered to consist of three predominant parts: the cerebrum, cerebellum and brainstem.

2.1.1 Cerebrum

The cerebrum is the largest part in the brain and is made up of the cortex. The cerebrum accounts for approximately 80% of total brain weight. The cerebrum is split into two hemispheres, which are linked via white matter (WM) called the corpus callosum. The cerebral hemispheres are divided into five lobes: the frontal lobe, parietal lobe, occipital lobe, temporal lobe and insula (Stephani et al., 2011) (Figure 2:3). The cerebral cortex has several features including gyri, sulci and fissures. The gyri are elevated ridges around the brain. The sulci are small grooves separating the gyri. For example the central sulcus splits the frontal lobes from the parietal lobe. Fissures (deep grooves) in the cerebrum divide the cerebrum into lobes. The two cerebral hemispheres are divided by a longitudinal fissure. The cerebrum is split from the cerebellum by a transverse fissure.

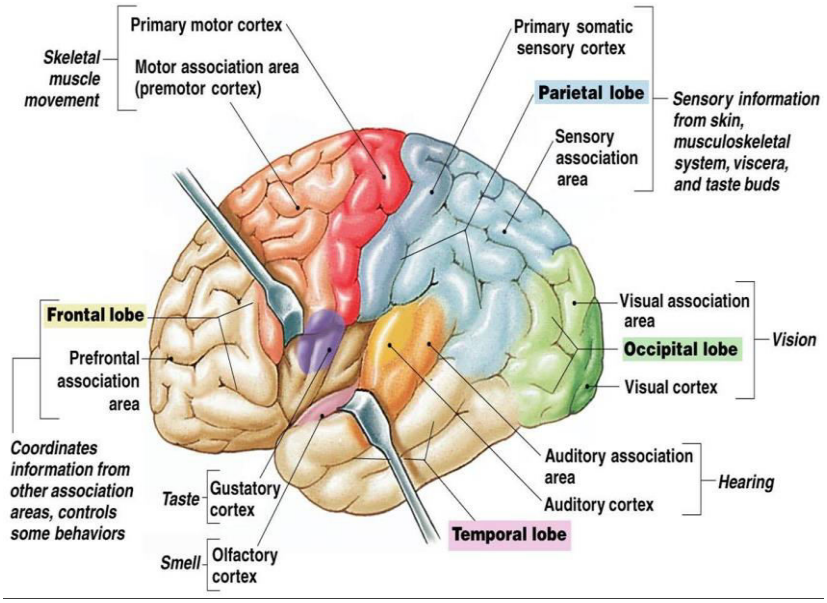


Figure 2:1 The lobes of the brain including the frontal, parietal, occipital and temporal lobe. Adapted from http://www.austincc.edu/rfofi/NursingRvw/PhysText/CNS.html

2.1.2 Frontal lobe

The frontal lobe is located deep in the (Figure 2:1) frontal bone of the skull (Tamraz, 2006). The frontal lobe is the largest part of the human brain and occupies one third of the cortical region of the cerebral hemisphere (Johns, 2014). The frontal lobe plays a very important role in the following functions: formation of memory, emotions, decision making, motor control, reasoning, personality, vision, cognitive function, language and neuropsychiatric functions (Chayer and Freedman, 2001). For example, the primary motor cortex (precentral gyrus) cortical site is involved with monitoring the movements of the body. Broca's region, also known as the left posterior inferior frontal cortex, controls facial neurons, speech and language function. The prefrontal cortex in the frontal lobe has an

important role in cognitive functions (e.g. attention, executive function, planning, regulation of emotion and working memory). The orbitofrontal cortex is involved in emotion, social behaviour, and impulse control (Bechara and Van Der Linden, 2005).

2.1.3 Parietal lobe

The parietal lobe is the portion of the cerebral cortex between the frontal and occipital lobes (Sveinbjornsdottir and Duncan, 1993). The location of parietal lobe is adjacent to the parietal bone of the skull and posterior to the central sulcus (Johns, 2014). The parietal lobe is described by three anatomical structures (Figure 2:1). The central sulcus separates the parietal lobe from the frontal lobe. The parieto-occipital sulcus divides the parietal and occipital lobes. The lateral sulcus (Sylvian fissure) divides the parietal lobe from the temporal lobe. The medial longitudinal fissure divides the two hemispheres. The parietal lobe contains three cortical regions: the primary somatosensory cortex, the somatosensory association cortex and the primary gustatory cortex (Johns, 2014) . The primary somatosensory cortex plays an important role in the processing of tactile and proprioceptive information. The somatosensory association cortex supports the integration and interpretation of sensations related to body position and orientation in space. The primary gustatory cortex is involved in the interpretation of the sensation of taste.

2.1.4 Occipital lobe

The smallest lobe in brain is the occipital lobe (Figure 2:3). The location of the occipital lobe is adjacent to the occipital bone of the skull (Sveinbjornsdottir and Duncan, 1993). The occipital lobe is separated from the parietal lobe by the parieto-occipital sulcus. The main function of the occipital lobe is the processing, integration and interpretation of vision and visual stimuli (Bender et al., 1957, Zeki and Moutoussis, 1997). The visual association area interprets information acquired through the primary visual cortex.

2.1.5 Temporal lobe

The temporal lobe is the anterior ventral lobe in the brain and the most heterogeneous of all cerebral lobes (Figure 2:3). The temporal lobe is located on the side of the brain and adjacent to the temporal bones in the skull. The Sylvian/lateral fissure separates the temporal lobe from the frontal lobe. Temporal lobe gross anatomy is typically split into three parallel gyri: the superior temporal gyrus, the middle temporal gyrus and the inferior temporal gyrus .The superior temporal gyrus includes the primary auditory cortex, which is responsible for hearing. In addition, the superior temporal gyrus contains Wernicke's area, which is responsible for language comprehension, and is located on the left temporal lobe (Bigler et al., 2007, Jou et al., 2010). The middle temporal gyrus is located on lateral surface of the temporal lobe, ventral to the superior temporal gyrus

(Onitsuka et al., 2004). The inferior temporal gyrus is located on the lateral and inferior surfaces of the temporal lobe, ventral to the middle temporal gyrus (Onitsuka et al., 2004). The temporal lobe is engaged in a diverse range of functions, including: speech, balance, perception of time, language, visual processing, emotional processing, auditory processing and memory (Hugdahl et al., 2009), (Staiman, 1998).

2.1.6 Insula

The insula (also known as the insula cortex) is considered the fifth lobe. The insula is hidden by the frontal temporal and parietal operculum (Stephani et al., 2011). The location of the insula is adjacent to the Sylvian fissure, which splits the frontal lobes and parietal lobes from the temporal lobe (Nieuwenhuys, 2012). The insula is in close communication with the thalamus, limbic system and cerebral cortex (Augustine, 1985, Augustine, 1996). The insula is separated into two parts: the large anterior insula and the smaller posterior insula (Nieuwenhuys, 2012, Stephani et al., 2011). The insula cortex is involved in emotional parts of pain, negative emotions, procedural memory, gustatory processing, olfactory processing, salutatory conduction, visceral processes, effective functioning and motor control responses (Naqvi et al., 2007, Soros et al., 2009, Wicker et al., 2003). In addition, the insula plays a role in network mediating cognition, attention, meditative states and salience (Lutz et al., 2009, Menon and Uddin, 2010).

2.1.7 Cerebellum

The cerebellum (Latin: diminutive of cerebrum) is also known as the “little brain” (Saab and Willis, 2003). The cerebellum is the largest structure in the hindbrain (Figure 2:2) The location of the cerebellum is posterior to the cranial fossa of the skull and dorsal to the pons and medulla oblongata. The cerebellum is separated into three major lobes: the anterior lobe, the middle lobe and the flocculonodular lobe. The cerebellum is responsible for balance maintenance, coordination of muscles tone and voluntary movement control. Other functions of the cerebellum include memory, attention, emotion, language, cognition and learning (Andreasen et al., 1999, Adamaszek et al., 2016, Barrios and Guardia, 2001, Ivry and Baldo, 1992, Norden and Blumenfeld, 2002, Schmahmann and Caplan, 2006).

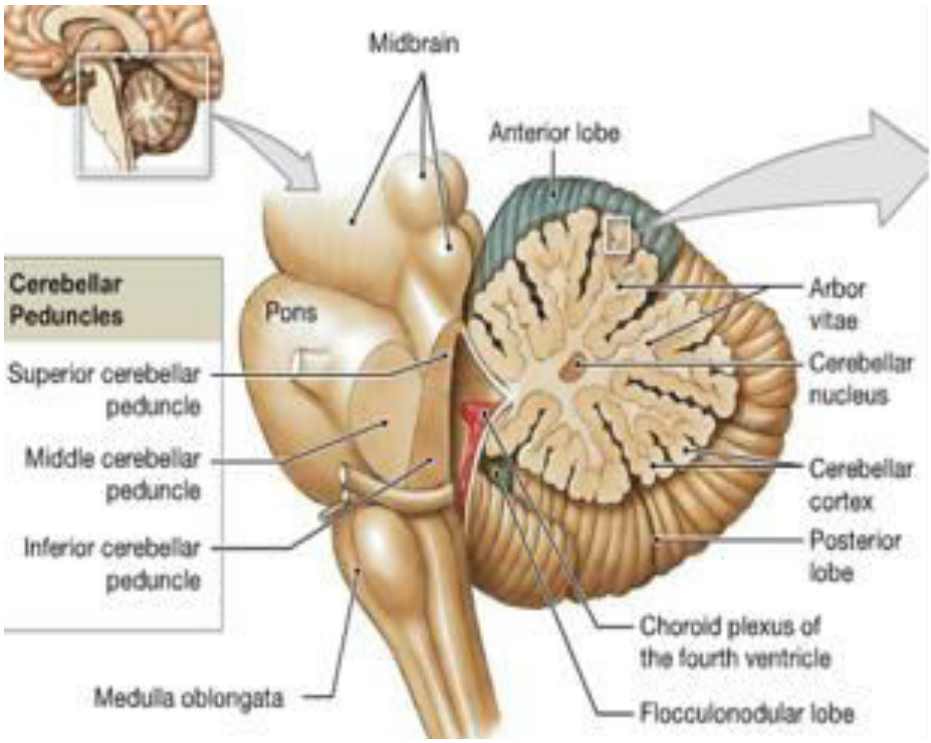


Figure 2:2 Illustration of the sagittal section through the brainstem and the vermis of the cerebellum, adapted from (Clinical Neuroanatomy, 7th ed., Snell, 2010).

2.1.8 Other subcortical brain structures

The basal ganglia consist of the caudate nucleus, putamen and globus pallidus .The function of the basal ganglia is movement regulation and skill leaning. The hippocampus (Latin: sea horse) is the main part of limbic system (Figure 2:4). The hippocampus is located in the medial aspect of the temporal lobe and is bordered laterally by the temporal horn of the lateral ventricle (Johns, 2014). The hippocampus is divided into three sub-regions including the hippocampal head, the hippocampal body and the

hippocampal tail (Johns, 2014). The hippocampus plays important roles in long-term memory and spatial navigation (Watanabe et al., 2008).

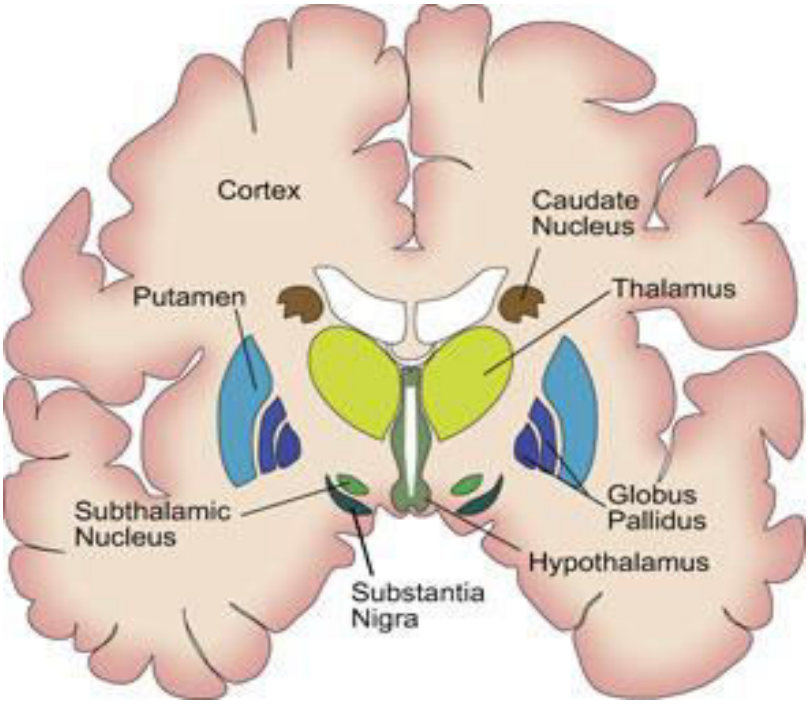


Figure 2:3 Coronal view of the main basal ganglia structures including the caudate nucleus, putamen and globus pallidus, adapted from (http://www.dana.org/news/brainwork/detail.aspx?id=6028).

The thalami (Greek: thalamos, inner chamber) are two egg-shaped masses (Figure 2:3) of grey matter (GM) in the middle of the brain (Johns, 2014). The thalamus is separated into anterior nuclei, medial nuclei and lateral nuclei (Johns, 2014). It sends and receives information from the cortex, and its functions include relaying sensory and motor signals to the cortex and the regulation of consciousness, sleep and alertness (McCormick and Bal, 1997, Norden and Blumenfeld, 2002).

2.1.9 White matter of the cerebral hemispheres

The white matter (WM) tracts are categorized into five functional groups (Wakana et al., 2004) including: (1) tracts in the brainstem, (2) projection fibres (cortex-spinal cord, cortex-brainstem, and cortex-thalamus connections), (3) association fibres (cortex-cortex connections), (4) limbic system tracts and (5) commissural fibres (right-left hemispheric connections) (Figures 2:4, 2:5).

2.1.9.1 Brainstem white matter tracts

In the brainstem, the WM includes the following tracts the superior cerebellar peduncles, the middle cerebellar peduncles, the inferior cerebellar peduncles, the cortical spinal tract and the medial lemniscuses (Wakana et al., 2004). The superior cerebellar peduncle tract is connected to the midbrain and contains a main projection from the dentate nucleus to the opposite thalamus and frontal lobe(Johns, 2014). The middle cerebellar peduncle tract is the largest of all the cerebellar peduncles tracts (Figures 2:4). It extends from the pons and represents the major afferent supply to the cerebellar hemispheres, which mainly originates from the opposite frontal lobe The inferior cerebellar peduncles tract is linked to the medulla and transfers afferent sensory information from the spinal cord (Johns, 2014, Wakana et al., 2004). The inferior cerebellar peduncles tract transmits many types of input and output fibres that are

mostly concerned with integrating proprioceptive sensory input with motor vestibular functions, including balance and posture maintenance (Johns, 2014).

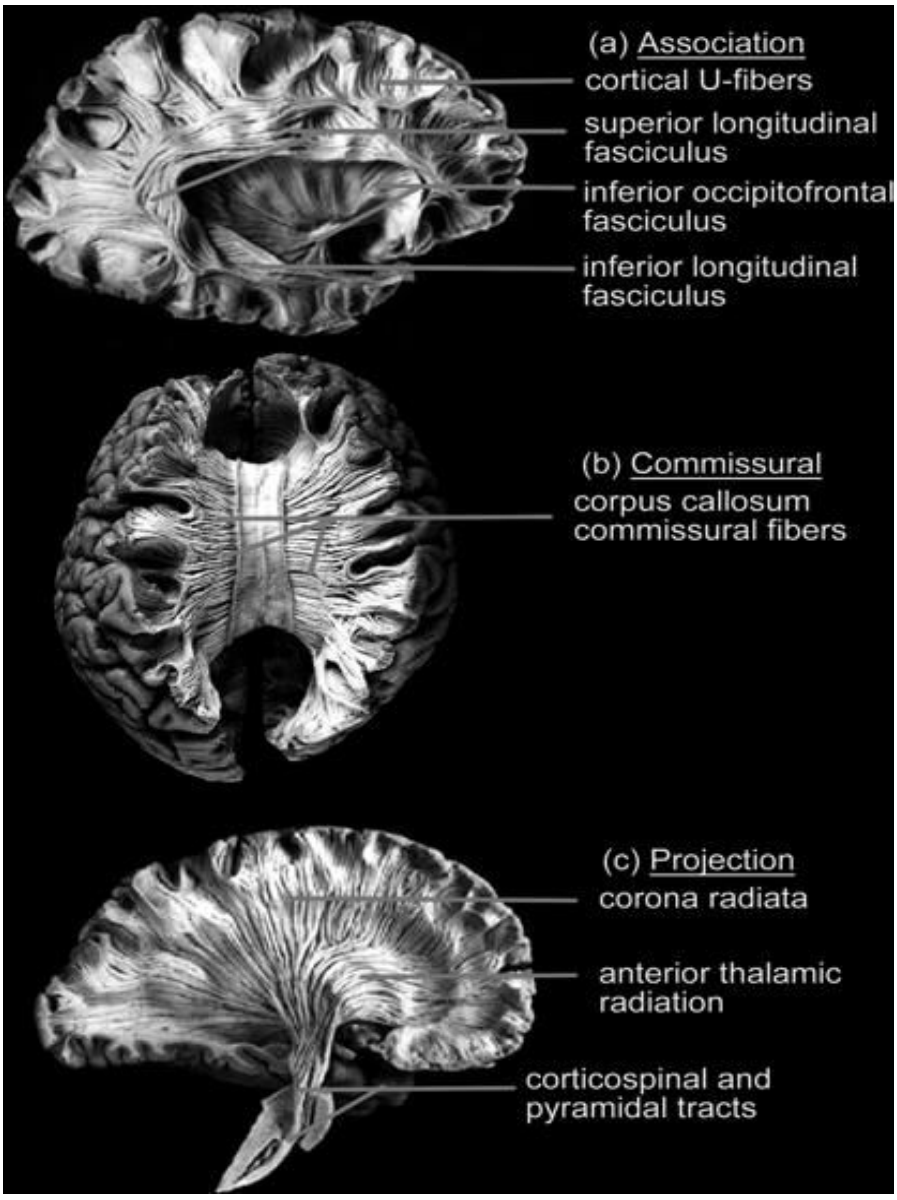


Figure 2:3 White matter tracts association , commissural and projections, adapted from (Terence H. Williams et al., 1997).

2.1.9.2 Projection fibres

Projection fibres are WM tracts from the cerebral cortex to the lower part of brain and spinal cord in both directions (Johns, 2014, Wakana et al., 2004). There are two types of projection fibres: (a) corticofugal, which terminate in the basal nuclei, brainstem or spinal cord, and (b) cortiopedal, which originate in the thalamus and terminate in the cerebral cortex (Wakana et al., 2004). The projection fibre tracts have two types of fibres: afferent (ascending) and efferent (descending) nerve fibres. Projection fibre ascending and descending tracts include corticothalmic fibres, corticofugal fibres and internal capsule fibres . Projection tracts connect hemispheres to other brain regions and the spinal cord (Wakana et al., 2004). Thalamic radiations are divided to three tracts: anterior thalamic radiations, superior thalamic radiations and posterior thalamic radiations (Figure 2:15). Thalamic radiations connect thalamic with cortical brain regions (Kenich et al., 2011).

The anterior thalamic radiation (Figures 2:4) contains fibres which link the mediodorsal thalamic nuclei to the cortex (Kahle W et al., 2002). The anterior thalamic radiation is the pathway of fibres that associate the anterior nuclear thalamus and the midline nuclear thalamus with the frontal lobe through the anterior thalamic peduncle (Wakana et al., 2004). The superior thalamic tract originates from the thalamus and projects to the postcentral gyrus of the parietal cortex (Wakana et al., 2004). The superior thalamic radiation is a pathway connecting the ventral nuclear of thalamus

with the precentral gyrus and the postcentral gyrus through the superior thalamic peduncle (Kenich et al., 2011). The posterior thalamic radiation connects the caudal of the thalamus with the occipital and the parietal lobes through the posterior thalamic peduncle (Wakana et al., 2004). The posterior thalamic radiation contains the optic radiation, which associates the lateral geniculate nucleus of the thalamus to the occipital lobe (Kenich et al., 2011). The thalamic radiation WM tracts join to the internal capsule placed between the putamen and the thalamus caudate nucleus areas (Wakana et al., 2004) The Corticofugal tracts consist of the corticobulbar, corticopontine, corticoreticular and corticospinal WM tracts (Figure 2:5) (Kenich et al., 2011). Corticobulbar tracts connect the cerebral cortex to the brainstem (Johns, 2014). The primary motor pathway consists of corticospinal and corticobulbar WM tracts which primarily originate from the motor and premotor regions of the frontal lobe (Johns, 2014). The corticospinal WM tract is the main somatic motor pathway and the longest continuous WM tract in the CNS (Johns, 2014). The internal capsule is a massive WM tract composed of 20 million projection fibres (Johns, 2014). The internal capsule is a WM tract located in the inferomedial part of every hemisphere of the brain. The internal capsule tract divides the caudate nucleus and the thalamus from the putamen and the globus pallidus.

2.1.9.3 Association fibres

The association fibre WM tract, also known as arcuate fibres, is limited to

a single hemisphere. Association fibres connect regions of the cerebral cortex within one hemisphere. These fibres are subdivided into two major groups: short arcuate fibres, also called U fibres (connecting adjacent gyri), and long arcuate fibres, which connect distant gyri in different lobes (Figure 2:4) (Wakana et al., 2004). There are two types of short arcuate fibres: (a) intracortical fibres located in the deeper portions of the WM and (b) subcortical fibres located just underneath the cortex. The long association fibres connect more broadly separated regions. The types of long association fibres include the superior longitudinal fasciculus, inferior longitudinal fasciculus, occipitofrontal fasciculus, uncinate fasciculus, cingulum and arcuate fasciculus (Wakana et al., 2004). The superior longitudinal fasciculus is the major bundle of nerve fibres (Schmahmann et al., 2008). The superior longitudinal fasciculus connects parts of the frontal lobe with the occipital and temporal regions (Figures 2:4,2:5). The superior longitudinal fasciculus plays a very important role in language-related functions (Madhavan et al., 2014).

The inferior longitudinal fasciculus fibres are smaller than the superior longitudinal fasciculus association fibres (Figures 2:5, 2:6). The inferior longitudinal fasciculus runs anterior from the occipital lobe, transient lateral to the optic radiation, and spreads to the temporal lobe. The inferior longitudinal fasciculus connects the temporal and occipital lobes. The inferior longitudinal fasciculus fibres contribute to visual recognition (Latini, 2015). The occipitofrontal fasciculus extends from the ventrolateral areas

of the frontal lobe to the temporal and occipital lobes (Figures 2:5, 2:6). The fibre is located deep within the cerebral hemisphere and is associated with the lateral border of the caudate nucleus. The occipitofrontal fasciculus consists of the superior occipitofrontal fasciculus and the inferior occipitofrontal fasciculus (Figures 2:4).

The superior occipitofrontal fasciculus is also called the subcallosal fasciculus (Figures 2:4). The fibre connects the frontal lobe to the ipsilateral parietal lobe. The superior occipitofrontal fasciculus plays a role in special awareness and symmetrical processing (Rizzolatti et al., 1990, Rizzolatti and Matelli, 2003). The Inferior occipitofrontal fasciculus (Figures 2:15) connects the ipsilateral frontal and occipital lobes, the ipsilateral frontal and posterior parietal and temporal lobes and intermingles with the uncinate fasciculus (Wakana et al., 2004). The function of the inferior occipitofrontal fasciculus is the integration of auditory and visual association cortices with the prefrontal cortex (Kier et al., 2004, Petrides and Pandya, 1988). The uncinate fasciculus is the major WM tract connecting the inferior frontal lobe gyri with the anterior temporal lobe (Catani et al., 2002, Papagno et al., 2011, Schmahmann et al., 2007). The uncinate fasciculus fibre plays a role in language, emotion processing and memory integration (Catani and Mesulam, 2008, Gaffan and Wilson, 2008).

2.1.9.4 Limbic system fibres

The limbic system plays an important role in memory, behaviour and emotions (Mori and Aggarwal, 2014). The limbic fibres include primarily the cingulum, fornix and stria terminalis trajectory (Wakana et al., 2004). The Cingulum WM tract (Figure 2:5) is the main fibres tract in the limbic system and offers the main route between the anterior thalamus and hippocampus (Burgel et al., 2006). This limbic fibre connects the prefrontal cortex and other parts of the frontal lobe to more posterior structures involving the temporal lobe and hippocampus (Burgel et al., 2006). The cingulum fibres form a WM tract that spreads longitudinally over the corpus callosum (Jones et al., 2013). The cingulum fibre is involved in the processing of emotions and cognitive executive (Ardekani et al., 2003, Matthews et al., 2008). Fornix is a fibre WM C-shaped tract located on the medial parts of the cerebral hemispheres (Thomas et al., 2011). The fornix fibre is a major output tract interconnecting the hypothalamus and hippocampus (Thomas et al., 2011). The fornix fibre is an important component of the limbic system and involved in the function of memory (Thomas et al., 2011). The stria terminalis fiber connects the hypothalamus to amygdala and the fibre is major output pathways of the amygdala (Johns, 2014).

2.1.9.5 Commissural fibres

Commissural (Latin: commissūra, a joining together) fibres interconnect

the two cerebral hemispheres (Johns, 2014). Commissural (transverse fibers) include the following: corpus callosum, anterior commissure, posterior commissure, and hippocampal commissure. The corpus callosum is the largest WM structure and is located between the two cerebral hemispheres, connecting them (Fitsiori et al., 2011). The corpus callosum is the largest of the commissure fibres, containing between 200 and 300 million myelinated axons (Johns, 2014). The corpus callosum includes four areas: (a) the genu, which is the arched anterior end of the corpus callosum, (b) the body, which curves and terminates in the posterior portion and (c) the splenium and (d) rostrum, which are thin sections at the anterior end of the corpus callosum (Leichnetz, 2006). The anterior commissure (AC), also called the precommissure, is a thin tract of WM between the olfactory regions of both hemispheres (Snell, 2010). The AC connects the right and left amygdalas and numerous cortical areas of the two temporal lobes (Leichnetz, 2006). The AC is located on the anterior wall of the third ventricle at the upper portion of the lamina terminalis (Snell, 2010). The AC connects the middle and inferior temporal gyri of the two hemispheres. The Talairach coordinate system is used to locate key landmarks in the AC.

The posterior commissure (PC), also called the epithalamic commissure, connects the right and left pretectal areas and connects cell groups of the mesencephalon (Leichnetz, 2006). The hippocampal commissure (i.e., the commissure of the fornix) contains transverse fibres (Leichnetz, 2006).

The hippocampal commissure connects the hippocampal formations of both sides (Snell, 2010).

Chapter 3 Introduction to epilepsy

3.1 Introduction

3.2 Basic concepts of epilepsy

3.2.1 Definitions

Epilepsy is a chronic brain disorder defined according to (Fisher et al., 2014) by any of the following clinical phenomena: (a) “at least two unprovoked (or reflex) seizures occurring >24 h apart”; (b) “one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years”; and/or (c) “diagnosis of an epilepsy syndrome”. According to the International League Against Epilepsy (ILAE), an **epileptic seizure** “is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain’’ to abnormal excessive or synchronous neuronal activity in the brain’’ (Fisher et al., 2014). According to the ILAE, a **focal seizure** is a seizure whose initial semiology indicates, or is constant with, early activation of only part of one cerebral hemisphere. The types of seizure include (a) simple partial seizures, (b) complex partial seizures and (c) secondary generalised tonic-clonic seizures. **A generalised seizure** is a seizure whose initial semiology indicates, or is constant with, more than minimal contribution of both cerebral hemispheres. These types of seizure will include the following: (a) absence seizures, (b) myoclonic seizures and (c) generalised tonic-clonic seizures (GTCS). **A provoked**

seizure is produced by somatic disorders beginning outside the brain for example, fever, infection, syncope, traumatic brain injury, brain tumours, hypoxia, toxins, cardiac arrhythmias or stroke, (Misra and Kalita, 2011)

3.3 Classification of epileptic seizures and syndromes

In epilepsy, there are two classifications frequently used for diagnosis and patients' treatment plans. Epileptic seizures can be described by a variety of clinical manifestations with motor, sensory, cognitive or psychological symptoms and signs. Every seizure is described by the duration of the seizure and the unique feelings and perceptions it is associated with. Epilepsy (or epileptic syndrome) is a characteristic clinical entity described by epidemiological factors, types of seizures, response to medication and disease prognosis (Urbach, 2013). Together, the identification of epileptic seizures and syndromes is very important for diagnosis and management.

3.3.1 Classification of epileptic seizures

In 1981 the ILAE introduced a standardised system of classification and terminology for epileptic seizures. In this system, epileptic seizures were divided into two types: partial (focal) seizures that spread within the one hemisphere and generalised seizures that mainly affect both hemispheres (Figure 3:1). Focal (partial) seizures were further categorized as (1) simple partial (focal) seizures where the awareness of patients is not affected and (2) complex partial (focal) seizures, which were identified by reduced consciousness. Classification was dependent on the clinical signs and

symptoms of the seizure based on electroencephalogram (EEG) results. Generally speaking, focal seizures were split into simple partial and complex partial seizures and further classified according to motor signs (focal motor without march, focal motor with versive, postural, phonatory), somatosensory or special sensory symptoms (visual, auditory, olfactory, gustatory, vertiginous), autonomic signs or symptoms (epigastric sensation, pallor, sweating, flushing, piloerection, pupillary dilation), and psychologic symptoms (dysphasic, dysmnesic, cognitive, affective, illusions, structured hallucinations). Generalised seizures were divided into absence seizures, myoclonic seizures, tonic-clonic seizures, clonic seizures, tonic seizures and atonic seizures. Table 3-1 shows the original international classification of seizures from 1981.

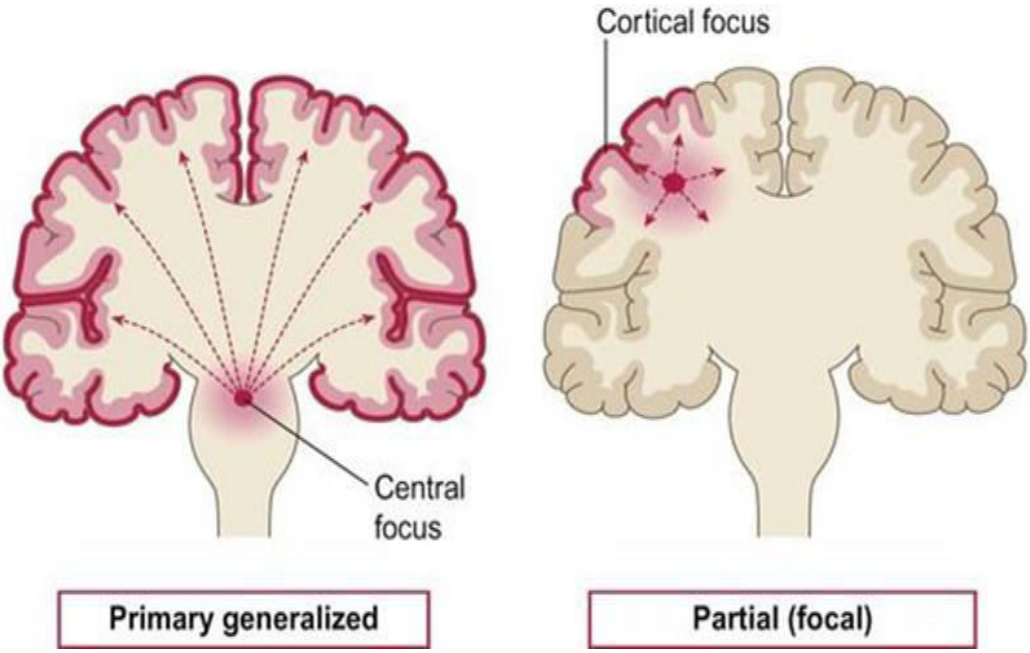


Figure 3:1 Types of seizure. Adopted from(Johns, 2014)

Table 3-1 International classification of seizures 1981

I**. Partial (focal, localization-related) seizures:**

A. Simple partial seizures
1. With motor signs
2. With somatosensory or special-sensory symptoms
3. With autonomic symptoms or signs
4. With psychic symptoms

B. Complex partial seizures
1. With impairment of consciousness at onset
2. Simple partial onset followed by impairment of consciousness

C. Partial seizures with secondary generalization
1. With simple partial seizures evolving to generalized seizures
2. With complex partial seizures evolving to generalized seizures
3. With simple partial seizures evolving to complex partial seizures evolving to generalized seizures.

II. Generalized seizures

A. Absence seizures
1. With impairment of consciousness only
2. With mild clonic components
3. With atonic components
4. With tonic components
5. With automatisms.

B. Myoclonic seizures
C. Clonic seizures
D. Tonic seizures
E. Tonic-clonic seizures
F. Atonic (astatic) seizures

adapted with permission from Proposal for revised clinical and electrographic classification of epileptic seizures. From the Commission on Classification and Terminology of the ILA Epilepsy.1981.

The current classification of seizures introduced by the ILAE In 2010 (Berg et al., 2010) is similar to the previous classification with respect to generalized seizures, however the new classification eliminates the subcategories of partial seizures, alternatively considering changed awareness and generalized convulsive activity to be descriptors for focal seizures with development into generalized convulsive activity.

Table 3-2 ILAE 2010 Classification of Seizures

I. Focal seizures Descriptors of focal seizures : A. Without impairment of consciousness or awareness 1. With observable motor or autonomic components 2. Involving subjective sensory or psychic phenomena only B. With impairment of consciousness or awareness C. Evolving to a bilateral, convulsive seizure
II. Generalized seizures A. Tonic-clonic seizures B. Absence seizures 1. Typical 2. Atypical 3. Absence with special features a. Myoclonic absence b. Eyelid myoclonia C. Myoclonic 1. Myoclonic 2. Myoclonic atonic 3. Myoclonic tonic D. Clonic E. Tonic F. Atonic G. Unknown 1. Epileptic spasms

adapted from (Berg et al., 2010). Revised terminology and concepts for organization of seizures and epilepsies.

The ILAE through the Commission for Classification and Terminology, has developed a working classification of seizures and epilepsy. In 2017, the ILAE announced a new classification of seizure types, mainly based upon the current classification formulated in 1981. The main differences include specific listing of certain new focal seizure types that may previously only have been in the generalized category, use of awareness as a surrogate for consciousness, emphasis on classifying focal seizures by the first

clinical manifestation (except for altered awareness), a few new generalized seizure types, ability to classify some seizures when onset is unknown, and renaming of certain terms to improve clarity of meaning. Figure 3:2 provide a new classification of seizures types by ILAE.

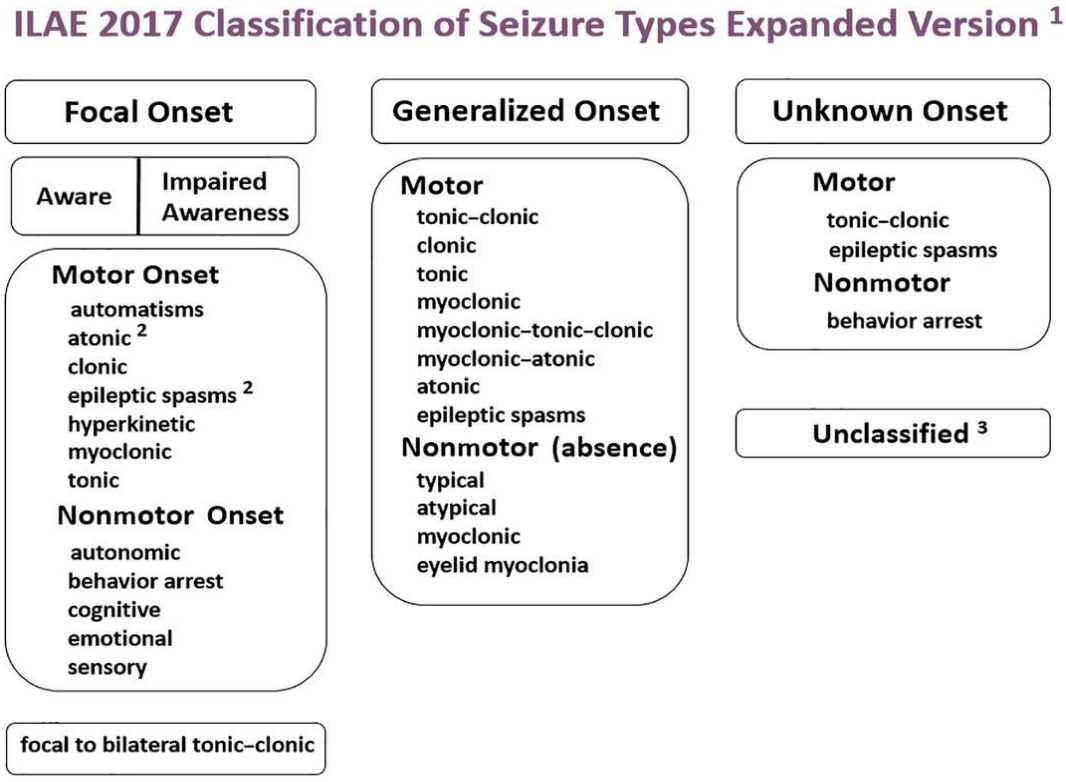


Figure 3:2 New classification of seizures type ILAE 2017

3.3.2 Classification of epileptic syndrome

There are numerous different categories of epilepsy classifications. Currently, most of epilepsy patients are given individual diagnostic or name of the epilepsy. The classification of epilepsies is a important clinical features on every patient with epilepsy disorder. Syndrome of seizure is type of epilepsy that based on the following : type of seizure,

EEG results , prognosis, pathophysiological , aetiological data , age of onset , neuroimaging findings , response of medication (AEDs). This new classification has kept the principal three seizures types (generalised, partial and unclassified). In 1989, ILAE proposed (Table 3-3) a new classification taking into account additional issues than the previous classification in 1981. ILAE described three category of syndrome epilepsies including; Idiopathic, Symptomatic, and Cryptogenic epilepsy syndrome. The Idiopathic epilepsy is syndrome that with a confirmed or supposed to be genetic cause (Urbach, 2013). Idiopathic epilepsy syndrome is characterized only by the symptoms of epilepsy with no fundamental structural brain lesions or other neurological symptoms. Symptomatic epilepsy is a syndrome with a demonstrated structural cause (Urbach, 2013). Cryptogenic epilepsy is syndrome the cause has not discovered (Urbach, 2013). In 2010, this classification was revised with an extra three-tiered classification (Berg et al., 2010) , in which (a) genetic, (b) structural and/or metabolic, and (c) unknown epilepsies are distinguished (Urbach, 2013). In 2001, the ILAE diagnostics scheme was split in to five axes (Table 3-4). The task force suggested a diagnostic scheme to direct treatment management and describe individual patients (Engel, 2001). Table 3-2 and Figure 3.1 provide a summary of the revised classification of focal and generalised seizures and how electroclinical syndromes can be organized according to the 2010 classification.

Table 3-3 International classification of epilepsies and epileptic syndromes

Localisation-related (focal, local, or partial) Idiopathic epilepsy with age-related onset - benign childhood epilepsy with centrotemporal spikes (benign rolandic epilepsy) - childhood epilepsy with occipital paroxysms - primary reading epilepsy Symptomatic epilepsy Cryptogenic epilepsy
Generalised Idiopathic epilepsy with age-related onset (listed in order of age at onset) - benign neonatal familial convulsions - benign neonatal non-familial convulsions - benign myoclonic epilepsy in infancy - childhood absence epilepsy - juvenile absence epilepsy - juvenile myoclonic epilepsy - epilepsy with generalised tonic-clonic seizures on awakening - other idiopathic epilepsies Cryptogenic or symptomatic epilepsy (listed in order of age at onset) - West syndrome (infantile spasms) - Lennox Gastaut syndrome (childhood epileptic encephalopathy) - epilepsy with myoclonic-astatic seizures - epilepsy with myoclonic absence seizures Symptomatic epilepsy - Non-specific syndromes (early myoclonic encephalopathy, early infantile epileptic encephalopathy) - Specific syndromes (epileptic seizures as a complication of a disease, such as phenylketonuria, juvenile Gaucher's disease or Lundborg's progressive myoclonic epilepsy)
Epilepsies undetermined whether focal or generalised With both generalised and focal features - neonatal seizures - severe myoclonic epilepsy in infancy - epilepsy with continuous spike waves during slow-wave sleep - acquired epileptic aphasia (Landau-Kleffner syndrome) - without unequivocal generalised or focal features
Special syndromes Situation-related seizures - febrile convulsions - seizures related to other identifiable situations, such as stress, hormonal changes, drugs, alcohol withdrawal or sleep deprivation Isolated, apparently unprovoked epileptic events Epilepsies characterised by specific modes of seizure precipitation Chronic progressive epilepsia partialis continua of childhood

Adopted from (Commission on Classification and Terminology , ILAE 1989).

Table 3-4 A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology

Axis 1	**Ictal phenomenology,** from the Glossary of Descriptive Ictal Terminology, can be used to describe ictal events with any degree of detail needed.
Axis 2	**seizure type**, from the List of Epileptic Seizures. Localization within the brain and precipitating stimuli for reflex seizures should be specified when appropriate
Axis 3	**Syndrome**, from the List of Epilepsy Syndromes, with the understanding that a syndromic diagnosis may not always be possible.
Axis 4	**Etiology**, from a Classification of Diseases Frequently Associated with Epileptic Seizures or Epilepsy Syndromes when possible, genetic defects, or specific pathologic substrates for symptomatic focal epilepsies
Axis 5	**Impairment**, this optional, but often useful, additional diagnostic parameter can be derived from an impairment classification adapted from the WHO ICIDH-2.

Adopted from ;a proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology (Engel, 2001)

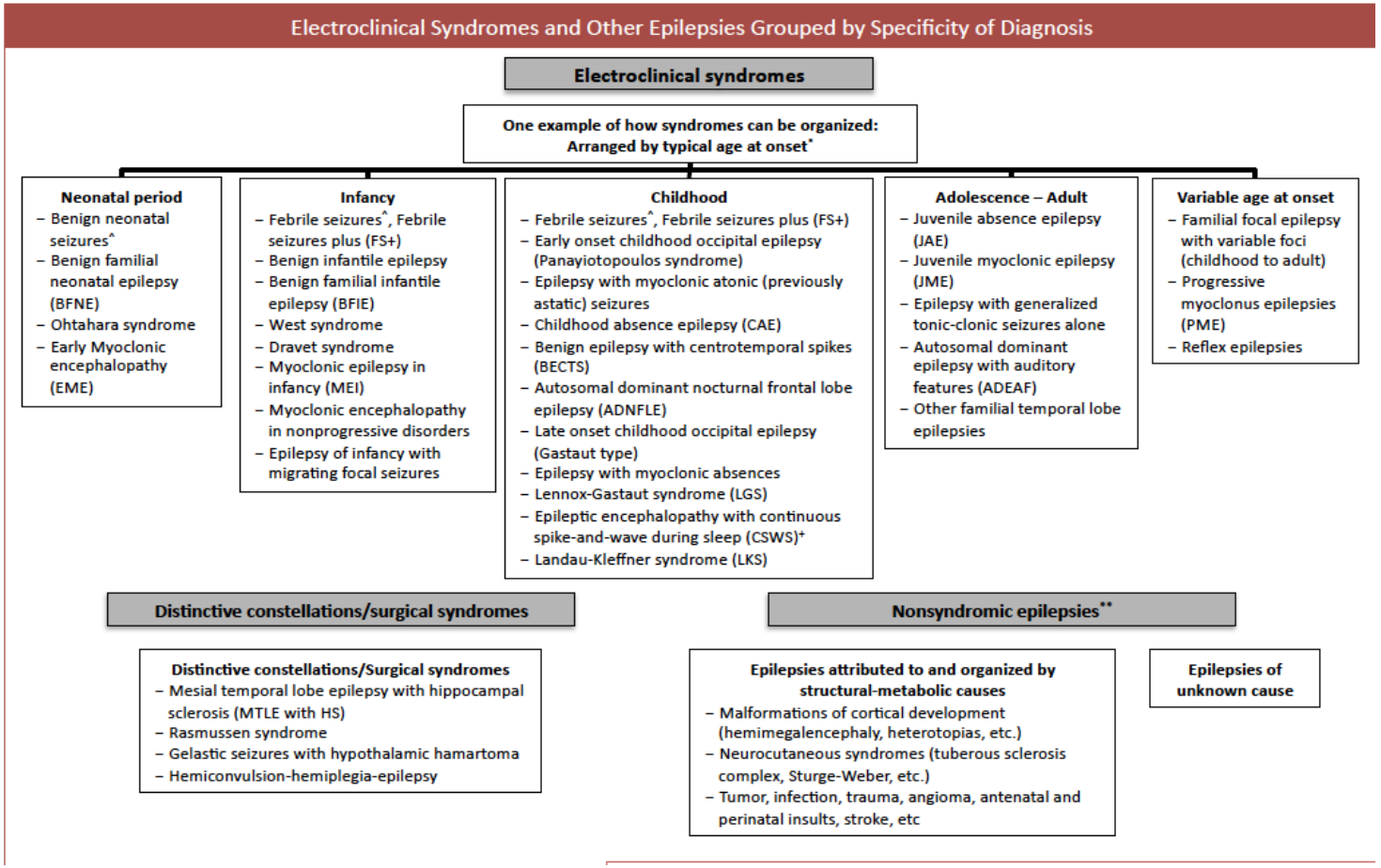


Figure 3:3 ILAE Proposal for Revised Terminology for Organization of Seizures and Epilepsies 2010.

3.4 Epidemiology morbidity and mortality

Epilepsy is one of the most common neurological disorders in the world, affecting all ages and genders. According to the Joint Epilepsy Council (JEC), 600000 people in the UK have active epilepsy. Approximately 87 people are diagnosed with epilepsy daily in the UK, and 31755 new cases are diagnosed annually (Epilepsy Actions website, 2016), translating to a prevalence of 9.7 cases per 1000 people. In the UK, ~1 person has active epilepsy per 103 people in general population. Individuals with active epilepsy have a premature death risk factor between 2 and 3 times greater than the general population (Tellez-Zenteno et al., 2005). Epilepsy is the 5th most common preventable cause of years of life lost in males and the 8th most common in females (Wheller et al., 2007).

Chapter 4 Principles of magnetic resonance imaging

4.1 Introduction

MRI is a powerful medical imaging technology that has been used for clinical diagnostic purposes for over 30 years in the medical field (Ai et al., 2012). The history of MRI development and use can be divided into three phases (Ai et al., 2012): (1) nuclear magnetic resonance (NMR) phenomenon, (2) MRI in clinical diagnosis and (3) functional MRI. The phenomenon of NMR was defined by Isidor Isaac Rabi, an American physicist working at New York City's Columbia University in 1938 (Rabi et al., 1938). In 1944, Rabi was awarded the Nobel Prize in Physics for his work surrounding NMR phenomenon (Ai et al., 2012). Later, in 1946, the phenomenon of NMR in liquids and in solids was discovered by Felix Bloch, a Swiss physicist working at Stanford, and Edward Mills Purcell, an American physicist working at the Massachusetts Institute of Technology. Bloch and Purcell were awarded in the Nobel Prize in Physics in 1952 for their contributions regarding NMR phenomenon in condensed matter (Ai et al., 2012). The spin echo was discovered by Erwin Louis Hahn, an American physicist, in 1951 (Hahn, 1950). Fourier transform NMR spectroscopy was developed by Richard Robert Ernst, a Swiss physical chemist, in 1966. Later, in 1991, Ernst was awarded the Nobel Prize in Chemistry for his contribution in NMR spectroscopy (Ernst, 1966). In 1973, Paul Christian Lauterbur (Lauterbur, 1973), an American chemist, and Sir Peter Mansfield, an English physicist (Mansfield and Grannell,

1973), defined magnetic field gradients to localize NMR signals. This development laid the foundation for modern MRI technology (Ai et al., 2012). In 2003, Lauterbur and Mansfield were awarded the Nobel Prize in Physiology for their innovations in MRI technology (Ai et al., 2012). Raymond Vahan Damadian, an Armenian-American physician working at the State University of New York as an associate professor of medicine in 1977 produced the first whole-body MR images (Ai et al., 2012). The first MRI scan took nearly 5 hours to produce an image (Ai et al., 2012). In 1977, English scientists Sir Peter Mansfield and Michael Maudlsey published an article (Mansfield and Maudsley, 1977) in The British Journal of Radiology to report the first MR image in humans: a cross-sectional image of the human finger (Ai et al., 2012). Other human anatomical images of the wrist, abdomen, head and body were captured between 1977 and 1980 (Ai et al., 2012).

Subsequently, in 1980, the first commercial MRI scanners were produced by FONAR Corporation, an American and British (from Oxford University) company (Ai et al., 2012). General Electric and Siemens started to produce MRI scanners in 1983 (Ai et al., 2012). Later, MRI technology advanced and the image quality improved. A new imaging method, fMRI, used to distinguish oxygenated blood from deoxygenated blood (BOLD) was discovered in 1990 by (Ogawa et al., 1990). The fMRI method allows for the mapping of the function of regions of the brain.

4.2 Atomic structure

The atom involves of a nucleus spinning its own orbiting the nucleus and spinning on their axis. The structure of atom including the nucleus, (Figure 4:1) Electron (+Ve charge), Proton (positive –Ve change) and Neutrons with no net charge (Pooley, 2005).

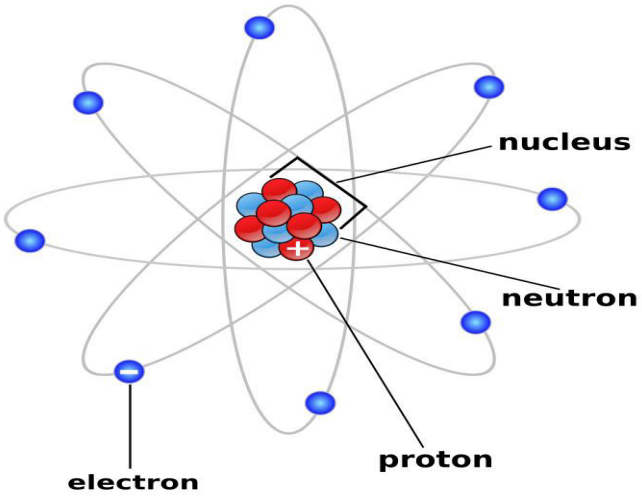


Figure 4:0:1 The nucleus, proton and neutron within the atom

4.3 Basic elements of magnetic resonance

There are three basic elements of magnetic resonance (Pooley, 2005);

(1) Hydrogen proton (H).
(2) Main magnetic field (B_0).
(3) Radio frequency signal (RF).

4.3.1 Hydrogen

Hydrogen is an important element in MRI for two reasons (Pooley, 2005). First, H is the most abundant element in human body (see Table 4-1 for

useful nuclei in MRI studies). Second, H has the strongest magnetic moment of any substance. The hydrogen proton is positively charged (Figure 3:2 A). Figure 3:2 B shows an H proton in a magnetic field spinning in random directions.

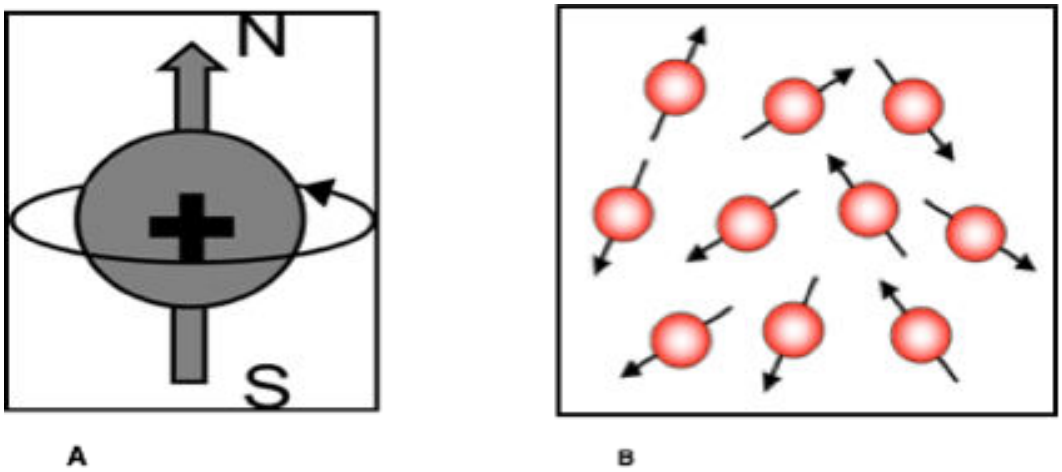


Figure 4:0:2 (A) Hydrogen proton . The positively charged hydrogen proton(+) spins about its axis and acts like a tiny magnet. N=north , S= south. (B) Hydrogen proton spinning in random directions. (Pooley, 2005).

4.3.2 Main magnetic field.

The second element of magnetic resonance is the main magnetic field (B_0) (Pooley, 2005). The main magnetic field is symbolized as $\mathbf{B_0}$, measured by a unit called a Tesla (T) (Pooley, 2005). The Tesla is the main unit used to measure magnetic field strength. In MRI, the electric current in the wires produces a mini magnetic field (Figure 3). In the main magnetic field ($\mathbf{B_0}$) there are three principles. First, the strength of main magnetic field must

be stable and not fluctuate. Second, the homogeneous main magnetic field should be uniform from the bore of the magnet (scan area) to the border fields (outside the isocenter) in all directions. Third, the main magnetic field should be large enough to create simple protons (Pooley, 2005).

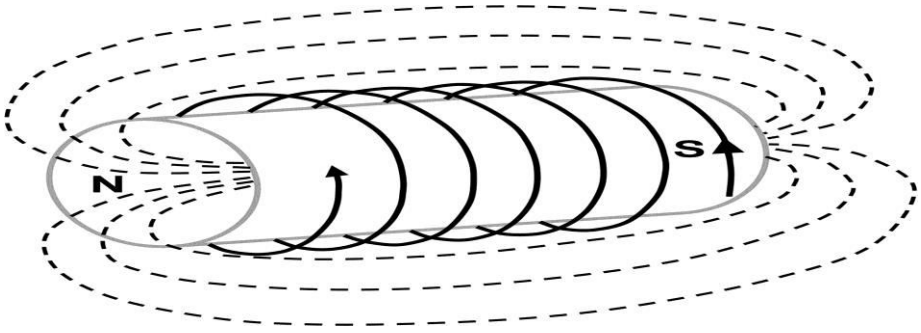


Figure 4:0:3 Main magnetic field. A large electric current in loops of wire at superconducting temperatures will produce a very magnetic field (Pooley, 2005)

4.3.3 Radio frequency

RF is symbolized by a sine wave (Hashemi et al., 2012). The RF consists of electromagnetic radiation lower in energy than interference. The energy (pulse) of the RF extends from 10 MHz to 100 MHz (Hashemi et al., 2012). The RF is the signal received and transmitted from protons. Larmor frequency is also known as precession frequency should the pulse of the RF present a smaller frequency when the H protons are processing (Pooley, 2005).

4.4 Larmor equation and precession

All active MRI nuclei spinning on their own axis and the secondary spinning occurring by external magnetic fields take place in a step called precession (Figure 4:4). Precession is type of turning defined as a wobble. The H_1 nuclei precess in the main magnetic field.

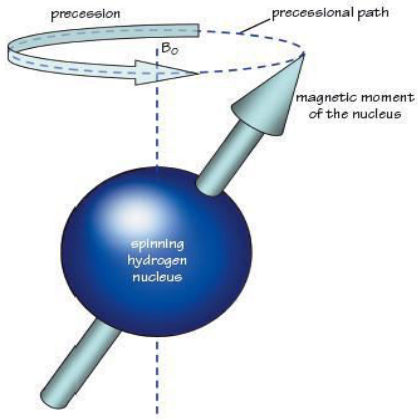


Figure 4:0:4 Precession of the protons and proton precession resembles the wobble of a top as it spins (Westbrook, 2015)

Larmor equation : The relationship between the main magnetic field strength ($\mathbf{B_0}$) and the gyromagnetic ratio (g) (Hashemi et al., 2012). Larmore equation explained below .

$$\mathbf{w = \gamma B_0} \quad \textbf{Equation 1}$$

w = Angular frequency

γ = Gyromagnetic ratio ; frequency of spine (ratio of magnetic moment) constant for nuclei . hydrogen ($H_{1)}$ gyromagnetic 42.58 MHz.

$\mathbf{B_0}$ = External magnetic field in tesla.

Tesla unite describe the filed strength of the isocenter of the large static main magnetic field **(B_0).**

4.5 Basic MRI imaging technique

A subject is first positioned in an MRI scanner in the bore of the MRI magnet ($\mathbf{B_0}$). The protons of hydrogen in the body can align in two directions (Westbrook, 2015). According to classical theory (Westbrook, 2015), the first direction is in parallel alignment (i.e., the same direction) with the magnetic field. The second direction is anti-parallel (i.e., the opposite direction) to the main field (Westbrook, 2015). Figure 4:4 shows hydrogen protons in alignment with the magnetic field.

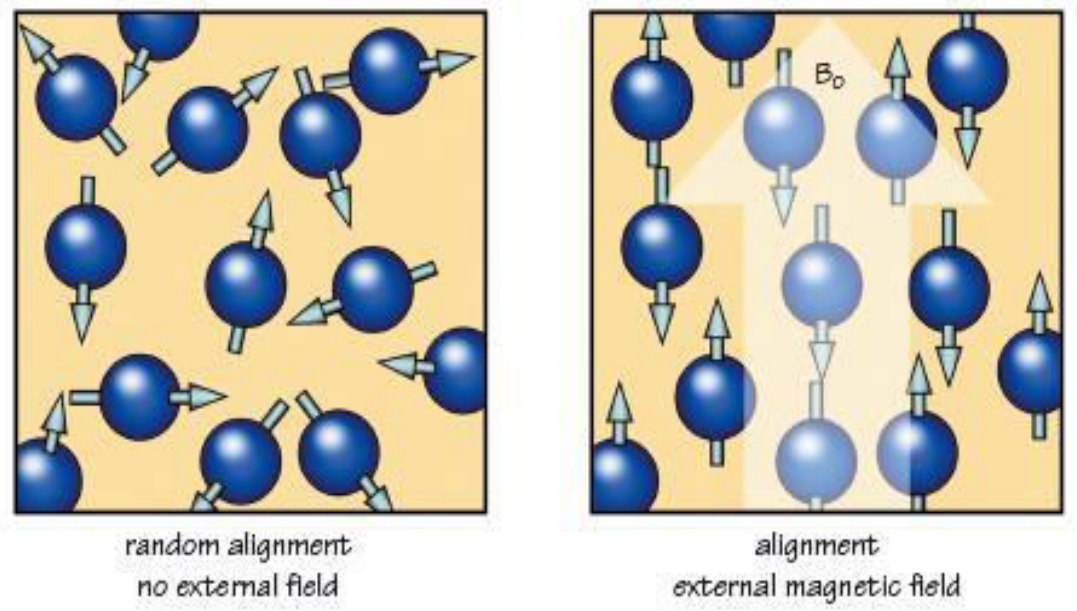


Figure 4:0:5 Hydrogen protons alignment. Adopted from (Westbrook, 2015)

There are continually a greater number of protons in the parallel direction than the anti-parallel direction (Westbrook, 2015). The number of protons

increasing in the parallel direction depends of the strength of the magnetic field. Subsequently, the protons with anti-parallel directions are forced by the magnetic field, causing the anti-parallel protons to cancel an equal number of protons in the parallel directions. The outstanding magnetic forces will be parallel to the main magnetic ($\mathbf{B_0}$) field. The previous explanations describe the net magnetization vector (NMV). The NMV is a quantity vector including the strength of magnet and the main magnetic ($\mathbf{B_0}$) field (Hashemi et al., 2012).

The NMV contains two directions: the longitudinal magnetization direction and the transverse magnetization direction (Figure 4:5 for NMV directions). The longitudinal magnetization is the magnitude in the direction of B_0. Conversely, the transverse magnetization is the magnitude in the transverse direction or vertical to B_0. All magnetization lies in the longitudinal plane and parallel to the main magnetic field before applying the RF pulse (Hashemi et al., 2012). Then, the RF pulse excitation is applied 90° to the NMV. The longitudinal magnetization is reversed to be transverse to the magnetization plane and the magnetization in the longitudinal plane is reduced to zero. Then, the RF pulse is turned off. Immediately after being switched off, the RF pulse in the longitudinal magnetization recovers and the decay stage starts in transverse magnetization. The recovery and decay happen at the same time but individually. This step is called time of relaxation or relaxation time. The relaxation time T1 recovery is 63% of the time it takes the longitudinal

magnetization to recover. Decay time (T2 decay) is the time it takes for 37% of the transverse magnetization to decay. Through the recovery time, the transvers magnetization is decreased and the longitudinal magnetization is increased. Then the signal is received by the coil and sent to the K-space for storage and reconstruction of the MRI image (Hashemi et al., 2012).

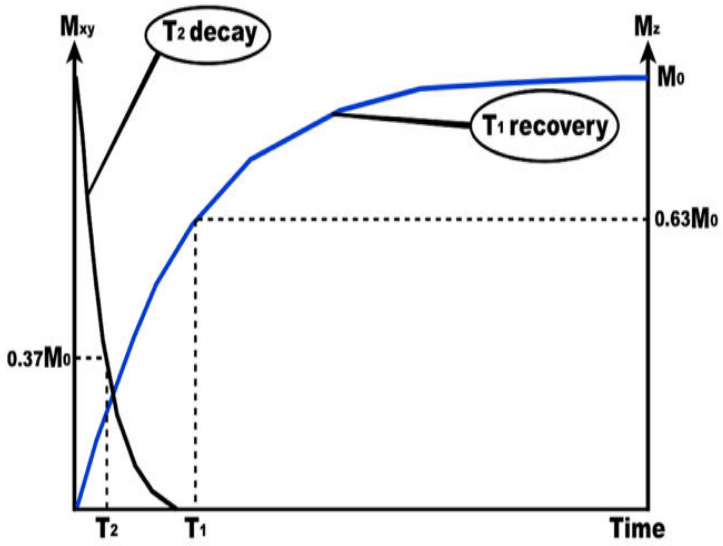


Figure 4:0:6 T1 recovery and T2 decay

4.6 MRI coils

MRI coils are electrical devices consisting of various loops of wire (Hashemi et al., 2012). In principle, MRI coils are divided into two types: gradient coils and transmit/receive coils. Gradient coils include the following: imaging gradient coils and shim coils. Gradient imaging coils have at least three couples of small electromagnets that create gradient magnetic fields. Shim coils are made up of small electromagnets working

to generate an additional uniform main magnetic field. Transmit/receive coils are small devices located on the subject to transmit and receive the RF signal. There are many types of coils: single-phase, quadrature (receive and transmit), surface or volume and single or phased array.

4.7 Imaging Parameters

All MRI sequences are planned with specific set methods to acquire MRI images. The following parameters make up the sequence for an MRI scan: repetition time (TR), echo time (TE), flip angle (FA), number of acquisitions (NEX), field of view (FOV), slice thickness, phase encoding and bandwidth. Any change in the MRI parameters can affect the resolution, signal to noise ratio and contrast of the MRI image.

4.7.1 Time repetition

Time repetition (TR) is the period of time between two excitations and is measured in milliseconds (ms). For example, in spin echo sequences, the repetition time is interval time between 90° RF excitation pulses. Figure 4:7 shows the time period between two 90° excitations. TR is involved in the contrast of MRI images and the time of the scan.

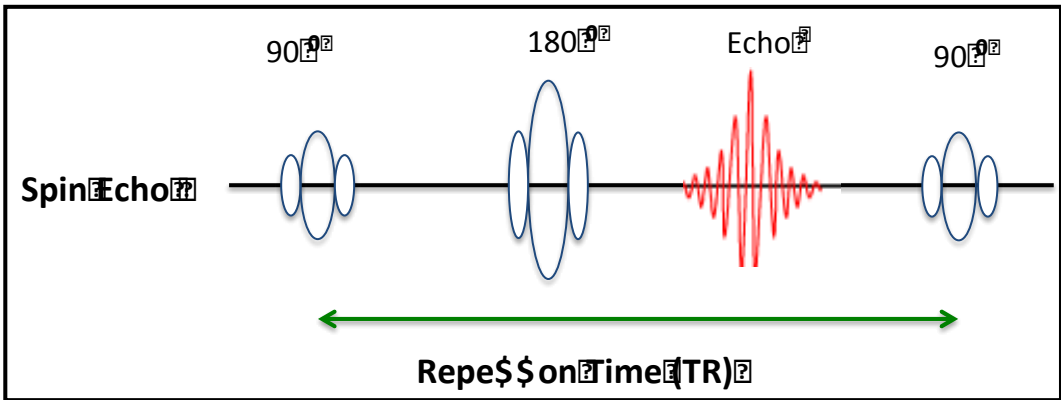


Figure 4:0:7 Show time period between two 90 excitation

4.7.2 Time of echo

Time of Echo (TE) is the period of time between the excitation pulse and echo production. TE measured in milliseconds (ms). TE is a very important parameter in determining the contrast of the image and the scan time for the MRI procedure. Figure (4:8) shows the time between the excitation pulse and echo.

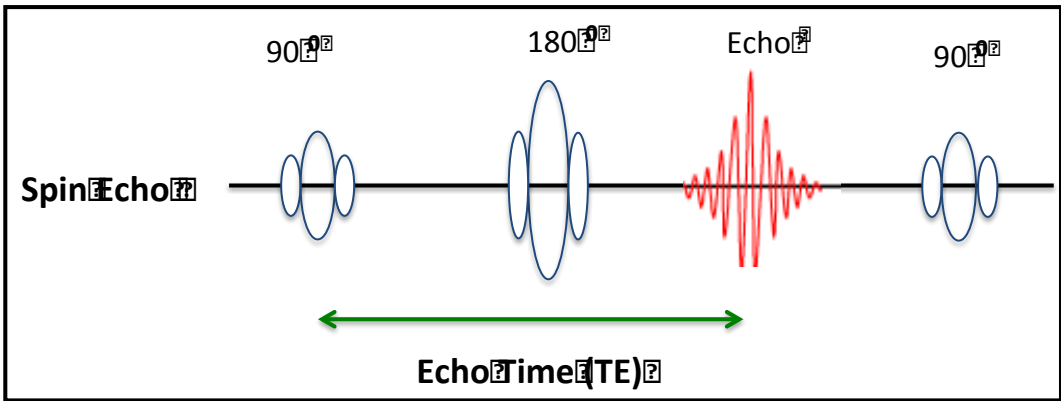


Figure 4:0:8 Time between excitation pulse and echo

4.7.3 Bandwidth

Bandwidth is the frequency range that is created across the subject by gradients throughout the acquisition. Bandwidth is involved in determining in signal-to-noise ratio (SNR).

4.7.4 Flip angle

Flip angle (FA) is the amount of rotation away from the longitudinal magnetization exerted by the NMV during the radiofrequency pulse application. In other words, the FA is the angle between the NVM and the main magnetic field ($\mathbf{B_0}$). The FA contributes to the contrast of the image and the scan time.

4.7.5 Number of acquisitions

Number of acquisition (NEX) is the number of acquisitions or the average signal number, also known as the number of excitations. NEX is involved in SNR and the time of the scan.

4.7.6 Field of view

Field of view (FOV) is the area of structural anatomy (i.e., the object) to be scanned in an MRI. FOV is involved in image resolution and SNR.

4.7.7 Slice thickness

The slice thickness is the thickness of the selected slice which influences the coverage and signal intensity.

4.7.8 Phase encoding

The phase encoding is the generation of phase differences along a particular direction of a slice of tissue in spatial localization of the MRI signal.

4.8 Pulse sequences

In this section the most common pulse sequences used in MRI imaging are described, including the following: spin echo, inversion recovery, gradient echo and echo planar imaging. Pulse sequences are ruled by two pulse parameters: TR and TE (Hashemi et al., 2012)

4.8.1 Spin echo sequence

The spin echo sequence is the most commonly used pulse sequence. The RF pulse sequence consists of a 90° excitation pulse followed by an 180° rephasing or refocusing pulse to eliminate field inhomogeneity. Figure (4:9) shows the effect of the spin echo pulse sequence on the magnetic moment. The two important points in the spin echo sequence are the 90° excitation pulse and the 180° rephrasing pulse. In Spin Echo The T1 – weighted image (short TR, short TE). Proton Density image (long TR, short TE) and T2 – weighted image (long TE, long TR).

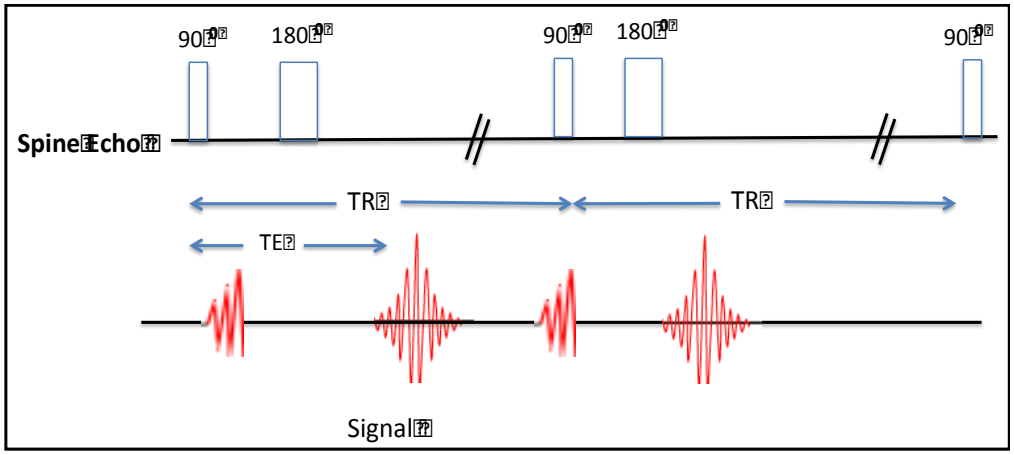


Figure 4:0:9 A simple representation of a pulse sequence in which the signal is refocused using a spin echo.

4.8.2 Inversion recovery sequence

The inversion recovery sequence (IR) involves an early 180° RF pulse to invert the magnetization followed by a 90° RF null time (inverting time) and 180° RF pulse to produce an echo. Figure (4:10) diagrams the inversion recovery sequence.

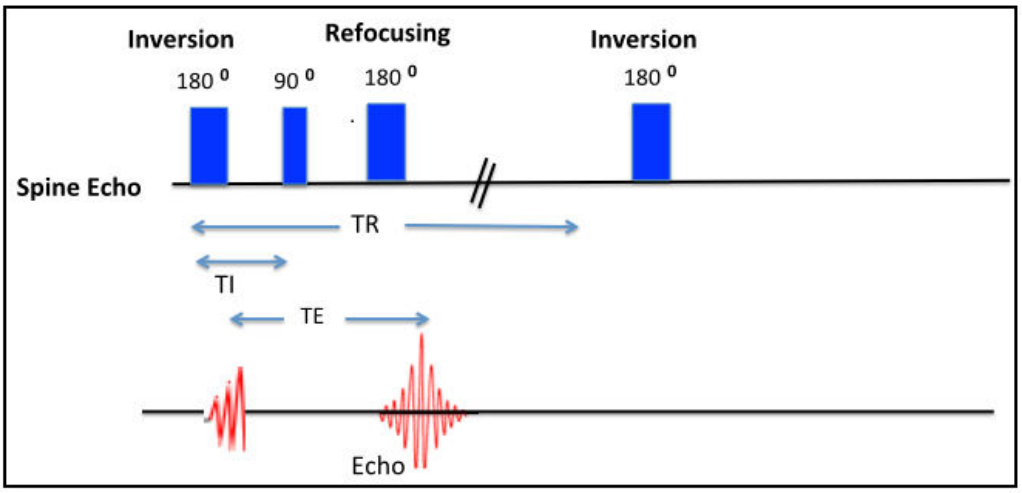


Figure 4:0:10 Illustration of Inversion recovery pulse sequence

4.8.3 Gradient echo sequence

The gradient echo sequence is the sequence in which the signal is refocused using a gradient. The gradient echo sequence generally uses an excitation FA of less than 90° and a gradient reversal to rephrase the protons Figure (4:11) The two important points in the gradient echo sequence are the variable flip angle and gradient reversal. In gradient echo pulse sequenceT1– weighted large Flip Angle short TR short TE, T2* - weighted small Flip Angle long TR long TE and Proton Density Weighted Small Flip Angle Long TR Short TE. The advantages of gradient echo sequences are much shorter scan times than SE pulse sequences, the low FA allows for faster recovery of longitudinal magnetization, gradients rephrase faster than 180° RF pulses and TR and TE values are shorter than SE. In contrast, the disadvantages of gradient echo sequences are that they are the susceptible to magnetic field inhomogeneity and susceptible to magnetic artefacts.

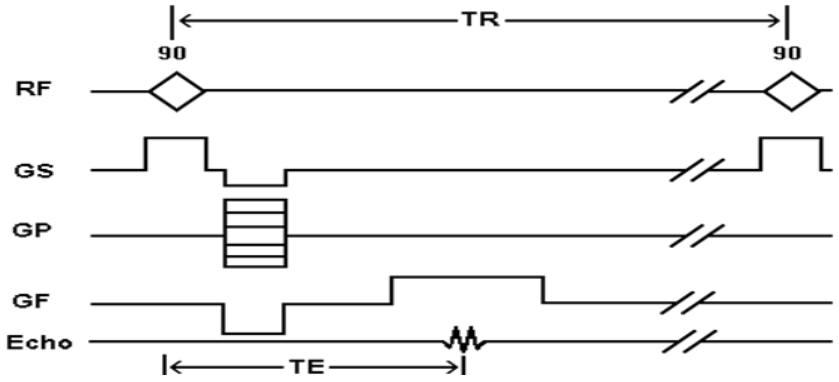


Figure 4:0:11 basic gradient echo sequence. Adopted from.www.mritutor.org

4.8.4 Echo planner imaging sequence

Echo planar imaging (EPI) was introduced by Sir Peter Mansfield from Nottingham in 1977 (Figure 4:12). EPI uses faster MRI imaging sequences and allows MRI data to be collected and reconstructed in less than a second (20-1 mm/ms) (Poustchi-Amin et al., 2001). The main concept behind EPI sequences is to complete the K-space in a single shot. As such, EPI is a single-shot technique with a time of imaging of 100 ms for a 128x128 matrix. There are two types of EPI sequences: single-shot EPI and multi-shot EPI. The advantages of EPI include reduced scanning time and motion artefacts. In contrast, EPI sequences are very sensitive to losing the signal.

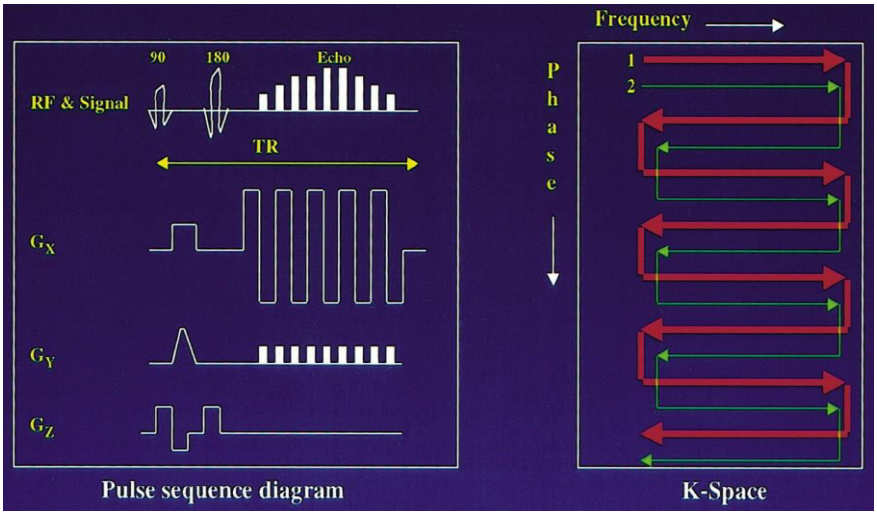


Figure 4:0:12 Echo-planar imaging. Within each TR period, multiple lines of imaging data are collected. G_x = frequency-encoding gradient, G_y = phase-encoding gradient, G_z = section-selection gradient (Poustchi-Amin et al., 2001).

4.9 Structural MRI

Structural MRI currently used investing and evaluation the patients who is suffering from epilepsy. Because of its high sensitivity and specificity for reporting the structural abnormalities in brain (Commission on Neuroimaging of the ILAE, 1998). The primary role of structural MRI is identifying the structural alternations in the brain that cause seizure. In patients with TLE the most common abnormality underlying epilepsy is hippocampal sclerosis and the report it by T1-weighted. The International League against Epilepsy proposed the structural MRI is not essential in case of absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy and benign focal epilepsy of childhood. Usually do not display any alterations and the majority of patients become seizures free when treated with AEDS (Commission on Neuroimaging of the ILAE, 1998). Nevertheless, applying advanced MRI protocols and techniques it has been exposed that even in these patients, structural abnormalities are present proposing focal abnormalities even in patients with IGE, which may be of prognostic or therapeutic relevance (Vollmar et al., 2011; Woermann et al., 1999). The structural MRI sequences include T1-weighted and T2-weighted 3D and 3D FLAIR used in NDE study. The MRI structural data may be post processed in order to allow statistical comparison of morphometric structures in patients groups or individual patients with healthy controls. This method permits for objective discovery of brain alterations associated with epilepsy disorders. These

approaches vary according to the morphometric feature that is measured and compared between groups. T1-weighted and T2-weighted 3D scans used to investigate the quantitative techniques to determine region-specific alterations in gross morphology in groups of patients with epilepsy relative to healthy controls. Gross morphological changes include tissue atrophy, pathological enlargement, and focal disruptions to the normal development of the brain, all of which may be connected to the onset of epileptic seizures. The 3D FLAIR sequences used to permit the determine of alterations in regional tissue integrity through unbiased quantification of voxel-wise FLAIR values using techniques originally developed for voxel-based analyses of T1-weighted data (Ashburner and Friston, 2000). The 3D T1-weighted sequences used for spatial normalisation, tissue segmentation, and quantitative morphometric analyses of brain structure, volume, thickness, surface area, curvature and shape analysis.

4.10 Functional MRI and resting state methods

The term fMRI frequently refers to the imaging of brain activation that was introduced by Dr. Seiji Ogawa in 1990s (Ogawa et al., 1990). The basic concept of fMRI is that the changes of neuronal activity produce a measurable change in MR signal. The oxygen (O_2) is transported to neurons through the hemoglobin. Once neuronal activity increases, there is an increased demand for O_2 and the local response is an increase in

blood flow to areas of increased neural activity (Ogawa et al., 1990). Hemoglobin is diamagnetic when the blood is filled with O_2 (oxygenated) and paramagnetic when the blood is devoid of O_2 (deoxygenated) (Ogawa et al., 1990). The change in magnetic properties is indicated to change in the MR signal of blood based on the level of oxygenation. These changes in signals can be identified using MR pulse sequence as blood-oxygen-level-dependent (BOLD) contrast (Ogawa et al., 1990).

Recently, the methods of neuroimaging have evolved to advanced examinations of the differences in structures and functions of the brain. As such, fMRI classically uses a task or stimulus to investigate changes in the BOLD signal (Lee et al., 2013). Recently, the BOLD fMRI method has been practically used for brain imaging when the subject is not involved in any task or activity; these are known as resting state methods (Lee et al., 2013). The resting state fMRI method measures spontaneous low frequency fluctuations (<0.1 Hz) in the BOLD signal to explore the functional connectivity of the brain (Lee et al., 2013). Resting state fMRI can be used to detect changes in functional connectivity in many neurological disorders (Barkhof et al., 2014). The resting state fMRI method was introduced by (Biswal et al., 1995). The resting state fMRI method measures spontaneous low frequency fluctuations (<0.1 Hz) in the BOLD signal to explore the functional connectivity of the brain (Lee et al., 2013). Resting state fMRI can be used to detect changes in functional

connectivity in many neurological disorders (Barkhof et al., 2014). The resting state fMRI method was introduced by (Biswal et al., 1995).

Chapter 5 Magnetic resonance imaging in epilepsy

5.1 Introduction

MRI sequences used in advanced imaging research employ several tests in a specific order: high resolution structural MRI, MRI Diffusion, fMRI, MRS and ASL. First, structural MRI includes high-resolution sequences with advanced imaging analysis to compare volumes; distributions and morphology difference in cortical and subcortical structures in the brain from T1 weighted images. Second, MRI Diffusion sequences give a measure of the diffusion of water in tissue. Third, fMRI utilises the differing magnetic properties of deoxygenated and oxygenated haemoglobin to map hemodynamic differences associated with the activity of neural blood oxygen level dependent (BOLD) response. MRS non-invasive methods then provide information on the composition of chemical brain tissue by detecting the resonance signals of metabolites. Finally, ASL is a non-invasive method capable of measuring the quantitative perfusion of an MRI. Significant improvement has been made in the last 20 years regarding the structural and functional imaging of the brain in epilepsy

(Urbach, 2013).

This chapter provides an overview of the MRI research undertaken regarding newly diagnosed epilepsy cases and the literature on chronic epilepsy in different imaging analysis methods, including, rsfMRI, fMRI and MRS.

5.2 Magnetic Resonance Imaging in newly diagnosed epilepsy

There is substantial literature on MRI in patients with chronic epilepsy. In contrast, only a few studies have applied quantitative MRI to evaluate structural brain tissue in patients with NDE (Liu et al., 2001, Liu et al., 2002, Liu et al., 2003a, Schmidt and Pohlmann-Eden, 2011, Van Paesschen et al., 1998). Moreover, most of the MRI studies about NDE were focused on the hippocampus (Briellmann et al., 2002, Salmenperä et al., 2005, Van Paesschen et al., 1998).

A quantitative hippocampal MRI study (Van Paesschen et al., 1997) on ethology and early prognosis of newly diagnosed partial seizures in adults provides the prospective of patients. The objective of the study was to discover MRI-based ethnologies and significant prognostics to evaluate how the information affected management by individual patients. The results of the study demonstrated that 48 out of 63 patients had normal MRI findings, accounting for 76% of the study population. Further, 10% of patients reported hippocampal sclerosis (HS), and another 14% of patients reported different abnormalities including lesions. A longitudinal quantitative MRI study by (Van Paesschen et al., 1998) was planned to evaluate the change of the hippocampus in patients with newly diagnosed partial seizures after experiencing a seizure just after diagnosis. Study

findings show 78% of patients had normal MRI scans, 11% of patients had HS and 11% of patients had other abnormalities.

Another longitudinal quantitative MRI study (Liu et al., 2002) with chronic and newly diagnosed seizure patients investigated the hippocampal volume difference, cerebellum and structures of the neocortical area over 3 years using MRI quantitative methods. Study findings showed no change in hippocampal volume and cerebellar differences at baseline. In a study of newly diagnosed patients and chronic epilepsy (Liu et al., 2002), the objective was to determine the damage to the hippocampus, cerebellum and the neocortical area in epilepsy patients over 3 years. Study outcomes reported no significant progressive changes in any of these three areas.

A prospective longitudinal quantitative MRI study (Briellmann et al., 2002) that included 24 patients with newly diagnosed lateralised temporal lobe epilepsy (TLE) identified a 9% reduction in volume of the hippocampus over 3.5 years. Quantitative MRI analysis techniques were applied to evaluate the cerebellar volume differences between newly diagnosed and chronic epilepsy patients (Hagemann et al., 2002). The techniques demonstrated cerebellar changes only in patients with chronic epilepsy.

Similarly, in a prospective research longitudinal follow up study of 122 chronic epilepsy patients including 68 patients with newly diagnosed epilepsy and 90 healthy controls, Liu et al. (2003) reported that patients

with chronic epilepsy have more progressive change in neocortical volume at 54% relative to healthy controls . Similarly, generalised volume loss in a few patients with NDE was examined in patients with NDE over 1, 2, 3 and 5 years (Salmenperä et al., 2005), and no difference in hippocampal volumes between patients with NDE and healthy controls was identified at baseline or at any of the follow up points. Eight per cent of patients with NDE had hippocampal damage at the diagnosed time, and 13% developed volume decreases two to three years after the follow up (Salmenperä et al., 2005). See the Table 5-1, Summary of Magnetic Resonance Imaging in newly diagnosed epilepsy studies, for an overview of these studies.

In study (Park et al., 2015) 22 patients with newly diagnosed partial epilepsy of unknown etiology, 36 healthy subjects and 24 patients with chronic partial epilepsy of unknown. Freesurfer whole brain analysis methods were applied to evaluate the cerebellar volume. In (Park et al., 2015) study the volumes of hippocampus, amygdala, thalamus, caudate, putamen, pallidum, brainstem, cerebellar gray and white matter, compared between the groups. The main result of this study was that there was significant volume reduction of cerebellar white matter in patients with NDE.

In addition, a brain morphology study was undertaken in children with newly diagnosed epilepsy (Saute et al., 2014) using FreeSurfer software measures of cortical morphology and subcortical and cerebellar volumes

relative to healthy controls. Patients with epilepsy demonstrated diffusion in the frontal, parietal and temporal lobes with volume reductions in the brainstem and subcortical structures (bilateral caudate, left thalamus, and right hippocampus). As mentioned previously, there is a growing body of literature regarding chronic epilepsy (D'Arcy et al., 2011, Gross, 2011, Pohlmann-Eden, 2011, Schmidt and Pohlmann-Eden, 2011). However, little attention is paid to structure and function in NDE studies. The primary aim of NDE research is to establish and develop new management techniques to prevent patients with NDE from developing chronic stage epilepsy disorders (Pohlmann-Eden, 2011). According the First Halifax International Epilepsy Conference, “there is a need for a unified theory of epileptogenesis, addressing the interplay of functional and structural brain changes’’ (Schmidt and Pohlmann-Eden, 2011). Furthermore, there is an urgent call for advanced MRI longitudinal studies in NDE (Schmidt and Pohlmann-Eden, 2011). The majority of fMRI studies have focused on chronic epilepsy. Conversely, there are relatively few studies regarding newly diagnosed epilepsy (Federico, 2011), and there is a need for fMRI research studies in patients with NDE. The limited studies in newly diagnosed epilepsy examine the language area and cognitive outcomes in children with new onset TLE (Sogawa et al., 2010). In addition, a study of MRS used hippocampal N-acetyl aspartate (NAA) to creatine ratio (NAA/Cr) in patients with newly diagnosed partial epilepsy (Li et al., 2000)

and showed significantly lower NAA/Cr values in 5 out of 8 patients (62.5%) with NDE

compared to 40 healthy subjects.

Table 5-1 Summary of structural MRI in newly diagnosed epilepsy studies

Authors	Subjects information	Study information's	Results
Van Paesschen at al (1997) Prospective study	63 patients with NDPS. 31 M, 32 F, Mean 26 Y, Age range 15-50 Y	1.5T MRI T1 and T2 weighted Hippocampal T2 (HCT2), HCV volumetric measurement,	76% normal MRI, 10% HS, 14% other abnormalities.
Van Paesschen at al (1998) Prospective study	36 patients with NDPS 15 M, 12 F Mean 26 Y, Age range 14-50 Y. 12 HC, 5 M, 7 F, Mean 29 Y, Age range 21-38 Y	1.5 T MRI T1 and T2 Weighted Hippocampal T2 (HCT2) and HCV volumetric Measurement.	78% normal MRI, 11% HS, 11% Other Abnormalities. No normal MRI patient Progressed to HS after 1 year
Liu et al (2001) Longitudinal prospective study	154 patients with chronic epilepsy and 90 NDPS. 20 HC, 13 M, Mean 35 Y, Age range 18-81 Y	1.5 T MRI T1, T2, FLAIR. HCV Measurement TBV, CBV, GMV, WMV, ICV.	No hippocampal and cerebellar changes at baseline. No significant progressive changes over 3.5 years
Liu et al. (2002) Prospective study	68 patients with NDPS 37 M, 32 F, Mean 31 Y, Age range 15-70 Y. HC 90, 49 M, 41 F, Mean 45 Y, Age range 14-77 Y	1.5 T MRI T1, T2, FLAIR. HCV Measurement TBV, CBV, GMV, WMV, ICV.	No progressive changes over 3.5 years
Hagemann et al(2002) Prospective study	3 patient groups and a control group Age range 17 - 50 Y. 94 NDE patients, 279 chronic epilepsy patients and 36 HC	1.5 T MRI, T1, T2, FLAIR Measurement TBV, CBV, and ICV.	Cerebellar changes in-patient with chronic epilepsy.
Liu et al.(2003) Longitudinal prospective study	53 newly diagnosed patients 28 M, 25 F, Mean 30 Y, Age range 15-56 Y. 114 patients with chronic epilepsy 53 M, 61 F, Mean 34.5 Y, Age range 14-74 Y. 90 controls 49 M, 41 F, M 35 Y, Age range 14-74 Y	1.5 T MRI T1, T2, FLAIR. HCV Measurement TBV, CBV, GMV, WMV, ICV	Generalised volume loss in n=small NDE patients Progressive changes more in chronic patients
Salmenperä et al. (2005) Prospective study	103 patients with newly diagnosed focal epilepsy, 52 M, 51 F, Mean 36 Y, Age range 15-65 Y. HC 20, 10 M,10 F, Mean 31 Y, Age range 21-50 Y	1.5 T MRI T1 and T2 Weighted Hippocampal T2 (HCT2) and HCV volumetric Measurement.	No difference in hippocampal volumes between patients with NDE and controls at baseline or at 1, 2, 3 or 5 years follow up 8% hippocampal atrophy at diagnosis,13% developed volume decrease 2-3 years follow up Patients who developed volume loss had more seizures before diagnosis & treatment

M = Male, **F** = Female, **Y** = years, **HC** = Healthy controls**, HS** = hippocampal sclerosis, **CB**V = cerebellar volume, **TBV** = Total brain volume, **GMV** = grey matter volume**, WMV** = white matter volume, **HCV** = hippocampal volume, NDPS = Newly diagnosed partial seizures

5.1 Magnetic Resonance Imaging in chronic epilepsy

There is a very large Magnetic Resonance Imaging literature describing chronic epilepsy using advanced applied sophisticated image analysis approaches to structural and functional MRI data. The structural and functional MRI studies report different alterations in brain regions in patients with chronic epilepsy. The quantitative MRI analysis methods are used to measure the change in cortex and subcortical structures between neurological patients and healthy controls.

Voxel based Morphometry **(**VBM) has been commonly applied in epilepsy imaging studies to investigate the differences in the morphology of the brain in different types of epilepsy. Several VBM studies have shown cerebral Grey Matter (GM) and white matter abnormalities in patients with TLE. In some examples, patients with TLE studies reported brain alterations including hippocampal, parahippocampal gyrus and anterior temporal lobe white matter atrophy in patients with TLE (Bonilha et al., 2004, Keller and Roberts, 2008). VBM study findings showed patients with TLE exhibited significantly increased or decreased GM and WM (Keller and Roberts, 2008).

5.1.1 Morphology in chronic epilepsy

Numerous epilepsy studies using VBM have focused on patients with refractory epilepsy (Bonilha et al., 2004, Keller et al., 2002, Yasuda et al.,

2010b, Yasuda et al., 2010a). In addition, patients with TLE in different VBM studies have exhibited grey matter reduction in different regions including: in ipsilateral to hippocampal atrophy, the parahippocampal gyrus, the superior temporal gyrus, the amygdala, the fusiform gyrus, in basal ganglia, the insula, the thalamus, the frontal lobe, parietal lobes, occipital lobes and the cerebellum (Bernasconi et al., 2004, Bonilha et al., 2005, Keller et al., 2002, Keller et al., 2004, Keller et al., 2009, Labate et al., 2008, Mueller et al., 2006, Riederer et al., 2008, Yasuda et al., 2010b). Moreover, Contralateral GM decrease has also been identified in many TLE studies in frontal lobes, parietal lobes , occipital lobes, and the cerebellum (Keller et al., 2002, Yasuda et al., 2010b, Yasuda et al., 2010a). Other TLE studies showed patients with TLE reported bilateral atrophy in the thalamus (Labate et al., 2008, Mueller et al., 2006, Yasuda et al., 2010b).

In fact, VBM tools are commonly used in idiopathic generalized epilepsy (IGE) to investigate the morphological difference in whole brain between patients with IGE and healthy controls. The most reliable outcome is the decrease in thalamus volumes found in most VBM studies (Ciumas and Savic, 2006, Chan et al., 2006, Helms et al., 2006, Kim et al., 2007, Lin et al., 2009b, Mory et al., 2011, Pardoe et al., 2008, Seneviratne et al., 2014). In addition, there is some change in frontal grey matter; either an increase or decrease (Seneviratne et al., 2014). For example, for frontal GM change, an IGE study showed decrease GM in the supplementary

motor area and posterior cingulate cortex (O'Muircheartaigh et al., 2011). Other VBM studies have found reduced GM volume in frontal, parietal, and temporal cortex, and the cerebellum of patients with IGE relative to healthy controls (Ciumas and Savic, 2006, Kim et al., 2007). Other studies have reported that patients with IGE demonstrate smaller GM volume in different regions; the subcallosal gyri of the frontal lobe, basal frontal lobe, anterior and posterior cingulate, and insula compared to healthy controls (Betting et al., 2010, Ciumas and Savic, 2006, Chan et al., 2006, Lin et al., 2009b). In contrast, a few VBM studies on patients with IGE have shown that they exhibit increased GM volume in different area: the frontobasal, superior mesiofrontal, and mesial frontal lobe relative to healthy controls (Betting et al., 2006, Kim et al., 2007, Woermann et al., 1999).

A study examining volumetric and shape analysis in patients with IGE using First-FSL shape analysis tools, FreeSurfer and VBM compared to healthy controls (Kim et al., 2013), reported reductions in thalamus volume and bilateral thalamus shape changes, relative to healthy controls. Another study examining patients with juvenile myoclonic epilepsy (JME) showed that they exhibited significant differences in both thalamus volume and subcortical shape in the left and right thalamus in patients with JME relative to healthy controls (Saini et al., 2013). There are differences in abnormalities in the brain structures of patients with chronic epilepsy including cortical thickness and thalamus volumes showing increased GM compared to healthy controls (Kim et al., 2007, Lin et al., 2009b,

Woermann et al., 1999). Other studies have found decreased cortical thickness and thalamus volumes (Bernhardt et al., 2009, Chan et al., 2006, Ciumas and Savic, 2006, Kim et al., 2007, Lin et al., 2009b, Woermann et al., 1999). Some chronic epilepsy studies have found no change in cortical thickness and a normal thalamus volume (Bernasconi et al., 2003, Natsume et al., 2003, Seeck et al., 2005). In addition, the different outcomes among studies of patients with epilepsy are restricted by different methods of analysis, demographic and physiological differences (for example total intracranial volume, age and gender), and may also be affected by sample size and the populations being studied.

5.1.2 Magnetic resonance spectroscopy in chronic epilepsy

The purpose of MRS in epilepsy is to assist the identification of the epileptogenic lesion. Numerous MRS studies have been applied in patients with IGE and JME. MRS methods are able to identify metabolite abnormalities in chronic patients. NAA is the most sensitive metabolite marker of chronic pathology. MRS methods have been applied in many studies in patients with TLE, and the majority of studies have examined their role in pre-evaluation surgery in patients with refractory TLE (Antel et al., 2002, Duncan, 1996, Duncan, 1997, Willmann et al., 2006). On other hand, there is a body of literature describing MRS in patients with chronic epilepsy; including IGE and JME. Most MRS studies of IGE and JME identify reduced NAA/Cr in the thalamus and frontal lobe (Bernasconi et al., 2003, de Araujo Filho et al., 2009, Doelken et al., 2010, Fojtikova et

al., 2006, Haki et al., 2007, Helms et al., 2006, Kabay et al., 2010, Lin et al., 2009a, Mory et al., 2003, Savic et al., 2000, Savic et al., 2004).

5.1.3 Functional magnetic resonance imaging in chronic epilepsy

fMRI methods were applied in patients with IGE and JME within multiple studies (Archer et al., 2003, Berman et al., 2010, Gotman et al., 2005, Hamandi et al., 2006, Liu et al., 2008, Moeller et al., 2008b, Moeller et al., 2008a, Moeller et al., 2009, Moeller et al., 2010, Moeller et al., 2011, Szaflarski et al., 2010, Szaflarski et al., 2013, Seneviratne et al., 2014, Vollmar et al., 2011). fMRI studies have identified activation during cognitive tasks in many regions of the brain (Roebling et al., 2009, Seneviratne et al., 2014), including in the thalamus regions with GSWDs (Seneviratne et al., 2014). However, some studies in IGE and JME identified activation in other regions of the brain including the frontal, partial, and temporal lobes and the insular (Seneviratne et al., 2014).

5.1.4 Resting state functional MRI in chronic epilepsy

In recent years, Resting-State functional MRI (rs-fMRI) study has increased in different neurological disorder and psychiatric patients to examine alterations in functional brain networks (Cataldi et al., 2013, Fox and Greicius, 2010, Lang et al., 2014, Pittau and Vulliemoz, 2015, Voets et al., 2012). The research in resting state functional connectivity adds more information on multifocal facets in networks of both focal and

generalized epilepsy (Pittau and Vulliemoz, 2015). The main resting state brain networks in humans include: visual, auditory, sensorimotor, default mode, attention, salience, control emotion and language networks (Lang et al., 2014).

Resting state fMRI is used to examine alterations in functional connectivity and networks, which are associated with alterations in structural connectivity and networks, which are modeled using DTI/DKI methods. Similar changes cannot be identified using conventional clinical MRI sequences, and advanced post processing image analysis studies are needed instead to measure reconstructed connections and networks. Based upon the recent changes in epilepsy classification, to consider the importance of brain networks for onset of seizure, even in focal epilepsies (Berg et al., 2010, Berg and Scheffer, 2011). There has been a change to identify how seizures begin and end through the study of macro-structural networks using neuroimaging data (Terry et al., 2012). The altered connectivity between brain areas is a legitimate hypothesis for the aetiology in focal and generalised seizures.

There are a large number of resting state studies applied to patients with TLE (Caciagli et al., 2014, Cataldi et al., 2013). The first was undertaken by (Zhang et al., 2010). It investigated resting-state data in patients with mTLE including left and right hippocampal sclerosis relative to healthy controls. Patients with mTLE demonstrated significantly decreased connectivity in the dorsal mesial prefrontal cortex, mesial temporal lobe

and inferior temporal lobe. In addition, patients with right mTLE compared to healthy subjects demonstrated bilaterally decreased functional connectivity in the mesial temporal lobe and increased functional connectivity in the posterior cingulate cortex (Liao et al., 2010). They also had increased functional connectivity in the medial temporal lobe, and the frontal lobe relative to healthy controls.

Other work has identified patients with mTLE as having decreased functional connectivity in the default mode between the parietal and frontal lobes. Resting state functional connectivity studies reported significant abnormalities in default mode networks for different neurological disorders including Alzheimer's, Parkinson's, and schizophrenia (Greicius et al., 2004, Garrity et al., 2007, van Eimeren et al., 2009). These default mode networks demonstrated reductions in functional connectivity in patients with TLE relative to healthy controls (Pittau et al., 2012, Pittau and Vulliemoz, 2015, Zhang et al., 2010). TLE studies showed patients had decreased functional connectivity in attentions networks compared to normal subjects (Bocquillon et al., 2009, Liao et al., 2010, Yang et al., 2010, Zhang et al., 2009b). In patients with absence epilepsy, default mode networks exhibited decreased functional connectivity relative to healthy controls (Luo et al., 2011, McGill et al., 2012, Song et al., 2011). Nevertheless, (Moeller et al., 2011) studied patients with IGE using EEG recordings with generalized spike wave discharges (GSWDs). The group with GSWD presented BOLD activation in different regions including the

thalamus, frontomesial cortex, and cerebellum.

In addition, the default mode regions exhibited BOLD deactivation. In healthy controls compared to patients with IGE in (Moeller et al., 2011), alterations in seed connectivity in left thalamus were identified in patients with IGE . Patients with IGE had increased connectivity in WM and decreased connectivity in the cerebellum. Studies on functional magnetic imaging in patients with IGE reported activation in several brain areas when cognitive task were undertaken (Seneviratne et al., 2014). The majority of fMRI studies showed activation in both thalamus in IGE patients with GSWDs (Berman et al., 2010, Gotman et al., 2005, Hamandi et al., 2006, Moeller et al., 2008b). Moreover, GSWDs are related to activation of cortical regions including the frontal lobe, parietal lobe, temporal lobe and insular (Seneviratne et al., 2014).

Table 5-2 Studies on thalamus in chronic epilepsy

Authors	Subjects information	Results
Ciumas et al (2006)	19 patients IGE, 52 HC (33.6±8.6)	VBM analysis showed reduced GM in frontal, parietal, temporal cortex, thalami and cerebellum
Chan et al (2006)	CAE 13 (17 ±8), HC 109 (29±9).	VBM analysis CAE patients showed areas of GM decrease in both thalami and in the subcallosal gyrus
Helms et al (2006)	43 patients IGE (age 32±8 years, 38 (age 29±8 years)	VBM showed the thalamic grey matter fraction was reduced in IGE. MRS reported IGE patients showed elevated Glx and reduced NAA concentrations in the thalamus
Muller et al (2006)	26 TLE-MTS (age, 35.6±9.7 years), 17 TLE-no (age, 35.6±11.1 years), and 30 HC (age, 30.3±11.1 years).	In TLE-MTS, GM/WM volume and concentration reductions were found in the ipsilateral limbic system, ipsi- and contralateral neocortical regions, thalamus, cerebellum, internal capsule, and brainstem when compared with controls
Kim et al (2007)	25 JME patients (mean age=22 ±5.1 years),43 HC mean age=23.1±4.3 Y	VBM showed GM volume reductions in the thalamus bilaterally in JME patients
Labate et al (2008)	95 TLE patients (mean age 36.6±15.8) years, 37 HC 37.3±10.6	VBM analysis provided evidence of a reduction in grey matter volume in the hippocampus and thalami
Pardoe et al (2008)	44 CAE mean age 15±2 years, 257 HC 34±13 years	VBM analysis showed reduced grey matter volume in thalamus
Lin et al (2009)	60 patients JME (mean 26.6±8.8 years), 30 HC 30.1±9.0 years	VBM revealed significantly reduced bilateral grey matter volume in thalamus
Kim et al (2013)	50 IGE (mean 26.0±7.2 years), 50 HC 26.8±5.6 years	VBM showed significant regional grey matter volume reduction in thalami in IGE, FSL-FIRST shape analysis revealed regional atrophy on the thalamus bilaterally in IGE
Saini et al (2013)	40 JME (mean 22.8±5.3 years), 19 HC 24.5±4.2 years.	Vertex-wise shape analysis showed significant reductions in the thalami bilaterally in JME, Significant volume loss in both the thalamus
Bernascon et al (2003)	20 IGE (mean 37±9 years),11HC 34±5 years	MRS study; Reduced NAA/Cr in thalamus
Fojtikova et al (2006)	18 IGE (mean 32.3±6.63 years) HC 25 27.6±3.70 years	MRS study; Reduced NAA, GLX, CHO and MI in frontal lobe, reduced NAA in thalamus
Haki et al (2007)	15 JME (mean 20.3 years) , HC 16 24.5 years.	MRS study; Reduced NAA/Cr in thalamus
Lin et al (2009)	60 JME (mean 26.6±8.85 years), 30 HC 30.1±9.0 years.	MRS study; Reduced NAA/Cr in thalamus
Doeken et al (2010)	9 AE average 28 years, 9 HC average 27 years	MRS study; Reduced NAA/Cr in thalamus
Hamandi et al (2006)	IGE 32, SGE 14	Activation in thalamus; deactivation in frontal, parietal, and temporal cortices
Moeller et al (2011)	IGE 27, Co 30	Activation in thalamus, mesial frontal cortex, cerebellum; deactivation in default mode areas

AE, absence epilepsy; **CAE**, childhood absence epilepsy; **Co**, controls; **GM**, grey matter; **GTCSO**, generalized tonic–clonic seizures only; **IGE**, idiopathic generalized epilepsy; **JME**, juvenile myoclonic epilepsy; **WM**, white matter, **MRS** ,MR spectroscopy, **GLX**, glutamine , **NAA,** *N*-acetyl aspartate, **TLE**, temporal lobe epilepsy, **MTS**, mesial temporal sclerosis, **CHO**, choline; **MI**, myo-inositol

Chapter 6 Subjects and methods

6.1 Study Population

6.1.1 Ethics

The present study was approved by the Local Group Committee NRES North West – Liverpool Central; reference number, 14/NW/0332; protocol number, ADV-MRI-PRO-V3; IRAS project ID, 140420. (appendices for ethics-related documents).

6.1.2 Recruitment and scanning

Patients with NDE were recruited from the Walton Centre National Health Service (NHS) Foundation Trust Liverpool. Patients who had attended epilepsy clinics and had been diagnosed with focal epilepsy within 12 months were among those recruited. The neurologist explained the study to patients with NDE in the clinic. If a patient was interested in participating in the NDE study, the clinical staff provided an information sheet and consent forms for the patient. After one week, the research team (consisting of a research nurse and myself) contacted the patient by phone to discuss participation in the NDE study. Further explanation of the study was provided by sending the participants information sheets (appendices). Each subject provided written informed consent to take part in the study. Then, I arranged MRI appointment scans at the Magnetic Resonance and Image Analysis Research Centre (MARIARC). The MRI scanning for patients with NDE and healthy controls was performed by

myself under the supervision of a research radiographer who was registered in the NHS. I am primarily responsible for collection of data and analysis in this thesis. (appendices for participant information sheet and MRI safety screening).

6.1.3 Patients

All patients participating in the NDE study were matched by age and gender to healthy controls. All patients were diagnosed with focal epilepsy by a consultant neurologist and recruited for MRI scanning within 12 months of diagnosis. A series of inclusion criteria were identified for the NDE study. First, patients had to be currently attending clinics at the Walton Centre and had to have been diagnosed with epilepsy by a neurologist <12 months prior to inclusion. Second, patients had to be diagnosed with focal seizure onset disorder (e.g. temporal or frontal lobe epilepsy). Third, patients had to be aged between 18 and 60 years.

The exclusion criteria for patients with NDE were: (a) patients diagnosed with non-epileptic seizures; (b) patients with single seizures; (c) patients with provoked seizures only or patients with acute symptomatic seizures (e.g. acute brain haemorrhage or brain injury); (d) patients with any progressive neurological disease (e.g. known brain tumour); (e) patients diagnosed >1 year prior to recruitment; and (f) patients aged <18 years or >60 years. Patients were recruited from the Walton Centre NHS Foundation Trust, Liverpool. We recruited 27 patients with NDE (14 male,

13 female, mean age (±SD) 33.2±12.13 years), including 2 left-handed patients. See Table 6-1 for demographic and clinical details of patients with NDE. After completing the MRI scan for each patient I transferred the MRI clinical Images from MARIARC to the Walton Centre NHS Foundation Trust and imported the images into the picture archiving and communication system (PACS) for radiology reporting.

Table 6-1 Demographic and clinical details of patients with NDE

Patient	Age Gender	Scan Date	EEG	MRI Report	Dx Date	Current Medications	Number Months between Dx &MRI	Number of seizures between Dx and MRI	Neurological History
P01	18 M	**10/14**	N	**Cortical dysplasia**	04/14	**LMT 400mgs**	6	**Multiple complex partial seizures**	No neurological history
P02	37 F	**10/14**	N	**Normal**	09/14	**LMT100mgs**	2	**TCS**	Concussive SZ
P03	39M	**11/14**	N	**Focal Gliosis**	05/14	**LMT 100mgs**	7	**No Seizures**	2 TCS & Brain injury age of 15
P04	57 M	**11/14**	N	**Cortical dysplasia**	03/14	**LEV1000mgs**	8	**Complex partial SZ seizure**	TCS& small cyst in Pituitary gland
P05	43 F	**11/14**	N	**Normal**	10/14	**LEV 1000 mgs**	1	**Complex partial SZ**	Headaches & 7 episodes.
P06	30 M	**11/14**	N	**Normal**	04/14	**LAM 150mgs**	7	**Single SZ**	No neurological history
P07	28 F	**11/14**	N	**Normal**	06/14	**LEV 1000mgs**	5	**No Seizures**	TCS & simple partial SZ
P08	37 M	**11/14**	A	**Normal**	03/14	**ZNS 200mgs**	8	**2 Partial SZ**	TCS & complex partial SZ
P09	30M	**11/14**	N	**L hippo <R**	03/14	**LMT 500 mgs**	8	**Partial Seizures**	Von Willebrand's disease
P10	22 M	**11/14**	N	**Normal**	10/14	**ZNS 150mgs**	1	**No Seizures**	TCS
P11	37M	**11/14**	N	**Normal**	10/14	**LMT 150mgs**	2	**No Seizures**	Febrile convulsions
P12	38 F	**11/14**	N	**WM change**	06/14	**ZNS 250mgs**	5	**TCS & Complex partial SZ**	Previous Hypoxic brain injury
P13	37 F	**12/14**	N	**Normal**	11/14	**ZNS 500mgs**	1	**No Seizures**	Simple partial seizure
P14	18 F	**12/14**	N	**Normal**	09/13	**LMT 150mgs**	11	**4 TCS & Complex partial SZ**	No neurological history
P15	54 F	**12/14**	N	**Normal**	11/14	**LMT 100mgs**	1	**Six seizure**	Single TCS and history febrile
P16	41 F	**01/15**	A	**Normal**	08/14	**LEV 500mgs**	5	**Partial seizure &TCS**	TCS
P17	25 F	**02/15**	N	**Normal**	12/14	**LMT 200mgs**	3	**Single seizure**	No neurological history
P18	18 M	**02/15**	A	**Normal**	112/14	**LMT 50mgs**	2	**Single seizure**	TCs
P19	56 M	**02/15**	N	**Normal**	01/15	**LMT 150mgs**	1	**No Seizures**	TCS & simple partial SZ
P20	41 F	**03/15**	N	**Normal**	01/15	**LMT 300mgs**	2	**No Seizures**	TCS & complex partial SZ
P21	22M	**03/15**	N	**Rt. hippo change**	01/15	**LMT 50mgs**	3	**No Seizures**	TCS
P22	23 M	**06/15**	N	**Normal**	03/15	**LMT l50mgs**	3	**No Seizures**	TCS
P23	20 F	**09/15**	N	**Normal**	08/15	**LMT 100mgs**	1	**No Seizures**	No neurological history
P24	32 M	**09/15**	N		08/15	**LEV 1000mgs**	1	**No Seizures**	TCS & previous Brain injury
P25	38 F	**09/15**	N	**Normal**	07/15	**LEV 1000 mgs**	2	**No Seizures**	TCS
P26	28 M	**10/15**	N	**Normal**	10/15	**LMT 150 mgs**	1	**No Seizures**	2 TCS
P27	24M	**10/15**	N	**Normal**	08/15	**Unknown**	2	**No Seizures**	No neurological history

A=Abnormal=Normal**; Dx=**Diagnosed **; TCS=**tonic clonic seizures**; LAM=**Lamictal **:LEV=**levetiracetam; **LMT=**lamotrigine**;Rt.hippo=**Right Hippocampus**; SZ=**seizure
WM=White matter**; ZNS=**Zonisamid

6.1.4 Healthy controls

Healthy controls were recruited from the University of Liverpool by sending emails to all staff and students. The inclusion criteria for healthy controls were: (a) no history of any neurological disease; (b) aged between 18 and 60 years; and (c) no use of drugs or >4 units of alcohol consumed in the preceding 48 hours. The exclusion criteria for healthy controls included: (a) any neurological diseases; and (b) use of drugs or >4 units of alcohol consumed in the preceding 48 hours. I recruited 32 healthy controls [14 male and 18 female, mean age 33.07±11.5 years (±SD)], including 2 left-handed subjects matched by age and gender to patients with NDE. Table 6-3 demographic for healthy controls information's. All participants provided written informed consent to participate in the MRI scan and NDE study. Table 6-2 summarizes the inclusion and exclusion criteria for the recruitment of patients and healthy controls.

Table 6-2 Summary of the inclusion and exclusion criteria for recruitment of NDE epilepsy patients and healthy controls

Criteria	Inclusion	Exclusion
NDE Patients	• Diagnosed with epilepsy by a neurologist less than 12 months previously • Diagnosis of a focal seizure onset disorder • Between ages 18-60 years	• Non-epileptic seizures • Provoked seizures • Acute symptomatic seizures • Progressive neurological disease • Diagnosis over one year
Healthy Controls	• No history of neurological or psychiatric disease • No use of drugs or over four units of alcohol consumed in the preceding 48 hours • Between ages 18-60 years	• Any neurological disease • Drug use or five or more units of alcohol consumed in the preceding 48 hours

Table 6-3 Healthy controls information's

ID	Gender	Age	Handedness	Notes
NDEC01	Male	19	Right	
NDEC02	Female	37	Right	
NDEC03	Female	29	Right	
***NDEC04**	Female	43	Right	Patient shows lesion on Rt frontal lobe.
NDEC05	Female	28	Right	
NDEC06	Male	31	Right	
NDEC07	Female	19	Right	
NDEC08	Male	38	Right	
NDEC09	Female	19	Right	
NDEC10	Male	37	Right	
NDEC11	Female	18	Right	
NDEC12	Male	21	Right	
NDEC13	Female	37	Right	
NDEC14	Female	54	Right	
NDEC15	Male	19	Right	
***NDEC16**	Female	38	Right	Patient shows lesions slightly more than expected of the patient's age.
NDEC17	Male	23	Right	
NDEC18	Female	40	Right	
NDEC19	Female	43	Right	
NDEC20	Female	25	Left	
***NDEC21**	Female	39	Right	A couple of lesions seen close to the genus of the corpus callosum
NDEC22	Female	31	Right	
NDEC23	Female	20	Right	
NDEC24	Male	38	Right	
NDEC25	Female	52	Right	
NDEC26	Female	45	Right	
NDEC27	Male	58	Left	
NDEC28	Female	58	Right	
***NDEC29**	Male	31	Right	Patient shows lesions slightly more than expected of the patient's age.
NDEC30	Male	21	Left	
NDEC31	Female	25	Right	
NDEC32	Male	54	Right	
NDEC33	Female	37	Right	
NDEC34	Male	32	Right	
NDEC35	Male	31	Right	
NDEC36	Male	33	Right	

Subjects were excluded from study for incidents findings based on radiologist report

6.2 Edinburgh Handedness Inventory

The Edinburgh Handedness Inventory was established by Oldfield (1971) as a test to evaluate hand preference. More specifically, it is used to measure the dominance of a participant's right or left handedness in

activities (Oldfield, 1971). Patients and healthy controls answer ten questions to evaluate their handedness preference when undertaking the following activities: writing, drawing, throwing, cutting using scissors, tooth brushing, cutting with a knife without use of a fork, using a spoon, using a broom/speed (upper hand), striking a match and opening a box (lid) (see the appendices Edinburgh Handedness Inventory forms).

Patients and healthy controls were requested to respond to each question depending on how frequently they use each hand for each task. The subjects were instructed to write “RR” letters if they always used their right hand, “R” for activities where they usually used their right hand, “RL” if they used ether their right or left hand, “LL” if they always used their left hand and “L” if they usually used their left hand. Handedness was calculated using the following values: RR=+2 score, R=+1 score, RL=0 score, L=–1 score and LL=–2 score. The sum was determined, taking note of positive or negative values, and then multiplied by 5. The final score of the Edinburgh Handedness Inventory should be somewhere between +100 (exclusive right handed) and –100 (exclusive left handed). All participants in our study were matched according to handedness.

6.3 MRI data acquisition

All participants were scanned using a Siemens 3T Trio whole-body scanner (Siemens, Erlangen, Germany) with an 8-channel radiofrequency (RF) head coil, together with foam padding to comfortably restrict head

motion at the MARIARC, University of Liverpool. Patients with NDE and healthy controls underwent a safety-screening form for research scans by myself, which was subsequently reviewed by a professional radiographer at MARIARC. Participants were screened for the following contradictions to MRI scanning: cardiac pacemaker or mini-defibrillator, brain clips (cerebral aneurysm clips), metal fragments in the eye or head, artificial heart valve, shrapnel and neurostimulators. Any patient with one or more of these contraindications was omitted from MRI scanning for to safety reasons.

6.3.1 Research MRI sequences

In this thesis I used four MRI research sequences to analysis MRI data and the total scan time for research sequences was 36 minutes. The research sequences including: structural T1 weighted and resting state

6.3.1.1 Structural magnetic resonance imaging

T1 structural magnetic resonance imaging sequences used spatial normalisation, segmentation of tissue, and quantitative morphometric analyses of brain structure including volume, thickness, surfaces and curvature. T1-weighted anatomical data 1 mm isotropic resolutions were obtained on a 3T magnet using 3D magnetization-prepared rapid gradient echo (M-PRAGE) sequence. Imaging parameters were: 160 slices, voxel resolution of 1.0×1.0×1.0 mm; pulse sequence parameters were: TR=1970 ms, TE=4.38 ms, number of averages=1, flip angle=90, FOV=256x256

mm^2, slice thickness=1 mm, matrix size=256 X 256 and a scan time of eight minutes.

6.3.1.2 Resting state sequences

Resting state functional MRI sequences were used to evaluate brain resting functional connectivity. A gradient echo EPI sequence was used during functional scans with the following parameters: TR=2000 ms, TE=30 ms, flip angle=90°, FOV=192 x 192 mm, slice thickness=3.5 mm, and matrix size=64 x 64. During the scanning, participants were told to close their eyes and relax but remain awake, and not to think about anything in particular. The scan duration was six minutes and four seconds.

6.3.2 MRI clinical sequences

In the NDE study, research sequence protocols included clinical sequences for patients with NDE and healthy controls. The purpose of clinical sequences is for clinical diagnostics, for reporting brain lesions and for incidental findings of healthy controls to be excluded from the research. Clinical sequences were reported by the expert neuroradiologist at the Walton Centre NHS Foundation Trust Hospitals, Radiology Department. The clinical scanning protocol included 3D T1-weighted high resolution volume with isotropic 1 mm voxels, 3D T2-weighted higher resolution volume with isotropic 1 mm voxels, 3D T2-fluid attenuated inversion recovery (FLAIR) with isotropic 1 mm voxels and 2D coronal T1-FLAIR.

The majority of MRI scans for epilepsy must include the previous sequences and be obtained on high field 3 tesla to provide more detail of brain structures than the MRI scans obtained on 1.5 tesla (Bernasconi and Bernasconi, 2011). The 3D T2-weighted sequences provide high resolution for the whole brain to detect focal cortical dysplasia (Vézina, 2011). FLAIR high-resolution whole brain sequences are used to evaluate hippocampal sclerosis and FCD.

6.4 Imaging analysis methods

In this thesis, I used the most popular neuroimaging analysis software, including FSL, FreeSurfer, Statistical Parametric Mapping (SPM) and CONN toolbox, to study the structural and functional alterations in patients with NDE and healthy controls. The method of medical imaging analysis for individual experiments is described independently in another chapter.

6.4.1 FSL software library

In this thesis, I used three methods of analysis from the FSL software. The first method is called FIRST FSL (Patenaude et al., 2011) and was used to evaluate the shape differences in subcortical structures of the brain in patients with NDE relative to healthy controls. FIRST techniques are explained in Chapter 7 (Section 7.3.3.1). The last method used in this thesis from the FSL software is called multivariate exploratory linear optimized decomposition into independent components (MELODIC), which

uses independent component analysis (ICA) to examine the functional connectivity of the whole brain in patients with NDE relative to healthy subjects (Beckmann and Smith, 2004, Beckmann et al., 2005). The technical procedures for ICA method is explained in chapter 9 (section 9.3.3.1).

6.4.2 FreeSurfer software

FreeSurfer is a free neuroimaging analysis tool used to analyse structural and functional brain MRI images (Fischl, 2012). FreeSurfer provides fully automatic analysis of cortical and subcortical brain anatomy (Fischl, 2012). FreeSurfer tools can be applied in many types of analyses of subcortical segmentation, cortical surface reconstruction, cortical segmentation, cortical thickness estimation, fMRI analysis and tractography (Fischl, 2012). In this thesis I used FreeSurfer tools to provide a full processing stream for structural MRI images to evaluate the subcortical brain structures of patients with NDE relative to healthy controls (Fischl, 2012). The technical application of FreeSurfer is described in Chapter 7 (Section 7.3.3.2).

6.4.3 Statistical parametric mapping

SPM is a statistical method developed by Friston et al. (Friston et al., 1990) at the Wellcome Department of Imaging Neuroscience (University College, London) to investigative changes in brain activity through neuroimaging experiments using neuroimaging methods including fMRI,

PET, SPECT, EEG and magnetoencephalography (MEG). I used SPM software in this thesis, as described in Chapter 7 (Section 7.3.3.3) to run voxel-based morphometry tools to distinguish alterations in brain morphology between patients with NDE and healthy controls. The procedure for the use of VBM tools is described in the same section in Chapter 7 (7.3.3.3). A description of the use of SPM software to run the CONN functional connectivity toolbox to investigate functional connectivity in ROI in both thalami in patients with NDE and healthy controls can be found in Chapter 9 (9.3.3.2). The procedure for the use of the CONN functional connectivity toolbox is also described in Chapter 9. CONN functional connectivity toolbox. The CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012) is an SPM-based toolbox for functional connectivity analyses in resting state fMRI techniques. The CONN functional connectivity is a free toolbox used to measure seed-to-voxel connectivity maps, region to region connectivity, graph theory and independent component analysis networks. In this thesis I used the CONN toolbox in Chapter 9 to examine functional connectivity in ROI in both thalami in patients with NDE compared to healthy controls. The analysis procedure for the CONN toolbox process is explained in Chapter 9 (Section 9.3.3.2).

Chapter 7 Brain morphology in patients with newly diagnosed epilepsy

7.1 Introduction

A number of neuroimaging research studies have been undertaken in patients with chronic epilepsy (Bernhardt et al., 2013, Bonilha et al., 2004, Bonilha et al., 2005, Chan et al., 2006, Dabbs et al., 2012, Hagemann et al., 2002, Hermann et al., 2005, Keller et al., 2002, Keller and Roberts, 2008, Keller et al., 2015, Labate et al., 2008, Lin et al., 2009b, Labate et al., 2013, Mueller et al., 2006, Pardoe et al., 2008, Woermann et al., 1999, O'Muircheartaigh et al., 2011, Saute et al., 2014, Seneviratne et al., 2014, Yasuda et al., 2010a). Many of these studies used advanced quantitative MR image analysis methods including VBM, shape analysis of subcortical structures, and measurement of subcortical volume and cortical thickness

Morphological results in patients with chronic epilepsy have previously been reported including several alterations in GM and WM, specifically in the hippocampus, parahippocampal gyrus, temporal gyrus, amygdala, fusiform gyrus, basal ganglia, insula, thalamus, frontal lobe, parietal lobes, occipital lobes and cerebellum (Bernasconi et al., 2004, Bonilha et al., 2005, Keller et al., 2002, Keller et al., 2004, Keller et al., 2009, Mueller et al., 2006, Labate et al., 2008, Riederer et al., 2008, Yasuda et al., 2010b). In addition, several studies have shown changes in brain volume and shape including in the thalamus (Kim et al., 2013, Saini et al., 2013).

There are relatively few quantitative MRI studies investigating morphometric alterations in patients with NDE (Liu et al., 2001, Liu et al., 2002, Liu et al., 2003a, Schmidt and Pohlmann-Eden, 2011, Van Paesschen et al., 1998). Those, which have been undertaken typically, applied volumetric

techniques. The results revealed no differences between patients with NDE and healthy controls. However, further work suggests that there are very few patients with volumetric abnormalities at diagnosis (Liu et al., 2001, Liu et al., 2002, Liu et al., 2003a, Salmenperä et al., 2005, Van Paesschen et al., 1998). The purpose of this thesis chapter was to improve upon previous work by quantitatively investigating the morphological differences in whole brain between patients with NDE and healthy controls using vertex wise shape analysis to evaluate the shape of subcortical structures, subcortical Volumes Analyses segmentation to evaluate the subcortical structure volume in the brain and VBM to investigate differences in the whole brain. This chapter provides a brief overview of the three quantitative MRI imaging analysis methods utilized within the work, explains the principle process for each method and describes the study population.

7.2 Subjects and methods

7.2.1 Participants

All study participants were recruited from the Walton Centre NHS Foundation Trust, Liverpool. All had been diagnosed with focal epilepsy by a consultant neurologist and recruited for MRI scanning within 12 months of diagnosis. Twenty-seven patients with NDE were recruited (14 male, 13 female, with mean age (M)=33.2, standard deviation (SD)=12.13) and 32 healthy matched controls (14 male, 18 female, M=33.07, SD=11.5). Control and NDE study participants were matched for age, handedness (whether they are left- or right-handed) and gender. The recruitment procedure is

explained in more detail in section 6.2.2; Table 6-1 and 6-3 describes the demographics and clinical details of study participants with NDE; and Table 6-2 defines the inclusion and exclusion criteria for the recruited healthy and control patients. The local NHS Research Ethics Committee North West - Liverpool Central, approved the study (Reference 14/NW/0332).

7.2.2 Image acquisitions and structural MRI data

(see section 6.3.1.1)

7.2.3 Structural MRI analysis

This section provides a brief overview of the quantitative MRI imaging analysis methods used, and explains the principles process for each method. Structural analyses were performed on a Mac Pro (Version OS X 10.11.6 16 GB, 2.9 GHz Intel Core i5).

7.2.3.1 Vertex shape analysis (FSL-FIRST)

First, structural T1 data (DICOM format) for each study participant was converted to a compressed NIfTI file format by MRIcron (http://www.cabiatl.com/mricro/mricron). FSL-Integrated Registration and Segmentation Toolbox using FSL-FIRST software (http://www.fmrib.ox.ac.uk/fsl/first/index.htm) was utilized for the work. Second, brain extraction to remove all non-brain tissue from images was undertaken using the (BET) tool (Smith, 2002). Then, automated segmentation was undertaken using the FSL-FIRST tool applied to the subcortical brain (brainstem, bilateral thalami, hippocampi, amygdalae, putamen, caudate nucleus, accumbens nucleus and pallidum) in both hemispheres

(Patenaude et al., 2011).

FSL-FIRST toolbox is based on a template created using manually segmented images with subcortical labels parameterized as surface meshes. Images from after the brain was extracted by BET were registered to the template via 12 degrees of freedom affine transformation (Jenkinson and Smith, 2001). After that, the images were registered to a subcortical mask of standard MNI 152 template. The subcortical mask labelled and segmented the subcortical brain structure dependent upon the T1 Image. A multivariate Gaussian model of vertex region and power variety was then used to create surface work for all subcortical structures in every research participant examined. Then, a fully automated segmentation using FSL-FIRST tools was used to visually check the segmentations for all subcortical structures, to confirm the steps for segmentation had been undertaken the correct way. The same number and naming of vertices were utilized to indicate point examinations for all study participants. Comparable vertex surfaces were adjusted to the mean surface of the layout for all subcortical structures in MNI152 space; F statistics were then calculated to compare the shape change alterations between patients with NDE relative to healthy controls (Patenaude et al., 2011). Fractional dimension (FD) analysis is method applied to a quantitative shape metric (Zhang et al., 2008). FD is method to estimate fractal dimensions of subcortical structures based on the counting of box and was applied onto subcortical structures images to explore the vertex displacement of subcortical structures (Zhao et al., 2016). Positive values of FD show the outward vertex displacement (expansion), whereas negative values show the inward vertex displacement

(contraction).

7.2.3.2 Volumetric FreeSurfer analysis

FreeSurfer software, used in the Centre for Biomedical Imaging, Harvard Medical School (Charlestown, MA), is freely downloadable from http://surfer.nmr.mgh.harvard.edu. Its fully automated methods were introduced by (Fischl, 2012), and it is frequently used to study cortical and subcortical brain anatomy. FreeSurfer (version 5.3.0) was utilized in this study and applied to segment the subcortical structure and produce volumes for all brain subcortical structures. Volumetric analysis was then performed based on 3D T1- weighted images. For each study participant, all MRI data (DICOM) was converted to an mgz format.

The MRI data was subjected to a series of steps to identify the appropriate area of the brain. First, correction of motions and averaging of volumetric T1 images was undertaken (Reuter et al., 2010). Then, non-brain tissue was removed using a hybrid watershed/surface deformation procedure (Segonne et al., 2004), followed by automated Talairach transformation, segmentation of the subcortical WM and deep GM volumetric structures (including thalamus, hippocampus, caudate nucleus, putamen, amygdala, accumbens and brainstem) (Fischl and Dale, 2000, Fischl et al., 2004a), normalisation of intensity (Sled et al., 1998), tessellation of the GM and WM boundary, and automated topology correction (Fischl et al., 2001, Segonne et al., 2007). Subsequently, surface deformation following intensity gradients was used to optimally place the GM, WM and cerebrospinal fluid (CSF) borders at locations where the greatest shift in intensity defines the transition to other tissue classes (Dale et al., 1999, Dale and Sereno, 1993,

Fischl and Dale, 2000).

When the models describing the cortical structures are completed, a number of procedures can be undertaken which require more data processing and analysis with surface inflation (Dale et al., 1999). The registration to a spherical atlas is based on individual cortical folding patterns to match cortical geometry between patients (Fischl et al., 1999), parcellation of the cerebral cortex into units with respect to gyral and sulcal structures (Desikan et al., 2006, Fischl et al., 2004b), and creation of a variety of surface-based data including maps of curvature and sulcal depth. This technique utilizes both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the GM/WM boundary to the GM/CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000). Then, using spatial intensity gradients across tissue classes to create maps means that the results are not simply reliant on absolute signal intensity. The maps produced are not limited to the voxel resolution of the original data and are thus, capable of detecting submillimeter differences between study groups. FreeSurfer software measures all subcortical volumes including: thalamus, hippocampus, caudate nucleus, putamen, amygdala, accumbens and pallidum collected for each study participant.

Volumes of the subcortical regions were analyzed using SPSS (Version 22, Chicago). Student's independent sample *t*-tests were used to examine whether differences in the volumes between patients with NDE and healthy controls were different at a statistically significant level (using a threshold *P*

< 0.05).

7.2.3.3 Voxel-Based Morphometry

VBM tools were established by Ashburner and Friston (Ashburner and Friston, 2000). VBM is a fully automated method to distinguish differences in brain morphology of two or more groups of study participants. The structural data were processed using Statistical Parametric Mapping software (SPM12) (Wellcome Department of Cognitive Neurology, London, UK) and its VBM8 toolbox, which are available at http://dbm.neuro.uni-jena.de/vbm. These tools boxes were processed using Matlab version R 2015 (The Mathworks, Inc., Natick, MA, USA). Using VBM, there are four steps to undertake analysis of structural MR data. These include spatial normalisation, tissue segmentation, spatial smoothing and statistical analysis. First, all MRI T1 images are co-registered or spatially normalized into the same stereotactic space (MNI asymmetrical average template of 305 subjects). This is undertaken by minimising differences between the residual sum of squares for MRI images and the template image, using a 12-parameter linear affine transformation and non-linear basis function for normalisation. Then, spatially normalised MRI images are automatically segmented into GM, WM and CSF. The segmentation is completed based upon a change in intensity between types of tissue and the distribution of GM, WM and CSF. Thereafter, the normalized segmented images are smoothed (8mm FWHM) by convolving with a Gaussian kernel. The spatial processing of MRI images was undertaken for study participants with NDE and for healthy controls. The MRI image results for the two study groups were compared on a voxel-by-voxel basis using generalised linear

modelling (and a statistically significant threshold of $P < 0.05$). Analyses of covariance were performed, with global mean voxel value as a confounding covariate. This covariate corrected for the effect of summed differences in voxel intensity across scans, while preserving district differences in GM. Grouped analyses were performed using parametric statistical procedures (2-sample *t*–test). Two contrasts were defined to detect whether each voxel had a greater (or lesser) probability of being GM in the NDE group than had the equivalent voxel in the healthy control group, representing an equivalent change in local GM density in patients with NDE. Using the results of the analysis, the anatomical locations of significant clusters were evaluated by localising their centre of gravity coordinates on template atlases using FSL view 5.0.7.

7.3 Results

7.3.1 Clinical characteristics

Twenty-seven NDE patients (14 male, 13 female, M=33.2, SD=12.13) and 32 healthy controls (14 male, 18 female, M=33.07, SD=11.5) were MRI scanned in this study. NDE patients and healthy controls were matched based on their age, handedness and gender.

Clinical magnetic resonance imaging reports examined by a neuroradiologist at the Walton Center indicated that six patients (out of 27, 22.2%) had abnormalities in their MRI brain scans including in the cortical dysplasia, slight changes in the size of the left or right hippocampus and changes in the WM region. The remaining 21 (77.8%) patients had MRI

scans negative for abnormalities. The anti-epileptic drugs (AEDs) in use at the time of NDE patient scans included: lamotrigine in 15 patients (out of 27, 59.25%), levetiracetam in six patients (22.22%), lamictal in one patient (3.70%), and zonismide in four patients (14.82%). See table 6-1 section 6.2.3 for the demographic and clinical details of patients.

7.3.2 Subcortical shape analysis

Subcortical structures were investigated in 27 patients with NDE and 32 healthy controls in regions including the: bilateral thalamus, bilateral hippocampus, bilateral caudate nucleus, bilateral putamen, bilateral amygdala and bilateral pallidum. Patients with NDE had significant differences in the shape of the left and right thalami compared to healthy controls. However, the other grey matter subcortical structure failed to exhibit any significant differences in subcortical shape between patients with NDE and healthy controls.

7.3.2.1 Left thalamic shape change

Shape analysis suggested regional areas where shape differences were significantly different (FDR) corrected P = 0.01) in the left thalamus in patients with NDE compared to healthy controls. (The Figures 7:1,7:3 below illustrate the differences in the left thalamus).

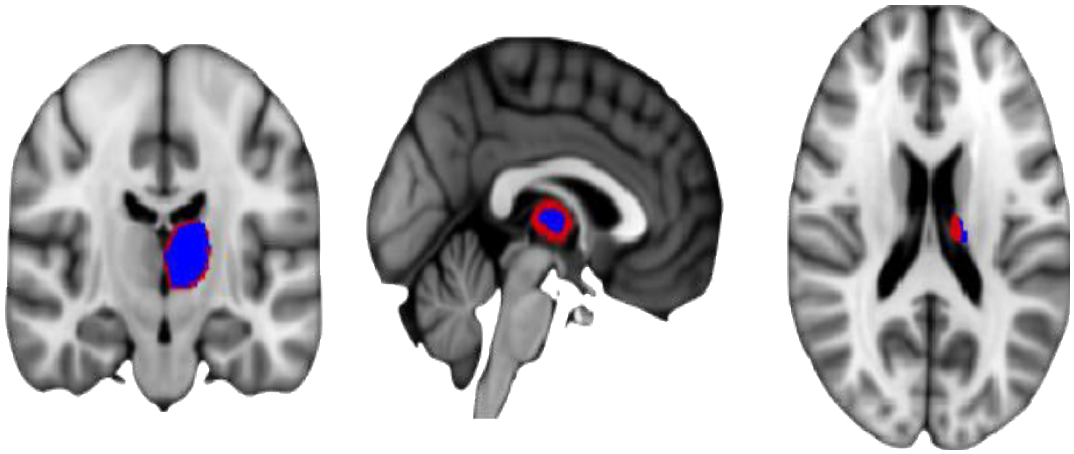


Figure 7:1 Illustration of the left thalamus shape change in patients with NDE compared to healthy controls *P* = 0.01 corrected register in MNI152 T1 1mm brain. The red colour represents the difference in thalamus shape

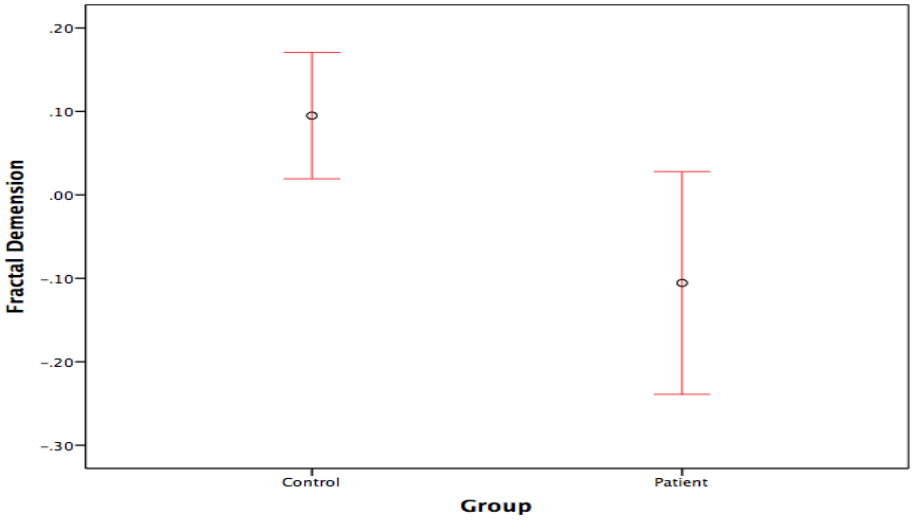


Figure 7:2 Error bar exhibited fractal dimension shape analysis change in left thalamus in patients with NDE compared to healthy controls

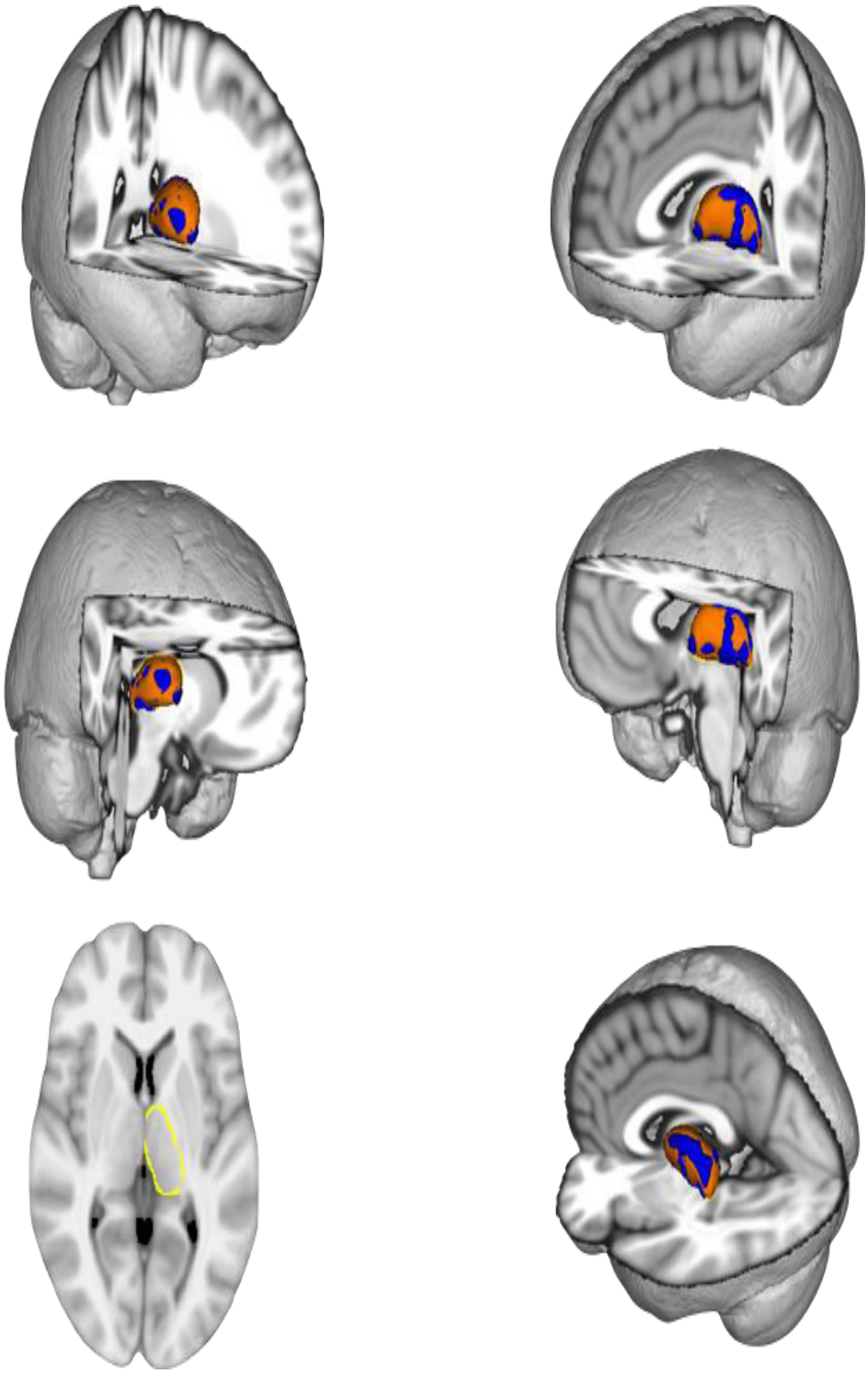


Figure 7:3 Comparison of patients with NDE and healthy controls using vertex shape analysis of the thalamus. The results are viewed from different directions, and are statistically significant at P< 0.01 (FDR corrected). Within the 3D view, orange represents the difference in thalamus shape and yellow represents thalamus locations

7.3.2.2 Right thalamic Shape change.

Shape analysis suggested regional areas where shape differences were significantly different (FDR corrected *P* = 0.01) in the right thalamus in patients with NDE compared to healthy controls. (The Figures 7:4, 7:6 below illustrate the differences in the right thalamus).

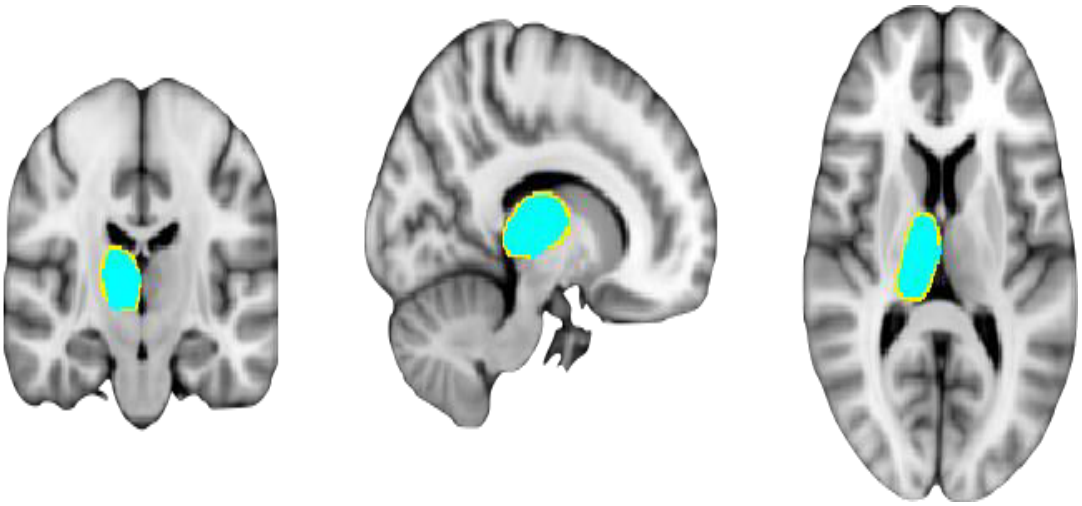


Figure 7:4 The right right thalamus shape change in patients with NDE compared to healthy controls (*P* = 0.01 corrected) register in MNI152 T1 1mm brain. The yellow colour represents the difference in thalamus shape

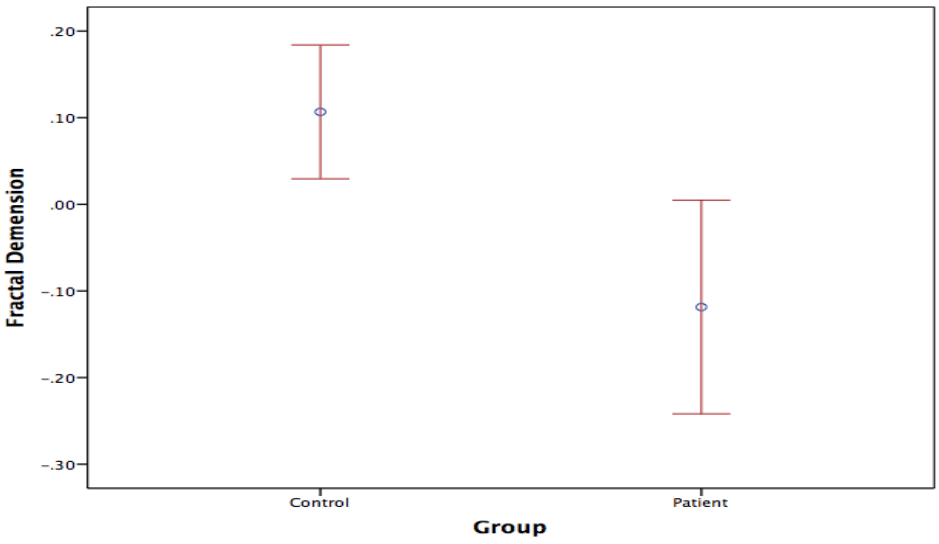


Figure 7:5 Error bar exhibited fractal dimension shape analysis change in right thalamus in patients with NDE compared to healthy controls.

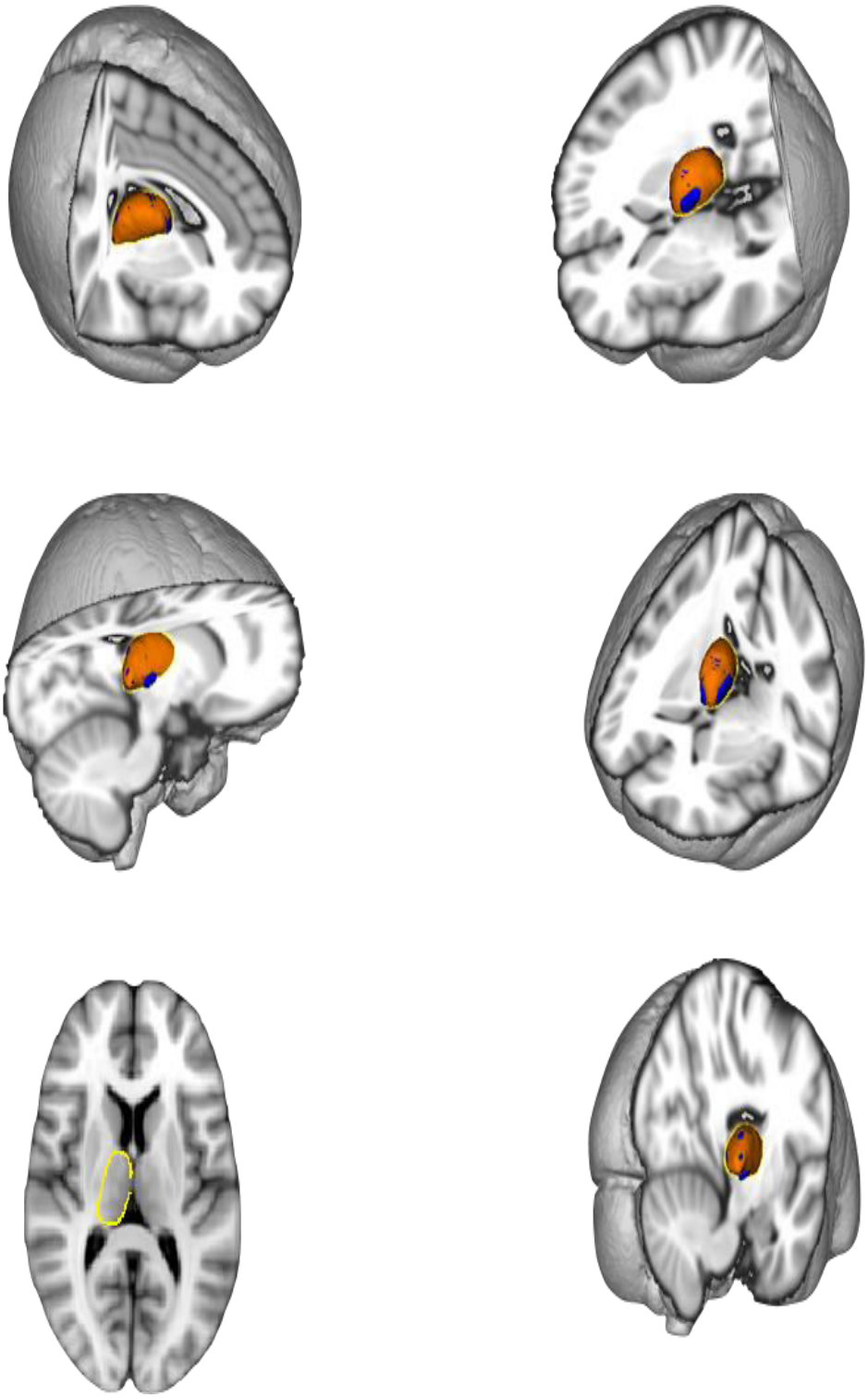


Figure 7:6 Comparison of patients with NDE and healthy controls using vertex shape analysis of the right thalamus. The results are viewed from different directions, and are statistically significant at P< 0.01 (FDR corrected). Within the 3D view, orange represents the difference in right thalamus shape and yellow represent thalamus locations

7.3.3 Subcortical volumes analyses

FreeSurfer software was used to perform volumetric segmentation in patients with NDE and healthy controls. Volumes were automatically measured in seven regions of the grey matter in both hemispheres including the: thalamus, hippocampus, caudate nucleus, putamen, amygdala, accumbens and pallidum The volumes were compared for NDE patients relative to healthy controls. Comparison of the volumes was undertaken using Student's independent sample t-tests (threshold for significant $P < 0.05$) with analyses undertaken using the SPSS (Version 22, Chicago) statistical software. There were significant volume reductions in the left thalamus, right thalamus of patients with NDE compared with the age- and sex-matched healthy control group. Figure7:7 Bar chart summarizes the volume characteristics of eight subcortical GM in both hemispheres, in 27 patients with NDE compared to 32 healthy controls

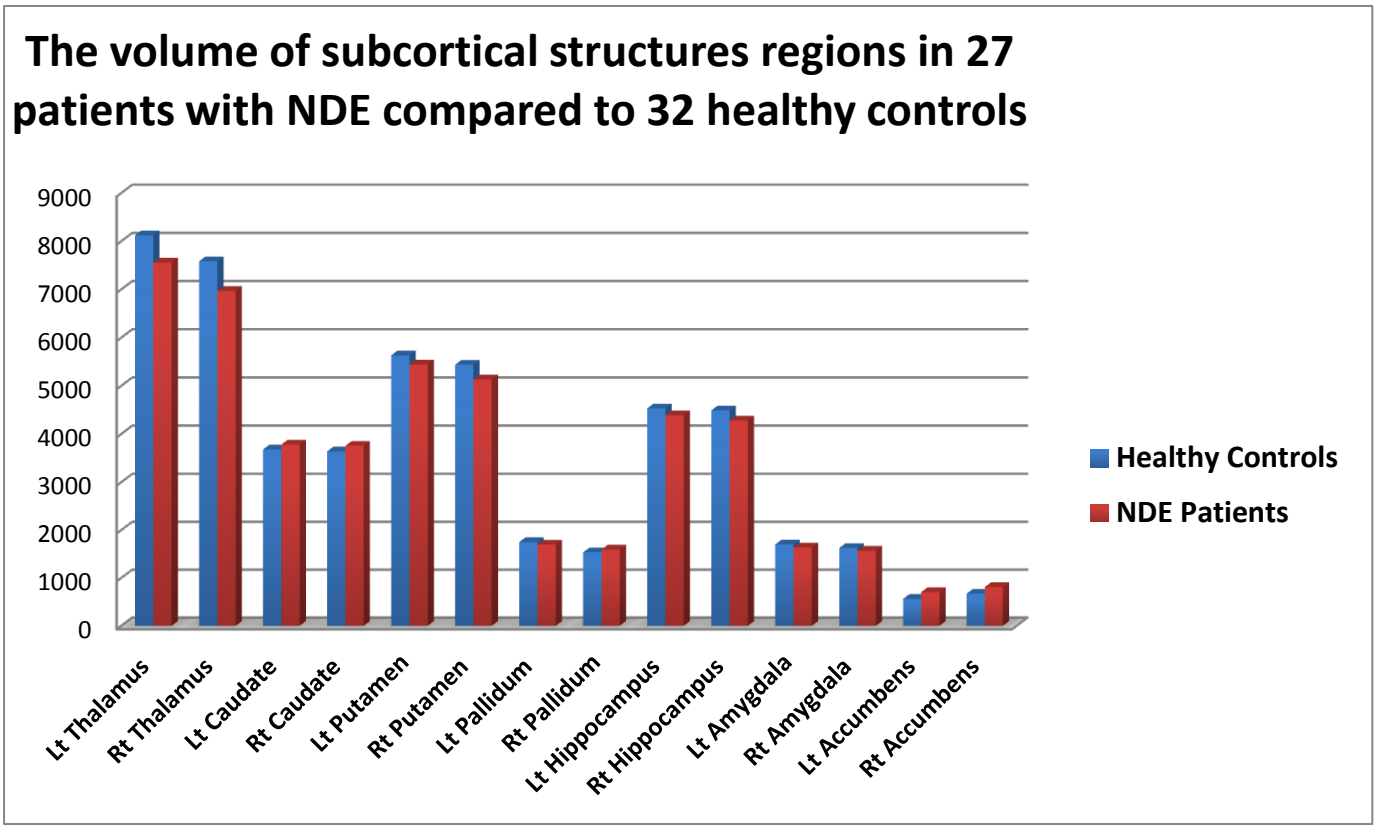


Figure 7:7 The volume of subcortical structures regions in 27 patients with NDE compared to 32 healthy controls

7.3.3.1 Left thalamus volumes

Compared to healthy subjects; patients with NDE exhibited significantly smaller volumes in left thalamus. (P = 0.016). The volumes in left thalamus in patients with NDE and healthy controls were (NDE; 7555.5 mm3),healthy controls ; 8118.7 mm3). The Error bar Figure show Left thalamus volumes distribution between patients with NDE and healthy controls.

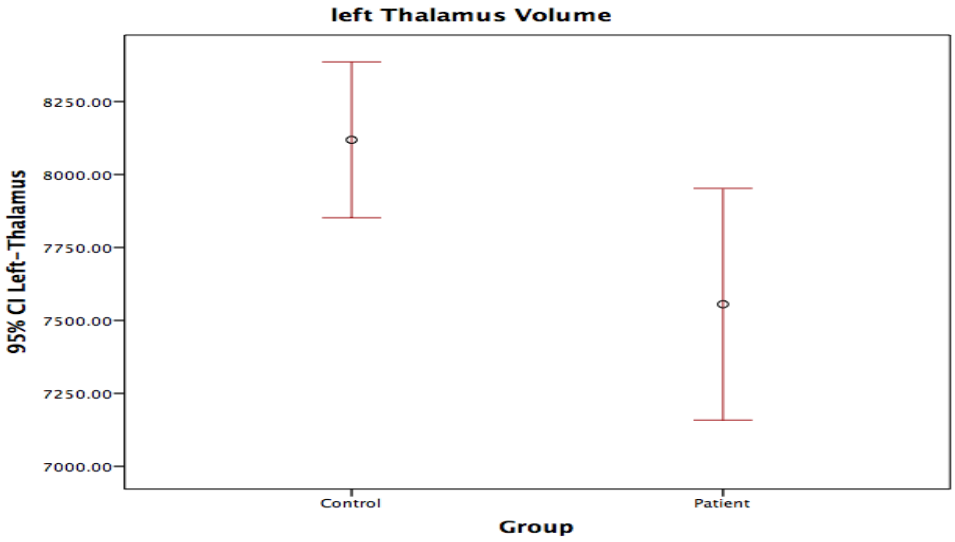


Figure 7:8 Error bar demonstrated the differences in Left thalamus between the patients with NDE and healthy subjects. Decreased in Left thalamus volumes between patients and control.

7.3.3.2 Right thalamus volumes

In comparison to healthy controls and patients with NDE in right thalamus volumes. Patients with NDE showed significantly decreased in volume of thalamus. Volumes of right thalamus in patients with NDE (6967.6 mm3) and healthy controls (7583.1 mm3) were (P corrected = 0.002).Figure 7:8 below exhibited the differences of right thalamus between patients with NDE relative to healthy controls.

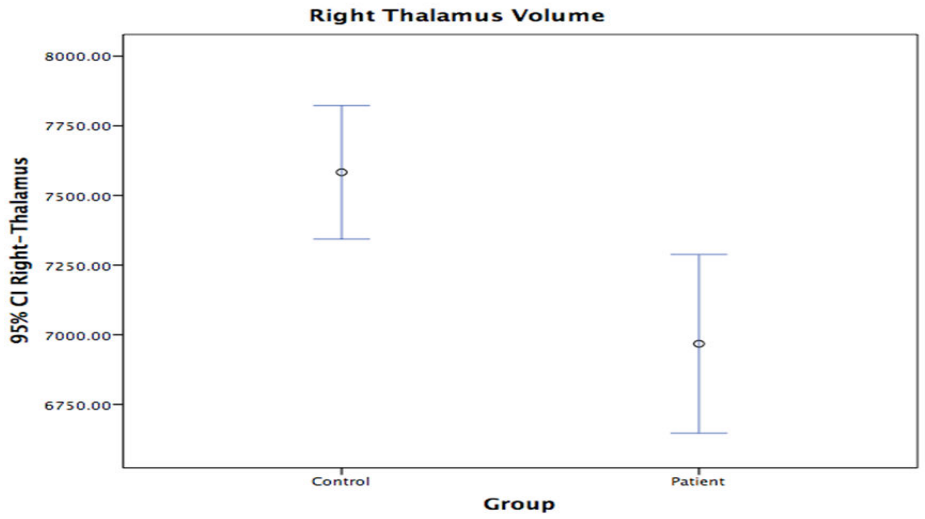


Figure 7:8 Error bar demonstrated the differences in Right thalamus between the patients with NDE and healthy controls. Decreased in Right thalamus volumes between patients and control

7.3.4 Voxel Based Morphometry analysis

To study variations between patients with NDE and healthy controls, the whole brain was analysed used SPM 12 and VBM8 tool, and differences in GM distribution were examined on a voxel by voxel basis. Two-sample *t*-tests were used to examine the use of total intracranial volume (TIV) as a global variable at every voxel. Two contrasts were assessed: 1) identifying local increases in GM distribution in patients with NDE and healthy controls; 2) identifying local increases in GM distribution in healthy controls, relative to patients with NDE. Each test was carried out on smoothed images (smoothed with an 8mm FWHM smoothing kernel). Statistically significant clusters were corrected for repeated measures at the cluster level (using a threshold of $P < 0.05$ and if they had a spatial extent of >20mm^3). Cluster level correction was undertaken using a 'region of interest' approach to improve statistic power, with 'regions' defined by the aal toolbox

implemented in SPM12 software. VBM analysis showed that patients with NDE had lower grey matter volume in the left thalamus compared to healthy controls (Talairach coordinate, -12, -18, 4, *P* value = 0.030, FWE corrected, z=3.71). Table 7-2 and Figure 7: 10 illustrate the location of the left thalamus in the standard.

Table 7-1 An overview of cluster characteristics (Regions, *P* value and MNI coordinates)

Cluster index	Regions	Cluster P-Value (corrected)	Peak Level Z max	MNI Co-ordinates X, Y,Z
1	Left thalamus	0.030	3.71	-12, -18, 4

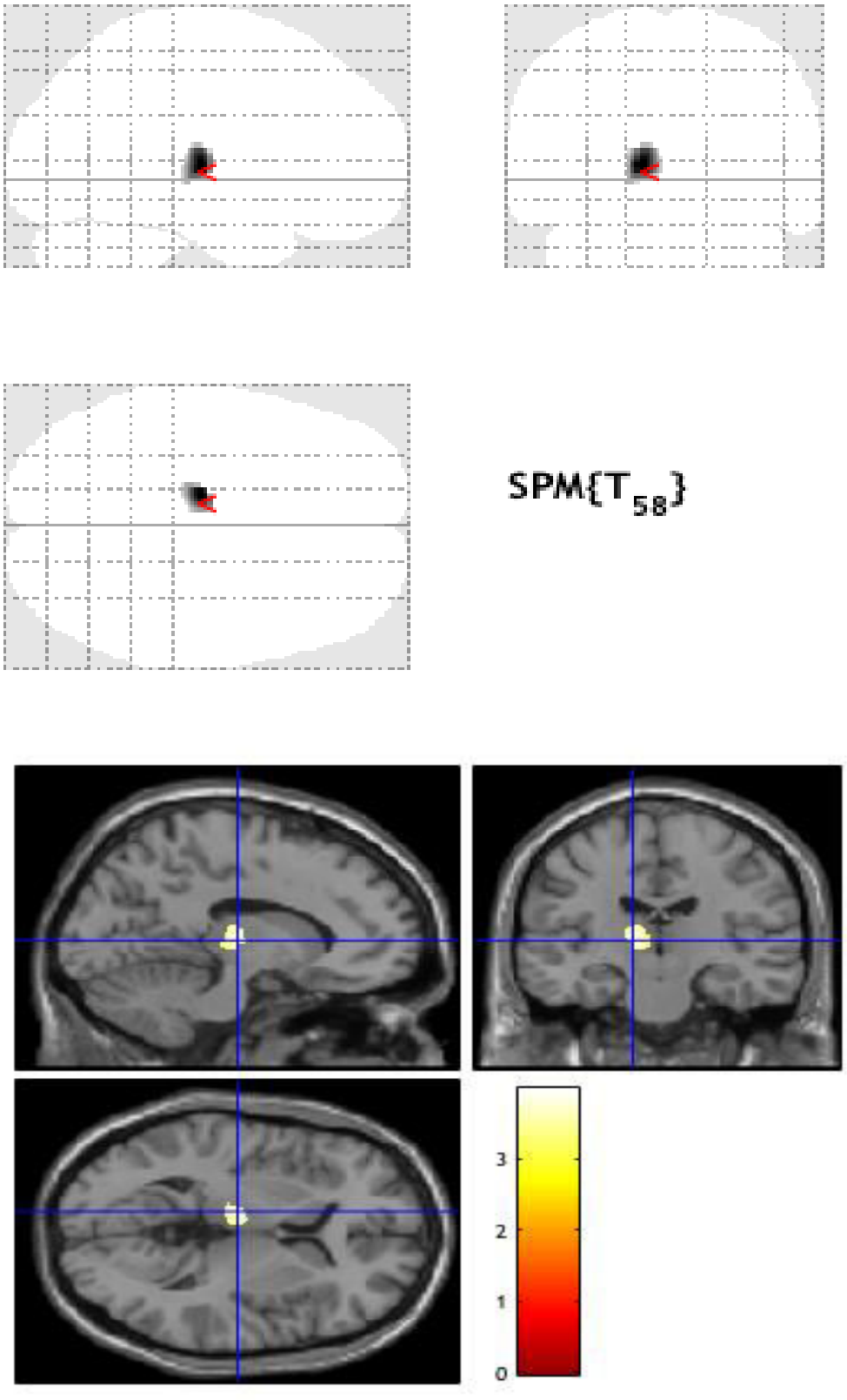


Figure 7:9 Statistical parametric map (extent threshold *P* corrected < 0.05) showing clusters with statistically significant of decreased in grey matter volume in Lt thalamus between NDE and healthy control.

7.4 Discussion

7.4.1 Summary of results

This study aimed to investigate the morphological differences in whole brain between patients with NDE and healthy controls. Multiple MRI structural techniques were applied including: shape analysis of subcortical structures in the brain using the FSL-First toolbox, using FreeSurfer software to performed subcortical volume analyses segmentation and VBM analysis to evaluate the morphometric differences in patients with NDE compared to healthy controls. The results suggest that patients with NDE have statistically significant differences in shape of their left and right thalamus compared to healthy controls, as suggested by analysis using FSL-FIRST. Between group comparisons of subcortical volumes of patients with NDE showed statistically significant smaller volumes of the left and right thalamus compared to healthy controls. Finally, the VBM analysis suggested that patients with NDE have a statistically significant regional GM reduction in the left thalamus compared to healthy controls.

In the previous, the cortex has for the most part been considered to be the site of seizure origin in the different types of epilepsy (Badawy et al., 2013). Results from histopathologic, electrophysiological, and imaging of brain studies now deliver sufficient indication demonstrating that like normal cerebral function, epileptic seizures involve widespread network interactions between cortical and subcortical structures (Badawy et al., 2013). These research studies display that different forms of generalized and focal epilepsy discharges and seizures engage various subcortical structures in

varying ways. Furthermore, there is some evidence to indicate that subcortical structures play a crucial role in the propagation of human epileptic seizures (Norden and Blumenfeld, 2002). Also, Many studies not paid attention to investigate role of subcortical structures in patients with epilepsy (Badawy et al., 2013). This why I focused and paid attention in this chapter to study subcortical structures volume and shape analysis . However, I used VBM analysis to explore differences in the whole brain in patients with NDE compared to healthy controls.

Also, there is some evidence to indicate that subcortical structures play a crucial role in the propagation of human epileptic seizures (Norden and Blumenfeld, 2002), including in the thalamus (Norden and Blumenfeld, 2002). The thalamus is the main source of connections to the cortex, however, these connections are exceeded in number by mutual connections from the cortex back to the thalamus (Norden and Blumenfeld, 2002). Many sources including from the primary sensory, basal ganglia, cerebellum and system of limbic, send afferent inputs, which are received by the thalamus; these inputs reach the specific relay nuclei thalamic and the information is integrated and processed before being transmitted to the cortex (Norden and Blumenfeld, 2002). The thalamic reticular nucleus (TRN) is frequently suggested to act as a shell for GABAergic cells. These cells (neurons) have powerful inhibitory connections which relay nuclei and might encourage a ‘burst’ firing mode that influences a slow wave in the cortex (Norden and Blumenfeld, 2002), which is normally found in sleep; the signals arriving at the thalamus are therefore suggested to be transferred to the cortex (Sejnowski and Destexhe, 2000). Moreover, the activity of desynchronization is seen when study participants are awake and in a

state of attention, indicating that uninhibited transference of information through the thalamus is possible (McCormick and Bal, 1997, Norden and Blumenfeld, 2002). In general, the thalamus is critical for sleep regulation and when waking, since it has the ability to transition between synchronized and desynchronized states (McCormick and Bal, 1994, McCormick and Bal, 1997, Norden and Blumenfeld, 2002, Sejnowski and Destexhe, 2000). Lastly, thalamus functions involve relaying sensory and motor signals to the cortex regulating consciousness, sleep, and alertness (McCormick and Bal, 1997, Norden and Blumenfeld, 2002). There is evidence that the thalamus is a critical component in neuronal circuits, involved in the spread of seizures and initiation in TLE (Li et al., 2014) and there are indications of thalamic contribution to seizures of TLE (Guye et al., 2006), however, the role of the thalamus in full seizures is poorly understood (Li et al., 2014) and not well described (Guye et al., 2006). Comparison of rat study models that experienced limbic status epilepticus followed by the succeeding progress of spontaneous seizures with rats that had experienced many seizures without becoming epileptic (Bertram et al., 1998, Bertram et al., 2001), suggested the thalamus plays a role in the amplification and distribution of limbic seizures (Bertram et al., 1998). Histopathologic findings from one of the studies showed that significant neuronal loss in cells in the medial subdivisions of the thalamus, combined with alterations of synaptic in the midline thalamus regions, might have improved the excitability of thalamic seizure circuits in epileptic rats (Bertram et al., 2001). This indicates that thalamic alterations could be specific to the epileptic condition, rather than merely a consequence of recurrent seizures (Bertram et al., 2001). These

animal studies also showed GABA activity in the medial dorsal nucleus (MD) of the thalamus, indicating a significant role in the primary seizure circuits of limbic seizures, and reduction in seizure duration (Bertram et al., 2001, Bertram et al., 2008).

Evidence also suggests that electrical stimulation plays a role in seizures, as in the reticular nucleus it was shown to suppress limbic motor seizures in a model of TLE (Nanobashvili et al., 2003). In another TLE model, electrical stimulation of the anterior nucleus of thalamus (ANT) caused an enhanced exhibition of seizure control in the status epilepticus (SE) (Hamani et al., 2008). Many studies have proposed that the thalamus contributes to TLE, particularly in the initiation of epileptic seizures (Li et al., 2014). In patients with intractable epilepsy, thalamic stimulation may significantly decrease the frequency of seizures in patients who failed to respond antiepileptic drugs and surgery (Fisher et al., 2010, Lim et al., 2007, Keller et al., 2014). Nevertheless, the mechanism which reduce seizures after stimulation treatment is still unknown (Keller et al., 2014).

Other studies using animal models with an absence of epilepsy showed a supporting 3Hz spike and wave seizure initiation in the thalamus (Amberson, 1954, Spiegel et al., 1951, Williams, 1953). The role of thalamus has been shown in animal models of IGE (McCormick and Contreras, 2001). In addition, animal work has suggested a role of the thalamus in the spread of seizures, in the primary seizure circuits of limbic seizures, in reducing seizure duration and demonstrated an enhancement in seizure control in the status epilepticus (Bertram et al., 2001, Bertram et al., 2008, Langlois et al., 2010, Li et al., 2014, McCormick and Contreras,

2001, Turski et al., 1984).

7.4.2 Neuroimaging discussion

To the best of my knowledge, this is the first study which has applied sophisticated image analysis to patients with NDE and reported both thalamus shape alterations and volume reductions relative to healthy controls used three methods structural analysis. However, (Park et al, 2015) study the volume of brain in 22 patients with newly diagnosed partial epilepsy of unknown etiology using FreeSurfer method. Little attention is paid to advanced neuroimaging when patients have newly diagnosed epilepsy. As a result, the first Halifax International Epilepsy conference highlighted an urgent need for a longitudinal study using neuroimaging in the newly diagnosed stages of epilepsy (Schmidt and Pohlmann-Eden, 2011).

The majority of studies in the neuroimaging field focus on patients with chronic epilepsy (Pohlmann-Eden, 2011). Nonetheless, a few studies have used applied quantitative MRI methods in newly diagnosed epilepsy (Liu et al., 2001, Liu et al., 2002, Liu et al., 2003a, Van Paesschen et al., 1998). The quantitative MRI studies of NDE typically applied hippocampus volumetric analysis (Briellmann et al., 2002, Salmenperä et al., 2005, Van Paesschen et al., 1998). Previously undertaken community MRI research with new partial seizures found ‘normal’ MRI results in 65-78% of patients, hippocampal sclerosis in 2-11% of patients and other MRI abnormities in 6-11% of patients; these findings resulted from the study of a small number of NDE patients with heterogeneous clinical syndromes.

Moreover, much of the previous research undertaken on newly diagnosed epilepsy has had limitations. First, most NDE studies have used conventional MRI standard sequences T1 weighted and T2 weighted and a MRI magnetic 1.5 Tesla for scanning; this cannot detect some of epileptogenic lesion and networks which cause seizures in epilepsy (Phal et al., 2008). Second, studies have used different MRI scanners for baseline and follow up scans, which is likely to affect the quality of images from an image resolution perspective. Third, there has been a lack of longitudinal data for comparative healthy control subjects (Briellmann et al., 2002, Hagemann et al., 2002, Liu et al., 2001, Liu et al., 2002, Liu et al., 2003b, Liu et al., 2003a, Salmenperä et al., 2005, Van Paesschen et al., 1998, Van Paesschen et al., 1997). Finally, previous quantitative MRI studies in patients with NDE have struggled to identify structural alterations in patients at the point of diagnoses, possibly due to previous studies focussing on the hippocampus.

The results from the present study, which exhibit both thalamus shape alterations and volumes reductions in NDE patients relative to healthy controls do not match the previous results (Briellmann et al., 2002, Hagemann et al., 2002, Liu et al., 2001, Liu et al., 2002, Liu et al., 2003b, Liu et al., 2003a, Salmenperä et al., 2005, Van Paesschen et al., 1998, Van Paesschen et al., 1997) potentially for many reasons. The previous research used a MRI 1.5 Tesla and standard MRI sequences where my study I used a MRI 3 Tesla scanner including MRI sequences 3D T1-weighted volume with isotropic 1mm voxels and type of sequences MPRAGE; this provides a better contrast between grey matter, white matter

and CSF. I also used 3D T2-weighted higher resolution volume with isotropic 1mm voxels, 3D T2, FLAIR, with isotropic 1mm voxels and 2D coronal T1-FLAIR sequences, which provide more detailed brain structures than the MRI scan obtained on 1.5 Tesla (Bernasconi and Bernasconi, 2011) and a higher resolution to identify focal cortical dysplasia and hippocampal sclerosis in the brain (Vézina, 2011). 3D MPRAGE sequences in the current study were developed for good contrast between GM, WM and CSF per unit of acquisition time and to improve SNR for image quality (Deichmann et al., 2000). The high filed MRI scanner 3 Tesla provided a better quality of images than a MRI 1.5 Tesla (Bernasconi and Bernasconi, 2011, Patenaude et al., 2011). Other differences between my work and that undertaken previously include that the previous work used hippocampal volume measurements and only focused on the hippocampus as the region of interest, whereas in my study I applied sophisticated image analysis on the whole brain of patients with NDE and I used novel methods including neuroimaging software; FSL-FIRST, FreeSurfer and VBM (Ashburner and Friston, 2000, Fischl, 2012, Patenaude et al., 2011) to process structural data and to evaluate morphological alterations in patients with NDE.

My research findings are also in agreement with the results of other studies examining similar clinical conditions. For example, my results suggest differences in both thalamic volume and bilateral thalamus shape in patients with NDE relative to healthy controls. Previous neuroimaging studies in patients with TLE have shown bilateral atrophy in the thalamus on both hemispheres (Labate et al., 2008, Mueller et al., 2006, Yasuda et al., 2010b), which agrees with the results from the present study for patients

with NDE. In the results of studies examining other types of epilepsy, MRI data in patients with IGE has suggested that thalamus volume is smaller (Ciumas and Savic, 2006, Chan et al., 2006, Helms et al., 2006, Kim et al., 2007, Lin et al., 2009b, Mory et al., 2011, Pardoe et al., 2008, Seneviratne et al., 2014), which is also in agreement with the results for thalamus volume in NDE patients in this study, compared to healthy controls. Morphological MRI studies of patients with chronic epilepsy showed thalamus volumetric reduction and shape alterations in patients with IGE using FSL-First, FreeSurfer and VBM tools compared to healthy controls (Kim et al., 2013). Additionally, patients with JME demonstrated significant thalamus volume and subcortical shape analysis changes, and changes in the shape of the left and right thalamus, relative to healthy controls (Saini et al., 2013). Several structural MRI studies in chronic epilepsy patients have used FSL-FIRST, FreeSurfer and VBM methods and showed morphologic changes in both the volume and shape of the thalamus (Mueller et al., 2006, Labate et al., 2008, Yasuda et al., 2010b).

Most MRS studies have suggested abnormally low concentrations of thalamus NAA/Cr in IGE and JWE patients relative to healthy controls (Bernasconi et al., 2003, de Araujo Filho et al., 2009, Doelken et al., 2010, Fojtikova et al., 2006, Helms et al., 2006, Haki et al., 2007, Kabay et al., 2010, Lin et al., 2009a, Mory et al., 2003, Savic et al., 2000, Savic et al., 2004). The reduction in NAA in epilepsy patients might be due the loss of neurons or shrinkage and dysfunction of metabolic (Bernasconi et al., 2003), which support the results of my current study.

Most of fMRI studies applied to IGE and JWE patients showed thalamic

activation (Archer et al., 2003, Berman et al., 2010, Gotman et al., 2005, Hamandi et al., 2006, Liu et al., 2008, Moeller et al., 2008b, Moeller et al., 2008a, Moeller et al., 2009, Moeller et al., 2010, Moeller et al., 2011, Seneviratne et al., 2014, Szaflarski et al., 2010, Szaflarski et al., 2013, Vollmar et al., 2011), which would also be in support of the morphometric MRI alterations in both thalamus in NDE patients in my study.

My study results are consistent with most previous work examining the volume and shape of the thalamus in chronic epilepsy patients. Overall, my results suggest that morphological changes in the brain alter the volume and shape of both thalamus in NDE patients, contributing to epileptic activity and seizures. Subcortical morphological alterations in patients with NDE may be relevant to epilepsy pathophysiology. Seizures in NDE patients could be a result of differences in the biochemical and neurobiological environment of the brain (Pohlmann-Eden, 2011). The consequences of seizures could cause damage to the thalamus throughout the lifetime of the NDE patient. The alterations exhibited in subcortical brain in patients with NDE could be a side effect of antiepileptic drugs (Coan and Cendes, 2013). The novel neuroimaging methods for morphology used in the current study shows morphological abnormalities in thalamus may be involved in epilepsy and could play a significant role in the initiation of epileptic seizures (Li et al., 2014). Furthermore, longitudinal neuroimaging research studies could help to understanding the role of thalamus in epilepsy disorder.

In FreeSurfer, each subject's volume is transformed into Talairach space by computing the translations, rotations, and, where the volume itself is not

resampled into Talairach space. Only the transformation is computed where data is normalized to MNI305 space. Freesurfer does not report true "Talairach" coordinates, which actually transformed from MNI 305 space. In FIRST toolbox, two-stage affine registration to MNI152 space at 1mm resolution is conducted. The first stage is a standard 12 degrees of freedom registration to the non-linear MNI152 template. The second stage applies a 12 degrees of freedom registration using an MNI152 sub-cortical mask to exclude voxels outside the sub-cortical regions. Finally, a reconstruction of the surfaces in MNI152 space is run. In VBM toolbox, segmentation of data set is run by Appling a registration to MNI Space and subsequently a non-linear deformation.

7.4.3 Limitations

The sample size of study participants is a limitation of this work: I examined 32 healthy controls and 27 patients with NDE using advanced methods. It would have been difficult to improve the study size because of the specificity of the criteria for recruitment, for example I needed individuals with focal epilepsy and date of diagnosis of less than 12 month from when patients were recruited. There are no previous advanced quantitative MRI studies including for structural and resting sate for NDE patients, I was unable to undertake power calculations to estimate the sample size needed for my work. I therefore consider my work to be a pilot study, as previously suggested under these circumstances (Billingham et al., 2013). A further limitation of the work is that the quality of images could affected by artifacts due to inhomogeneity intensity and head motion during the scan (Marques

et al., 2010).

Furthermore, for each Neuroimaging software analysis there are specific technical limitations. The VBM method has some limitations, for example it is sensitive to methodological choices in normalisation, smoothing kernel and template so using the VBM default settings for processing steps may not create the best results. Also, the quality of normalised and segmented images was visually inspected rather than being able to use an improved procedure. VBM tools show similar findings using two different smoothing kernels (6 and 8mm) offering optimal sensitivity for regions of interest. VBM techniques are not sensitive to deep atrophy in the GM region, and nor is the FSL-First method (Ashburner and Friston, 2000, Bookstein, 2001, Mechelli et al., 2005).

The FSL-FIRST tool was used to evaluate subcortical structures shape analysis (Patenaude et al., 2011) and FreeSurfer software was used for volumes analyses segmentation. The Segmentation algorithms methods is sensitive to changes in contrast in boundary voxels (Morey et al., 2009). The segmentation image quality therefore needs to be visually and manually checked to identify any major defects in how the segmentation was undertaken. The last limitation, leaking knowledge and unclear understanding the epileptogenesis in epilepsy disorder (Schmidt and Pohlmann-Eden, 2011).

7.5 Conclusion

In conclusion, previous quantitative MRI research projects have had difficulty in detecting brain changes in patients with epilepsy at the point of

diagnosis (Briellmann et al., 2002) possibly because they have concentrated on the hippocampus. In this study I report that structural differences exist in regions outside the temporal lobe. Excluding the difficulty in identifying MRI volumetric abnormalities in patients with NDE (Liu et al., 2002), this is the first study which has combined multiple analysis methods and found both thalamic shape alterations and volume reduction in patients with NDE relatively to matched healthy controls. These findings suggest that the thalamus plays a very important role in epilepsy (Li et al., 2014)

Chapter 8 Resting state functional connectivity in patients with newly diagnosed epilepsy

8.1 Introduction

Recently, BOLD fMRI method has been practically applied in brain imaging while the subject is not involved in any task or activity (i.e. resting state methods) (Lee et al., 2013). The resting state fMRI method measures spontaneous low frequency fluctuations (<0.1 Hz) in the BOLD signal to explore the functional connectivity of the brain (Lee et al., 2013). Resting state scans have various advantages over conventional MRI and fMRI (Lang et al., 2014). Resting state techniques could offer further detail on functional brain disturbances in many neurological disorders and psychological illnesses. Resting state methods are not limited by fMRI paradigm tasks, such as finger tapping, lip puckering, tongue movement, semantic decisions, motor task and other similar activities. Also, Resting state fMRI more comfortable for children and patients with cognitive constraints more than fMRI task. The goal is to explore spontaneous fluctuations in brain activity between two or more anatomically distinct time-series of brain districts (Friston, 2011). It is beneficial to recognize functional connectivity changes in brain activity patterns between two groups during rest of brain. There is three methods used to study and analysis resting state sequences. The analysis methods are Independent component analysis (ICA), Seed based analysis region of interest and Graph theory analysis. ICA analysis apply to analysis whole brain and not require a prior hypothesis. In recent years, resting state fMRI methods have experienced a dramatic increase in use for the investigation of functional networks in healthy and disease-specific populations (Biswal et al., 1995, Biswal et al., 2010, Caciagli et al., 2014, Greicius et al., 2003). Resting state fMRI imaging can be used to detect changes in functional connectivity in many neurological

disorders and psychological illnesses to assess the changes in functional brain networks (Cataldi et al., 2013, Fox and Greicius, 2010, Lang et al., 2014, Pittau and Vulliemoz, 2015, Voets et al., 2012).

Resting state fMRI has been used widely to evaluate the abnormalities in functional brain networks with independent component analysis (ICA) methods in different patients with neurological disorders. Examples include ICA in patients with Alzheimer's disease or dementia (Greicius et al., 2004, Rombouts et al., 2009, Seeley et al., 2009), depressive disorders (Anand et al., 2005, Greicius et al., 2007) and amyotrophic lateral sclerosis (ALS) (Mohammadi et al., 2009). The resting state fMRI method has been commonly applied to examine synchronous brain activity in a variety of functional connectivity networks, including the auditory, attention, emotional, language and visual networks (Cordes et al., 2001, Fox et al., 2006, Hampson et al., 2002, Lowe et al., 1998). The investigation of resting state networks (RSNs) may assist in elucidating the mechanism of neural activity in patients with epilepsy (Cao et al., 2014). Resting state fMRI provides an opportunity to identify the functional brain networks that are configured abnormally in patients with epilepsy and may provide insight into the underlying causes of pharmacoresistance (Caciagli et al., 2014, Cendes, 2011, Centeno and Carmichael, 2014).

A large number of resting state studies exist among patients with TLE (Cataldi et al., 2013, Caciagli et al., 2014). There is also extensive literature on neuroimaging in patients with a variety of chronic epilepsy disorders. However, there are only a handful of studies that have used quantitative MRI to explore structural brain alterations in patients with NDE and none, to my knowledge, that have used advanced image analysis approaches for MRI data specifically

acquired to determine alterations in brain connectivity.

In this chapter, I had two aims. Firstly, to examine the functional connectivity in the whole brain using ICA FSL in patients with NDE relative to healthy controls. Secondly to examine structural alterations in the shape and volume of both thalami in patients with NDE compared to healthy controls using the CONN functional connectivity toolbox to explore the functional connectivity in regions of interest (ROI) of both thalami other regions of the brain. Subjects and methods

8.1.1 Participants

(see section 7.2.1 Page)

8.1.2 Image acquisitions and functional magnetic resonance imaging data

All participants were scanned using a Siemens 3T Trio whole-body scanner (Siemens, Erlangen, Germany) with an 8-channel RF head coil with foam padding to comfortably restrict head motion at the MARIARC, University of Liverpool.

8.1.2.1 Structural MRI sequences

(see section 7.2.3)

8.1.2.2 Resting state functional sequences

(see section 6.3.1.2)

8.1.3 Functional MRI analysis

8.1.3.1 Independent component analysis FSL analysis

Resting state data were collected using the FMRI Expert Analysis Tool (FEAT), part of FSL (FMRIB's Software Library; www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). Pre-processing resting state raw data included motion correction, spatial normalization and smoothing via FEAT (Smith, 2002, Jenkinson et al., 2002). In spatial normalization using a Gaussian kernel of 4-mm full width at half maximum, a grand-mean intensity normalization of the entire 4D dataset was achieved using a single multiplicative factor with high-pass temporal filtering. Then, registration to the T1 high resolution and MNI-152 standard space was performed. Next, normalized 4D data sets were resampled to 2-mm isotropic voxels to reduce computational burden in the subsequent analysis stages. ICA was carried out at a dimensionality of 20. RSNs of interest covered the whole brain (Beckmann et al., 2005). The subsequent steps involved extracting RSNs. Briefly, standard group ICA was carried out via probabilistic ICA (PICA) (Beckmann and Smith, 2004) using FSL MELODIC (Oxford Centre for Functional MRI of the Brain [FMRIB], University of Oxford, UK). Group PICA steps of processing were utilized for the individual pre-processed and normalized sets of data. Then, non-brain tissue voxels were removed, the data were demeaned voxel-wise and all sets of data were normalized in a voxel-wise manner. Next, data sets from patients with NDE and healthy controls were connected in time to produce a single 4D data set. The data set was then deconstructed into independent components. The order of model selection demonstrated that most of the frequently observed large scale RSNs could be

distinguished from the data when applying this technique (Abou-Elseoud et al., 2010).

Next, subject-specific statistical maps were produced to examine the differences between patients with NDE and healthy controls using a dual-regression method (Filippini et al., 2009). Spatial maps of the ICA groups were used in a linear model fit against every single resting state fMRI data set (spatial regression) to create matrices that described the temporal dynamics for every component and subject individually. Multiple linear regression of these time courses was transferred out against the pre-processed single 4D data sets in the standard space resolution, thus offering better spatial correlation maps. The dual-regression spatial maps of all subjects were next compiled into single 4D files for every original independent component. The statistical difference was calculated non-parametrically using the FSL randomization tool (5000 permutations) to detect statistically significant changes between patients with NDE and healthy controls within the boundaries of the spatial maps obtained with MELODIC. Finally, FWE correction for multiple comparisons was completed, implementing TFCE and using a significance threshold of $P < 0.05$ (Smith and Nichols, 2009).

8.1.3.2 Resting-state fMRI: ROI-to-ROI analysis

Data were pre-processed and analysed using SPM12 (Wellcome Department of Cognitive Neurology, London, UK, running Matlab R.2015a). Pre-processing of the resting state data included motion correction, slice timing correction, co-registration to structural scan, spatial normalization to MNI space and spatial smoothing (8-mm Gaussian kernel default setting). Every subject's structural

high-resolution T1-weighted scan was segmented into grey matter, white matter and CSF tissue classes using the unified segmentation approach implemented in SPM12 with default settings. Resting state functional connectivity analyses were carried out using the CONN-fMRI functional connectivity toolbox (http://www.nitrc.org/projects/conn; (Whitfield-Gabrieli and Nieto-Castanon, 2012).

To reduce the artefacts' impact in resting state data, the post-processing artifact Detection Tool toolbox for fMRI data was employed (http://www.nitrc.org/projects/artifact_detect). To further assess functional connectivity between thalamic and cortical stuttering from structural changes in patients with NDE relative to healthy controls, the BOLD time series obtained from each seed ROI was correlated with the extracted time-series from all other seeds in the corresponding network. These values were entered into second-level GLM analysis, implemented in the CONN toolbox, and a two independent samples *t*-test was completed to assess the difference between patients with NDE and healthy controls based on changes in ROI-ROI connectivity for each seed region. Significant results were interpreted using a threshold of $P < 0.05$, and FDR correction was applied to correct for the multiple tests required.

8.2 Results

8.2.1 Clinical characteristics

(see section 7.3.1 Page)

8.2.2 Independent component analysis

Resting state MRI data was analyzed using FSL MELODIC (Beckmann et al., 2005). We utilized ICA to investigate whole brain functional connectivity in

patients with NDE relative to healthy controls. I performed 20 component ICA using fMRI resting state data from 59 participants, including 27 patients with NDE and 32 healthy controls. Abnormal functional connectivity was found within two resting-state networks in patients with NDE compared to healthy controls. In this study, we identified one component as artifacts out of 20 ICA components.

Primary visual network

Patients with NDE demonstrated decreased connectivity in their primary visual network, (*P* = 0.03, corrected) compared to healthy controls (in the left putamen, MNI center of mass coordinates [x, y, z]: -20, -4, 6, *P* = 0.03, corrected) (Figure 8:1 and table 8-1)

Table 8-1 Visual resting state anatomical regions, *P* value and MNI coordinates

Resting-State Network	**Anatomical Regions**	**Cluster *P* Value (corrected)**	**MNI Co-ordinates X, Y, Z**
Primary visual network	Left putamen	0.03	-20,-4, 6

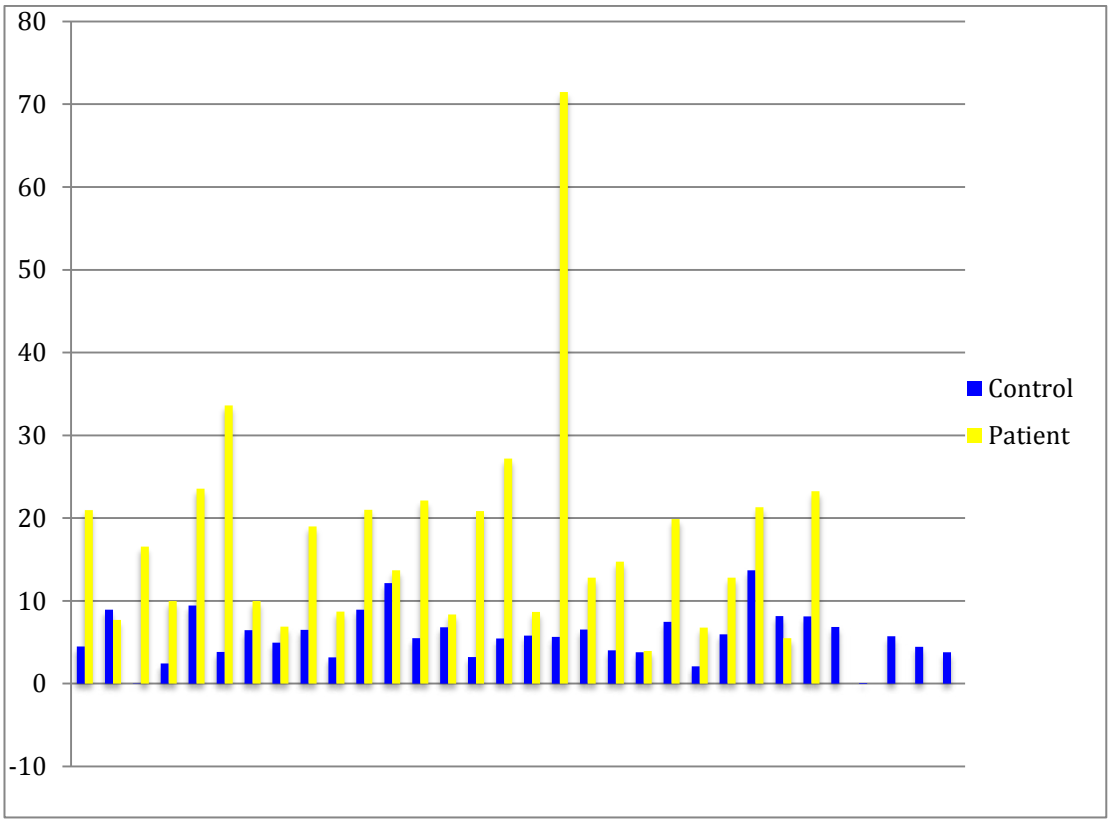


Figure 8:1 Distribution of the mean individual *z*-scores within healthy controls (blue) and NDE patients (yellow), both sorted from smallest to highest *z*-value for visual resting state network

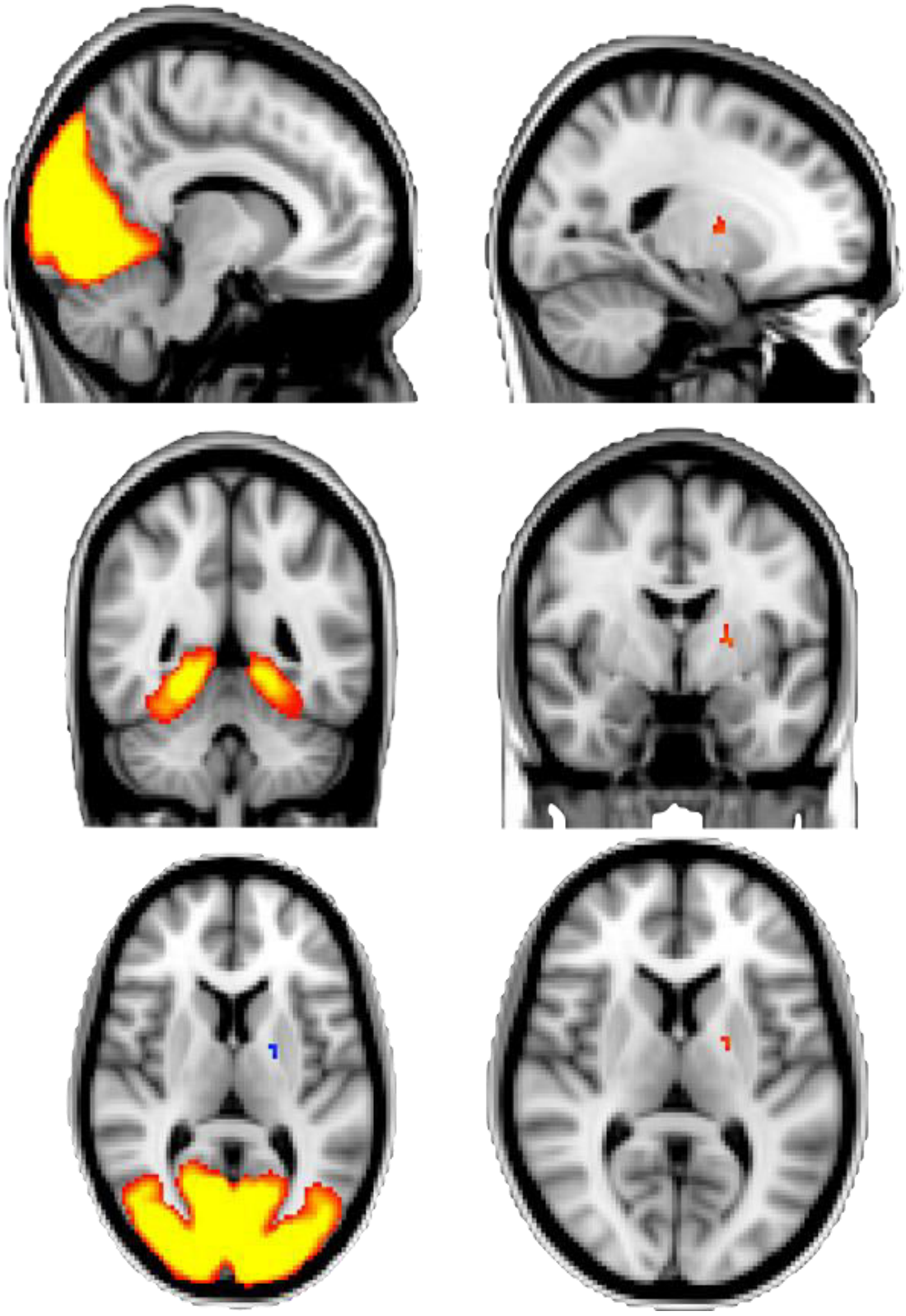


Figure 8:2 Group differences in functional connectivity between patients with NDE and healthy controls. Patients with NDE demonstrated decreased connectivity in primary resting state visual network

Right attention network

Patients with NDE exhibited reduced connectivity in the right resting state attention network (in the left supramarginal gyrus, Montreal Neurological Institute [MNI] center of mass coordinates [x, y, z]: -54, -40, 30) *P* = 0.03, corrected compared to control subjects (Figure 9:4 and table 9-2).

Table 8-2 Attention resting state network, anatomical regions, *P* value and MNI coordinates.

Resting-State Network	Anatomical Regions	Cluster *P* Value (corrected)	MNI Co-ordinates X, Y, Z
Right attention network	Left supramarginal gyrus	0.03	-54,-40,30

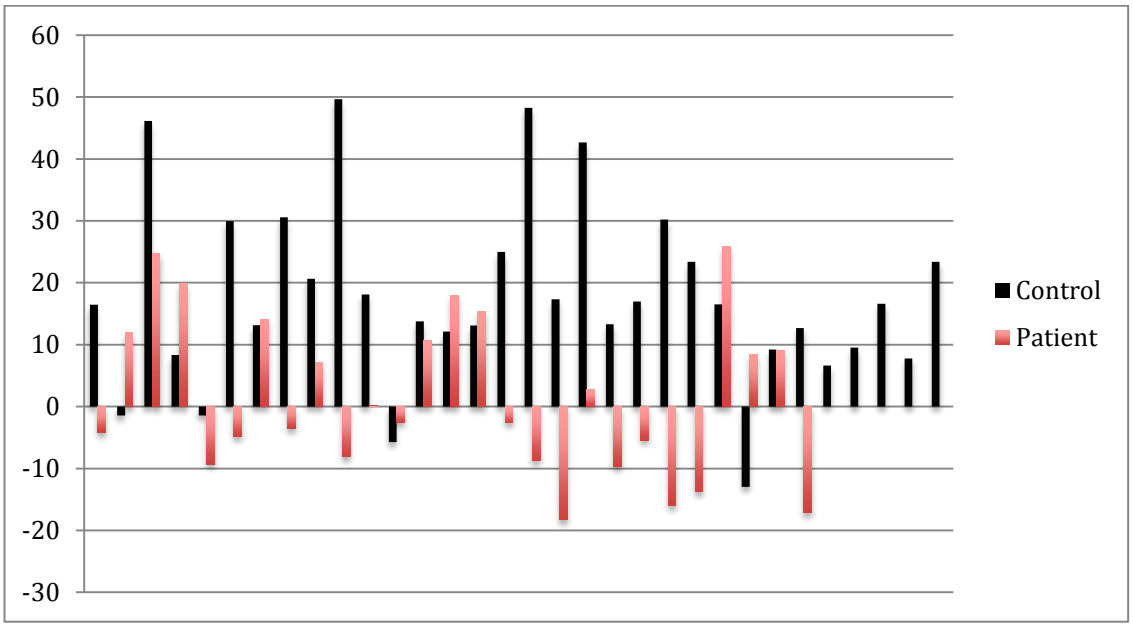


Figure 8:4 Distribution of the mean individual *z*-scores in healthy controls (black), and NDE patients (red), both sorted from smallest to highest *z*-value for attention resting sate network

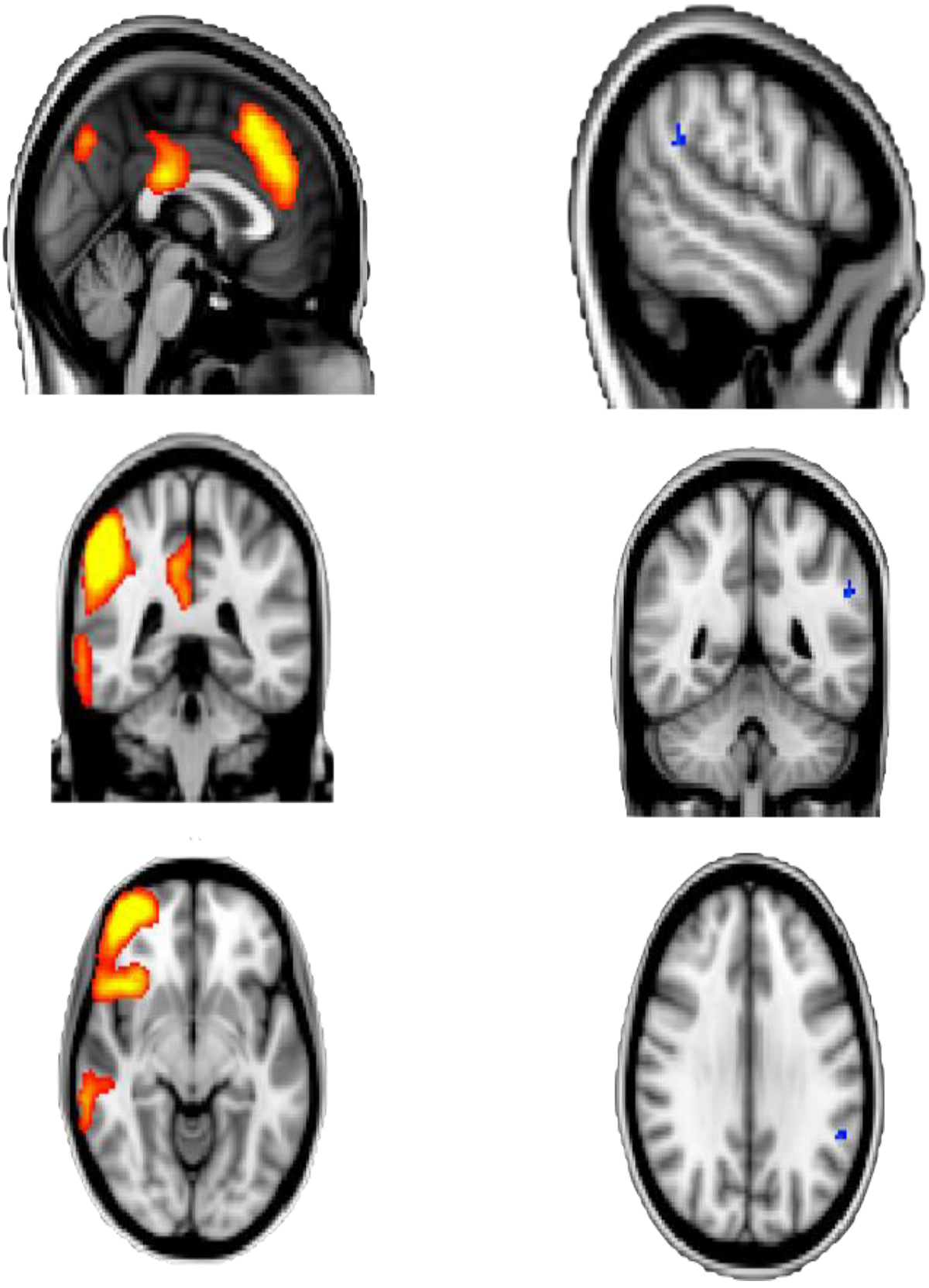


Figure 8:5 Group differences in functional connectivity between patients with NDE and healthy controls. Patients with NDE exhibited reduced connectivity in the right attention resting state network

8.2.3 ROI-to-ROI Analysis

ROI-ROI functional connectivity was compared between patients with NDE and healthy controls using two-sided independent *t*-test analysis implemented in the CONN functional connectivity toolbox. In (Figure 9:5) the red colour indicates the positive connectivity in controls (negative in patients), while the blue colour represents the negative connectivity in controls (positive in patients). Darker colours represent strong statistic while lighter colours illustrate week statistic ($P < 0.05$, FDR corrected).

Patients with NDE demonstrated increased functional connectivity between subcallosal cortex and both thalami (Figures 9:6 and 9:7). Patients with NDE relative to healthy controls displayed significant ($P < 0.01$, FDR corrected) functional connectivity between the subcallosal cortex and both thalami (Figures 9:6 and 9:7).

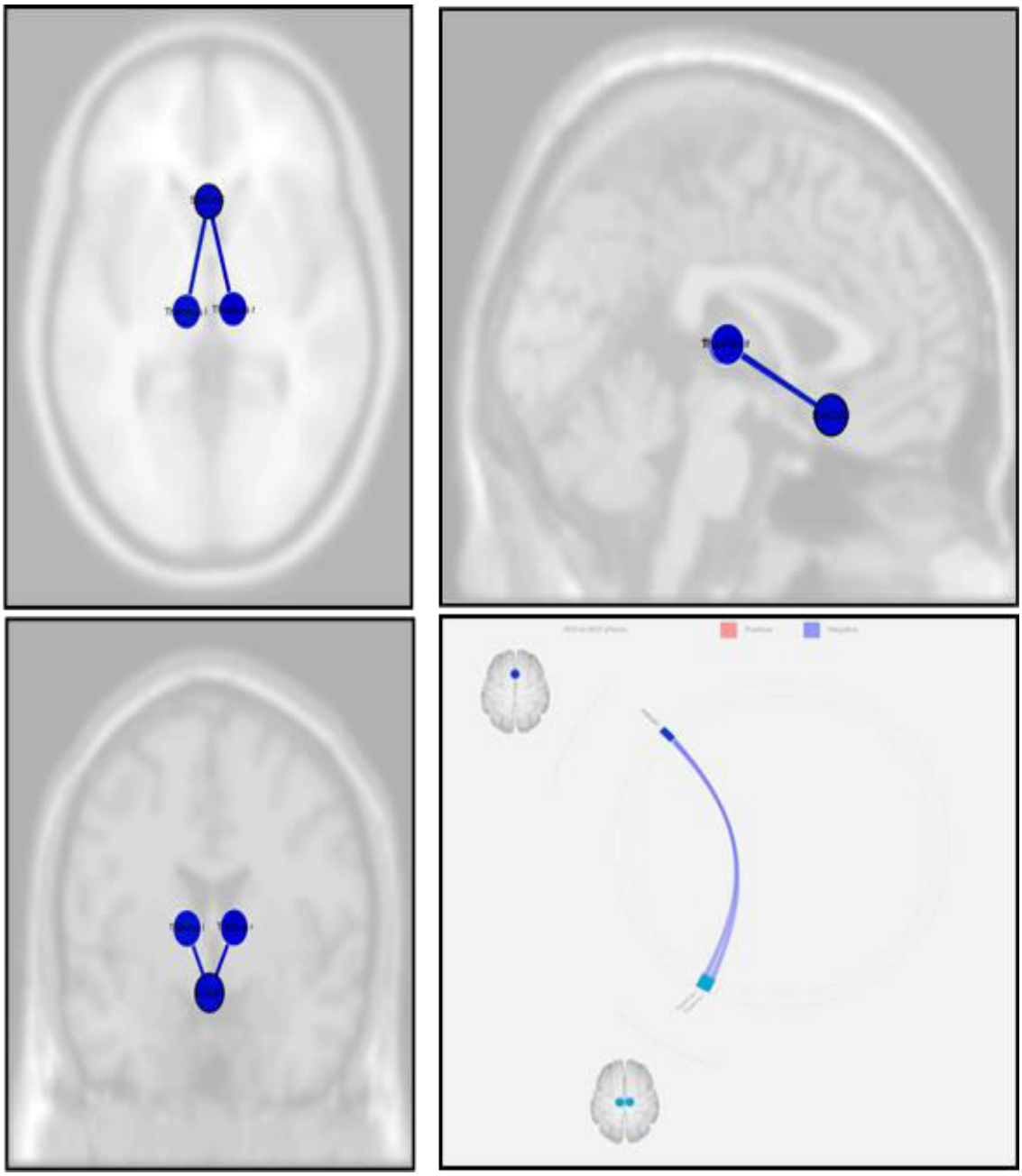


Figure 8:6 Patients with NDE showing positive functional connectivity between the subcallosal cortex and both thalamus. The blue colour represents the negative connectivity in controls (positive in patients).

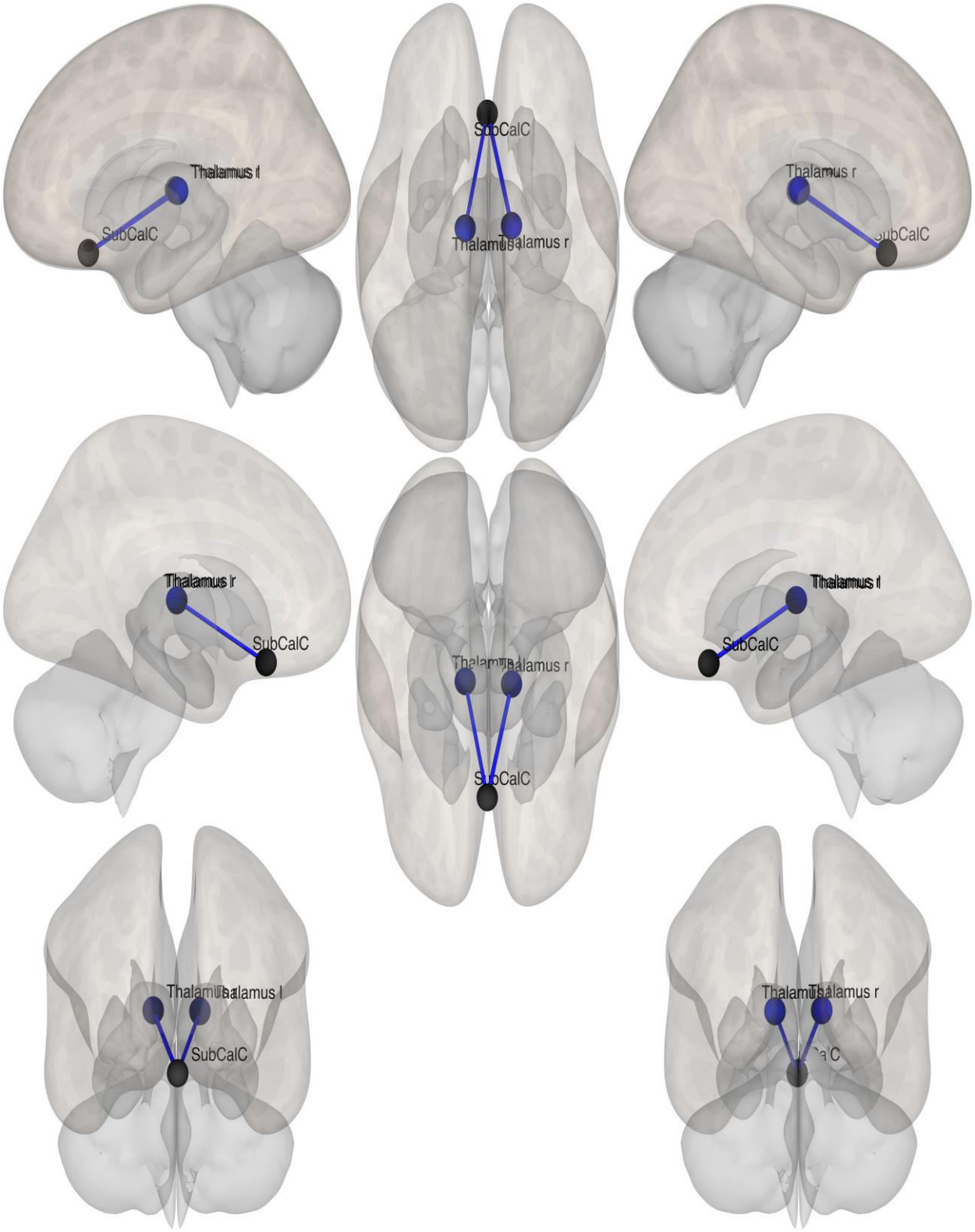


Figure 8:7 Resting state functional connectivity increases in patients with NDE compared to health controls between the subcallosal cortex and both thalamus. The blue colour represents the negative connectivity in controls (positive in patients)

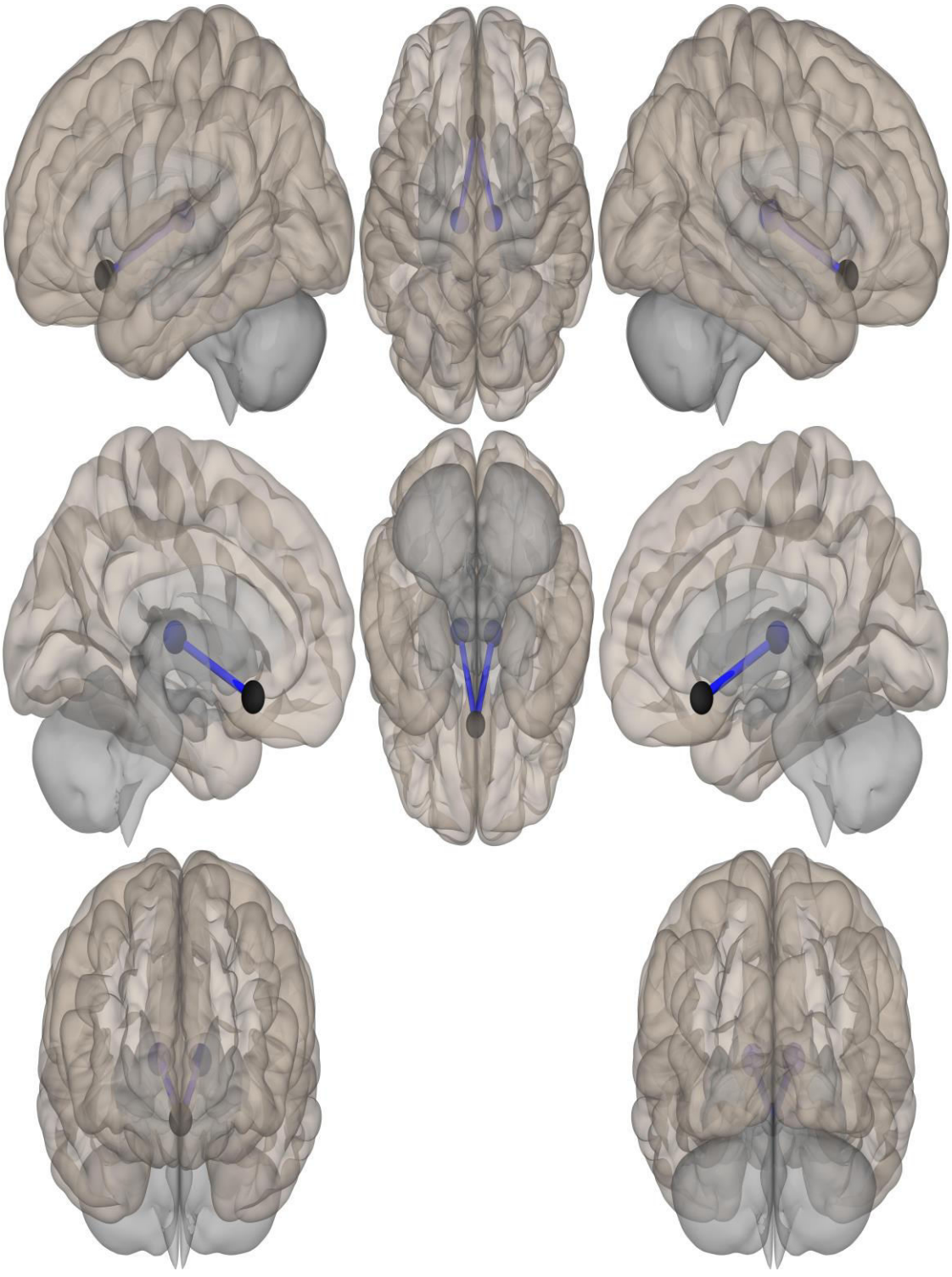
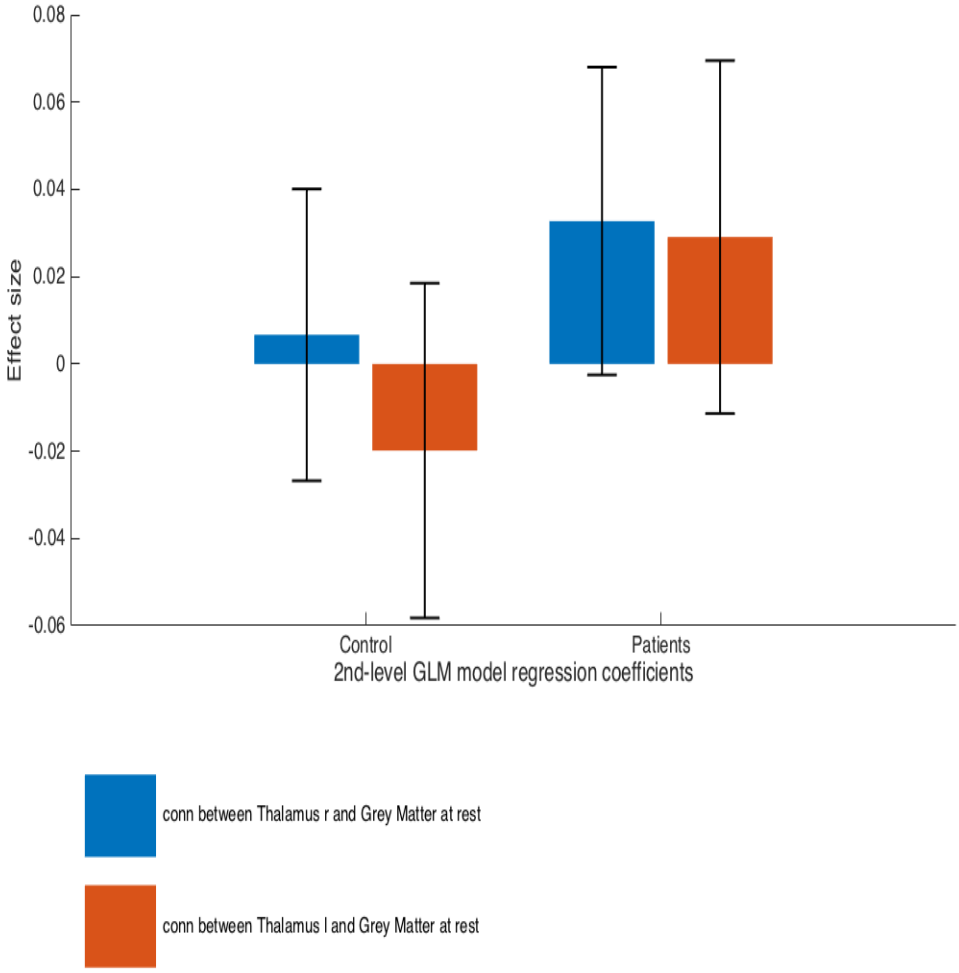


Figure 8:8:8 3D view illustrating the greater functional connectivity in patients with NDE compared to health controls between the subcallosal cortex and thalamus. The blue colour represents the negative connectivity in controls (positive in patients)


0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
Effect size
Control
Patients
2nd-level GLM model regression coefficients
conn between Thalamus r and Grey Matter at rest
conn between Thalamus l and Grey Matter at rest

8.3 Discussion

The multimodal MRI data reported here represent whole brain functional connectivity analyses and functional connectivity in thalamic ROI in patients with NDE compared to healthy controls. Summary of results

To the best of my knowledge, this study is the first to describe functional network abnormalities in patients with NDE. Both methods of data analysis, ICA functional connectivity and CONN ROI showed changes in patients with epilepsy. Compared with healthy controls, patients with NDE demonstrated alterations in visual and attention functional connectivity networks using ICA methods. In CONN analysis, patients with NDE exhibited increased functional connectivity between the subcallosal cortex and both thalami.

8.3.1 Resting state fMRI in epilepsy

The majority of resting state studies in patients with focal epilepsy have examined patients with TLE (Caciagli et al., 2014, Cataldi et al., 2013, Pittau and Vulliemoz, 2015) and focused on the functional connectivity of limbic structures (Caciagli et al., 2014). The attention network affected by disconnected neural circuitry could be influenced by brain damage and drugs (Raz, 2004). The attention network is divided into two main subnetworks: the ventral and the dorsal subnetworks (Fox et al., 2006, Corbetta and Shulman, 2002). The dorsal subnetwork references top-down attention (Corbetta and Shulman, 2002); the process by which a subject voluntarily directs attention, for example, during cognitive tasks (Corbetta and Shulman, 2002). Conversely, the

ventral subnetwork references bottom-up attention, which focuses on processes activated by sudden and unexpected sensory stimulations, for example, a ringing bell (Corbetta and Shulman, 2002, Cataldi et al., 2013). In several studies (Liao et al., 2010, Yang et al., 2010, Zhang et al., 2009b), patients with mesial TLE (mTLE) exhibited reduced functional connectivity in the dorsal attention network via a top-down attention task compared with healthy controls. In addition, patients with TLE have demonstrated dysfunction in the ventral attention network (Bocquillon et al., 2009). These findings suggest that deficiency in the attention networks may be responsible for the poor performance of patients with TLE in attention tests compared with healthy controls (Fleck et al., 2002, Stella and Maciel, 2003, Zheng et al., 2012). The functional connectivity disturbance in attention networks may also be associated with behavioural abnormalities in patients with epilepsy (Luo et al., 2012, Zhang et al., 2009b).

The results from the present study, which suggest changes are exhibited in attention functional connectivity networks in patients with NDE relative to healthy controls, are in agreement with other studies of patients with TLE (Bocquillon et al., 2009, Liao et al., 2010, Yang et al., 2010, Zhang et al., 2009b). Likewise, results from (Saute et al., 2014), in which children with NDE and ADHD demonstrated changes in attention networks, are in agreement with the findings of the present study. My findings also suggest reduced functional connectivity in attention networks, which is in agreement with previous studies of children with epilepsy (Widjaja et al., 2013). In (Wang et al., 2011), patients with generalized tonic-clonic seizures (GTCS) showed reductions in the functional connectivity of attention networks, further supporting my findings.

The visual cortex is the part of the cerebral cortex that is located in the occipital lobe in the brain and involved in the important task of processing visual information (Bock et al., 2011, Damoiseaux et al., 2006). The primary visual network is one of the main networks in the visual cortex and includes the medial and lateral visual networks (Damoiseaux et al., 2006). Studies by (Grant, 2005, Mazzucchi et al., 1985) suggest that patients with epilepsy may have hyperfunction in visual processing caused by epileptic activity, with increased occurrence in the epileptic hemisphere (Zhang et al., 2009a). The reduced functional connectivity in the visual network was a significant result in the present study of patients with NDE. In a study by (Zhang et al., 2009a), patients with TLE demonstrated changes in the visual network, similar to the findings of (Luo et al., 2012), which also showed changes in the visual network of patients with epilepsy relative to healthy controls. (Widjaja et al., 2013) demonstrated alteration in the functional connectivity of the visual network between patients with NDE and healthy controls. Decreased functional connectivity in the visual network was also demonstrated by (Wang et al., 2011) in patients with epilepsy and GTCS relative to healthy controls. The results of my study are consistent with the results of these previous studies, demonstrating alteration in the visual network of patients with NDE relative to healthy controls.

ROI resting state analysis involves using morphological findings to investigate functional connectivity in both thalami. In my study, patients with NDE showed increased functional connectivity between the subcallosal cortex and both thalami. The thalamus is involved in the important role of filtering or gating information exchange between different regions of the brain (Wang et al.,

2015). In addition, the thalamus plays a very important role in epilepsy. There is evidence suggesting that it is involved in the initiation and spread of seizures in TLE (Li et al., 2014). Animal studies in the absence of epilepsy support this notion and showed a 3-Hz spike and wave of seizure initiation in the thalamus (Amberson, 1954, Spiegel et al., 1951, Williams, 1953). In addition, a thalamus role has been exhibited in animal models of IGE and it has been suggested that the thalamus is involved in the spread of seizures, the primary seizure circuits of limbic seizures, reducing seizure duration and enhanced seizure control in the status epilepticus (Bertram et al., 2001, Bertram et al., 2008, Langlois et al., 2010, Li et al., 2014, McCormick and Contreras, 2001, Turski et al., 1984).

A number of other fMRI studies have reported thalamic activation in patients with IGE and JME compared with healthy controls (Archer et al., 2003, Berman et al., 2010, Gotman et al., 2005, Hamandi et al., 2006, Liu et al., 2008, Moeller et al., 2008b, Moeller et al., 2008a, Moeller et al., 2009, Moeller et al., 2010, Moeller et al., 2011, Seneviratne et al., 2014, Szaflarski et al., 2013, Szaflarski et al., 2010, Vollmar et al., 2011). My results are consistent with previous studies, and demonstrate the activation of the thalamus in patients with epilepsy. In some studies (Chen et al., 2015, Masterton et al., 2012), patients with childhood absence epilepsy reported decreased connectivity in the thalamus, which was not in agreement with the present study's findings. However, there were alterations in functional connectivity between cortex regions and the thalamus that either decreased or increased functional connectivity. My results are consistent with morphological alterations in patients with NDE (in Chapter 7), and show changes in the volume and shape of both thalami in patients with NDE. These are also in agreement with previous

studies, and demonstrate the relationship between structural and functional changes in patients with NDE. In patients with focal epilepsy the alterations in resting state fMRI and changes in functional connectivity appear to be similar to alterations in morphological structural MRI (Liao et al., 2011, Voets et al., 2012). The change in volume and shape of the thalamus in the current study could explain why both thalami showed alterations in functional connectivity in resting state fMRI analysis. The present structural MRI study findings are consistent with previous structural MRI studies in patients with TLE, which suggested bilateral atrophy in the thalamus (Labate et al., 2008, Mueller et al., 2006, Yasuda et al., 2010b). My findings were also in agreement with other studies of patients with IGE that demonstrated a reduction in thalamus volume (Ciumas and Savic, 2006, Chan et al., 2006, Helms et al., 2006, Kim et al., 2007, Lin et al., 2009b, Mory et al., 2011, Pardoe et al., 2008, Seneviratne et al., 2014). The connectivity differences between the thalamus and cortical districts could contribute to the clinical symptoms of epilepsy patients.

Overall, my results suggest that structural and functional changes in both thalami in NDE patients may contribute to epileptic activity and could be relevant to epilepsy pathophysiology. Brain structural and functional impairment may play a role in cognitive dysfunction in patients with epilepsy. Functional connectivity network studies in patients with epilepsy could have several clinical implications. Initially, these studies could be used to explore the pathophysiology underlying cognitive decline. Previous structural and functional connectivity network studies have revealed that cognitive decline in epilepsy patients is related to a loss of efficiency in the various networks of the brain (Vaessen et al., 2012, Vlooswijk et al., 2011). The findings of studies by

(Vaessen et al., 2012, Vlooswijk et al., 2011) demonstrated reason for speculation that functional and structural networks may possibly be used to detect patients with a risk of developing cognitive impairment. It is very important for future work to focus on developing a better understanding of how functional connectivity relates to the structure of brain (Caciagli et al., 2014). Also, to increase an understanding of the functional connectivity in patients with NDE, a comparison should be made between patients who were seizure-free one year after diagnosis with patients who continue to experience seizures over the same time period.

8.4 Limitations

Several limitations of my study should to be mentioned. First, the sample size was relatively small for a study of patients with epilepsy; however, the recruitment of patients with NDE is very challenging. Second, the resting state fMRI scanning time of the present study (6 minutes) is relatively short considering a suggestion by some researchers to increase scan time from nine to 12 minutes in order to improve the reliability of results (Birn et al., 2013). Third, antiepileptic drugs can influence the functional connectivity in patients with NDE. A study by (Kiviniemi et al., 2005) demonstrated that midazolam increases functional connectivity in primary sensory and sensorimotor neural networks. In addition, in fMRI, motion is the most common cause of artefacts and produces signal changes (Robinson et al., 2013). Head motion has demonstrated an influence on functional network connectivity and the strength of functional connectivity networks; however, most of the brain network alteration across subjects was not associated with motion in the present study

(Robinson et al., 2013, Van Dijk et al., 2012).

8.5 Conclusion

In summary, this study conducted functional analyses of resting state MRI using ICA analysis and ASL sequences to examine functional connectivity networks and measure CBF in patients with NDE. Using ICA analysis, patients with NDE showed alterations of functional connectivity in visual and attention networks relative to healthy controls. Also, in this study, ROI analysis suggested increased functional connectivity in both thalami and parallel with morphological alterations in patients with NDE, demonstrated changes in the shape and volume of both thalami.. Although sophisticated quantitative MRI analysis techniques provide important knowledge regarding underlying pathology in epilepsy, future studies with larger sample sizes would further elucidate the major pathology evolving to epileptogenesis in patients with NDE, and increase understanding of how brain structures affect functional connectivity.

Chapter 9 Summary, strengths, limitations, future work and conclusions

9.1 Summary of findings

The main goal of this thesis was to determine the nature and extent of structural and functional brain alterations in patients with newly diagnosed epilepsy (NDE).

Within my work, I used sophisticated magnetic resonance imaging sequences and multimodal neuroimaging methods to explore structural and functional brain alterations in patients with NDE compared to healthy controls. To my knowledge, this is the first study in which such work has been undertaken. Figure 10:1 is a visual summary of the main findings I have obtained whilst studying for my thesis.

My main objectives were:

1. **To determine any morphometric changes in patients with NDE compared to healthy controls using quantitative MRI analysis.**

My first experiment employed a novel geometric shape analysis technique examining subcortical structures (Figure 10:1D,F) and subcortical volume measurement using the Freesurfer method and voxel-based morphometry (VBM) analysis (Figure 10:1G) to investigate morphological alterations in subcortical structures and cortical grey matter (GM) in patients with NDE compared to healthy controls. 3D high-resolution T1-weighted sequence scans were analysed, and it was hypothesised that patients with NDE would display subcortical shape and volume abnormalities relative to healthy controls. FIRST FSL shape analysis showed patients with NDE had shape changes in both thalami relative to healthy controls. Freesurfer method analysis showed volume

changes in patients with epilepsy in both thalami. In addition, VBM SPM analyses demonstrated local GM volume reductions in the thalamus in patients with NDE compared to healthy controls.

2. **To identify functional connectivity differences (in the whole brain and regions of interest) in patients with NDE and healthy controls using novel resting state methods.**

In my third experiment I used independent component analysis (ICA) (Figure 10:1A,C) to investigate the whole brain. Patients with NDE exhibited reduced functional connectivity in the visual and attention networks relative to healthy controls. Also, ROI-ROI functional connectivity analysis (Figure 10:1B and E) using the CONN statistical parametric mapping (SPM) toolbox in patients with NDE suggested increased functional connectivity between the subcallosal cortex and both thalami compared with healthy controls.

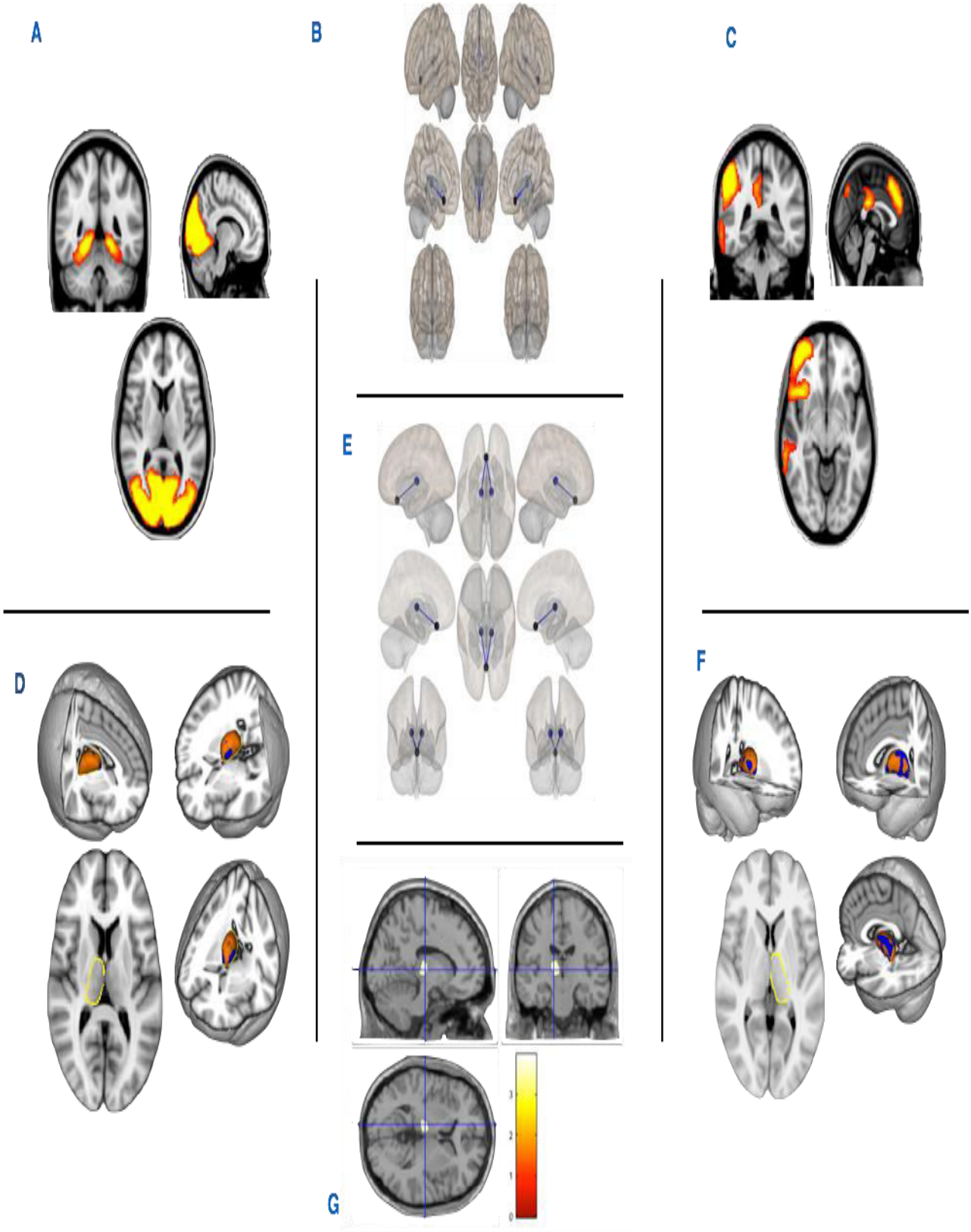


Figure 9:9:1 A visual summary of the main significant findings obtained in this thesis.

Table 9-1 Summary of the main significant results in NDE study

Reference number	Results
A	Group differences in functional connectivity between patients with NDE and healthy controls. Patients with NDE demonstrated decreased connectivity in the primary visual resting state network
B	3D view illustrating the greater functional connectivity in patients with NDE compared to health controls between the subcallosal cortex and thalamus. The blue colour represents the negative connectivity in controls (positive in patients)
C	Group differences in functional connectivity between patients with NDE and healthy controls. Patients with NDE demonstrated decreased connectivity in the right attention resting state network
D	Comparison of patients with NDE and healthy controls using vertex shape analysis of the right thalamus. The results are viewed from different directions, and are statistically significant at $P < 0.01$ (FDR corrected). Within the 3D view, orange represents the difference in right thalamus shape and yellow represents thalamus locations
E	Resting state functional connectivity increased in patients with NDE compared to health controls between the subcallosal cortex and both thalamus
F	Comparison of patients with NDE and healthy controls using vertex shape analysis of the thalamus. The results are viewed from different directions, and are statistically significant at $P < 0.01$ (FDR corrected). Within the 3D view, orange represents the difference in thalamus shape and yellow represents thalamus locations
G	Statistical parametric map (extent threshold $P < 0.05$ corrected) showing clusters with decreases in grey matter volume in the left thalamus between NDE and healthy controls

Table 9-2 Summary of consistent findings of NDE studies

Methods	Findings
Vertex shape analysis FSL First	FIRST FSL shape analysis showed patients with NDE had shape changes in both thalami relative to healthy controls
Freesurfer method analysis	Freesurfer method analysis showed volume changes in patients with epilepsy in both thalami
VBM SPM analyses	VBM SPM analyses demonstrated local GM volume reductions in the thalamus in patients with NDE compared to healthy controls
Resting state functional connectivity Conn Toolbox	CONN statistical parametric mapping (SPM) toolbox showed increased functional connectivity between the subcallosal cortex and both thalami compared with healthy controls

9.2 Study strengths and limitations

9.2.1 Strengths

1. This is the first study to use a number of sophisticated MRI sequences, including structural, and resting state methods, in patients with NDE.
2. The present study used novel techniques to analyse the MRI data of patients with NDE and employed a combination of advanced structural and functional analyses.
3. This study demonstrated novel findings with respect to structural and functional alterations in both thalami in patients with NDE relative to

healthy controls.

4. The functional alterations in the thalamus were parallel with morphological alterations in patients with NDE.

9.2.2 Limitations

1. Some of the patients with NDE refused to have their MRI scan performed at the MRI centre at the University of Liverpool because it was far away from the Walton Centre. Eight patients wanted the scan performed at the Walton, which is where their treatment provider was located. This point should be considered in any future projects, especially in patients with NDE, since some patients with recently diagnosed epilepsy have a greater degree of trust in their treatment providers' centres than research centres.
2. There remains a paucity of knowledge and unclear understanding regarding epileptogenesis.
3. Considering the small sample of patients with NDE as well as healthy controls, it is not known whether the alterations found before seizure onset in patients with NDE were the result of seizures, AEDs or other disease-related factors. Future longitudinal studies in patients with NDE should seek to confirm that seizure duration correlates with changes in thalamic volume and functional connectivity over time due to the unclear mechanisms in epilepsy. There were numerous biological and clinical variables not controlled for in medical imaging analyses of the effects of epilepsy on brain morphology, for example, the frequency of seizures, severity of seizures, status epilepticus and AED use, all of which have

previously been associated with alterations in the brain.

9.3 Future research

The results presented in this thesis highlight the need for future sophisticated quantitative MRI analysis studies of NDE to understand the progression of brain damage in epilepsy based on underlying aetiology, duration and the frequency of seizures, genetic predisposition and other environmental factors.

Future work should consider whether the nature and extent of brain alterations for NDE patients are related to seizure control by AEDs or natural remission. Advanced MRI data for patients with NDE should be compared between patients who have been seizure-free one year since diagnosis and patients who have not responded to AEDs and continue to experience seizures over the same time period. This approach represents the first step towards the development of prognostic imaging markers of seizure control in patients with NDE (Bilevicius et al., 2010). In addition, future research should compare patients with NDE with patients with intractable epilepsy and negative MRIs from the Walton Centre to examine the possible degenerative effects of chronic epilepsy on brain structure and function.

Finally, this thesis demonstrated the use of advanced MRI sequences and imaging analysis to examine structural and functional alterations in both thalami; future research should explore the thalamus in patients with NDE using magnetic resonance spectroscopy.

9.4 Conclusion

In conclusion, this thesis describes the first time in which novel techniques and advanced MRI sequences have been employed to study structural and functional alterations in patients with NDE compared to healthy controls. In previous quantitative MRI studies there have been difficulties in identifying brain alterations in patients with epilepsy at the point of diagnosis (Briellmann et al., 2002), possibly because the work has focused on the hippocampus. In my work I have combined multiple analysis methods and reported shape alteration and volume reduction in both thalami in patients with NDE relative to matched healthy controls. Functional analysis showed alterations in the visual and attention networks in patients with NDE using ICA analysis. Furthermore, in this study, ROI analysis displayed increased functional connectivity in both thalami in patients with NDE compared to healthy controls.

The present thesis is demonstrate functional and structural alterations in both thalami in patients with NDE. These findings suggest that the thalamus plays a very important role in epilepsy pathophysiology (Li et al., 2014). My results offer further understanding regarding the role of structural and functional alterations in NDE. They highlight the need for future quantitative MRI analysis studies to help patients avoid the chronic stage of epilepsy, thus improving their quality of life.

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Appendices

9.5 Ethics NHS Permission

Non-CTIMP Permission

The Walton Centre NHS
NHS Foundation Trust

Excellence in Neuroscience

Professor Anthony Marson
Professor of Neurology
University of Liverpool
Neurological Science, Clinical Sciences Centre
Aintree University Hospital, Lower Lane
Liverpool

Date: 13th August 2014

Dear, Tony

Study title:	**Advanced MRI in newly diagnosed and intractable epilepsy**
REC reference:	**14/NW/0332**
Protocol number:	**ADV-MRI-PRO-V3**
IRAS project ID:	**140420**
RD&I reference;	**RG127-14**

Thank you for providing all of the documentation for the above study.

I am pleased to inform you that the above study has been given full R&D permission and you may begin this at the Walton Centre NHS Foundation Trust. This has been granted for the duration of the REC approval for your study.

Permission is granted on the understanding that the study is conducted in accordance with the Research Governance Framework, Trust policies and procedures, and all applicable legislation including, but not limited to, the Data Protection Act, the Health and Safety at Work Act, Human Tissue Act. As Chief Investigator you retain overall responsibility for compliance with these requirements by all members of the research team. The recruitment target is **85** patients for this study.

You must ensure that you read and understand the enclosed conditions of approval

Should you have any queries, or feel that we can be of assistance, please do not hesitate to contact a member of the R&D office on 0151 529 8854.

I would like to take this opportunity to wish you well with your research

Yours Sincerely,

DAVID WATLING
R+DI manager

Dr M. Steiger
Director of Research, Development & Innovation

Non-CTIMP Permission

CONDITIONS FOR RESEARCH APPROVAL

This study is subject to external performance management of recruitment on time and to target. You are responsible for ensuring that recruitment targets are met. The Trust expects the first participant to be recruited within 30 days of receipt of R&D approval. The R&D department must be informed of the date on which the first participant is recruited. You may delegate this responsibility to the CTU or a responsible member of your research team. If there is a delay in starting recruitment, you must inform the R&D office and give a reason for this. You are also required to provide recruitment data to the R&D office upon request. This information will be used to inform performance targets, which will be reported to the Trust Board.

Note that for non-questionnaire type studies you are required to document patient participation in a clinical trial on the orange card in the patient casenotes. This is required to ensure that the Trust retains the case notes for the period of time required for research records. If you are unsure as to the requirement of this in relation to your trial please contact the R&D office prior to commencement.

You must obtain R&D approval (or acknowledgement) for all amendments during the course of the study. You must not implement an amendment until this has been granted. You should submit all amendment documentation to the R&D office unless the study has been adopted onto the UKCRN portfolio. If the study has been adopted onto the UKCRN portfolio and has been processed through CSP, *all amendments must be submitted through the Lead CLRN. In such cases please do not send any amendments to the R&D office directly*. The Lead CLRN will inform us of any amendments and will supply us with the required documentation.

You must ensure that annual reports are submitted to the REC in a timely fashion, and a copy should also be sent to R&D. On completion of the study you must ensure that the End of Study Report is submitted to the REC, and a copy sent to R&D. More information on post-approval requirements is available from
http://www.nres.nhs.uk/applications/after-ethical-review/

You must inform the R&D office of any changes to the management of your project, any extensions to the study, and any changes in funding, if applicable.

The Trust may monitor the progress or audit the conduct of your study at any time. The Trust reserves the right to suspend or withdraw R&D approval if you do not adhere to the requirements outlined. You must abide by all determinations of the R&D office and accept their final authority.

9.6 Participant information sheet

The Walton Centre NHS
NHS Foundation Trust

The Walton Centre NHS Foundation Trust
Lower Lane, Fazakerley
Liverpool, L9 7LJ, UK
Tel: 0151 525 3611
Fax: 0151 529 5500

Professor Anthony Marson
Professor of Neurology
2nd Floor, Neurological Science, Clinical Sciences Centre
Aintree University Hospital and The Walton Centre NHS Foundation Trusts
Lower Lane, Liverpool, L9 7LJ
Telephone: 0151 529 5770
Email: a.g.marson@liv.ac.uk

PARTICIPANT INFORMATION SHEET (ADV-MRI-EPp-V1, 03/12/13)

Advanced MRI in epilepsy

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

What is the purpose of the study?
As part of the investigation of your epilepsy, you may be coming to the hospital to have a Magnetic Resonance Imaging (MRI) scan, or you may have had a scan previously. At the moment, we find that many people with epilepsy have normal MRI scans. We think this is because we do not yet know the best way to carry out MRI scanning for people with epilepsy, and that the current use of MRI scanning may not be detailed enough. We have developed some new MRI scanning techniques, and would like to try them out, to compare the new methods with "standard" scans. We hope that these new scans will provide us with a more detailed picture of the structure of the brain, which will be very important for people with epilepsy.

Why have I been chosen?
You have been chosen because we know you have been diagnosed with epilepsy arising from a particular part of the brain ("focal epilepsy").

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
If you take part, the investigation and treatment of your epilepsy will not be changed in any way. We will send you an appointment to have an MRI brain scan at the Walton Centre NHS Foundation Trust, which is located at Aintree University Hospital, or at the Magnetic Resonance Image Analysis Research Centre (MARIARC), at the University of Liverpool. Your "standard" scan, which you may be having anyway, takes about 30 minutes. We would like you to stay in the scanner for an extra 15-30 minutes for the new scans, totalling 45-60 minutes. Prior

The Walton Centre NHS Foundation Trust

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Liverpool, L9 7LJ, UK
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Fax: 0151 529 5500

to the scan we would also like to run two short assessments, one for your handedness and one for IQ. These will last approximately 15 minutes.

Expenses and payments.
We will reimburse you £30 if you decide to take part.

What do I have to do?
All you need to do is lie still in the scanner for the duration of the scan.

What are the other possible disadvantages and risks of taking part?
The technique of MRI has been in use in medicine for about 25 years, and has shown to be safe. It does not involve any radiation. In some people, there are times when it is not safe to be scanned. For example, in the first three months of pregnancy, or when there are surgical clips inside the brain, or if there is a heart pacemaker or Vagus Nerve Stimulator fitted. Furthermore, the scanner may get warm and noisy, and may not be suitable for sufferers of claustrophobia. However, rest assured, you will be contacted by telephone prior to the scan to identify any reasons why you should not have the scan. We will also thoroughly review with you on the day of scanning whether there is any possible risk for you. Earplugs are used to reduce the impact of scanner noise.

What are the possible benefits of taking part?
There may be no direct benefit to you from taking part. We hope that we will gain useful information about your epilepsy from these new scanning techniques, but we cannot be certain about this.

What if there is a problem?
Any complaint about the way you have been dealt with during the will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

The Walton Centre NHS
NHS Foundation Trust

The Walton Centre NHS Foundation Trust
Lower Lane, Fazakerley
Liverpool, L9 7LJ, UK
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PART 2

What will happen if I don't want to carry on with the study?
You can withdraw from this research study at any time, even while you are having the tests done. This will not affect your treatment in any way. Even if you withdraw from the study, we would still like to use any information we might already have collected. However, if you want us to destroy the information we have collected, we will do so.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from The Walton Centre NHS Foundation Trust. In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the hospital, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?
Yes. We will collect information about you that could identify you personally (for example, because the information includes your name or date of birth). We will also collect information about you because we believe it might be relevant to understanding the research. This will include: information about your epilepsy and its treatment, any previous scans and EEGs, dates and times of seizures near to the MRI scan. This information will be stored in anonymised form (which means that your name, address and other personal details will not be associated with the information we use) on computers owned by the hospital and on computers owned by the University of Liverpool, the university associated with The Walton Centre. These computers will be securely controlled by the research team under the direct responsibility of Dr. Simon Keller and Dr. Vanessa Sluming, and no-one outside the team will have access to your information. We will use the information we collect to answer the questions relevant to this research project. In the future it is possible we might have new research questions that could be answered by looking at your information in new ways. We would seek approval from the Research Ethics Committee to use your information for new research projects. If the Research Ethics Committee believed we should contact you again to ask your permission to re-use your information, we will do so. The information we collect in this project will be kept for 10 years and then destroyed securely. The hospital has a duty to ensure research conducted here is of a high standard and auditors from the hospital may need to review any information we hold about you. The auditors will maintain the highest standards of confidentiality. Procedures for handling, processing, storage and destruction of your data are compliant with the Data Protection Act 1998.

Involvement of your doctor
The doctor looking after you in the hospital will be aware of your participation in this research study.

What will happen to the results of the research study?
The scientific results of this research study will be published in scientific and medical journals and may be discussed at scientific meetings. You will not be personally identified in any way. A summary of the findings of this study will be made available to you, should you request them via the consent form.

The Walton Centre NHS
NHS Foundation Trust

The Walton Centre NHS Foundation Trust
Lower Lane, Fazakerley
Liverpool, L9 7LJ, UK
Tel: 0151 525 3611
Fax: 0151 529 5500

Contact Details:
For further information, please contact any of the team running this research project:

Prof Anthony Marson, Professor of Neurology
2nd Floor, Neurological Science, Clinical Sciences Centre
Aintree University Hospital and The Walton Centre NHS Foundation Trusts
Lower Lane, Liverpool, L9 7LJ
Telephone: 0151 529 5770; Email: a.g.marson@liv.ac.uk

Dr. Simon Keller, Lecturer in Neuroimaging
2nd Floor, Neurological Science, Clinical Sciences Centre
Aintree University Hospital and The Walton Centre NHS Foundation Trusts
Lower Lane, Liverpool, L9 7LJ
Telephone: 0151 529 5943; Email: simon.keller@liv.ac.uk

Dr. Vanessa Sluming
Senior Lecturer in Medical Imaging
Whelan Building, Quadrangle, Brownlow Hill
The University of Liverpool, Liverpool, L69 3GB
Telephone: 0151 529 5461
Email: vanessa.sluming@liv.ac.uk

This completes Part 1 of the Information Sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

The Walton Centre NHS
NHS Foundation Trust

The Walton Centre NHS Foundation Trust
Lower Lane, Fazakerley
Liverpool, L9 7LJ, UK
Tel: 0151 525 3611
Fax: 0151 529 5500

Who is organising and funding the research?
This research is being organised Prof Anthony Marson and is sponsored by The Walton Centre NHS Foundation Trust. There is no outside funding for this research and neither the research team nor your doctor receives any payment if you take part.

Who has reviewed the study?
The Northwest Liverpool Central Research Ethics Committee has reviewed this study and given a favourable ethical opinion for this research.

You will be given a copy of the information sheet and a copy of your signed consent form to keep.
Thank you for considering taking part in this research project, and thank you for taking the time to read the information sheets.

The Walton Centre NHS Foundation Trust
Excellence in Neuroscience

The Walton Centre NHS Foundation Trust
Lower Lane, Fazakerley
Liverpool, L9 7LJ, UK
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Professor Anthony Marson
Professor of Neurology
2nd Floor, Neurological Science, Clinical Sciences Centre
Aintree University Hospital and The Walton Centre NHS Foundation Trusts
Lower Lane, Liverpool, L9 7LJ
Telephone: 0151 529 5770
Email: a.g.marson@liv.ac.uk

[insert date]

Mr / Mrs **[insert patient name]**
[Insert full postal address here]

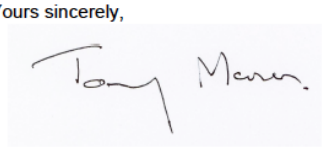
Dear Mr / Mrs **[insert patient name]**,

As part of the work at The Walton Centre and University of Liverpool we conduct research with the ultimate aim of improving health care for people who have been diagnosed with epilepsy. My research team and I are aiming to recruit people with a recent diagnosis of epilepsy to take part in our MRI study. I am contacting you to ask whether you would be interested in having a new MRI scan at The University of Liverpool. I have included an information sheet with this letter so that you may read about the project. A member of our research team will call you after you have had some time to look through the information sheet to determine whether or not you would like to participate.

You are free to decline this request, which will have no impact on your quality of care.

Many thanks for your consideration.

Yours sincerely,



Professor Anthony Marson
Consultant Neurologist

Version 01
29/04/2014

Institute of Translational Medicine, University of Liverpool
The Walton Centre NHS Foundation Trust

Healthy Volunteers (age 18-60 years) Needed for Brain MRI Scan Research

We are undertaking a study that will help us to understand brain structure and function in epilepsy. To assist in this we are seeking to recruit healthy volunteers between the ages of 18 and 60 years of age. The study will involve:

1. Prior to the MRI scan, being screened by the radiographer to ensure that it is safe for you to be scanned, and undertaking brief handedness and IQ questionnaires (taking approximately 15 minutes in total).

2. Having a Magnetic resonance (MRI) scan of your brain, normally of no more than 1 hour duration, at the Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool, or The Walton Centre NHS Foundation Trust.

No adverse effects are known to result from magnetic resonance. If you (or anyone you know) would like to more information about this study to allow you to decide whether to participate details, please contact the researchers.

Thank you!

Researchers:

Batil Alonzi, Institute of Translational Medicine, University of Liverpool, MARIARC, Pembroke Place, University of Liverpool, L69 3GE. Tel: 015179454627, Email: alonazi@liv.ac.uk

Barbara Kreilkamp, Institute of Translational Medicine, University of Liverpool, Clinical Sciences Centre, University Hospital Aintree and The Walton Centre NHS Foundation Trusts, Lower Lane, Liverpool, L9 7LJ. Tel: 01515295417, Email: B.Kreilkamp@liv.ac.uk

Dr Simon Keller, Institute of Translational Medicine, University of Liverpool, Clinical Sciences Centre, University Hospital Aintree and The Walton Centre NHS Foundation Trusts, Lower Lane, Liverpool, L9 7LJ. Tel: 01515295943, Email: simon.keller@liv.ac.uk

Dr Vanessa Sluming, Institute of Translational Medicine, Whelan Building, The Quadrangle, University of Liverpool, Liverpool L69 3GB. Tel: (+44) 0151-794 5760, e-mail: vanessa.sluming@liverpool.ac.uk

9.7 Protocol review King's college London

Institute of Psychiatry

at The Maudsley

Professor Mark Richardson MA PhD FRCP
Paul Getty III Professor of Epilepsy
Head, Department of Clinical Neuroscience
P043 Institute of Psychiatry
Camberwell
LONDON SE5 8AF
UK

Tel: +44 (0)20 7848 5429
Fax: +44 (0)20 7848 0988
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KING'S College LONDON

University of London

Mr Alex Astor
Head of Research Support, Health and Life Sciences
University of Liverpool
Research Support Office
3 Bronlow Street
Liverpool L69 3GL

21st January 2014

Dear Mr Astor,

Re: Advanced MRI in newly diagnosed and intractable epilepsy. PI: Prof A Marson

Thank you for asking me to review this protocol. I am very familiar with current best practice in neuroimaging research in epilepsy and have run a research programme in this area for 14 years.

The protocol is extremely clearly written, making evaluation very straightforward.

The research question is important. The designs of both the retrospective and prospective studies are appropriate. Known risks are described accurately and all the expected safeguards have been included. Inclusion and exclusion criteria and recruitment methods are appropriate.

The MRI protocol and analysis methods reflect the state-of-the-art and are likely to provide valuable new information about the biological basis of epilepsy and treatment response.

There are no difficult ethical issues.

Please do not hesitate to contact me if any further information is required.

Yours sincerely,

Mark Richardson

Professor Mark Richardson
Head, Department of Clinical Neuroscience
King's College London

9.8 Protocol review university of South Carolina

MUSC Health
NEUROLOGY SERVICES

Department of Neurosciences
Division of Neurology
96 Jonathan Lucas St.
CSB 301 • MSC 606
Charleston, SC 29425-6160
Phone: 843-792-1414
MUSC.edu/neurosciences

January 20th, 2014.

Mr. Alex Astor
Head of Research Support
Health and Life Sciences
University of Liverpool
Research Support Office
3 Bronlow St. L69 3GL
Liverpool, UK

RE: Independent peer review of proposal:
"***Advanced MRI in newly diagnosed and intractable epilepsy.***"
Principle Investigator: Professor Anthony Marson
Department of Molecular and Clinical Pharmacology
Institute of Translational Medicine
University of Liverpool

Dear Mr. Alex Aston:

I have reviewed the research protocol for the project entitled "Advanced MRI in newly diagnosed and intractable epilepsy", submitted for support to the Joint Research Office Sponsorship Committee at the University of Liverpool.

I believe this is a well-suited research study, aiming primarily to determine the nature and extent of brain structural and functional alterations in patients with focal epilepsy.

In fact, after carefully reviewing the research plan, I believe this is a highly relevant project with great probability of successfully completing its scientific objectives. The excellence of the project is apparent through the clinical importance of the topic, the quality of the research team, and the carefully designed research methodology.

Please do not hesitate to contact me if I can be of any further assistance.

Sincerely,

Leonardo Bonilha MD PhD
Assistant Professor
Division of Neurology
Medical University of South Carolina
Phone 843 792 3383, Fax 842 792 8626
bonilha@musc.edu

9.9 Intention to sponsor form University Of Liverpool

LIVERPOOL HEALTH PARTNERS

Professor Tony Marson
Clinical Sciences Centre
Lower Lane
Liverpool
L9 7LJ

Mr Alex Astor
Head of Research Support – Health and Life Sciences

University of Liverpool
Research Support Office
2nd Floor Block D Waterhouse Building
3 Brownlow Street
Liverpool
L69 3GL

Tel: 0151 794 8739
Email: astor@liverpool.ac.uk

06 March 2014

Sponsor Ref: UoL001021

Re: Intention to Sponsor

"Advanced MRI in newly diagnosed and intractable epilepsy"

Dear Professor Tony Marson

After consideration by the Chair of the Joint Research Office Sponsorship Committee I am pleased to confirm that the University is prepared to act as Sponsor under the Department of Health's Research Governance Framework for Health and Social Care 2nd Edition (2005) for the above study.

The following documents have been received by the Research Support Office

Document title	Version	Date
Protocol	Version 2	03/12/13

Please note this does **NOT** constitute final Sponsor approval to allow you to commence recruitment. Sponsor Notification to Proceed will be given when final NRES REC Favourable Opinion, financial and other regulatory requirements have been met. Please see Appendix 1 to this letter for further information and a list of the documents required.

If you have not already applied for regulatory approvals through IRAS you may now do so at https://www.myresearchproject.org.uk/Home.aspx.

In order to meet the requirements of the Research Governance Framework 2nd Ed 2005, the University requires you to agree to the following Chief Investigator responsibilities: -

1. Inform the Research Support Office (RSO) as soon as possible of any SAE's within the University

2. Provide copies to the RSO of annual progress and safety reports sent to NRES and any other regulatory authorities

3. Comply with the Research Governance Framework 2nd Ed 2005 and all relevant legislation, including but not limited to the Data Protection Act 1998 and the Human Tissue Act 2004

4. Inform the University of any amendments to, or changes of status in the study **prior to** submission to NRES or any other regulatory authorities

5. Maintain the study site file

6. Make available for review any study documentation so requested by the sponsors and regulatory authorities

7. University professional indemnity and clinical trials insurances will apply to the study as appropriate. This is on the assumption that no part of the clinical trial will take place outside of the UK. If you wish to conduct any part of the study in a site outside the UK or you wish to sub-contract any part of the study to a third party specific approvals and consideration of appropriate indemnity would be required.

If you have any queries regarding the sponsorship of the study or the above conditions please do not hesitate to contact the Research Support Office on 0151 794 8373 (email sponsor@liverpool.ac.uk).

Yours sincerely

Mr Alex Astor
Head of Research Support – Health and Life Sciences
Research Support Office

9.10 Edinburgh Handedness Inventory form

SUBJECT ID:

EDINBURGH HANDEDNESS INVENTORY

Please answer the following questions about your handedness using the responses listed

RR = ALWAYS RIGHT

R = USUALLY RIGHT

RL = EITHER

L = USUALLY LEFT

LL = ALWAYS LEFT

With which hand do you:

Write ..... : _____

Draw ..... : _____

Throw ..... : _____

Cut using scissors ..... : _____

Toothbrush ..... : _____

Cut with knife (without fork) ..... : _____

Use a spoon ..... : _____

Use a broom/spade (upper hand) ..... : _____

Strike a match? ..... : _____

Open a box (lid)? ..... : _____

9.11 Consent form

CONSENT FORM

Title of Project: Advanced MRI in epilepsy.
Version 1: ADV-MRI-V1 **03/12/13**

Name of Researcher: Professor Anthony Marson

	Please initial box
1. I confirm that I have read and understand the information sheet dated 03/12/13 (ADV-MRI-EPp-V1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4. I agree to my GP being informed of my participation in the study.	☐
5. I would like to receive a summary of the findings from this study.	☐
6. I agree to take part in the above study.	☐

Name of Patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

9.12 MRI Safety screening form

UNIVERSITY OF LIVERPOOL

Report Disc..... Scanning ID.....

Date of Screening.....

Magnetic Resonance and Image Analysis Research Centre (MARIARC)

SAFETY SCREENING FORM FOR RESEARCH SCANS

Surname..... Forenames.....

Address.....

E-mail address..... Telephone number.....

Date of Birth ..... Sex ..... Weight ..... Height .....

G. P.'s Name & Address .....

.....

It is very important that you give full and accurate answers to the questions on this form. The information you give will be treated in strict confidence.

Our policy on unexpected abnormal findings in research studies

Occasionally research studies using magnetic resonance imaging (MRI) reveal significant unexpected abnormalities which require medical follow-up, either for further investigation or (more rarely) treatment. The MRI scans we do are for research, but we review them carefully to avoid missing any such abnormality. For studies of brain, we take a high-quality image (which adds a few minutes to the research study for which you have volunteered) and have them reviewed by a consultant radiologist. If any significant unexpected abnormality is found we will send the report to your GP, who will be able to take it further with you. Studies of organs other than brain are dealt with slightly differently, but in any case a health professional will review an MRI scan and report any significant unexpected abnormality to your GP.

WARNING **Do NOT enter the scan rooms with ANY metal objects such as:-**

Glasses	Jewellery/piercings	Safety pins
Coins	Removable Dentures	Hairpins/hairclips
Keys	Eye make-up	Mobile phone
Watch	Hearing aid	Magnetic strip cards e.g. credit cards

Lockers are provided in the waiting area for the safe-keeping of your belongings. Do not take anything through to the scanning area. A changing area is provided where you can change out of your clothes into a gown. Underwear with metal fastenings must be removed and left in the changing room. You may wear a t-shirt under the gown as long as it has no metal. If you have an inhaler or other emergency medication please alert the researcher and leave it in the changing room where it can be located in an emergency.

The following are hazardous during an MR scan.
You should not undergo an MR scan if these apply.

Please tick YES or NO boxes as appropriate:

Yes	No	
		Cardiac Pacemaker or mini-defibrillator
		Brain clips (cerebral aneurysm clips)
		Metal fragments in the eye or head
		Artificial heart valve
		Neurostimulators
		Shrapnel

Yes	No	
		Do you have any internal metal at all?

If YES, where, is it?

The following may interfere with and perhaps preclude an MR scan

Please tick YES box if any of these apply:

Yes	
	Surgical Clips
	Vascular clips or stents
	Shunt, spinal or ventricular
	Insulin Pump
	Electrodes
	Hearing aids or cochlear implant
	Orthopaedic Surgery
	Prosthesis e.g. limb, eye
	Metal mesh implants
	Wire sutures
	Tattoos
	Colostomy , ileostomy or drainage bag
	Any history of injuries to the eyes
	Other implants, including dental work

The following may make it impossible for us to scan you:			
Yes	**No**	**Please tick YES or NO boxes as appropriate**	**If YES, please give details**
		Have you had any operations or procedures in which metal may have been implanted in your body?	
		Have you suffered from any back or neck pain?	
Are you suffering, from or receiving treatment (e.g. medication) for any disease, illness or medical condition?			
		• Epilepsy, fits/faints/dizzy spells, migraine	
		• Renal (kidney) disease	
		• Cardiac (heart) disease	
		• Hypertension (high blood pressure)	
		• Chest (lung disease), including asthma	
		• Diabetes	
		• Other illness	
		Do you carry any emergency medicine with you?	
		Have you had symptoms in the region we are scanning ?	
		Is there any possibility you could be pregnant? Do you have an IUD? Are you breast feeding?	
		Do you suffer from claustrophobia (fear of tight spaces)?	

MARIARC volunteer screening form – Feb 2014 2

If you have any queries or concerns, please ask.

If you are willing to continue please tick the boxes and sign below:

I confirm that the information given by me on this form is complete and accurate, to the best of my knowledge	☐
If an unexpected significant abnormality is discovered I consent to my GP being contacted	☐
I understand that I must not take any metal objects into the scan room with me.	☐

Signature..... **Date**.....

To be completed by an authorised member of MARIARC personnel

Proceed with MR? YES / NO

Name..... Signature: .....

Position at MARIARC..... Date:.....

When more than one study is scheduled, the table below **MUST** be filled in for all MR scan dates subsequent to the first. If there have been no changes to any conditions above since the previous screening including operations, metal in the eye or likelihood of pregnancy then tick **NO CHANGE** and sign as indicated.

NO CHANGE	Signature of volunteer	Signature of screener	Scanning ID
	To confirm that the answers to the questions asked on this form are unchanged		