

Investigation of cortical excitability in epilepsy
using
Transcranial Magnetic Brain Stimulation

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This thesis is being submitted for the degree of Ph.D.

I Mary-Anne Sarah Yorkstone Wright, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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My thanks go to the patients who kindly agreed to participate in these studies. These patients all suffer from refractory epilepsy and are a reminder, if one is needed, of the continued importance of research into neurological disorders.

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PRESENTATIONS, PUBLICATIONS AND AWARDS

- Paper publication: “Cortical excitability predicts seizures in acutely drug-reduced temporal lobe epilepsy patients” Wright M.A., Orth, M. Patsalos, P., Smith, S.J. & Richardson, M.R. *Neurology* 2006, 67(9): 1646-51.
- Poster presentation at Electrophysiological Technologists Association, Institute of Child Health, London, 2003: “Transcranial Magnetic Stimulation during Drug Reduction in Localisation Related Epilepsy: Preliminary Results”.
- Poster presentation at the 25th International Epilepsy Congress, Lisbon 2003: “Transcranial Magnetic Stimulation during Drug Reduction in Localisation Related Epilepsy: Preliminary Results”.
- Poster presentation at the Queen Square Symposium, London 2004. “Transcranial Magnetic Stimulation during Drug Reduction in Localisation Related Epilepsy.
- Oral presentation for the Department of Clinical and Experimental Epilepsy, Institute of Neurology, London 2004: “Cortical Excitability in Human Temporal Lobe Epilepsy”.
- Poster presentation at the International League Against Edinburgh Conference, Edinburgh 2006. “Cortical excitability & seizure prediction in temporal lobe epilepsy”.
- Poster presentation at the 28th Annual Epilepsy Conference, Lisbon 2003: “Motor threshold in mesial temporal lobe epilepsy: effect of seizures”. For this poster, I won the young investigators award in the category of ‘interdisciplinary, complementary interaction’, presented by Dr. Simona Codrea representing the YNT Epilepsy Groups Steering Committee, sponsored by UCB Pharma.

ABSTRACT

This thesis describes a study of 70 patients with epilepsy and a normal control group. Subjects were studied with Transcranial Magnetic Brain Stimulation using a variety of parameters including measurement of motor evoked potentials, active and resting motor threshold, paired pulse inhibition and facilitation and cortical silent period. Data from normal subjects, 40 patients with temporal lobe epilepsy (TLE) and 17 patients with neocortical epilepsy was collected. The following data were acquired from some or all of each group: resting motor threshold (RMT), active motor threshold (AMT), cortical silent period (CSP), and conditioning-test stimulation data from which were derived measures of intracortical inhibition (SICI) and facilitation (SICF).

Data were also obtained regarding drug treatment and seizure timing. Groups of subjects were compared. TLE patients were studied serially to examine the effects of seizures on TMS parameters. The main findings were that the groups differed regarding RMT and AMT, probably reflecting drug treatment; the patients groups differed in SICF; serial studies of the TLE patients showed changes in SICI and SICF which preceded seizures. RMT and AMT were elevated following seizures. Comparing the TLE and neocortical patient groups, there was higher SICF in the mTLE group. The difference between the patient groups demonstrates that epilepsies arising from distinct areas of the brain effect cortical excitability measured from the motor cortex in different ways.

Repetitive TMS (rTMS) was undertaken in 11 subjects with refractory localisation related epilepsy. No effect of rTMS on the number of EEG spikes was seen as a result of a single session of rTMS, suggesting a variety of possibilities; rTMS may have no beneficial effect on focal seizures; the stimulus parameters may have been unsuitable; the chosen outcome measure – number of spikes – may be too inherently variable to show an effect after a relatively brief period of rTMS.

TABLE OF CONTENTS

Acknowledgements.....	3
Presentations, Publications and Awards	4
Abstract	5
List of Abbreviations	9
LIST OF FIGURES	13
LIST OF TABLES	15
CHAPTER 1	16
1. Introduction to Epilepsy	16
1.1 Seizure Types	16
1.1.1. Refractory Focal Epilepsy.....	17
1.2 The EEG in Epilepsy.....	18
1.2.1. Spike Frequency	19
1.2.2 The EEG in mesial temporal lobe epilepsy (mTLE)	20
1.2.3. Long Term EEG Monitoring (LTM) in Epilepsy	20
1.3 Basic Neurobiology of Epilepsy	22
1.3.1. Basic Physiology	22
1.3.2. Ion Channels	24
1.4 Cortical Excitability and Temporal Lobe Epilepsy	25
1.4.1. Cerebral connections and Epileptiform Activity	27
1.5 Remote effects of Mesial Temporal Lobe Epilepsy	29
1.6 Medical Treatment of Epilepsy	31
1.6.1 Antiepileptic Drugs.....	32
1.7 Alternative Treatment of Epilepsy	34
1.7.1 Surgical Treatment of Epilepsy	34
1.7.2. Alternatives to AEDs and epilepsy surgery.....	35
CHAPTER 2.	37
Introduction to Transcranial Magnetic Stimulation	37

2.1 History and Basic Techniques.....	37
2.1.1 Principles of TMS.....	38
2.1.2. Sites of Stimulation	38
2.2 Measurements of Corticospinal Output	42
2.2.1 Motor Evoked Potential.....	42
2.2.2 Motor Threshold	44
2.2.3 Conditioning Stimuli: Paired Pulse Stimulation.....	45
2.2.4 Cortical Silent Period.....	47
2.3 Pharmacological Effects on TMS	47
2.3 Variability of TMS Measures.....	51
2.5 TMS in Focal Epilepsy.....	53
2.5.1 Motor Threshold	54
2.5.2. Paired Pulse TMS	57
2.3.5. Cortical Silent Period.....	61
2.3.6. Summary.....	64
2.6. TMS and Idiopathic Generalised Epilepsy.....	65
2.6.1. Motor Threshold	65
2.6.2. MEP Amplitude	68
2.6.3. Paired Pulse Investigations	69
2.6.4. Cortical Silent Period.....	70
2.6.5. Summary.....	72
2.7: Hypotheses tested in this thesis	73
CHAPTER 3. Motor Threshold Measurements in Patients with Mesial Temporal Lobe Epilepsy.....	74
3.1 Aim of study.....	74
3.2 Results	79
3.3 summary	82
3.3. Discussion	85
CHAPTER 4: Paired Pulse Inhibition.....	90
4.1 Methods.....	90

4.2 Results	95
4.3 Discussion	109
CHAPTER 5: Cortical Silent Period.....	115
5.1 Methods	115
5.2 Results	120
5.3 DISCUSSION	129
CHAPTER 6: Comparing cortical excitability in patients with mTLE versus other partial epilepsies.....	132
6.1 Methods	132
6.2 Results	133
6.3 Discussion	138
CHAPTER 7. Repetitive TMS in Patients with Refractory Partial Epilepsy	141
7.2 Methods	143
7.3 Results	145
7.4 Discussion	145
CHAPTER 8. Discussion.....	150
Summary of significant results.....	151
9. References.....	158

LIST OF ABBREVIATIONS

ADFLE	Autosomal Dominant Frontal Lobe Epilepsy
AED	Antiepileptic Drug
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
AMT	Active Motor Threshold
AP	Action Potential
BZP	Benzodiazepine
Ca ⁺⁺	Calcium ion
CBF	Cerebral Blood Flow
CBZ	Clobazam
Cl ⁻	Chloride ion
CPS	Complex Partial Seizure
CSP	Cortical Silent Period
cTLE	Cryptogenic Temporal Lobe Epilepsy
DTI	Diffusion Tensor Imaging
DZP	Diazepam
EEG	Electroencephalogram
EMG	Electromyogram
EPSP	Excitatory Postsynaptic Potential
ESM	Ethosuximide
HS	Hippocampal Sclerosis

FBM	Felbamate
FDG-PET	Fludeoxyglucose Position Emission Topography
FGPE	Feline Generalised Penicillin Epilepsy
FLAIR	Fluid Attenuated Inversion Recovery
FLE	Frontal Lobe Epilepsy
fMRI	Functional MRI
GABA	Gamma-Aminobutyric Acid
GAERS	Generalised Absence Epilepsy Rats from Strasbourg
GTCS	Generalised Tonic Clonic Seizure
HS	Hippocampal Sclerosis
IDMT	Interhemispheric difference in motor cortical threshold
IED	Interictal Epileptiform Activity
IGE _{VC}	Idiopathic Generalised Epilepsy with versive or cycling movements
IPSP	Inhibitory Post Synaptic Potential
JAE	Juvenile Absence Epilepsy
JME	Juvenile Myoclonic Epilepsy
K ⁺	Potassium ion
LICI	Long Intracortical Inhibition
LTD	Long term depression
LMT	Lamotrigine
LRE	Localisation Related Epilepsy
LTM	Long Term Monitoring
LVT	Levetiracetam

LZP	Lorazepam
M1	Primary Motor Cortex
MCD	Malformation of Cortical Development
MEG	Magnetoencephalography
MEP	Motor Evoked Potential
MT	Motor Threshold
mTLE	Mesial Temporal Lobe Epilepsy
MRI	Magnetic Resonance Imaging
Na ⁺	Sodium ion
NEA	Non Epileptic Attack
NMDA	N-methyl-D-aspartic acid
OXC	Oxcarbazepine
PB	Phenobarbitone
PET	Position Emission Tomography
PHT	Phenytoin
PSP	Postsynaptic Potential
ppTMS	Paired Pulse Transcranial Magnetic Stimulation
PWE	People with epilepsy
RMP	Resting Membrane Potential
RMT	Resting Motor Threshold
rTMS	Repetitive Transcranial Magnetic Stimulation
SICI	Short Intracortical Inhibition
SICF	Short Intracortical Facilitation

SPECT	Single Photon Emission Computed Tomography
SPS	Simple Partial Seizure
S/R	Stimulus Response
SUDEP	Sudden Unexpected Death in Epilepsy
TLE	Temporal Lobe Epilepsy
TMS	Transcranial Magnetic Stimulation
VBM	Voxel Based Morphology
VNS	Vagal Nerve Stimulation
VPA	Valproic acid/ sodium valproate
WAG/Rij	Wistar Albino Glaxo from Rijswijk
ZNS	Zonisamide

LIST OF FIGURES

Figure 1: schematic illustration of a typical neuron.....	22
Figure 2: paroxysmal depolarising shift	24
Figure 3: schematic illustration of mesial temporal sclerosis	26
Figure 4: demonstrating the principles of transcranial magnetic stimulation.....	40
Figure 5: magnetic flux of circular versus butterfly coil	41
Figure 6: motor evoked potential.....	43
Figure 7: measurement of EMG activity during tms at patients bedside.....	76
Figure 8: study design day one	77
Figure 9: study design day three:	77
Figure 10: motor thresholds, day one	83
Figure 11: motor thresholds day three	84
Figure 12: study design for seizure timing data.....	93
Figure 13: change in cortical excitability between day 1 and day 3 in the ipsilateral hemisphere according to occurrence of seizures following day 3.	99
Figure 14: change in cortical excitability in contralateral hemisphere according to timing of seizures.....	100
Figure 15: change in cortical excitability in ipsilateral hemisphere according to timing of seizures.....	101
Figure 16: SICI and SICF in patients with no seizure within 48 hours after day 3.	102
Figure 17: SICI and SICF in patients with seizures within 48 hours after day three	103
Figure 18: difference between MEP sizes correlates with time until next seizure.	104
Figure 19: Intracortical inhibition and intracortical facilitation on day 3 in contralateral hemisphere, divided according to occurrence of the subsequent seizure.	105
Figure 20: intracortical inhibition and facilitation in ipsilateral hemisphere.....	106
Figure 21: SICI and SICF on day 1 and day 3, divided according to hemisphere of seizure onset in patients who did not have seizures.	107
Figure 22: SICI and SICF on day 1 and day 3, divided according to hemisphere of seizure onset in pateints who did have seizures.	108

Figure 23: Automated measurement of CSP duration (seconds) for all 29 subjects, divided according to SIDE and PREVIOUS SEIZURE; data are shown for day 1 and day 3.	123
Figure 24: Automated measurement of CSP duration plotted against “by eye” measurement (both measured in seconds). A linear regression line is plotted.	124
Figure 25: Shows automated measurement of CSP/MEP ratio plotted against “by eye” measurement (both measured in s/mV). A linear regression line is plotted. The measures are highly correlated (Pearson $R=0.834$, $P<0.001$).	125
Figure 26: Automated measurement of CSP/MEP ratio plotted against “by eye” measurement, using the logarithm of these measurements (both measured in s/mV). A linear regression line is plotted.	126
Figure 27: Figure 26: comparing the log of CSP/MEP ratio in both hemispheres. There were no significant effects of NEXT SEIZURE (Hypothesis 4).	128
Figure 28: MT in normal controls and entire patient group.	135
Figure 29: SICI and SICF in entire patient group.	135
Figure 30: MT in the two patient groups and normal subjects.	137
Figure 31: SICI and SICF in the two patient groups and normal subjects.	137
Figure 32: Standard 10-20 electrode placement system with adaption of right and left superficial sphenoidal electrodes in place of A1 and A2 (Jasper 1958).	147

LIST OF TABLES

Table 1.	Summary of the effects of certain antiepileptic drugs on different parameters of transcranial magnetic stimulation.	p. 50
Table 2.	Demographic information on the study of motor thresholds including information in doses changes of AEDs.	p. 87-89
Table 3.	Demographic, clinical and experimental details for all patients undergoing ppTMS.	p. 113-114
Table 4.	Mean, standard deviation (SD) and coefficient of variation (CV) of the various CSP measurements.	p. 127
Table 5.	Patient demographic data for CSP study, including seizure types, imaging data and drug doses on 2 days of measurement.	p.130-131
Table 6.	Patient demographic data for neocortical patients.	p. 139-140
Table 7.	Patient demographic data for rTMS study.	p. 148-149

CHAPTER 1

1. INTRODUCTION TO EPILEPSY

Epilepsy is the most common serious neurological disorder, and has recently been reported to have a prevalence of 5-8 per 1000 in developed countries, 15.4 per 1000 in rural developing countries and 10-3 per 1000 in urban developing counties (Ngugi 2009). An individual is considered to have epilepsy if they have had more than one unprovoked seizure. Epilepsy can be classified into several separate seizure types and syndromes (Commission of ILAE 1981; Commission of ILAE 1989; Engel, Jr. 2001; Engel, Jr. 2006). While the classification of epilepsy and seizure types is very complex, one of the ways that epilepsy can be broadly defined is whether seizures are focal/ partial, or idiopathic generalised in origin. Focal epilepsy accounts for approximately 60% of total numbers of people with epilepsy (PWE) (Hauser et al. 1993; Sidenvall et al. 1993). This section will concentrate mainly on focal epilepsy, including evaluation techniques and treatment options.

1.1 SEIZURE TYPES

An epileptogenic zone within the brain is responsible for the initiation of the abnormal neuronal activity that results in the generation of a focal seizure (Rosenow and Luders 2001); an imbalance between cortical inhibitory and excitatory processes occurs resulting in an epileptiform discharge, and the degree of spread of abnormal neuronal activity determines the severity and duration of a seizure. The symptomatogenic zone is the cortical area activated by the epileptiform discharge (Rosenow and Luders 2001), and this determines the seizure semiology: ictal symptoms vary in localising value, with some symptoms providing stronger evidence than others. The cause of focal epilepsy is often an epileptogenic lesion visible on neuroimaging, e.g. hippocampal sclerosis or an area of focal cortical dysplasia. Some forms of focal epilepsy such as autosomal dominant frontal lobe epilepsy (ADFLE) have been shown to have a genetic basis (Steinlein et al. 1995), while others occur as a result of insult, infection or neoplasm. Focal epilepsy can also occur with no known focal lesion and normal neuroimaging; this is termed cryptogenic epilepsy (Commission of ILAE 1981).

Focal epilepsy may arise from the cortex of any lobe of the brain, or from deeper structures such as the limbic system, but most commonly arise from either the frontal or temporal lobes. Seizures arising from the temporal lobe may begin with an aura or ‘warning’, including déjà vu, jamais vu, fear, unusual tastes or smells, and rising epigastric sensations. Frontal lobe seizures, often arising from sleep, can present with hypermotor activity or adverse motor activity, and are often brief with quick recovery. Seizures arising from parietal and occipital lobes are less common: the former may present with paraesthesiae or other sensory symptoms, while seizures arising from the occipital lobe typically present with visual symptoms.

In the 1981 ILAE classification of seizures, partial or focal seizures are classified as a spectrum of simple partial seizures (SPS), complex partial seizures (CPS) and seizures with secondary generalisation (GTCS) (Commission of ILAE 1981). SPSs involve an aura with a variety of possible symptoms but with no loss of awareness, while CPSs involve loss of awareness and symptoms reflecting the area(s) of the brain that are involved. CPS may arise with or without a preceding SPS, and may evolve into a secondary GTCS if the seizure activity spreads to both hemispheres and becomes generalised. Patients with focal epilepsy can also have a GTCS with no clear preceding SPS or CPS.

1.1.1. REFRACTORY FOCAL EPILEPSY

For many people with epilepsy (PWE), medication will control seizures and allow the individual to lead a full life. Approximately one third of PWE, however, is refractory to medication and continues to experience seizures. Epilepsy may be considered refractory if seizures have not been well controlled by at least 2 AEDs at therapeutic or maximally tolerated doses; seizures occur frequently and without long periods of remission; seizures have been refractory for at least one year (Brodie 2005). Before a patient can be considered to have refractory epilepsy, compliance with medication must be established and there must be no doubt as to the nature of the seizures, ruling out the possibility of psychogenic non-epileptic attacks (NEA), either as co-existing disorder or as the sole cause of seizure-type episodes. NEA account for approximately 20% of patients presenting to specialist epilepsy centres with apparently intractable epilepsy (Krumholz 1999).

Pharmacoresistant epilepsy is more common in patients with focal epilepsy compared to idiopathic epilepsy, and is associated with an increase in physical and psychological morbidity as well as an increased risk of mortality (Brodie 2005). For these patients with refractory focal epilepsy, alternative treatment options should be explored as the implications of poorly controlled epilepsy are wide-ranging and include reduced quality of life (Vickrey et al. 2000) and an increased risk of sudden unexplained death in epilepsy (SUDEP) (Tomson et al. 2005). Refractory epilepsy is also associated with side effects of antiepileptic drugs (AEDs) e.g. cognitive impairment, possible toxicity and teratogenicity, social stigma, difficulty in obtaining or maintaining employment; concerns regarding pregnancy and family life, increased risk of injuries, loss of driving license and overall independence, (Brodie 2005). Refractory epilepsy also represents a significant socio-economic burden (Heaney and Begley 2002).

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1.2 THE EEG IN EPILEPSY

The electroencephalogram (EEG) of a patient EEG of a PWE is often unremarkable for the majority of time unless there is an underlying neurological condition such as encephalitis. A routine 20 minute EEG recording in an otherwise healthy patient may be entirely normal; indeed, prolonged EEG monitoring over several days may not reveal any definite signs of epilepsy. Most PWE, however, have a degree of epileptiform activity occurring intermittently between seizures. These abnormalities are termed interictal epileptiform abnormalities (IEDs) and incorporate spikes and sharp waves that represent the irritative zone (Rosenow and Luders 2001). They result from the summation of excitatory and inhibitory postsynaptic potentials in apical dendrites of pyramidal neurons, and synchronous activation of a few squares centimetres of cortex is required in order to record a spike or sharp wave from scalp electrodes (Smith 2005). IEDs have distinctive morphology, and are classified according to waveform duration: spikes transients have a duration of 20-70 msec and sharp waves a duration of 70-200 msec.

The EEG has higher specificity than sensitivity. The first EEG in a PWE has approximately 50% yield of IED, and repeated recordings including periods of drowsiness and sleep

increases this to 80% (Binnie 1996). Provocative procedures such as hyperventilation and sleep deprivation (and photic stimulation in certain idiopathic epilepsies) may increase the likelihood of recording IED, but caution must be used as these procedures can also provoke seizures and this is undesirable in patients with well controlled epilepsy. The EEG has been shown to be more likely to pick up epileptiform activity if performed within 24 hours of the first seizure compared to EEGs performed later (King et al. 1998).

Rarely, IEDs can be seen in the EEG of people with no history of epilepsy. While only 0.5% of healthy adults without epilepsy have IEDs in their EEG (Gregory et al. 2003), this increases to 10-30% in patients with cerebral pathology such as previous head injury or neuro-surgery (Zivin and Marsan 1968). IEDs can also be seen in patients taking antipsychotic medicine such as clozapine, olanzepine and amisulpride who have no history of seizures (Pogarell et al. 2004; Treves and Neufeld 1996). Hence, EEG must be interpreted in the context of the clinical history and overall presentation. Other waveform abnormalities can be seen in the EEG, such as paroxysmal theta activity and slow activity; these are considered to be non-specific but can be of significance in the evaluation of established epilepsy.

1.2.1. SPIKE FREQUENCY

There is wide intraindividual and interindividual variation in the frequency of spikes. Some PWE may have an entirely normal EEG between seizures while others may have very frequent, almost continuous spikes. IEDs are often enhanced by drowsiness and non-REM sleep, and this is why it is advantageous to perform EEG recordings during sleep. There are many factors that can influence IED frequency: spikes can increase in frequency postictally, for example (Gotman and Marciani 1985). The time since the most recent seizure can also affect the EEG: one study of patients with different forms of epilepsy showed that spikes are more likely to be seen in patients who have had a seizure within the past 2 days compared to those who have not had a seizure in the same time frame; the authors also found that spikes were more frequent in patients with a higher seizure frequency, and more frequent spikes were seen in patients with focal epilepsy compared to generalised epilepsy (Sundaram et al. 1990).

1.2.2 THE EEG IN MESIAL TEMPORAL LOBE EPILEPSY (MTLE)

The interictal EEG is of significant value in classifying epilepsy syndromes and the location of spikes and sharp waves is important in the study of focal epilepsy. The interictal EEG is not only of use in localising the irritative zone but can also provide predictive information regarding surgical outcome. Several studies have looked at the interictal EEG in patients with TLE in relation to outcome of resective surgery. The presence of extratemporal EEG abnormalities in patients undergoing anterior temporal lobectomies has been shown to be a predictor of poor outcome (de Curtis and Avanzini 2001). In a retrospective study of patients with normal neuroimaging who underwent both scalp and invasive EEG recordings, bilateral and precentral spikes and/or slow waves were found to be significantly more common in patients with seizures involving the temporal lobe and neighbouring areas (i.e. multilobar epilepsy) than in patients with purely temporal lobe seizures (Barba 2007). Relatively infrequent interictal spike frequency in mTLE has also been shown to be a predictor for positive outcome following resective surgery compared to patients with frequent spikes (Krendl et al. 2008). The same study found that there was no significant difference in outcome between patients who had bilateral compared to unilateral IEDs. In contrast a study using implanted subdural strip electrodes showed that patients with unilateral interictal epileptiform activity had a significantly higher success rate compared to those with bilateral epileptiform activity, with 89% becoming seizure free versus 40-56% (Hufnagel et al. 1994), and that spike frequency had no effect on outcome following anterior temporal lobectomy.

1.2.3. LONG TERM EEG MONITORING (LTM) IN EPILEPSY

Once a patient is established as having refractory focal epilepsy, surgery may be considered as a treatment option. Almost all patients being assessed for epilepsy surgery will undergo LTM (Engel, Jr. 1996). Reduction of AED dose is widely used during LTM to increase the likelihood of recording seizure(s) (Scott et al. 2000). AED reduction may be omitted for safety purposes, if the patient has experienced previous episodes of status epilepticus or when there is a history of frequent secondary generalised seizures. AED reduction should be carried out according to a set protocol with the safety of the patient being the foremost concern.

Other provocative procedures such as sleep deprivation (Rajna and Veres 1993), exercise and alcohol consumption may also be carried out.

The primary aim of LTM is to record the patients habitual seizure type(s). Simple partial seizures often occur with no clear scalp EEG changes and no outward clinical signs (Devinsky et al. 1988). In the setting of LTM, patients can be reluctant to report simple partial seizures and may choose not to press an alarm or event button for fear of wasting staff time; they may also be unsure if the symptoms they are experiencing are indeed an aura or a reflection of the anxiety they feel towards having a more pronounced seizure; these factors can make the objective documentation of simple partial seizures complicated and possibly inaccurate. Complex partial seizures, on the other hand, are usually accompanied by clear EEG and clinical changes, and they often provide more information than secondary generalised seizures since during the latter the EEG is largely obscured by myogenic and movement artefact. Both the electrographic data and detailed seizure semiology are carefully assessed to help lateralise and localise the epileptogenic zone.

The data collected during LTM are used in combination with other investigations including neuroimaging and neuropsychometry to build a picture of an individual's suitability for surgery. If the data identify an area of the brain that can be surgically removed without causing significant deficit, the patient may undergo a resection after being informed of the chance of becoming seizure free as well as possible neurological deficits (Engel, Jr. 1996). The most frequent type of refractory epilepsy in developed countries is that arising from the mesial temporal lobe, and hippocampal sclerosis is the most common underlying pathology (Margerison and Corsellis 1966). Other possible aetiologies of surgically treatable epilepsy include cortical dysplasia, neoplasms such as low grade gliomas or dysembryoplastic neuroepithelial tumours, vascular malformations and areas of old injury or stroke (Frater et al. 2000).

1.3 BASIC NEUROBIOLOGY OF EPILEPSY

Epileptic seizures occur due to an imbalance between cortical excitation and inhibition, which can result in hypersynchronous and excessive neuronal firing. The mechanisms of epileptogenesis differ between focal and idiopathic generalised epilepsies, and between different subgroups of epilepsy. The mechanisms are not fully understood and research continues in both animal models and humans. This section introduces the basic physiology of neuronal activity followed by a summary of the pathophysiological mechanisms of focal and idiopathic epilepsies.

1.3.1. BASIC PHYSIOLOGY

A neuron consists of a cell body or soma with many dendrites and one axon that extend away from the soma (Hof et al. 2008). Neurons connect to surrounding neurons at synapses; most synapses connect axons with dendrites, but there are also axon to soma synapses, axon to axon synapses and dendrite to dendrite synapses. The cell can be either depolarised or hyperpolarised depending on whether there is an overall reduction or an increase in membrane potential respectively. Excitatory inputs typically occur at synapses on the dendrites, while inhibitory synapses are located on both the cell body and on dendrite (McCormick 2008).

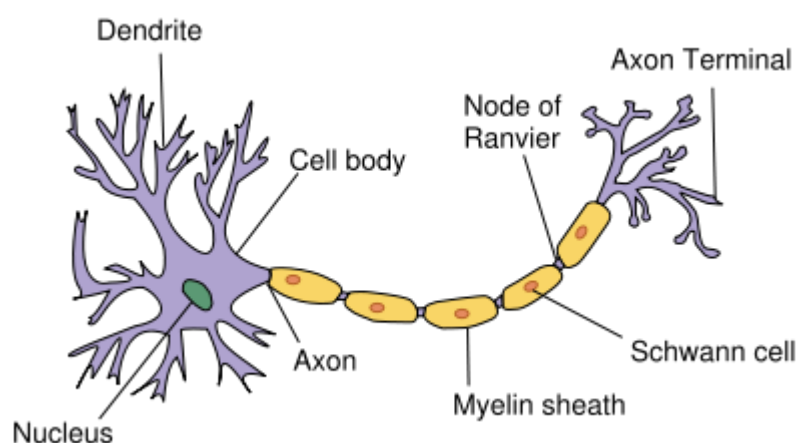


FIGURE 1: SCHEMATIC ILLUSTRATION OF A TYPICAL NEURON.

Source: <http://training.seer.cancer.gov/anatomy/nervous/tissue.html>

At an excitatory synapse, a neurotransmitter binds to a specific receptor and there is an influx of sodium ions (Na^+) and efflux of potassium ions (K^+), leading to depolarisation and an action potential (AP) (Rutecki 1992), and subsequently to an excitatory postsynaptic potential (EPSP). At an inhibitory synapse, release of a neurotransmitter results in influx of negatively charged chloride ions (Cl^-), and a large hyperpolarisation of the membrane resulting in an inhibitory post synaptic potential (IPSP). IPSP and EPSPs from the cell body and dendrites summate to produce an overall postsynaptic potential (PSP) (Heinemann et al. 2008). Background EEG activity is formed by such summated EPSPs and IPSPs in large cortical pyramidal neurons (Freeman 2004a; Freeman 2004b; Freeman 2005; Freeman 2006).

If there is overall depolarisation at the initial segment of the axon reaching a certain threshold, an AP occurs and propagates down the axon. Once an AP reaches the synaptic terminal, there is depolarisation of the membrane away from the resting membrane potential (RMP) and presynaptic channels open: Ca^{2+} enters and cause the release of neurotransmitters from vesicles into the synaptic cleft (Reuter 1983). If neurotransmitter receptors located at the postsynaptic membrane are activated by the neurotransmitter, a PSP occurs in the postsynaptic neuron due to inward current of Na^+ and a resulting AP. Epileptic discharges are thought to occur when, rather than a single action potential occurring, there is overt depolarisation or a 'paroxysmal depolarising shift' (PDS) with a burst of APs superimposed upon prolonged depolarisation; these may be represented as spikes or sharp waves on a scalp EEG recording (Ayala et al. 1973).

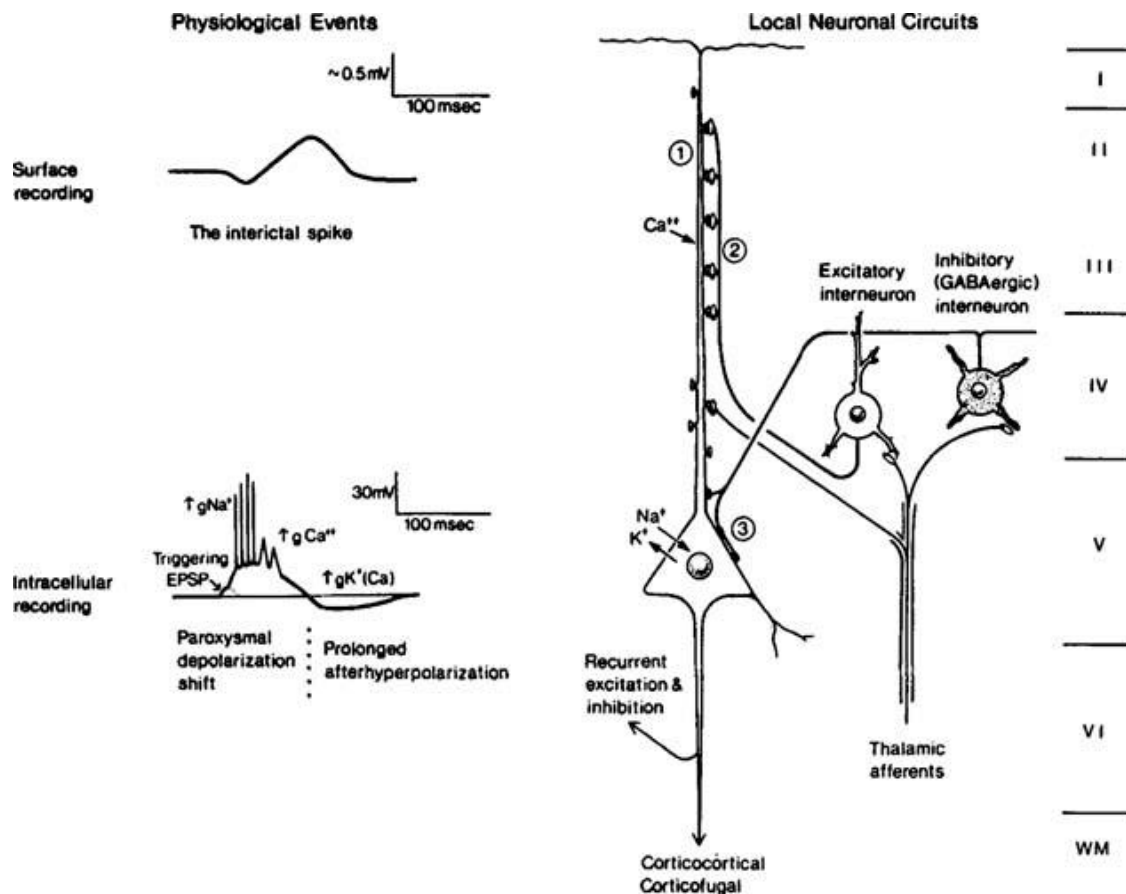


FIGURE 2: PAROXYSMAL DEPOLARISING SHIFT

This figure demonstrates a paroxysmal depolarising shift (below left) and the effect on the EEG recording (above left). Local neuronal circuits are depicted on the right, showing excitatory and inhibitory interneurons at different cortical layers. This diagram is reproduced with permission from the Handbook of Physiology (Collins 2011).

1.3.2. ION CHANNELS

Ion channels are present at pre and post synaptic membranes, and they are selective for the ions that they allow to cross. These are either voltage gated or non-gated, and the gated ion channels either respond to a neurotransmitter (ligand gated) or respond to changes in membrane potential (Kullmann and Schorge 2008). Non-gated ion channels are always in the open state and are important in maintaining the RMP. There are several types of voltage gated ion channels. Transmembrane voltage gated potassium channels influence the RMP and are crucial in determining neuronal excitability and in returning a cell that has been depolarised by an action potential to resting state; the rate at which the potassium channel is closed can determine whether a neuron is capable of rapid and repetitive firing. Voltage gated

sodium channels are divided into inactivating and non-inactivating; the former open quickly during an action potential and depolarisation and then close as rapidly, while the latter open with less depolarisation and stay open for a longer time, making the neuron more susceptible to depolarisation. Voltage gated calcium channels are located at both pre and post synaptic terminals, and are subdivided into L-, P/Q-, N- and T-type. N- and P/Q-type calcium channels are located at synaptic terminals and mediate the entry of calcium into the presynaptic membrane, allowing release of neurotransmitters. L-type channels are mostly located postsynaptically and allow influx of calcium. They are slowly inactivated, allowing further influx of Ca^{2+} and further depolarisation. Voltage gated chloride channels are present in neurons inhibited by GABA (Hille and Catterall 1999).

Ligand gated channels are numerous, and also vary in function. Acetylcholine channels allow passage of sodium, potassium and calcium ions, which depolarises the cell membrane and can trigger voltage gated channels to open. GABA_A receptors are located on postsynaptic neurons and they bind GABA, barbiturates, ethanol and benzodiazepines and inhibit neuronal activity. GABA_B receptors are found both pre- and post- synaptically, and they underlie slow inhibitory post synaptic potentials (MacDonald and Mody 2008). Glutamate receptors are divided into NMDA, AMPA and kainate receptors. NMDA receptors are found mainly postsynaptically and gate sodium and calcium ions. AMPA receptors are found postsynaptically and also gate sodium and calcium ions. Kainate receptors are expressed both pre and postsynaptically and gate sodium ions (Wilcox et al. 2008).

1.4 CORTICAL EXCITABILITY AND TEMPORAL LOBE EPILEPSY

As mentioned, the most common form of focal epilepsy is that arising from the temporal lobe. Mesial temporal lobe epilepsy is also the most studied type of focal epilepsy, and the most common pathological finding in TLE is hippocampal sclerosis, visible on MRI with hippocampal atrophy on T1 and increased mesial temporal signal on T2 weighted MRI (Woermann et al. 1998). The introduction of 3-Tesla MRI has revealed focal abnormalities in 20% of patients with refractory focal epilepsy, including hippocampal sclerosis, who previously had normal or equivocal imaging (Hogan et al. 2008; Strandberg et al. 2008). Hippocampal sclerosis can arise as a result of complicated febrile convulsions, status

epilepticus, head injury or encephalitis (Mathern et al. 1995), and as well as being the underlying cause of epilepsy HS can also arise after sustained epileptiform activity at a separate, primary epileptogenic zone (Simons et al. 2004). Hippocampal sclerosis is also seen in patients with other neurological disorders such as multiple sclerosis (Sicotte 2008), and may be found in otherwise normal subjects.

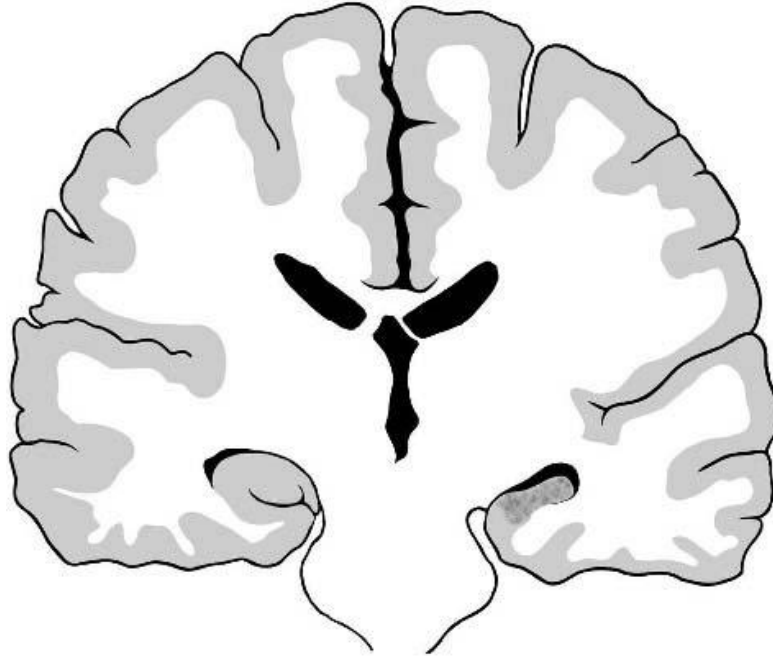


FIGURE 3: SCHEMATIC ILLUSTRATION OF MESIAL TEMPORAL SCLEROSIS

Note asymmetry of hippocampi, temporal lobes/cortex, and fornices (reproduced with permission from Hadjikoutis and Smith 2005).

The mesial temporal lobe includes the limbic system, which lies on either side of the thalamus at the top of the brainstem. The limbic system consists of the hippocampus, amygdala, insula, anterior thalamic nuclei and olfactory cortex. It is functionally involved in emotion, behaviour/ motivations, long term memory and olfaction. The hippocampal formation consists of the hippocampus proper, the dentate gyrus and the subiculum. There is a trisynaptic excitatory pathway from the entorhinal cortex to the dentate granule cells; this projects to CA3 pyramidal neurons via mossy fibres, and then to the CA1 regions via Schaffer collaterals. There are also local circuits in each region with interneurons that are either excitatory or inhibitory (McIntyre and Schwartzkroin 2008).

The emotions linked to the hippocampus include fear and anger and feelings related to survival. Memory encoding is linked to the amygdala and the hippocampus (Squire and Zola-Morgan 1991), and damage to these areas can result in significant cognitive deficit. Memory function is lateralised, with verbal memory being processed in the dominant (usually left) hemisphere while visual or non-verbal memory is processed in the non-dominant (usually right) hemisphere (Smith and Milner 1981). Memory function is often affected in patients with chronic TLE associated with hippocampal sclerosis, and psychometry to assess verbal and non-verbal memory is an essential part of the presurgical work up (Engel, Jr. 1996), assisting not only in providing concordant data regarding the epileptogenic zone based on cognitive deficits, but also providing some prediction of the likely extent of postoperative cognitive decline by assessing existing capabilities (Ivnik et al. 1987; Spiers et al. 2001).

Experimental models of mTLE provide insight into the neurobiological and neurophysiological changes that can result from seizures. Many animal models have used kainic acid or kindling to induce epileptogenesis, and the kindling model closely resembles human TLE regarding seizure semiology. Stimulation of the perforant pathway – an afferent pathway to the hippocampus – also leads to hippocampal spikes, epileptiform discharges and non-convulsive seizures in rodent models (Sloviter 1983), and causes changes in the structure of the hippocampus similar to those seen in autopsy tissue of the brain in humans with epilepsy (Dam 1980; Margerison and Corsellis 1966). Changes in the hippocampus in animal models include alterations in glutamate receptor function, changes in the GABAergic circuitry, synaptic reorganisation and formation of aberrant neuronal networks. The sclerotic process includes reactive gliosis, granule cell dispersion and segmental loss of neurons in the hilar polymorphic region and CA1 and CA3 pyramidal region of the hippocampus, with relative sparing of the CA2 pyramidal region and dentate granule cells.

1.4.1. CEREBRAL CONNECTIONS AND EPILEPTIFORM ACTIVITY

Neurons are linked together as part of circuits in the brain, which communicate with other circuits via white matter connections consisting of transverse fibres, projection fibres and association fibres. Transverse commissural fibres connect the cerebral hemispheres. The

anterior commissure connects the two temporal lobe structures and the hippocampal commissure joins the two hippocampi. Projection fibres connect the cortex to lower parts of the brain and to the spinal cord and association fibres form connections between different parts of the cerebral hemisphere. Epileptiform activity can spread to cortical areas either locally and preferentially within layer V of the neocortex (Chagnac-Amitai et al. 1990). More distal spread also occurs, which can determine the appearance of seizures semiologically and electrographically.

In terms of neural networks and epileptogenesis, the thalamocortical and the limbic systems have both been studied extensively. The former explores the mechanisms of idiopathic epilepsy, in particular absence seizures. Early work used the feline generalised penicillin epilepsy (FGPE) model, and found both the thalamus and cortex to be involved during generalised seizures (Avoli and Gloor 1982a). Moreover, the thalamocortical network must be intact for seizures to arise (Avoli and Gloor 1981; Avoli and Gloor 1982a; Avoli and Gloor 1982b). Generalised Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo from Rijswijk (WAG/Rij) are genetic rodent models that have been used to explore pathophysiological changes in the thalamocortical system in idiopathic epilepsy. Studies have shown that during spike wave discharges, certain regions are involved more intensely than others, namely in somatosensory and motor regions in the anterior cortex and corresponding thalamic nuclei (Nersesyan et al. 2004; Vergnes et al. 1990). There also is evidence that a cortical focus may drive the discharge with the thalamus playing a secondary role (Meeren et al. 2002). Blumenfeld (2005) summarises that there is on-going debate as to whether initial voltage changes are seen first in the thalamus or the cortex, and that the many different findings in experimental models suggest that there may not be a single onset region; rather that there is an abnormal oscillating corticothalamic network that can be triggered into producing epileptiform discharges in different ways and in different areas of the network.

The neural networks of seizures that initiate in the limbic system are thought to extend beyond the temporal lobe. Seizures are initiated when there is an abnormal, excessive, hypersynchronous discharge of a population of cortical neurons, occurring as a result of an imbalance between excitation and inhibition (McCormick and Contreras 2001).

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Spencer (2002) describes human epilepsy as a disorder of large neuronal networks, with seizures propagating in an extensive and variable manner to that can involve any region/neural structure that has anatomic connections to the primary seizure network; this variability is reflected in clinical seizures and intracranial EEG recordings. Seizure propagation occurs when neighbouring neurones are involved; the recruitment of a sufficient number of neurons leads to a loss of the surrounding inhibition and propagation of seizure activity into contiguous areas via local cortical connections, and to more distant areas via long commissural pathways such as the corpus callosum.

The connectivity of the limbic system involves numerous afferents and efferents (McIntyre and Schwartzkroin 2008). The hippocampus, entorhinal cortex, amygdala, parahippocampal / piriform cortex and perirhinal cortex are all thought to be significantly involved in temporal lobe complex partial seizures: these regions are closely connected, have low thresholds for seizure induction and tend to show some degree of damage associated with long term seizure activity; they should be viewed as an interactive system rather than isolated structures (McIntyre and Schwartzkroin 2008). The major input to the hippocampal formation is an excitatory input from the entorhinal cortex. There are also numerous subcortical fibre inputs to the hippocampus, including a strong excitatory input from the thalamus to the CA1 region (Bertram and Zhang 1999). Efferents include cortical and subcortical projections. Cortical projections arise in the subiculum and the CA1 region of the hippocampus and project to the entorhinal cortex. The CA1 region and the subiculum send axons to widespread prefrontal cortices. Imaging studies using various techniques have shown that limbic seizures propagate beyond the primary epileptogenic zone to other areas of the temporal lobe and to extratemporal areas, both ictally and interictally, as described below.

1.5 REMOTE EFFECTS OF MESIAL TEMPORAL LOBE EPILEPSY

Experiments in this thesis are built on the premise that measurements of cortical excitability made in the motor cortex are relevant to epilepsy originating in the mesial temporal lobe. Above I have discussed the initiation of seizures in the mesial temporal lobe, and potential pathways of propagation. Considerable evidence in human mTLE supports the premise that

mTLE has remote effects, which may underpin the alterations of motor cortex excitability detected with TMS.

Ictal SPECT studies show focal increases in blood flow associated with the increase in neuronal metabolic activity that occurs during a seizure (Schwartz and Bonhoeffer 2001). Van Paesschen and colleagues have used SPECT to study patients with TLE under the premise that changes in cerebral perfusion reflect changes in neuronal activity (Van Paesschen et al. 2003). They performed ictal SPECT on 24 patients with unilateral HS and demonstrated a cluster of hyperperfusion in the ipsilateral frontal lobe, at the border of the ipsilateral middle gyrus and the precentral gyrus, as well as 8 clusters of hypoperfusion in frontal lobes. The frontal lobe hypoperfusion was more consistent in the ipsilateral hemisphere. The authors suggest that the frontal lobe changes could reflect ictal surround inhibition, a mechanism designed to limit seizure propagation (Prince and Wilder 1967), or an ictal steal phenomenon whereby increased blood flow to the temporal lobe is at the expense of frontal lobes. Ipsilateral hypoperfusion was noted in two other ictal SPECT studies of patients with unilateral temporal lobe epilepsy (Menzel et al. 1998; Rabinowicz et al. 1997).

A study coordinating ictal SPECT and interictal FDG-PET in patients who have been surgically treated for mesial temporal lobe epilepsy also demonstrated frontal lobe changes (Nelissen et al. 2006). FDG-PET studies measure the level of glucose metabolism in different regions of the brain. Nelissen and colleagues demonstrated interictal ipsilateral frontal lobe hypometabolism and ictal hypoperfusion compared to control subjects. FDG-PET recordings also demonstrated ipsilateral temporal hypometabolism, and thalamic hypometabolism was also seen to a lesser extent. The authors postulate that the findings represent “surround inhibition” (Prince and Wilder 1967).

Voxel based morphometry (VBM) is a quantitative MRI analysis technique that looks at subtle structural changes that may not be visible in an individual, but may be seen when comparing patient groups. VBM looks at the distribution and volume of grey or white matter in the brain (Duncan 2009). A recent study of the temporal lobe network in mesial temporal lobe epilepsy has shown changes in brain matter beyond the temporal lobe (Riederer et al. 2008): patients with left sided HS showed widespread changes including the contralateral parahippocampal and superior temporal gyrus, the cerebellum, frontal regions and the right cingulum; patients with right HS showed decreased grey matter volume in the ipsilateral

hippocampus and right medial thalamus. In patients with cryptogenic TLE (cTLE), regional brain atrophy was seen in patients with right or left cTLE, and this was more pronounced in the patients with left cTLE. A subsequent study also found that left sided mesial TLE is associated with more extratemporal grey matter changes than right sided TLE, and the degree of change was associated with both the number of seizures and the duration of epilepsy (Coan et al. 2009). This study also found changes in frontal regions.

Diffusion tensor imaging (DTI) is an MRI technique that allows imaging of white matter in terms of fibre orientation and integrity. This technique has demonstrated widespread changes in the brains patients with TLE. Patients with unilateral TLE have significant changes in frontal-temporal white matter tracts affecting the epileptic hemisphere (Lin et al. 2008), and widespread changes in areas including the ipsilateral temporal lobe, the inferior frontal gyrus and articulate fasciculus have also been demonstrated (Focke et al. 2008).

In summary, there are well documented changes in cortical excitability in both generalised and focal epilepsy. The changes in focal epilepsy are not restricted to the epileptogenic zone but extend to distal areas both ictally and interictally. In particular, changes in both the thalamus and frontal regions have been noted in epilepsy arising from the hippocampus using a variety of modalities.

1.6 MEDICAL TREATMENT OF EPILEPSY

Before treatment for epilepsy is started, it is vital that the diagnosis is correct. This can often be established by careful and detailed history taking in the clinic, ideally with both the patient and a person who has witnessed a typical attack. The most common differential diagnosis for epilepsy is dissociative/ psychogenic non-epileptic seizures. Other differentials include hypoglycaemia, panic attacks, sleep disorders, hypnic jerks, transient global ischaemia and transient ischaemic attacks (Benbadis 2009). While verifying the diagnosis, tests should be carried out to establish the underlying cause of the condition, not least because this may influence how the epilepsy is treated.

After the diagnosis of epilepsy has been made, and preferably before AEDs are introduced, it should be considered whether there are any triggers that can be avoided. For example, an

individual may present with a seizure following sleep deprivation and excessive alcohol consumption; in such a case, lifestyle changes may be sufficient to raise a lowered seizure threshold and avoid further seizures. If this cannot be achieved and seizures continue, the next step in treatment is to introduce a first line AED at a low dose with gradual incremental increases. The aim is to gain full control of seizures with no troubling side effects, allowing the patient to lead a full life.

Patients should be counselled regarding the use of AEDs. A patient is likely to have to continue taking medication in the medium to long term and sometimes indefinitely; the side effects should be explained as well as the fundamental requirement of adhering to the treatment regimen. Non-compliance can be assessed by measuring serum AED levels; this also serves to guide future dosage changes. Women of child bearing age should be counselled as to possible teratogenic side effects of AEDs and should be carefully monitored before and during pregnancy.

The first choice AED may depend on the seizure type, as some AEDs are better suited to certain syndromes, and some AEDs can exacerbate certain seizure types; for example, idiopathic types of epilepsy may be aggravated by the use of carbamazepine (Genton et al. 2000). No AED is without side effects, and somnolence, sedation, dizziness and cognitive impairment may come to light with higher doses and when more than one AED is used (Mula and Trimble 2009). Efficacy of the AED must therefore be balanced against side effects, and the dose adjusted appropriately. In patients who do not respond to the first AED, a second drug may be added with the original AED gradually tapered down or kept in place. In some cases, a patient may be treated with more than two AEDs, either continuously or as add-on therapy during a cluster, e.g. the use of a benzodiazepine in patients with catamenial seizures.

1.6.1 ANTIEPILEPTIC DRUGS

There are many drugs available for the treatment of seizures. The first anticonvulsant – phenobarbitone – was discovered serendipitously in 1912. This was followed by the next generation of AEDs: ethosuximide, carbamazepine, phenytoin, sodium valproate and benzodiazepines. These drugs are still in common use today. Carbamazepine and sodium valproate have also been modified and made available as ‘controlled release’ versions. These

have been joined by a new generation of drugs over the past three decades that are often better tolerated and have fewer drug interactions. For some patients, the newer drugs are also more efficacious. AEDs differ in their mechanisms of action. There are three main ways in which AEDs work, although many drugs have more than one mode of action. AEDs are anticonvulsant rather than anti-epileptic; they increase the seizure threshold and are prophylactic, but are not a cure or a failsafe solution.

Drugs that modulate voltage gated ion channels can be subdivided according to whether they primarily target the sodium or the high or low voltage calcium channels. The former include phenytoin (PHT), carbamazepine (CBZ) and oxcarbazepine (Huuskonen et al. 1997), lamotrigine (LTG), zonisamide (ZNS), felbamate (FBM) (for review see Czapinski et al. 2005) and more recently rufinamide (Hussar and Bilbow 2009) and lacosamide (LCM) (Perucca et al. 2007). Sodium valproate targets the sodium channel in addition to the low voltage calcium channel, while ethosuximide primarily targets the low voltage calcium channel (Patsalos 2005). Drugs acting at the voltage gated sodium channels bind to the protein in the inactivated state and reduce channel conductance, therefore preventing repetitive neuronal firing (Sills 2009). High voltage gated calcium channels are located on pre-synaptic nerve terminals and they are thought to regulate neurotransmitter release. The low voltage calcium channels are located post-synaptically and predominantly in the thalamocortical relay neurones; this may explain the efficacy of ethosuximide and sodium valproate in absence seizures (Coulter et al. 1989), which are thought to arise from the thalamocortical system.

Drugs that enhance GABA-mediated inhibitory transmission, either at the GABA_A receptors or by altering GABA turnover, include phenobarbitone, benzodiazepines, vigabatrin and tiagabine. GABA is the main inhibitory neurotransmitter in the nervous system. It is released from GABAergic nerve terminals and acts on post-synaptic GABA_A and GABA_B receptors. When GABA_A receptors are bound by GABA, the result is fast neuronal hyperpolarisation and inhibition. GABA_B receptors affect slow neuronal hyperpolarisation post-synaptically, and also limit GABA release presynaptically. Phenobarbitone and benzodiazepines both bind to the GABA_A receptor to increase neuronal inhibition, and felbamate, topiramate and levetiracetam also have some influence on function of the GABA_A receptor. GABA turnover is modulated by both vigabatrin and tiagabine, which increase the amount of GABA both globally in the brain and at the level of the synapse (Sills 2009).

Drugs that attenuate glutamate-mediated excitatory neurotransmission include felbamate and topiramate, although both drugs have complex modes of action involving both GABA receptors and sodium and calcium channels. Glutamate receptors are divided into NMDA, AMPA and kainate receptors. The anticonvulsant mechanism of felbamate and topiramate is thought to be via blockade of the NMDA receptor. Phenobarbitone may block AMPA receptors.

Levetiracetam has an unusual mode of action. The binding site is at presynaptic vesicle protein 2A (Lynch et al. 2004). This AED has a broad spectrum, and has both anticonvulsant and anti-kindling effects. Levetiracetam has been reported to work via inhibition of high voltage calcium channels (Niespodziany et al. 2001) and reduction of voltage-gate potassium channels (Madeja et al. 2003).

The selective sodium channel blocking drugs – carbamazepine, phenytoin and oxcarbazepine – have been shown to be particularly efficacious in partial epilepsies. Selective calcium channel blockers – gabapentin and pregabalin – are also efficacious in partial epilepsy. AEDs that have more than one mode of action such as sodium valproate, lamotrigine, and topiramate can be used with most seizure types.

1.7 ALTERNATIVE TREATMENT OF EPILEPSY

1.7.1 SURGICAL TREATMENT OF EPILEPSY

Some patients with refractory partial epilepsy may be suitable for resective epilepsy surgery, which can result in significant reduction in seizure frequency with consequent increased quality of life and reduced morbidity and mortality. Typically, patients with lesional epilepsy and those with mesial temporal lobe epilepsy are considered to be good candidates for epilepsy surgery (Cascino 2004). The majority of patients with mTLE have HS, and the extent of damage and cell loss may be apparent on an MRI scan. Moreover, T2-weighted MRI may show increased signal intensity using FLAIR in patients with mesial temporal sclerosis. Removal of the mesial temporal structures - the amygdala and hippocampus – is associated with a good postsurgical outcome, with approximately 80% of patients being rendered seizure-free.

Patients with epilepsy arising from other areas of the brain and with other underlying pathologies are also surgical candidates, and even those with normal imaging may be considered. Epilepsy surgery involving resection of a low-grade glial neoplasm or cavernous hemangioma tends to be associated with a favourable outcome (Cascino 2004); in other cases of focal resection, the chances of substantially reducing seizure frequency depends on how much of the epileptogenic tissue is able to be safely removed.

The possible risks of surgery must be carefully assessed before the option is given to the patient, and this involves careful study of all data by a multidisciplinary team. The patient should be counselled regarding likely negative effects of surgery in addition to the likelihood of having a good outcome. In some cases, particularly if resection involves an eloquent area of cortex, surgery may be carried out with the patient awake in order to carry out functional mapping of that area of the cortex and minimise the risk of disability (Sahjpal 2000). In patients who have focal epilepsy but no structural MRI abnormality, additional tests are required before resection can be considered. This may include intracranial EEG recording with subdural and/or depth electrodes; the placement of the electrodes will be determined by a variety of factors including seizure semiology and scalp EEG, FDG-PET, MEG and ictal SPECT. These patients have a less favourable outcome, but a reduction in seizures number or severity may still have a positive impact on patient quality of life. Patients who have previously been turned down the possibility of surgery because they have normal imaging should be reassessed with advancements in technology; i.e. a 3 Tesla MRI may reveal an abnormality that was not visible on a 1.5 Tesla MRI.

1.7.2. ALTERNATIVES TO AEDS AND EPILEPSY SURGERY

When a patient with refractory epilepsy is not suitable for resective surgery, there are a few alternatives. Vagal nerve stimulation (VNS) is an add-on treatment that has been available since 1997 for patients with focal epilepsy. Although unlikely to render a patient seizure free, VNS can reduce seizure frequency by at least 50% in a third to a half of patients (Cramer et al. 2001), and it also has a positive effect on quality of life independent of effects on seizure control (Cramer 2001). Dietary manipulation, using a modified version of the high fat low carbohydrate Atkins diet, has also been shown to be efficacious in adults with refractory epilepsy, although it is not always well tolerated and can have significant side effects such as

increased cholesterol levels (Kossoff et al. 2003; Kossoff et al. 2008). Two forms of brain stimulation are currently in development: the Responsive Neurostimulation System and stimulation of the anterior nucleus of the thalamus (Cascino 2008). Meanwhile, new anticonvulsant drugs are also being developed which may help patients who have previously responded poorly to medical treatment. With rational design of drugs designed using improved knowledge of the pathophysiology of different types of epilepsies, focal or generalised, new drug treatment aimed at specific molecular targets may offer hope to patients who are refractory to available medication and not suitable for epilepsy surgery (Meldrum and Rogawski 2007).

CHAPTER 2.

INTRODUCTION TO TRANSCRANIAL MAGNETIC STIMULATION

2.1 HISTORY AND BASIC TECHNIQUES

The history of transcranial magnetic stimulation (TMS) lies in the prior development of transcranial electrical stimulation (TES) as a method for investigating the human motor system. Initial studies involved direct electrical stimulation of the brain (Bartholow 1874; Penfield and Jasper 1954), and it was not until 1980 that Merton and Morton were able to successfully stimulate the brain through the intact skull and scalp. Although this new non-invasive technique was successful, it also had drawbacks; TES is an uncomfortable procedure that requires high voltage stimulation to penetrate the skull; TES commonly causes contraction of scalp muscles and stimulation of nociceptors, and was therefore not considered to be a suitable tool for routine clinical use. Nonetheless, Merton and Morton demonstrated that it is possible to stimulate both the motor and occipital areas of the cortex using transcranial stimulation, resulting in contralateral muscle twitch or in phosphenes, respectively. Shortly after this, TMS of the brain was developed and proved to be a more tolerable procedure (Barker et al. 1985). Since the initial studies that focused on studying corticospinal motor conduction, for example in multiple sclerosis and motor neuron disease (Barker et al. 1987), TMS has developed into a widely used technique in studies of the physiology of the human cortex. Although research to date has included various areas of the cortex, the majority of work has focused on the primary motor area, since the output - a muscle twitch - can be measured accurately and objectively with electromyographic (EMG) recordings from the contralateral target muscle. This section will focus on the use of TMS over the primary motor cortex for the measurement of cortical excitability and inhibition. The principles and techniques of magnetic stimulation, and how the effects of stimulation can be measured, will be discussed.

2.1.1 PRINCIPLES OF TMS

TMS is based on the principles of magnetic induction, first described by Faraday in 1831. Unlike electrical stimulation, which is significantly attenuated by the skull and tissue before reaching the cortex, the magnetic field produced by TMS is largely unaffected as it passes through bone and tissue, and as a result, stimulus intensities are lower than those that are required for TES. The procedure is therefore more comfortable, and except at very high stimulus intensities, does not result in significant scalp muscle contraction or pain. Magnetic stimulation utilises a magnetic coil that is placed over the scalp. Intense pulses of electrical current, which are discharged by a bank of capacitors within the coil, induce the magnetic field. As the current is discharged, a 'click' is heard within the coil. The stimulator coil is effectively the primary circuit, with current flowing from the coil to the secondary circuit (the body). The changing magnetic field in the brain causes an eddy current, which in turn leads to depolarisation of neuronal membranes, culminating in an action potential or a post synaptic potential (Terao and Ugawa 2002).

2.1.2. SITES OF STIMULATION

The current induced by the magnetic stimulator coil flows in parallel to the surface of the brain and to horizontal cortical fibres, which are preferentially stimulated (see below). The depth of stimulation is limited by the sharp fall off of the magnetic field, reaching an average penetration of 19mm from the coil (Hess et al. 1987; Marg and Rudiak 1994). When the motor cortex is chosen as the site of stimulation, as is often the case, the site of stimulation is the premotor cortex and stimulation results in a myogenic response in the contralateral hand. Stimulation occurs across a region of about 5mm (Cohen et al. 1990b). Patton and Amassian (1954) took direct recordings from the pyramidal tract of monkeys following a single electrical stimulus to the surface of the motor cortex, and found that a series of volleys arises. The initial wave results from the direct activation of pyramidal tract axons, probably at the initial axon segment or at proximal internodes in subcortical white matter, and is called the D-wave. Further waves with longer latency can also be observed; these are thought to arise from indirect, transsynaptic activation of pyramidal tract neurons, and are termed I-waves. These are called I-1, I-2 and I-3, named in order of their latency, and they occur at intervals

of approximately 1.5msec. While D-waves have a lower threshold than I-waves with low-intensity electrical stimulation, the opposite is true for TMS in which I-waves are more readily seen at lower stimulus intensities (Edgley et al. 1990). The explanation for the preferential activation of I-waves with TMS is that that magnetic stimulation is thought to excite corticospinal neurones transsynaptically rather than directly (Day et al. 1987). The preferential transsynaptic activation by TMS may be due to the parallel orientation of the interneurons with respect to the surface. This would make them more prone to excitation than corticospinal neurons, which lie perpendicular to the precentral gyrus. Indeed, the conduction time of motor evoked potentials (MEPs) is affected by the orientation of the coil with respect to the scalp, suggesting that different populations of nerves are stimulated with certain coil positions (Sakai et al. 1995). Patton and Amassian (1954) also showed that D-waves, but not I-waves, can be elicited after removal of the cortex. Hence, there is an important hypothetical difference between the effects of TES and TMS: TES stimulates corticospinal outputs directly at a cortical or just subcortical level, and hence will not be influenced by intracortical modulation of cortical excitability; elements of TMS such as MEP amplitude or latency, on the other hand, may be subject to intracortical modulation because it relies on intracortical transsynaptic pathways (Kujirai et al. 1993).

Using needle electrode recordings of single motor units (rather than surface EMG which is usual in TMS experiments), Oh et al. (1997) showed that the direction of current across the motor cortex influences the I-waves observed: a current flowing posterior-anterior (usual in TMS experiments) resulted predominantly in I₁ waves, whereas a current in the opposite direction resulted predominantly in I₃ waves. Direct epidural recordings over the spinal cord in man did not appear to confirm this however, with I₃ waves being rarely seen at low intensity with current flow in either direction (Di Lazzaro et al. 2001).

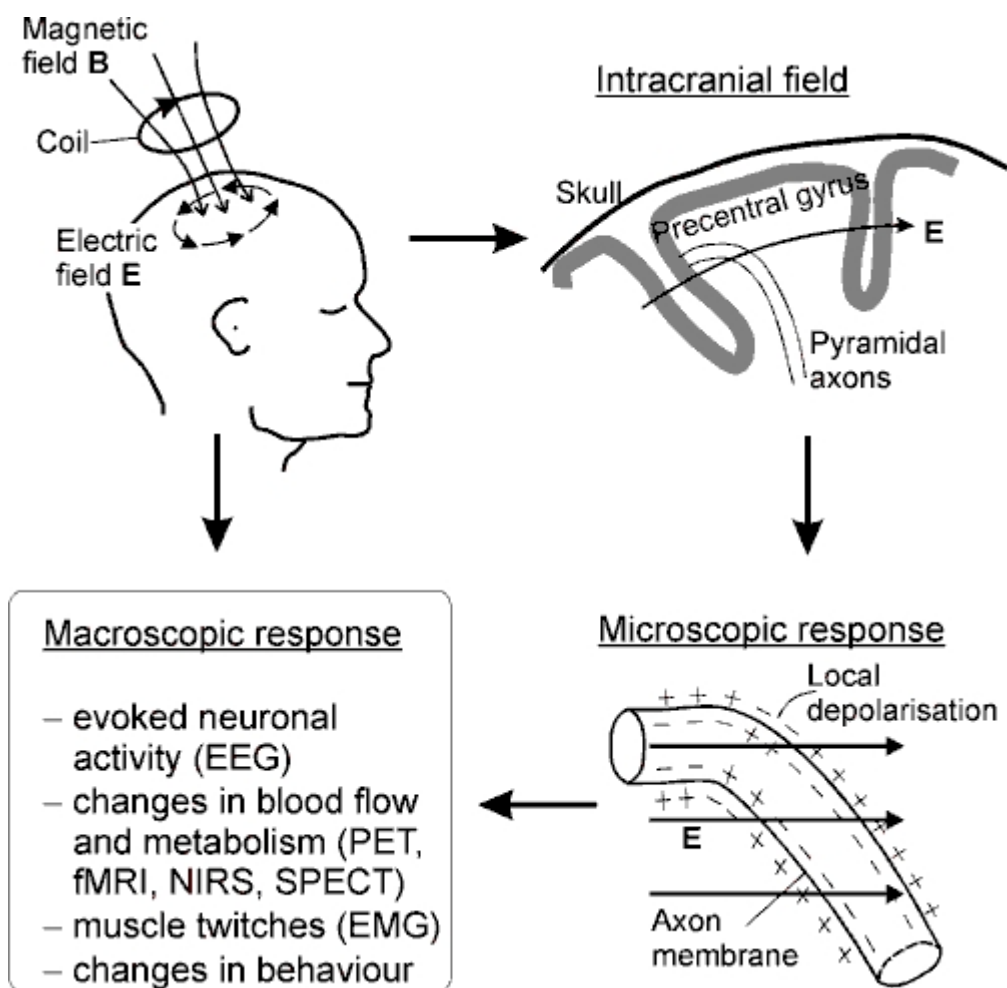


FIGURE 4: DEMONSTRATING THE PRINCIPLES OF TMS

Current in the coil generates a magnetic field (**B**) that induces an electric field (**E**). The lines of **B** go through the coil; the lines of **E** form closed circles. The upper-right drawing illustrates schematically a lateral view of the precentral gyrus in the right hemisphere. Two pyramidal axons are shown, together with a typical orientation of the intracranial **E**. The electric field affects the transmembrane potential, which may lead to local membrane depolarisation and firing of the neurone. Macroscopic responses can be detected with functional imaging tools, with surface EMG, or as behavioural changes (e.g. muscle twitch). This diagram is reproduced with kind permission from the author (Ruohonen 1998).

The first coils to be developed were circular in shape. Although this shape is easy to construct and useful in that the coils can be placed readily over most areas of the body, one major setback is the lack of certainty as to the precise site of stimulation, because the magnetic field diverges as it leaves the coil. Coils generate a maximum magnetic field of 1.5-2 Tesla. The

maximum induced electric field occurs around the edge of the coil, and cannot be focused accurately. Although smaller circular coils do reduce this lack of certainty regarding the site of stimulation, they overheat readily. Moreover, the depth of penetration is reduced with smaller circular coils, so that higher intensity stimulation is required, and this can lead to discomfort (Barker 1999). Figure-of-eight coils consist of two coils attached side by side; the current flows in opposite directions in the two coils, with the maximum induced electric field occurring beneath the point of intersection of the two coils (Cohen et al. 1990a). Stimulation can therefore be more focused with figure-of-eight coils than with circular coils (see figure 1); however it is still not possible to determine precisely the area that is being stimulated (Barker 1999).

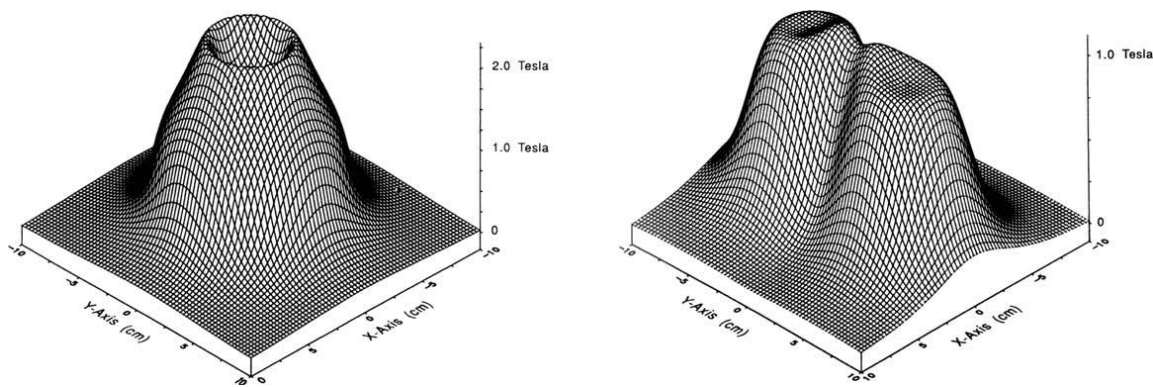


FIGURE 5: MAGNETIC FLUX OF CIRCULAR VERSUS BUTTERFLY COIL

The diagram above shows three dimensional representation of the peak magnetic flux produced on the surface for a circular coil (right) and a butterfly coil (left). (Figure reproduced with the kind permission of Magstim Company, Whitland, U.K.)

The current flowing through the coil can be monophasic or biphasic. These different current profiles have different physiological effects. A monophasic pulse has a stronger effect if the current induced by the upstroke of the pulse flows posterior-anterior across the motor strip (i.e. the coil handle towards the back of the head) whereas a biphasic stimulus is weaker if the first upstroke produces a posterior-anterior current (Kammer et al. 2001). Furthermore, the most effective component of the pulse for stimulating cortex is the end of the first phase of a monophasic pulse, but the end of the second phase of a biphasic pulse (Corthout et al. 2001),

suggesting it is charge accumulation in the cortex rather than the induced current itself which is effective.

A further practical limitation of stimulator coils is their tendency to overheat. This occurs as a result of the current passing through electrical resistance in the coil. If the coil is being used repetitively, with insufficient time between pulses to allow the coil to cool down, the heat will continue to rise and, in commercially made systems, a sensor within the coil will cause it to shut down when 41 degrees Celsius is reached (Epstein 2008). Therefore, coil heating may prove problematic during prolonged periods of paired-pulse stimulation and particularly during repetitive stimulation. The solution is often to replace one coil with another when it overheats; however, this requires having a second coil ready to use and is not ideal. Attempts continue to be made in order to improve the design of stimulator coils. For example, water-cooled coils have been developed (Cadwell Laboratories Inc.; Kennewick, W.A.), but these require safety consideration. Air-cooled coils are also available (Magstim Company; Whitland, Dyfed, UK) but are bulky and noisy. More recently, coils with reduced internal resistance have been shown to delay overheating without affecting efficacy (Weyh et al. 2005).

2.2 MEASUREMENTS OF CORTICOSPINAL OUTPUT

2.2.1 MOTOR EVOKED POTENTIAL

The motor evoked potential is the compound muscle action potential that arises as a result of stimulation of the 'hotspot' for a chosen contralateral target muscle. As described previously, low intensity TMS is thought to stimulate corticomotoneurons indirectly, or transsynaptically, whereas direct stimulation occurs mainly at higher stimulus intensities. The corticomotoneurons then excite anterior horn cells, which in turn stimulate the target muscle, culminating in a contraction. The extent of muscle contraction, and hence the size of the MEP, varies according to certain stimulus parameters, including the intensity of stimulation, voluntary facilitation and conditioning stimulus. The size of the MEP can also be affected by factors such as hand preference, indicating interhemispheric changes in the cortical motor representation (Triggs et al. 1999).

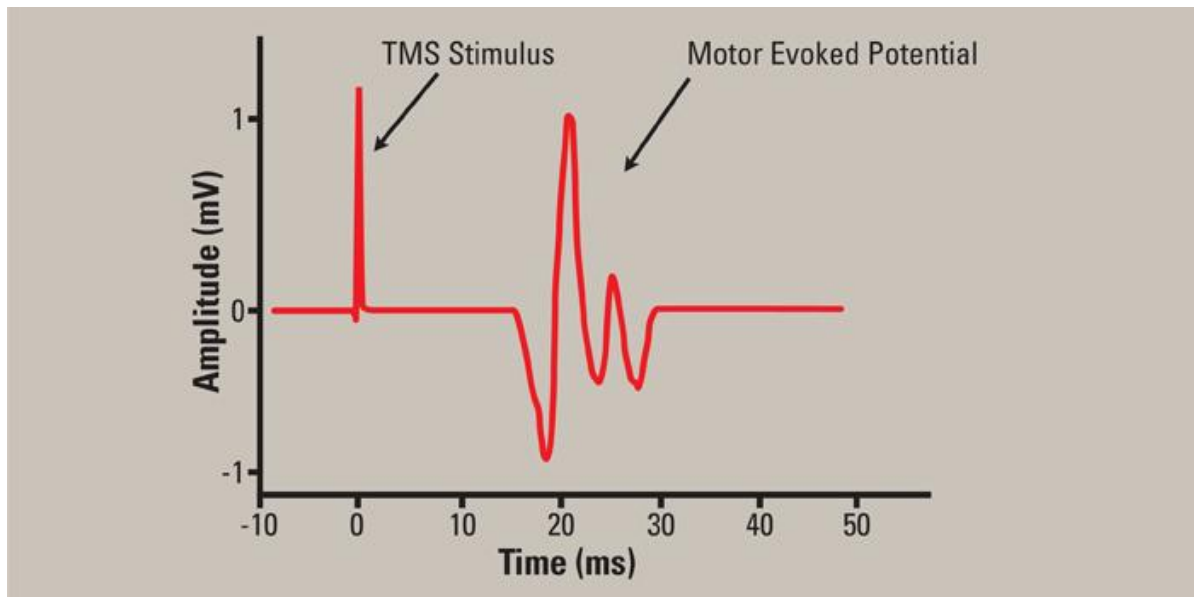


FIGURE 6: MOTOR EVOKED POTENTIAL

Graphic representation of a motor evoked potential (modified image from Rosa et al. 2010).

An input-output curve, also known as a recruitment or stimulus response curve, demonstrates that the size of the MEP increases in a sigmoid shape as the intensity of TMS is increased, from rest until reaching a plateau at maximal contraction (Devanne et al. 1997). The slope of the curve gives an indication of the number of corticospinal motoneurons that can be activated for a given muscle, and therefore the excitability of that area (Ridding and Rothwell 1997; Valls-Sole et al. 1994). For example, the curve is steeper in the muscles of the hand, which have larger cortical representation (Abbruzzese et al. 1999; Chen et al. 1998). The slope of the curve is increased by voluntary contraction of the target muscle. It is also increased in upper limbs by teeth clenching (Boroojerdi et al. 2000).

As mentioned above, various forms of voluntary facilitation have been shown to increase the size of the MEP to a set stimulus. These include contraction of the target muscle, which causes a general increase in corticospinal excitability, as reflected by a rise in the slope of the recruitment curve. The threshold for stimulation is concomitantly decreased in cortical and spinal motoneurons during voluntary contraction, because of facilitatory feedback from the contracted muscle and associated skin and joints (Houk 1974; Rossini et al. 1996). Mental imagery can also facilitate responses. Gandevia and Rothwell first demonstrated this

phenomenon in using electrical stimulation in subjects who were instructed to focus on a particular intrinsic hand muscle; EMG recordings of the focus and a non-focus muscle were made, and it was demonstrated that facilitation was restricted to the focused muscle (Gandevia and Rothwell 1987).

Further studies have shown that MEPs can be facilitated using alternative types of imagery. For example, Rossini et al. (1999) showed that while actual motor tasks enhance MEP amplitude the most, there is also facilitation during non-motor mental activity. The authors demonstrated that although spinal motoneuronal excitability is increased by mental imagery, the main effect was on cortical excitability. Rossini and colleagues suggest that these cortical facilitatory effects might be due to an increased firing level of motor cortex pyramidal neurons, as is seen during overt movements. The facilitation could additionally or alternatively be due to an increased spatial awareness of the muscle being targeted, as has been observed with somatosensory evoked potential facilitation (Garcia-Larrea et al. 1991). Facchini et al. (2002) demonstrated that simple motor imagery tasks result in a rapid decrease in motor threshold and facilitation of the MEP in contralateral muscles. They suggest that the decrease in motor threshold represents an increase of excitability within the 'neural core region' for the selected muscle. Facilitation of the MEP recruitment curve was seen in the target muscle, but not in a different muscle in the same hand that was used as a control. The results were therefore selective for the target muscle, with the imagery perhaps modulating certain motor pathways within the corticospinal tract for individual hand muscles; i.e. involving the same motoneurons that are involved in executing a specific task, but without any significant movement. Tinazzi et al. (2003) demonstrated that cortical excitability is increased more during complex tasks than simple ones, reflecting the greater number of muscles involved in the movement.

2.2.2 MOTOR THRESHOLD

The motor threshold (MT) is commonly defined as the intensity of stimulation required to elicit a small motor evoked potential (50-100 μ V), in at least 5 of 10 TMS pulses (Rossini et al. 1994). The MT can be defined with the muscle at rest (RMT) or during active voluntary

contraction (AMT), when – as described above – excitation is facilitated and the threshold lowered. Stimulus intensity is commonly defined as a percentage of the maximum output of the stimulator. The motor threshold varies between muscles groups, and is lowest in the intrinsic hand muscles (Wasserman et al. 1992), which are commonly chosen for TMS studies.

2.2.3 CONDITIONING STIMULI: PAIRED PULSE STIMULATION

Conditioning stimuli can have a dramatic effect on the MEP, depending on the intensity of the first (conditioning) stimulus and the interval between it and the second (test) stimuli. A frequently used approach to study intracortical excitability is paired-pulse TMS (ppTMS). The first studies used conditioning stimuli and test pulses of equal intensity, but it was not possible to accurately determine the site(s) responsible for these effects from these experiments (Claus et al. 1992; Valls-Sole et al. 1992). In 1993, Kujirai and colleagues developed an alternative technique in which the intensity of the conditioning stimulus was less than the motor threshold; i.e. not sufficient to elicit any corticospinal volleys or a muscle twitch (Kujirai et al. 1993). If there are any effects on the test stimulus as a result of the conditioning stimuli, they are therefore likely to be due to intracortical mechanisms rather than spinal mechanisms. These parameters are thus suitable for investigating the excitability of inhibitory and excitatory circuits within the motor cortex (Ziemann et al. 1996c). Kujirai et al. (1993) also showed that there is no H-reflex change with ppTMS and therefore no indication that this is a spinal phenomenon, and that a conditioning TMS pulse had no effect on a test TES pulse (which, as outlined above, is not susceptible to intracortical modulation). Both of these are strong arguments supporting a cortical site for these phenomena. Furthermore, a combined TMS and PET study revealed increased cerebral blood flow (CBF) in the ipsilateral primary motor cortex correlating with increasing short intracortical inhibition (SICI) and short intracortical facilitation (SICF), indicating synaptic activation within the M1. The sites producing these effects were not identical, suggesting that different areas of the motor cortex are responsible for the inhibitory and facilitatory effects. With short intracortical inhibition (SICI) but not short intracortical facilitation (SICF), distal changes in CBF were seen in the contralateral primary motor area as well as the ipsilateral premotor cortex (Strafella and Paus 2001).

The parameters typically used for paired-pulse TMS are a conditioning stimuli of 80% RMT with a suprathreshold test stimuli of approximately 110-120%, or such that produces a MEP with peak to peak amplitude of approximately 1mV. The resultant MEP is usually expressed as a percentage of the mean unconditioned MEP size. The hand should be completely at rest during measurements, since even minor muscle contraction can reduce both inhibitory and facilitatory effects (Ridding et al. 1995). The interstimulus interval (ISI) is the time between the conditioning and the test pulses. With an ISI of 1-5 ms, the effect of the conditioning stimuli is inhibitory, and the effect is therefore termed short intracortical inhibition (Paschal et al. 2008). Facilitation of responses occurs with ISIs of 7-20 ms (SICF). These effects are proposed to be due to separate excitatory and inhibitory interneuronal circuits activated by the conditioning stimuli (Kujirai et al. 1993; Ziemann et al. 1996c). Ziemann and colleagues (1996c) demonstrated that changing the coil position could have conflicting effects on inhibitory and facilitatory processes; while inhibition was largely unaffected by changing the orientation of the coil, facilitatory effects disappeared if the coil was rotated by 90°, suggesting that different populations are involved in the two processes. The population of neurons responsible for inhibitory effects is not sensitive to the orientation of the coil relative to the skull, whereas the opposite is true for the neurons responsible for facilitation. As with the H-reflex studies of inhibitory effects by Kujirai and colleagues, Ziemann and colleagues found that conditioning stimuli producing clear facilitation of test responses had no effect on the H-reflex in the same muscles. They therefore propose that facilitation takes place within the cortex, possibly involving cortico-cortically projecting pyramidal cells and their axons, in light of their superficial location and the fact that they are orientated mainly horizontally and in an anterior-posterior direction (Gatter and Powell 1978); both properties would make these cells and axons particularly sensitive to low-threshold magnetic stimulation.

Long intracortical inhibition (Hamer et al. 2002) occurs with ISIs of 50-200ms and with suprathreshold conditioning and test pulses (Claus et al. 1992; Valls-Sole et al. 1992). Unlike SICF, which is thought to be related to GABA_A function, LICF is linked with GABA_B function (for review see Chen 2004), and the two forms of inhibition are thought to be mediated by different cell populations (Sanger et al. 2001).

2.2.4 CORTICAL SILENT PERIOD

The cortical silent period (CSP) refers to the period of EMG attenuation, or 'silence', which can be seen following a motor evoked potential in a voluntarily contracted target muscle. The CSP can also be seen in the absence of a preceding MEP, indicating that a descending volley in corticospinal pathways and a muscle twitch are not necessary for generation of the CSP (Davey et al. 1993). The duration of the silent period tends to increase in proportion with the size of the muscle contraction, reaching a maximum duration of around 200 ms (Cantello et al. 1992). The CSP offers a further opportunity to probe intracortical inhibitory processes. The first 50 ms period of the CSP partially reflects spinal inhibitory mechanisms: the H-reflex is strongly attenuated during this early phase of the CSP, but not during the later phase and the silent period seen following peripheral nerve stimulation is much shorter than the CSP (Cantello et al. 1992). This suggests that the later part of the CSP results exclusively from cortical inhibitory processes (Fuhr and Agostino 1991). The CSP following TES is shorter than that seen using TMS, reinforcing the idea of a cortical mechanism (Inghilleri et al. 1993). Studies of patients with lesions located within, or projecting to, the primary motor cortex suggest that the processes responsible for the silent period are largely generated within this area (von Giesen et al. 1994). Presuming then that the silent period is cortical in origin, it could be due to either an inhibition of cortical motoneurons, or to a loss of excitatory cortical drive to spinal motoneurons (Abbruzzese and Trompetto 2002).

2.3 PHARMACOLOGICAL EFFECTS ON TMS

Neuropharmacological studies help to increase our understanding of the physiological basis of the responses to TMS. Such studies have shown that voltage-dependent sodium channel blockers such as PHT increase the motor threshold, with the maximum increase being seen at the time of peak plasma levels of the AED (Ziemann et al. 1996a). GABA enhancing drugs such as diazepam have been shown to have no effect on MT in the majority of studies; however, some changes in motor threshold have been noted with in patients with chronic intake of diazepam or episodes of benzodiazepine overdose (Palmieri et al. 1999). The involvement of the glutamate excitatory system has been studied using an NMDA-channel blocker, and it was shown that this had no significant effect on motor threshold (Ziemann et

al. 1998b). It is thought that motor threshold largely depends on sodium channel function, which in turn reflects membrane excitability (Ziemann et al. 1995).

Several neuropharmacological studies have investigated the inhibitory and facilitatory effects of ppTMS. Ion-channel blockers have no effect on ppTMS (Boroojerdi et al. 2001; Chen et al. 1997b; Schulze-Bonhage et al. 1996; Ziemann et al. 1996a). GABAergic agonists tend to increase intracortical inhibition and decrease facilitation (Ziemann et al. 1995; Ziemann et al. 1996a; Ziemann et al. 1996b). In contrast, tiagabine, which is a blocker of GABA uptake, causes a reduction of intracortical inhibition at an ISI of 3 ms (Werhahn et al. 1999). The authors suggest that this effect is due to the stimulation of presynaptic GABA_B receptor, which in turn results in inhibition of the postsynaptic GABA_A receptor-mediated inhibitory postsynaptic potential (IPSP). This theory is based on the observation that in vitro studies of tiagabine demonstrate a prolongation of the GABA_B receptor-mediated component of the IPSP (Thompson and Gahwiler 1992). The glutamatergic, noradrenergic and dopaminergic systems also influence the effects of ppTMS. For example, the NMDA-receptor blockers dextromorphan and memantine have been shown to decrease intracortical facilitation and increase inhibition (Schwenkreis et al. 1999; Ziemann et al. 1998b). Dopamine agonists and antagonists have also been shown to affect the responses to ppTMS in healthy volunteers, with dopaminergic agonists tending to increase intracortical inhibition and antagonists tending to reduce inhibition (Ziemann et al. 1997). Herwig et al. (2002) demonstrated the effects of the noradrenaline-reuptake inhibitor reboxetine on intracortical excitability. Reboxetine not only lowers the resting motor threshold, but also increases facilitatory effects with ISIs of 8-20 ms, suggesting an increase in intracortical excitation. The authors suggest that this effect may be due to either induction of slow excitatory postsynaptic potentials or increased excitability of pyramidal tract neurons. Ion channel blockers such as the AEDs carbamazepine and phenytoin have no significant effect on either inhibitory or facilitatory processes (Chen et al. 1997b; Ziemann et al. 1996a). Overall, these results support the hypothesis that the effects of intracortical inhibition reflect the excitability of interneuronal GABAergic pathways within the primary motor cortex. Moreover, they suggest that GABAergic mechanisms also play an important role in the facilitatory effects seen with ISIs of 7-20 ms. The mechanisms are clearly more complicated than simply depending on GABAergic function, however; the dopaminergic system is also involved in intracortical

inhibition, while the glutamatergic and noradrenergic systems influence intracortical facilitation.

The effects of AEDs and other drugs on the CSP have also been investigated. Drugs with different mechanisms of action can have similar effects on the CSP; the Na⁺ channel blockers CBZ , PHT and LMT cause no effect on the silent period (Chen et al. 1997;Schulze-Bonhage et al. 1996;Ziemann et al. 1996a), while GABA_A-agonists including ethanol, LZIP and TGB lengthen the CSP (Werhahn et al. 1999;Ziemann et al. 1995; Ziemann et al. 1996b). An NMDA agonist has also been shown to increase the duration of the CSP (Ziemann et al. 1998a). These results demonstrate the complexity of the CSP, and imply that it is not solely influenced by intracortical GABAergic inhibition, as had been previously thought (Hallett 1995).

Drug	Mode of Action	MT	CSP	SICI	SICF	References
Carbamazepine	Na ⁺ channel blocker	↑	↑	—	—	(Ziemann et al. 1996a)
Lamotrigine	Na ⁺ channel blocker=	↑		—	—	(Boroojerdi et al. 2001;Tergau et al. 2003)
Phenytoin	Na ⁺ channel blocker	↑	—	—	—	(Chen et al. 1997;Mavrouidakis et al. 1994)
Valproic Acid	GABA/ Na ⁺ channel blocker	—	—	—	—	(Ziemann et al. 1997)
Diazepam	GABA _A agonist	—	—			(Palmieri et al. 1999)
Ethanol	GABA _A agonist	—	↑	↑	↓	(Ziemann et al. 1995)
Lorazepam	GABA _A agonist	—	↑	↑	↓	(Ziemann et al. 1996b)
Vigabatrin	GABA agonist	—	—	—	↓	(Ziemann et al. 1996a)
Tiagabine	GABA agonist	—	↑	↓	↑	(Werhahn et al. 1999)
Gabapentin	?GABA/ Ca ⁺⁺ channel	—	↑	↑	↓	(Rizzo et al. 2001;Ziemann et al. 1996a)
Topiramate	Na ⁺ / GABA _A -agonist/ NMDA antagonist	—	—	↑	—	(Reis et al. 2002)
Levetiracetam	Binds synaptic protein SV2A	↑	↑	—	—	(Solinas et al. 2008)
Pregabalin	Binds to subunit of the voltage-dependent Ca ⁺⁺ channel	—	↑	↓	—	(Lang et al. 2006)

TABLE 1: SUMMARY OF THE EFFECTS OF SOME ANTIEPILEPTIC DRUGS OF TMS MEASURES.

2.3 VARIABILITY OF TMS MEASURES

Motor thresholds and MEPs are both subject to substantial interindividual variability, and to a lesser extent intraindividual variability. The variability of motor threshold amongst individuals makes it less than ideal for group studies. However, because the intraindividual variability is relatively low, MT measurements can be a worthwhile measure in longitudinal studies. Although paired-pulse TMS results show lower variability than single-pulse TMS, inter-subject variability still tends to be high in paired-pulse studies.

In a large study of variability of MEP results, (Wasserman 2002) looked at recordings taken from 151 healthy individuals. No significant effects of age or sex were found on either resting or active motor thresholds. However, it was confirmed that recordings from individuals differ to a “striking degree”. The author suggests that experimental error may account for as much as 36% of the interindividual variability with the target muscle at rest, and up to 50% during active contraction. Factors such as coil positioning and electrode placement are named as possible sources of error in motor threshold determination and MEP recordings. Since a circular coil that encompasses the circumference of the head was used in the experiments, it is probable that the variability may be even higher when figure-of-eight coils are used, since these have a greater propensity to rotate, therefore shifting the exact site of stimulation. As a further potential source of error, Wasserman cites the possibility of motoneurons firing asynchronously with the consequence of potentials being cancelled out on the surface EMG. This would cause variations in the size and the morphology of MEPs (Magistris et al. 1998). The study also found a significant correlation between recordings taken from siblings, suggesting a significant genetic input. This could be due to either similarities in skull thickness and cortical sulcation patterns, since a significant cause of variation in motor threshold measurements may be differences in the distance from the coil to the brain: i.e. interindividual variations in skull thickness and cortical sulcation. A further possible reason for the reduced variability between siblings could be genetically determined similarities in the intrinsic properties of the target motoneurons.

Paired-pulse recordings are also subject to large levels of variability, again with no correlations with age or sex. Since the stimulation levels used for ppTMS are determined by the MT, the factors mentioned above must be partially responsible. However, Wasserman also proposes that biological differences may account for the variability, for example the tendency of individuals to experience negative emotions such as anxiety (Ziemann 2001); this may be a particularly important source of variation in male subjects. Maeda et al. (2000) have also studied the variability of ppTMS, and found that the effects are reproducible, with less variability in the inhibitory effects seen at shorter ISIs.

Sommer et al. (2002) looked at motor evoked potentials and intracortical inhibition and facilitation in 6 healthy subjects, and like Wasserman also found a large interindividual variability of MEP amplitude. The authors suggested that the variability stems from supraspinal sites, since comparatively little variability is seen in H-reflex responses (Kiers et al. 1993). As possible sources of variability, Sommer and colleagues suggest interindividual differences in the precise localisation of the motor cortex representation for given hand muscles, as well as differences in the distances from the skull to the brain.

Pitcher et al. (2003) studied the variability of stimulus-response curves in 42 subjects. Once again, there were no effects of age on the MT, the maximum amplitude of the MEP or the maximum slope of the curve. However, it was shown that larger stimulus intensities were needed in order to achieve maximal MEPs and recruitment curves in older subjects. Moreover, trial-to-trial variability was higher in older subjects, particularly when lower stimulus intensity was used. Variability tended to decrease in all age groups as the stimulus intensity was increased, although this was less marked in the older subjects. Variability in MEP measurements also tended to be higher in females.

Summary of TMS as Tool for Measuring Cortical Excitability

It is clear that TMS is a very useful tool for the noninvasive study of the human nervous system. The technique can help to shed light on functional and anatomical aspects of the

central nervous system, in particular the cortical excitatory and inhibitory systems. The technique is therefore of use in investigating a range of neurological conditions in which these systems may be affected. TMS can also be used to further our understanding of the pharmacology of neuroactive drugs. TMS is a useful tool in the investigation of cortical excitability, particularly when used in longitudinal studies. Further studies investigating ways to reduce experimental error and overall variability will help to improve the standards of this technique.

2.5 TMS IN FOCAL EPILEPSY

Transcranial magnetic stimulation provides a non-invasive method of measuring cortical excitability in control subjects as well in patients with neurological disorders. One of the initial uses of TMS in epilepsy was investigating whether single pulse or repetitive TMS could activate the epileptic foci in order to aid and expedite presurgical evaluation of patients with partial epilepsy (Hufnagel et al. 1990a; Hufnagel et al. 1990b) but the results were not reproducible (Schuler et al. 1993) and TMS was not considered to be sufficiently sensitive or specific as an activating procedure in patients with focal epilepsy (Jennum and Winkel 1994). TMS has since been used as a tool to further our understandings of epilepsy and cortical excitability interictally using single, paired pulse or repetitive stimulation, or by measuring the cortical silent period.

Single or paired pulse TMS is considered to be safe to use in patients with epilepsy without triggering seizures (Steinhoff et al. 1993) as long as exclusion criteria are used, e.g. presence of metal in the skull or mouth, skull deformity or cardiac pacemaker (Tassinari et al. 1990). Patients who are pregnant and those with cochlear implants are also unsuitable for TMS studies, and the recommendation has been made that all subjects should be protected against possible auditory damage by using hearing protection such as ear muffs or ear plugs (Rossi et al. 2009). Repetitive TMS (rTMS) has the potential to cause seizures, but with the use of approved parameters as well as careful EEG and EMG monitoring rTMS is a tool that can be safely used in patients with epilepsy.

There are many studies of TMS in epilepsy using a variety of TMS parameters and investigating many different seizure types and syndromes (for review see Tassinari et al. 2003). This section will focus on group studies of TMS in focal epilepsies. Most of these studies include patients who are treated with one or more AEDs, and this must be taken into account when analysing the results since AEDs are known to affect TMS parameters (for review see Ziemann 2004).

2.5.1 MOTOR THRESHOLD

The motor threshold refers to the minimal stimulus intensity required to elicit a motor evoked potential (MEP) in the target muscle. The motor threshold can be measured at rest (RMT) or during active contraction of the muscle (AMT); the threshold is lowered during muscle contraction (Hess et al. 1987). As discussed previously, MT reflects neuronal membrane excitability and a number of AEDs increase the MT (Ziemann et al. 1996a).

Several studies have looked at the effects of AEDs on motor threshold (MT) in patients with medically refractory partial epilepsy. The majority of studies include patients who are taking at least one AED, but Varrasi et al. (2004) found no difference in RMT compared to controls in their study of 21 patients with untreated, drug naïve partial epilepsy. A more recent study of drug naïve patients with partial epilepsy revealed higher motor threshold in the ipsilateral hemisphere only (Badawy et al. 2007). Two studies have measured MT in heterogeneous and mostly treated populations of patients with refractory partial epilepsy (Hufnagel et al. 1990c; Michelucci et al. 1996a). All patients in the study by Hufnagel et al. had temporal lobe epilepsy, either cryptogenic or symptomatic, while Michelucci et al. studied patients with cryptogenic partial epilepsy thought to be arising from temporal or frontal regions. In both studies, AED doses were reduced in a minority of patients. Hufnagel et al. also investigated the effects of seizure frequency and interictal epileptiform abnormalities on the selected parameters. Both studies showed that RMT is higher in patients treated with AEDs compared to controls. This increase was bilateral in those patients with unilateral epilepsy for whom hemispheres could be classified as either ipsilateral or contralateral to the side of seizure

focus, a finding that has since been replicated by Wischer et al. (1997) in patients with TLE. Both Hufnagel et al. and Michelucci et al. also showed that patients on monotherapy had significantly lower thresholds to those taking 2 or more AEDs. Drug reduction resulted in significant reduction of RMT, as would be expected, while a separate study showed that AED withdrawal leaves RMT at a similar level to control values (Werhahn et al. 2000). Hufnagel et al. noted that drug reduction also made measurement of MEPs possible following drug reduction in patients in whom this was previously impossible. These studies therefore demonstrate that while MT may not be normal in drug naïve patients, it is increased in patients taking anticonvulsant medication and the level of increase is dependent of the amount of medication taken.

Hufnagel and colleagues also looked at the effect of seizure frequency in a subgroup of patients with complex partial seizures who were taking 2 or 3 AEDs and found that RMT was significantly lower in patients with high seizure frequency compared to those with lower seizure frequency.

Cicinelli et al. (2000) studied 16 patients with cryptogenic partial epilepsy and 16 age-matched controls. All patients were treated with AEDs, and 7 of the patients were receiving polytherapy. As expected, MT was increased in AED treated patients compared to untreated controls. There was no significant difference between the presumed ipsilateral and contralateral hemispheres; patients all had ictal and interictal video-EEG recordings along with MRI and neuropsychometry to lateralise the ictal focus. Hamer et al. (2005) studied 23 patients with medically refractory temporal or extratemporal lobe (non-motor) epilepsy and also demonstrated symmetrical resting motor threshold between ipsilateral and contralateral hemispheres; all patients took at least one AED with sodium channel blocking function, and had higher RMT compared to controls. Cantello et al. (2000) also found that RMT is higher in patients receiving more AEDs in their study of patients with cryptogenic localisation related epilepsy.

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A large study of 110 patients with epilepsy looked at MT in patients with cryptogenic partial epilepsy and those with primary generalised epilepsy (Tataroglu et al. 2004). Results were compared with control values. Patients were classified into three groups depending on seizure

frequency. A small subgroup of patients who were newly diagnosed had TMS performed before and after initiation of treatment; the remainder of patients were taking a variety of AEDs, most commonly carbamazepine and sodium valproate. As expected, the patients with epilepsy had higher MT compared to controls and patients taking more than one AED had higher thresholds than those on monotherapy. When the epilepsy group was subcategorised into partial and generalised, it was found that the patients with primary generalised epilepsy did not have a significantly different MT to the controls, while those with partial epilepsy did have a significantly higher threshold than both the controls and the patients with primary generalised epilepsy. The authors also found reduced motor thresholds in patients with controlled epilepsy than those with refractory or partially controlled seizures. Both observations could be explained by the amount of drugs taken in each group: patients with partial epilepsy may be less likely to respond favourably to AEDs and may take more than one medication, resulting in higher thresholds; similarly, patients with partial epilepsy who are well controlled may represent individuals who respond better to AEDs than the other categories of partially controlled or refractory epilepsy and as such may be taking less medication. The study also compared data between hemispheres and found no difference between the epileptic and non-epileptic hemispheres, but as the lateralisation was based on interictal EEG only this result is of uncertain significance.

Badawy et al. (2007) measured MT amongst other parameters in 27 drug naïve patients with focal epilepsy. These patients had epilepsy arising from various regions of the brain but all were thought to have unilateral foci, and epilepsy arising outside the primary motor cortex. They found a significant difference in MT, with increased threshold in the hemisphere ipsilateral to presumed epileptogenic zone. This finding was reproduced in a later study (Badawy et al. 2010), which also showed that introducing AEDs resulted in a similar increase in MT in both hemispheres. There are no other studies of MT in drug naïve patients.

These studies show that AEDs – particularly sodium channel blockers – increase the motor threshold. MT is further increased with the use of more than one AED, and this effect is reversible when doses are reduced. No studies have found interhemispheric differences in MT comparing either the right with the left hemisphere, and only Badawy and colleagues have compared the hemisphere ipsilateral or contralateral to the seizure focus and found

significant differences. It is notable that the studies by Bawady and colleagues included large numbers of patients, and although the patients were clinically heterogeneous in terms of ictal onset zone, several other possible variables were excluded (e.g. sleep deprivation, differences in menstrual cycle in repeated measurements) and large numbers of patients were studied.

2.5.2. PAIRED PULSE TMS

Wischer et al. (1997) used paired pulse TMS to investigate intracortical inhibition and facilitation in 14 patients with TLE, making comparisons between ipsilateral and contralateral hemispheres as well as between controls. All patients were taking sodium channel blockers, and as expected had raised RMT compared to control subjects. The study demonstrated a reduction in SICI in patients with TLE compared to control subjects. This reduction was similar in both the ipsilateral and contralateral hemispheres, whereas SICF was reduced only in the ipsilateral hemisphere. The authors interpreted the changes as SICI as representing a reduction in inhibitory cortical mechanism seen in patients with intractable epilepsy that cannot be explained by the effects of AEDs. The interesting asymmetry in SICF is not expanded upon by Wischer et al, but it is possible that the finding of reduced ipsilateral SICF may represent a compensatory mechanism to counteract hyperexcitability and spread of seizure activity in these refractory patients.

Werhahn et al. (2000) studied 15 patients with cryptogenic or symptomatic partial epilepsy of mixed aetiology including temporal and extratemporal lobe epilepsies. Measurements of SICI and SICF were made at a time when the patients had been off anticonvulsant medication for at least 48 hours, and were seizure free for at least 4 hours. In patients who had a unilateral focal epileptic syndrome, comparisons were made between the ipsilateral and contralateral hemispheres. Comparisons were also made between those with temporal and extratemporal epilepsy. The principal result of this study was a significant reduction in both SICI and SICF in patients compared to controls. SICF was significantly reduced in the ipsilateral hemisphere, and in the contralateral hemisphere there was marked reduction in SICI. These findings were independent of the effects of timing of the most recent seizure, with 14 hours being arbitrarily chosen as the cut off period, and were also independent of time since last

dose of AED. There was also no significant effect of duration of epilepsy. They found no difference in SICI and SICF between the patients with TLE and those with extratemporal epilepsy, implying that the effect of partial epilepsy on the excitability of the motor cortex is similar whether the epilepsy is originating from temporal or extratemporal regions. There were no significant differences in SICI or SICF between right and left hemispheres. The alterations in SICI and SICF could not be attributed to alterations in membrane excitability since these would be accompanied by changes in RMT, which was not observed. The observed alterations in SICI and SICF in this study are therefore more likely to involve indirect transsynaptic mechanisms acting on interneurons, which have a remote effect on the motor cortex. The fact that the CSP was also unchanged in this group of patients suggests that the effects on SICI and SICF are independent of the GABA_B postsynaptic receptors which are thought to be responsible for the generation of the silent period (Siebner et al. 1999). The authors therefore suggest that alterations in GABA_A dependent interneurons may be responsible for the changes seen, and suggest an interaction between local and remote mechanisms leading to chronic changes in excitability. The authors suggest that SICF may be of use in presurgical assessment of the lateralisation of seizure onset, but suggest further investigations due to the small numbers included in their study.

Cantello et al. (2000) studied a group of 18 patients with cryptogenic localisation related epilepsy and used direct comparison of patients and controls in addition to cluster analysis to compare results between subgroups of patients. All patients had their seizure type classified according to ILAE criteria (1981) following examination including MRI and video-EEG classification with ictal and interictal recordings. Patients had a variable foci including both the left and right hemispheres, and temporal, rolandic, frontal or occipital areas of ictal onset. Comparison of patients and controls demonstrated significantly higher SICI in the right hemisphere, but direct comparison between ipsilateral and contralateral hemispheres showed mixed results. Cluster analysis of ipsilateral and contralateral hemispheres revealed 7 “pathologic” patients with defective inhibition and increased facilitation: the changes were ipsilateral in 2 patients, contralateral in 1 patient and bilateral in the remaining 4. Importantly, there was no difference in AED treatment between the patients in cluster and those with normal SICI and SICF. Most interesting in this study is perhaps the finding of differences based on seizure frequency: patients with relatively more frequent seizures (>2 per month) largely predominated among the cluster group of patients with pathologic paired pulse TMS

(ppTMS) results. The pathologic cluster group also had significantly more interictal epileptiform activity in their EEG recordings. The authors point out that five of the seven patients in the “pathologic cluster” group had seizures arising from outside the rolandic area, and postulate that this reflects functional changes that spread intrahemispherically and interhemispherically from the ictal onset zone to the primary motor cortex.

Varrasi et al. (2004) studied 21 patients with partial epilepsy who were all untreated and showed that when patients were assigned to “pathologic clusters” using cluster analysis based on their EEG and clinical findings, there was significant a difference in the pattern of SICI. One third of the patients were included in the pathologic cluster, and this was subdivided into right or left hemisphere or bilateral. The other two thirds of the patients had similar TMS results compared to controls, and other TMS parameters (MT, SICF and CPS) were unremarkable in the pathologic cluster group. Varrasi et al. found reduced SICI in the patients with more frequent IEDs in the EEG, and attribute this to defective GABA inhibition, and the authors suggest that this is due to distant epileptic activities influencing the primary motor area.

Hamer et al. (2005) found significantly increased SICF in ipsilateral hemispheres in 23 patients with extratemporal epilepsy compared to temporal epilepsy, with a strong trend to increased SICF in the contralateral hemisphere. They found no significant difference in SICI or SICF between ipsilateral and contralateral hemispheres, however, or between right and left hemispheres. There was no significant difference in SICI between the patient subgroups (temporal versus extratemporal). There was no significant correlation between duration of epilepsy or seizures frequency.

Work from this thesis has already been published and is relevant to this context: Wright et al. (2006) studied a group of patients with mesial temporal lobe epilepsy, specifically examining the effect of AED reduction and seizure timing on SICI and SICF. This is described in detail in chapter 4. 18 patients with heterogeneous epilepsy in terms of ictal onset zone (refractory unilateral mesial temporal lobe epilepsy) were studied before and after acute AED withdrawal, with a 2-day interval between recordings. Ipsilateral and contralateral

hemispheres were directly compared. Data collected prior to drug reduction did not show any correlation with previous or subsequent seizures, and there was no effect of laterality.

However, data collected on day 3 showed weaker SICI and SICF in both hemispheres with data combined in patients who went on to have a seizure within the next 48 hours. This was explained by reduced SICI and SICF in the ipsilateral hemisphere, and the effect was found to be independent of the timing of the last seizure. There were no changes in MT. However, the study shows that paired pulse data collected after acute AED withdrawal does have a strong correlation with the time to the next seizure. Since the patients had seizures arising from an area remote to the primary motor cortex, the results may indicate that there are graded changes in the physiological function of the CNS in patients with epilepsy that occur within an epileptic network.

Badawy and colleagues have recruited a series of drug naïve patients from the First Seizure Clinic in order to study patients prior to commencement of treatment with antiepileptic medication, allowing study of TMS measures without the possible interference of drug effects. Patients with focal epilepsy were studied, and in addition to the changes in motor threshold mentioned above, they found differences in paired pulse results in 27 patients with focal epilepsy arising outside the motor cortex (Badawy et al. 2007). Specifically, they demonstrated hyperexcitability in the ipsilateral hemisphere compared to the contralateral hemisphere, and suggest that this represents a disturbance in intracortical networks that extends beyond the focus to the primary motor cortex but remains lateralised. Badawy et al. (2009) also found state-dependent changes in cortical excitability in a large cohort of patients with epilepsy. They studied drug naïve patients with either idiopathic generalised epilepsy or focal epilepsy and measure baseline TMS in addition to periictal measurements. The main finding was increased excitability in patients who had a seizure within the following 24 hours compared to interictal measurement, manifesting as decreased MT and SICI and increased SICF. Looking specifically at the data from the 35 patients with focal epilepsy (21 temporal and 14 extratemporal), these changes were seen in both hemispheres if the patients had seizures with secondary generalisation. In patients with focal epilepsy who did not have generalised seizures, similar changes were only seen in the ipsilateral hemisphere while there were complex changes in the contralateral hemisphere including increased SICF and reduced SICI. Postictally there was a reduction in cortical excitability. The preictal differences in the contralateral hemisphere in the patients with focal epilepsy may represent increased

intracortical inhibition which could either serve as a preventative mechanism in terms of spread of seizure activity, or reflect structural and physiological changes that contributes towards involvement of the contralateral hemisphere during seizures. The postictal changes may reflect depression of cortical activity within the epileptic network that lasts hours or days and which may serve to prevent further seizures. Next, Badawy et al (2010) looked at the effect of AED treatment on patients with focal or idiopathic generalised epilepsy, and compared those whose seizures became well controlled with those who continued to have seizures. In the focal epilepsy group, they again found that in untreated patients there is a reduction in SICI and LICI in the ipsilateral hemisphere. After treatment, the patients who became seizure free had significant reduction in cortical excitability in the ipsilateral hemisphere, with the appearance of recovery curves resembling those of the contralateral hemisphere and non-epilepsy controls. Patients who continued to have seizures had no change in cortical excitability in the ipsilateral hemisphere after taking AEDs, while there were changes in the contralateral hemisphere with an increase in cortical excitability leading to a loss of the difference between ipsilateral and contralateral hemispheres that was seen prior to introduction of AEDs. Some patients went on to receive an additional AED, and they had additional TMS measurements which revealed, in patients with focal epilepsy, a bilateral reduction in cortical excitability. The lack of change in the ipsilateral hemisphere and changes in the contralateral hemispheres in patients who continue to have seizures after treatment with AEDs suggest that the AED had little or no effect on the ipsilateral (epileptic) hemisphere, and that the contralateral hemisphere is affected by the epileptic process. The authors suggest that TMS could be a useful tool in predicting outcome following AED treatment: this was more pronounced in the patients with IGE, but was also suggested in patients with focal epilepsy.

2.3.5. CORTICAL SILENT PERIOD

CSP measurements showed little difference between patients and controls in the study by Werhahn et al.(2000), where patients had been off AEDs for at least 48 hours prior to TMS, and were not investigated by Wisner et al. (1997). Cantello et al. (2000) also failed to find any significant differences in CSP, and Badawy et al. (2007) found no significant differences between contralateral and ipsilateral hemispheres in untreated patients with focal epilepsy.

Hamer et al. (2005) demonstrated significantly shorter CSP in the ipsilateral hemisphere compared to the contralateral hemisphere in 23 patients with partial epilepsy: this effect was more pronounced in patients with extratemporal lobe epilepsy compared to temporal lobe epilepsy, and was not correlated with duration of epilepsy or with seizure frequency. The authors propose that their findings support the view that epileptogenic zones outside the primary motor area can lead to inhibition of inhibitory interneurons and therefore overall disinhibition of the primary motor area (von Giesen et al. 1994).

Ertas et al. (2000) studied 25 patients with epilepsy, including simple partial, complex partial, absence, myoclonic and generalised seizures. Patients were categorised into subgroups depending on whether they were controlled with medication, uncontrolled with medication, or untreated; this categorisation was irrespective of seizure type. Not all patients were treated with AEDs, and those who were treated used a variety of different AEDs. Longer silent periods were seen in untreated patients with seizures; these were significantly longer than in the control group, in contrast to the findings of Werhahn et al. (2000) who observed normal CSP in untreated patients with partial epilepsy. Meanwhile, Ertas et al. (2000) found no significant difference in the CSP between the untreated patients with seizures and the other patient groups (i.e. those who were treated, with either controlled or uncontrolled seizures): this is suggested to be due to the mixture of different syndromes and seizure types in each subgroup, which may also be responsible for the discrepancy between this study and that of Werhahn et al. (2000). Nevertheless, the authors propose that prolonged CSP in patients with epilepsy represents an overactive cortical inhibitory mechanism the function of which is to suppress epileptiform activity: this is less pronounced or absent in treated patients with controlled seizures, but is seen in patients with uncontrolled epilepsy whether or not they are taking AEDs. It would be of use to extend this study focusing on individual epilepsy syndrome(s), and look at interside differences in patients with unilateral partial epilepsy.

Cincotta and colleagues have investigated the CSP in patients with epilepsy arising from the motor cortex and in patients with remote seizure foci. They initially compared TMS results of 8 patients with cryptogenic partial epilepsy and seizures beginning with clonic jerking in the right upper limb with 10 patients with cryptogenic partial epilepsy and seizures without clonic activity (Cincotta et al. 1998). All patients had longstanding, refractory epilepsy and were taking at least one AED. The motor threshold was elevated in both patient groups compared to controls. The study found bilateral prolonged CSP in the patients with clonic jerks compared to both the other patients and a control group. There was no difference

between patients with non-clonic seizures and controls. They found an interhemispheric difference in SP duration that was greater in the patients with clonic seizures than in those without, with prolonged silent periods in the contralateral hemisphere. There was a trend towards a negative correlation between the duration of the silent period and the time since the last seizure. The authors suggest that the results may represent interictal hyperexcitability of cortical inhibitory neurons in patients with epilepsy involving the primary motor cortex; this may be a compensatory mechanism akin to surround inhibition described by Prince and Wilder (1967). The authors suggest that the shorter SP in the ipsilateral hemisphere may be due to overall counterbalance of epileptogenic hyperexcitability. In a further study of 3 patients with cortical dysgenesis not involving the primary motor cortex, prolonged silent periods were seen each with interhemispheric difference in CSP duration (Cincotta et al. 2000). This study demonstrates that pathology distal to the primary motor cortex can affect the silent period.

Cicinelli et al. (2000), investigating CSP in 16 patients with cryptogenic partial epilepsy, found a difference between ipsilateral and contralateral hemispheres when using incrementally increased stimulus: the silent period increased linearly in the contralateral hemisphere, but was significantly 'flatter' in the ipsilateral hemisphere, demonstrating less inhibition. This was observed in all patients; of note is that most patients in the study had frontotemporal EEG abnormalities and only one had involvement of central regions electrographically. In addition, the CSP duration was longer in the contralateral hemisphere than the ipsilateral hemisphere at higher stimulus intensities (160% or 180% of MT). The increasing length of CSP with higher intensity stimulus is a normal physiological response (Cantello et al. 1992), and the lack of this response in the ipsilateral hemisphere indicates dysfunction of inhibition which could lead to overall hyperexcitability and a propensity to uncontrolled seizures.

Tataroglu et al. (2004) in their large study of patients with partial or generalised epilepsy found that patients had longer CSPs than control subjects. There was no significant difference in SP between patients with partial epilepsy and controls, but patients with primary generalised epilepsy and primary myoclonic epilepsy both had prolonged silent periods. Unlike the MT, CSP was not influenced by the number of AEDs taken. The authors report no significant effect of hemisphere on TMS parameters; however, since all patients with partial epilepsy had normal neuroimaging and only interictal EEG recordings (almost half of which were normal), the value of this is uncertain. The CSP was prolonged after administration of

AEDs in 15 de novo patients in whom TMS was performed before and after treatment with carbamazepine was commenced.

2.3.6. SUMMARY

The studies discussed above demonstrate that measurement of cortical excitability in epilepsy has shown variable findings, perhaps resulting from certain study limitations such as studying heterogeneous populations of patients, small numbers of patients, and patients taking a variety of AEDs that are known to affect TMS parameters. However, there are also some clear findings. TMS is able to demonstrate changes in cortical excitability that are remote from the area of seizure onset, since most studies have investigated patients with epilepsy not involving the motor strip. This provides support to the concept of an epileptic network that extends beyond the immediate area or lobe to areas including the primary motor cortex. This epileptic network may facilitate seizure propagation outside the seizure onset zone (Hamer et al. 2005). Changes outside the seizure onset zone may otherwise reflect surround inhibition (Cincotta et al. 2000).

Because most patients will already be treated, and assuming that AEDs have the same effect on both hemispheres, a compromise is to study interside differences using the contralateral hemisphere as a control. This method was used by Wright et al. (2006) to demonstrate postictal changes in cortical excitability in a clinically homogenous group of patients who all had mesial temporal lobe epilepsy, but differed in terms of medication, seizure frequency and other factors. Badawy and colleagues have performed extensive studies on untreated patients with IGE and focal epilepsy, and have found interhemispheric differences in the motor threshold, as well as remote effects of the epileptogenic zone supporting the concept of a widespread epileptic network. They have also demonstrated that there are significant changes in cortical excitability before and after seizures, and that there is a difference in TMS parameters between patient who respond well to AED and those who continue to have seizures. Further TMS studies would certainly benefit from similar studies with strict inclusion criteria and the study of drug naive patients where possible.

2.6. TMS AND IDIOPATHIC GENERALISED EPILEPSY

2.6.1. MOTOR THRESHOLD

Several studies have investigated TMS in patients with idiopathic generalised epilepsy (IGE), with variable results. Motor threshold (MT) was found to be significantly lower in untreated epilepsy patients compared to controls, while treated patients had significantly higher thresholds than control subjects (Reutens and Berkovic 1992). The increase in threshold in treated patients was thought to represent effects of AEDs since most of the patients in the study were treated with sodium valproate (VPA). A further study by Reutens and colleagues (1993) showed that MT increases in patients after commencing treatment with VPA and that this increase correlates with plasma levels. The study also showed that untreated patients with IGE had lowered MT compared with controls, implying increased cortical excitability in these patients. Conversely, increased MT compared to controls was found in 20 patients with IGE manifesting as absence seizures (Gianelli et al. 1994); this increase occurred regardless of whether or not the patients were treated with AEDs but was slightly more pronounced in treated patients; 12 patients were taking either VPA, phenobarbitone, or a mixture of both while 8 patients were untreated. It is not obvious why there is a discrepancy in results between these studies.

Delvaux et al. (2001) studied 18 patients with presumed IGE within 48 hours of an initial generalised seizure and before receiving any anticonvulsant medication; most patients went on to have more generalised seizures and had normal MRIs. They found that RMT and AMT values were significantly higher in these patients compared to control values. Half of the patient group was retested after two to four weeks, when the MT measurements were significantly reduced and no longer different from the control group. This study shows that changes in TMS parameters can vary according to the proximity of seizures. The results may represent dysfunction within the motor cortex that occurs following a seizure, and/ or a protective mechanism that counterbalances the epileptogenic activity within the cortex.

Caramia et al. (1996) studied 7 patients with juvenile myoclonic epilepsy (JME), a subgroup of IGE characterised by myoclonic jerks in addition to two patients with ‘sporadic grand mal’ (presumed to be primary generalised) seizures, who were treated with phenobarbitone. All but one of the JME patients was treated with VPA. JME is of particular interest in TMS studies because of the direct involvement of the motor cortex and presumed dysfunction within this area. The study revealed an increase in MT in the patients with JME, which was attributed to anticonvulsant therapy. In the single untreated JME patient, the MT was lower than for both treated patients and controls; the MT was also lower in the 2 non-JME patients; this finding is difficult to interpret given the very small number of patients and the fact that both patients were taking phenobarbitone – as a GABA-enhancing drug, it is unlikely to cause an increase in MT (Ziemann et al. 1995). Nevertheless, the authors propose that a future use of TMS may be in distinguishing different forms of IGE.

Brodtmann et al. (1999) measured MT in a heterogeneous group of 7 patients with IGE and controls; 3 patients had JME, 1 had juvenile absence epilepsy (JAE) and 3 had generalised tonic clonic seizures only. All patients were untreated at the time of TMS measurement. The study found no difference in MT between patients and controls – or between the two patient groups – but did demonstrate interesting results in recovery curve analysis, and these are discussed in the paired pulse section. Manganotti et al. (2000) also studied 15 patients with JME. Like Reutens and Berkovic (1992), they found that untreated patients had significantly lower thresholds compared to treated patients and controls. There was no significant difference in threshold between treated patients and controls, however; the treated patients took either VPA or phenobarbitone, and the former group would have been expected to have raised MT.

Aguglia et al. (2000) studied an interesting group of patients with IGE, which included 10 patients with seizures that included versive or cycling motor symptoms (IGE_{VC}). Patients with IGE without asymmetric features were included for comparison. Most of the patients had JME or juvenile absence epilepsy, and the majority was treated with VPA. No significant asymmetries were seen in MT between hemispheres in either patient group. Nor were there any differences in MT between the contraversive and ipsiversive hemispheres in the IGE_{VC} group, in either treated or untreated patients. However, the interhemispheric difference in

motor cortical threshold (IDMT) demonstrated values beyond the normal range in 7 of the 10 IGE_{VC} group, and in only one of the 13 patients with IGE and no asymmetrical symptoms. Although the change in IDMT values was not related to the side of the versive/ circling seizures or to handedness overall, in three patients for whom there was EEG telemetry data - and therefore, unambiguous data on seizure semiology - there was a lower threshold in the contraversive hemisphere. The authors suggest that this asymmetry of cortical excitability may reflect structural changes that have previously been observed in patients with JME (Woermann et al. 1998), and that these changes may be responsible for the asymmetrical motor activity that can occur during seizures. The lack of similar findings in the remaining IGE_{VC} patients is possibly due to the lack of precise seizure semiology, which could be provided by video EEG telemetry.

Cantello et al. (2006) have studied the effects of VPA on a heterogeneous population of patients with focal or generalised epilepsy by measuring various TMS parameters before commencement of treatment and again after three months of treatment. The initial baseline recordings similar RMT compared to controls, but this was significantly increased after treatment. The increase in RMT was greater in the patients with focal epilepsy compared to those with generalised epilepsy, and there was no interhemispheric difference in the patients with focal epilepsy. Kazis et al. (2006) also found reduced mean threshold in their study of 30 drug naive patients with IGE. They measured lower threshold (LT) and upper threshold (UT), i.e. the highest intensity and lowest intensity stimuli required to elicit a MEP, as described by Mills and Nithi (1997); the mean threshold (MT) is the arithmetic mean of UT and MT. Kazis et al. found that UT, LT and MT are all significantly lower than controls in untreated patients, and that all of these parameters increased significantly after chronic administration of VPA. Nine patients who received increasing doses of VPA were repeatedly studied, and the increase in mean motor threshold correlated moderately with plasma levels.

The reduction in motor threshold observed in some of these studies is suggestive of cortical hyperexcitability. An increase in motor threshold, on the other hand, can be accounted for in some instances by the effects of AEDs. There is the additional issue of possible changes in MT occurring in relation to the timing or frequency of seizures. It is also possible that the MT is affected in different ways by the different subgroups of IGE. This suggests that IGE should

not be considered as homogeneous group in terms of underlying pathophysiology, and that cortical membrane excitability might differ between the subgroups of IGE.

2.6.2. MEP AMPLITUDE

The MEP recruitment curve is created by plotting the amplitude of MEPs against increasing stimulus intensities. Studies of the MEP amplitude in patients with IGE are fairly sparse. However, Gianelli et al. (1994) studied 8 untreated patients with absence epilepsy and found that MEP amplitude was reduced when TMS was triggered during the slow wave of a spike-wave discharge. This suggests a transient decrease in cortical excitability related to this specific EEG change. Caramia et al. (1996) noted that patients with JME showed marked potentiation of MEP size during TMS sessions, with no increase in stimulus threshold. This potentiation was especially marked in the single unmedicated JME patient. No potentiation was observed in the group of non-JME IGE patients; rather, stimulus intensity had to be increased in order to elicit MEPs in this group. Indeed, in the majority of normal subjects, there is a gradual decrease in MEP size as stimulation continues, which is thought to represent a protective mechanism (Rossini et al. 1991). The opposite findings in the JME patients may represent hyperexcitability of the motor cortex, due to an increase in excitatory function and/ or a deficiency in inhibitory function. The contrasting observations in the non-JME group suggest an increase in background cortical inhibition, possibly due to phenobarbitone effects, or alternatively - and as mentioned above - reflecting the different pathophysiology in different types of IGE. The findings of Manganotti et al. (2000) did not corroborate these findings in their study of patients with JME. MEP amplitudes were not significantly different from those of control subjects. Delvaux et al. (2001) also found no significant difference in MEP amplitude between patients with presumed IGE and control subjects.

Kazis et al. (2006) assessed the MEP recruitment curve using stimulus levels of 5-100% of maximum stimulator output, in 5% steps. After patients received chronic treatment with VPA, there was a significant decrease in the maximum amplitude value. The curve suggested that the effect of VPA on MEP size was greatest at the higher range of stimulus intensities.

This is a novel finding, and the authors suggest that the findings may be attributable to the action of VPA on sodium channels and/or GABA_A receptors

2.6.3. PAIRED PULSE INVESTIGATIONS

Many studies have looked at paired-pulse responses in patients with IGE. Caramia et al. (1996) found a reduction in short latency intracortical inhibition (SICI) in JME patients treated with VPA, particularly using ISIs of 2, 3 and 4 msec. Two other patients with IGE but not JME showed no significant change in SICI compared to controls; although inhibition tended to be enhanced in the patients, these results did not reach significance. Both patients were taking phenobarbitone. The results in the JME patients suggest a loss of intracortical inhibition. The study also found that there was an incremental increase in MEP size during TMS sessions such that thresholds had to be lowered. This effect was not seen in the non-JME patients. The results suggest a loss of intracortical inhibition in patients with JME in addition to either hyperexcitability or deficient inhibition.

Manganotti et al. (2000) also found a significant reduction in SICI using short ISIs of 1-4ms in patients with JME. This effect was seen bilaterally, and was present in both treated and untreated patients. However, inhibition was significantly less in the untreated patients, whether taking VPA or phenobarbitone. There was no difference in long-latency SICI (using ISIs of up to 400ms), or in intracortical facilitation (SICF). The authors propose that GABA_A-mediated inhibition is affected in these JME patients, since the ISIs of early inhibition coincide with the duration of the inhibitory post synaptic potentials mediated by GABA_A receptors (Kang et al. 1994).

In the study of untreated patients with presumed IGE by Delvaux et al (2001), slightly different parameters were used for ppTMS. Instead of using below active MT stimuli, the conditioning stimulus was actually slightly above active MT. The reason for this alteration was to enhance facilitatory effects; however, the consequence of this is possible involvement of subcortical structures confounding the results. This study did not find any alteration in

SICI, but SICF measurements were significantly reduced compared to control subjects. These results differ from those seen by Caramia et al. (1996) and Manganotti et al. (2000), and the discrepancies may be due to changes in stimulation parameters, or due to differences in the underlying pathophysiology of the patient groups. The authors, however, state that despite the unorthodox methodology of using a suprathreshold conditioning stimulus, the observed effects on facilitation are likely to be largely cortical in origin. These results are thought to represent either a lack of SICF, or abnormally prolonged inhibition which may represent a protective mechanism against the spread or recurrence of epileptogenic activity.

Cantello et al. (2006) found an increase in SICF in 8 drug naive patients with IGE compared to controls but no difference in SICI. The study included patients with absence epilepsy, GTCS on awakening and JME. After treatment with VPA, there was a significant reduction in SICF. In contrast, Badawy et al. (2007) found increased SICI in 35 drug naive patients with IGE.

2.6.4. CORTICAL SILENT PERIOD

The cortical silent period has been less extensively investigated in patients with IGE. CSP measurements were made by Manganotti et al. (2000), who failed to find significant changes in cortical silent period either between hemispheres in the epilepsy patients, or between the epilepsy and control groups. Because changes in SICI were observed in this study, the authors suggest that SICI and CSP are governed by different intracortical inhibitory mechanisms. Delvaux et al. (2001) also recorded CSP in their study of patients with de novo generalised seizures. They found no significant difference in CSP duration, although the mean of results from the patients with epilepsy was lower than that from the control subjects. Cantello et al. (2006) also found no significant difference between patients with IGE and controls whether drug naïve or taking VPA.

Macdonnel et al. (2001) studied 21 patients with IGE; 9 with JME, 1 with JME and the remainder had tonic clonic seizures alone with no specific syndrome. No patients had taken

any AEDs for a least one week prior to the TMS recording. CPS was measured at three stimulus intensities. CSP duration increased in both patients and control subjects with increasing stimulus intensity, and the CSP was significantly longer in the patients with epilepsy at each stimulus intensity. This is thought to reflect enhanced intracortical inhibition, and the authors suggest that there may be an increase in the facilitating effect of the transthalamic motor circuit on the motor cortex, thereby increasing the excitability of both inhibitory and excitatory interneurons, or an intrinsic hyperexcitability of interneurons in the motor cortex may exist.

Kazis et al. (2006) measured silent period before and after chronic administration of VPA. Stimulus/ response (S/R) curves of CSP duration against stimulus intensity were constructing, using a wide range of stimulus intensities. At baseline, before patients were treated with VPA, the S/R curve was significantly different to controls; specifically, the maximum SP value of the curve was significantly longer in the IGE patients, and the 'V50' – where the CSP duration is halfway between minimum and maximum – was significantly shorter. After chronic use of VPA, the maximum CSP value was significantly decreased. This is a novel finding, and the authors speculate that the shortening of the SP reflects VPA-induced potentiation of the GABA_A receptor leading to curtailing of the GABA_B IPSP and overall reduced cortical excitability since the CSP is thought to be mediated in the main part by GABA_B receptors, and the amplitude of these receptors IPSPs is compromised by concurrent activation of GABA_A receptors (Crunelli et al. 1988; Lopantsev and Schwartzkroin 1999; Thomson and Destexhe 1999).

Akgun et al. (2009) studied the CSP in treated patients with JME and their asymptomatic siblings, numbering 21 subjects in each group. The patients were all treated with anticonvulsants, the majority with VPA monotherapy but a minority with a combination of VPA, lamotrigine and phenobarbitone or lamotrigine and phenobarbitone. The duration of CSP was found to be significantly longer in the patient group than in the control group. The asymptomatic siblings also tended to have a longer CSP than controls but this did not reach significance.

2.6.5. SUMMARY

In summary, the studies outlined above have shown a variety of results and the inconsistency between certain results may reflect a) differences in drug treatments, and b) differences in pathophysiological mechanisms between different forms of IGE. However, the studies of TMS in generalised epilepsy to date certainly provide insight regarding the possible pathophysiological mechanisms of different types of IGE, and of particular interest is the study of JME since this syndrome directly involves the motor cortex. It is also important to note that TMS parameters can be influenced by timing of recent seizures; this is not only an area that could be further explored but should also be borne in mind for future studies of TMS and epilepsy.

2.7:HYPOTHESES TESTED IN THIS THESIS

The following hypotheses were tested in the following chapters:

Hypothesis 1: Motor cortex excitability, measured using resting and active motor threshold with TMS, is not constant in patients with mTLE, but is affected by multiple factors including recent seizures, future seizures, hemisphere of seizure onset and antiepileptic drug changes. This hypothesis is explored in Chapter 3.

Hypothesis 2: Motor cortex excitability, measured using short interval intracortical inhibition and intracortical facilitation with TMS, is not constant in patients with mTLE, but is affected by multiple factors including recent seizures, future seizures, hemisphere of seizure onset and antiepileptic drug changes. This hypothesis is explored in Chapter 4.

Hypothesis 3: Motor cortex excitability, measured using cortical silent period with TMS, is not constant in patients with mTLE, but is affected by multiple factors including recent seizures, future seizures, hemisphere of seizure onset and antiepileptic drug changes. This hypothesis is explored in Chapter 5.

Hypothesis 4: Motor cortex excitability, measured using resting motor threshold, active motor threshold, short interval intracortical inhibition and intracortical facilitation with TMS, differs between patients with mTLE, patients with neocortical focal epilepsies and normal controls. This hypothesis is explored in Chapter 6.

Hypothesis 5: 1Hz repetitive TMS administered over the seizure focus reduces EEG interictal epileptiform activity. This hypothesis is explored in Chapter 7.

CHAPTER 3. MOTOR THRESHOLD MEASUREMENTS IN PATIENTS WITH MESIAL TEMPORAL LOBE EPILEPSY

3.1 AIM OF STUDY

The aim of this section of the study was to measure resting and active motor thresholds in patients with mesial temporal lobe epilepsy and to test whether there was any effect of recent seizure activity on these measures. Changes brought on by changes in dosage of antiepileptic medication were expected.

Subjects

In this section, 35 subjects with refractory unilateral mesial temporal lobe epilepsy were studied during routine pre-surgical admission to the Jules Thorn Telemetry Unit, National Hospital for Neurology and Neurosurgery, London UK. Eighteen of these subjects were included in our previous study (Wright et al. 2006). The epilepsy syndrome and presumed hemisphere of seizure onset were determined on the basis of clinical assessment, EEG (ictal and interictal), ictal video and neuroimaging data. Drug reduction was performed according to departmental protocol. Patients with any additional neurological or motor disorders, dual pathology underlying epilepsy, learning difficulties, or pregnancy were excluded. The study was approved by the institutional research ethics committee, and all patients gave written informed consent.

Each subject had their antiepileptic drugs reduced following admission on to the telemetry unit, after giving informed consent. The first TMS session took place on the day of admission at 3pm, prior to drug reduction. On the first evening of admission, the dose of each AED was reduced by half according to our telemetry unit protocol, with a further halving of dose on the second evening where appropriate (i.e. to 25% of original AED dose). No further reductions were made. The second TMS session took place on day 3 at 3pm. Medication was

administered at 0800 and 2000 each day by nursing staff on the unit. Since the timing of drug administration and TMS session was kept fairly constant, there was little variation in the time between taking medication and the recordings.

TMS Recordings

All equipment was housed in mobile units, allowing data collection to take place at the patient bedside (see figure 2). Patients were seated in a comfortable armchair, and instructed to remain alert throughout the recordings. EMG recordings were taken from the first dorsal interosseus muscle (FDI) of each hand in a belly tendon montage, using silver/silver chloride surface disc electrodes. EMG signals were filtered and amplified (CED 1902, Cambridge Electronic Design, Cambridge UK) using a bandwidth of 15-5000 Hz and a gain of 1000 and collected using Signal 4.05 software (Cambridge Electronic Design, Cambridge UK). A Magstim (Dyfedd, Wales, UK) figure-of-eight coil with a 9cm external loop diameter, connected to a Magstim 200 stimulator was used to deliver magnetic stimulation. The coil was positioned tangentially to the scalp with the handle pointing backwards at an angle of approximately 45° to the midline, resulting in a current flow of posterior to anterior across the motor cortex. The optimal site for stimulation of the FDI was established for each hemisphere in turn and was marked on the scalp. The mark stayed in place between days 1 and 3, thus ensuring accurate placement of the coil throughout both sessions. All patients were undergoing EEG monitoring at the time of the TMS study, and electrodes were removed when necessary in order to avoid heating of the electrodes and/or attenuation of the magnetic pulse. EEG recordings were paused during TMS recordings to minimise interference.



FIGURE 7: MEASUREMENT OF EMG ACTIVITY DURING TMS AT PATIENTS BEDSIDE.

Motor Threshold

Motor threshold (MT) was defined as the intensity of stimulation required to elicit a small motor evoked potential (MEP) of 50-100 μ V in at least 5 of 10 TMS pulses (Rossini et al. 1994), and expressed as a percentage of the maximum output of the stimulator. MT was determined in the FDI at rest when possible (resting motor threshold, RMT), and during voluntary contraction (active motor threshold, AMT). Visual feedback was provided during AMT measurement using an analogue display connected to a force transducer, which the patient squeezed between thumb and first finger in order to keep the level of contraction of FDI constant at approximately 20% of maximum contraction.

Seizure Timing

Seizure timing was recorded in order to examine possible change in RMT and AMT following drug reduction, and the relationship between RMT and AMT and the occurrence of seizures. On day 1, the time since the last seizure (prior to admission) was recorded according to patients' prospectively recorded seizure diaries. Patients were assigned to one of two

groups: Group Aprev included those who experienced a seizure more than 7 days before admission, group Bprev within less than 7 days before admission; the cut-off point of 7 days was selected as it provided a similar number of patients in each group. The time to the next seizure, and whether or not it occurred within the next 4 days was also recorded (i.e. while still on the telemetry unit). Patients were assigned to one of two groups: Group Anext included those who experienced a seizure more than 4 days after day 1, group Bnext within less than 4 days after day 1. Similarly on day 3, the timing of seizures before and after TMS was examined, in the following categories: Group Aprev included those who experienced a seizure more than 9 days before day 3, group Bprev within less than 9 days before day 3 (i.e. the cut-off date was the same as for day 1); Group Anext included those who experienced a seizure more than 2 days after day 3, group Bnext within less than 2 days after day 1 (i.e. again the cut-off date was the same as for day 1). Note that a patient might move between categories between day 1 and day 3, because of the occurrence of any seizures between days 1 and 3.

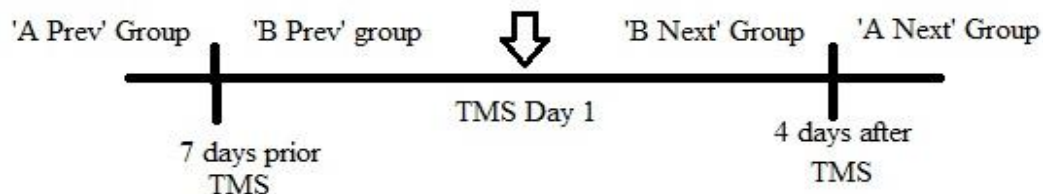


FIGURE 8: STUDY DESIGN DAY ONE

Patients are assigned to the particular groups depending on the timing of their most recent seizure.

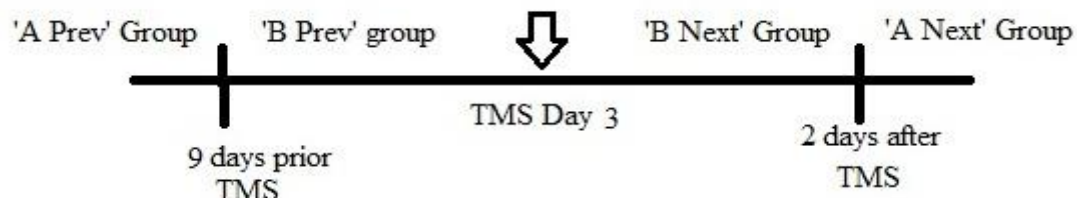


FIGURE 9: STUDY DESIGN DAY THREE:

Patients are assigned to new groups depending on the timing of seizures.

Antiepileptic drug (AED) dose changes

28 of the 35 subjects underwent AED reduction, and the AED doses were recorded at each TMS session. This information is shown in table 1.

Data Analysis

With these data, the following hypotheses were tested using repeated measures of analysis of variance (rmANOVA):

1) Thresholds fall with AED reduction; this fall in threshold is different for the two hemispheres and for RMT vs. AMT.

The study aimed first to establish whether AED reduction led to a fall in MT, and whether this fall was similar in the two hemispheres, and for both RMT and AMT. This was tested using rmANOVA with DAY, LATERALITY and RMT-AMT as within-subjects factors.

2) On day 1 or on day 3, the time since the last seizure determines motor threshold; this effect is different for the two hemispheres and for RMT vs. AMT.

The study next aimed to examine whether MT was related to the time since the previous seizure. This relationship was tested using rmANOVA with LATERALITY and RMT-AMT as within-subjects factors, and PREVIOUS SEIZURE as a between-subjects factor. The data from the data from day 1 and day 3 was examined independently.

3) On day 1 or on day 3, the time to the next seizure determines motor threshold; this effect is different for the two hemispheres and for RMT vs. AMT.

This was tested using rmANOVA with LATERALITY and RMT-AMT as within-subjects factors and NEXT SEIZURE as a between-subjects factor.

4) On day 1 or on day 3, the effect of previous seizures on threshold may depend on the time to the next seizure.

This was tested using rmANOVA with LATERALITY and RMT-AMT as within-subjects factors and PREVIOUS SEIZURE and NEXT SEIZURE as between-subjects factors.

5) The relationship between motor threshold and seizures is a dynamic phenomenon which will change between day 1 and day 3.

This was tested using rmANOVA with DAY, LATERALITY and RMT-AMT as within-subjects factors and PREVIOUS SEIZURE and NEXT SEIZURE as between-subjects factors.

3.2 RESULTS

In this section, 35 patients who were being assessed for epilepsy surgery were studied (14 women) with mean age of 38 years (range 21-65). Three patients were left handed and one was ambidextrous. Demographic data is provided in Table 1. All of the subjects had refractory mTLE with refractory complex partial seizures, and 18 patients also had secondarily generalised tonic clonic seizures. Twenty six of these represented hippocampal sclerosis (16 affecting the right hemisphere). One of these subjects was thought to have an additional dysembryoplastic neuroepithelial tumour (DNT) in the mesial temporal lobe. Of the remaining 8 subjects with mTLE, 2 had a mesial temporal DNT, 1 subject had a mesial temporal cavernoma, and 2 subjects an amygdala tumour. Three patients had normal neuroimaging, and the diagnosis of mesial temporal lobe epilepsy was based on scalp video-EEG telemetry data in two patients (seizure semiology and electrographic changes) and on invasive video-EEG telemetry in one.

All of the subjects were studied on day 1, and 25 underwent TMS on both days. Of those who were only studied on day 1, six did not undergo drug reduction (subject #'s 1, 3, 5, 9, 19 and 23) so were not studied on day 3. One subject (# 33) did undergo drug reduction but had his drugs reinstated before day 3, following a series of seizures. One subject (#17) declined to participate on day 3.

A hierarchical series of planned multivariate rmANOVA models were examined. In all instances, the distribution of measurements of RMT and AMT satisfied Mauchly's test of sphericity and no correction of P-values was needed.

The study first aimed to establish whether AED reduction led to a fall in MT and whether this fall was similar in the two hemispheres for both RMT and AMT. As expected, motor thresholds dropped between day 1 and day 3 as AEDs were reduced, in the 24 patients with complete data for both days; RMT and AMT were similarly affected and there was no difference between hemispheres. This was shown using rmANOVA with DAY, LATERALITY and RMT-AMT as within-subjects factors: there was a main effect of DAY ($F(1,23) = 25.51$, $P < 0.001$) but no effect of LATERALITY and no interactions involving LATERALITY or RMT-AMT.

The study next examined whether MT was related to the time since the previous seizure; the data for day 1 and day 3 was examined separately. Examining all of the data collected on day 1, patients with seizures within the last 7 days had higher thresholds than patients without recent seizures, in the 31 patients with a complete set of data for day 1. This was shown using rmANOVA with LATERALITY and RMT-AMT as within-subjects factors and PREVIOUS SEIZURE as a between-subjects factor, which revealed a main effect of PREVIOUS SEIZURE ($F(1,29) = 4.20$, $P = 0.050$). Looking just at only RMT, patients with recent seizures had higher RMT compared to patients without recent seizures. This was shown by a significant interaction between RMT-AMT and PREVIOUS SEIZURE ($F(1,29) = 4.88$, $P = 0.035$). Examination of the data suggested this effect was similar in both hemispheres, with mean RMT being higher in Bprev patients having recent seizures within the last 7 days compared to Aprev having the most recent seizure longer ago than this (post hoc unpaired T-

tests, uncorrected, equal variances not assumed, ipsilateral: $T = 2.22$, $P = 0.035$; contralateral: $T = 2.16$, $P = 0.040$).

The study next examined whether MT was related to the time until the next seizure, and the data for day one and day three was examined separately. Examining all of the data collected on day 1, there was no overall difference between thresholds in patients having their next seizure within the next 4 days and those with seizures at a more distant time. However, in the hemisphere ipsilateral to seizure onset, there was a trend for thresholds to be higher in patients having their next seizure within the next 4 days compared with those with seizures at a more distant time. This was shown using rmANOVA with LATERALITY and RMT-AMT as within-subjects factors and NEXT SEIZURE as a between-subjects factor, which revealed an interaction between LATERALITY and NEXT SEIZURE ($F(1,29) = 3.89$, $P = 0.058$). Examination of the data suggested this effect was similar for RMT and AMT in the hemisphere ipsilateral to seizure onset, with weak trends that mean RMT and AMT were higher in Anext patients having the next seizure after more than 4 days compared to Bnext patients having the next seizure within the next 4 days (post hoc unpaired T-tests, uncorrected, equal variances not assumed, RMT: $T = 1.39$, $P = 0.18$; AMT: $T = 1.46$, $P = 0.16$).

Therefore, from these data, RMT was increased in patients who had recent seizures within the previous 7 days, and both RMT and AMT may be somewhat increased in patients having the next seizure in more than 4 days. The study next aimed to determine whether these were independent effects. In the next planned rmANOVA model, all the data collected on day 1 was examined again in order to determine whether there were independent effects of recent and next seizures on motor thresholds. Taking into account the time to the next seizure, thresholds were higher in patients with seizures within the previous 7 days than in patients without recent seizures. This was shown using rmANOVA with LATERALITY and RMT-AMT as within-subjects factors and PREVIOUS SEIZURE and NEXT SEIZURE as between-subjects factors, which revealed a main effect of PREVIOUS SEIZURE ($F(1,27) = 5.47$, $P = 0.027$). Taking into account the time since the previous seizure, there was no significant effect of the time to the next seizure ($F(1,27) = 2.02$, $P = 0.17$). Therefore, the

effect of PREVIOUS SEIZURE on MT was enhanced by taking into account NEXT SEIZURE and was independent of NEXT SEIZURE.

The study next examined all of the data collected on day 3, applying three planned rmANOVA models analogous to those applied to the day 1 data. On day 3, there was no significant effect of previous or subsequent seizures.

The final planned rmANOVA examined all of the data from day 1 and day 3 for the patients with complete data for both days who also did not have seizures between days 1 and 3 and therefore did not move between categories ($n = 19$). This confirmed an effect of previous seizures on motor thresholds, and that this effect was different for RMT and AMT. This was shown using rmANOVA with DAY, LATERALITY and RMT-AMT as within-subjects factors and PREVIOUS SEIZURE and NEXT SEIZURE as between-subjects factors. This revealed an interaction between RMT-AMT and PREVIOUS SEIZURE ($F(1, 15) = 7.16, P = 0.018$). There were no additional interactions with LATERALITY or DAY, suggesting this effect was not significantly different between hemispheres or between days 1 and 3, despite the lack of significant effects when day 3 data were examined in isolation.

3.3 SUMMARY

In summary, this study of 35 patients with mesial temporal lobe epilepsy undergoing drug reduction looked at changes in active and resting motor threshold while factoring in the timing of recent seizures. The results are as follows :

- A fall in MT between days 1 and 3 following drug reduction was seen, as expected. This fall was seen in both hemispheres.

On day 1 before drug reduction there was:

- Higher resting thresholds in patients with recent seizures; this effect was independent of timing of the next seizure.

- This effect was **not** lateralised regarding ipsilateral and contralateral hemispheres
- There was a trend for higher thresholds in the hemisphere ipsilateral to seizure onset, in patients having their next seizure within the next 4 days compared with those with seizures occurring later.

On day three, after drug reduction, there was:

- Higher RMT In patients with recent seizures, although this was not of statistical significance

No effects of previous or subsequent seizures on MT in the data from days 1 and 3 where no seizures occurred between these days confirmed an effect of previous seizures on motor threshold ($P = 0.018$)

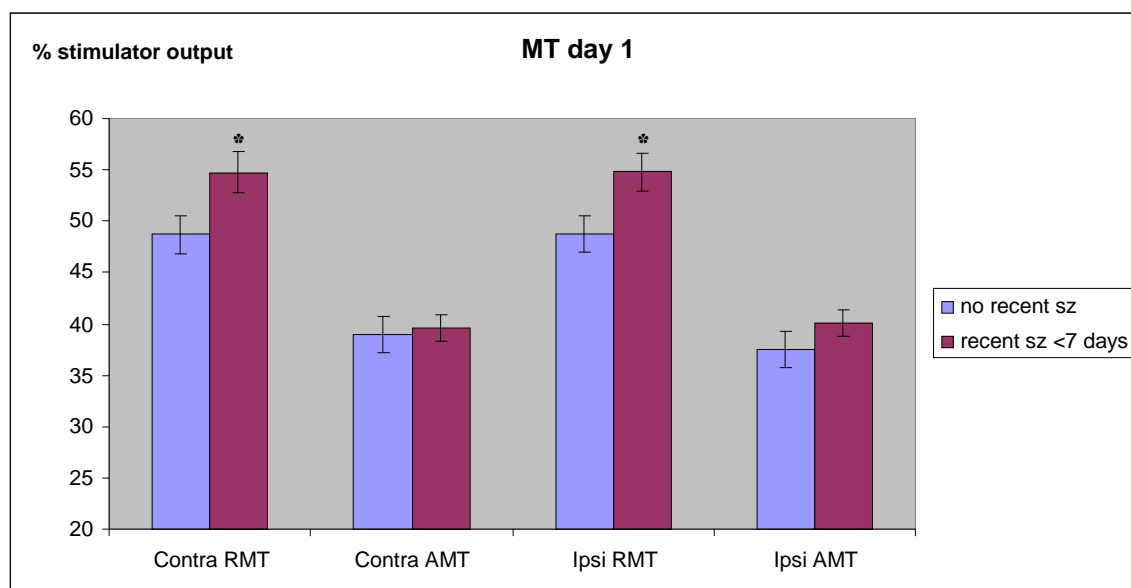


FIGURE 10: MOTOR THRESHOLDS, DAY ONE

Motor thresholds on day 1, divided according to hemisphere and previous seizures (31 patients with complete day 1 data). RMT, is significantly higher in patients with recent seizures compared to those without, and this effect is seen in both hemispheres. Contra = hemisphere contralateral to seizure onset ($P = 0.035$).; Ipsi = hemisphere ipsilateral to seizure onset ($P = 0.040$).

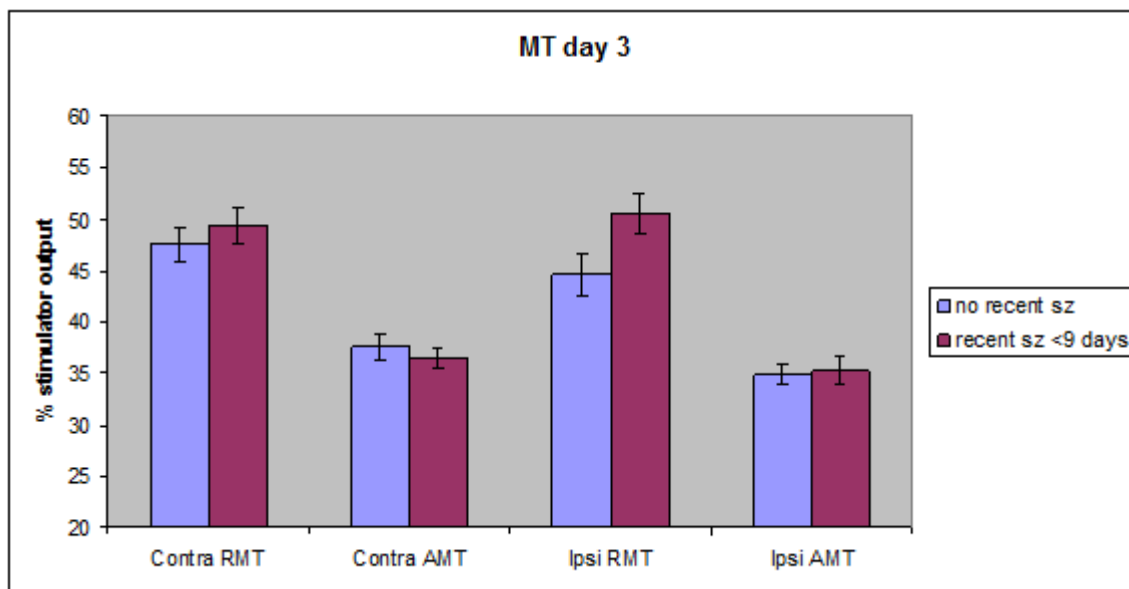


FIGURE 11: MOTOR THRESHOLDS DAY THREE

Motor thresholds on day 3, divided according to hemisphere and previous seizures (25 patients with complete data 3 data). There were no significant effects of previous or subsequent seizures.

3.3. DISCUSSION

The finding that motor thresholds are reduced by AED reduction is in keeping with previous studies (Hufnagel et al. 1990c; Michelucci et al. 1996b; Werhahn et al. 1999).

The principal finding in this section is that resting motor threshold was elevated bilaterally in patients who had recent seizures compared to those who did not. This effect was not statistically different between the same subjects studied on day 1 and studied again after drug reduction on day 3, although data for day 3 in isolation did not reach significance. In conclusion, the effect of recent seizures on motor threshold may be prolonged and not abolished by reducing AEDs.

It is important to note that the two groups of patients (those with recent seizures, and those without) may differ in other ways, and these other differences might explain the difference in thresholds. In particular, it is conceivable that patients with recent seizures may have more frequent seizures, and therefore take higher doses of AEDs. Given the extreme heterogeneity of AED treatment in this group of patients, it is extremely difficult to determine whether this might be the case; a qualitative inspection of the AED information does not support this, however. More convincingly, patients with more frequent seizures would not only be more likely to have had a seizure recently, but would also be more likely to have subsequent seizures sooner. Therefore one might expect a similar effect of subsequent seizures on motor thresholds (i.e. patients whose next seizure happens sooner would also have higher thresholds). However, this effect was not seen; moreover, the effects of recent and subsequent seizures on motor threshold were independent. Although this cannot be regarded as incontrovertible evidence that difference in AEDs do not explain the differences between those with recent seizures and those without, this evidence is nonetheless supportive of the conclusion that AED differences do not explain the motor threshold differences. In support of this, Hufnagel et al (1990c) found that motor thresholds are lower in patients with frequent seizures.

Our finding of symmetrical threshold elevation (i.e. effects not different between ipsilateral and contralateral hemispheres) is supported by Cicinelli et al (2000), Hamer et al. (2005) and Tataroglu et al. (2004).

More recently, Badawy et al. (2007) found thresholds to be different between hemispheres, with higher thresholds in the ipsilateral hemisphere in patients with focal epilepsy. However their patients were new-onset AED naïve patients; it is conceivable that chronic epilepsy, or chronic AED treatment, or both, may abolish this asymmetry; or that the threshold elevation caused by AEDs may mask this difference between hemispheres.

	Sex	Age	Handedness	Seizure types	MRI focus	Tx on day 1	Tx on day 3
1	M	41	R	CPS	L HS & DNT	CBZ 1200	CBZ 1200
2	M	41	R	CPS, GTCS	R HS	CBZ 1200 PGB 170 LMT 250	CBZ 600 PGB 75 LMT 125
3	F	47	R	CPS	L HS	CBZ 1000 TPM 125	CBZ 1000 TPM 125
4	F	65	R	CPS, GTCS	R HS	TPM 300 CBZ 1400	TPM 75 CBZ 400
5	F	34	R	CPS	R HS	LVT 2000 OXC 1200 CLB 10	LVT 2000 OXC 1200 CLB 10
6	F	24	R	CPS, GTCS	R HS	LVT 750 OXC 1200 PHT 200	LVT 250 OXC 300 PHT 50
7	F	21	L	CPS	R HS	LMT 250 TPM 200	LMT 150 TPM 100
8	M	27	A	CPS, GTCS	R HS	LVT 2000	LVT 1000
9	M	27	R	CPS	L HS	LVT 1000 LMT 300	LVT 1000 LMT 300
10	F	28	R	CPS, GTCS	L HS	VPA 1800 LVT 1500	VPA 900 LVT 750
11	M	33	R	CPS, GTCS	L HS	CBZ 1600 LVT 3000	CBZ 800 LVT 1500
12	M	46	R	CPS, GTCS	R HS	LVT 500 CBZ 800 PHT 300	LVT 250 CBZ 200 PHT 150
13	F	45	R	CPS, GTCS	R DNT	CBZ 1200 LVT 1500	CBZ 350 LVT 750
14	M	23	L	CPS	L HS	CBZ 800 VPA 1000	CBZ 200 VPA 800

	Sex	Age	Handedness	Seizure types	MRI focus	Tx on day 1	Tx on day 3
15	F	30	R	SPS	R HS	LMT 600	LMT 150
16	M	37	R	CPS	Normal imaging	LMT 400 LVT 1000	LMT 200 LVT 1000
17	F	41	R	CPS	L HS	CBZ 1200 VGB 1750 LVT 250	CBZ 300 VGB 1000 LVT 0
18	M	35	R	CPS, GTCS	L HS	LVT 500 CLB 20	LVT 250 CLB 10
19	M	49	R	CPS	Normal Imaging	LVT 3500 LMT 500	LVT 3500 LMT 500
20	F	59	R	CPS	L DNT	CBZ 900 TPM 250	CBZ 250 TPM 75
21	F	41	R	CPS, GTCS	L HS	CBZ 1200 CLB 30	CBZ 900 CLB 20
22	M	34	R	CPS	R HS	CBZ 1200 LMT 400	CBZ 300 LMT 200
23	M	30	R	CPS	R HS	VPA 2000	VPA 2000
24	M	45	L	CPS, GTCS	R HS	VPA 2500 LMT 350	VPA 1300 LMT 100
25	M	27	R	CPS	Normal Imaging	LMT 400 LVT 1000	LMT 200 LVT 1000
26	F	31	F	CPS, GTCS	R HS	LVT 2000 CBZ 1600	LVT 1000 CBZ 400
27	F	26	R	CPS, GTCS	R HS	LVT 2000 CBZ 600	LVT 1000 CBZ 200
28	F	22	R	CPS	L amygdala tumour	LMT 350	LMT 175

	Sex	Age	Handedness	Seizure types	MRI focus	Tx on day 1	Tx on day 3
29	M	31	R	CPS, GTCS	R amygdala tumour	LVT 3000 LMT 400	LVT 750 LMT 100
30	M	53	R	CPS, GTCS	L HS	LVT 3000 CBZ 1400	LVT 1500 CBZ 400
31	M	59	R	CPS	R cavernoma	LVT 1000 PHT 300	LVT 500 PHT 75
32	M	52	R	CPS, GTCS	R HS	LVT 3000 CBZ 1400	LVT 750 CBZ 350
33	M	38	R	CPS	R HS	LVT 1000 CBZ SR 1200	LVT 1000 CBZ SR 1200
34	M	43	R	CPS, GTCS	L HS	VPA 1600	VPA 800
35	M	31	R	CPS, GTCS	R HS	LVT 1800 CBZ 800	LVT 400 CBZ 400

TABLE 2.DEMOGRAPHIC, CLINICAL AND EXPERIMENTAL DETAILS FOR THE 20 SUBJECTS IN CHAPTER THREE.

R: right; L: left; A: ambidextrous; HS: hippocampal sclerosis; DNT: dysembryoplastic neuroepithelial tumour; CPS: complex partial seizure; GTCS: generalised tonic-clonic seizure; LVT: Levetiracetam; OXC: oxcarbazepine; PHT: phenytoin; LTG: lamotrigine; CBZ: carbamazepine; TPM: topiramate; CLB: clobazam; VPA: sodium valproate; VGB: vigabatrin Tx: treatment.

CHAPTER 4: PAIRED PULSE INHIBITION

The aim of the next study was to examine whether simple non-invasive measurement of motor cortex excitability using paired pulse TMS in patients with unilateral mTLE would reveal changes in excitability over a similar long time course prior to seizures.

4.1 METHODS

Subjects

This section of the thesis included 18 patients with refractory unilateral mTLE during routine admission to the National Hospital for Neurology and Neurosurgery, London UK. The motor threshold data from these patients was also studied in chapter 3. The epilepsy syndrome and presumed hemisphere of seizure onset were determined on the basis of clinical assessment, EEG (ictal and interictal), ictal video and neuroimaging data. Patients with any additional neurological or motor disorders, dual pathology underlying epilepsy, learning difficulties, or pregnancy were excluded. The study was approved by the research ethics committee of the National Hospital for Neurology and Neurosurgery, and all patients gave written informed consent.

Antiepileptic drug reduction

All patients had their antiepileptic drugs reduced according to the protocol of the seizure monitoring unit, with the first reduction taking place on the evening of the day of admission (therefore after the first TMS recording). In six patients, serum drug levels were measured on both days, i.e. before and following drug reduction, with blood samples taken immediately after the TMS measurement session. Since TMS took place at a similar time for each patient and for each day of recording, there was little variation in the time between taking morning doses of AEDs and the recordings.

Electromyography recording

Electromyography (EMG) was recorded using silver/silver chloride surface EMG disc electrodes positioned over the first dorsal interosseous (FDI) muscle of each hand, in a belly tendon montage. EMG signals were filtered and amplified (CED 1902, Cambridge Electronic Design, Cambridge UK) using a bandwidth of 15-5000 Hz and a gain of 1000 and collected using Signal 4.05 software (Cambridge Electronic Design, Cambridge UK).

Transcranial Magnetic Stimulation

TMS recording sessions took place on the afternoon of day 1 and the afternoon of day 3. Patients were seated alert and relaxed. Surface electromyography (EMG) was recorded over the first dorsal interosseous (FDI) muscle of each hand. A figure-of-eight coil (9cm external loop diameter) connected via a Magstim BiStim unit (Magstim Company, Dyfed UK) to two Magstim 200 stimulators was used to deliver magnetic stimulation. The coil was positioned tangentially to the scalp with the handle pointing backwards at an angle of 45° to the midline, producing a current flow from posterior to anterior across the motor cortex. Resting and active motor thresholds (RMT, AMT) were measured on both days in a standard manner (Rossini et al. 1994). Visual feedback was provided during AMT measurement using an analogue display connected to a force transducer, which the patient squeezed between thumb and first finger in order to keep the level of contraction of FDI constant at approximately 20% of maximum contraction. During measurement of resting MT, relaxation was monitored using continuous visual feedback from the EMG recording.

All equipment was housed in mobile units, as shown in figure 2, allowing data collection to take place at the patient bedside. The optimal site for stimulation of the FDI was established for each hemisphere in turn and was marked to ensure correct positioning during and between TMS sessions. This mark remained in place between sessions. Since all patients were undergoing scalp EEG recordings during the TMS sessions, electrodes were temporarily removed from the vicinity of the sites of stimulation where necessary, to avoid possible

attenuation of the magnetic pulse and electrode heating. The EEG recording was paused during stimulation in order to reduce electrical interference with the EMG recording.

Paired pulse stimulation (ppTMS) was carried out using a standard approach (Kujirai et al. 1993). In order to measure short intracortical inhibition (SICI) and facilitation (SICF), paired pulse stimulation (ppTMS) was carried out following MT measurements, using a subthreshold conditioning stimulus followed by a suprathreshold test stimulus (Kujirai et al. 1993). The suprathreshold stimulus was set at approximately 120% of RMT to produce an unconditioned MEP of 0.5 – 1.5mV. Two blocks of ppTMS were acquired for each hemisphere with interstimulus intervals (ISI) of 2, 3, 12 and 15 ms, with 10 conditioned motor evoked potentials (MEPs) at each ISI and 20 unpaired unconditioned MEPs in each block; one block used a conditioning stimulus set at 80% AMT, the other at 90% AMT. Stimuli were delivered in random order every 4s. The order of testing of the hemispheres was randomised. The mean MEP amplitude for each ISI was calculated, combining data from both blocks, and expressed as a percentage of the mean amplitude of the unconditioned MEP.

Data Analysis

Seizure timing data regarding complex partial and secondary generalised seizures was included; simple partial seizures were not included since the occurrence and exact timing of these was more subjective. According to patients' prospectively recorded seizure diaries, patients were assigned to one of two groups: Group Aprev included those who experienced a seizure more than 5 days before admission, group Bprev those with any seizure within 5 days before admission. This cut-off of 5 days was chosen a priori in the expectation that this would give approximately equal sized groups. At the end of the hospital admission, patients were classified on a separate axis according to the occurrence of seizures following drug reduction: Anext included patients who experienced a seizure more than 48 hours after the day 3 TMS session, and Bnext included patients who experienced a seizure within less than 48 hours. This cut-off of 48 hours was chosen because most patients would be routinely discharged from hospital at the end of this period.

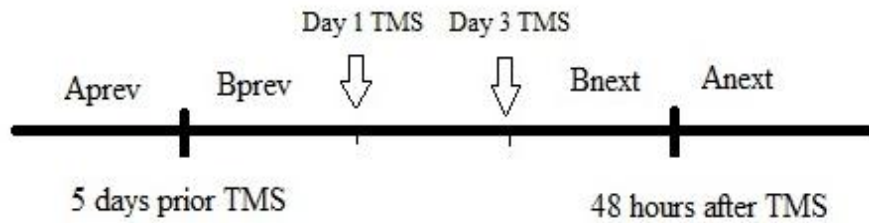


FIGURE 12: STUDY DESIGN FOR SEIZURE TIMING DATA.

TMS was performed on days 1 and 3, and data assigned to one of two groups for day 1 data (Aprev and Bprev) and one of two groups for day 3 data (Bnext and Anext).

The following hypotheses were tested:

1) On day 1, prior to drug reduction, motor threshold is correlated with time since last seizure.

This was tested using repeated-measures analysis of variance (ANOVA_{rm}) of the threshold data with the within-subjects factors CONDITION (two levels: resting or active motor threshold) and LATERALITY (two levels: ipsilateral or contralateral to seizure focus) and the between subjects factor PREVIOUS SEIZURE (two levels: Aprev, Bprev).

2) On day 1, prior to drug reduction, SICI and SICF are correlated with time since last seizure.

This was tested using ANOVA_{rm} of the MEP data with the within-subjects factors ISI (four levels: ISIs of 2ms, 3ms, 12ms, 15ms) and LATERALITY and the between subjects factor PREVIOUS SEIZURE.

3) On day 3 following drug reduction, the time to the next seizure is predicted by motor threshold.

This was tested using ANOVArm with the within-subjects factors CONDITION and LATERALITY and the between subjects factor NEXT SEIZURE (two levels: Anext, Bnext).

4) On day 3 following drug reduction, the time to the next seizure is predicted by SICI and SICF.

This was tested using ANOVArm with the within-subjects factors ISI and LATERALITY and the between subjects factor NEXT SEIZURE.

TMS and seizure data for all subjects were collated in SPSS for Windows version 11 (SPSS Inc., Chicago, IL). $P < 0.05$ was used as the threshold for significance. Mauchly's test was used to examine sphericity, and Greenhouse-Geisser correction was used for non-spherical data in ANOVArm.

In addition to testing multifactorial a priori hypotheses using repeated-measures ANOVA as described above, a multivariate data-led approach to characterise the differences between patients who had seizures following day 3 and those who did not was also used. A stepwise discriminant function analysis was used to identify the variables which most strongly contributed to discrimination between patients who had seizures within 48hrs of day 3 and those who did not, entering all of the SICI and SICF data from day 3 (maximum number of steps 16; minimum partial F to enter was 3.84, maximum partial F to remove was 2.71). Finally, using the variables identified in the discriminant function analysis, a correlation between these discriminant variables and time to next seizure after day 3 was examined.

4.2 RESULTS

This study included 18 patients (6 female) with a mean age of 34 (range 21-53). All patients had refractory mTLE with complex partial seizures, and 8 patients had also experienced generalised tonic clonic seizures. Neuroimaging revealed unilateral mesial temporal lobe lesions in all patients except #17, who had negative neuroimaging but whose interictal and ictal EEG and seizure semiology was supportive of unilateral mTLE. In the majority of cases (n=14), neuroimaging revealed hippocampal sclerosis. Ten patients had right sided seizure onset, 8 left. Clinical and demographic information, AED doses, tests performed and seizure timing for each patient is provided in table 2. Not all subjects underwent all tests on both day 1 and day 3, for the following reasons: thresholds too high to make any measurements (subject #17 on day 1); thresholds too high to permit ppTMS (#15 and #18 on day 1); and patient preference (#13, #14, #16 day 3). There was no correlation between the timing of the most recent seizure and the next seizure on either day 1 or day 3 (Spearman's $\rho = 0.063$, $P = 0.782$ 2-tailed and $\rho = 0.141$, $P = 0.493$ respectively for day 1 and day 3). Membership of groups Aprev, Bprev, Anext or Bnext was not predicted by duration of epilepsy or seizure frequency prior to admission (Mann-Whitney U, all $P > 0.05$ 2-tailed). There was no difference in mean drug dose reduction between groups Anext and Bnext (Mann-Whitney U, $P = 0.235$ 2-tailed). There was no difference between percentage reduction in AED dose and percentage change in blood AED concentration in each subject (n = 6, Wilcoxon signed ranks test, $P = 0.182$ 2-tailed). There was a non-significant positive correlation between percentage change in AED dose and percentage change in blood AED concentration (n = 6, Spearman's $\rho = 0.255$).

Relationship between seizures and TMS parameters before AED reduction

The study initially assessed whether TMS data before AED reduction (on day 1) differed between patients who had a seizure in the 5 days prior to admission and those who did not. The study also assessed whether data on day 1 differed between patients who subsequently had a seizure following AED reduction and those that did not. 10 subjects had a seizure within the previous 5 days (Bprev), and 7 had not had a seizure in this time (Aprev).

Following AED reduction on day 3, 8 patients experienced a seizure within 48 hours (Bnext), and 7 did not (Anext).

Thresholds, either in hemispheres ipsilateral or contralateral to the seizure focus, were similar between patients with a seizure within 5 days of admission and those without (ANOVArm, no main effect of PREVIOUS SEIZURE or NEXT SEIZURE and no interactions involving PREVIOUS SEIZURE or NEXT SEIZURE). SICI and SICF then examined. Two Bprev patients had thresholds too high to permit paired-pulse measurement of SICI and SICF on day 1; therefore 8 patients who had a seizure within the previous 5 days (Bprev), and 7 who had not had a seizure in this time (Aprev) were examined. There was no difference in SICI or SICF between the hemispheres, and no difference between SICI and SICF accounted for by the occurrence of recent or next seizures (ANOVArm, no main effect of LATERALITY, PREVIOUS SEIZURE or NEXT SEIZURE, and no interactions involving these factors). In summary, data collected on day 1, at baseline prior to drug reduction, showed no correlations with previous or subsequent seizures.

Relationship between seizures and TMS parameters after AED reduction

The study then evaluated data obtained after AED reduction (day 3) and assessed in a similar way whether this data collected on day 3 would differentiate between patients that had a seizure following AED reduction from those that did not. 8 patients subsequently had a seizure within the next 48 hours following day 3 (Bnext), 7 did not have a seizure in this time (Anext). Thresholds at day 3 were similar in both groups of patients and did not predict seizures (ANOVArm, no main effect of NEXT SEIZURE and no interactions involving NEXT SEIZURE).

Combining data from both hemispheres, SICI and SICF were both weaker in patients (Bnext) who had a seizure in the next 48hrs compared with patients (Anext) who did not have a

seizure in the next 48hrs (ANOVA, interaction between ISI and NEXT SEIZURE: $F[3,39] = 4.934$, $P = 0.005$, corrected P (Greenhouse-Geisser) = 0.033, figure 15). This effect was not accounted for by the time since the previous seizure (an additional factor PREVIOUS SEIZURE was added to the model as a confound: interaction between ISI and NEXT SEIZURE $F[3,33] = 5.502$, $P = 0.004$, corrected P (Greenhouse-Geisser) = 0.026). This effect was not accounted for by any difference in seizure frequency prior to admission between the groups of patients (seizure frequency prior to admission was added to the model as a confounding covariate: interaction between ISI and NEXT SEIZURE $F[3,36] = 5.185$, $P = 0.004$, corrected P (Greenhouse-Geisser) = 0.030). This effect was also not accounted for by any difference in motor threshold between the groups of patients (RMT and AMT from both hemispheres on day 3 were added to the model as confounding covariates: interaction between ISI and NEXT SEIZURE $F[3,27] = 4.013$, $P = 0.017$, corrected P (Greenhouse-Geisser) = 0.064). The difference between patients according to the time until the next seizure was explained by reduced SICI at 2ms ISI and reduced SICF at 15ms ISI in the ipsilateral hemisphere of patients (Bnext) who had a seizure in the next 48hrs (post hoc T-tests, unpaired, equal variances not assumed, comparing Bnext patients with Anext patients, ISI 2ms in the ipsilateral hemisphere: $T = 2.415$, $P = 0.033$; ISI 15ms in the ipsilateral hemisphere: $T = 2.709$, $P = 0.018$; differences for all other ISIs in both hemispheres were not significant).

Multivariate discriminant function analysis to identify the factors most predictive of next seizure

In order to identify the variables most predictive of whether the patient would have a seizure within the next 48 hours after day 3, the MEP data collected on day 3 was entered into a stepwise discriminant function analysis, in order to identify the variables most predictive of whether the patient would have a seizure within the next 48hrs after day 3. The discriminant function identified only ipsilateral SICI at 2ms ISI and ipsilateral SICF at 15ms ISI as having significant discriminatory value. The discriminant function identified in this manner was:

$$0.835(\text{MEP size for ipsilateral SICF at 15ms}) - 0.771(\text{MEP size for ipsilateral SICI at 2ms})$$

These parameters showed a clear difference between subjects who had seizures within 48hrs of day 3 and those that did not. It can be seen that patients who did not have seizures within 48hrs after day 3 showed a marked difference between SICI and SICF, which was symmetrical between hemispheres and stable between days 1 and 3. In contrast, patients who had seizures within 48hrs of day 3 had relatively impaired SICI and SICF in the contralateral hemisphere on day 1 (little difference between SICI and SICF), which both became more normal on day 3; the ipsilateral hemisphere showed the opposite effect, with relatively normal SICI and SICF on day 1 becoming less normal (little difference between SICI and SICF) on day 3.

The discriminant function identified that the difference between the MEP sizes for SICI at 2ms ISI and SICF at 15ms ISI in the ipsilateral hemisphere on day 3 was the best discriminator between patients who had seizures within 48hrs of day 3 and those who did not. Finally therefore the correlation between this difference and time until next seizure after day 3 (figure 26) was examined. Firstly only from the 8 patients who had seizures within 48hrs of day 3 was examined; these seizures were all while the patients were on the seizure monitoring unit, hence times of seizures were known precisely. Also, all of these patients only had seizures after day 3. For this subgroup, there was a strong correlation between difference between the MEP sizes for SICI at 2ms ISI and SICF at 15ms ISI in the ipsilateral hemisphere on day 3 and the time until the next seizure after day 3 (Spearman's $\rho = 0.810$, $P = 0.015$); a smaller difference between SICI and SICF was associated with a shorter time until next seizure. If the remaining 7 patients who did not have seizure within 48hrs of day 3 were also included, the correlation was even stronger (Spearman's $\rho = 0.866$, $P < 0.001$). This correlation remained significant even after partialling out the effects of time since previous seizure or preadmission seizure frequency.

Two patients studied on day 3 (#3 and #5) had seizures between days 1 and 3; exclusion of these patients from the above analyses did not alter the results. In summary, paired-pulse TMS data collected on day 3 in acutely AED-reduced mTLE patients very strongly correlated with time until the next seizure.

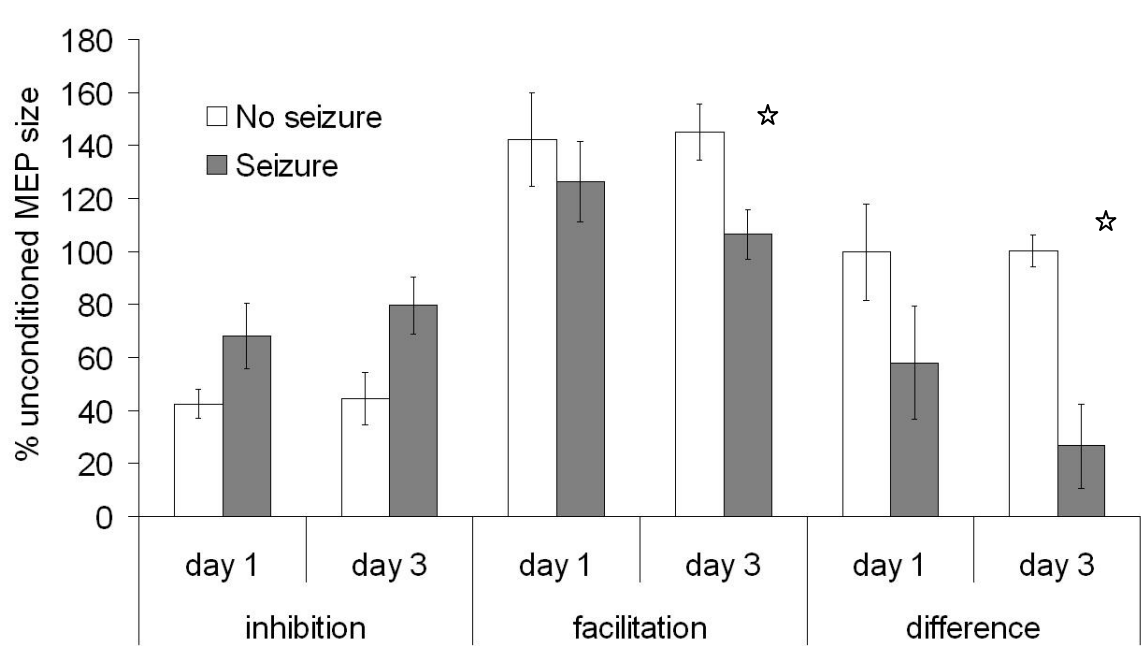


FIGURE 13: CHANGE IN CORTICAL EXCITABILITY BETWEEN DAY 1 AND DAY 3 IN THE IPSILATERAL HEMISPHERE ACCORDING TO OCCURRENCE OF SEIZURES FOLLOWING DAY 3.

Bars show mean \pm SEM.

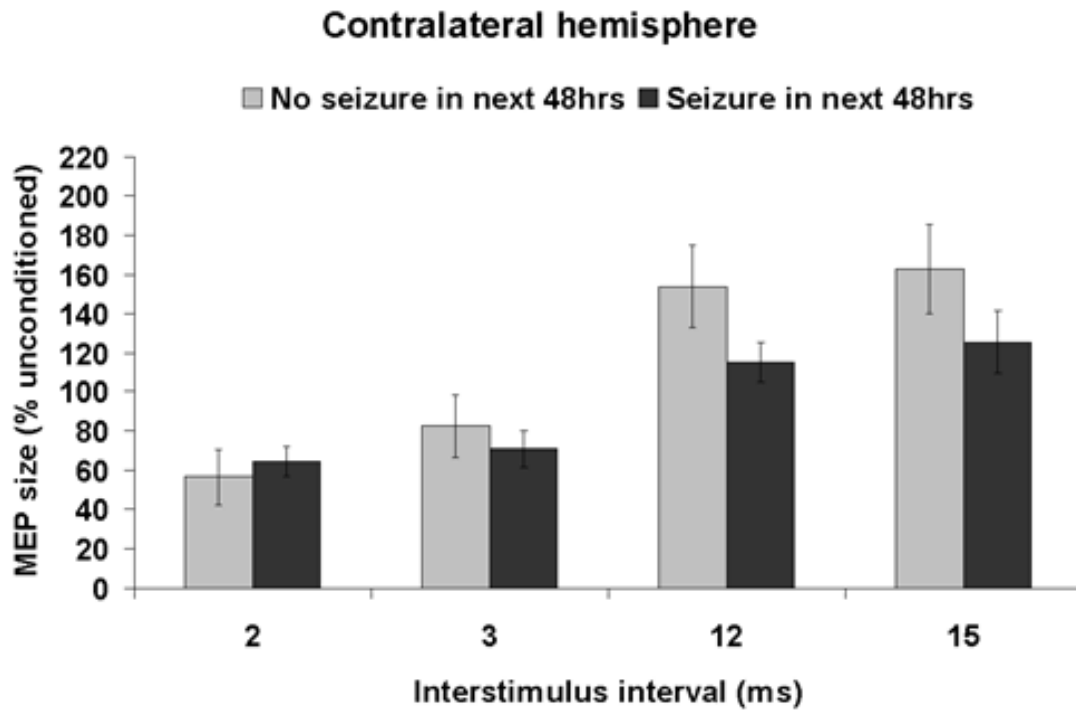


FIGURE 14: CHANGE IN CORTICAL EXCITABILITY IN CONTRALATERAL HEMISPHERE ACCORDING TO TIMING OF SEIZURES

(SICI at ISIs of 2 and 3ms) and intracortical facilitation (SICF at ISIs of 12 and 15ms) on day 3 divided according to occurrence of the subsequent seizure.

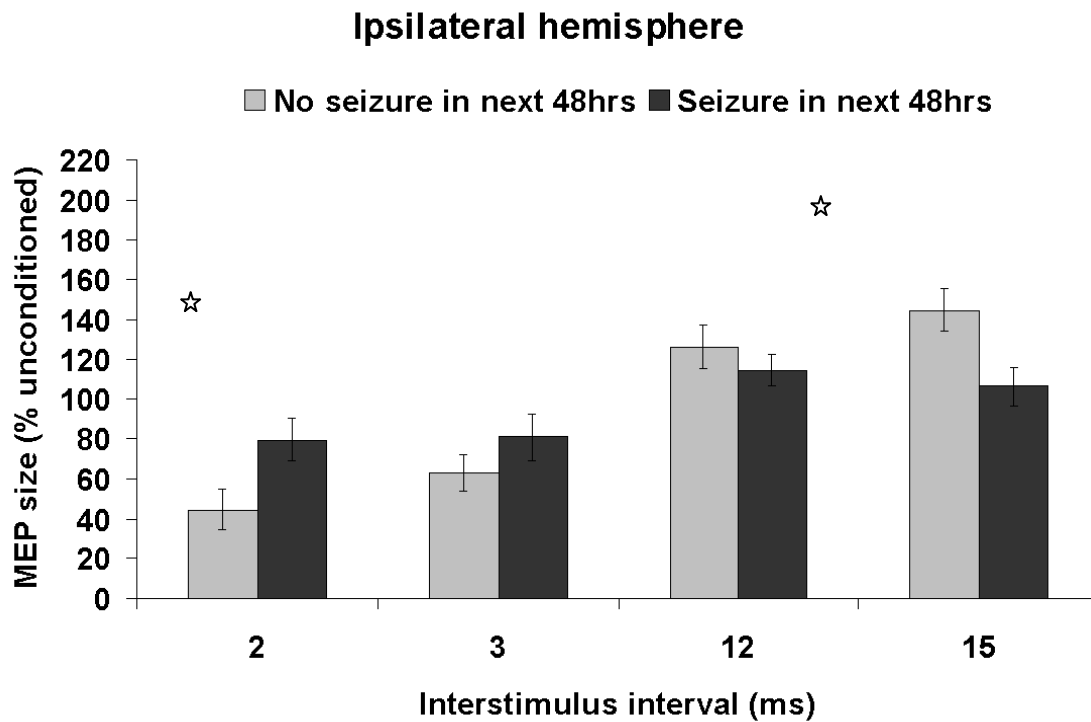


FIGURE 15: CHANGE IN CORTICAL EXCITABILITY IN IPSILATERAL HEMISPHERE ACCORDING TO TIMING OF SEIZURES

(SICI at ISIs of 2 and 3ms) and intracortical facilitation (SICF at ISIs of 12 and 15ms) on day 3 divided according to occurrence of the subsequent seizure. There was impaired SICI at 2ms ISI and SICF at 15ms ISI in the ipsilateral hemisphere in patients having a seizure in the next 48hrs compared to patients who did not have a seizure in the next 48hrs.

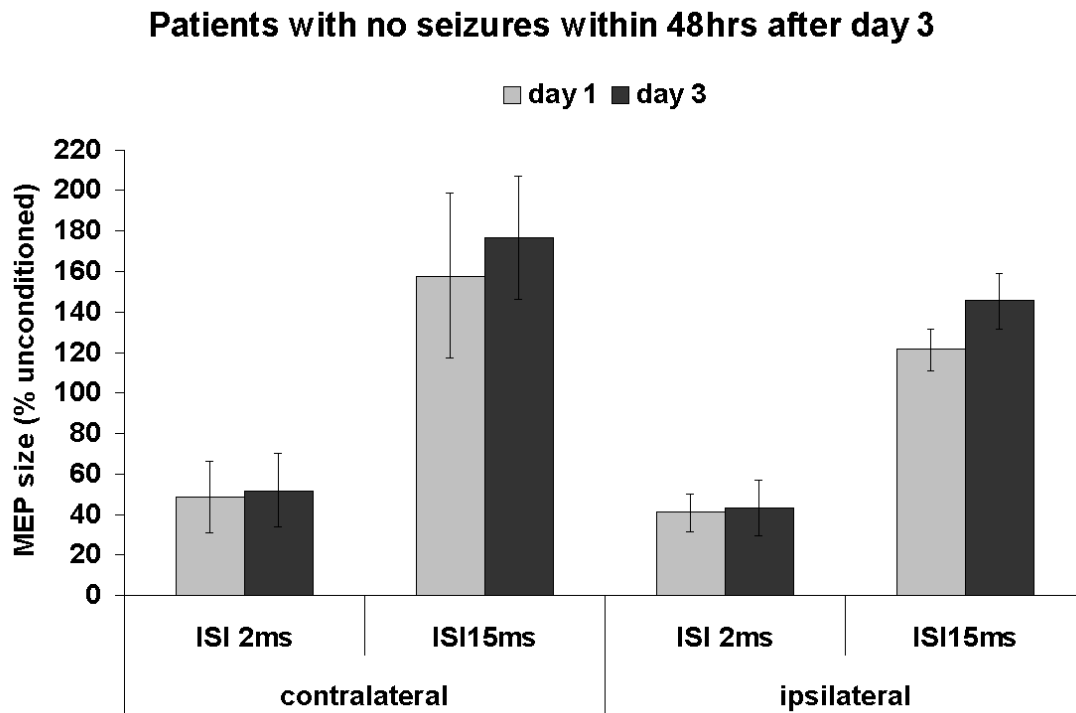


FIGURE 16: SICI AND SICF IN PATIENTS WITH NO SEIZURE WITHIN 48 HOURS AFTER DAY 3.

Patients who did not have seizures in the 48hrs following day 3 had stable SICI and SICF which did not change between day 1 and day 3 and was similar in both hemispheres.

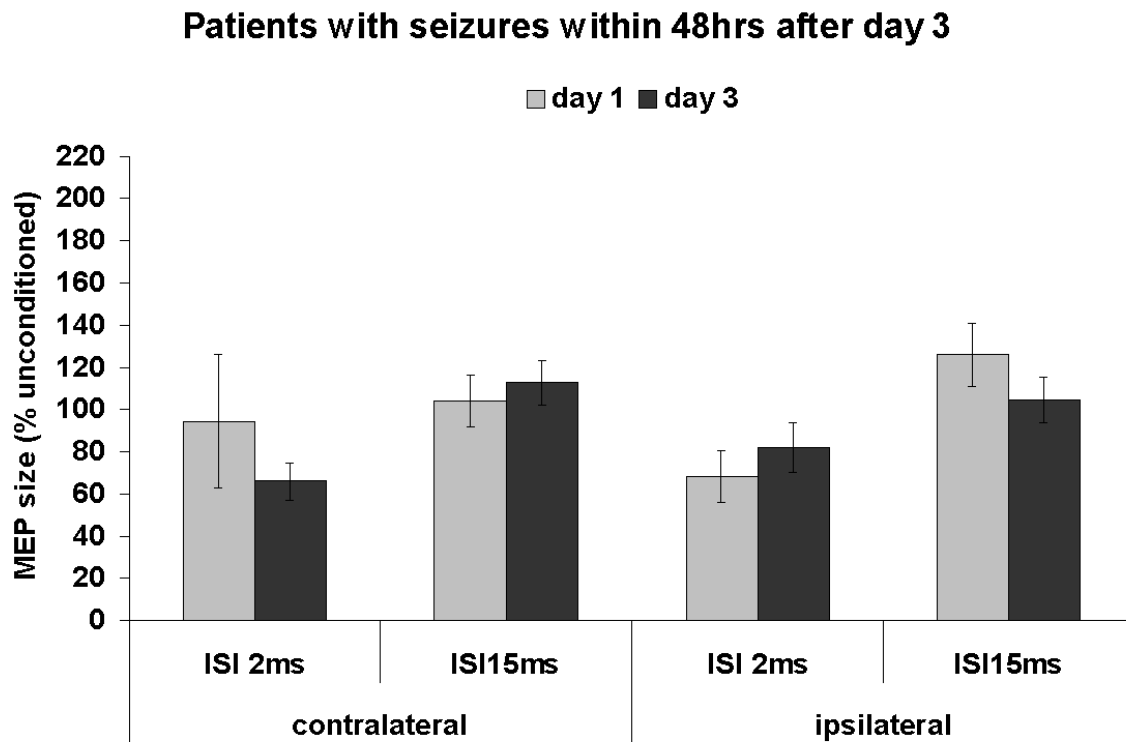


FIGURE 17: SICI AND SICF IN PATIENTS WITH SEIZURES WITHIN 48 HOURS AFTER DAY THREE

Patients who had seizures following day 3 had unstable SICI and SICF which changed between day 1 and day 3: in the contralateral hemisphere, relatively impaired SICI on day 1 became more normal on day 3; in the ipsilateral hemisphere, relatively normal SICI and SICF on day 1 became impaired on day 3

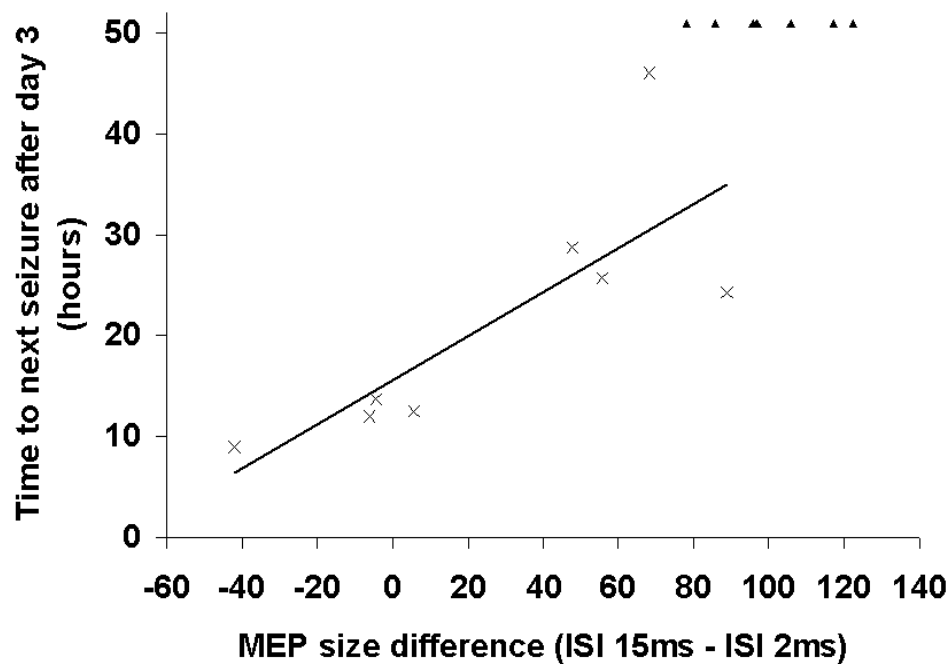


FIGURE 18: DIFFERENCE BETWEEN MEP SIZES CORRELATES WITH TIME UNTIL NEXT SEIZURE.

The difference between MEP sizes for SICI at 2 msec ISI and SICF at 15 msec ISI on day 3 in the ipsilateral hemisphere correlates with the time until the next seizure. This variable was most predictive of whether patients would have a seizure within 48 hours after day 3.

Crosses: patients having the next seizure within 48 hours of testing on day 3; triangles: patients having the next seizure beyond 48 hours. The linear regression line pertains only to the patients having seizures in the next 48 hours.

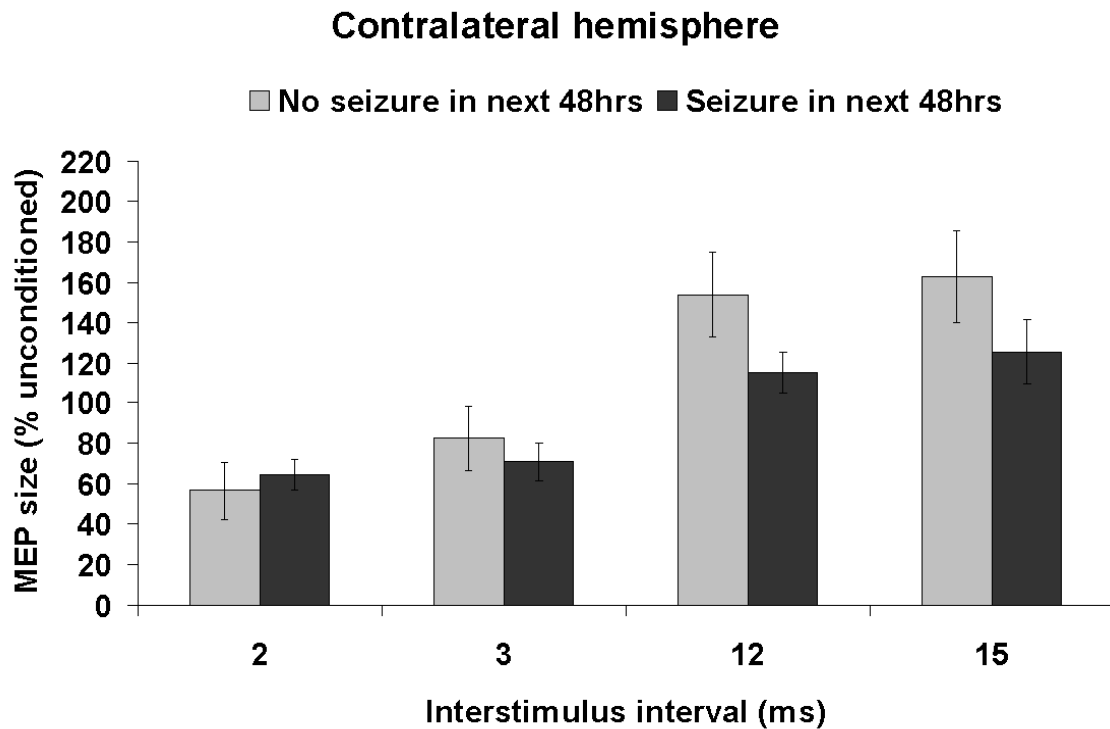


FIGURE 19: INTRACORTICAL INHIBITION AND INTRACORTICAL FACILITATION ON DAY 3 IN CONTRALATERAL HEMISPHERE, DIVIDED ACCORDING TO OCCURRENCE OF THE SUBSEQUENT SEIZURE.

Intracortical inhibition (SICI at ISIs of 2 and 3ms) and intracortical facilitation (SICF at ISIs of 12 and 15ms) on day 3 in the contralateral hemisphere, divided according to occurrence of the subsequent seizure.

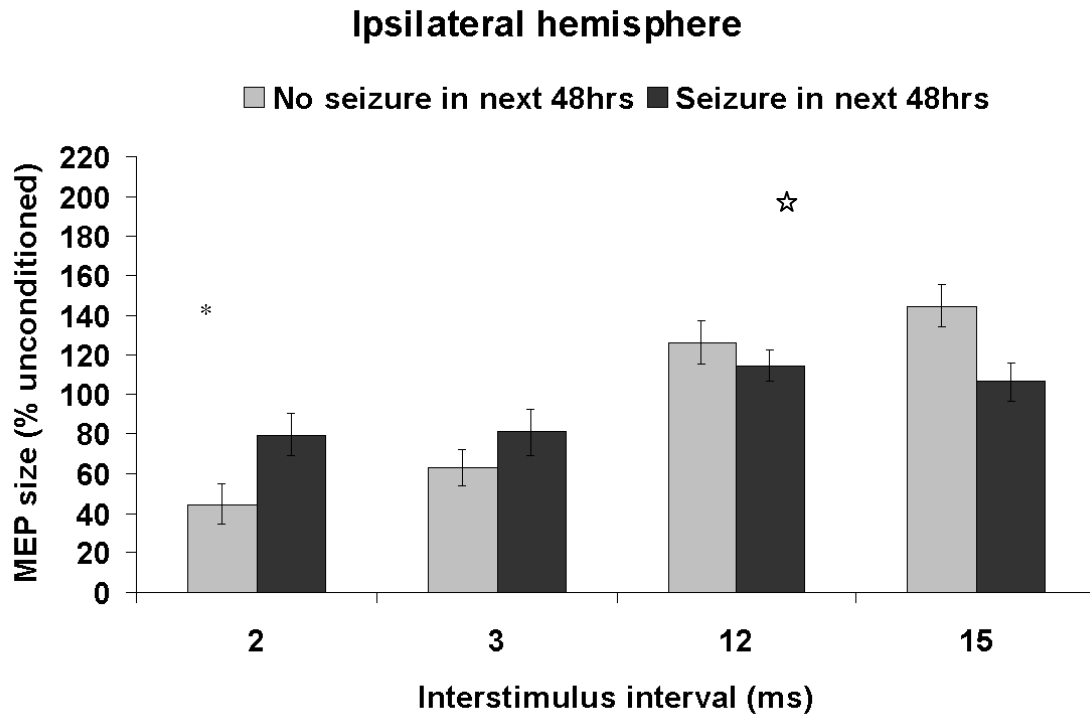


FIGURE 20: INTRACORTICAL INHIBITION AND FACILITATION IN IPSILATERAL HEMISPHERE.

Intracortical inhibition (SICI at ISIs of 2 and 3ms) and intracortical facilitation (SICF at ISIs of 12 and 15ms) on day 3 in ipsilateral hemisphere, divided according to occurrence of the subsequent seizure. There was impaired SICI at 2ms ISI and SICF at 15ms ISI in the ipsilateral hemisphere in patients having a seizure in the next 48hrs compared to patients who did not have a seizure in the next 48hrs.

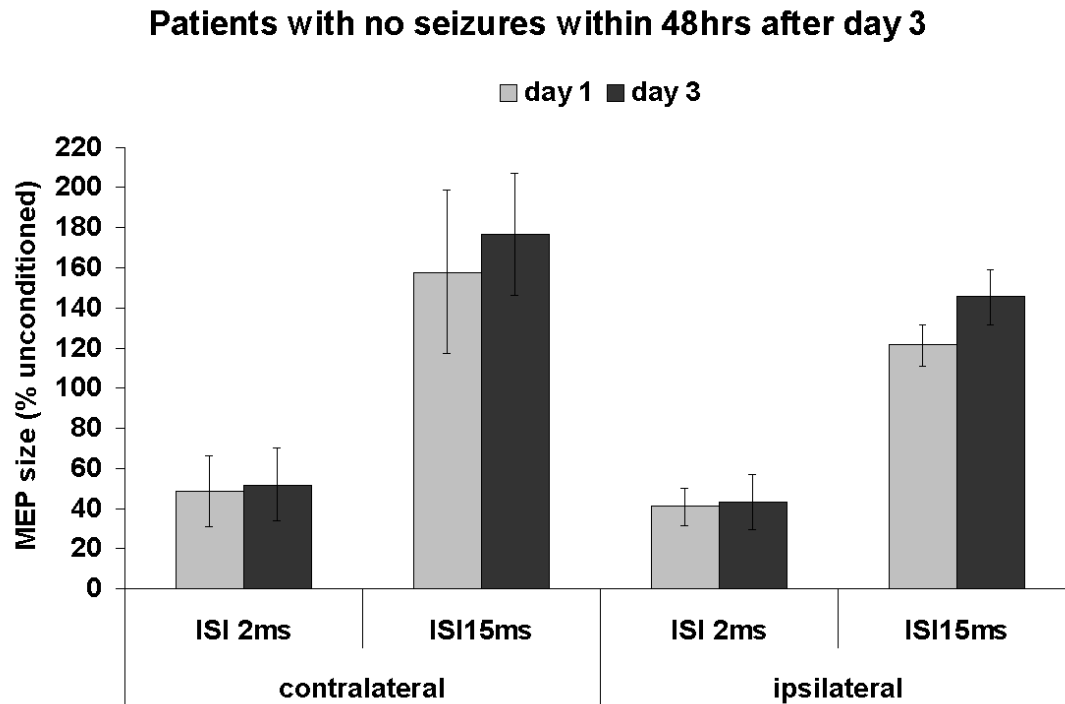


FIGURE 21: SICI AND SICF ON DAY 1 AND DAY 3, DIVIDED ACCORDING TO HEMISPHERE OF SEIZURE ONSET IN PATIENTS WHO DID NOT HAVE SEIZURES.

SICI at 2ms and SICF at 15ms on day 1 and day 3. Patients who did not have seizures in the 48hrs following day 3 had stable SICI and SICF which did not change between day 1 and day 3 and was similar in both hemispheres.

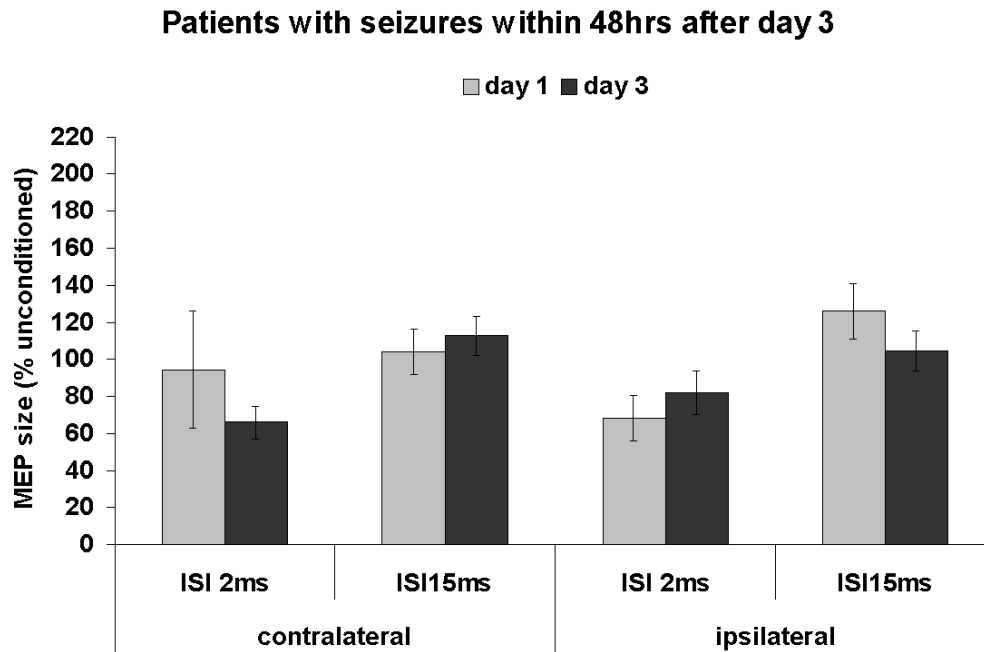


FIGURE 22: SICI AND SICF ON DAY 1 AND DAY 3, DIVIDED ACCORDING TO HEMISPHERE OF SEIZURE ONSET IN PATIENTS WHO DID HAVE SEIZURES.

SICI at 2ms and SICF at 15ms on day 1 and day 3. Patients who had seizures following day 3 had unstable SICI and SICF which changed between day 1 and day 3: in the contralateral hemisphere, relatively impaired SICI on day 1 became more normal on day 3; in the ipsilateral hemisphere, relatively normal SICI and SICF on day 1 became impaired on day 3.

4.3 DISCUSSION

The principal novel finding of this study is that the difference between SICI (at 2ms ISI) and SICF (at 15ms ISI), measured using TMS in the hemisphere ipsilateral to seizure onset, is highly correlated with the time until the next seizure in acutely AED-reduced patients with mTLE. The correlation spans a long period of time; at least 48hrs. This specific finding is supported by both hypothesis-led and data-led analyses of the same data – that is, the predictive value of ipsilateral SICI at 2ms and SICF at 15ms was found using an a priori ANOVA model, and by a multivariate discriminant function. A particular strength of this data is that we show these findings are related specifically to the time until the next seizure; the effect of the previous seizure was shown not to affect the findings by including time since previous seizure as a confounding covariate. Most published seizure-prediction studies have not taken previous seizures into account, so that seizure clustering may lead to effects of previous seizures confounding the effects due to an impending seizure. Furthermore, these findings relating to SICI and SICF are not explained by motor threshold changes. Additionally, the findings are not explained by pre-admission seizure frequency. Importantly, by measuring the same variables on two separate occasions (before and after AED-reduction), the findings are shown to be state-specific (i.e. relate to the acute state of being seizure-prone), since the same effects could not be demonstrated on day 1, prior to AED-reduction. It should be noted that 4 of the 18 patients studied (2 of the 15 patients studied on day 3) had seizures between days 1 and 3; excluding these patients from the analyses did not change the findings. The observation of loss of normal SICI and SICF prior to seizures is in accord with the fundamental hypothesis that seizures result from abnormal cortical excitability.

An unavoidable weakness of many clinical studies is that patients may be heterogeneous in terms of diagnosis and treatment. This study only included patients with a uniform clinical syndrome of mTLE, established by extensive investigation including MRI and seizure monitoring. However, it was not possible to find a group of subjects taking identical AED treatment; indeed it would be impossible to do so. Nonetheless, AED reduction in this study followed a uniform protocol and differential effects of AED reduction are unlikely to explain the findings; there was no correlation between parameters of drug reduction and seizure

occurrence, although our study was not powered to fully account for differential AED reduction effects. However, the observation that reduction of many different AED regimens results in a similar group finding could be regarded as a strength of the present study – the implication is that AED reduction resulted in facilitation of seizures via a common mechanism involving cortical excitability.

Previous studies have hinted at changes in TMS parameters that correlate with the occurrence of seizures and provide support for our findings. In a TMS study of the cortical silent period (CSP) in LRE, there was a weak non-significant negative correlation between the duration of the ipsilateral CSP and the time since the last seizure prior to TMS (Cincotta et al. 1998). In another study of LRE, those with higher seizure frequency showed weaker SICI and stronger SICF than those with fewer seizures, although of inconsistent lateralisation (Cantello et al. 2000). The present study has shown more consistent differences according to seizure occurrence, and also a dynamic change in cortical excitability between TMS measurement sessions which predicts subsequent seizures. A recent study of epilepsy patients treated with vagal nerve stimulation (VNS) examined SICI and SICF (Di Lazzaro et al. 2004); in this study, five patients were examined twice, with stimulator switched on and switched off. In the stimulator ‘on’ condition, there was a marked increase in SICI compared with the stimulator ‘off’ condition; there was no effect on SICF. Although small, this study adds weight to our argument that a loss of SICI is related to the increased likelihood of occurrence of seizures.

More recently, Badawy et al. (2009) demonstrated changes in cortical excitability in patients who underwent TMS recordings 24 hours before and after seizures. They studied 23 patients with IGE and 35 patients with focal epilepsy arising from either temporal, frontal or occipital lobes (i.e. non motor cortex onset). They demonstrated that in the 24 hours before a seizure, there is an increase in cortical excitability which is demonstrated by a decrease in motor threshold, an increase in intracortical facilitation and reduction in intracortical inhibition. Interestingly, these changes are bilateral in the patients with generalised seizures, whether primary or secondarily generalised, but are restricted to the ipsilateral hemisphere in patient with focal seizures who had complex partial seizures with secondary generalisation. In the patient with focal epilepsy and no generalised seizures, changes were also seen in the

contralateral hemisphere with an increase in motor threshold. In the 24 hours after a seizure, they found a significant decrease in cortical excitability measured with SICI and SICF in the ipsilateral hemisphere. In patients with focal epilepsy who had generalised seizures, there was also a change in the contralateral hemisphere with reduced excitability. Badawy et al. note that the preictal increase in excitability and postictal reduction in excitability is the same in patients with generalised seizures whether they have primary or secondary generalised seizures. In the patients with focal epilepsy, different changes are seen in the contralateral hemisphere when the seizure does not generalise. Badawy et al. suggest that this may represent a protective mechanism. Their study appears to show that there are fairly long lasting (at least 24 hour) transitional phases before and after seizures.

Not only has the present study suggested long-lasting pre-ictal changes in cortical excitability, but it also suggests that these changes in focal epilepsy are widespread, since the changes were detected in motor cortex while the seizure onset is assumed to be in the mesial temporal lobe. Techniques used to anticipate or predict seizures based on non-linear EEG analysis also suggest long-lasting widespread changes prior to seizures (Le Van Quyen M et al. 2000; Le Van Quyen M et al. 2001; Navarro V et al. 2002). Two kinds of hypotheses have been evolved in the context of this literature: firstly, that the preictal changes in EEG are due to subthreshold epileptic recruitment across a large-scale network; or, secondly, that the preictal state is not related to an epileptic process, but represents a brain state which facilitates the evolution of seizures. A possible candidate for this long-term facilitation process could be a decrease in remote inhibitory control of the epileptogenic zone (Depaulis et al. 1994) which might be paralleled by changes in inhibitory processes elsewhere in the brain, such as the motor cortex as seen in our study. Although the timescale of preictal changes detected by such EEG methods is shorter than that suggested by our data here, a recent study showed changes in cortical excitability prior to seizures over a similar timescale. This study by Kalitzin et al. (2005) used direct stimulation of the hippocampus during EEG recording via implanted depth electrodes to show that the phase clustering of certain frequency components exhibited changes across time. These changes correlated with the future occurrence of seizures; using a probability distribution method, an index of phase clustering was shown to reliably predict seizures, with changes in this index evolving over several tens of hours. The patient group, study setting, effects of AED reduction and time-course of preictal cortical excitability changes are very similar between this previous study and ours reported here.

In summary, this present study of mTLE patients has shown that:

- Reduction in AED dose, which subsequently results in seizures, is accompanied by a dynamic loss of SICI and increase of SICF in the ipsilateral hemisphere
- The magnitude of SICI and SICF in these seizure-prone AED-reduced patients correlates with the time to the next seizure.
- This preictal change observed is remote from the seizure focus and may cast light on the nature of widespread preictal brain states
- This may play a future role in seizure anticipation.

ID	Duration of epilepsy (years)	Imaging finding	Seizure type	Seizure frequency/month	Day 1 tests	Day 1 drug dose (mg)	Day 1 group	Day 3 tests	Day 3 drug dose (mg)	Day 3 group
1	12	R HS	CPS GTCS	1	RMT AMT ppTMS	LEV 750 OXC 1200 PHT 200	Aprev	RMT AMT ppTMS	LEV 250 OXC 300 PHT 50	Bnext
2	3	R HS	CPS GTCS	8	RMT AMT ppTMS	LEV 2000	Aprev	RMT AMT ppTMS	LEV 1000	Anext
3	22	L HS	CPS	2	RMT AMT ppTMS	LEV 1000 LTG 300	Aprev	RMT AMT ppTMS	LEV 500 LTG 150	Anext
4	33	RHS	CPS GTCS	2	RMT AMT ppTMS	LEV 500 CBZ 800 PHT 300	Bprev	RMT AMT ppTMS	LEV 250 CBZ 200 PHT 150	Bnext
5	22	R medial temporal DNET	CPS GTCS	3	RMT AMT ppTMS	CBZ 1200 LEV 1500	Bprev	RMT AMT ppTMS	CBZ 350 LEV 750	Anext
6	15	L HS	CPS	4	RMT AMT ppTMS	CBZ 800 VPA 1500	Bprev	RMT AMT ppTMS	CBZ 200 VPA 800	Bnext
7	30	L HS	CPS GTCS	14	RMT AMT ppTMS	LEV 500CLB 20	Aprev	RMT AMT ppTMS	LEV 250 CLB 10	Anext
8	31	R HS	CPS	6	RMT AMT ppTMS	VPA 2500g LTG 350	Bprev	RMT AMT ppTMS	LTG 100 VPA 1300	Anext
9	12	R HS	CPS GTCS	10	RMT AMT ppTMS	LEV 2000 CBZ 1600	Aprev	RMT AMT ppTMS	LEV 1000 CBZ 400	Bnext

ID	Duration of epilepsy (years)	Imaging finding	Seizure type	Seizure frequency/month	Day 1 tests	Day 1 drug dose (mg)	Day 1 group	Day 3 tests	Day 3 drug dose (mg)	Day 3 group
10	15	R HS	CPS	4	RMT AMT ppTMS	CBZ 600 LEV 2000	Bprev	RMT AMT ppTMS	CBZ 100 LEV 1000	Bnext
11	5	R amygdala tumour	CPS GTCS	2	RMT AMT ppTMS	LEV 2250 LTG 300	Bprev	RMT AMT ppTMS	LEV 750 LTG 100	Bnext
12	14	L HS	CPS GTCS	2	RMT AMT ppTMS	LEV 3000 CBZ 1400	Aprev	RMT AMT ppTMS	LEV 1500 CBZ 400	Bnext
13	7	L HS & L temporal DNT	CPS	8	RMT AMT ppTMS	CBZ 1200	Bprev	none	N/A	N/A
14	33	L HS	CPS	4	RMT AMT ppTMS	CBZ 1200 VGB 1750 LEV 500	Bprev	none	N/A	N/A
15	23	R HS	SPS CPS	8	RMT AMT	CBZ 1200 LTG 400	Bprev	RMT AMT ppTMS	CBZ 300 LTG 200	Anext
16	18	R HS	CPS	10	RMT AMT ppTMS	VPA 2000	Aprev	none	N/A	N/A
17	6	Normal	CPS	2	none	LTG 400 LEV 1000	N/A	RMT AMT ppTMS	LTG 200 LEV 1000	Bnext
18	17	L amygdala tumour	CPS	6	RMT AMT	LTG 350	Bprev	RMT AMT ppTMS	LTG 100	Anext

TABLE 3: DEMOGRAPHIC, CLINICAL AND EXPERIMENTAL DETAILS FOR ALL PATIENTS UNDERGOING PPTMS (CHAPTER 4).

CHAPTER 5: CORTICAL SILENT PERIOD

The aim of this study was to measure cortical silent period duration in patients with refractory mesial temporal lobe epilepsy, with measurements taken from both ipsilateral and contralateral hemispheres. Two measurements were made during a 3 day period. The hypothesis was that changes in seizure timing, both before, between and after TMS measurements may have a significant effect on CSP measurements. We also examined whether or not the CSP would be sensitive to changes in AED dosages.

5.1 METHODS

Subjects

The study included 29 subjects with refractory unilateral mesial temporal lobe epilepsy during routine pre-surgical admission to the Jules Thorn Telemetry Unit, National Hospital for Neurology and Neurosurgery, London UK. Twelve of these subjects were included in our previous study using paired pulse TMS (Wright et al. 2006) as described in chapter 4, and all subjects are included in chapter 3 on motor threshold changes in patients with epilepsy. The epilepsy syndrome and presumed hemisphere of seizure onset were determined on the basis of clinical assessment, EEG (interictal and, where possible, ictal), ictal video and neuroimaging data. Drug reduction was performed according to departmental protocol. Patients with any additional neurological or motor disorders, dual pathology underlying epilepsy, learning difficulties, or pregnancy were excluded. Patients with any in dwelling metallic objects such as cardiac pacemakers were also excluded. The study was approved by the institutional research ethics committee, and all subjects gave written informed consent.

Each subject was considered for antiepileptic drugs reduction following admission on to the telemetry unit, after giving informed consent. Drug reduction was carried out when deemed necessary to precipitate seizures, but was not performed in patients with frequent seizures or patients with recent secondary generalised seizures and/ or episodes of status epilepticus. The first TMS session took place on the day of admission at 3pm, prior to drug reduction. On the

first evening of admission, the dose of each AED was reduced by half according to the telemetry unit protocol, and there was usually a further halving of dose on the second evening where appropriate (i.e. to approximately 25% of original AED dose). No further reductions were made. The second TMS session took place on day 3 at 3pm. Medication was administered at 0800 and 2000 each day by staff on the unit. Since the timings of both drug administration and TMS session was kept fairly constant, there was little variation in the time between taking medication and the recordings.

TMS Recordings

All equipment was housed in mobile units, allowing data collection to take place at the patient bedside. Patients were seated in a comfortable armchair, and instructed to relax but remain alert throughout the recordings. EMG recordings were taken from the first dorsal interosseus muscle (FDI) of each hand in a belly tendon montage, using silver/silver chloride surface disc electrodes. EMG signals were filtered and amplified (CED 1902, Cambridge Electronic Design, Cambridge UK) using a bandwidth of 15-5000 Hz and a gain of 1000 and collected using Signal 4.05 software (Cambridge Electronic Design, Cambridge UK). A Magstim (Dyfedd, Wales, UK) figure-of-eight coil with a 9cm external loop diameter, connected to a Magstim 200 stimulator was used to deliver magnetic stimulation. The coil was positioned tangentially to the scalp with the handle pointing backwards at an angle of approximately 45° to the midline, resulting in a current flow of posterior to anterior across the motor cortex. The optimal site for stimulation of the FDI was established for each hemisphere in turn and was marked on the scalp. The mark stayed in place between days 1 and 3, thus ensuring accurate placement of the coil throughout both sessions. All patients were undergoing EEG monitoring at the time of the TMS study, and electrodes were removed when necessary in order to avoid heating of the electrodes and/or attenuation of the magnetic pulse. EEG recordings were paused during TMS recordings to minimise interference.

Motor Threshold

Before CSP measurements were taken, measurements of active and resting motor threshold (MT) were made since the stimulus intensity for CSP was based on the active motor threshold. Measurements of MT were taken from both hemispheres during both recording sessions; therefore the stimulus amplitude was adjusted accordingly between sessions and for each hemisphere. The technique for measuring motor threshold is provided in chapter 2. MT was determined in the FDI at rest when possible (resting motor threshold, RMT), and during voluntary contraction (active motor threshold, AMT). Visual feedback was provided during AMT measurement using an analogue display connected to a force transducer, which the patient squeezed between their thumb and first finger in order to keep the level of contraction of FDI constant at approximately 20% of maximum contraction.

Cortical Silent Period

CSP data was collected after motor threshold measurements, and after ppTMS measurements in a subgroup of 12 subjects. During measurement of CSP, magnetic stimulation was set at 120% of active motor threshold. The subject was instructed to hold the force transducer between thumb and first finger. The amplitude of maximal contraction was noted and thereafter the subject was instructed to maintain a tonic contraction at 20% of maxima. To help keep the level of contraction constant, both the subjects and operators were able to view an analogue display. Before stimulation was delivered, subjects were advised that the stimulation would cause a muscle contraction in the hand. A train of approximately 15 stimuli was then delivered, or as many necessary in order to have 12 clear MEP and silent period complexes for each hemisphere on each session.

The silent period was measured by eye with the placement of cursors, and all measurements were carried out by the same operator. Measurements were carried out in a random order with regard to ipsilateral and contralateral hemisphere, although the operator was not blinded. In-house software was used to measure silent period duration and MEP amplitude (NuCursor

software, Sobell Research, Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College of London, London, UK).

A second analysis was also carried out by a second investigator who had not been involved in data collection and was blind to patient group and outcome. This analysis used an automated method (King et al 2006), in which a cumulative sum of the rectified EMG was calculated; the end of the CSP is defined as the point where the gradient of the cumulative sum time series is zero. This automated method allows CSP to be calculated objectively.

Seizure Timing

Seizure timing was recorded in order to examine possible change in RMT and AMT following drug reduction, and the relationship between RMT and AMT and the occurrence of seizures. On day 1, the time since the last seizure (prior to admission), and to the following seizure was recorded, according to patients' prospectively recorded seizure diaries. Patients were assigned to one of two groups: Group Aprev included those who experienced a seizure more than 7 days before admission, group Bprev within less than 7 days before admission; the cut-off point of 7 days was selected as it provided a similar number of patients in each group. The time to the next seizure, and whether or not it occurred within the next 4 days (i.e. while still on the telemetry unit) was also recorded. Patients were assigned to one of two groups: Group Anext included those who experienced a seizure more than 4 days after day 1, group Bnext within less than 4 days after day 1. Similarly on day 3, the timing of seizures before and after, in the following categories was examined: Group Aprev included those who experienced a seizure more than 9 days before day 3, group Bprev within less than 9 days before day 3 (i.e. the cut-off date was the same as for day 1); Group Anext included those who experienced a seizure more than 2 days after day 3, group Bnext within less than 4 days after day 3 (i.e. again the cut-off date was the same as for day 1). A subject might move between categories between day 1 and day 3, because of the occurrence of any seizures between days 1 and 3.

Antiepileptic drug (AED) dose changes

23 of the 29 subjects underwent AED reduction, and the AED doses were recorded at each TMS session. This information is shown in table 1.

Calculation of CSP

4 different methods were used to calculate CSP:

1. Placement of cursors by eye, using the following criteria: first cursor at the beginning of MEP onset, second cursor at the point at which sustained, baseline level of EMG was returned. The MEP was included in the total duration to reduce error. Unclear complexes, with breakthrough EMG activity for example, were not included in measurement. The area of the MEP curve was also measured.
2. An automated computerised “cumulative sum” method to calculate the CSP duration (described by (King et al. 2006)
3. Calculation of CSP duration divided by MEP amplitude (CSP/MEP) using “by eye” CSP duration, justified by Orth and Rothwell’s findings that this measure is much less variable between subjects and between stimulus intensities (Orth and Rothwell 2004).
4. Calculation of CSP/MEP using automated CSP duration.

The mean was calculated from approximately 12 MEP and silent period complexes in each hemisphere and for each patient.

Method 4 was used for statistical testing. There was also post hoc exploration of the effects using the other 3 methods. The correlation between automated and “by eye” measurement of CSP duration and CSP/ MEP ration was also examined.

Statistical methods

It was anticipated that CSP measurements would be very variable, reducing the statistical power of the data, hence simple statistical models were deliberately used. Based on the

findings from the studies of paired-pulse and thresholds described in the previous sections, the following 4 hypotheses were tested:

1. Silent periods would be different between ipsilateral and contralateral hemispheres.

2. Silent periods would be affected by AED reduction.

- These two hypotheses were tested using repeated measures ANOVA with the within-subjects factors DAY (day 1, day 3) and SIDE (ipsilateral, contralateral).

3. On day 1, silent periods would be different between patients who had not had seizures in the preceding 7 days (Aprev) and patients who had seizures in this time period (Bprev); and that this effect might differ between hemispheres. This was tested using repeated measures ANOVA of day 1 data, with a within-subjects factor SIDE and a between-subjects factor PREVIOUS SEIZURE (Aprev, Bprev).

4. On day 3, silent periods would be different between patients who did not have seizures in the next 2 days (Anext) and patients who did go on to have seizures in this time period (Bnext); and that this effect might differ between hemispheres. This was tested using repeated measures ANOVA of day 3 data, with a within-subjects factor SIDE and a between-subjects factor NEXT SEIZURE (Anext, Bnext).

A significance threshold of $P < 0.05$ was used.

5.2 RESULTS

CSP ratio (automated method) (figure 20)

Hypotheses 1 and 2:

In the 17 patients with full data from both day 1 and day 3, there was no main effect of DAY ($F[1,16]=1.404$, $P=0.253$) or SIDE ($F[1,16]=2.882$, $P=0.109$), and no interaction between these ($F[1,16]=0.376$, $P=0.548$). Therefore CSP is very stable across the period of AED

reduction, and not different between ipsilateral and contralateral hemispheres for the group as a whole.

Hypothesis 3:

In the 27 patients with full data for day 1, there was no significant main effect for either PREVIOUS SEIZURE or SIDE, but there was an interaction between these ($F[1,25]=6.236$, $P=0.019$). This suggests that CSP/MEP ratio is affected differently by recent seizures in the ipsilateral and contralateral hemispheres. Post hoc analyses was used to examine whether this could be accounted for by the intensity of stimulation used; therefore ipsilateral and contralateral AMTs were included as covariates in the model. The effect was not altered ($F[1,22]=7.435$, $P=0.012$). In addition to AMTs, we also included the factor NEXT SEIZURE in the model, but the effect was unchanged ($F[1,20]=6.380$, $P=0.020$). Therefore the conclusion is that the interaction between PREVIOUS SEIZURE and SIDE is not explained by differences in AMT between Aprev and Bprev groups, and nor is it accounted for by future seizure occurrence.

A post hoc T-test showed that this effect is explained by a longer silent period on the contralateral side in patients having the most recent seizure more than 7 days ago ($T = 2.402$, $P=0.024$).

Hypothesis 4:

There were no significant effects of NEXT SEIZURE.

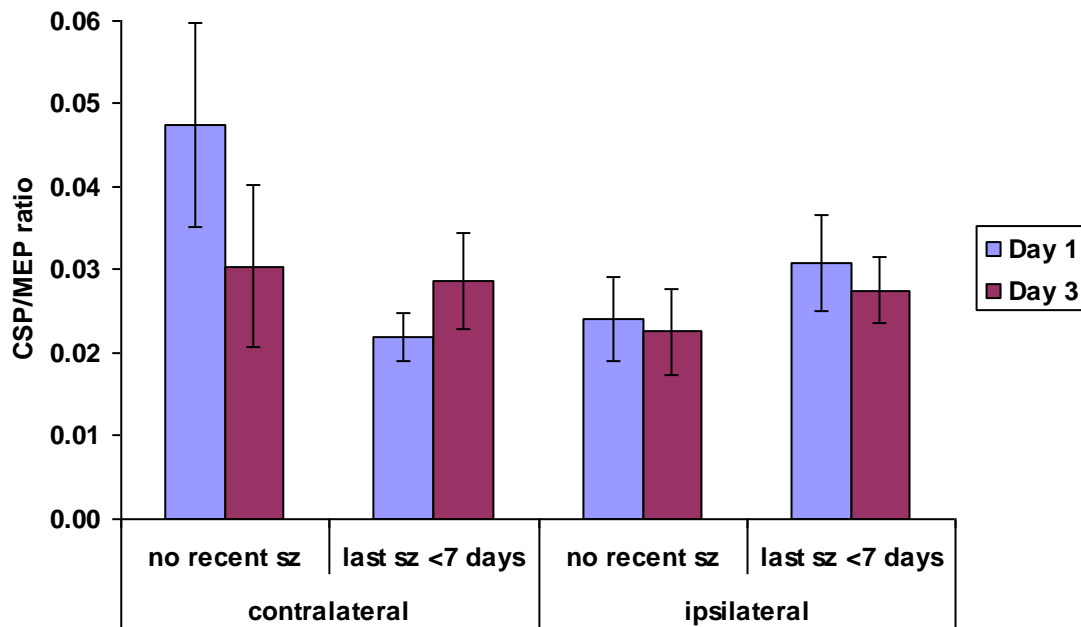


FIGURE 22: AUTOMATED MEASUREMENT OF CSP/MEP RATIO (S/MV) FOR ALL 29 SUBJECTS, DIVIDED ACCORDING TO SIDE AND PREVIOUS SEIZURE; DATA ARE SHOWN FOR DAY 1 AND DAY 3.

CSP duration (automated method)

Hypotheses 1 and 2:

In the 17 patients with full data from both day 1 and day 3, there was no main effect of DAY ($F[1,16]=0.333$, $P=0.572$) or SIDE ($F[1,16]=0.313$, $P=0.584$), and no interaction between these. Therefore CSP is very stable across the period of AED reduction, and not different between ipsilateral and contralateral hemispheres for the group as a whole.

Hypothesis 3:

In the 27 patients with full data for day 1, there was no significant main effect for either PREVIOUS SEIZURE or SIDE, and the interaction between these showed only a trend ($F[1,25]=3.006$, $P=0.095$).

Hypothesis 4:

There were no significant effects of NEXT SEIZURE.

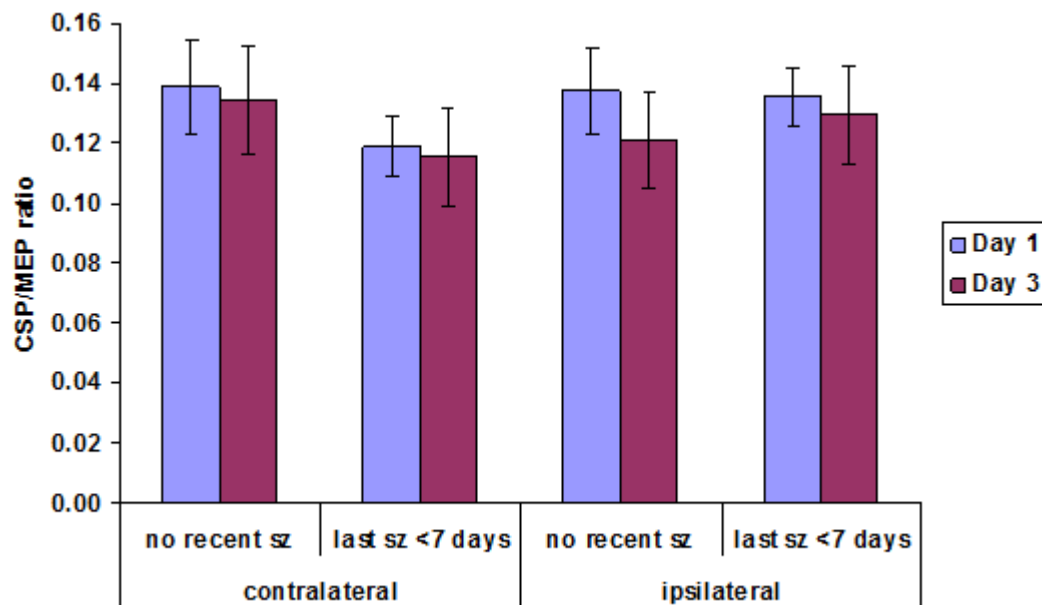


FIGURE 23: AUTOMATED MEASUREMENT OF CSP DURATION (SECONDS) FOR ALL 29 SUBJECTS, DIVIDED ACCORDING TO SIDE AND PREVIOUS SEIZURE; DATA ARE SHOWN FOR DAY 1 AND DAY 3.

CSP duration (“by eye” method)

Correlations between automated and “by eye” methods

Figure 11 shows automated measurement of CSP duration plotted against “by eye” measurement. The measures are correlated well above chance (Pearson $R=0.279$, $P=0.021$).

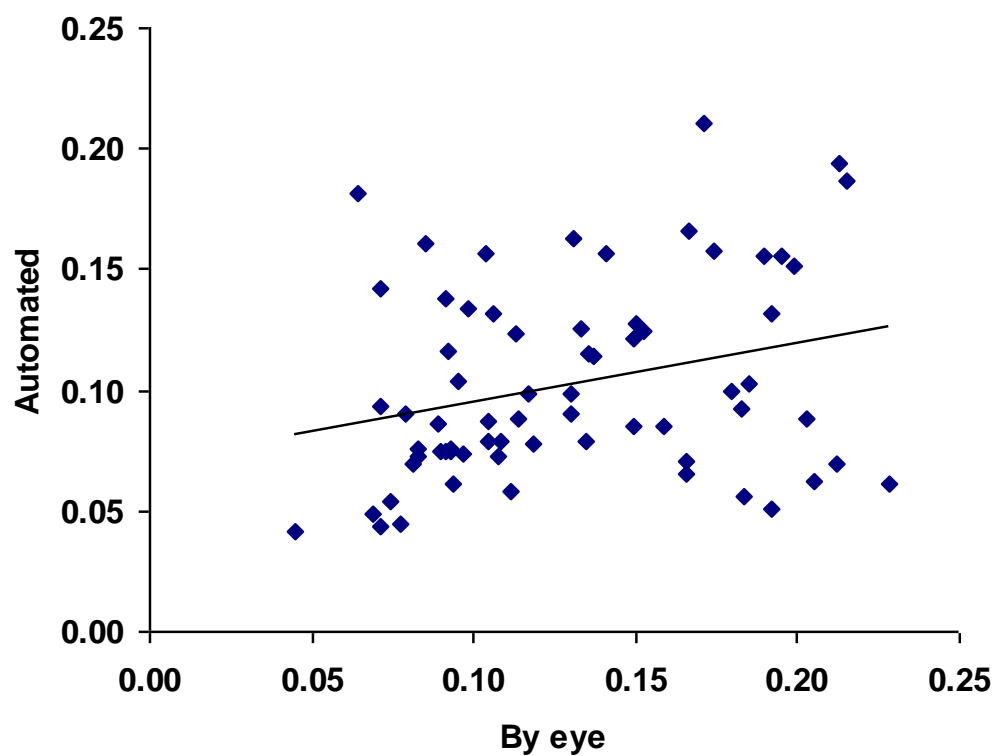


FIGURE 24: AUTOMATED MEASUREMENT OF CSP DURATION PLOTTED AGAINST “BY EYE” MEASUREMENT (BOTH MEASURED IN SECONDS). A LINEAR REGRESSION LINE IS PLOTTED.

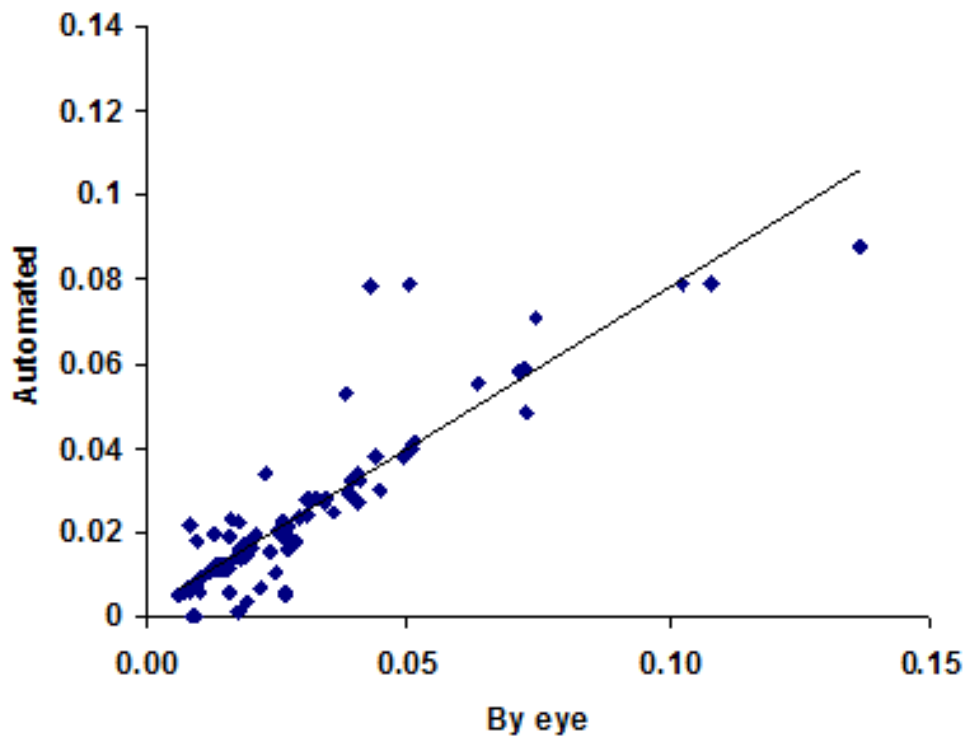


FIGURE 25: SHOWS AUTOMATED MEASUREMENT OF CSP/MEP RATIO PLOTTED AGAINST “BY EYE” MEASUREMENT (BOTH MEASURED IN S/MV). A LINEAR REGRESSION LINE IS PLOTTED. THE MEASURES ARE HIGHLY CORRELATED (PEARSON $R=0.834$, $P<0.001$).

It is very obvious that measures are clustered towards the origin, with scattered outliers. Therefore these are plotted in Figure 23 on log-log axes, which redistribute the data points more evenly. A very good correlation is still apparent.

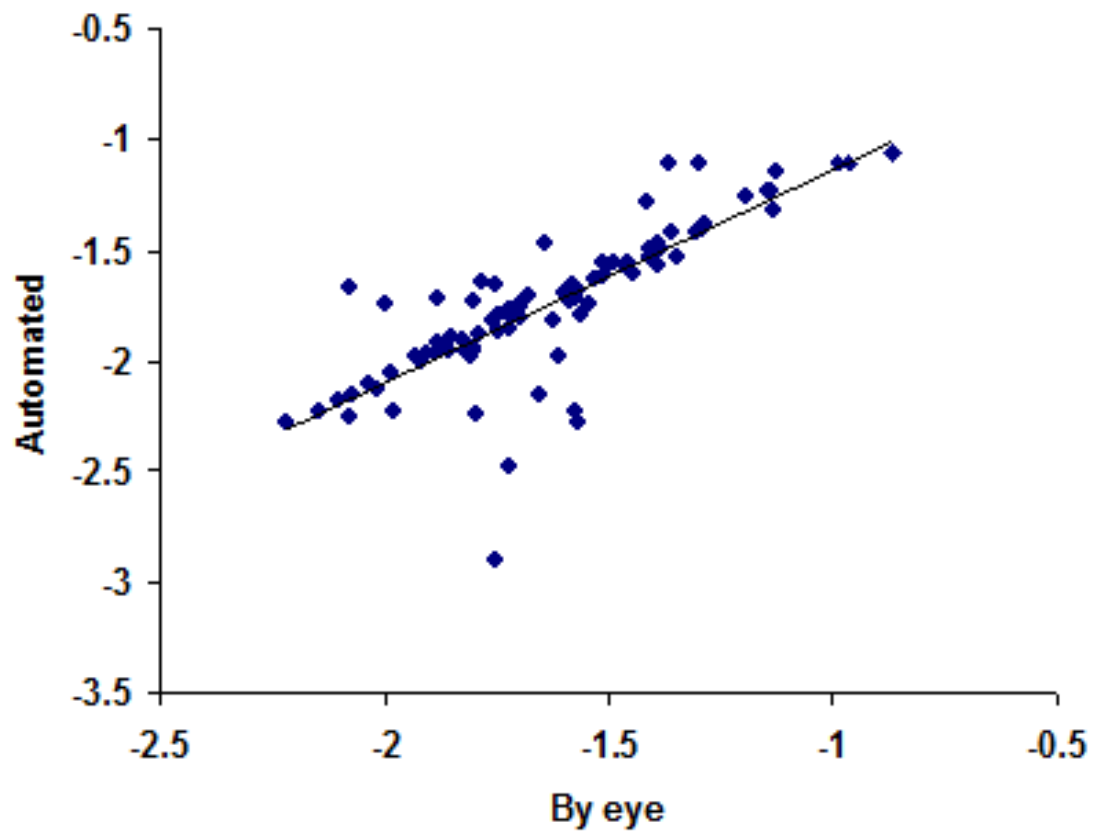


FIGURE 26: AUTOMATED MEASUREMENT OF CSP/MEP RATIO PLOTTED AGAINST “BY EYE” MEASUREMENT, USING THE LOGARITHM OF THESE MEASUREMENTS (BOTH MEASURED IN S/MV). A LINEAR REGRESSION LINE IS PLOTTED.

Variability of measures

Means, standard deviations (SD) and coefficients of variation (SD/mean) were calculated for the “by eye” measurements of CSP duration and CSP/MEP ratio; for the automated measurements; and for the logarithms of automated measurements (table 1). The log values were least variable; and the automated methods most variable.

Measure	Units	Method	Mean	SD	CV
duration	seconds	by eye	0.102	0.041	0.399
duration	seconds	automated	0.129	0.046	0.359
ratio (CSP/MEP)	seconds/mV	by eye	0.024	0.019	0.804
ratio (CSP/MEP)	seconds/mV	automated	0.029	0.022	0.781
log ratio (CSP/MEP)	seconds/mV	by eye	-1.739	0.34	0.195
log ratio (CSP/MEP)	seconds/mV	automated	-1.629	0.283	0.173

TABLE 4: MEAN, STANDARD DEVIATION (SD) AND COEFFICIENT OF VARIATION (CV) OF THE VARIOUS CSP MEASUREMENTS.

Logarithm of CSP ratio (automated method)

Given that the log of CSP/MEP measured using the automated method was least variable, appeared normally distributed, and was strongly correlated with log of “by eye” CSP/MEP ratio, a final posthoc analysis was performed to address hypotheses 1-4 using log CSP ratio (automated method). These results were essentially identical to the first analysis, confirming that effects were not carried by outliers.

Hypotheses 1 and 2:

In the 17 patients with full data from both day 1 and day 3, there was no main effect of DAY ($F[1,16]=1.074$, $P=0.315$) or SIDE ($F[1,16]=3.638$, $P=0.075$), and no interaction between these ($F[1,16]=0.054$, $P=0.820$). Therefore CSP is very stable across the period of AED reduction, and not different between ipsilateral and contralateral hemispheres for the group as a whole.

Hypothesis 3:

In the 27 patients with full data for day 1, there was no significant main effect for either PREVIOUS SEIZURE or SIDE, but there was an interaction between these ($F[1,25]=5.940$, $P=0.022$). This suggests that CSP/MEP ratio is affected differently by recent seizures in the ipsilateral and contralateral hemispheres. In post hoc analyses we examined whether this could be accounted for by the intensity of stimulation used; therefore we included ipsilateral and contralateral AMTs as covariates in the model. The effect was not altered ($F[1,22]=6.683$, $P=0.017$). In addition to AMTs, we also included the factor NEXT SEIZURE in the model, but the effect was unchanged ($F[1,20]=4.566$, $P=0.045$). Therefore we conclude that the interaction between PREVIOUS SEIZURE and SIDE is not explained by differences in AMT between Aprev and Bprev groups, and nor is it accounted for by future seizure occurrence.

A post hoc T-test showed that this effect is explained by a longer silent period on the contralateral side in patients having the most recent seizure more than 7 days ago ($T=2.360$, $P=0.0246$).

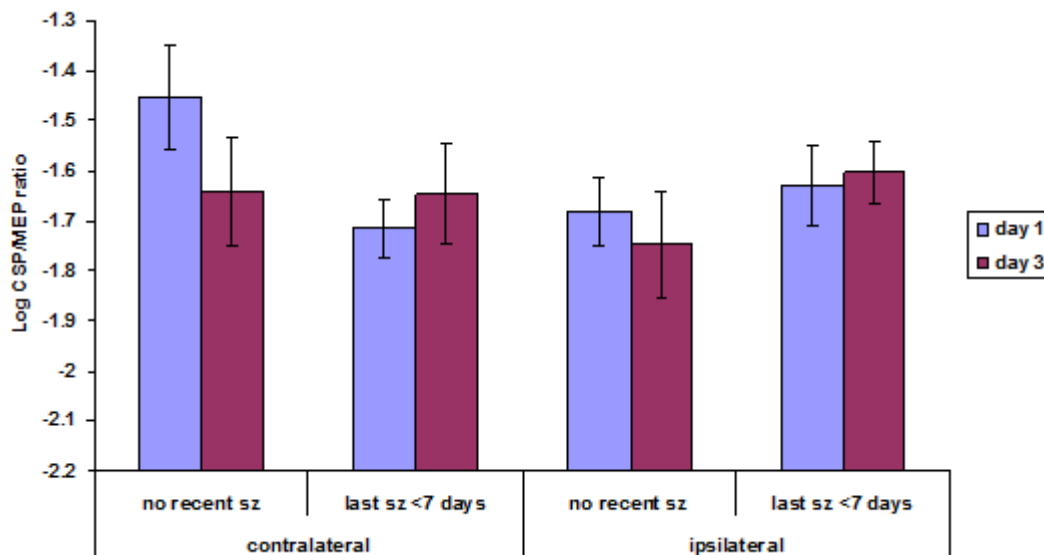


FIGURE 27: FIGURE 26: COMPARING THE LOG OF CSP/MEP RATIO IN BOTH HEMISPHERES. THERE WERE NO SIGNIFICANT EFFECTS OF NEXT SEIZURE (HYPOTHESIS 4).

5.3 DISCUSSION

In this chapter, various measurement methods for CSP were explored. A priori, the CSP/MEP ratio as proposed by Orth and Rothwell, coupled with the automated cumulative sum CSP measurement methods of King et al (2006) was chosen. This was expected to provide the most reproducible and objective measures, and maximise the possibility to detect differences between groups of patients. This was indeed the case, with the CSP/MEP ratio method showing a significant effect, whereas CSP duration showed only a trend.

The principal finding in this section was that CSP/MEP was greater in the contralateral hemisphere of patients who had seizures more than 7 days ago compared to patients whose seizures were more recent. From these data it cannot be certain whether the long CSP/MEP ratio is “more normal”, or reflects a pathological process specific to the contralateral hemisphere, or reflects a compensatory process which is effective in the contralateral hemisphere but fails in the ipsilateral hemisphere. Speculatively, this latter explanation is preferred. Normal control data would be needed to address this question.

The effects of recent seizures on CSP were independent of the stimulus intensity used. Indeed, we found CSP duration and CSP/MEP ratio to be very stable during this experiment, with no change in CSP or CSP/MEP ratio despite drastic changes in AED doses. This is substantially different from the effects of AED reduction on MT.

Automated measures detected an interaction between the timing of previous seizure and hemisphere, whereas “by eye” measures did not,

CSP/MEP ratio was quite variable with some outliers with high ratio (see figure 4). Log data were much better distributed (see Figure 5). Log CSP/MEP data showed exactly the same effects as CSP/MEP data, suggesting the effects were not carried by these outliers with high CSP/MEP ratios.

ID	Sex	Age	Sz Type	Aetiology	Treatment Day 1	Treatment Day 3
1	M	41	CPS	L HS, ?DNET	CBZ 1200	CBZ 1200
2	M	41	CPS, GTCS	R HS	CBZ 1200 PGB 170 LMT 250	CBZ 600 PGB 75 LMT 125
3	F	47	CPS	L HS	CBZ 1000 TPM 125	CBZ 1000 TPM 125
4	F	65	CPS, GTCS	R HS	TPM 300 CBZ 1400	TPM 75 CBZ 400
5	F	34	CPS	R HS	LVT 2000 OXC 1200 CLB 10	LVT 2000 OXC 1200 CLB 10
6	F	24	CPS, GTCS	R HS	LVT 750 OXC 1200 PHT 200	LVT 250 OXC 300 PHT 50
7	F	21	CPS	R HS	LMT 250 TPM 200	LMT 150 TPM 100
8	M	27	CPS, GTCS	R HS	LVT 2000	LVT 1000
9	F	28	CPS, GTCS	L HS	VPA 1800 LVT 1500	VPA 900 LVT 750
10	M	33	CPS, GTCS	L HS	CBZ 1600 LVT 3000	CBZ 800 LVT 1500
11	F	45	CPS, GTCS	R mesial DNT	CBZ 1200 LVT 1500	CBZ 350 LVT 750
12	M	23	CPS	L HS	CBZ 800 VPA 1000	CBZ 200 VPA 800
13	F	30	SPS	R HS	LMT 600	LMT 150
14	M	37	CPS	Normal Imaging	CBZ 1600 VPA 800 CLB 10	CBZ 400 VPA 400 CLB 0
15	F	41	CPS	L HS	CBZ 1200 VGB 1750 LVT 250	CBZ 300 VGB 1000 LVT 0
16	M	35	CPS, GTCS	L HS	LVT 500 CLB 20	LVT 250 CLB 10
17	M	22	CPS	L HS	LVT 3000 OXC 1800	LVT 1500 OXC 900
18	M	49	CPS	Normal Imaging	LVT 3500 LMT 500	LVT 3500 LMT 500
19	F	59	CPS	L temporal DNT	CBZ 900 TPM 250	CBZ 250 TPM 75

ID	Sex	Age	Sz Type	Aetiology	Treatment Day 1	Treatment Day 3
20	M	30	CPS	R HS	VPA 2000	VPA 2000
21	M	45	CPS, GTCS	R HS	VPA 2500 LMT 350	VPA 1300 LMT 100
22	M	27	CPS	Normal Imaging	LMT 400 LVT 1000	LMT 200 LVT 1000
23	M	31	CPS, GTCS	R amygdala tumour	LVT 3000 LMT 400	LVT 750 LMT 100
24	M	59	CPS	R cavernoma	LVT 1000 PHT 300	LVT 500 PHT 75
25	M	52	CPS, GTCS	R HS	LVT 3000 CBZ 1400	LVT 750 CBZ 350
26	M	53	CPS, GTCS	L HS	LVT 3000 CBZ 1400	LVT 1500 CBZ 400
27	M	38	CPS	R HS	LVT 1000 CBZ SR 1200	LVT 1000 CBZ SR 1200
28	M	43	CPS, GTCS	L HS	VPA 1600	VPA 800
29	M	31	CPS, GTCS	R HS	LVT 1800 CBZ 800	LVT 400 CBZ 400

TABLE 5: PATIENT DEMOGRAPHIC DATA FOR CSP STUDY

Patient demographic data for CSP study, including seizure types, imaging data and drug doses on 2 days of measurement. CBZ = carbamazepine; CLB = clobazam; CPS = complex partial seizure; DNT = dysembryoplastic neuroepithelial tumour; F = female, GTCS = generalised tonic clonic seizure; HS = hippocampal sclerosis; L = left; LVT = levetiracetam; M = male; OXC = oxcarbazepine; PHT = phenytoin = right; VPA = valproic acid.

CHAPTER 6: COMPARING CORTICAL EXCITABILITY IN PATIENTS WITH MTLE VERSUS OTHER PARTIAL EPILEPSIES

The aim of this section was to compare data between patients with mTLE (for which the data was included in previous chapters) and data from patients with neocortical epilepsies and controls. Data for some of the neocortical epilepsy patients and controls were acquired by Dr. Michael Orth whilst working at the Sobell Department of Motor Neuroscience and Movement Disorders, UCL, and are included in this thesis with his permission. Dr. Orth collected data with the assistance of my supervisor Mark Richardson, who ensured identical data collection methods and quality control between investigators. The data analyses here are the first analyses using these data, and all findings are novel and not previously described elsewhere.

6.1 METHODS

Data was collected from 20 patients with neocortical epilepsies, 36 patients with mesial temporal lobe epilepsy and 11 normal controls. Of the neocortical patients, 10 patients were male, and the average age was 34.6 (range 24-60) (table 5). 15 of these patients had normal MRI and localising and lateralising information was then compiled from each individual's seizure semiology and interictal/ ictal EEG where available. In three patients, additional lateralising data was provided by studies including MEG, ictal SPECT and EEG-fMRI. All 20 of these patients had either neocortical temporal or frontal lobe epilepsies and all were taking at least one AED. In four patients, the ictal onset zone could not be lateralised. In the remaining 16 patients, 11 had left sided onset and 5 had right sided onset. Of the patients with mesial temporal lobe epilepsy, 22 were male, and the average age was 37 (range 21-65). 16 patients had left temporal lobe epilepsy and the remainder had right TLE.

All patients and control subjects underwent the following measurements: RMT, AMT, and paired pulse measurement of SICI and SICF. Measurements were taken from both hemispheres. The methodology for measurement of each of these parameters is as described in previous chapters. For the mTLE patients, all data was collected at the patient bedside. For

the patients with neocortical epilepsy and the normal controls, measurements were made in a specialist TMS lab using the same type of Magstim equipment and the same techniques. The inpatient data was taken from recordings made on day 1 (in most cases, this was prior to any drug reduction).

Measurements for SICI with conditioning pulses at 80% and 90% were averaged, and measurements at 2ms and 3ms were averaged – i.e. all the SICI data were averaged together to give one “SICI” value per hemisphere.

Measurements for SICF with conditioning pulses at 80% and 90% were also averaged, and measurements at 12ms and 15ms were averaged, to give one “ICF” value per hemisphere.

6.2 RESULTS

First analysis

Measures between ipsilateral and contralateral hemispheres were compared for all patients whose seizure onsets could be lateralised using paired T-tests.

There were no significant differences between ipsilateral and contralateral hemisphere data for the following: RMT, AMT, SICI, SICF (all P –values > 0.5)

There were also no significant differences between ipsilateral and contralateral hemisphere data for RMT, AMT, SICI, or SICF considering the mTLE group alone or for the neocortical group alone.

Similarly, there were no significant differences comparing left and right hemispheres (as opposed to ipsilateral versus contralateral) for any of the measures; this was the case for the whole group, as well as for mTLE group alone and neocortical group alone.

Second Analysis

Having found no difference between hemispheres – either left versus right or ipsilateral versus contralateral – patients and normal controls were then compared. Because the measurements of RMT, AMT, SICI, and SICF were no different between hemispheres, the data between hemispheres was averaged (i.e. each subject therefore has just one average

value for RMT, AMT, SICI, SICF). This method allows direct comparison of data between patients and normal subjects (who of course do not have ipsilateral or contralateral hemispheres); and also allows the inclusion all subjects, including those neocortical patients who lack lateralising data.

A comparison was then made of the entire patient group versus normal subjects using unpaired t-tests regarding $p < 0.05$ as significant. Because 4 T-tests were performed (one each for RMT, AMT, ICI, ICF), a Bonferroni correction was used to avoid false-positives. Using this correction, the significance level was $0.05/4 = 0.0125$.

RMT and AMT were significantly different between patients and normal subjects (uncorrected $p < 0.001$ in both instances). RMT and AMT were higher in patients compared to control subjects. SICI was significantly reduced in patients (uncorrected $p = 0.0070$). SICF was not different between patients and normal subjects (uncorrected $p = 0.44$).

Because RMT and AMT and SICI were different between patient and normal groups, possible correlations between SICI and RMT or AMT which might explain this difference were explored. SICF was not correlated with either RMT or AMT, nor was SICI correlated with AMT, but SICI was weakly correlated with RMT (Pearson's $R = 0.386$). Therefore, there was post hoc comparison of SICI between patients and normal using a univariate ANOVA, including RMT and AMT in the model. The difference between patients and normal was still significant ($P = 0.0164$).

The conclusion is therefore that SICI is relatively reduced in patients; that it is not different between hemispheres; and that it is not explained by any correlation between SICI and MT.

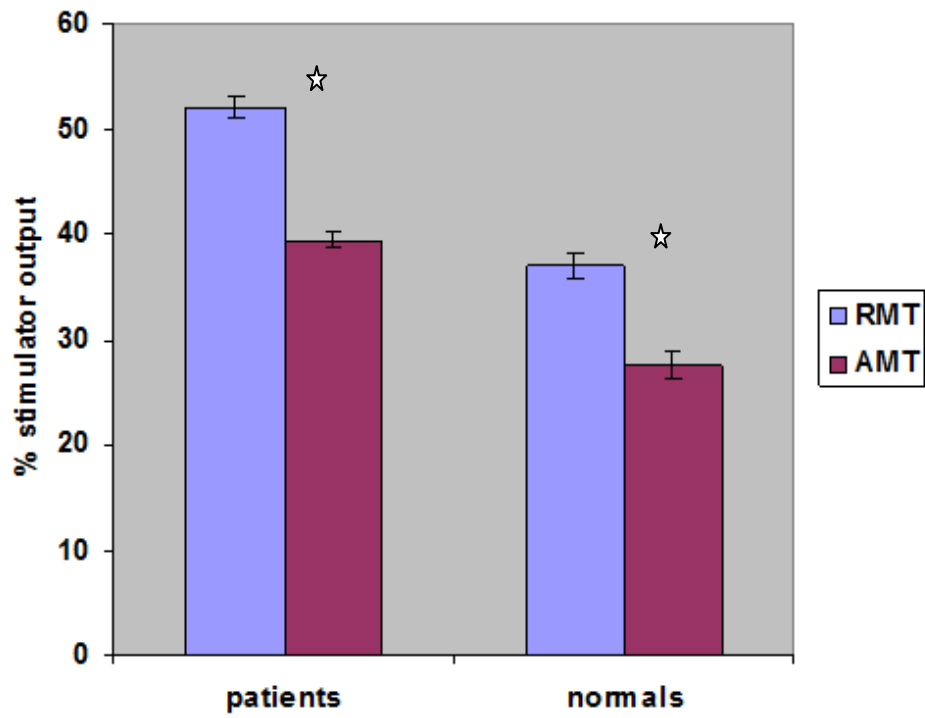


FIGURE 28:MT IN NORMAL CONTROLS AND ENTIRE PATIENT GROUP.

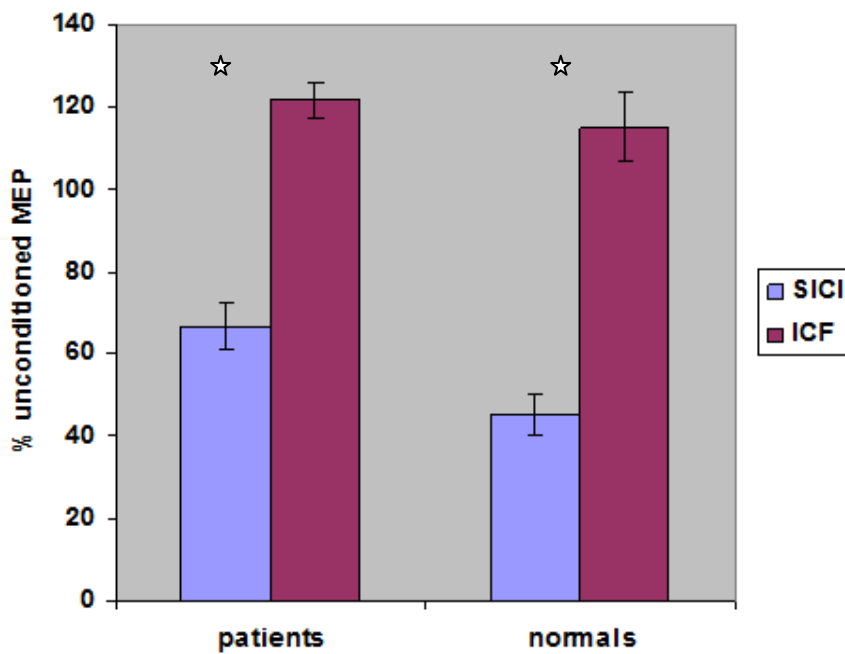


FIGURE 29: SICI AND SICF IN ENTIRE PATIENT GROUP

Third Analysis

Next compared the three groups were compared (normal subjects, neocortical patients, mTLE patients) using ANOVA, which included 3 groups (normal, neocortical, mTLE) and all 4 measures (RMT, AMT, SICI, SICF). A post hoc correction for multiple comparisons was applied (Least Significant Difference test).

This showed:

- 1) Significant main effects for RMT and AMT (both $p < 0.001$) explained by normal subjects having lower MTs than either patient group (all comparisons $p < 0.001$); no differences between patient groups (Figure 27).
- 2) Significant main effect for SICF ($p = 0.046$) explained by higher SICF in the mTLE group compared to the neocortical group ($p = 0.019$; figure 30).

There was a trend towards a significant difference in SICI ($p = 0.088$), explained by less SICI in mTLE compared to normal subjects ($p = 0.029$); there was no difference in SICI between the patient groups. (Figure 30).

In conclusion, although this supports the finding above of a difference between SICI in patient and controls, it shows that this effect might be more marked in the mTLE group. It also shows that the two patient groups differ in SICF.

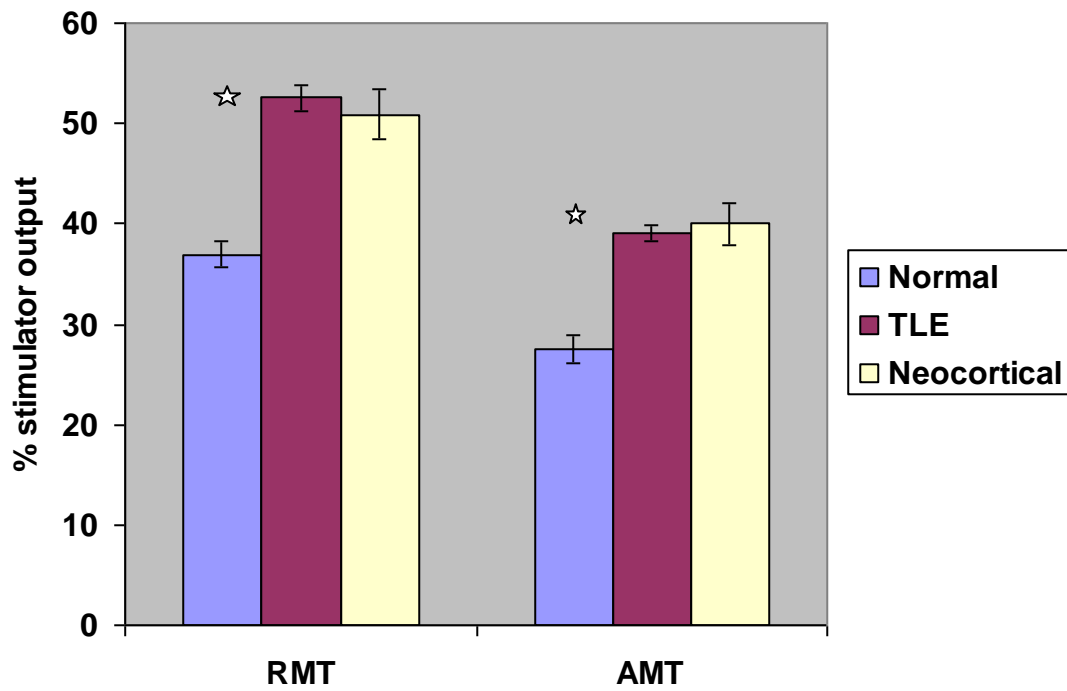


FIGURE 30: MT IN THE TWO PATIENT GROUPS AND NORMAL SUBJECTS.

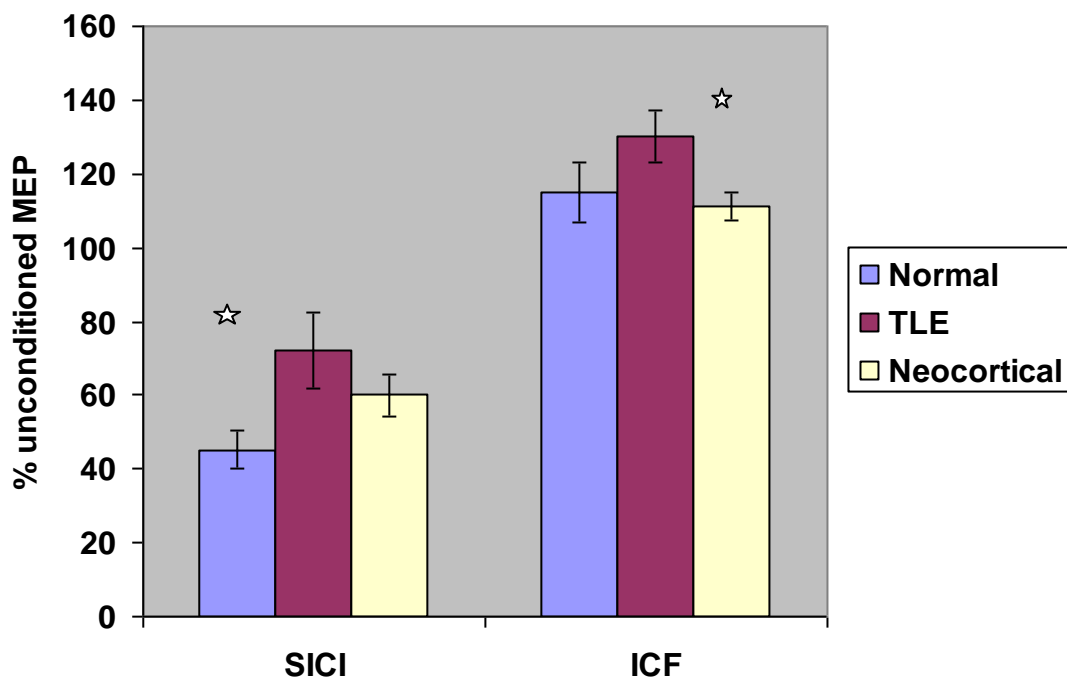


FIGURE 31: SICI AND SICF IN THE TWO PATIENT GROUPS AND NORMAL SUBJECTS.

6.3 DISCUSSION

This study comparing cortical excitability measures between control subjects, patients with mesial temporal lobe epilepsy and neocortical epilepsy has shown significant differences between patients and controls.

The changes in motor threshold are almost certainly due to the effects of antiepileptic medication, which is known to increase both active and resting motor threshold (Ziemann et al. 1996a). There were no changes in any measures in the patient group when comparing hemispheres as ipsilateral versus or contralateral or right versus left.

Significant differences were found comparing SICI and SICF between patients and normal controls with a reduction in SICI in patients suggesting impaired cortical inhibition in these patients. Comparing the mTLE and neocortical patient groups, there was higher SICF in the mTLE group and a trend towards a decrease in SICI.

It was not possible to factor in the timing of previous seizures in this section as the data was not available for the neocortical group.

The differences between the neocortical and mTLE groups demonstrate that epilepsies arising from distinct areas of the brain effect cortical excitability measured from the motor cortex in different ways. The majority of the patients in the neocortical group had epilepsy arising from frontal regions, with a minority having temporal lobe epilepsy **not** arising from mesial structures. In this study, the timing of previous or next seizure was not taken into account as the data was not available for the neocortical group. As demonstrated in chapter 4, the timing of seizures before and after TMS measurements are made can affect cortical inhibition and facilitation (Wright et al. 2006).

In summary the present study demonstrated:

- Increased motor threshold in patient groups, likely caused by antiepileptic medication
- No significant differences between ipsilateral and contralateral hemispheres, or right and left hemispheres, in the patient groups
- Reduced SICI in patients compared to normal controls
- Higher SICF in the mTLE group compared to the neocortical group

ID	SZ SEMIOLOGY	INTERICTAL EEG	ICTAL EEG	MRI/ other imaging
1	Nocturnal: wakes, choking sensation, lip smacking	Bilateral independent sharp waves.	Probable right frontal lobe seizures based on electroclinical data.	Non lesional
2	shortness of breath > tonic seizure	Spikes and sharp waves bifrontal, right or left	Lending support to L frontal onset	Non lesional
3	Motor arrest > pallor > head turning to L > GTCS	Spike and slow wave bursts anterior emphasis	No information	Non lesional
4	taste in mouth, tightness in stomach, head turns to R	bifrontal abnormalities more marked on R	Non localised	Non lesional
5	motionless staring>hypermotor features	frequent multifocal independent spikes	Spiking L temporal lobe	Ictal SPECT: L frontotemporal involvement. Left ictal SPECT
6	secondary generalised seizures from sleep	bilateral anterior predominant spikes and slow wave	No information	Non Lesional
7	Sensory disturbance R hand	Small polyspikes over both hemispheres L>R	No information	Non lesional
8	Fear>speech and hearing problems > head deviates to the R	Left mid temporal	Left temporal lobe focus	MEG L superior temporal gyrus focus. FDG-PET: focal L frontal hypometabolism
9	R arm goes down > L arm moves w jerk > Legs stiffen.	bilateral frontal slow	anterior onset, poss. max at F3	Left frontal cortical dysplasia
10	Blank>stares>R arm dystonia, L arm automatisms.	Left sided slow waves	Ictal non lateralising	Heterotopic nodules left frontal white matter
11	Lapses in awareness	Brief frontal polyspikes	Frontal onset spike wave discharges	Non lesional

12	From light sleep, buzzing and ringing sound.	Spikes L>R	obscured by myogenic activity	Non Lesional
13	Seizures described by Consultant Neurology as 'frontal' in semiology.	No information	No information.	Non lesional
14	Hyperkinetic motor	Bifrontal abnormalities with L sided predominance	Left frontal spikes.	Focal cortical dysplasia near left operculum
15	SPS: paraesthesia and clenching of L hand	Suggestive of right frontal.	Underwent invasive EEG recording. R frontal lobe onset.	Non lesional.
16	Loss of awareness, staring, R eye deviation	Bifrontal abnormalities	Generalised with bifrontal maximum	MEG and EEG/fMRI suggest left frontal lobe epilepsy
17	Lapses of awareness	Generalised polyspike and slow wave activity.	Electroclinical characteristic are most consistent with partial epilepsy. This and the interracial abnormalities are suggestive of mesial frontal epilepsy.	Non lesional
18	Oral & manual automatisms; nonsensical speech; confusion	Left temporal sharp waves and spikes.	Electroclinical characteristics of are consistent with a seizure arising from the left temporal lobe.	Left anterior temporal DNT
19	SPS: small flushing feeling. Unsure of CPS semiology.	F8, A2	Electroclinical characteristics of three of the seizures are typical for left frontal lobe seizures	Non lesional
20	Speech arrest	Right temporal sharp waves.	Complex partial seizures arising from the right temporal lobe, probably neocortical in origin	R superior temporal gyral atrophy & sulcal widening

TABLE 6: DEMOGRAPHIC DETAILS OF PATIENTS WITH NEOCORTICAL EPILEPSY

CHAPTER 7. REPETITIVE TMS IN PATIENTS WITH REFRACTORY PARTIAL EPILEPSY

7.1 Introduction

Transcranial magnetic stimulation, in addition to being of use in recording certain parameters of cortical inhibition and excitation, has the potential of being a therapeutic tool. TMS, when delivered in repetitive pulses (rTMS), has been shown to reduce cortical excitability when applied at low frequencies: stimulation with frequencies of 0.9 Hz was demonstrated to decrease MEP amplitudes and therefore decrease cortical excitability, with the effect lasting at least 15 minutes (Chen et al. 1997a). Cincotta et al. (2003) showed that stimulation at 0.3Hz with a suprathreshold stimulus intensity caused significant lengthening of the cortical silent period, while suprathreshold stimulation at 1Hz has been shown to both increase motor threshold and reduce MEP size (Muellbacher et al. 2000). Chen et al suggested that the underlying mechanism may be similar to long term depression. Repetitive TMS therefore appears to have the ability to modulate neuronal behaviour.

Further studies of rTMS in epilepsy have measured the effects of stimulation using a variety of different parameters and different patient populations. The outcomes measures are typically changes in seizure frequency or changes in the EEG (for review, see Kimiskidis 2010). In this respect, an early pilot study using low frequency (0.33Hz) rTMS over the vertex for 5 consecutive days demonstrated exciting results with a temporary reduction in seizure frequency in patients with refractory LRE (Tergau et al. 1999). A follow up study was performed with a crossover, controlled design of 17 patients with refractory epilepsy, involving stimulation over the vertex for 5 consecutive days (Tergau et al. 2003). Frequencies of 1Hz and 0.33Hz were compared. There was a trend towards decreased seizure frequency in the group receiving stimulation at 0.33Hz but this did not reach significance. Both studies included patients with temporal and extratemporal epilepsy. EEG analysis was not included in these studies.

Cantello et al. (2007) studied 43 patients with drug resistant epilepsy in a double-blind, crossover study with sham and active stimulation. The patient group was heterogeneous but most patients had neocortical epilepsy. Using a stimulus frequency of 0.3Hz, they delivered

1000 stimuli over the vertex every day for 5 continuous days. They used the same methods as Tergau and colleagues, and the outcome measures included both seizure frequency and changes in severity of IEDs. EEG was recorded before the first TMS session and again after the last session on day 5. In the group receiving active stimulation, there was a trend towards a reduction in the average number of IEDs that gradually returned to baseline after 4 weeks. This effect was seen in one third of patients. There was no effect on seizure frequency.

A randomised, double blind and sham controlled study with a more homogenous patient group with targeted stimulation has been carried out by Fregni et al. (2006). All 21 patients had malformations of cortical development. Stimulation at 1Hz was targeted towards the MCD, with the stimulation site determined by the site of the lesion and according to the 10-20 international electrode placement system. Stimulation was delivered at 1Hz with a fixed intensity (70% of maximum stimulator output) for five consecutive days. Each session lasted 20 minutes. EEG recordings were carried out before and immediately after the 5 days of stimulation, and again after 30 and 60 days. Recordings lasted 20 minutes and patients were kept awake. There was no significant difference in the number of IEDs at baseline between the active and sham groups. In the patients receiving active stimulation, there was a significant reduction in IEDs comparing the baseline EEG to the EEG recording performed immediately after the course of stimulation. This reduction was still present at week 4 but not at week 8, suggesting a temporary but still fairly long lasting effect. The authors attribute the positive findings to the fact that they stimulated areas where the IEDs were arising from – a cortical convexity rather than from a deeper structure. This theory may be supported by the findings of Theodore et al (2002), who studied seizure frequency before and after one week of 1Hz rTMS; they found a trend towards a short term decrease in seizures in the ‘active’ group, and this was more marked in patients with lateral compared to mesial temporal foci. The EEG was not included in the study by Theodore and colleagues.

Joo and colleagues (2007) performed rTMS at 0.5Hz on 35 patients with either focal epilepsy or multifocal/ non-localised epilepsy including EEG recordings. Like the studies above, patients underwent 5 days of consecutive rTMS. This was delivered at 0.5 Hz and an intensity of 100% RMT. Patients received either 3000 or 1500 pulses. In 18 patients with focal epilepsy, stimulation was directed towards the foci and guided by ictal EEG or distribution of IEDs. In the remainder, stimulation was delivered over the vertex. The study found a non-significant decrease in seizure frequency and a significant decrease in IED immediately after the course of stimulation, with total attenuation of IED in 6 of the 35

patients. This reduction was not specific to the subgroup of patients with focal epilepsy, however. The possible long term effect of this was not studied by follow up EEG recordings.

Aim of Study

This was a pilot study in patients with refractory epilepsy carried out in order to study the effects of 1Hz stimulation on the EEG with tailored area of stimulation. The stimulus intensity was adjusted for each patient and set at 80% of RMT. The majority of the patients recruited were inpatients on the video-EEG monitoring unit who had been admitted with the specific intention of recording seizures, and therefore the aim of this pilot study was not to produce a therapeutic effect but to study acute effects of rTMS on the EEG.

7.2 METHODS

11 patients with refractory partial epilepsy were selected for an unblinded pilot study to examine the effects of rTMS on the number and distribution of spikes on scalp EEG. Most patients (10) were studied while in patients on the video-EEG telemetry unit, and 1 was studied as an outpatient (subject #9). All patients were able to give written consent. Patients with any metallic object in the head or large CSF spaces in head were excluded from the study. While all patients had partial epilepsy, the location of the lesion differed with 2 patients having definite mesial temporal lobe epilepsy and 5 others having extratemporal lesions. 4 patients had cryptogenic partial epilepsy with normal or equivocal neuroimaging. Full demographic details are given in table 1.

Stimulation and EEG recordings took place either at the patient's bedside in the case of inpatients, or in a quiet recording room in the case of outpatients. At least 30 to 60 minutes of EEG data was recorded prior to rTMS stimulation. The acquisition time before and afterwards was kept as similar as possible for each patient. 24 channels of EEG were recorded, with electrodes positioned according to the 10-20 International Placement System (Jasper 1958). Patients were instructed to relax with their eyes closed. They were encouraged to stay awake so that the 'before' and 'after' state could be controlled; even though interictal epileptiform discharges are typically potentiated by sleep, one cannot guarantee that a patient

who drowns prior to rTMS would drowse again afterwards, therefore drowsiness/ sleep was discouraged.

Repetitive TMS was performed using a Magstim Rapid stimulator. (Magstim Company, Whitland, U.K). Before rTMS was performed, the ipsilateral resting threshold was measured. The patients then underwent 30 minutes of rTMS at 1Hz using a figure of 8 coil held in position throughout by an operator with stimulation at 80% of RMT. The position of the coil depended on the location of lesion. For patients with mTLE, stimulation was positioned over the premotor cortex, 3cm anterior to the 'hotspot' used for measurement of threshold, with the exception of patient #4, who had stimulation superior to electrode T3 reflecting the interictal spike maxima (spikes in patients with mTLE are more often maximal at anterior temporal and superficial sphenoidal electrodes rather than at the mid temporal electrode). The reasoning for stimulating over the premotor cortex was the finding that stimulating this area has an inhibitory effect over the motor cortex (Chen et al. 2003; Gerschlagier et al. 2001; Munchau et al. 2002; Schlaghecken et al. 2003), in addition to the evidence of significant pathways between mesial temporal and frontal areas as discussed in section 1.5. For all other patients, stimulation was targeted over the presumed seizure foci; this was based on imaging and EEG data. Patient #2 had previously undergone resection of a right frontal tumour, but because his current EEG showed maximal interictal spikes at F3, this area was chosen for stimulation. Any electrodes positioned near to, or over, the site of stimulation were removed to avoid electrode heating or interference with stimulation. Patients were again kept alert throughout the stimulation. Coil overheating was a common problem, and two coils were used alternately during each experiment in order to minimise disruption to the stimulation.

After rTMS, patients were again instructed to relax with their eyes closed but to maintain wakefulness. The EEG recordings both before and after TMS were visually inspected by myself, an experienced clinical physiologist, to ensure the continued wakefulness of the patient.

Spikes for both pre- and post-rTMS were then counted using in-house software (SpikeAnalysis v2.10c). This software allows the user to accept or reject waveforms (i.e. reject artefactual waveforms) and is more sensitive than specific. The software then generates a report detailing the number and distribution of spikes. The number of spikes at each

electrode is provided, according to maximum amplitude of each spike, and the distribution of spike fields is provided as either unilateral left, unilateral right or bilateral.

7.3 RESULTS

This study included 11 patients (7 female) with an age range of 24-54 (average age 36). See table 1 for patient demographics. For statistical analysis of the data, repeated measures ANOVA, with two factors: "session" (pre-rTMS, post-rTMS) and "side" (ipsilateral, contralateral) was used. There was a significant main effect of "side" with significantly more spikes on the hemisphere ipsilateral to the epileptic foci ($F[1,9]=7.298$, $P=0.024$). There was no effect of "session" i.e. the overall numbers of spikes did not change $P=0.404$ after rTMS. In addition, there was no interaction between "session" and "side" i.e. there was no effect of rTMS just on one hemisphere $P=0.551$. These results were confirmed by T-test: there was no difference between "before" and "after" data for either ipsilateral spikes ($P=0.466$), contralateral spikes ($P=0.353$), bilateral spikes ($P=0.686$) or total spikes ($P=0.414$).

7.4 DISCUSSION

This pilot study failed to show any significant effects of repetitive transcranial magnetic stimulation at 1Hz on the interictal EEG in patients with refractory partial epilepsy. Although the numbers of the study were small, this is not thought to be the main reason for the lack of significant results.

In this study I strove to minimise any variation between EEG collection "before" and "after" in order to reduce the variability in spike numbers: patients were in the same state of arousal (awake with eye closed); recordings took place on the same day and in the same environment; the patients' medication was not altered; the patients were not in an acutely postictal state. The lack of positive findings can be partially explained by the intrinsic variability in spike frequency. Spike frequency is known to be enhanced in certain situations, e.g. during sleep and in the postictal period (Kaibara and Blume 1988). Apart from these times, the number of spikes can be variable with no clear external influences.

The limitations of the study are clear. Apart from the small numbers of patients studied, spike counting was carried out by myself, and as such was not blinded to 'before' and 'after' states. Any future studies should address this issue. However, since spike counting was performed using an automated system (with necessary adjustments for false positives or missed spikes), it is not thought that this affected the findings.

The present study did not use the repeated rTMS protocol that was described in the introduction, which typically involves 5 days of stimulation. The intention was not to lower patients' seizure thresholds or prevent seizures but to measure the acute effect of rTMS on the EEG. It may be that a single session of rTMS is not sufficient to cause modulate cortical excitability to an extent that the EEG is significantly affected.

An important factor when considering rTMS, EEG and epilepsy is the positioning of the coil and the location of the epileptic foci: studies have indicated that stimulation of more superficial lesions is more efficacious than that of deep structures, which would correlate with the limited depth of penetration of TMS at approximately 1.5-2cm (Epstein et al. 1990). This could partially explain our negative findings. Due to the lack of significant findings in this pilot study, a planned randomised study of 'active' rTMS versus 'sham' to eliminate a possible placebo effect using a sham coil was abandoned.

A further factor in the negative findings could be that rTMS-induced changes are too subtle to be detected by the methods used in the present study. Alternatively, the impact of rTMS may be dependent on an individual's susceptibility, such that no major group effect or consistent effect across many patients may emerge. This theory is supported by the recent findings of Brodbeck et al. (2010), who studied not only spike numbers but spike strength and spike topography in 5 patients with focal epilepsy. They found a reduction in spikes in just one patient, and a change in spike topography in another.

No patients reported negative effects of rTMS during this study and no seizures occurred during stimulation.

In summary, in this small open pilot study, it was not possible to replicate the effects of rTMS on interictal spikes found in previous published studies.

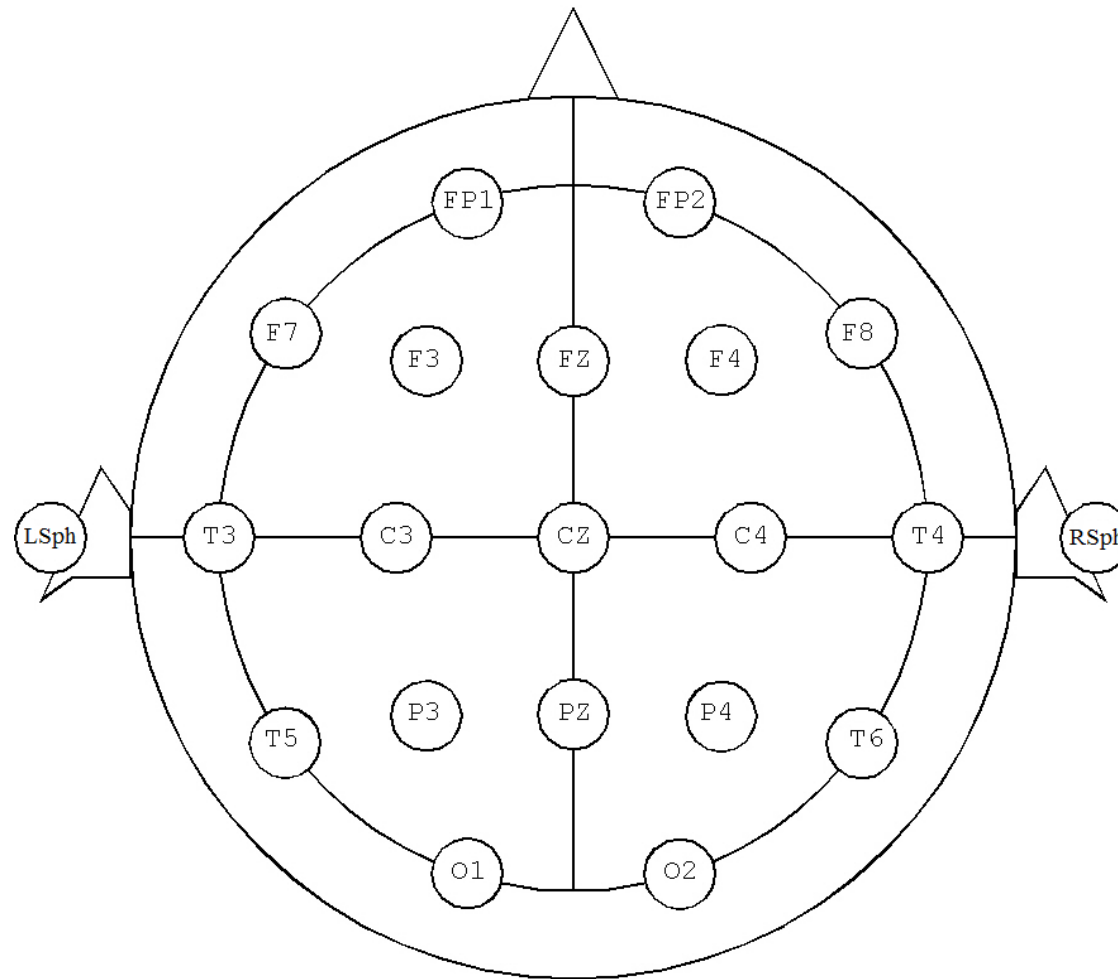


FIGURE 32: STANDARD 10-20 ELECTRODE PLACEMENT SYSTEM WITH ADAPTION OF RIGHT AND LEFT SUPERFICIAL SPHENOIDAL ELECTRODES IN PLACE OF A1 AND A2 (JASPER 1958).

ID	sex	Age	D.O.B.	Sex	Interictal EEG focus	Ictal EEG focus	MRI
1	M	30	29/10/1976	M	Right frontotemporal	Not available	R HS
2	M	33	19/12/1969	M	F3*	Not available	Previous surgery for R frontal tumour
3	F	53	23/03/1950	F	F8, right superficial sphenoidal	Not available	? Small R HS
4	M	38	23.07.1966	M	T3	left temporal	L HS
5	F	28	27/10/1978	F	F4, Fz, S/W	widespread	Normal MRI
6	F	24	12/01/1979	F	Widespread/ bifrontal and R frontotemporal	widespread R>L	R frontal small scar
7	F	51	07/11/1952	F	Generalised spikes with bifrontal bias, R>L	Unclear	R medial frontal FCD
8	F	34	11.02.1968	F	F3, F7, Fp1, T3	Not available	Normal MRI
9	M	54	24/01/1949	M	F8	Not available	R superior temporal gyrus
10	F	25	15/04/1977	F	F3	Not available	DNT (partially excised)
11	F	23	16/09/1979	F	F4, F8	Not available	Normal MRI

TABLE 7: PATIENT DEMOGRAPHICS, CHAPTER 7

DNT = dysembryoplastic neuroepithelial tumour. FCD = focal cortical dysplasia, HS = hippocampal sclerosis

ID	Area Stimulated	Pre TMS L	Pre TMS R	Pre TMS Bilateral	Post TMS L	Post TMS Right	Post TMS Bilateral
1	3cm ant to L FDI hotspot	0	182	0	0	121	0
2	F3	15	0	6	4	1	16
3	F8	24	127	13	17	157	12
4	1 cm above T3	13	1	0	7	0	1
5	between F4 and Fz	0	21	18	0	67	23
6	F4	1	109	49	4	108	60
7	Between Fz & F4	-	-	108 S&W	-	-	95 S&W
8	F3	30	1	6	3	0	0
9	T6	6	0	1	1	1	0
10	2cm above Fp1	77	24	33	25	12	12
11	F4	5	4	1	11	6	3

TABLE 28: STIMULATION DETAILS FOR EACH SUBJECT.

FDI = first dorsal interosseus; S&W = spike and wave discharge.

F3, F8, F4, Fz, T6 and Fp1 are electrode positions (see figure 32).

CHAPTER 8. DISCUSSION

The aim of this thesis was to measure cortical excitability in patients with focal epilepsy using transcranial magnetic stimulation. The motivation of the work was to study a group of patients with focal epilepsy who were heterogeneous in terms of the site of epileptogenic zone, and patients with unilateral, mesial temporal lobe epilepsy were chosen. As discussed in chapter one, temporal lobe epilepsy is the most common form of focal epilepsy, and the most common pathological finding in TLE is within the mesial structures (hippocampal sclerosis). Various imaging studies of patients with mTLE have demonstrated that there are changes in areas remote from the mesial temporal structures, largely involving the ipsilateral frontal lobe as well as connections between the ipsilateral frontal and temporal lobes. These studies demonstrate that seizures arising in limbic structures can affect areas outside the temporal lobe. Perhaps of particular interest in the context of this thesis are the findings of Van Paesschen and colleagues, who demonstrated hyperfusion at the border of the ipsilateral middle gyrus and the precentral gyrus in addition to clusters in the frontal lobes using ictal SPECT in patients with unilateral hippocampal sclerosis (Van et al. 2003). In this thesis, TMS was used to stimulate the motor cortex and record from a hand muscle, with the hypothesis that evaluating the excitability of the motor cortex of both hemispheres and comparing between the hemispheres ipsilateral and contralateral to the areas of seizure onset may demonstrate significant differences.

The hypothesis was that this noninvasive technique would be able to detect variability in motor thresholds, paired pulse inhibition and excitation, and the cortical silent period, according to the occurrence of seizures, before and after TMS measurement sessions. The thesis also investigated differences between the ipsilateral (affected) and contralateral hemispheres. It also looked at differences between groups of patients with focal (mesial versus non mesial temporal/ extratemporal) epilepsies and normal controls with the hypothesis that epilepsy arising from different area of the brain may have different effects on cortical excitability measured from the motor cortex. In addition, the acute effect of rTMS on the EEG in patients with epilepsy was studied, with the hypothesis that low frequency rTMS may cause a modulation in neuronal excitability that could be measured at the level of scalp EEG. All patients included in this thesis were recruited from a tertiary referral hospital and all patients studied had refractory epilepsy that was being treated with at least one antiepileptic

drug. The effects of AEDs on TMS parameters has been included in the introductory chapter (section 2.3), and this is an important confound to be taken into account when analysing and interpreting the results.

The significant findings are presented below, followed by more detailed discussion of each chapter.

SUMMARY OF SIGNIFICANT RESULTS

- Motor threshold in patients with focal epilepsy is reduced following AED withdrawal
- Motor threshold is increased following seizures
- The difference between SICI at 2ms and SICF at 15ms correlates with the time until the next seizure, demonstrating active changes in cortical excitability preceding seizures
- CSP/MEP ratio is greater in the contralateral hemisphere of patients who have seizures more than 7 days before TMS recording
- There is increased cortical excitability in patients compared to controls but no differences between temporal and extratemporal patients
- A single session of repetitive, low frequency rTMS does not affect EEG spiking

The aim of the study described in chapter 3 was to measure resting and active motor thresholds in patients with unilateral mesial temporal lobe epilepsy undergoing AED dose reduction and to test the effect of recent and subsequent seizures before and after the TMS measurement sessions. Patients on the EEG telemetry unit were chosen for the study several reasons: firstly, the timing of seizures with respect to the TMS measurement session would be very precise (assuming any seizures occurred during the admission); secondly, it is well-recognised that AED reduction, part of the routine on most telemetry units, is associated with a markedly increased likelihood of seizure occurrence. Hence it was likely to obtain seizures

within hours or a few days of TMS sessions and to have a precise time of seizure occurrence – neither of these factors would have been likely if we had only studied outpatients. One relatively homogeneous clinical subgroup of epilepsy was chosen for this study – patients with mesial temporal lobe epilepsy – in order to reduce between-subjects variability as much as possible. Despite this, although the patient group was homogeneous in terms of the ictal onset zone (mesial temporal), there were unavoidable differences in AED treatment between the patients. Many studies of patients with epilepsy can be criticised on the grounds that AED treatment is often variable in terms of specific drug and dosages. This confound can only be avoided by studying patients taking the same drug at similar doses (very difficult to achieve), or through studying treatment-naïve patients. There was no access to a sufficient population of patients taking the same medication, nor did was there access to a sufficient number of treatment-naïve patients.

In Chapter 3, it was found that resting motor thresholds were higher in patients with recent seizures and that this was the case in both ipsilateral and contralateral hemispheres. This was seen on day one, prior to any changes in AED dose. On day three, following drug reduction to 50% or 25% of baseline levels, the resting motor threshold was still significantly higher in patients with recent seizures. There was no effect of subsequent seizures. Since MT is thought to reflect membrane excitability, the results suggest that the elevated motor thresholds may represent a neuro-protective mechanism to prevent further seizures; this inhibitory mechanism would be widespread since the effect is bilateral and remote from the area of epileptogenicity. The study could have been improved by taking in to account the nature of recent seizures as was done by Badawy and colleagues (2009); they found bilateral changes in cortical excitability in patients with recent secondary generalised seizures, while patients who had recent focal seizures without generalising had similar changes in the ipsilateral hemisphere only. In the present study, it is assumed that the group of patients will have included some with generalised and some with focal seizures, hence the finding of bilateral increased MT is entirely in keeping with the work of Badawy and colleagues. It is instructive to compare Badawy et al.'s findings with mine:

- Both studies independently show:
 - Measurements of motor cortex physiology using TMS show differences between the preictal, postictal and interictal states

- Intracortical mechanisms responsible for paired-pulse inhibition and facilitation (probably involving synaptic processes and small networks) are impaired in the pre-ictal period, producing “increased excitability” or “reduced inhibition”, or both
 - Mechanisms responsible for motor threshold (probably involving neuronal ion channels) result in increased threshold in the postictal period
- Badawy, but not this thesis, showed:
 - Mechanisms responsible for motor threshold (probably involving neuronal ion channels) result in decreased threshold in the preictal period
 - Badawy’s patients were untreated; Wright’s patients were treated, which results in substantial increase in MT, which perhaps prevented this effect being detected

This part of the thesis was published in 2006, prior to Badawy’s paper, hence the Badawy paper is a reassuring confirmation of the prior novel finding in an independent group of patients, and carried out by an entirely independent team.

In the subsequent study (chapter 4), paired pulse TMS was used to investigate cortical inhibitory and excitatory processes in patients with unilateral refractory mesial temporal lobe epilepsy. SICI and SICF are thought to provide information on GABA_A- and glutamate-mediated circuits in the primary motor cortex (for review see Tassinari et al. 2003). SICI and SICF were measured before and after drug reduction. It was demonstrated that drug reduction leading to seizures was accompanied by a dynamic loss of SICI and an increase in SICF in the ipsilateral hemisphere which correlated with the time until the next seizure. The study demonstrated that preictal changes can be recorded from areas remote from the seizure focus, suggesting widespread preictal brain changes. This was another novel finding published in 2006, and also confirmed in the subsequent study of Badawy and colleagues (2009).

This study did not show any preictal change in MT, which was demonstrated by Badawy and colleagues. Their patients were untreated; the patients in the present study were all treated which results in significant MT elevation caused by AEDs; we speculate that the substantial

elevation of MT caused by AEDs may have masked any preictal drop in MT, which Badawy's study identified.

Chapter 5 studied the effects of seizures on cortical silent periods in the same patient group. Measurement of the cortical silent period in patients with unilateral mesial temporal lobe epilepsy again involved taking two measurements made over a 3 day period with AED reduction to 50% or 25% of baseline. The cortical silent period has been shown to be a complex phenomenon, although GABA_B –mediated inhibitory mechanisms at the cortical level are thought to play an important role. The hypothesis of the study was that changes in seizure timing, both before, between and after TMS measurements, may have a significant effect on CSP measurements. An additional hypothesis was that changes in AED dosage may have an effect on CSP measurements. The results revealed that CSP/MEP ratio was greater in the contralateral hemisphere of patients who had seizures more than 7 days ago compared to patients whose seizures were more recent. It is difficult to explain an effect specific to the contralateral hemisphere. One can speculate that this reflects a compensatory process which serves to reduce the likelihood of further seizures, which is effective in the contralateral hemisphere but fails in the ipsilateral hemisphere.

The study also showed that the effects of recent seizures on CSP were independent of the stimulus intensity used. CSP duration and CSP/MEP ratio were found to be very stable during this study, with no change in CSP or CSP/MEP ratio despite drastic changes in AED doses. This is substantially different from the effects of AED reduction on MT, perhaps reflecting the different neurobiological processes underlying MT and CSP. Finally, the study demonstrated that by analysing CSP/MEP ratio values as used by Orth and Rothwell (2004) was more sensitive than analysing CSP duration alone; and that automated analysis contributed to sensitivity compared to using “by eye” measurement.

There are no other data examining the effects of seizures on CSP. Badawy and colleagues did not include this measurement in their dataset. Hence currently this remains a novel but unconfirmed finding, and there is no equivalent data from drug naïve patients.

Comparing results between the patients with unilateral mesial temporal lobe epilepsy, patients with neocortical epilepsy and control subjects was next carried out (chapter 6). There was a significant elevation in motor threshold in both patient groups that was put down to the effects of antiepileptic medication, which is known to increase resting and active motor thresholds (Ziemann et al. 1996a). Despite the differences between the patient groups, and the extreme variability of AEDs, MT was identical between the patient groups, confirming that the MT elevation is likely due to treatment effects rather than being disease-related. Additionally, there may be a generic mechanism underlying both types of epilepsy that result in elevated motor thresholds; that would have to be explored in groups of patients who are drug naïve, however, which has practical limitations. There were no changes in any measures in the patient groups when comparing hemispheres as ipsilateral versus or contralateral or right versus left. Significant differences were found comparing cortical excitability between patients and normal controls, however, with a reduction in SICI in patients suggesting impaired cortical inhibition in these patients. When comparing the mTLE and neocortical patient groups, there was higher SICF in the mTLE group and a trend towards a decrease in SICI.

The differences between the neocortical and mTLE groups demonstrate that epilepsies arising from distinct areas of the brain effect cortical excitability measured from the motor cortex in different ways. The majority of the patients in the neocortical group had epilepsy arising from frontal regions, with a minority having non-mesial temporal lobe epilepsy. The study did not take into account timing of previous or next seizure as the data was not available for the neocortical group. As was demonstrated in chapter 4, the timing of seizures before and after TMS measurements are made can effect cortical inhibition and facilitation (Wright et al. 2006). However, we have no reason to expect that this would explain the between-group differences. No other study by a single group of investigators has divided patients according to the site of focus between mesial temporal and neocortical groups, applying identical measurement protocols to both groups and to normal controls. Hence currently the finding of differences between neocortical and mesial temporal groups remains unconfirmed by others. The finding of unilateral ipsilateral reduction in SICI is highly plausible, and broadly in keeping with the findings of others. However, the group of patients is relatively large ($n = 56$) and the effect strong, hence the data is regarded as reliable and strong evidence for failure of ipsilateral SICI in focal-onset epilepsy.

The variability of TMS measurements over time was measured, with the aim of understanding how cortical excitability might vary before and after seizures. For logistical reasons, it was only practical to carry out only two studies per patient during a stay on the telemetry unit. Future studies with using TMS in patients with epilepsy would benefit from a higher number of measurements than the two that were typically carried out on each patient in this thesis. There would be extreme logistical obstacles to studying patients with TMS very frequently, but even in a small group of subjects, demonstrating the time-course of pre- and post-ictal changes in excitability would be of very high interest.

The study of patients who are taking AEDs is not ideal but is unavoidable when studying patients with long standing epilepsy as compared with *de novo* epilepsy. This thesis has shown that changes in cortical excitability can be detected despite complex differences in antiepileptic drug regimen between patients and comparing patient groups.

In this thesis, I undertook multiple measurements in the same subjects, increasing the possibility of Type 1 error (false positive findings) because of multiple non-independent statistical tests. To protect against this, in all cases I set *a priori* hypotheses, and in most instances the statistical model was a repeated measures ANOVA incorporating multiple factors. Having found a significant effect of the ANOVA, it is statistically legitimate to deconstruct the complex model into simpler comparisons (e.g. T-tests) in order to identify the specific factor(s) which account for the significant effect in the ANOVA.

Previous studies suggest that rTMS might have an effect on EEG spikes and on seizures. Nonetheless the quality of such studies is variable (e.g. many were unblinded) and the reliability of the effect remains uncertain. Hence, although a more substantial study of rTMS and its effects on EEG was planned, the initial plan set out to undertake a small unblinded pilot to confirm that an effect is possible, and to determine an effect size. However, the pilot study demonstrated that single-session rTMS at 1Hz has no significant effect on spike frequency, when comparing the EEG before and immediately after stimulation at 1Hz.

Because of this, the planned cross over study with sham stimulation was abandoned. Nonetheless neuro-modulation using electrical stimulation of the brain (deep brain stimulation or direct focal stimulation at the cortical focus) has been shown to be effective in controlling epilepsy, hence positive findings from studies conducted by others are plausible. It is conceivable that failure to detect an effect in the present study was a chance-driven error, or that the site of stimulation, or method of spike-counting, were inadequate. There are further on-going studies elsewhere, and no doubt more data will become available in future to enable stronger conclusions to be reached

This thesis has shown that cortical excitability is different between focal epilepsy patients and normal controls; and between different groups of focal epilepsy patients. This thesis has shown that certain parameters of cortical excitability differ between patients who have had recent seizures and those who have not; and, of great interest, this thesis have shown that cortical excitability differs between patients who will have seizures soon, and those who will not. Some of these findings are already published, and, gratifyingly, have already been replicated by others. I hope that these findings may contribute to a better understanding of preictal changes in brain physiology.

In terms of future direction for TMS in epilepsy, Badawy et al. (2010) have recently detected significant differences in cortical excitability in patients with epilepsy according to their response to antiepileptic medication by performing TMS before and after initiation of treatment. They have found significant differences in both focal and generalised epilepsy groups, and it is possible that in the future TMS may be used to help guide the management of seizures.

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