Non-Invasive Methods To Investigate Brain Function In Health And Disease

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ABSTRACT

Non-invasive methods to investigate brain function have been used in research laboratories for many decades, however their popularity has increased in recent years given the ease of use and broad application. Such methods have proved valuable in improving our knowledge about numerous areas of basic brain function.

Many non-invasive techniques have also been applied to patient groups to allow further identification of pathological mechanisms, but critically a new role has been found for some as biomarkers of disease. Neurodegenerative disease is fast becoming one of the biggest medical problems in the first world. An aging population has caused the relative incidence of many conditions to rise dramatically and studies suggest that this trend will continue. Although our knowledge surrounding these conditions has improved significantly, most remain notoriously difficult to diagnose and to treat. The recent introduction of neuroprotective drugs offers the potential to slow the progression of some diseases. However, to take full advantage of these disease-modifying treatments, administration must occur early in the disease course which fuels the demand for selective and specific diagnostic tests.

There is currently a great need to enhance the clinical diagnostic repertoire with reliable, robust and specific biomarkers of neurodegenerative disease. However, careful, rigorous studies are required to validate the use of non-invasive techniques in this role. The same level of care should also be applied to techniques used in basic research; without a fundamental understanding of the mechanisms underpinning these techniques, their utility in the investigation of specific processes or pathways is questionable. This thesis aims to address specific cases to evaluate existing techniques and to screen potential new disease biomarkers.

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ABBREVIATIONS

ABPB	Abductor Pollicis Brevis
ALS	Amyotrophic Lateral Sclerosis
AMT	Active Motor Threshold
BD	*Bis die* or *twice a day*
CM	Corticomotoneuronal
CMC	Corticomuscular Coherence
CMCT	Central Motor Conduction Time
CNS	Central Nervous System
CSF	Cerebrospinal fluid
CST	Corticospinal Tract
DBS	Deep Brain Stimulation
DCN	Deep Cerebellar Nuclei
DMT	Direct Motor Threshold
EDB	Extensor Digitorum Brevis
EDC	Extensor Digitorum Communis
EEG	Electroencephalogram
EMG	Electromyogram
FDI	First Dorsal Interosseous
FDS	Flexor Digitorum Superficialis
FFT	Fast Fourier Transform
GABA	Gamma-Aminobutyric Acid
Gi	Gigantocellular Reticular Nucleus
GS	Gastrocnemius

HSP	Hereditary Spastic Paraplegia
IMC	Intermuscular Coherence
iMEP	Ipsilateral Motor Evoked Potential
LED	Light Emitting Diode
LEV	Levetiracetam
LFP	Local Field Potential
LMN	Lower Motor Neurone
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MLF	Medial Longitudinal Fasciculus
MND	Motor Neurone Disease
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MSA	Multiple System Atrophy
%MSO	% Maximum Stimulator Output
MT	Motor Threshold
NCS	Nerve Conduction Study
ODRT	Objective Direct Response Threshold
PBP	Progressive Bulbar Palsy
PD	Parkinson’s Disease
PF	Posterior Fossa
PLS	Primary Lateral Sclerosis
PMA	Progressive Muscular Atrophy
PMRF	Pontomedullary Reticular Formation
PnC	Caudal Pontine Reticular Nucleus

PnO	Oral Pontine Reticular Nucleus
PSP	Progressive Supranuclear Palsy
PSTH	Post Stimulus Time Histogram
RMT	Resting Motor Threshold
RST	Reticulospinal Tract
rTMS	Repetitive Transcranial Magnetic Stimulation
SCA	Spinocerebellar ataxia
TA	Tibialis Anterior
tDCS	Transcranial Direct Current Stimulation
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
UMN	Upper Motor Neurone
VEMP	Vestibular Evoked Myogenic Potential

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CHAPTER I: INTRODUCTION

The last few decades have seen the introduction of a wide range of techniques which facilitate the non-invasive investigation of brain function in human subjects. These accessible methods have proved invaluable at developing our understanding of motor control as well as other neurological processes.

Many non-invasive techniques have been applied to patient groups to allow further identification of pathological mechanisms, but critically a new role has been found for some as biomarkers of disease. The ability to detect disease before this can be achieved by other clinical scales or investigations is vital at a time when the best treatments we have at our disposal can only slow the relentless progression of neurological disease.

There is currently a great need to enhance the clinical diagnostic repertoire with reliable, robust and specific biomarkers of neurodegenerative disease. The incidence and prevalence of many neurological conditions is increasing in association with an aging population and detailed genetic and pathological investigations have revealed that many diseases are not a single entity but actually fall within a complex spectrum of similar pathological processes. Despite this huge progress in understanding the nature of neurodegenerative disease, our ability to detect it in the earliest stages remains completely inadequate. Patients often have to wait many months and sometimes years to receive an accurate diagnosis; often by this point substantial irreversible damage has accumulated and there is little which can be done in the way of treatment. Although, diagnostic criteria have improved over the past two decades, this gap between the onset of pathology and accurate diagnosis is not closing at a rate sufficient enough to facilitate useful clinical intervention. This problem needs to be addressed if we are to improve patient care and make the most effective use of neuroprotective treatments.

Neurodegenerative disease

Neurodegenerative diseases form a diverse spectrum of chronic neurological disorders which are associated with progressive damage to the nervous system. Since neurons are

difficult to regenerate or repair in adults, the damage is cumulative and permanent. In all conditions, neurodegeneration begins long before symptoms arise, therefore considerable, irreversible damage has already accumulated before patients present to a neurology service. This feature makes neurodegenerative diseases particularly devastating for patients and also incredibly difficult for clinicians to treat.

Historically, most neurodegenerative diseases were considered to be rare. However, over the last half century there has been a colossal improvement in acute phase medical treatment for common causes of death such as stroke and myocardial infarction. As a result, the population are living much longer with the average life expectancy in the UK now 81.5 years for females and 77.2 years for males (Office for National Statistics, 30 Oct 2008). With an aging population, the relative occurrence of neurodegenerative diseases is increasing rapidly (Day et al., 2007; Kondo, 1996; Riggs and Schochet, 1992). This rising prevalence represents a huge financial burden on society as well as presenting a significant medical challenge for the future.

In many common neurodegenerative diseases including Parkinson's disease and motor neurone disease, the underlying neuropathological processes are relatively well understood. Despite this, there are few specific diagnostic tests, sensitive to the biological hallmarks of these diseases. Instead, diagnosis relies largely upon clinical opinion and consensus diagnostic criteria. A key feature of these criteria is usually disease progression. Accurate diagnoses cannot therefore be applied at a very early stage, limiting the opportunity for early intervention.

Over the past decade, there has been an emerging interest in neuroprotection; developing drugs to limit neuronal death and therefore slow progression in chronic neurodegenerative disease. Neuroprotective agents such as Riluzole and Rasagiline have been released onto the market with good effect in patients with a disease duration of approximately two years (Bensimon et al., 1994; Lacomblez et al., 1996; TEMPO, 2002). It is reasonable to believe that these agents would have an even greater neuroprotective effect if administered earlier in the disease course, before significant cell loss has occurred. Theoretically this would enable progression of the disease to be slowed at a point when there is less damage and therefore more residual function, although this has not yet been formally demonstrated. If

we are to test and implement this kind of early interventional treatment however, there is clearly an urgent need for accurate early biomarkers of disease.

Genetics of neurodegeneration

Genetic mutations have been shown to play a significant role in the aetiology of many neurodegenerative disorders. Consequently, we now have molecular signatures that can selectively identify several of these diseases. This is not only useful in symptomatic patients; we can also pre-emptively screen populations at a higher risk of developing particular conditions. As a result, the onset of some hereditary diseases such as spinocerebellar ataxia (SCA) and Huntington's chorea can be accurately predicted many years before the first symptoms appear.

Pre-symptomatic screening is an incredible achievement of genetic research, however in practice it can only be realized in a minority of conditions. In fact, the majority of neurodegenerative diseases are by nature genetically complex; they are not usually linked to a single gene mutation. Instead, they form a heterogeneous spectrum of disorders which can share clinical features and underlying pathology. Moreover, diseases are being sub-divided and re-classified as there is more understanding of the processes underpinning their pathology. Such a complex system undoubtedly makes early, accurate diagnosis a significant challenge to clinicians who have few diagnostic tools at their disposal.

Current diagnostic complement

Traditionally, neurological diseases are identified by their clinical presentation – the signs and symptoms that they typically cause. However, this approach is highly subjective and largely dependent on clinical experience; in the case of neurodegeneration it is also complicated by the fact that many diseases present with similar features. Generally speaking, there is a current lack of specific diagnostic tests for almost all of the common neurodegenerative diseases. Instead, consensus criteria guide disease classification; these usually comprise a specification of clinical features with supporting laboratory data. The complexity and often conservative nature of these criteria can delay definitive diagnosis; in some cases, clinically definite disease can only be confirmed post mortem.

In support of the clinical examination, clinicians have a limited selection of standard laboratory tests at their disposal. Imaging techniques such as MRI are usually the first port of call. These are extremely useful for detecting gross anatomical abnormalities like those seen in stroke, although are not always able to reveal subtle changes. In many cases, standard brain imaging serves simply to exclude obvious differentials rather than to confirm a diagnosis.

Nerve conduction studies (NCS) are performed as standard for most patients with a suspected neuropathy. These involve stimulation of peripheral nerves to evaluate the function of motor or sensory nerve fibres. Often, NCS are performed in combination with electromyography (EMG) to help localize the source of the abnormality. This test usually involves fine needle electrodes inserted superficially into muscles, so can be a little painful. EMG and NCS in combination can certainly detect abnormalities associated with movement disorders, but they are not always sensitive to discreet differences between degenerative diseases.

Transcranial Magnetic Stimulation (TMS) is a non-invasive technique which is available in some hospital trusts, although it is usually restricted to research institutes given its expense and rather specialist nature. TMS can be helpful in locating lesions in the corticospinal tract (CST); indeed it has a supporting role to play in the diagnosis of neurodegenerative processes affecting the CST such as MS and MND (Chen et al., 2008; Eisen and Shtybel, 1990). For some patients however TMS is not appropriate as high stimulus intensities often have to be used in the presence of CST damage; this is not always tolerated.

In addition to the standard tests available, there are some highly specific disease biomarkers which can be employed when certain conditions are suspected. For example, there are genetic tests for inherited conditions, immunological assays and radiopharmaceutical tracers (eg DaTSCAN). Importantly, the availability of these specific tests is restricted to a relative minority of diseases; the most common neurodegenerative conditions such as Alzheimers disease and MND are still diagnosed largely on the basis of consensus clinical criteria.

Need for better diagnosis of neurodegenerative diseases

Many neurodegenerative diseases are not separate nosological entities but instead form part of a disease spectrum. As such, symptoms often overlap and diseases may also share pathologies or genetic mutations. With such a complicated spectrum of disease, a reliable method of differentiation becomes critical. The current collection of standard laboratory tests (detailed previously) are not sufficiently sensitive to inform diagnosis. In combination with clinical examination, current tests are still estimated to result in unacceptably high rates of misdiagnosis (Davenport et al., 1996; Rajput et al., 1991). Coupled with the increasing prevalence of neurodegenerative disease, the urgent need for better biomarkers of neurodegeneration is evident. There are obvious implications for prognosis, treatment, and also for future clinical trials.

It is known that early intervention in neurodegenerative disease is essential. Neuroprotective treatment can only be beneficial if it is implemented at a stage where there is still sufficient residual neuronal function. In stark contrast to this, it may take years to reach an accurate diagnosis for MND or Parkinsonism. By this time, symptoms tend to be prominent and often troublesome to the patient who is likely to have acquired some degree of disability. Uncertainty surrounding diagnosis can also have huge psychological implications for the patient; depression is common in neurodegenerative disease (Cummings, 1992; Taylor et al., 2010).

It is important to note that misdiagnosis or delays induced by consensus criteria will also impede experimental trials of novel treatments for neurodegenerative disorders. For example, it has been estimated that 20-25% of patients diagnosed with PD actually have an atypical form of Parkinsonism (Hughes et al., 1993; Litvan et al., 1997; Rajput et al., 1991). These diseases tend to be much more aggressive and importantly have very different underlying pathology. It is crucial therefore that patients are diagnosed accurately in order for the appropriate therapeutic strategy to be put in place. Accurate diagnosis would also prevent research being compromised by heterogeneous patient populations.

All neurodegenerative diseases have a long pre-clinical period during which there is considerable cell loss, but no overt clinical symptoms. For example in Parkinson's disease,

symptoms do not appear until 60-80% of dopaminergic neurons are lost (Bernheimer et al., 1973) and one report suggested that there may be up to 50% loss of motoneurones before clinical onset of MND (Aggarwal and Nicholson, 2002). An exciting possibility would be the potential for pre-symptomatic diagnosis in these conditions, something which is already possible for inherited disorders. This would optimise the therapeutic window for treatment, targeting the system before the majority of the damage has been done. Administration of disease-modifying therapy in the pre-symptomatic stages of disease may help delay disease onset and significantly slow progression.

Disease detection: what are biomarkers?

Biomarkers accurately measure the status of a particular biological system or process. They are objective, quantifiable measures which are independent of opinion. In essence, therefore they are a simple measure of a complex process. Consequently, they have a significant role to play during many stages of the clinical process.

Biomarkers can be particularly useful in the initial detection of disease; identifying an abnormal physiological measure which is indicative of a pathological process. But this is not their only role. Given that they reflect a physiological measure, monitoring a biomarker over time can give information about the rate of progression of a disease as well as the efficacy of treatment. In addition, initial measures may be able to give some prognostic information about the likely clinical course of the condition.

Electrophysiological techniques as biomarkers of neurodegenerative disease

Without sensitive biochemical markers of neurodegenerative disease, we must look for indirect evidence of pathology. The term *electrophysiology* refers to a group of techniques classically used to investigate the integrity of the central and peripheral nervous systems. As opposed to subjective clinical scoring or consensus criteria, electrophysiology offers a precise and objective method of interrogating these pathways. Currently, electrodiagnostic tests play a significant role in the diagnosis of peripheral disorders of muscle and nerves, but are less frequently utilised in the diagnosis of CNS disease.

Electrophysiological tests are usually non-invasive (occasionally involving needle electrodes) and simple to perform. In a standard clinical neurophysiology department, the primary aim is to look for evidence of abnormalities within the nerves and muscles. Here, EMG and NCS are particularly useful in helping to distinguish between processes which might otherwise look very similar - for example tremor and clonus. The high sensitivity and large collection of normative data means that many diseases can be identified early in the disease course.

In the basic science community, there is a rich collection of electrophysiological techniques which also have the advantage of interrogating central pathways. There are a diverse range of stimulation paradigms which probe particular pathways using techniques such as TMS, direct current (tDCS) and electrical stimulation (TES). There are also techniques which are known to probe the function of important brain structures such as the cerebellum and brainstem. Whilst some of these techniques have already found clinical utility (for example, measuring central motor conduction delays in MS with TMS), in most cases they have never been considered for diagnostic purposes. However, the simplicity and sensitivity of these techniques would make them suitable for this task.

Biomarkers may have a role in monitoring disease progression and drug side effects

Aside from the question of accurate diagnosis, biomarkers could have numerous other roles in the clinical setting. For instance, they could be useful in monitoring the course of neurodegenerative disease. This could potentially help clinicians with the difficult task of predicting disease milestones; ultimately, biomarkers may also provide an accurate prognosis as well as yield information on the efficacy of the treatment regimen. This is particularly important since we know that neurodegenerative disorders progress at very different rates and can be notoriously difficult to treat.

There may also be an important role for biomarkers in clinical trials. Assessing the efficacy of new drugs during phase III clinical trials is currently achieved using clinical measures. These are both time-consuming and subjective. A quantifiable biomarker for assessing drug efficacy would be much more robust and efficient. Not only would this streamline large

multi-centre trials, it could also significantly reduce the length and cost of clinical trials. Biomarkers implemented in earlier stages of drug development could also reduce the risks assumed by patients during clinical trials by picking up ineffective agents early in the process.

In addition, we know that many drugs can cause unwanted adverse effects in the nervous system. It is easy to overlook the importance of this, especially if the drugs are treating the primary disease effectively. However, some side effects can be severe and irreversible such as the cerebellar atrophy associated with some antiepileptic treatments. In such cases, identifying this problem early, before damage accumulates would be very useful. In particular a quantitative ratio of drug efficacy versus adverse effects would be an objective way of assessing the most appropriate treatment for individuals.

Developing biomarkers

Many sensitive indicators of nervous system dysfunction have been used in basic science for decades. Recently, some of these have become available in the clinical setting for diagnostic and therapeutic purposes. TMS, for example, has been used to identify central motor conduction delays associated with MS (Eisen and Shtybel, 1990) and excitability changes in MND (Vucic and Kiernan, 2006). However there are many more techniques which could be exploited successfully in this way.

Useful biomarkers must fulfil certain criteria. Firstly, they must be able to detect objectively a feature or hallmark of a particular disease process. Moreover, they must be able to do this with high confidence; that is they must be both sensitive and specific to that particular entity. Finally, the results generated using any biomarker must be highly reliable and reproducible. Without all of these features, a biomarker simply would not be sufficiently efficacious to justify use as a clinical diagnostic test.

In addition to the criteria above, there are also a number of features which are desirable for any clinical test. Ideally, they should be either non-invasive or minimally invasive to reduce the impact on patients. There should also be a clear economic justification; to warrant routine use, a cost-benefit analysis is often required to take into account the cost of

facilities, equipment and the skill base of the staff necessary to conduct and interpret any test result.

Thesis objective

The aim of this thesis is to explore the use of non-invasive techniques both in the investigation of normal and pathological brain function. Most of the paradigms discussed therefore will not be novel; it is only their application which is novel. I would like to help to facilitate the efficient and appropriate use of these techniques in both basic and clinical research. To that end, basic studies have been carried out in animals and healthy human subjects to help elucidate more information about the underlying mechanisms.

A major part of this thesis is concerned with improving the diagnosis of neurodegenerative disease by verifying the specificity of non-invasive methods and investigating their use as biomarkers. In the first instance these techniques have been applied to patients who already have an established diagnosis. This gives us the opportunity to test whether the techniques have the sensitivity and specificity necessary to be implemented as a biomarker of neurodegenerative disease. In the future, the ultimate aim is to extend this research into the non-invasive detection of sub-clinical disease by following patients longitudinally. An obvious way to test for this would be to use patients with a genetic disposition to a particular condition which would allow measures to be taken before and after the onset of symptoms. These patients are rare however, so larger scale studies might involve broad investigation of all patients presenting to movement disorder specialists with a particular range of symptoms. This type of study would enable us to determine at what stage in the disease course our techniques are effective and whether they will truly have clinical utility as biomarkers.

All experiments described in this thesis were approved by the Local Research Ethics Committee. Those involving patients were also sponsored by the Newcastle-upon-Tyne Hospitals NHS Trust. The clinical trial involving use of Levetiracetam required additional approval from the Medicines and Healthcare products Regulatory Authority. Animal procedures were carried out under UK Home Office personal and project licenses and in accordance with the Animals (Scientific procedures) Act 1986.

Patients were referred to the studies by the consultants responsible for their care. They were identified from specialist clinics within the Royal Victoria Infirmary, Newcastle General Hospital and Sunderland Royal Hospital. Age-matched control subjects were recruited from members of the Institute of Neuroscience and their friends and families. Participating subjects all gave written informed consent.

CHAPTER II: ASSESSING THE CORTICOSPINAL TRACT IN MOTOR NEURONE DISEASE

Motor neurone disease (MND) is a sporadic, fatal, neurodegenerative disorder. It is a rare condition with an estimated incidence of 1-6 people affected in every 100,000 (Alonso et al., 2009; Dean et al., 1994). The MND spectrum comprises four clinically defined subtypes between which there is some degree of overlap. These are: amyotrophic lateral sclerosis (ALS); progressive bulbar palsy (PBP); progressive muscular atrophy (PMA); and primary lateral sclerosis (PLS).

MND presents most commonly as ALS and is characterised by widespread degeneration of neurones within the ventral horn of the spinal cord and the motor cortex. This is typically an aggressive and rapidly progressive form of MND; average life expectancy is only 2-3 years from onset (Forbes et al., 2004; Hudson et al., 1986; Sorenson et al., 2002).

As with other neurodegenerative conditions, there are some well characterised genetic associations with MND. Of these, a mutation in the SOD1 gene on chromosome 21q is the most common; this is associated with ALS (Rosen et al., 1993). However, familial MND is only reported in an estimated 10% of cases. For the remaining 90% of patients, the disease process is considered to be sporadic and there is no known aetiology.

Early diagnosis is important in a disease in which the primary strategy of treatment is to halt, or slow the relentless degeneration of motor neurones. Unfortunately, even at symptomatic presentation, when the diagnosis is often uncertain, there is already extensive motoneurone degeneration. MND patients could have already lost 10-50% of motoneurones before muscles become symptomatically weak (Aggarwal and Nicholson, 2002). This presents problems for clinical trials of neuroprotective agents; changes in endpoints such as strength and disability are likely to be small and difficult to discriminate statistically because of the extensive neuronal destruction. Despite this, early trials of the neuroprotective agent riluzole demonstrated a small but significant improvement in life expectancy in patients with ALS (Bensimon et al., 1994; Lacomblez et al., 1996). This

benefit would likely be significantly enhanced if an earlier diagnosis could be achieved to open up the therapeutic window.

Current diagnostic criteria for MND

There are no specific diagnostic tests for any of the conditions on the MND spectrum. Instead, diagnosis involves first excluding the possibility of other diseases which can mimic MND such as cervical spondylotic myeloradiculopathy, primary progressive multiple sclerosis or hereditary spastic paraplegia. Then, doctors turn to clinical presentation; patients must fulfil criteria agreed by international committees. It may therefore take several months or even years to confirm diagnosis.

The first and probably still most widely used consensus criteria (El Escorial) were introduced by the World Federation of Neurologists (Brooks, 1994) and subsequently revised (Brooks et al., 2000) to increase sensitivity. Rapid progression of the condition is usually key to diagnosis, except in the case of PLS. Diagnosis is mainly on the basis of clinical evidence although neurophysiological tests were incorporated in the revision which is important since EMG is able to detect some of the chronic neurogenic changes associated with MND. To obtain a label of 'probable MND', patients must have a progressive history as well as presence of both upper and lower motoneurone signs in at least two spinal regions on examination. There must also be an absence of sensory signs which cannot be explained by any other differential diagnosis. The El Escorial criteria is useful in ALS which has diffuse degeneration across both central and peripheral nerves but does not account for the other variants of MND which have a more localized onset.

Although still in widespread use, the El Escorial criteria have been repeatedly criticized. Traynor et al. (2000) demonstrated poor sensitivity in their large cohort of ALS patients. Moreover, approximately 10% of their patients died without ever reaching a definite diagnosis. Consequently, further standards have been proposed. Specialist criteria were created by a subcommittee of the world federation of neurology explicitly to define patients appropriate for each phase of clinical trials (World Federation of Neurology Research Group on Neuromuscular Diseases Subcommittee on Motor Neuron Disease., 1995; Miller et al., 1999). These 'Airlie House' criteria also evaluated methods of assessment and rating

scales in terms of which were preferred tests and which were merely acceptable. The 'Awaji criteria' (de Carvalho et al., 2008) were also proposed which suggested that the criteria of denervation in multiple muscles and spinal regions could be fulfilled by a combination of findings on clinical examination and EMG studies. This was a minor modification to El Escorial which was designed to facilitate earlier diagnosis. A subsequent publication has demonstrated this method to be more sensitive than El Escorial in the diagnosis of ALS (Carvalho and Swash, 2009).

EMG studies have long had a central role in the diagnosis of MND and are an important feature of consensus criteria. Consequently, there are a number of specific neurogenic changes which support a diagnosis of MND. A standard clinical study in the UK consists of a 45 minute appointment window during which the clinician must be able to demonstrate significant changes in multiple muscles supplied by at least two of the four spinal regions (cervical, thoracic, lumbar and sacral). Intramuscular EMG recordings are performed which involve insertion of a needle electrode into muscle tissue and recording electrical potentials both at rest and during gentle muscle contraction.

Resting muscles are normally electrically silent in healthy people. However, in MND, active denervation gives rise to biphasic fibrillation and fasciculation potentials which reflect spontaneous discharge of both individual and groups of nerve fibres respectively. Positive sharp waves may also be observed early in the disease course in the absence of other electrical abnormalities. These are characterised by a rhythmic discharge in relaxed muscles with a sharp positive deflection followed by a slow negative phase which gradually returns to baseline. On EMG examination, positive sharp waves can often be seen in the presence of fibrillation potentials and although the exact origin is unclear, both are thought to arise as a direct consequence of individual muscle fibres losing their connection to the corresponding motor axon.

On voluntary muscle contraction in healthy subjects, there is normally a highly ordered recruitment of motor units from small to large as the effort is increased. However, in MND this recruitment is usually impaired due to the reduced number of motor units available. In addition, the remaining motor units are typically very large and polyphasic as a result of re-innervation of muscle fibres.

A wide range of muscles are usually sampled since a diagnosis of MND by current criteria can only be supported if multiple muscles and limb segments are involved. The detection of sensory changes or conduction block during NCS are also important findings since they do not support a diagnosis of MND.

There are also further techniques which though not incorporated into diagnostic criteria can provide useful information about the state of the system. TMS can be a useful adjunct; it is known that patients with MND typically have either absent or small amplitude MEPS alongside a prolonged CMCT (Cruz Martinez and Trejo, 1999; Mills and Nithi, 1998; Osei-Lah and Mills, 2004). As the disease progresses, MEPs become harder to elicit and eventually disappear altogether. A recent report further suggested that MND can be picked up before the obvious change in MEPs appear. Vucic and Kiernan (2006) described reduced motor threshold which is predictive of MND; this is followed by a significantly increased threshold as motoneurones begin to die.

Descending pathways controlling movement

In mammals, there are a number of descending motor pathways which influence movement control (Fig. 2-1A); their relative size is different amongst species, but the general function is preserved. The dorsolateral pathways of the corticospinal tract and rubrospinal tracts are chiefly associated with fine, dextrous control of distal muscles, in particular the control of hand movements. In contrast, the ventromedial pathways of the reticulospinal, vestibulospinal and tectospinal tract are predominantly responsible for balance and control of posture (Lawrence and Kuypers, 1968b).

The corticospinal tract

Primates have a unique ability amongst animals to perform very fine, skilled, dexterous hand movements. This ability has evolved relatively recently alongside the development of the direct corticomotoneuronal projection from the brain to the spinal cord. In humans and primates, this projection forms a very specialised component of the corticospinal tract (CST) which is the major pathway through which the cortex influences the spinal cord and therefore controls movements.

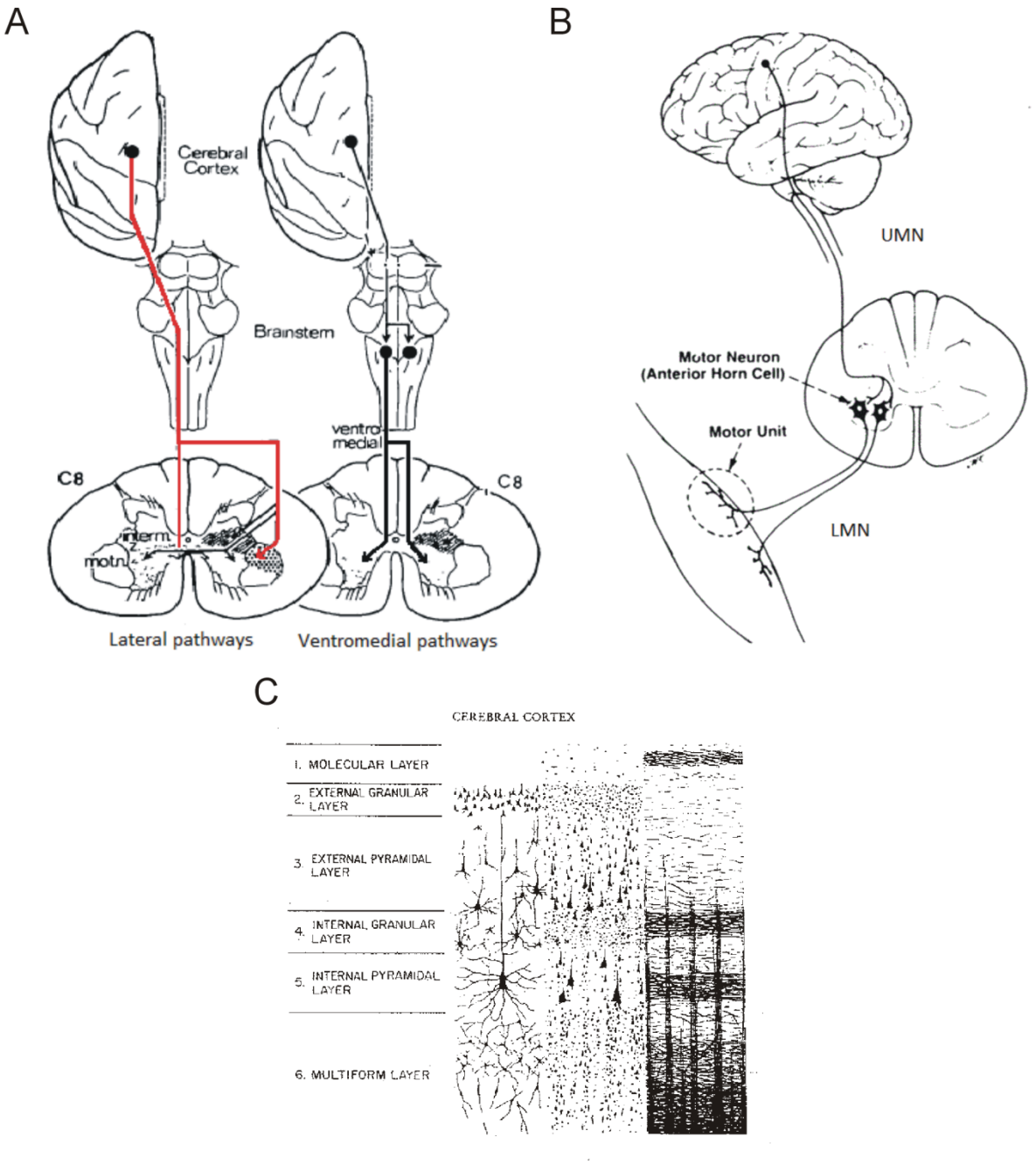


Figure 2-1 A: Schematic representation of the major descending motor pathways in primates. The corticospinal tract is highlighted in red. Adapted from Brinkman and Kuypers (1973). B: Schematic of the descending corticospinal tract showing the central connection between cortex and spinal cord and the peripheral projection to the muscle. C: Detailed anatomical structure of motor cortex. From www.brainmind.com.

The CST is a vast control network constituting approximately 1 million fibres in humans. Anatomical studies demonstrate that the CST mainly innervates distal musculature facilitating its role in shaping fine fractionated finger movements. It is more developed in higher apes (especially humans) than in any other mammal. These abundant direct corticomotoneuronal (CM) connections are known to exert powerful monosynaptic effects on hand muscles. Moreover digital dexterity is greatest in animals with a higher proportion of CM connections (Heffner and Masterton, 1983) indicating the importance of these projections in shaping precise movements.

Corticospinal tract anatomy

The CST originates primarily in the primary motor cortex, although a significant number of fibres also arise from the supplementary motor area, arcuate motor area and cingulate gyrus (Dum and Strick, 1991; Galea and Darian-Smith, 1994; He et al., 1993; He et al., 1995; Nudo and Masterton, 1990). These corticospinal neurones project to all levels of the spinal cord, but the majority of fibres terminate in cervical regions which innervate the neck and upper limbs (Weil and Lassek, 1929). CST neurones target the ventral horn; here they usually synapse onto interneurons but sometimes directly onto motoneurones. The corticospinal tract also projects to the medullary region; this is discussed in more detail in chapter V.

The CST is predominantly a crossed pathway; 85-90% of fibres cross the midline at the level of the medulla oblongata and descend in the lateral CST via the dorsolateral funiculus (Armand and Kuypers, 1980; Rosenzweig et al., 2009). These projections terminate in the lateral region of the ventral horn; this area of the spinal cord is particularly rich in motoneurones which innervate distal limb muscles. The remaining 10-15% of axons descend ipsilaterally in the medial CST, projecting predominantly to the axial musculature. Recent work has suggested an added layer of complexity in that there is also a re-crossing of a significant number of fibres (Rosenzweig et al., 2009).

Corticospinal tract fibres are myelinated and therefore fast conducting. The axons of the CST are highly collateralised within the brain; branches relay information about descending motor commands to other cortical areas. However, compared to other

descending pathways, the CST is not highly collateralised within the spinal cord; presumably this selective pattern of termination contributes to the facilitation of very fine, precisely controlled movements.

The corticospinal pathway is divided into 2 distinct sections (Fig. 2-1B). Anatomists describe the connection between motor cortex and the spinal cord as *corticospinal* with *motoneurons* projecting from the cord to the muscles. However, clinicians use different terminology and it is these that will be used within this chapter. *Upper motor neurones* (UMNs) have cell bodies located in the motor cortex and long axons which project to either the brainstem or ventral horn of the spinal cord. *Lower motor neurones* (LMNs) have axons arising from the ventral horn of the spinal cord or the brainstem and project to muscle fibres.

The principle cells of the CST are pyramidal tract neurones, the largest of which are commonly known as Betz cells. These are found in layer V of the primary motor cortex and were thoroughly described by Ramon y Cajal almost a century ago. The largest Betz cells can reach up to 120μm in humans, though the majority are small (<40 μm). The cortical layers and their corresponding cytoarchitecture are shown in figure 2-1C.

Distinct clusters of pyramidal cells are found throughout M1, particularly in caudal parts of the precentral gyrus. This pattern of distribution is distinct to M1 but the functional significance is unknown. In addition, M1 is not densely packed like other cortical areas and in particular layer V is the least densely packed with cells. Unlike the sensory cortices, motor cortex has only a very gross somatotopic organisation. The neurologists Penfield and Rasmussen (1950) originally revealed the ordered structure of M1 via their microstimation mapping studies. However this organisation has since been shown to be less ordered than previously thought (Liddell and Phillips, 1950; Schieber and Hibbard, 1993; Schieber, 2001). Although there are separate areas for large body segments, there is a distributed organisation of the smaller parts within these discrete zones. For example, there is overlap in cortical representation for the fingers within the area known to contain neurons projecting to the upper extremities. A similar level of somatotopy has also been demonstrated in premotor areas (Godschalk et al., 1995).

Motor cortex receives significant input from adjacent somatosensory areas and this sensory feedback has a significant role in motor control. Indeed, lesions of CST cause sensory deficits; Hamzei et al (2006; 2008) demonstrated altered activation of somatosensory areas in patients with damage to the CST. This was more pronounced in patients with the most extensive lesions. Significant corticomuscular coherence has also been identified between oscillations in somatosensory areas, motor cortex (Witham et al., 2007) and the periphery (Witham et al., 2010) suggesting there is sensorimotor integration in the cortex which informs the execution of new movements. Moreover, Riddle and Baker (2005) showed that introducing an afferent feedback delay by cooling the periphery could alter this corticomuscular coherence.

Corticospinal tract lesions

Natural, selective lesions of the corticospinal tract are rare (pure motor stroke, caused by a lacune selectively affecting the CST). Nevertheless, there have been some cases of pyramidal stroke and hemiplegia (Chokroverty et al., 1975; Ropper et al., 1979) which have helped us to assess the role of the CST. Such cases report unilateral weakness in voluntary movements with deficits most apparent in the distal musculature. Recovery is usually weighted towards proximal muscles (Lang et al., 2006) which is in agreement with reorganisation of brainstem motor pathways; these have relatively more connections with proximal motoneurones.

Controlled lesion studies in animals have been useful in revealing the functional role of the CST in movement control. Lawrence and Kuypers' (1968) seminal work demonstrated that damage to the CST in primates was not as catastrophic as previously thought. Although immediately after bilateral pyramidectomy animals were paralysed, they recovered relatively quickly. Indeed, after this period of recovery, it seemed that there were very few gross deficits in movements; animals could grip, walk and climb surprisingly normally. Significant motor deficits were restricted to the fine, fractionated finger movements; monkeys struggled to retrieve food from small wells and they had difficulty grooming each other. These deficits are thought to be related to the number of corticomotoneuronal fibres since they are greater in chimpanzees than macaques (Tower, 1940).

Reversible inactivation of motor cortex with muscimol has supported observations in lesioned animals. Injections into discrete regions of M1 resulted in weak finger movements and an inability to fractionate fingers (Brochier et al., 1999; Schieber and Poliakov, 1998). These problems were resolved once the effects of muscimol had worn off.

Current methods of corticospinal tract assessment

It is considerably easier to assess the integrity of the 'lower motor neurone' or the peripheral component of the system. Nerve conduction studies (NCS) assess the integrity of these pathways and there are hallmark features associated with motor neuron degeneration. This represents a simple and reliable test of peripheral nerve function and integrity.

In contrast, we are very poor at assessing the integrity of the 'upper motor neurone'; this is almost always the limiting factor in diagnosis since there are no consistent, robust and reliable methods to do this. The standard assessment of CST function for most clinicians is limited to assessing the deep tendon reflexes with a tendon hammer. Detection of hyperreflexia is a useful sign of a central neurological abnormality but is only a very gross test of the stretch reflex loop. It could certainly not be considered a specific test of CST integrity since there are many components of this loop which could contribute to the abnormalities observed. However, this is often used to infer CST damage given the simplicity of the test and its availability in clinic.

A second useful sign of CST damage is the Babinski reflex which is a primitive reflex normally observed in infants before maturation of the CST (Babinski, 1896; Lance, 2002). This manifests as a profound dorsiflexion of the toes when the sole of the foot is rubbed with a blunt instrument. The response normally disappears in ambulating infants from the age of ~12-18 months; thereafter the same stimulus elicits plantar flexion of the toes in healthy children and adults. A positive Babinski sign from the age of 2 years is indicative of a CST lesion which has unmasked the primitive reflex. Whilst this is a useful sign of dysfunction, it is rather non-specific and is abnormal in a number of neurological conditions.

Formal assessment of the integrity of the CST is challenging and can only be performed

using indirect methods. These techniques are highly specialised and not readily available to all clinicians in the UK. Nevertheless the methods are outlined below.

Transcranial Magnetic Stimulation (TMS)

TMS is a non-invasive method of activating brain structures which can be a useful diagnostic indicator of damage to the CST. Weak electric currents are generated in neural tissue by the discharge of rapidly changing magnetic fields over the surface of the skull. In this way, current is transmitted through the skull (which would normally act as an electrical insulator) without causing pain via activation of cutaneous skin receptors. TMS over the motor cortex activates the monosynaptic corticomotoneuronal connections which generate short-latency MEPs in contralateral muscles.

Throughout the last two decades, the parameters for TMS of the motor system have been well described. There is an enormous amount of data showing the standard limits in the normal population. Because of this thorough classification, abnormalities can be identified in patients with some neurodegenerative diseases relatively easily using single pulse TMS (Eisen and Shtybel, 1990). Indicators of pathology in TMS include delayed conduction times, abnormal threshold for evoked responses and abnormal waveform morphology.

One parameter that is difficult to measure with a standard single pulse TMS protocol is the recruitment of upper motor neurons by the stimulus. MEP response amplitude should correlate to this but is highly variable from trial to trial, even in healthy subjects, therefore reduced conduction as a result of underlying pathology can be difficult to determine. More recently, the triple stimulation technique has been developed to improve the sensitivity of TMS to loss of UMNs. This comprises a double collision test involving TMS over the motor cortex followed by electrical stimulation of the ulnar nerve and Erb's point. The timing is such that almost all of the available UMNs are recruited and respond synchronously producing a time-locked MEP in a contracting muscle. The triple stimulation technique provides a quantitative measure of the number of upper motor neurons that can be activated with TMS and has therefore increased the sensitivity for detection of UMN degeneration in conditions such as MND.

Whilst TMS is certainly a useful diagnostic aid, at high intensities of stimulation, there is

profound current spread which can cause discomfort through activation of muscles of the face and neck. This is particularly the case when a double cone coil is used to activate lower limb muscles. It is also the case with the triple stimulation technique which involves 3 high intensity stimuli in close succession. Often patients find TMS at these high intensities intolerable.

Unfortunately, TMS equipment is expensive and as a result is only available at limited clinical sites throughout the UK. Despite its clear utility in diagnosis, few hospitals have the technology; consequently TMS is not usually available as a standard clinical test. Its use is typically restricted to research establishments.

Magnetic Resonance Imaging (MRI)

MRI can be a useful method of revealing CST damage. High signal in motor regions of cortex and the pyramidal tracts have been related to diseases such as MND. However, MRI is not particularly sensitive to early subtle pathological changes and is usually only useful when there is already profound damage to the system. In fact, brain MRI is often normal in patients with corticospinal tract dysfunction (Luis et al., 1990; Udaka et al., 1992). Furthermore, small areas of signal change are common in the aging brain (Drayer, 1988; Hendrie et al., 1989).

MRI tractography

MRI tractography is a relatively new technique which aims to reveal the underlying integrity of brain pathways using non-invasive imaging technology. Diffusion tensor imaging, sensitive to diffusion of water in physiological tissue can produce 3-dimensional images of structures. Free diffusion occurs in all directions, but barriers such as axons cause uneven diffusion. Large axon tracts such as the CST cause diffusion along the orientation of the fibres. A number of recent publications have observed early degenerative changes in the CST using this method (Ciccarelli et al., 2006; Roccatagliata et al., 2009).

Assessing UMN dysfunction in MND

To establish a new test of UMN dysfunction in MND, we must first look at the rare cases

where there is a relatively selective degeneration of the CST. We are not interested in these *per se* since they only make up a very small part of the MND population. However, investigating the 'pure' UMN and LMN forms of MND enables us to detect whether the technique is sensitive to damage within the UMN and more importantly is able to differentiate between established cases. If a technique is able to do this effectively, it could then be used in ALS to show UMN dysfunction in the presence of LMN disease.

Primary Lateral Sclerosis (PLS)

PLS is a rare condition which forms part of the MND spectrum and accounts for as few as 3% of all patients with MND (Gordon et al., 2006). In PLS, there is selective degeneration of the layer V pyramidal neurones of the precentral gyrus and corticospinal tract with preservation of anterior horn cells (Hudson et al., 1993; Pringle et al., 1992). Patients usually present in the fifth or sixth decade with a slowly progressing ascending spastic tetraparesis. PLS is characterised by an insidious, slow progression. Unlike other MND variants, the disease duration is long which is an important distinction used in the diagnostic process. Median disease duration for PLS is 19 years (Pringle et al., 1992); consequently this particular form of MND does not significantly reduce life expectancy.

Anatomically, there is a striking absence of Betz cells from layer V of the motor cortex and a reduction in pyramidal cells from other layers (Pringle et al., 1992). Remaining cortical cells are often small in size compared to ALS patients or controls (Pringle et al., 1992). These fundamental changes within the CST can be detected through non-invasive methods. PLS patients have prolonged or absent MEPs (Brown et al., 1992; Zhai et al., 2003) (Brown et al., 1992) and localised cortical atrophy on MRI (Pringle et al., 1992).

There is little evidence for pathology in brain areas other than the corticospinal tract. The selective nature of PLS therefore provides the perfect platform to investigate the role of the CST in movement and to assess new methods of assessing CST integrity. Although in PLS hand function is impaired and foot movements are slow and weak, there is usually sufficient residual function to perform simple repetitive manual and ankle flexion tasks.

Progressive Muscular Atrophy (PMA)

Approximately 10% of MND patients only have lower motor neuron signs at symptom onset. In this condition, the degenerative process is initially limited to spinal motoneurones. Progressive degeneration of anterior horn cells within the spinal cord is the hallmark pathological feature of PMA at autopsy. This is a rapid process and any cells that are spared usually contain distinctive inclusion bodies.

In this 'pure' lower motor neuron disorder, there is initially no involvement of the corticospinal tract. Consequently, the upper motor neuron signs, spasticity and brisk reflexes, are absent. In many cases of PMA however, pathology eventually spreads to both upper and lower motoneurones (Ince et al., 2003), therefore this condition is considered to be a slowly progressing form of ALS with a significantly longer survival time.

Oscillations and coherence

Motor cortical cells modulate activity during skilled upper limb movements. Rhythmic oscillatory activity can be observed in sensorimotor cortex which typically falls into two frequency bands: alpha (8-12Hz) and beta (15-30Hz). The first demonstration of this pattern of activity in humans was performed by neurosurgeons using electrocorticogram recordings to identify epileptogenic tissue (Jasper and Penfield, 1949; Penfield, 1954). Subsequently, oscillations have been demonstrated in motor cortical local field potentials in animals (Baker et al., 1997; Donoghue et al., 1998; Murthy and Fetz, 1992) and electroencephalograms (EEG) or magnetoencephalograms (MEG) in human subjects (Pfurtscheller et al., 1996; Salmelin and Hari, 1994).

Similar oscillatory activity can also be observed in contralateral forearm and intrinsic hand muscles during both isometric and auxotonic contractions. Coupling between oscillations in two structures can be measured using coherence analysis. Coherence is a measure of correlation of two signals in the frequency domain and is bound between 0 (no correlation) and 1 (complete correlation). Coherence in the beta frequency band is present between the cortex and the periphery (Baker et al., 1997; Conway et al., 1995; Halliday et al., 1998; Kilner et al., 2000; Murthy and Fetz, 1996; Salenius et al., 1997) and has also been shown between different muscles. The function and the origin of these synchronous oscillations

has been the subject of much debate, however it is clear that they are propagated within the corticospinal tract to the periphery. Given this dependence of coherence on the CST, it may be a useful indicator of CST dysfunction.

Corticomuscular coherence

The prevailing view is that the source of corticomuscular coherence (CMC) is the primary motor cortex (M1) and that oscillations are propagated to muscle via the corticospinal tract (CST). Many groups have found phase lags between cortex and muscle which would support this notion (Gross et al., 2000; Mima et al., 2000). In addition, activity generated in motor cortex following TMS (Hansen and Nielsen, 2004) and pyramidal tract stimulation (Jackson et al., 2002) has been shown to re-set oscillations and modulate corticomuscular coherence.

It has been suggested more recently that coherence is propagated via a loop involving both ascending and descending tracts. Coherence between sensory cortices and the periphery can be observed (Witham et al., 2010). Moreover, directed coherence is significant both in the ascending and descending directions (Witham et al., 2010; Witham et al., 2011). It is certainly true that sensory disturbances have a detrimental effect on coherence (Kilner et al., 2004; Fisher et al., 2002) so this should be considered in patients who have known sensory neuropathies.

Over the last decade there has been a surge of interest into the functional significance of corticomuscular coherence, with only a limited improvement in our understanding. The phenomenon has been compared to the visual 'binding' process; here, 40Hz oscillations are hypothesized to be responsible for the integration of distributed information into a complete visual percept. However, this is unlikely to be the case in the motor system for a number of reasons, chiefly that 15-30Hz cortical oscillations are abolished upon movement execution (Kilner et al., 1999; Pfurtscheller et al., 1996) and therefore are unlikely to have a direct role in movement control. This has led some researchers to conclude that they simply form an 'idling rhythm' within the motor cortex (Pfurtscheller et al., 1996). An alternative hypothesis may be that coherent motor oscillations have a role in system recalibration following movement. Errors in movements are likely to confuse the system as

to where the limbs are in space; evaluation of proprioceptive information would therefore be crucial to the accurate planning of new movements. In support of this hypothesis, coherence amplitude in the beta frequency range has been shown to vary according to the size of the previous movement (Kilner et al., 2003). Moreover, manipulation of afferent feedback loops via cooling or ischaemic block reduces CMC (Fisher et al., 2002; Riddle and Baker, 2005).

Whilst beta band coherence is maximal during steady-state isometric tasks, this is not reported to be the case during dynamic tasks where there has been shown to be a frequency shift to gamma band coherence (Andrykiewicz et al., 2007; Kristeva et al., 2007). This increased coherence in the gamma band has been proposed to occur as a result of enhanced attention to a more complicated task and reflect the integration of multimodal information to optimise the motor response (Andrykiewicz et al., 2007). This process would therefore be analagous to the high frequency oscillations observed in the binding process of the visual system (Gray and Singer, 1989).

Information surrounding the importance of oscillations has also been inferred by observing patients with movement disorders. In Parkinson's disease, untreated patients have excess beta band activity in the basal ganglia which normalizes upon administration of dopamine or high frequency stimulation of the substantia nigra (Brown et al., 2001). The degree of clinical improvement in both cases correlates well to the change in beta band coherence (Kuhn et al., 2006; Silberstein et al., 2005). These patients also have increased coherence between cortex and the basal ganglia in the beta band (Fogelson et al., 2006; Williams et al., 2002). Moreover, beta frequency stimulation of basal ganglia slows movement (Chen et al., 2007; Eusebio et al., 2008), however, it must be noted that deficits in movement following beta frequency stimulation are very modest. Based on this collection of evidence, it has been suggested that the motor deficits found in patients with Parkinson's disease can be largely attributed to the abnormal and synchronous beta band oscillations (Brown, 2007). This view of excess beta oscillations as pathological is in disagreement with the traditional thinking that they subserve a positive state whether that be cortical idling or a form of system recalibration.

Intermuscular coherence

Coupling between two EMG signals can also be measured using the coherence method. As with CMC, in healthy subjects intermuscular coherence (IMC) is maximal during sustained muscle contraction (Norton and Gorassini, 2006). Intermuscular coherence however typically shows two distinct frequency bands centred around 10Hz and 15-30Hz (Grosse et al., 2002).

IMC in the 15-30 Hz range is dependent on supraspinal structures, including the CST, because it disappears after stroke and complete spinal cord injury (Farmer et al., 1993; Hansen et al., 2005). Moreover, in partial spinal cord injury, in which the CST may be intact, training in some patients not only increases functional recovery and MEP amplitudes, but also increases beta band IMC (Norton & Gorassini, 2006). In contrast, IMC in the 8-12 Hz range persists after complete spinal cord lesions (Norton et al., 2003; Norton et al., 2004), suggesting that it is largely dependent on segmental or spinal mechanisms.

Intermuscular coherence has many advantages over corticorticomuscular coherence; the most obvious being that an EEG is not required. Scalp EEG, although relatively simple to record and in widespread use has many associated difficulties. Signal to noise ratio tends to be very low and it can be contaminated by EMG which can make it difficult to use in patients with movement disorders. Taking advantage of the fact that the same cortical drive which generates CMC is likely to contribute to coherence between muscles, IMC is likely to be an easier technique to utilize clinically. It is a quick and easy method which could be recorded with the facilities available in a standard hospital neurophysiology department. Also, the variability in CMC may be due to scalp projection of cortical signals; with IMC, we have more confidence that we are recording from the correct place.

Methods.

Two assessments were performed on patients recruited to this trial. Firstly, a TMS study was carried out to determine motor threshold, MEP latency and amplitude as well as the central motor conduction time (CMCT). Since there is a large amount of data on TMS in MND in the literature, this enabled comparison of coherence measures to the results of

previous studies. Corticomuscular and intermuscular coherence was also measured in the upper and lower limbs.

Transcranial magnetic stimulation

Single pulse TMS was performed over motor cortex whilst EMGs were recorded to measure motor evoked potentials (MEPs) in the contralateral muscles. Motor threshold (MT) is defined as the lowest intensity of stimulation at which an MEP of defined size is observed; this is usually the intensity at which there are responses in 50% of stimuli (Rossini et al., 1994; Rothwell et al., 1999).

Active motor threshold (AMT) was identified using a circular coil over vertex whilst patients provided a background contraction of either the hand or foot muscles. This was to provide the maximum sensitivity for the detection of descending volleys which is especially important in patients with degeneration of the corticospinal tract who are known to have increased motor threshold (Triggs et al., 1992). The coil was oriented to activate the contralateral hemisphere to the limb under investigation (A side up: left; B side up: right). Stimulus triggered EMG activity for FDI was viewed on an oscilloscope. Stimulus intensity was increased in 5% increments until an approximate threshold was located for FDI. From there, intensity was altered in 1% increments accurately to pinpoint AMT.

MEPs were typically recorded at 10% MSO above the AMT. PLS patients are known to have very high thresholds so this stimulus intensity was chosen to allow a consistent comparison between patients and controls. Stimuli were delivered at 0.2Hz whilst subjects maintained a background contraction of the relevant muscles. At each intensity, approximately 10 responses were recorded.

In some cases, TMS was also performed over the spinal roots to allow measurement of peripheral conduction time. This was done at the level of the cervical cord for the upper limbs and the lumbar cord for the lower limbs. Subtraction of this peripheral conduction time from the MEP latency allowed calculation of central motor conduction time (CMCT).

Coherence task

Corticomuscular (CMC) and intermuscular coherence (IMC) testing was also performed in

our patient cohort. Coherence measurement requires only a weak muscle contraction; this means that even patients with profound muscle weakness have enough residual muscle activity to perform the task. Given the loss of Betz cells in PLS and the resultant loss of propagation of oscillations from M1 to muscles, a corresponding loss of coherence in the beta frequency band would be expected.

Subjects were asked to execute a simple repetitive task. They were required to perform an auxotonic contraction of the upper and lower limb. For the upper limb, they were required to perform a precision grip, guided by a small, compliant piece of plastic tube (Portex, external diameter 10mm, internal diameter 8mm, 19cm in length) attached to the finger and thumb with micropore tape. Subjects were instructed to oppose the two ends of the tubing, a movement which required a minimum force of 1N. The task followed the pattern of a 3 second hold, followed by a 2 second rest period; a computer generated auditory cues to instruct the subject. For lower limb investigations, the task took the same format but with dorsiflexion of the ankle.

Patients were required to perform 100 repetitions of each task. In one case, where a patient was particularly weak, this was divided into two smaller, more manageable segments.

TMS and coherence testing was also carried out in 20 age-matched control subjects. Not all subjects underwent both assessments; this is indicated in the figure legends.

Recordings

Electromyogram (EMG)

Bipolar surface EMGs were recorded using adhesive electrodes (101600, Bio-logic, Biosense Medical) arranged in a standard tendon-belly configuration (or with both electrodes on the muscle belly in larger muscles). The electrodes were positioned carefully over the muscles of interest since the differential recording is sensitive to populations of motor units directly underlying the electrodes. The skin was prepared prior to attachment of the electrodes using 100% ethanol solution and then allowed to dry. A ground electrode was used, usually placed on the wrist. Signals were amplified (gain:1-5k) and bandpass

filtered (30Hz-2kHz) via an isolated Digitimer amplifier (NL820).

The activity in 3 muscles from each limb under investigation was recorded. In the arm, the muscles used were first dorsal interrosseus (FDI), flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC). During the lower limb task, EMG activity was recorded from extensor digitorum brevis (EDB), tibialis anterior (TA) and gastrocnemius (GS).

Electroencephalogram (EEG)

Cortical activity generated by populations of neurons can be measured using non-invasive scalp EEG in humans. The EEG signal comprises summated inhibitory and excitatory post-synaptic potentials from a large number of synchronised pyramidal cells. EEG signals are dominated by neuronal activity occurring directly beneath the recording electrodes, but are also sensitive to more widespread activity.

A single channel differential EEG was recorded from sensorimotor cortex, contralateral to the muscles under investigation. The scalp was prepared using an abrasive paste (Nuprep, Digitimer Ltd) and adhesive electrodes (Ambu Neuroline 720, Biosense Medical) were used. During the upper limb task, a montage was used which has previously been identified as optimal for recording oscillatory potentials related to hand movements; this involved placement of electrodes 3cm lateral to vertex with the non-inverting electrode two centimetres anterior and the inverting electrode two centimetres posterior to this point. A ground electrode was positioned over the forehead.

A lower limb montage EEG was also implemented during ankle dorsiflexion. Here, electrodes were located more centrally as the leg area of the motor strip is known to be located on the bank of the longitudinal fissure. For this, the inverting electrode was positioned over vertex with the non-inverting electrode 2cm anterior to this position. The ground electrode remained on the forehead.

In some cases, a conductive paste was used (Ten20) along with a bandage over the electrodes to improve contact and minimise the effects of noise. Signals were amplified at 50k and bandpass filtered (3Hz-2kHz).

Recordings were digitized at a sampling rate of 5kHz using a CED Power1401 (Cambridge Electronic Design). Data were captured to a PC running Spike 2 software along with event markers and where necessary stimulus profiles.

Patients

Patients were recruited from the specialist MND centre at the Newcastle General Hospital by the consultant in charge of their care. Study inclusion was made on the basis of current diagnostic criteria for PLS (Pringle et al., 1992) and PMA (evidence of LMN deficits and exclusion of other differentials). Only patients with a definite diagnosis and normal sensory investigations were included.

Patient demographics are shown in tables 2-1 and 2-2. Eight patients with PLS and six patients with PMA were recruited from the MND service. Data were compared with the equivalent recorded from 20 age-matched controls (36-78 years; mean 60 years).

In addition to the patients recruited to the main trial, one patient was investigated early in the clinical process as part of a new prospective trial of intermuscular coherence as a diagnostic test for MND.

Primate model of PLS

Intermuscular coherence was also tested in a primate model of PLS. This involved a female macaque monkey (*M. Mulatta*) with a unilateral lesion of the left pyramidal tract at the level of the medulla. The lesion was made using a radiofrequency lesion maker (Model RFG 5, Radionics Inc.) whilst the animal was under general anaesthesia.

The animal had bilaterally implanted EMG patch electrodes over a range of hand and arm muscles. These were implanted using a technique developed by Miller & Houk (1993). In some cases, EMG recordings were supplemented using surface electrodes. All surgical procedures were carried out under aseptic conditions and general anaesthesia (3-5% sevofluorane inhalation in 100% O_2 with alfentanil infusion).

Recordings were made 3 months post-lesion. At this stage, the animal had recovered normal upper limb function. During the session, the monkey sat in a custom-made

	Sex	Age at Δ (years)	History at Δ (years)	Initial presentation	Past medical history	Drug history	Family history (HSP)	Bladder function	Investigations					
									Blood tests	CSF (OCBs)	EMG/ NCS/SEPs	MRI		MEPs
												Brain	c-spine	
1(RC)	M	49	4	RUL weakness	Nil	Nil	Nil	NAD	Normal B12 VDRL negative	NAD (absent)	NAD	Rolandic atrophy	NAD	UL & LL absent
2(AB)	F	66	4	LL and LUL spasticity	LVH	Bisoprolol Diclofenac Fluoxetine Baclofen Dantrolene Oxybutinin Quinine Riluzole Vitamin C & E	Nil	Frequency	Normal B12. HTLV1, VDRL, Borrelia burgdorferi serology negative.	NAD (absent)	NAD	NAD	NAD	RUL MEP ↓V LUL & LL MEPs absent
3(PD)	M	52	18	LL spasticity (R>L)	HD aged 20 [Rx DXT+ Splenectomy]	Riluzole Vitamin C Vitamin E	Nil	NAD	Normal VLCFA, WBC enzymes	NAD (absent)	NAD	NAD	NAD	R EDC & R GS absent. MEPs ↓V & ↑CMCT
4(AM)	M	45	3	LL spasticity (L>R) Pseudo-bulbar Dysarthria	DM II Hypertension R Sciatica	Lisinopril Quinine Baclofen Riluzole Vitamin C & E	Nil	NAD	Normal B12, VLCFA, WBC enzymes, VDRL negative	NAD (absent)	NAD (except L ulnar neuropathy, denervation L T7 paraspinal)	NAD	NAD	R FDS & R LL MEPs absent R FDI & R EDC MEPs ↓V & ↑CMCT
5(GM)	M	75	5	LL spasticity (L>R)	L sciatica	Bendro-flumethiazide Baclofen Riluzole Vitamin C Vitamin E	Nil	NAD	Normal B12, VLCFA, WBC enzymes	NAD (absent)	NAD (except chronic L L4/L5 radiculopathi c changes)	NAD	NAD	R EDC, R EDB & R GS MEPs absent R FDI MEP ↓V R TA MEP ↓V & ↑CMCT
6 (JH)	M	60	5	Pseudobulbar dysarthria L UL and LL spasticity	IHD Colonic Carcinoma 1999 Hypertension	Amitriptyline Baclofen Atorvastatin ?riluzole ?VitC/E	Nil	NAD		NAD (absent)	NAD (except L L5 radiculo-pathy)	NAD	NAD	R FDS, R EDC absent R FDI long latency and polyphasic
7(CC)	F	42	2	Progressive spastic paraparesis	Nil	Sertraline Femulen	Nil	NAD	B12 normal Normal VLCFA, WBC enzymes	Protein 0.54 g/L (absent)	-	NAD	C5/C6 disc-osteophyte complex (no neural compression)	R EDC & R GS MEPs absent R FDI, FDS, TA & EDB MEPs ↑CMCT & ↓V
8 (JT)	M	74	3.5	Progressive R LL weakness	BPH	Nil	Nil	BOO & Urgency	Copper normal Auto-antibodies negative B12 normal		NAD	Mild involutional change. Minor small vessel CVD	Multi-level degenerative changes, no neural compromise	Normal UL MEPs ↑CMCT in LL MEPs

Table 2-1: PLS patient demographics

	Sex	Age at Δ (years)	History at Δ (months)	Initial presentation	Past medical history	Drug history	Family history (MND)	Bladder function	Investigations				
									Laboratory tests	CSF (OCBs)	EMG/ NCS	Radiology	MEPs (SEPs)
1 (LB)	F	69	18	Bilateral foot drop Progressive LL and UL weakness Sympromatic fasciculations	IHD ↑BP	Clopidogrel Atenolol BFMTZ ISMN Lisinopril	Nil	NAD	Anti-GM1 Ab & anti-MAG Ab negative CK 328 UPEP normal	NAD (absent)	AHD (UL&LL)	CT head normal	NAD (-)
2 (HR)	F	67	24	Bilateral foot drop Progressive LL and UL weakness Sympromatic fasciculations	Non-toxic goitre	Ranitidine Lofepramine	Nil	NAD	Anti-GM1 Ab & anti-MAG Ab negative CK normal UPEP normal	NAD (absent)	AHD (UL&LL)	MRI brain and spine normal	NAD (-)
3 (GH)	M	61	10	Progressive LL weakness and cramps	Nil	-	Nil	NAD	Anti-GM1 Ab & anti-MAG Ab negative CK 372 UPEP normal	NAD (absent)	AHD (UL&LL)	MRI lumbar spine moderate canal stenosis (L3/4 & L4/5) without neural compression	NAD (-)
4 (JD)	M	73	12	Weakness R UL initially, progressing to bilateral UL & LL weakness with bulbar involvement	Cervical spondylosis IHD (MI 2001) CD Eczema Rhinitis Appendectomy RIH	Mesalazine Aspirin Ramipril Atenolol	Nil	NAD	Anti-GM1 Ab & anti-MAG Ab negative CK normal IgG lambda paraprotein (immuno-fixation) UPEP normal	NAD (absent)	AHD (UL&LL)	MRI cervical spine posterior disc osteophytes with C3/C4 and C6/C7 stenosis and ligamentous hypertrophy but no root or cord compression	NAD (-)
5 (PS)	M	65	60	Generalized cramps (legs, arms and abdomen) since 2003 and left foot drop since 2007	IHD ↑BP	Atenolol ISMN Aspirin Amlodipine GTN Simvastatin	Nil	NAD	Normal B12, autoimmune screen, SPEP, IgG, IgA, IgM CK 249	NAD (absent)	AHD (UL&LL)	Normal MRI spine	NAD (-)
6 (KM)	M			Progressive LL weakness & mild bilateral foot drop. Some UL weakness on examination.	↑BP Peptic ulcer disease		Nil				AHD (UL&LL)		Low threshold (-)

Table 2-2: PMA patient demographics. Abbreviations: Δ= diagnosis; HPC = history of presenting complaint; CSF cerebrospinal fluid; OCBs = oligoclonal bands; EMG/NCS = electromyogram/nerve conduction studies; MEPs = motor evoked potentials; LL = lower limb; UL = upper limb; L = left; R = right; LVH = left ventricular hypertrophy; HD = Hodgkin's disease; Rx= treatment; DXT = radiotherapy; DMII = type II diabetes mellitus; HTLV1= human T lymphotropic virus; VDRL = venereal disease research laboratory; VLCFA = very long chain fatty acids; V = voltage; ISC =intermittent self catheterization; BPH = benign prostatic hyperplasia; BOO = bladder outflow obstruction

recording chair and was head restrained via an implanted stainless steel headpiece. She wore a loose-fitting neck collar, and a sleeve which restrained the arm on one side. For coherence testing, the monkey was required to grip a plastic rod with the hand. The length of each trial was variable, however only grips of over 1.64 seconds were included in the analysis.

In addition to measuring coherence, MEPs from bilateral FDI muscles were also recorded from this animal. This was done under sedation with ketamine (10mg/kg intramuscular injection) with a double small 25mm TMS coil positioned over each motor cortex (Magstim Ltd).

Analysis

EMG recordings were full wave rectified and Fourier analysis was performed to examine the frequency characteristics of the data. This was done for each muscle in individual subjects and then data were combined to measure the population effect.

Analysis focused upon the central 1.64 seconds of the 3 second hold period, the phase when beta frequency oscillations are greatest. EEG and EMG power spectra, as well as CMC and IMC were calculated using two contiguous 0.82s-long sections of data from each trial and a 4096-point Fast Fourier Transform (Baker et al., 1997). This yielded a frequency resolution of 1.22 Hz.

The absolute level of power in either EEG or EMG has little meaning, since it will depend on such uncontrolled factors as precise electrode placement relative to muscle or cortical generators. Accordingly, power was expressed relative to the average total power seen in the recording period before task onset. The power spectrum at frequency *f* was calculated using a Fast Fourier Transform (FFT) algorithm as:

$$Power(f) = \frac{1}{n}\sum_{i=1}^{n} X_i(f)X_i^*(f)$$

where n is the number of disjoint sections, * denotes complex conjugates and $X_i(f)$ is the fourier transform for each segment of data within the signal.

Coherence was then calculated to measure the extent of the correlation between two signals in the frequency domain. This is defined as the normalised cross-spectrum and was calculated by:

$$coh(f) = \frac{\left|\frac{1}{n}\sum_{i=1}^{n} X_i(f)Y_i^*(f)\right|^2}{\frac{1}{n}\sum_{i=1}^{n} X_i^*(f)X_i(f) \,.\, \frac{1}{n}\sum_{i=1}^{n} Y_i(f)Y_i^*(f)}$$

where X(f) and Y(f) are the Fourier transforms of the two signals.

Significance levels were calculated using formulae from Rosenberg et al (1989) based on the number of disjoint sections used. Coherence is significantly different from zero if it is larger than Z, at P<0.05 where

$$Z = 1 - 0.05^{1/(n-1)}$$

Averages were calculated for subject groups to allow comparison of coherence across the population. This was achieved simply by calculating the mean coherence over the full spectrum. Data from muscles in the upper limb and the lower limb were pooled to increase sensitivity.

To compare coherence within the beta frequency band between patient groups, the relevant data was first extracted. The window of interest was set between 15-30Hz and the mean coherence within this range was calculated for each subject. These values were used to populate cumulative distribution plots for each group.

Unpaired t-tests allowed statistical testing between patient and control groups each of which consisted of different sample sizes.

There are intrinsic problems associated with the measurement of coherence. Averaging coherence across different datasets is difficult since there are inherent properties of the data which will affect the outcome. Firstly, the two datasets are collected under different conditions even when we control for as many variables as possible. Moreover, altering the

size of disjoint sections used in the FFT affects the frequency resolution. This did not affect the results reported here since all subjects and patients (with the exception of one PLS patient) performed the same number of trials and the number of data segments used in the analysis was uniform throughout.

Coherence estimates are positively biased (overestimated) and this bias depends on the sample size. Sampling bias is defined as the difference between the actual coherence value and the estimated coherence for a given subset of data. Working with physiological data, we only ever have a finite subset of data available to estimate coherence. In addition, the coherence magnitude probably changes with time. Using a larger number of trials within analysis reduces variability and gives an estimate with lower bias but it is difficult to eliminate this problem. It is also difficult to know exactly how much data is needed to get an accurate result where coherence values are small as these values are subject to more bias and are therefore less accurate.

To reduce the effect of bias on the results presented here, the number of trials (~100) and therefore disjoint sections (~200) used for analysis in every individual were kept within a strict range. This could have been increased further by asking subjects to complete more trials but for patients with muscle weakness it would be difficult to maintain the same level of effort for a longer period of time.

Coherence bias was calculated using equation (2) from Benignus (1969).

$$B_{coh} = \frac{1}{n}(1 - coh)^{115}(115)(115)$$

Bias-correction was then applied to the intermuscular coherence data by subtracting Bcoh from the initial coherence values. In almost all cases, the bias was approximately 0.005. This obviously has a bigger effect on the smaller values of the patients.

IMC analysis was also performed on the data collected from the upper and lower limb tasks described previously. The analysis itself was also the same except that it was limited to the EMG channels recorded. The base channel for the coherence analysis was either FDI or

EDB.

To test the application of intermuscular coherence as a biomarker, an odds analysis approach was taken. This allowed us to determine the relative likelihood of having MND given a particular coherence value. This is a useful method for the present application since it could be applied cumulatively; combination of information from all muscles in this study could give a more accurate prediction than one alone. However, it is acknowledged that the present dataset is not yet sufficiently comprehensive to provide definitive values; the method described here simply demonstrates the type of simple but effective approach which could be used.

Firstly, curves were fitted to the log-transformed cumulative probability data based on a Gaussian distribution. The odds ratio was then calculated by dividing the probability of each coherence value being from a PLS patient by the probability of the same value being a control. This calculation was done across the coherence spectrum for each muscle investigated.

Results

Figure 2-2 shows the results of investigations in a single patient with PLS (patient AB) followed longitudinally over a two year period. AB presented aged 64 with a two year history of progressive bilateral weakness. This began in the lower limbs and gradually ascended; the left arm and leg were more severely affected than the right and the investigations were thus limited to the left side only. AB had no lower motor neurone signs and normal nerve conduction studies. During the initial investigation, MEPs were small, delayed and poorly formed (Fig. 2-2A). Corticomuscular and intermuscular coherence in the beta band was present (Fig. 2.2D). The patient was re-assessed 22 months later during which time the disease had gradually progressed. There was particular deterioration in mobility as a result of lower limb weakness and the arms were notably weaker. At this point, there were no MEPs present, even when the stimulator output was maximal (Fig. 2-2E). Patient AB had no significant corticomuscular or intermuscular coherence in any of the muscles tested (Fig. 2.2H). As such, it appears that both TMS and coherence are

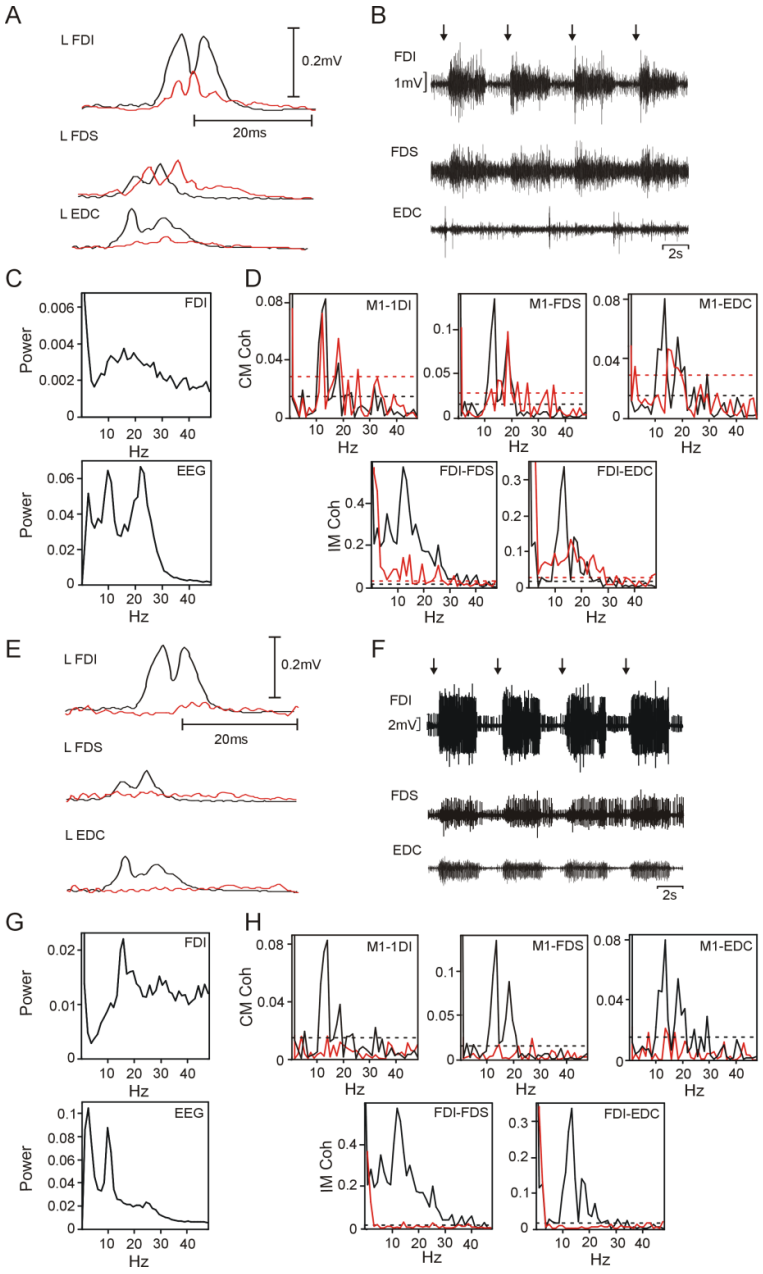


Figure 2-2 A-D: Results obtained from patient AB on her first assessment in the laboratory. A: MEPs are shown from 3 muscles in patient AB (red) and an age-matched control subject (black). B: Raw EMG records show modulation with the task; arrows indicate trial onset. C: Example power spectra for left FDI muscle and contralateral EEG. D: Corticomuscular and intermuscular coherence spectra. Significance levels are represented by dashed lines. Note the higher significance level for AB who completed fewer trials (this data was not used in the average in order to reduce coherence bias). E-G: As in A-D; data from the patient's second visit to the lab.

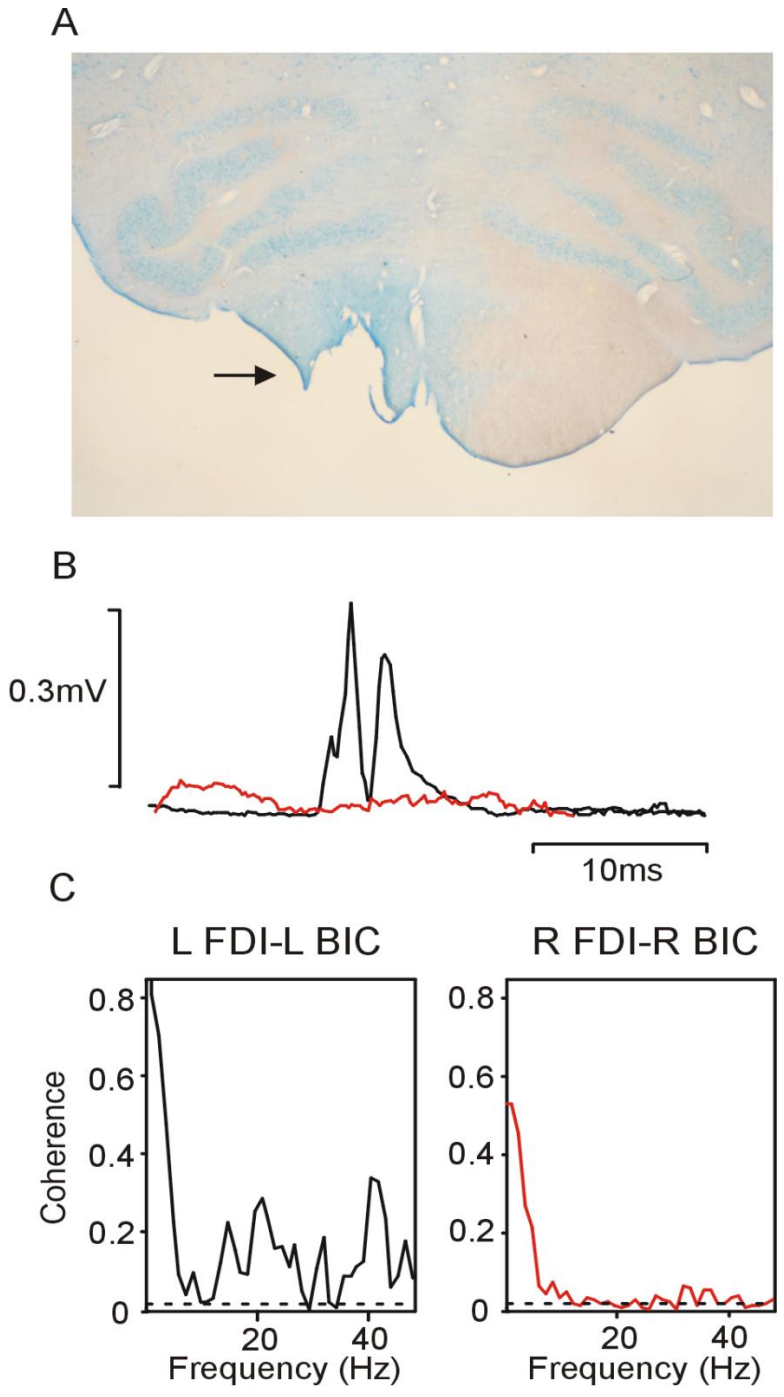


Figure 2-3 A: Transverse section through the pyramids at the level of the medulla showing the extent of the PT lesion in monkey M. B: MEPs evoked from stimulation of right (black) and left (red) motor cortex 3 months post lesion to the left pyramidal tract. C: Intermuscular coherence plots calculated from EMG data collected during a steady hold task. Dashed line indicates significance level.

measures which reflect the degeneration of the CST.

Figure 2-3 shows data recorded from a monkey with a lesion of the pyramidal tract. A transverse section through the pyramids at the level of the medulla shows the extent of the lesion and that it was confined to the pyramidal tract (Fig. 2-3A). Similar to the patient shown in figure 2-2, MEPs and significant intermuscular coherence could only be observed when the corticospinal tract was intact (Fig. 2-3B, C).

Active motor thresholds for TMS are shown in figure 2-4. There was a significant difference between PLS patients and both PMA patients and controls for the upper limb ($P<0.05$). However, for the lower limb, although the difference between PLS patients and controls was significant ($P<0.05$), thresholds for the PMA group failed to be significantly different to PLS patients ($P=0.053$). Interestingly, some of the PMA patients within the cohort exhibited particularly low AMTs; this measure of cortical hyperexcitability has previously been observed in the early stages of MND and may reflect a compensatory cortical adaptation (Mills and Nithi, 1997; Vucic and Kiernan, 2006).

There are very few systematic studies of active motor threshold in the literature so it is difficult to get normal values for a large cohort. However, mean threshold for upper limbs in control subjects was found to be the same as those previously shown in older subjects (Oliviero et al., 2006). In fact, most studies utilise resting motor thresholds. Whilst easier to determine than active threshold, this is less useful in patients with CST disease. Active muscles produce tonic motoneurone firing which increases the sensitivity for detection of a CST volley. This becomes critical in patients who have degeneration of pyramidal cells and motoneurones since they may have insufficient residual connections for TMS to activate muscles at rest.

MEP latencies (Fig. 2-5A) and central motor conduction times (CMCT; Fig. 2-5B) are presented for FDI and EDB. These parameters are largely outside normal limits for both PLS and PMA patients. On the basis of this data it would be difficult to separate patients with pure upper motor neurone and lower motor neurone degeneration using TMS.

Averaged corticomuscular coherence spectra are shown for the patient subgroups and

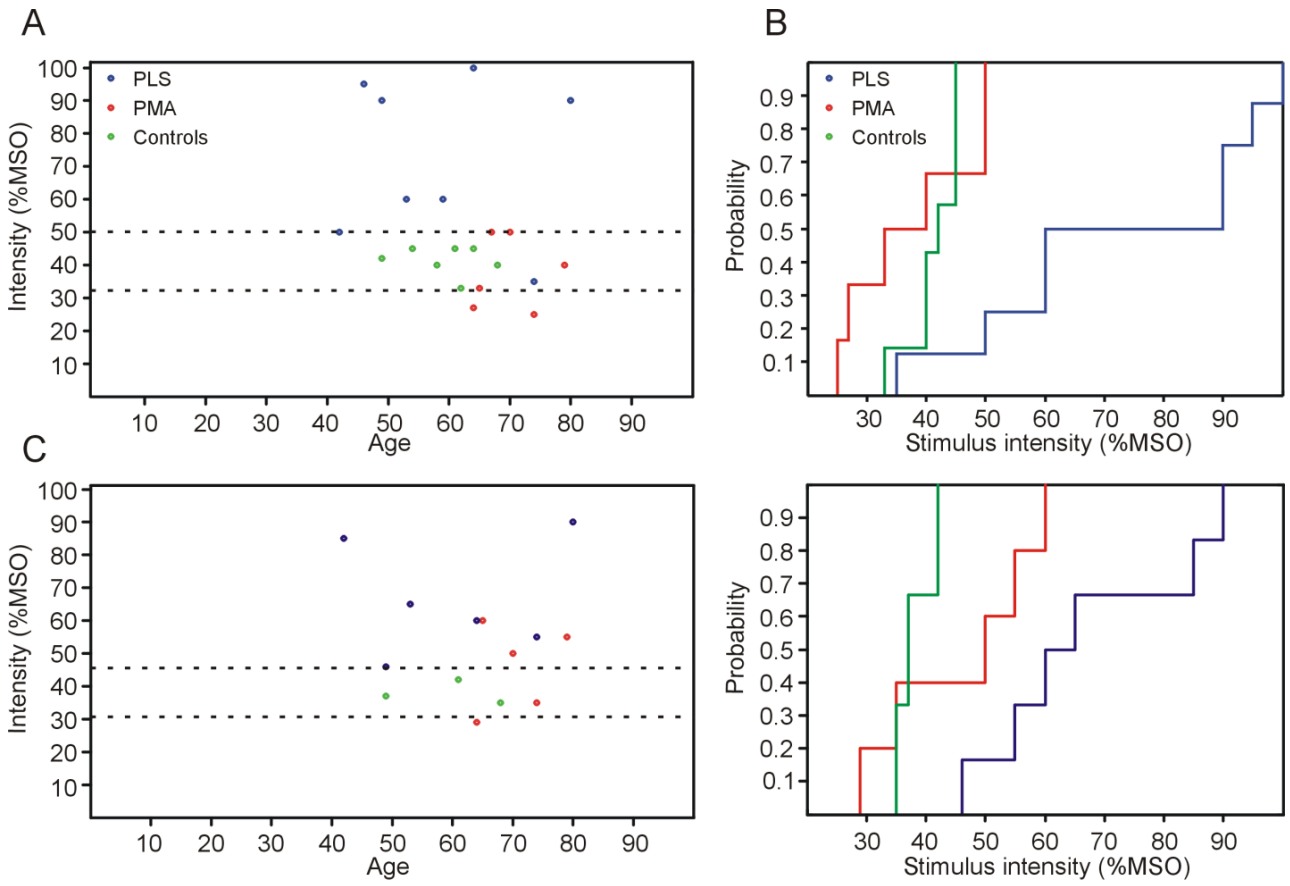


Figure 2-4: Scatter plots of active motor thresholds versus age for the upper limb (A) and lower limb (C). Dotted lines denote the upper and lower boundaries of control means +/- 2SD. Control subjects here were aged between 49-68 years. Corresponding cumulative probability plots show the population spread of active motor thresholds for the upper limb (B) and lower limb (D).

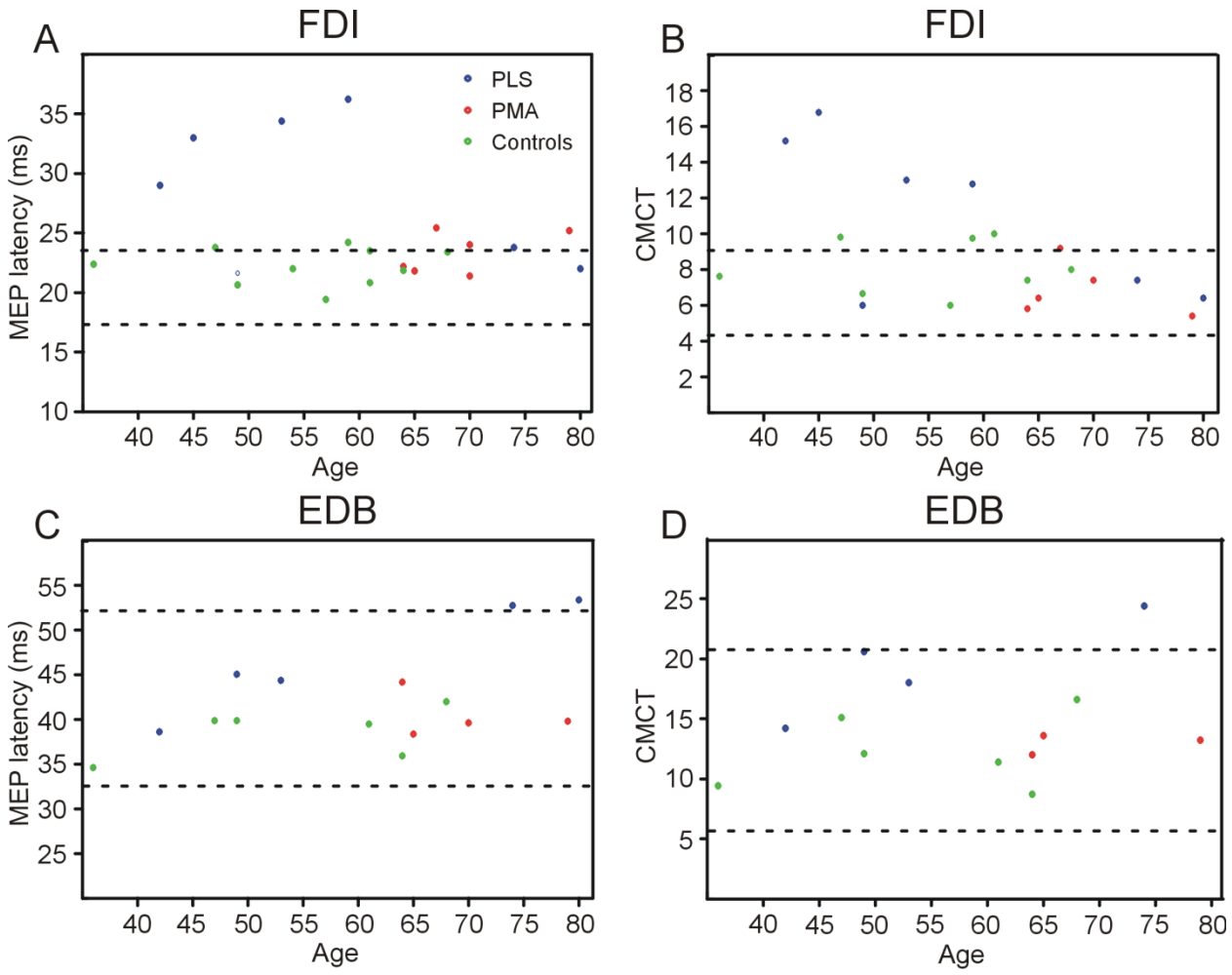


Figure 2-5: Scatter plots of MEP (A,C) and CMCT (B,D) latencies. Dashed lines in A and B denote normal values +/- 2SD obtained from Eisen and Shtybel (1990); subjects aged 20-83 years old. Dashed lines in C and D show control means +/- 2SD since normative data was not available for the distal foot muscles in the literature (subject ages: 36-68 years).

healthy controls in figures 2-6 and 2-7. There is a striking reduction in beta band power in the EEG of PLS patients. This probably reflects the loss of Betz cells in the motor cortex. There was also a notable absence of significant beta band corticomuscular coherence across the PLS population (Fig. 2-6). In fact, taking the bias into account, PLS patients have almost zero coherence. In contrast, beta range EEG power and CMC is relatively preserved in PMA patients, particularly in the upper limb (Fig. 2-7).

Intermuscular coherence between different muscle pairs are shown for our patient subgroups and healthy controls in figures 2-8 and 2-9. Population analysis reveals that on average PLS patients do not have significant beta band IMC (Fig. 2-8), but that it remains in patients with PMA (Fig. 2-9). This is less evident in the lower limb but importantly some IMC is still present in patients with PMA whereas it is completely absent in PLS.

Cumulative distribution plots for corticomuscular coherence are shown in figure 2-10. Significant differences between groups (P=<0.05) were only found between PLS patients and controls in FDI, and between PLS and PMA patients in EDB. A better separation of groups was found with intermuscular coherence (Fig. 2-11). Significant differences were found between PLS and PMA patients (P=<0.05; FDI-FDS, FDI-EDC, EDB-TA) and between PLS patients and controls (P=<0.05; FDI-FDS, EDB-TA, EDB-GS). In contrast, there were no significant differences in intermuscular coherence between PMA patients and control subjects.

Below each cumulative distribution plot in figure 2-10 are the fitted cumulative probability curves and the associated plots of the odds ratio for each muscle pair. This displays the odds of a person being normal or having MND given a particular value of coherence for that muscle pair. Odds of being diagnosed with MND are significantly higher with lower coherence values. Combination of the odds from different muscle pairs could increase the power of the prediction.

Figure 2-12 shows the results obtained from a patient studied prospectively. Patient AP is 30 year old male with a two year history of progressive weakness and muscle wasting in the lower limbs. He initially presented with a unilateral foot drop; symptoms then progressed in an ascending and asymmetric manner predominantly affecting the left leg.

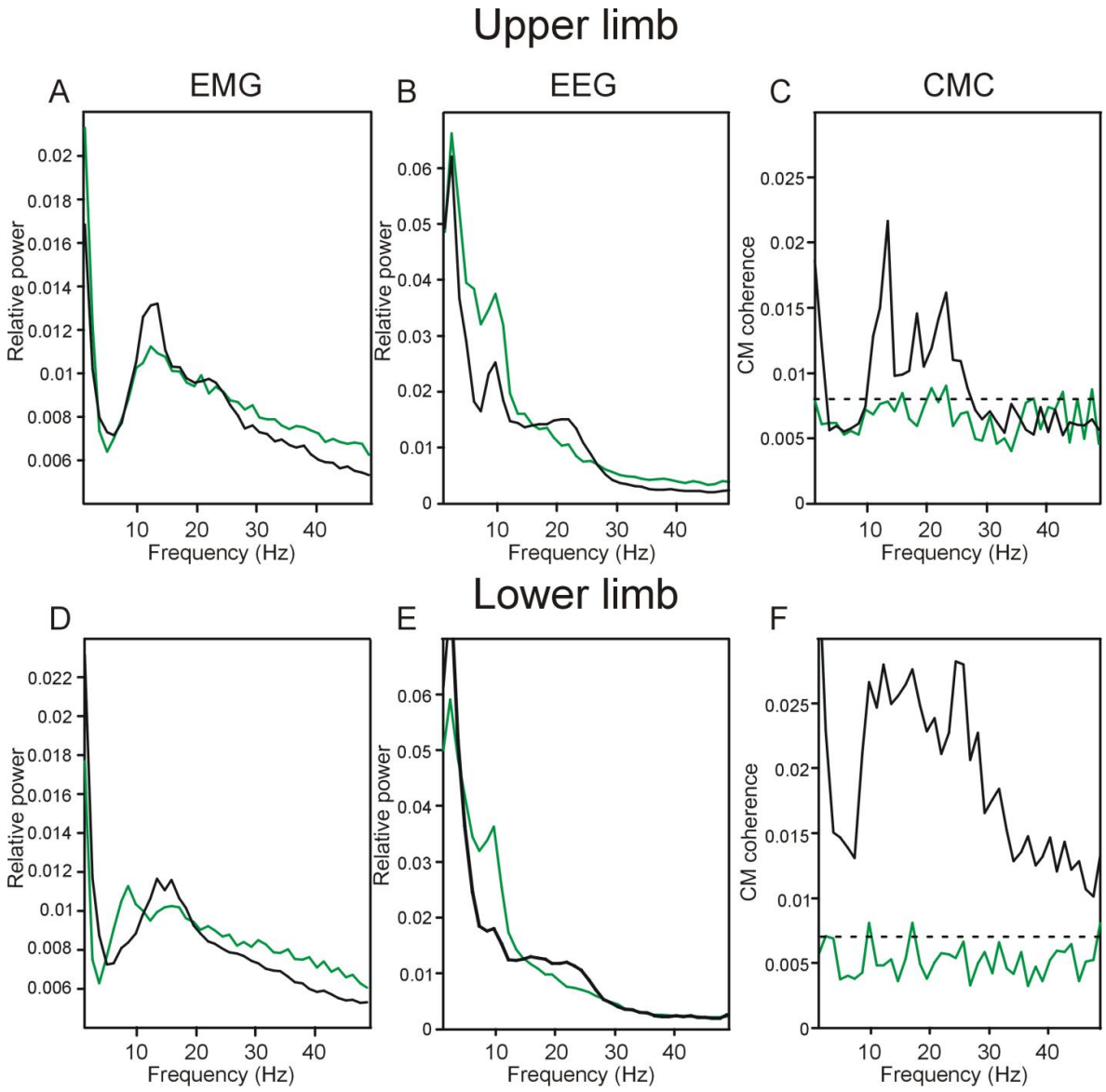


Figure 2-6: Population averages are shown for the PLS patients (green) and age-matched controls (black). Upper limb results are shown in A-C (8 patients & 16 controls) and lower limb results are shown in D-F (6 patients & 13 controls). A, D: EMG power across all muscles investigated. B, E: EEG power. C, F: Corticomuscular coherence combined across all muscles.

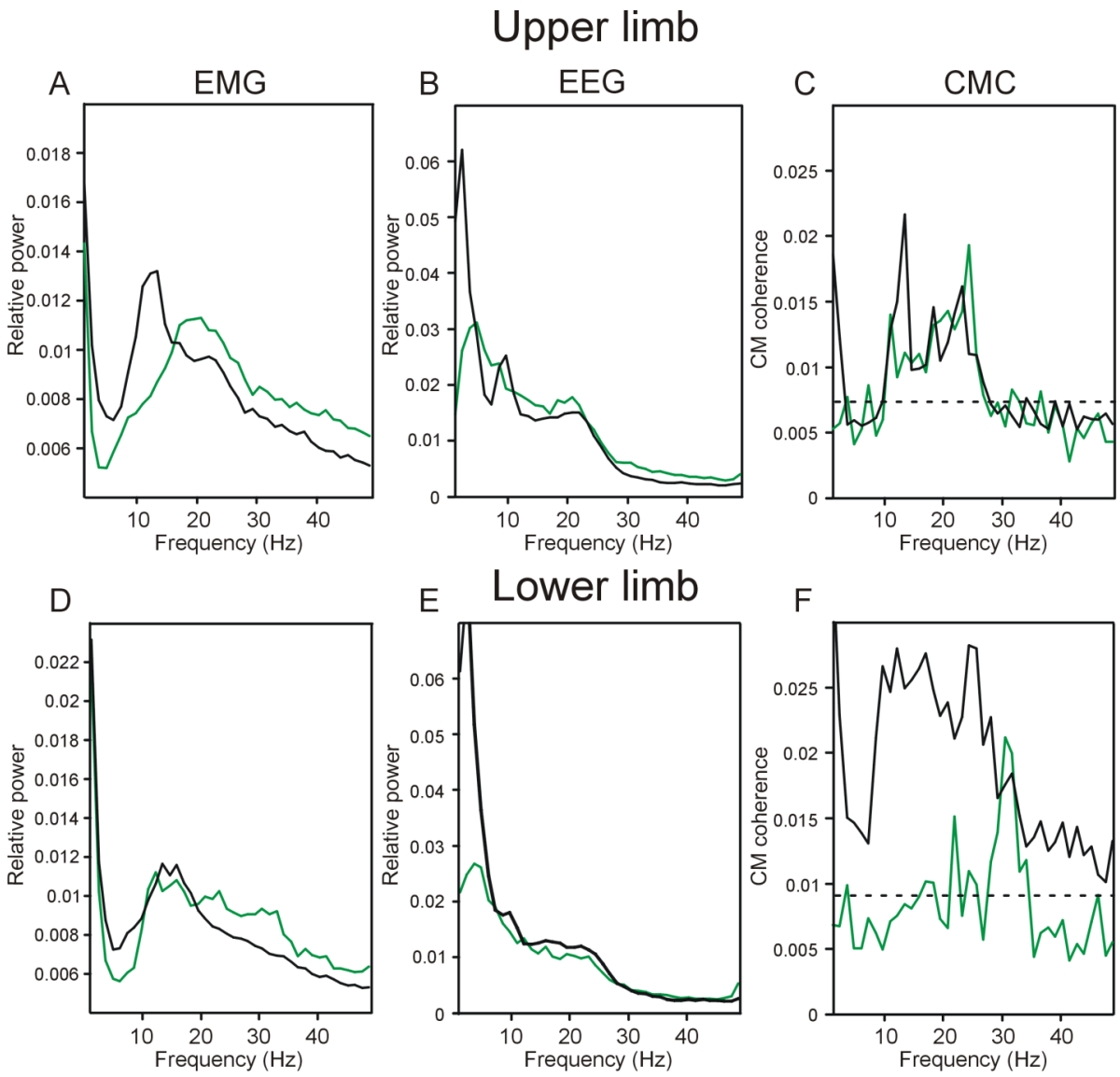


Figure 2-7: Population averages are shown for the PMA patients (green) and age-matched controls (black). Upper limb results are shown in A-C (6 patients & 16 controls) and lower limb results are shown in D-F (6 patients & 13 controls). A, D: EMG power across all muscles investigated. B, E: EEG power. C, F: Corticomuscular coherence.

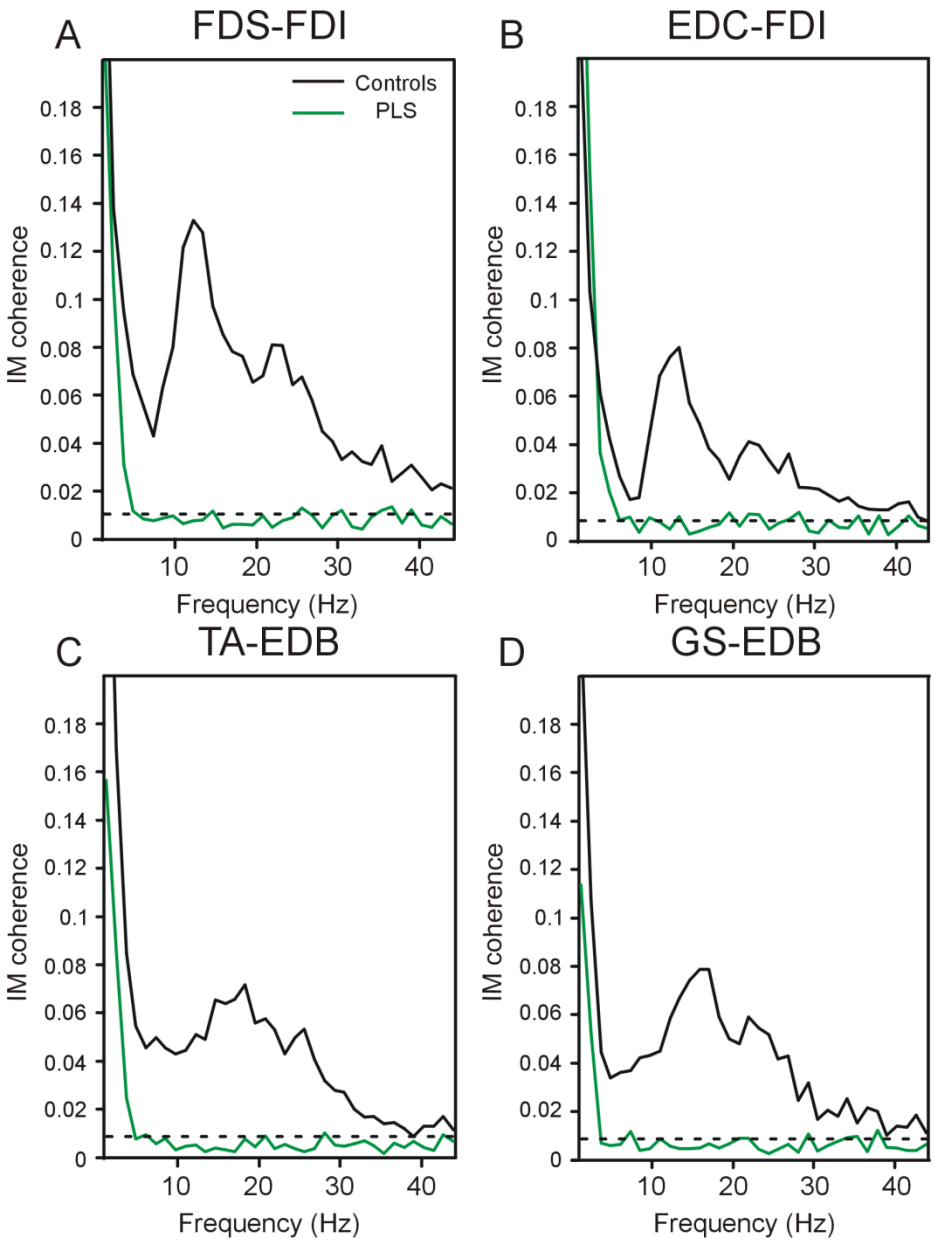


Figure 2-8: Population averages are shown for intermuscular coherence in PLS patients (green) and age-matched controls (black). Upper limb results are shown in A-B (8 patients (15muscles) & 16 controls (32 muscles)) and lower limb results are shown in C-D (6 patients (12 muscles) & 13 controls (24 muscles)).

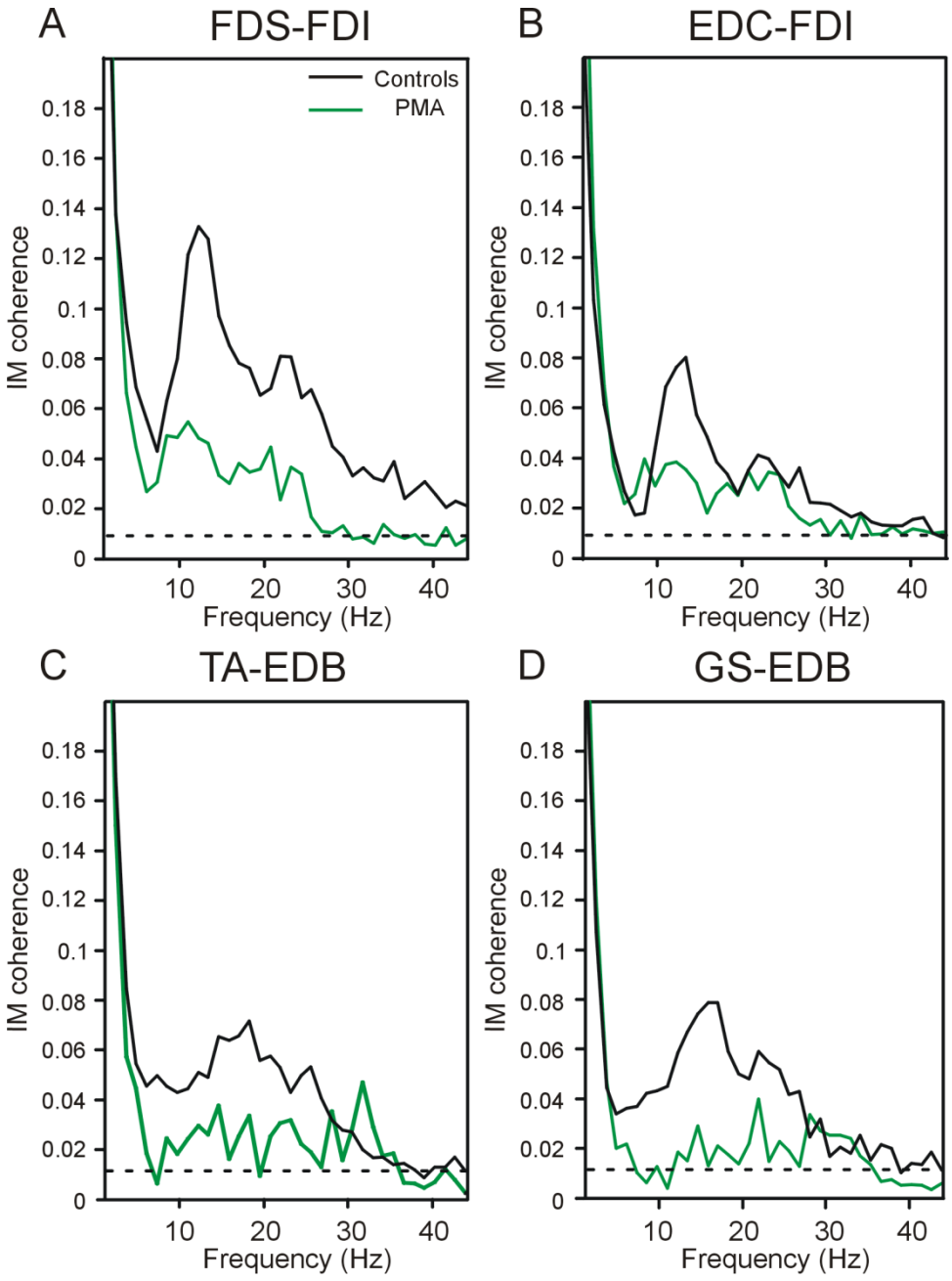


Figure 2-9: Population averages are shown for intermuscular coherence in PMA patients (green) and age-matched controls (black). Upper limb results are shown in A-B (6 patients (10 muscles) & 16 controls (32 muscles)) and lower limb results are shown in C-D (6 patients & 13 controls (24 muscles)).

There was no upper limb involvement and no upper motor motor neurone signs at this stage. Initial EMG studies revealed some neurogenic changes which were confined to the lower limbs. This was felt to be consistent with a lumbo-sacral polyradiculopathy. A follow up study was completed 10 months later since there was a clear progression in symptoms; the lower limb muscles became progressively more wasted and the patient became aware of fasciculations in both upper and lower limbs. The second neurophysiological examination revealed severe neurogenic changes in the lower limbs combined with mild changes in the upper limbs. These were thought to be indicative of diffuse anterior horn cell disease but criteria for ALS were not fulfilled since there was no evidence of upper motor neurone involvement. No significant IMC was found in patient AP between 15-30Hz in either leg or in the upper limb. Subsequently, genetic tests revealed this patient had a point mutation at c341T>C in the SOD1 gene on chromosome 21q; this has been shown to be linked to familial ALS (Rosen et al., 1993). The combined odds for patient AP as having MND (vs having no CST disease) across all muscle pairs was calculated as 280.5:1 (FDI-FDS: 8:1; FDI-EDC: 5.9:1; EDB-TA: 0.5:1; EDB-GS: 11:1). Detection of abnormal intermuscular coherence was completed 6 months prior to the positive genetic test. Moreover, 21 months later this patient still does not fulfil criteria for definite MND on any of the commonly used clinical scales (excluding the results of the coherence analysis and genetic tests which would not normally form part of the diagnostic process).

Discussion

Increased MEP latencies & relationship to disease duration

Prolonged MEP latencies and CMCTs can be observed in both PLS and PMA. These findings support previous observations showing that these TMS parameters are often abnormal in MND (Brown et al., 1992; Cruz Martinez and Trejo, 1999; Mills and Nithi, 1998). This study has also shown in one patient that MEPs disappear with progression of disease (Fig. 2-2). However, despite the clear abnormalities detected, these do not appear to be uniform. This likely reflects the subtle mix of upper and lower motor neuron signs exhibited by patients over the course of a long disease. Furthermore, some patients have MEP latencies and CMCTs within the normal range. The variable findings in TMS

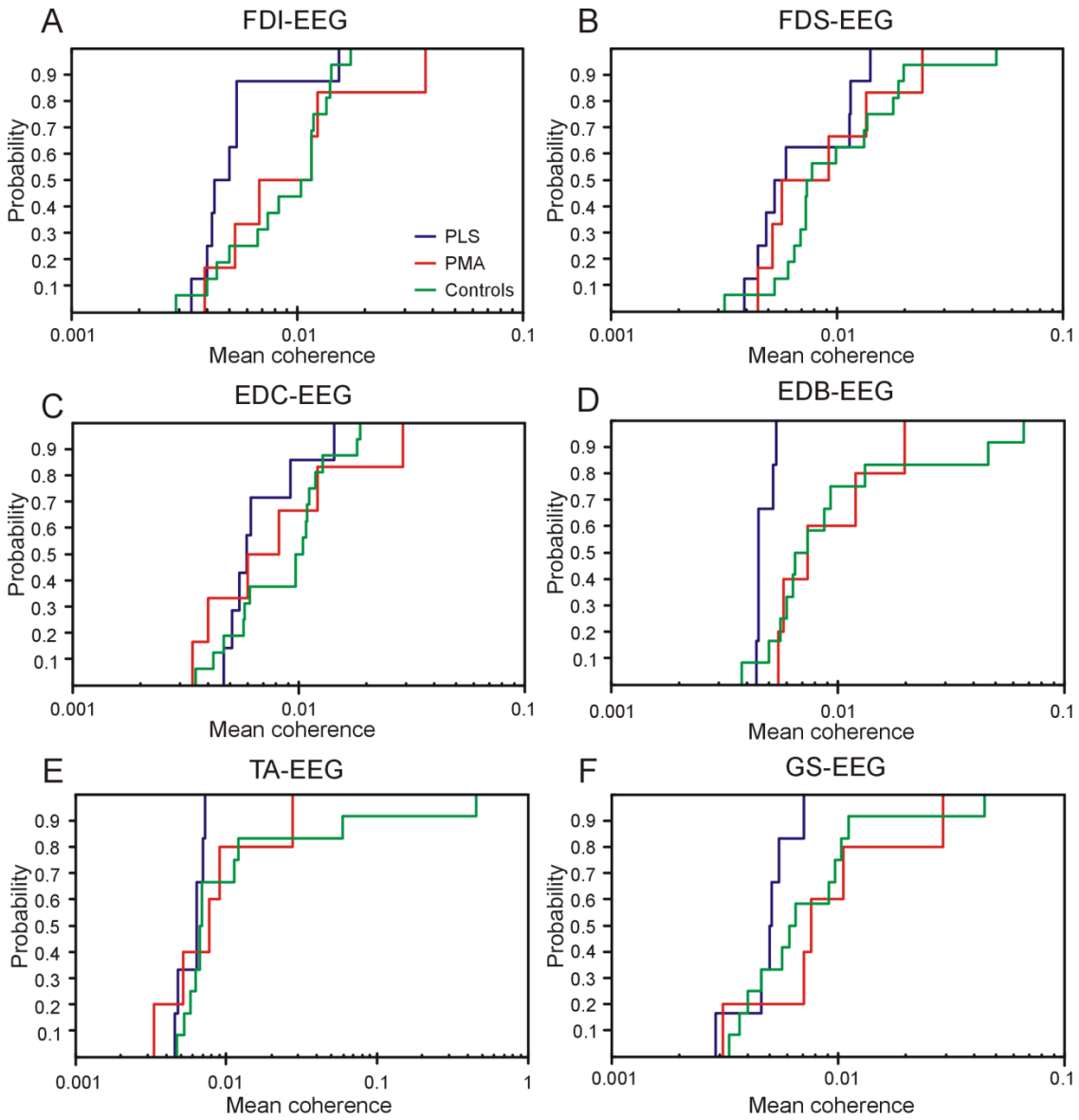


Figure 2-10: Cumulative distribution plots for corticomuscular coherence between FDI-EEG (A), FDS-EEG (B), EDC-EEG (C), EDB-EEG (D), TA-EEG (E) and GS-EEG (F). Coherence is plotted on a logarithmic scale.

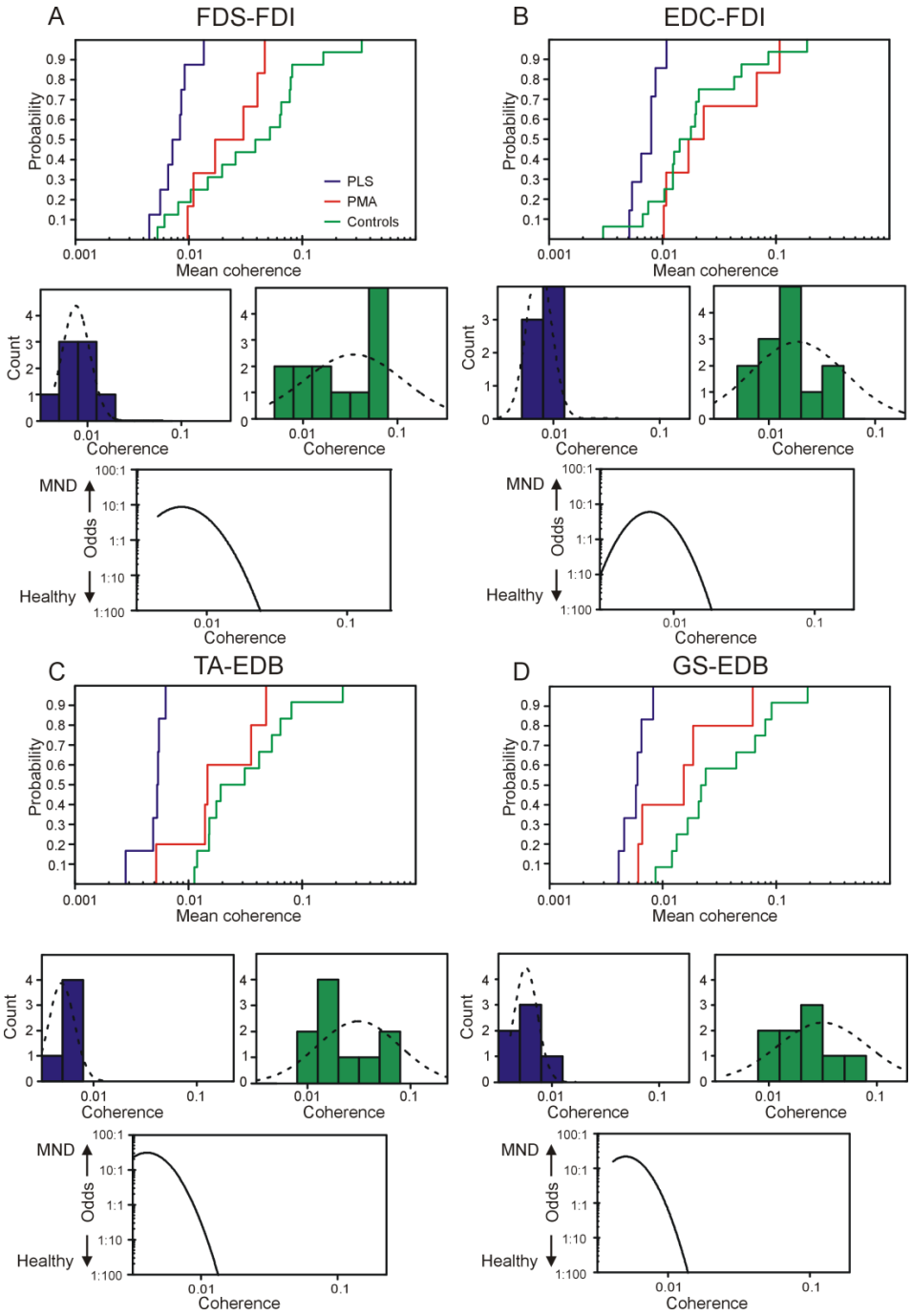


Figure 2-11: Cumulative distribution plots are shown for intermuscular coherence between FDI-FDS (A), FDI-EDC (B), EDB-TA (C) and EDB-GS (D). Coherence is plotted on a logarithmic scale. In each case, the individual histograms of coherence values are shown for PLS patients (blue) and controls (green) with the Gaussian curve fit (dotted line) superimposed. The odds ratio curve is also displayed for each muscle pair.

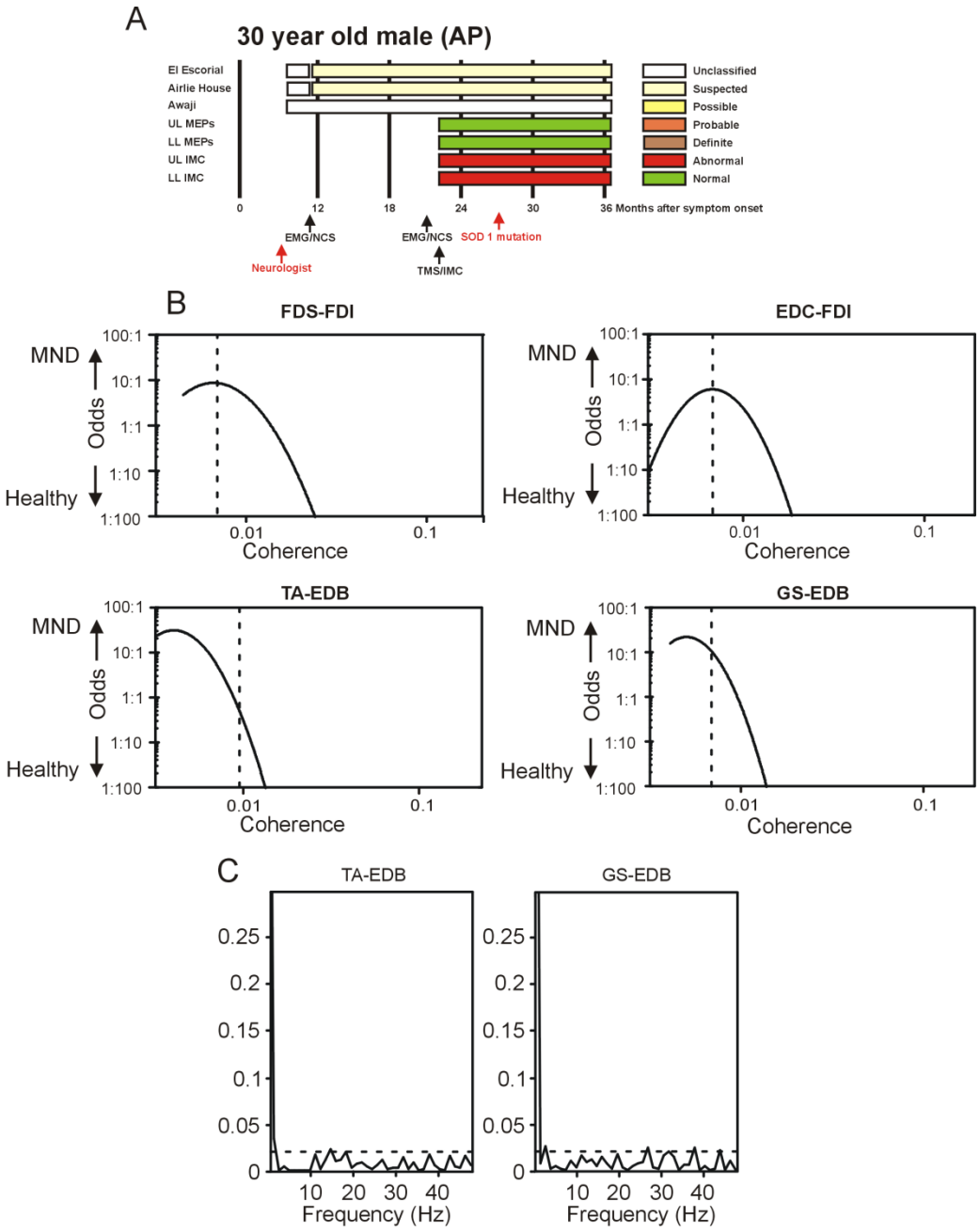


Figure 2-12: Prospective patient data from a 30 year old male patient with suspected anterior horn cell disease. A: Gantt chart showing timeline of diagnosis according to available consensus criteria (not considering coherence or genetic tests) and clinical tests. B: Odds ratio plots from figure 11. Dashed line shows log coherence values found in the left upper and lower limbs for patient AP. C: Intermuscular coherence plots for the lower limb muscles.

parameters are not sufficient to differentiate the conditions from each other, or from normal controls in many cases. Moreover, abnormalities within these parameters do not correlate to disease severity in MND (Mills and Nithi, 1998).

CST and 15-30 Hz corticomuscular coherence

In a group of 8 patients with PLS, this study has shown that significant 15-30 Hz corticomuscular coherence is absent and is associated with reduced beta band oscillations in the motor cortex. However, the study has also demonstrated that corticomuscular coherence is highly variable in healthy individuals. In fact, it was difficult to separate patients from control subjects using these values. There are many reasons why this was the case. Firstly, there may have been variability in the EEG recordings. This could have been due to a number of factors such as high electrode impedence or inaccurate electrode positioning. However this is not likely to introduce such high variability since the sensorimotor potential measured is large and easily recorded over a wide area of scalp. Moreover, EEG power was observed in the beta frequency band in all subjects.

Recent evidence from our laboratory suggests that the variability in coherence might not be as straightforward as previously thought (Witham et al., 2011). Using a Granger Causality method, they have shown that there is significant directed coherence both in the ascending and descending direction between EEG and EMG in humans. This suggests that oscillations are part of a sensorimotor loop, being propagated out to the periphery on a motor arm and returning information about the state of the system on a sensory arm. Corticomuscular coherence would therefore be a relative measure of both of these interactions. Significant coherence would be observed if the oscillations propagated in one direction were bigger than in the other. Importantly however, if oscillatory propogation were equal in both directions, cancellation could occur and the result would be no net corticomuscular coherence.

Since CMC could not be reliably and robustly obtained in healthy control subjects, it is probably not useful as a test of CST dysfunction.

15-30Hz intermuscular coherence

Beta band oscillations in the muscles are thought to occur as a result of a common cortical drive from the CST. These oscillations and the coupling between them disappear after stroke and complete spinal cord injury (Farmer et al., 1993; Hansen et al., 2005; Norton et al., 2003; Norton et al., 2004). Moreover, in partial spinal cord injury, in which the CST may be intact, training in some patients not only increases functional recovery and MEP amplitudes, but also increases beta band IMC (Norton and Gorassini, 2006). On the basis of this evidence from the literature and our findings, it seems to be that intermuscular coherence reflects the integrity of the corticospinal tract. The results presented here show a clear and significant separation of PLS patients from PMA patients and control subjects using this parameter. In the monkey, there is also a distinct absence of IMC following pyramidal tract lesion. Moreover, intermuscular coherence was absent in a patient with anterior horn cell disease before they met clinical criteria for MND (Fig. 2-12). IMC would therefore appear to be a simple, non-invasive method of assessing upper motoneurone function without the difficulty associated with EEG recording.

15-30 Hz EEG and EMG oscillations in PLS

The findings from this study provide evidence that the CST couples 15-30 Hz oscillations in motor cortex and EMG. Histological evidence from *post mortem* studies has confirmed that neuronal attrition is limited to the Betz cells of primary motor cortex in PLS (Pringle et al., 1992). This would suggest that it is specifically the large diameter, fastest conducting CST axons that are fundamental to 15-30 Hz corticomuscular coherence in man. Previous reports have shown that beta oscillations can originate in the motor cortex and be propagated via a descending tract to the periphery. Roopun et al (2006) showed evidence of beta oscillations (20-30Hz) generated in layer V pyramidal cells in cortical slices. These oscillations were not dependent on synaptic input, but were abolished by reducing gap junction conductance. Furthermore, Jackson et al (2002) demonstrated that the phase of beta oscillations could be reset by stimulation of the pyramidal tract in monkeys. This supports the suggestion that pyramidal tract neurones are involved in the generation of beta oscillations which are then propagated to the periphery.

The reduction of beta band EEG oscillations in the 15-30Hz range and the corresponding absence of significant corticomuscular coherence in PLS patients could support the hierarchical model of corticomuscular coherence; here, there would be a corticofugal propagation of 15-30Hz oscillations. In line with this thinking, it may be that in PLS oscillations are not propagated faithfully from the motor cortex to the muscles. However, recent evidence suggests that oscillations may be travelling in a loop with both an ascending and descending arm (Witham et al., 2007; Witham et al., 2010; Witham et al., 2011). Given that the sensory arm of the loop may be important in mediating coherence, the technique should only be used in patients who have normal sensory nerve conduction studies and sensory evoked potentials.

Patient medication

Five PLS patients who participated in this study were taking the neuroprotective agent riluzole (see table 2-1), which has a number of potentially confounding pharmacological actions. Riluzole blocks non-inactivating persistent inward sodium currents (Urbani and Belluzzi, 2000), activates potassium conductances (Duprat et al., 2000) and blocks protein kinase C (Noh et al., 2000), thereby reducing glutamate release (Wang et al., 2004), increasing glutamate re-uptake (Fumagalli et al., 2008) and enhancing the post-synaptic effects of $GABA_A$ mediated inhibition (He et al., 2004). Reduced extracellular glutamate concentrations are unlikely to affect beta-frequency oscillations in frontal cortex (Roopun et al., 2006), whereas enhanced $GABA_A$ transmission will increase oscillations in 8-12 and 15-30 Hz ranges (Baker and Baker, 2003). Although by blocking protein kinase C, riluzole could theoretically inhibit intracellular calcium oscillations (Kim et al., 2005), there is no evidence linking low frequency intracellular calcium oscillations and 8-30 Hz cortical oscillations. Reassuringly, in this study there were no significant difference between the results obtained from the five PLS patients who were taking riluzole and the three PLS patients who were not. Moreover, PMA patients, who were all taking riluzole, appeared to have normal corticomuscular and intermuscular coherence.

Clinical application of intermuscular coherence

MND is a clinical diagnosis, dependent on the presence of lower and upper motoneurone

disease in multiple regions, after excluding other, potentially treatable causes. The El Escorial criteria were revised in 1998 to include preclinical evidence on EMG of lower motor neuron disease outside regions identified clinically. This requires documentation of EMG changes indicative of denervation in at least two muscles innervated by different roots and nerves, in two different limbs. As a consequence, the revised criteria have permitted earlier and more accurate diagnosis of MND, particularly the ALS and PMA variants.

Despite the improvement brought about by consensus criteria, the diagnosis of MND is still not satisfactory. Mean time to diagnosis is 16 months from symptom onset (Donaghy et al., 2008; Househam and Swash, 2000). Many of these patients will have ALS which has a mean life expectancy of only a further 16 months post diagnosis (O'Toole et al., 2008). This is a very small time window in which to see any benefit of neuroprotective drugs. Moreover, by this stage, much of the cumulative damage to motoneurones has already taken place. The key to optimise the effects of neuroprotection is early detection of upper motoneurone dysfunction.

From this small study, we can conclude that 15-30Hz intermuscular coherence might be used as a simple, inexpensive and accessible test for assessing CST integrity. All that is required are facilities for EMG recording, a computer and analysis software. Most of this is readily available in standard neurophysiology departments, therefore the data required for coherence analysis could be collected routinely during an EMG or nerve conduction study. Intermuscular coherence appears to be a robust measure of CST function, however this should be confirmed in a larger patient sample (including other conditions presenting with progressive CST dysfunction).

It is important to note however that sensory afferents are likely to play an important role in both 15-30 Hz corticomuscular coherence (Baker et al., 2006; Riddle and Baker, 2005) and intermuscular coherence (Kilner et al., 2004). Therefore any diagnostic inference regarding CST function using coherence can only be made in the presence of normal somatosensory evoked potentials and sensory nerve action potentials.

Future plans

This is a small scale study which was designed to provide some clarification of the origin of coherence and its potential value as a diagnostic indicator. The results presented herein demonstrate that intermuscular coherence is a reasonable measure of CST integrity but a larger study is required which involves more patients and a larger control cohort with specific age-matched divisions. In addition to this, the specificity and selectivity of coherence for MND needs to be tested. Therefore we would also like to investigate this technique in some other neurological conditions which can mimic MND for example spinal muscular atrophy, multifocal motor neuropathy and hereditary spastic paraplegia.

We know that the technique works in a group of patients with established disease. But the aim is to use it to detect changes early on in the disease course; to be able categorically to diagnose MND before this can be done by consensus criteria. The next step therefore is to carry out a prospective study in a large group of patients who are referred to the MND clinic for investigation. This would enable us to test the sensitivity and specificity of coherence as a diagnostic test and to determine at what disease stage changes in coherence become apparent. Only then would coherence be useful as a diagnostic test, promoting early disease detection and facilitating earlier introduction of neuroprotective agents.

Recent work suggests that directed coherence may be a more useful indicator of CST integrity than intermuscular coherence. We may see little or no net coherence if ascending and descending synchronous oscillations are sufficient to cancel each other out. Perhaps therefore, descending directed coherence (EEG→EMG) would be abnormal in patients with CST degeneration whilst ascending directed coherence (EMG→EEG) remained unaffected. This should be further investigated in patients with both CST and sensory deficits.

CHAPTER III: CORTICOSPINAL ACTIVATION CONFOUNDS CEREBELLAR EFFECTS OF POSTERIOR FOSSA STIMULI

Since the ablation studies of the nineteenth century, it has been known that the cerebellum plays a critical role in movement control. It receives a vast number of inputs from all over the brain leading it to be well placed to co-ordinate complex multi-joint movements.

Given the importance of the cerebellum in movement control, disruption or dysfunction of this structure can be highly disabling. An objective way of assessing cerebellar function would therefore be invaluable for early diagnosis of degenerative processes and in monitoring disease progression.

Non-invasive electrophysiological study of the cerebellum is difficult because of its deep location within the skull. Current tests are indirect and rudimentary; most importantly, they are only applied when obvious cerebellar signs are evident. This is often too late for patients to begin neuroprotective treatment.

A sensitive test of cerebellar function would be useful to help diagnosis of severe degenerative disorders. This would in turn feed into recruitment of appropriate patients for clinical trials and help monitor disease progression. In addition, there are potential applications for clinical monitoring of drugs with known cerebellar side effects such as antiepileptics.

The cerebellum

Despite our extensive knowledge of cerebellar anatomy, we still do not fully understand the role of the cerebellum in motor control. At first glance, the structure has an extremely regular and simple organisation, but modelling the function of the cerebellum has proved surprisingly difficult. To date no one model of cerebellar function has been able to explain all of the phenomena observed in behavioural studies.

Much of our knowledge relating to cerebellar function has arisen from studying the effects of damage to it. Flourens (1824) observed the motor deficits arising from lesions of the

cerebellum in animals. He suggested that the role of the cerebellum is to co-ordinate movements; his pioneering experiments demonstrated that the cerebellum is not necessary for movement, but that without it, movements are inaccurate, dysmetric and poorly co-ordinated.

The first detailed description of the effects of cerebellar damage in humans was recorded by the eminent British Neurologist Gordon Holmes. During his time serving with the British Expeditionary Forces in the first world war, he observed soldiers with gunshot wounds to the cerebellum and described a characteristic 'decomposition of movement'. Following cerebellar damage, complex multi-joint movements were broken down into a series of separate, fractionated movements. Patients exhibited impaired movement timing, movement onset, and showed inappropriate movement acceleration. These problems were often most pronounced in movements which required sensory guidance. Holmes proposed therefore that the cerebellum 'tunes up the cerebral motor apparatus…so that they respond promptly to volitional stimuli…'.

Whilst the theory of cerebellar function is complex and contested, the general view is that the role of the cerebellum is not to initiate movement, but to regulate the precise timing and co-ordination of movements. It achieves this via a vast network of connections between the motor and sensory areas of the brain and spinal cord.

Anatomy of the cerebellum

The majority of the cerebellum is composed of a highly folded, convoluted layer of gray matter; this comprises the cerebellar cortex. Underneath is a layer of white matter, the *arbor vitae*, which contains all of the myelinated afferent and efferent fibres which relay information to and from other areas of the brain. Embedded within the white matter are the cerebellar output relays of the deep cerebellar nuclei (DCN).

The cytoarchitecture of the cerebellar cortex is very well documented. This is largely thanks to the detailed histological studies perfomed by Ramón y Cajal in the late nineteenth century. These remarkable descriptions and drawings of the cerebellar cytoarchitecture revealed the simplicity and uniformity of the cerebellar structure. Cerebellar cortex is

organized into three well defined layers: the molecular layer, Purkinje cell layer and deep granular layer (see Fig. 3-1). Highly localized within these layers are the cells of the cerebellar circuit.

Purkinje cells are the focus of activity within the cerebellar cortex. They are large and distinctive cells, characterized by their extensive dendritic tree. Their cell bodies reside in the narrow, single cell thick, Purkinje cell layer whilst long dendrites project into the molecular layer. Purkinje cells receive inputs from two main sources. Climbing fibres wrap around Purkinje cell dendrites as they wind their way up through the molecular layer from the white matter. The elaborate twisting structure of the climbing fibres around Purkinje cell dendrites means that many hundreds of synapses are present; climbing fibres therefore provide a very strong input. Parallel fibres course perpendicular to the Purkinje cell dendritic trees, running through them at a right-angle and making synapses on to multiple cells.

The granular layer is packed densely with small granule cells. As a result of this dense packing, the cerebellum contains 50% of all neurons in the brain despite only being 10% of the total brain volume. Granule cell axons project from their source through the Purkinje cell layer and into the molecular layer where they split into two and traverse the molecular layer as parallel fibres. Golgi cells are inhibitory interneurons in the granular layer which synapse onto granule cells. Input is received from mossy fibres and parallel fibres allowing for feedforward and feedback inhibition of granule cells.

The molecular layer is the outermost layer of cerebellar cortex and contains the Purkinje cell dendritic tree; here, the different input fibres converge. Stellate and basket cells are also distributed throughout the molecular layer; these are inhibitory interneurons which synapse directly onto Purkinje cells.

Purkinje cells are the sole output of the cerebellar cortex, providing inhibitory input to the deep cerebellar nuclei. The majority of output fibres from the cerebellum originate in the DCN. This symmetrical and bilateral group of nuclei consists of the lateral dentate nucleus, intermediate interpositus nucleus and medial fastigial nucleus. DCN receive a combination of inhibitory input from Purkinje cells and excitatory collateral input from mossy fibres and

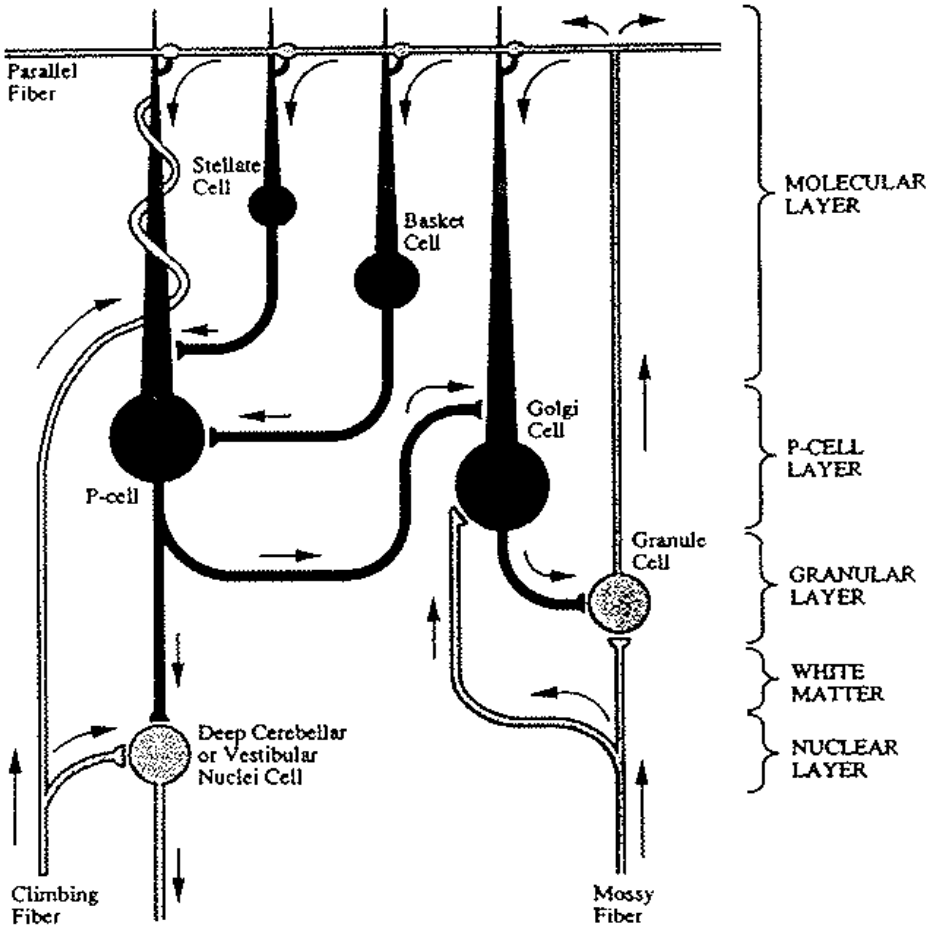


Figure 3-1: Schematic diagram showing the structure of the cerebellar cortex. From *Movements of the eyes (Carpenter, 1988)*.

climbing fibres.

Inputs to the cerebellum

Mossy fibres deliver inputs to the cerebellum from a variety of sources, however a large proportion arise from cortical areas via the corticopontocerebellar tract. This delivers an efference copy of motor commands to Purkinje cells and the DCN via the pontine nuclei, including information about the visual, motor and sensory aspects of intended movements. Mossy fibres are carried in the largest of the cerebellar peduncles, the middle cerebellar peduncle. The substantial volume of these inputs demonstrates the importance of the cerebellum in co-ordinating movements.

Mossy fibres synapse onto granule cells which are located within the cerebellar cortex; their axons are parallel fibres which project onto Purkinje cells. Parallel fibres have a very weak effect on Purkinje cells, therefore many mossy fibres must fire simultaneously and rapidly in order to excite them. When Purkinje cells are activated by this pathway, they produce characteristic simple spikes which typically have a very fast discharge rate.

Mossy fibres can also arise from the spinocerebellar tract. The dorsal aspect of this ipsilateral ascending pathway carries information about proprioception from golgi tendon organs and muscle spindles in the periphery. In contrast, the ventral spinocerebellar tract transmits an efference copy of the motor program to the cerebellum (Arshavsky et al., 1978).

Climbing fibres originating in the inferior olive have a much more direct and powerful effect on cerebellar Purkinje cells. These projections arrive into the cerebellar cortex via the inferior cerebellar peduncle. Each climbing fibre targets up to 10-15 Purkinje cells, however each Purkinje cell receives input from only one climbing fibre (Eccles et al., 1966). Activation of this pathway causes discharge of distinctive slow frequency complex spikes. This powerful input can suppress simple spike activity via mossy fibre input (Ekerot and Kano, 1985; Ito et al., 1982). Climbing fibres relay information from sensorimotor cortex, spinal cord, reticular formation, red nucleus, superior colliculus and the vestibular system.

Outputs from the cerebellum

There is a highly ordered, topographical pattern of projections from DCN (Hoover and Strick, 1999). These cerebellar efferent fibres are carried via the superior and inferior cerebellar peduncles crossing the midline in the caudal midbrain. The main target is the contralateral ventrolateral and ventroanterior regions of the thalamus. It was initially thought that the pathway beyond thalamus projected exclusively to primary motor cortex. In fact, central to the cerebellothalamocortical pathway is a very strong projection from dentate nucleus to motor and premotor cortices (Orioli and Strick, 1989; Sasaki, 1976). However, alongside these projections, anatomical studies have repeatedly shown that there are a wide range of non-motor cortical targets (Barbas et al., 1991; Dum and Strick, 2003; Goldman-Rakic and Porrino, 1985; Middleton and Strick, 2001; Miyata and Sasaki, 1983; Sasaki et al., 1979). These projections include red nucleus, superior colliculus, vestibular nuclei and prefrontal cortex. This suggests that the cerebellum has a more global role in the control of movement than previously thought.

Information gathered from cortical areas is relayed back to the cerebellum via reciprocal connections to the pontine nuclei (Glickstein et al., 1985) and inferior olive (Andersson and Nyquist, 1983; Baker et al., 2001) thereby forming a complete motor loop. The strongest cortico-pontine and cortico-olivary connections are from areas 4 and 6 but there are a wide range of cortical sources (Allen et al., 1978; Glickstein et al., 1985; Tsukahara, 1974).

The cerebellum also projects to both the reticular formation (Bantli and Bloedel, 1975; Cohen et al., 1958) and the red nucleus (Dekker, 1981); these are the origin of two major descending pathways from the brainstem. Stimulation of DCN in macaques produces short latency bilateral responses in forelimb muscles which are postulated to be mediated via either the reticulospinal or rubrospinal tracts (Soteropoulos and Baker, 2008). In monkeys, these pathways have been shown to have monosynaptic connections with motoneurones or spinal interneurons (Fujito et al., 1991; Riddle et al., 2009).

Measuring Cerebellar Dysfunction

Current measures of cerebellar function are by nature very crude. They lack sensitivity and

rely largely on either subjective assessment or indirect measures of movements. Clinically there are three main measures of cerebellar function. Firstly, MRI is available to detect any gross abnormalities although it is not able to identify subtle morphological changes. Pegboard tests are often used to probe motor co-ordination; the time to complete a series of movements is related to the degree of dysfunction. However this is a very crude and inaccurate way of measuring cerebellar function. Ataxia scales are used primarily for monitoring progression of conditions affecting the cerebellum. These are really the mainstay of assessing the cerebellum in clinic. However these scales are often highly subjective, being susceptible to both intra- and inter-rater variability.

In the research laboratory, there are many more tests which have been designed to investigate the role of the cerebellum in motor function. Often these are very robust and reliable techniques, for example motor learning and eyeblink conditioning, yet the transition between research investigation and clinical application has never been made.

There is an evident gap for a specific marker of cerebellar dysfunction - not necessarily for those with overt cerebellar disease but for detection of subclinical cerebellar disorders. We currently lack the ability to diagnose conditions early and also to detect cerebellar disease within a complex motor disorder. An example of this lies with the complicated tree of Parkinsonian disorders. An estimated 20-25% of patients with Parkinsonism are thought to have conditions other than Parkinson's disease (Hughes et al., 1993; Litvan et al., 1997; Rajput et al., 1991). One of these conditions, multiple system atrophy (MSA), critically involves degeneration of the cerebellum yet it is difficult to differentiate from PD in the early stages. The ability to detect subclinical cerebellar disease in these instances is crucial not only for patients but also for effective early recruitment to therapeutic clinical trials.

Non-invasive stimulation of the cerebellum

Non-invasive electrical stimulation of the cerebellum was first demonstrated by Ugawa and colleagues (1991). High voltage electrical stimuli were applied across the back of the skull; electrodes were positioned over the two mastoid processes. This did not have direct motor effects but was capable of suppressing MEPs elicited by non-invasive stimulation over motor cortex (Ugawa et al., 1991; Ugawa et al., 1994a). As with all forms of percutaneous

electrical stimulation, there is unavoidable local activation of cutaneous receptors. Consequently the method is very painful and therefore often not tolerated by subjects.

To overcome the problem of painful stimulation, magnetic stimulation of the cerebellum was subsequently implemented (Ugawa et al., 1994b; Ugawa et al., 1995; Ugawa et al., 1997; Werhahn et al., 1993). The introduction of a double cone coil which could activate deeper structures than conventional coils facilitated this move. Optimal coil position was investigated; this was found to be mid-way between the occiput and the mastoid and was proposed to be due to specific activation of the cerebellum. The effects observed at this stimulation site were the same as those reported from electrical stimulation over the occiput. Ugawa and colleagues (Ugawa et al., 1994a; 1995) suggested that the effects may result from inhibition of the DCN as a result of activation of Purkinje cells. This hypothesis is consistent with reports that MEP suppression was not present in patients with specific degeneration of cerebellar cortex (Ugawa et al., 1994b).

When cerebellar TMS was first introduced, the specificity of the technique was investigated. Ugawa and colleagues first considered activation of descending motor pathways; they demonstrated that stimulation over the occiput could produce a single descending volley in the spinal cord which could be collided out by stimulation of the peripheral nerves (Ugawa et al., 1994c). Moreover, collision of an antidromic spike elicited by stimulation over the occiput with descending volleys from motor cortex showed that this was likely to occur via the corticospinal tract (CST); (Ugawa et al., 1994c). The optimal site for direct activation of CST was found to be over the midline (Ugawa et al., 1994c) rather than the lateral site proposed for specific cerebellar stimulation.

Ascending sensory afferents could also be activated using this method. High intensity TMS causes contraction of neck and proximal muscles; this would activate a multitude of cutaneous afferents and muscle spindles. Appropriately timed sensory volleys could therefore influence motor output. Despite consideration of activation of these other structures, it was concluded that at the intensities used and given the specificity of stimulation site and polarity that the effects observed must be due to cerebellar stimulation.

Since the first descriptions of cerebellar stimulation, there have been many reports in the literature using this technique. Recently, there has been a surge of interest in low frequency repetitive TMS (rTMS). With this technique, it is possible to create 'virtual lesions', reversibly inactivating regions of cerebral cortex to investigate their normal function. A range of reports have emerged investigating cerebellar function using rTMS over the posterior fossa (Del Olmo et al., 2007; Jancke et al., 2004; Miall and Christensen, 2004; Theoret et al., 2001).

'Cerebellar inhibition' of MEPs

Electrical (Ugawa et al., 1991) or magnetic (Ugawa et al., 1995) stimulation over the posterior fossa (PF) has been shown to suppress MEPs elicited by motor cortical stimulation 5-7ms later. Evidence from patients with focal cerebellar lesions suggests that this is likely to occur via a cerebellothalamocortical pathway (Matsunaga et al., 2001; Ugawa et al., 1994b; Ugawa et al., 1995; Ugawa et al., 1997); reduced MEP suppression is seen in patients with lesions of the cerebellum or one of the inflow/outflow pathways, but is normal in ataxia of non-cerebellar origin. As a result, Ugawa et al (Ugawa et al., 1994b; Ugawa et al., 1997) suggested that cerebellar stimulation could be a useful tool to distinguish disease of cerebellar outflow (yielding impaired suppression) from that of cerebellar afferent pathways (suppression normal).

This paired stimulation technique is a promising technique for detecting sub-clinical cerebellar dysfunction. It could have potential applications in the early diagnosis of degenerative processes like MSA at a stage at when neuroprotective treatment could be implemented. However, reports have shown preserved MEP suppression in patients with cerebellar agenesis (Meyer et al., 1993; Meyer et al., 1994; Ugawa et al., 1994b; Ugawa et al., 1997). In addition, concerns remain over the specificity of the technique. It is known that stimulation over the posterior fossa region can directly activate descending motor structures such as the pyramidal tract. Collaterals of these CST neurons could potentially exhibit an inhibitory effect on motor cortical cells via recurrent inhibition (Renaud and Kelly, 1974).

Methods

Eleven healthy volunteers (age 23-54 years, 10 male and 1 female) consented to participate in this study. Subjects sat resting their head on a custom-made frame, modelled on an ophthalmic slit lamp (Fig. 3-2A, B). Straps immobilised the head with the neck flexed. Magnetic stimulation coils were clamped rigidly to the frame.

EMG recordings

Surface electromyogram (EMG) recordings were made from two intrinsic hand muscles (first dorsal interosseus, FDI; abductor pollicis brevis, AbPb) and two forearm muscles (flexor digitorum superficialis, FDS; extensor digitorum communis, EDC). EMG signals were amplified (gain 1-5K, bandpass 30 Hz-2 kHz) and digitised at a 5 kHz sampling rate by a Power1401 interface (CED Ltd, Cambridge, UK) running Spike2 software, together with signals indicating the time of trial onset and occurrence of auditory cues.

Transcranial Magnetic Stimulation

Stimulation over motor cortex was performed using a 13cm outside diameter round coil (Magstim 200 stimulator). This was oriented A side up for left hemisphere activation. Stimulus intensity was set to generate an MEP from FDI/AbPB at approximately 0.5 mV at rest (MEP sizes: FDI, 0.53±0.09mV; AbPB, 0.27±0.07mV; EDC, 0.17±0.03mV; FDS, 0.21±0.04mV, mean ± SEM across subjects). PF stimulation used a double cone coil and another Magstim 200 stimulator.

The threshold intensity for direct activation of CST fibres was first identified with the coil centred over the inion, oriented to produce a downward current in the brain. This was termed the direct motor threshold (DMT). Subjects produced a gentle background contraction, and stimulus-triggered EMG from FDI and AbPB were viewed on an oscilloscope. Stimulus intensity was increased in 1% increments until a response above background was observed. Threshold was defined as the intensity of stimulation which produced an MEP in 50% of stimuli. MEP latencies were a few milliseconds shorter than responses to motor cortical stimuli, consistent with brainstem CST activation. Mean threshold intensity was 75% MSO (% maximum stimulator output; range 60-100%). The

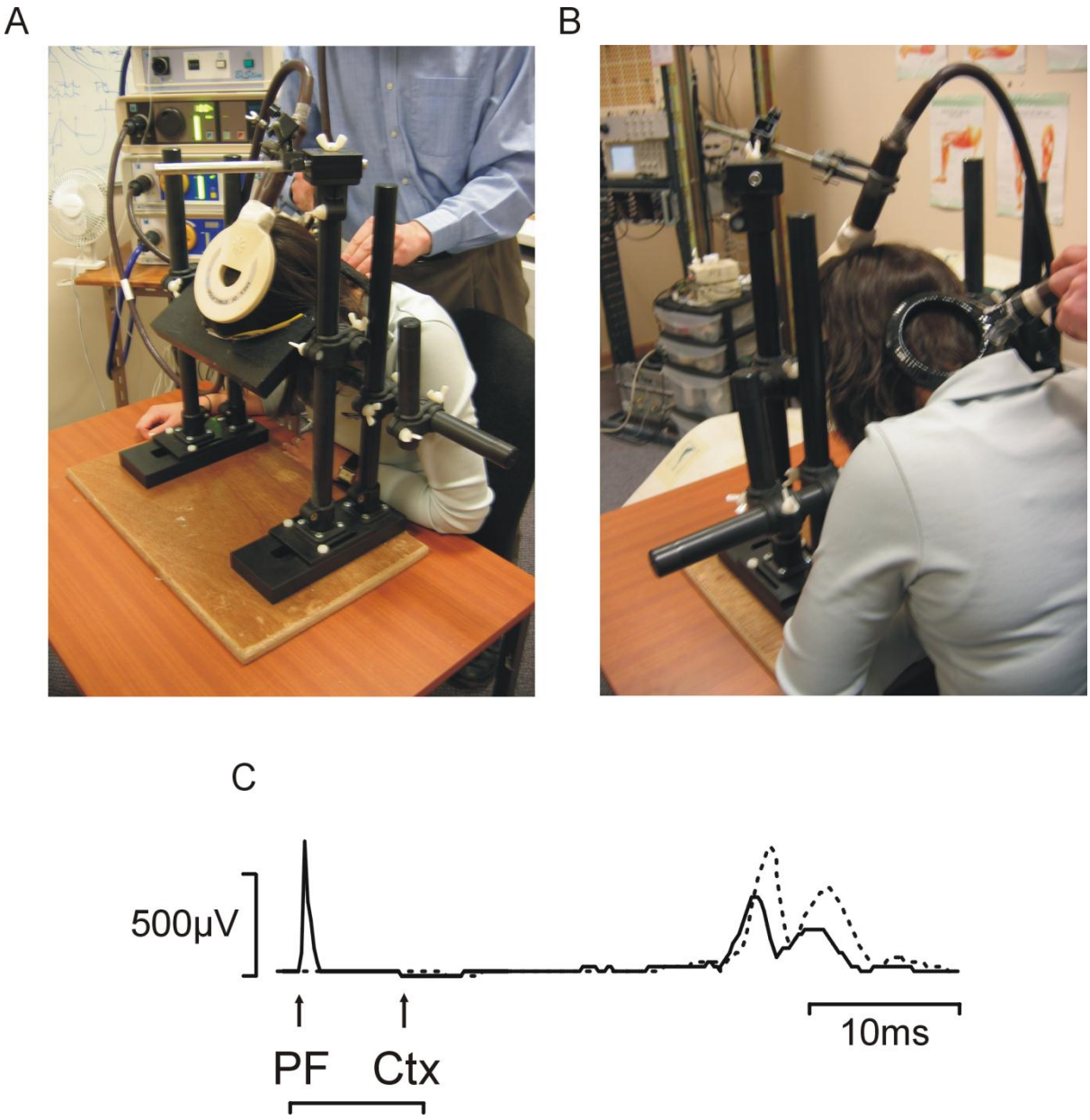


Figure 3-2: Photographs showing experimental set-up. A: Subject seated with forehead resting on the frame. A round TMS coil is mounted on a fixation bar to hold it in place over the M1 hotspot and a second coil (double cone) is held over the occiput. B: Rear view showing the position of the two coils relative to the head. C: Example MEP traces showing the relative timings of the conditioning (PF) and test (Ctx) stimuli. A test MEP is shown in the dashed example with a conditioned MEP denoted by the black line.

coil was then repositioned midway between the inion and the right mastoid incisura. Intensities used during the experiment ranged from DMT-20% to DMT, in 5% steps.

Motor threshold identification

Subjects made a background contraction and PF stimuli (0.2Hz) were given at a randomly chosen intensity. The coil remained oriented to produce a downward brain current since we found this to be the most sensitive method of detecting CST activation in our subjects. This experiment was performed with the muscle active to produce tonic motoneurone firing, providing the greatest sensitivity to detect a CST volley via its evoked EMG activity. Subjects were required to squeeze the levers of an auxotonic precision grip manipulandum (Riddle and Baker, 2005) between finger and thumb. Lever displacement had to exceed 17mm; below this, a tone sounded until the criterion displacement was achieved. It is reasonable to expect that CST activation will also occur at the same intensity when the subject is at rest, since stimulation is distant to the axon initial segment and hence insensitive to the level of cortical excitability. This is so, even though the weak CST volley produced by a near threshold PF stimulus is unlikely to generate a measurable MEP in resting motoneurons.

Paired pulse paradigm

Subjects were at rest. Motor cortex was stimulated either alone, or preceded by PF stimulation (3, 5 or 7ms intervals; intensities as before). Here, the coil was inverted to generate an upward brain current, previously found to be optimal for MEP suppression (Ugawa et al., 1995). Around 20 stimuli at each intensity were delivered.

Analysis

Off-line analysis in the MATLAB environment separated responses according to condition and compiled averages of rectified EMG. Single sweep responses were measured as the area between the MEP onset and offset, judged from the averaged response. Responses to PF stimulation alone were normalised as a percentage of the mean background level over the 20ms prior to the stimulus. Significance was assessed by comparing single trial responses with a similar duration of the pre-stimulus background (paired t-test).

Conditioned responses to motor cortical stimulation were expressed as a percentage of the unconditioned response; significant suppression was assessed with unpaired t-tests.

Results

Single subject results

Figures 3-3A-E show the results from an individual subject, in whom the initial estimate of the threshold for a direct response to PF stimulation was 70% MSO. Figure 3-3A shows a clear averaged EMG response at this intensity. No significant direct responses were present at lower PF intensities (Fig. 3-3C). Conditioned MEPs (7ms ISI) are illustrated for this subject in Fig. 3-3B. Suppression was elicited consistently at all intensities of PF stimulation. Therefore, for this individual, stimulating at intensities of DMT-5% and below seems to generate MEP suppression in the absence of direct CST activation.

Figures 3-3F-J show data from a subject whose PF threshold was initially estimated as 80% MSO. Significant suppression was seen as low as 65% MSO (Fig. 3-3I), however there was also a significant direct response to PF stimulation at this intensity (Fig. 3-3H). The observed suppression cannot therefore be unambiguously assigned to a cerebellar pathway. Moreover, although the response was not significant at 60%, the response region was above baseline, and the characteristic bifid peak produced by an MEP in rectified EMG was clearly visible (Fig. 3-3F, 60%). It would be unsafe to conclude that there was no CST activation at an intensity of 60% MSO in this subject.

ISI timecourses for individual subjects (Fig. 3-3E,J) confirmed that MEPs were suppressed at 5 and 7ms intervals, but not at 3ms, consistent with previous reports (Matsunaga et al., 2001; Ugawa et al., 1995). Previous work has used PF stimulus intensity 5-10% MSO below the threshold estimated with the coil in the midline (Matsunaga et al., 2001; Ugawa et al., 1994b; Ugawa et al., 1995). At this intensity, we found 9/11 subjects had significantly suppressed MEPs. However, all nine also had a direct response in at least one muscle.

Redefining threshold for accurate threshold identification

Working from the direct response threshold subjectively estimated with the coil at the

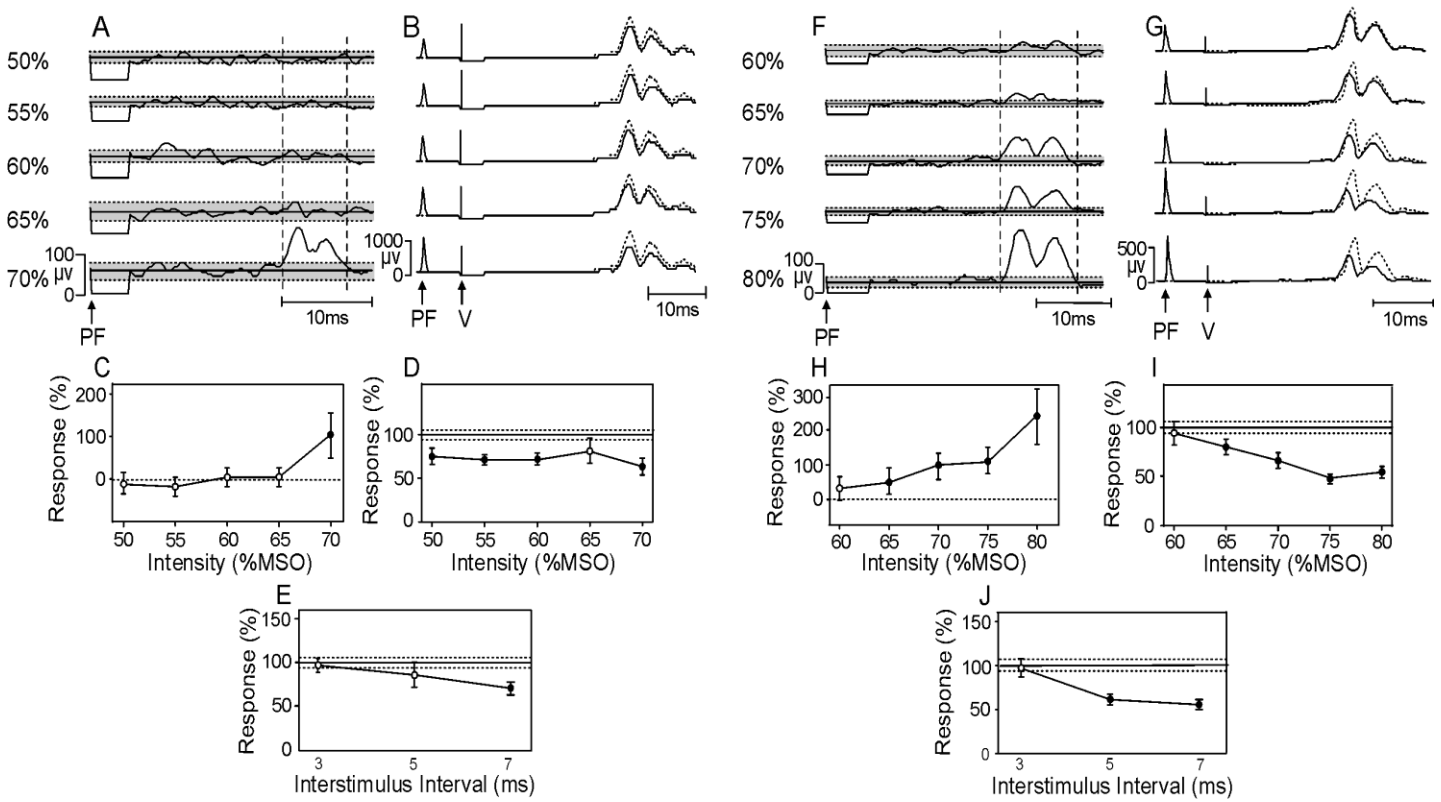


Figure 3-3: Single subject results. A: Averaged rectified EMG (FDI) following PF stimulation at different intensities. Vertical dashed lines show response region. Grey shading shows mean baseline ± 2 standard errors. B: MEPs following motor cortical stimulation (intensity 44% MSO). Dotted lines are unconditioned responses; solid lines show responses conditioned by PF stimulation. C: Response magnitude (expressed as a % of mean background EMG) versus intensity. D: Conditioned response magnitude as a percentage of unconditioned responses. E: Average time course of MEP suppression at DMT-10%. In (C, D and E), error bars show standard errors; horizontal dotted lines in (D and E) show standard error of unconditioned response. Filled circles indicate significant points ($P < 0.05$). F–I: As in (A)–(D) for a different subject. Motor cortical stimulation in this subject was performed at 36% MSO. J: Average time course of MEP suppression at DMT-5% for this subject.

midline is clearly unsound. Stimuli weaker than this with the coil placed more lateral do produce CST activation, confounding the interpretation of MEP suppression. It is unlikely that this was due to a lower threshold in the lateral position, as a previous study demonstrated that the midline was the optimal position for CST activation (Ugawa et al., 1994c). Alternatively, weak responses following near-threshold stimuli may be missed in single sweeps, causing threshold to be assigned higher than it actually is. Some previous reports determined threshold from averaged MEPs (Daskalakis et al., 2004), but many authors do not clearly describe the method used. Choosing the intensity of PF stimulation more carefully might therefore allow selectivity for cerebellar pathways. In order to test this, responses to PF stimuli were rectified and averaged; threshold was then redefined according to the presence of significant MEPs in these averages. This is a sensitive means of determining threshold and was designated the objective direct response threshold (ODRT).

Population data

Averaged data are presented as a function of intensity relative to ODRT in figure 3-4. For the 3ms interval (Fig. 3-4A), MEP suppression (circles) was not evident at any intensity. By comparison, a clear effect on motor cortical output was observed with the longer intervals. For 5ms, significant MEP suppression was generated at 15% MSO below ODRT (Fig. 3-4B), whilst suppression at the 7ms ISI was significant at all intensities tested (Fig. 3-4C). Overlain on the plots of figure 3-4 is the averaged direct response (squares). When data was averaged across subjects, small but significant responses were seen even 10% below ODRT. This suggests that even when we determined threshold using a highly objective, sensitive method in single subjects, weak responses were still present at lower intensities. These could only be revealed by the increased statistical power of pooling data across subjects.

The effect of current direction on PF stimulation

One important parameter of PF stimulation is the orientation of the TMS coil. It has previously been shown that an orientation yielding an upwards brain current (as used here) generates the greatest MEP suppression. In a subset of 7 subjects, the direct motor

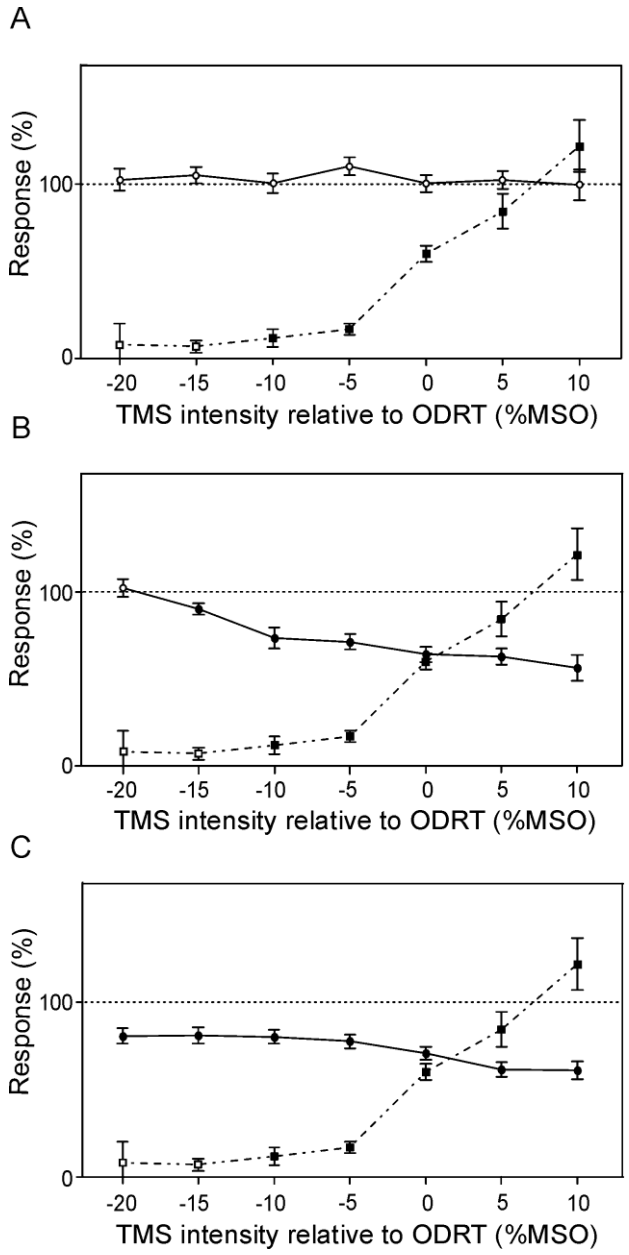


Figure 3-4: Average direct response and MEP suppression for each ISI is plotted versus stimulus intensity (expressed relative to ODRT). Solid lines plot average MEP suppression at ISI's of 3 ms (A), 5 ms (B) and 7 ms (C). Filled circles indicate significant points ($P < 0.05$). Dashed lines show average direct response size (expressed as a % of mean background EMG) following PF stimulation for comparison. Filled squares indicate significant points ($P < 0.05$). All curves are averaged over the 11 subjects who participated in this study. Error bars show standard errors.

responses were tested when the coil was oriented either to produce this current direction, or the opposite. Figure 3-5A plots the response amplitude averaged across subjects versus intensity relative to ODRT. The two plots indicate responses generated with a brain current oriented downwards (solid line) and upwards (dashed line). Significant responses were generated at 10% below ODRT with the downward brain current, but only at 20% above ODRT with the upward brain current. Figure 3-5B illustrates the response seen in one subject at 74% of maximal stimulator output with a downward brain current; by contrast there was no response at this level (Fig. 3-5C) or at a higher intensity (84%; Fig. 3-5D) with the coil inverted.

In a subset of subjects (2/11), we also conducted the conditioning-test experiment with both PF coil orientations (upward and downward brain current). Stimulation intensities were the same for both recordings though we identified the motor threshold in both orientations. Contrary to previous reports (Ugawa et al., 1995) an equal degree of MEP suppression was observed with both current directions (Fig. 3-5E).

Discussion

Magnetic stimulation of the PF probably has complex actions, and a variety of peripheral and central pathways could contribute to its effects (Meyer et al., 1993). Antidromic action potentials in the CST could plausibly produce MEP suppression, as corticospinal neurones are known to make recurrent collaterals to inhibitory interneurones in the cortex (Renaud and Kelly, 1974). Other brainstem pathways, such as the spinothalamic tract or dorsal columns, could also produce MEP suppression.

To be certain that MEP suppression is mediated by cerebellar pathways, we must exclude all other possibilities. This requires a particularly stringent standard of evidence: to exclude a CST contribution, for example, it is necessary to show with high confidence that there is *no* activation of corticospinal fibres. It is critical therefore accurately to measure the threshold for a direct response to corticospinal stimulation. When this was done using online observation of single sweep responses, threshold was generally overestimated. Subsequent observation of averaged traces revealed significant averaged responses at intensities below our initial threshold estimate. However, even correcting the threshold

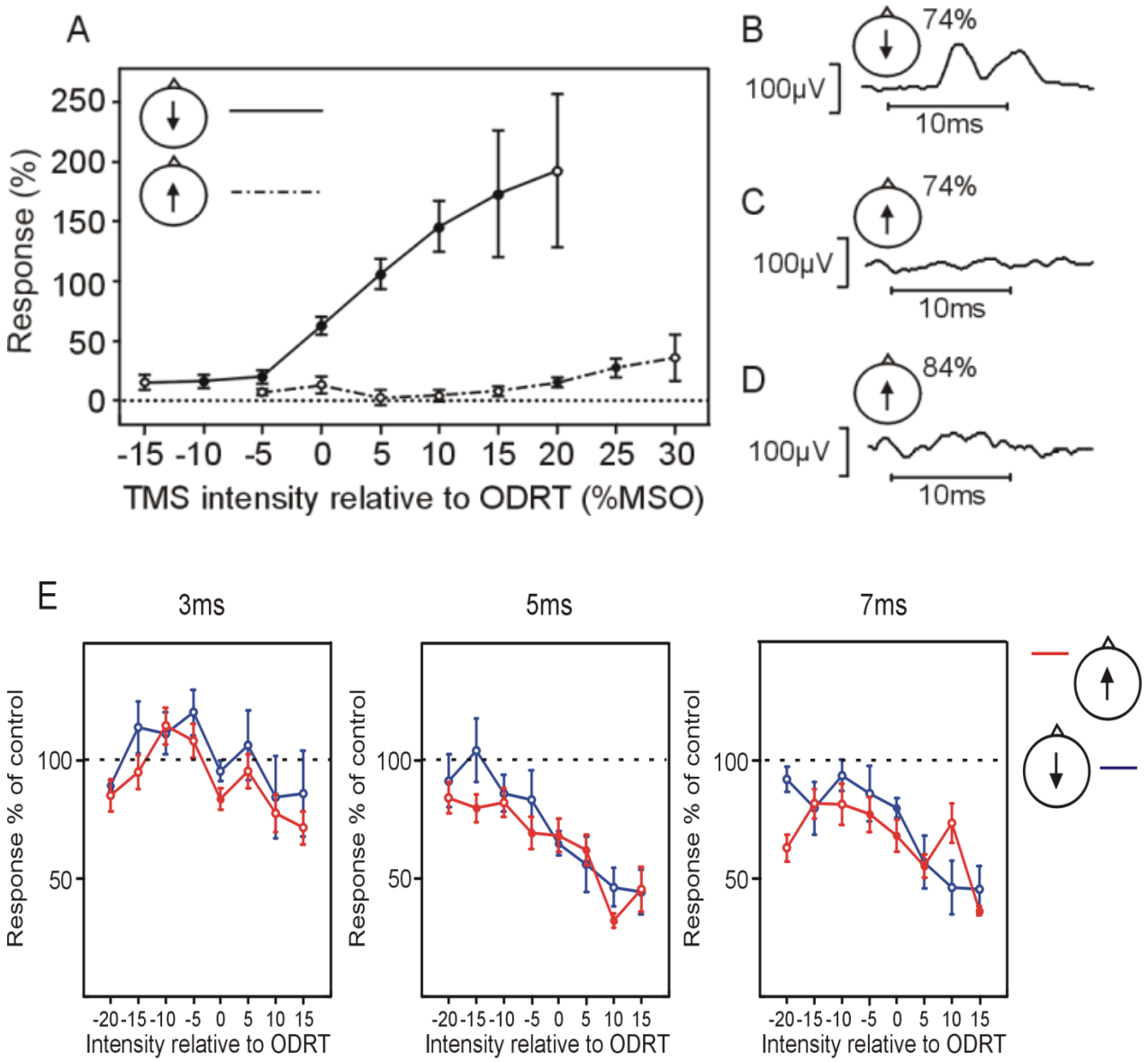


Figure 3-5: Effect of coil orientation on direct responses. A: Direct response size as a function of stimulus intensity relative to ODRT, averaged across seven subjects. Solid line indicates measurements with brain current downwards; dashed line indicates brain current upwards. Solid circles indicate responses significantly different from zero ($P < 0.05$). Error bars show standard errors. B-C: Example response from one subject (FDI muscle) with an intensity of 74%, brain current downwards (B) and upwards (C). (D) Same subject, intensity 84%, brain current upwards. E: Average MEP suppression in 2 subjects across all muscles recorded with both an upward and downward brain current. Filled circles denote datapoints which are significantly different from baseline.

measure using these averages (ODRT) did not give the required level of confidence that no response was present. When data was averaged across subjects, small but significant responses at intensities below ODRT could be revealed.

Antidromic activation of corticospinal axons has the greatest potential to generate MEP suppression following PF stimulation (via intra-cortical recurrent collaterals). Such activation cannot be assessed directly; we can only measure the generation of responses in muscle, which results from orthodromic activation of corticospinal axons and subsequent synaptic action on motoneurons. This study revealed a marked difference in the efficacy of upward versus downward orientations of stimulating current in generating muscle responses. The most plausible explanation for this difference is the presence of anode block with the upward current. Stimulation over the brainstem, where the tracts are small, may be sufficient to concentrate current and generate a focal hyperpolarisation. This would prevent transmission of MEPs, but suppression via antidromic activation and recurrent inhibition would remain unaffected. This notion is supported by the fact that MEP suppression was observed using either an upward or downward current for stimulation over the PF (Fig. 3-5E). Despite anode block affecting the orthodromic transmission of activity for the upward brain current, the observation of suppression suggests a role for recurrent inhibition in the phenomenon. Therefore, only if no direct response can be seen following PF stimulation with a downward brain current is it safe to assign MEP suppression seen with an upward brain current to non-CST pathways.

The future of PF stimulation

The deep location of the cerebellum beneath the skull means that high stimulus intensities are routinely used in TMS studies (mean threshold here was 75%). Due to current spread at high intensities, a stimulus of this size over the PF is likely to activate many structures, including part/all of the cerebellum. However, it is difficult to identify exactly how much each structure contributes to the effects on MEPs.

Preserved MEP suppression has been demonstrated in some patients with degenerative ataxia or cerebellar agenesis (Meyer et al., 1993; Meyer et al., 1994; Ugawa et al., 1994b; Ugawa et al., 1997). Disruption of the cerebellar outflow via thalamus to cerebral cortex is

likely to produce associated changes in cerebral cortical function, which could plausibly include the networks underlying recurrent inhibition from CST activation. Abnormal suppression would then be seen in patients with cerebellar dysfunction, but the test would not directly measure the cerebellar pathology. This distinction becomes of key importance when PF suppression of MEPs is used to investigate an unknown disease. In that case, the confounded cerebellar and CST responses to PF stimulation demonstrated in this study will make unambiguous interpretation of results impossible.

Unpublished data from our laboratory has failed to demonstrate conditioning of MEPs with cerebellar stimulation in an anaesthetised marmoset monkey (personal communication with Dr Demetris Soteropoulos and Prof. Stuart Baker). Epidural stimulation over motor cortex was performed in order to elicit a volley in the spinal cord. This was preceded with epidural stimulation of cerebellar cortex with ISIs between 1-10ms. No conditioning effects were observed at any of the intervals tested.

Given the non-selective nature of TMS over the PF, its utility as an experimental tool will depend on the purpose to which it is put. If all that is desired is to activate the cerebellum, this can probably be achieved. However, if coactivation of other structures would confound the results obtained, the method must be used with considerable caution. To conclude therefore, although some of the effects are likely to be mediated by cerebellar pathways, it is difficult to justify PF stimulation as a specific diagnostic test for subclinical cerebellar disease. In addition, the technique can be uncomfortable which would preclude use in frail or elderly patients.

Impact on other research

Data from this study has shown that CST activation occurs at intensities routinely used to stimulate over the PF. Although it remains possible that the cerebellum contributes to the observed suppression at this level, the confounding activation of other structures are likely to generate ambiguous results. Therefore, a highly conservative approach must be taken if this method is to be used as a specific assessment of cerebellar function. Intensities 15-20% below threshold should be used and suitable controls undertaken in order to verify the absence of activity in other motor pathways. The results presented here agree with reports

that demonstrate some patients with cerebellar defects continue to show motor cortical suppression following PF stimulation (Meyer et al., 1993; Meyer et al., 1994; Ugawa et al., 1994b; Ugawa et al., 1997).

The surge of interest into 'cerebellar' TMS is a concern. The latest wave of studies merely reproduce the standard methods without any consideration for the wider effects of TMS over this region. It may be that some of the consequences of PF rTMS do indeed result from disruption of cerebellar pathways. However, the profound recurrent inhibition from repetitive antidromic CST activation would also severely impair motor performance, limiting the conclusions which can be drawn from these studies.

Recent developments

A recent study has demonstrated that for two thirds of a large cohort of healthy subjects, the optimal site for CST activation is lateral to midline (Shirota et al., 2010a). The finding of heterogeneity within a large population supports the findings reported here; most subjects reported had MEP suppression with corresponding direct responses following TMS in a lateral position. This highlights the difficulty in finding a site for selective and specific activation of the cerebellum; especially one which is consistent across subjects.

Despite the potential for confounding activation of descending motor pathways, the interest in 'cerebellar' TMS remains strong. In particular, the potential role of this paired pulse paradigm as a diagnostic test is still under investigation. One Japanese group recently found MEP suppression to be abnormal in patients with PSP but normal in PD patients who were on medication (Shirota et al., 2010b). In addition, Ni et al (2010) showed motor cortical inhibition was reduced in PD patients off medication. These two studies in combination support the original hypothesis – that this technique could be used for the detection of subclinical cerebellar disease. However care should be taken when interpreting their results in terms of whether the effects described are mediated by the cerebellum. Both used a stimulus intensity which was only 5% below threshold and the coil was in the lateral position. Moreover, this particular group of patients have complicated movement disorders which are likely to affect a variety of brain systems.

CHAPTER IV: THE ROLE OF LEVETIRACETAM IN THE TREATMENT OF CEREBELLAR TREMOR

Multiple Sclerosis (MS) is the most common and disabling neurological disorder affecting young adults in the UK and as a result costs the NHS millions of pounds a year (Kobelt et al., 2006). It is a chronic and complicated disease with no cure. Onset is early, usually between the ages of 20 and 50; and disease duration is long, with patients on average living 40 years beyond diagnosis (Kesselring and Beer, 2005). A multitude of symptoms and progressive disablement means that there is a high prevalence of social and psychological problems, and that MS is therefore a complex disease to treat effectively.

The underlying pathophysiology of MS describes an autoimmune disease which primarily attacks oligodendrocytes within the central nervous system. This is characterized by multiple focal lesions to the white matter of the brain and spinal cord. The lesions are plaques of demyelination, mediated by B and T cells which appear to be sensitized to myelin antigens. As a result, histological analysis of lesions shows a selective loss of myelin and oligodendrocytes, with the preservation of nerve cells and axons. This feature distinguishes MS from other autoimmune disorders. Patients are responsive to immunosuppressive therapy, confirming the underlying pathology of this disease.

In over 85% of cases, multiple sclerosis follows the pattern of relapse and remission (Weinshenker et al., 1989a; Weinshenker et al., 1989b). Relapses develop acutely and are typically followed by several weeks or months of recovery. Sometimes this recovery is complete but often patients retain residual symptoms which become more troublesome as the disease progresses. Patients are usually neurologically stable between relapses. Disease modifying treatments such as beta interferon, natalizumab and campath can reduce the number and severity of relapses and slow the progression of disability (Coles et al., 2006; Miller et al., 2003; Paty and Li, 1993).

Many patients with relapsing-remitting MS go on to develop a progressive form of the disease; this is no longer characterised by discrete autoimmune attacks. Instead, the disease

follows a steady course characterized by a slowly progressing disablement; 50% of patients are reported to require a walking aid 15 years after diagnosis (Runmarker and Andersen, 1993; Weinshenker et al., 1989b) and 50-80% of patients also become unable to work during the first 10 years of disease onset (Rao et al., 1991). This combination of factors and a general loss of independence means that depression and anxiety (sometimes present in even the most benign cases) feature highly amongst MS patients. Pharmacological management is therefore desirable in order to help the patient to lead a relatively 'normal' lifestyle.

Current MS therapy has two aims; short term symptomatic relief and long term slowing of disease progression. Immunomodulatory treatments are disease-modifying and aim to reduce the severity and frequency of relapses. These are taken over long periods to help alter the time course of the disease. Drugs such as interferon-beta and natalizumab are very effective in many patients, however the long term effects have yet to be fully investigated since these drugs have only been widely available for less than twenty years.

Symptomatic drugs do not alter disease progression, but are used for the day-to-day management of an individual's specific symptoms. Given that some patients do not qualify for immunomodulatory therapy, especially those who have the progressive form of the disease (Rieckmann et al., 2004), symptomatic treatments are essential for most patients (Samkoff and Goodman, 2011).

In severe cases, more radical treatments have been attempted such as thalamotomy and thalamic deep brain stimulation. However, so far these therapies haven't shown evidence of any sustained long term benefit. In fact, results are highly unpredictable, often depending on careful patient selection and dedicated post-operative care. On average MS patients have a life expectancy only seven years shorter than normal (Stevenson and Thompson, 1998), so that specific symptomatic treatments remain highly desirable.

Tremor

Tremor is classified as a rhythmic, involuntary oscillatory movement. It is a common manifestation of multiple sclerosis (MS), estimated to affect between 26-58% of patients

(Alusi et al., 2001; Pittock et al., 2004). Typically tremor is not an isolated feature in MS, but is often coupled with other cerebellar symptoms which together form a complex motor disorder. Consequently MS tremor is usually associated with a poor prognosis (Pittock et al., 2004).

Traditionally, Holmes' (rubral) tremor was considered to be the characteristic severe tremor found in MS patients. This constitutes both a rest, postural and action tremor of low frequency and irregular pattern. However, Alusi et al (2001) found no patients out of a large cohort with true rubral or resting tremor. Instead, 58% exhibited an action tremor of either a postural, intention or kinetic type. Interestingly, 20 of these were asymptomatic despite exhibiting something similar to physiological tremor. Of the symptomatic patients, most described the tremor as originating in the arms before spreading to the other limbs (often bilaterally) and progressing in severity.

The origin of MS tremor is difficult to pinpoint due to the nature of the disease. Multiple diffuse white matter lesions are spread throughout the brain and spinal cord which means that tremor is rarely an isolated symptom. However, damage to the cerebellum or one of the cerebellar afferent/efferent pathways is generally considered to be the primary cause (Feys et al., 2005). Experimental inactivation of the cerebellar nuclei in animals reversibly generates tremor with similar properties to that seen in MS (Vilis and Hore, 1980). Moreover, Alusi et al (2001) found a strong positive correlation between tremor and the severity of other cerebellar signs such as dysmetria and dysdiadochokinesia, which supports this theory.

Similar tremor is also seen in many other neurological conditions such as essential tremor and stroke. There is hypothesised to be an overlap in the pathophysiology in that the cerebellum is implicated in most cases. For the purposes of this chapter, the tremor observed in this patient cohort will be referred to as either MS or cerebellar tremor.

MS (cerebellar) Tremor

Cerebellar tremor is usually associated with movement; patients present with discontinuities in their movements, which are more obvious when the movement is slow

and are amplified towards the end of the movement (intention tremor). In general, this type of tremor becomes worse as more precision is required for a movement, for example pointing to a target. Intention tremor in particular can be highly disabling; it is associated with severely impaired quality of life measures and is regarded as one of the most difficult symptoms of MS to treat. Postural tremor can also be a manifestation of cerebellar disease although this is much less common and can often be the result of combined cerebellar and brainstem lesions in MS.

Cerebellar tremor tends to occur at approximately 3-6Hz, but is highly irregular in frequency and amplitude. The underlying mechanism driving these rhythmic oscillatory movements is unclear. Figure 4-1 shows the extensive network of cortical areas that contribute to the preparation and co-ordination of movements. For each movement elicited, an efference copy is relayed to the cerebellar nuclei via olivary and pontine pathways. This carries information regarding the sensory, visual and motor aspects of the intended movement. In addition, interpositus receives peripheral feedback via spinocerebellar pathways to update the system as the movement progresses. Clearly therefore, this is a complex network with multiple feedback loops contributing to online error correction. Therefore, alteration of any part of this system could potentially give rise to tremor.

Many groups have attempted ablation studies to try and determine the particular region which is critical to the generation of cerebellar tremor. The crucial area is thought to be one of the deep cerebellar nuclei given that this type of intention tremor does not arise with lesions of the cerebellar cortex. However, there has been no firm conclusion with some groups stressing a role for dentate (Cooke and Thomas, 1976; Vilis and Hore, 1977), some for interpositus and others suggesting that both are implicated (Gemba et al., 1980). Such conflicting results are likely to arise because these two cerebellar nuclei are in such close proximity and the techniques used (cryoprobes, muscimol inactivation and electrolytic lesions) are not selective enough to inactivate one in isolation.

Despite the focus on identifying a central mechanism responsible for the generation of cerebellar tremor, many authors have proposed a role for peripheral factors. Vilis and Hore have shown that tremor amplitude and frequency depend on mechanical factors associated with movements. In a series of experiments, they cooled dentate and interpositus cerebellar

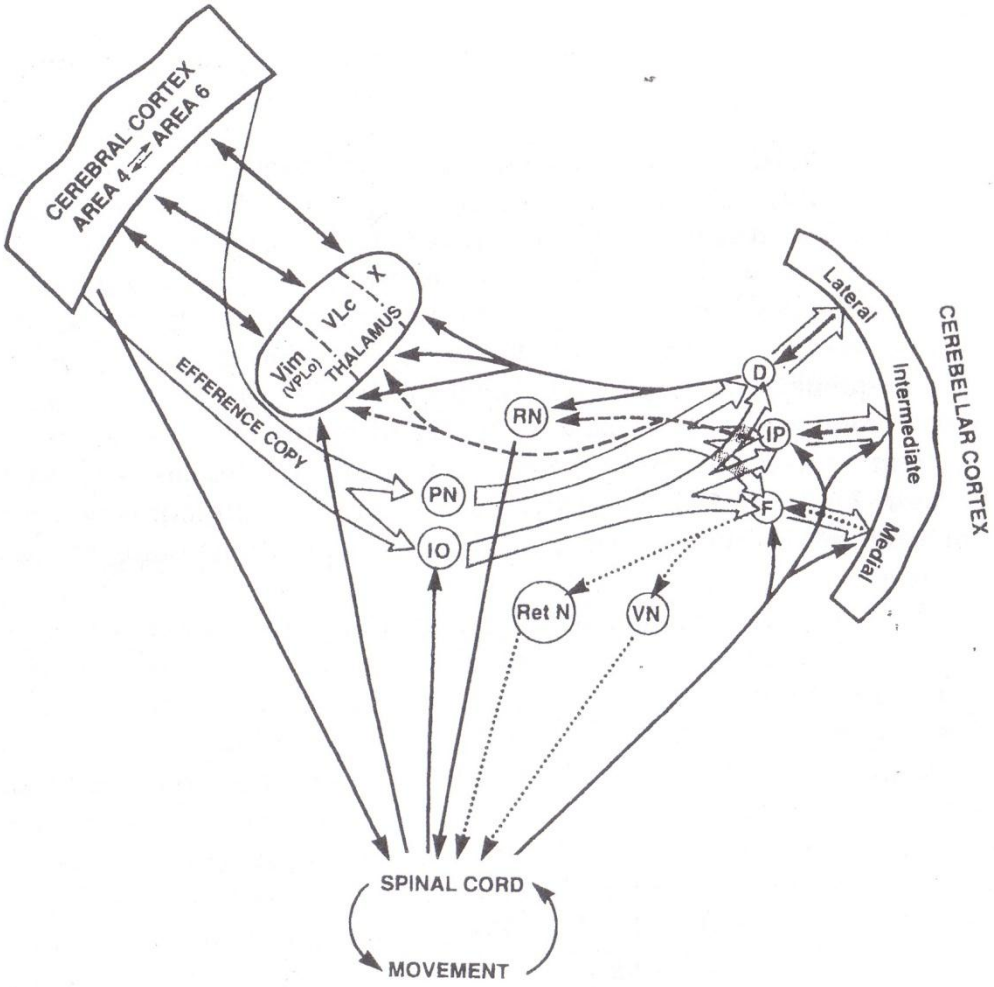


Figure 4-1: Schematic diagram showing the complex network of neural pathways associated with movement control and co-ordination. Theoretically, tremor could arise from a disturbance in the normal function of any of these loops. Reproduced from Tremor: Elble and Koller (1990).

nuclei in monkeys as a model of cerebellar tremor (Vilis and Hore, 1977; Vilis and Hore, 1980). Cooling generated ipsilateral discontinuities in acceleration traces which appeared as rhythmic oscillations. During performance of a flexion-extension tracking task, addition of a spring load to the forearm muscle increased the tremor frequency whilst adding an inertial load reduced tremor frequency. Additionally, tremor was resynchronised with a limb perturbation. In this way, tremor was influenced by mechanical conditions, which implies a possible role for the proprioceptive feedback reflex in its pathophysiology. A limb weighting study in humans has also shown that damping of the mechanical component of movement can reduce action tremor (Aisen et al., 1993).

A further theory for the generation of tremor implicates altered muscle spindle sensitivity. Cooling of peripheral muscles has been found to reduce cerebellar intention tremor (Feys et al., 2005). This is thought to be because temperature sensitive muscle spindles fire less frequently at lower temperatures, therefore decreasing the afferent feedback contribution to the stretch reflex. In addition, following cerebellectomy or cerebellar lesioning, reduced drive to motoneurones and reduced afferent feedback activity causes both static and dynamic spindle sensitivity to decrease (Gilman, 1969a; Gilman, 1969b). This could therefore account for the delayed movement onset latencies described by Vilis and Hore (1980). However, Vallbo (1971) demonstrated the onset of Ia firing to begin up to 50ms after initial EMG activity which does not fit with this theory. Moreover, muscle spindle sensitivity is restored with time (Van der Meulen and Gilman, 1965), and so although muscle spindles may contribute to tremor production, there are likely to also be other mechanisms involved. For instance, the increased motor unit twitch times observed by Riddle & Baker (2005) in peripheral cooling could be responsible for the EMG onset delay described by Vilis and Hore (1980).

As already described, many theories concerning the origin of cerebellar tremor implicate peripheral factors. However, it has been shown that cerebellectomized primates still exhibit tremor despite the removal of peripheral afferent feedback (Gilman et al., 1976). In addition, cerebellar tremor and discontinuities occur in the absence of visual feedback, therefore error recognition and correction by the cerebellum do not depend upon the visual system (Flament et al., 1984). Although it is likely that there is a peripheral

contribution, this is probably only by way of modulating activity from a central rhythm generator.

Treatment of MS tremor

Tremor is regarded as one of the most difficult symptoms of MS to treat and also one of the most frustrating for the patient. Severity varies according to time of day, and is worsened by high temperature (Uhthoff's phenomenon), excitement, physical exertion and fatigue.

Aside from pharmacological treatment, compensation techniques can be used to help patients manage tremor. Movements can be stabilised by bracing the reaching hand, or limbs can be fixed in a 'position of function' to allow necessary daily tasks such as eating. Occupational therapy can also help train patients to learn basic movement patterns with the aim that eventually they will become more automatic and controlled. In addition, studies have shown that weighting a limb can help reduce tremor (Aisen et al., 1993). This works by lowering the mechanical resonant frequency and therefore damping the oscillations. However, this may promote weakness and fatigue in some patients. Physiotherapy can help reduce tremor in some patients, although effects are usually temporary.

Surgeons have attempted to alleviate MS tremor through stereotactic techniques for over 40 years; initially, by way of thalamotomy, but more recently by thalamic stimulation. The optimal target site for such procedures is controversial, with some teams aiming for the nucleus ventralis intermedius (cerebellar input nucleus) (Hirai et al., 1983) and others, the nucleus ventralis oralis posterior (basal ganglia output) (Alusi et al., 1998; Nguyen and Degos, 1993). In fact, the target site may vary according to the particular tremor subtype exhibited by the patient and indeed some surgical studies have shown thalamic stimulation to be more beneficial for patients with postural rather than kinetic tremor (Montgomery et al., 1999; Waubant et al., 2001).

There are concerns over the long-term effects of surgery; tremor reappears in 20% of patients after 12 months (Alusi et al., 2001). There is also a substantial risk of complications, including hemiparesis and seizure. As a result, surgery is usually only considered for the most severely affected; a careful selection process identifies those most

likely to benefit. The selection criteria usually distinguishes those with a stable disease history and minimal disability. Recent studies have explored possible tests which could help to select these patients (Alusi et al., 2003; Liu et al., 2000).

Pharmacological therapy is the mainstay of treatment for MS tremor. Clinical trials have explored the use of drugs such as glutethimide (Aisen et al., 1991), izoniazid (Hallett, 1985; Koller, 1984), carbamazepine (Sechi et al., 1989), primidone (Henkin and Herishanu, 1989), propranolol (Koller, 1984) and cannabis (Fox et al., 2004). Although these agents can prove beneficial for some patients, results are not consistent. Moreover, patients often experience intolerable side effects, notably hepatotoxicity with high doses and many drugs exacerbate common MS symptoms such as fatigue.

All current techniques to treat MS tremor show only limited success. Drug therapy is certainly a useful tool, but efficacy is subjective. Occupational therapy is not effective in all patients and limb weighting promotes weakness and fatigue (Hewer et al., 1972), two common MS symptoms. In addition, surgical therapy has shown limited success, principally due to the relatively small numbers of patients investigated, but also as long-term effects are largely unexplored. A combinatory approach to treatment is therefore desirable until further progress is achieved in this area.

Leviteracetam

Recent studies have suggested that Levetiracetam (LEV) could be effective in the treatment of MS tremor (Feys et al., 2009; Mandler, 2003; Solaro et al., 2008; Striano et al., 2006). LEV was licensed by the FDA in 1999 for use as an adjunctive anticonvulsant therapy. Since then, it has been found to have a unique mode of action amongst anti-epileptic agents. This has led to a multitude of off-label trials of LEV for indications ranging from psychiatric disorders (Grunze et al., 2003) to migraine (Brighina et al., 2006) and chronic pain management (Rossi et al., 2009b).

Antiepileptics in general have a wide range of pharmacological actions. Some are known to inhibit GABA uptake and breakdown such as vigabatrin, thereby increasing inhibitory neurotransmission. Others alter ionic conductances such as the calcium and sodium

currents associated with action potentials. In fact, most antiepileptic drugs have multiple actions upon the central nervous system which are not fully understood. Indeed, the antiepileptic mechanism of action of LEV is yet to be fully elucidated. Preclinical studies suggested that Leviteracetam has no effect on sodium, calcium or potassium channels, and does not alter the neurotransmission of GABA or glutamate (Sills et al., 1997; Zona et al., 2001).

More recently, an *in vitro* study demonstrated that LEV is involved in the partial inhibition of N-type calcium currents (Lukyanetz et al., 2002). These ionic conductances are present predominantly on presynaptic cell membranes, and their modulation affects the level of intraneuronal calcium and consequently overall cell excitability. *In vitro* studies have also demonstrated that LEV binds to a specific site on synaptic vesicle protein 2A which is found predominantly in cerebral cortex, hippocampus and the cerebellum (Lynch et al., 2004; Noyer et al., 1995). This molecule is involved in the process of neurotransmitter vesicle fusion and exocytosis.

It is unlikely however that these are the only effects mediated by LEV. Poulain and Margineanu (2002) suggested that the drug may affect postsynaptic GABA function and modulate GABA-gated currents by acting indirectly on $GABA_A$ receptors. Further biochemical testing is required to confirm whether this is the case.

The specific localisation of the LEV binding site to the cerebellum has raised interest in the prospect of treating cerebellar disorders. An anecdotal report by Mandler et al (2003) showed reduction of severe cerebellar tremor in one patient following treatment with LEV. Subsequently, several clinical trials have investigated the effect of LEV on cerebellar tremor in MS. Striano et al (2006) found a significant reduction in outcome measures following a 5-6 week titration. Contrary to this, neither Feys et al (2009) nor Solaro et al (2008) reported any reduction in tremor severity or improvement in motor function during their respective trials. The conflicting results may be a consequence of differing dosing regimen; LEV dose was much higher in the Striano study group (3-4000mg/day), as opposed to 1-2000mg/day administered by Feys et al (2009) and a maximum of 1500mg/day used by Solaro et al (2008).

Aims/outline

The aim of this study was to evaluate and quantify the efficacy of LEV in the treatment of upper limb cerebellar tremor. Previous studies have used a range of clinical outcomes including tremor/ataxia scores, quality of life assessments and some quantification of reaching movements. This study utilised accelerometry and 3-dimensional motion tracking to facilitate quantification of tremor amplitude within the cerebellar tremor frequency band.

Methods

Patients & study design

Twenty patients with a diagnosis of multiple sclerosis (McDonald et al., 2001) and clinical evidence of cerebellar tremor were initially recruited to the study. All patients provided informed consent prior to the start of the trial. The study was approved by the local research ethics committee and the MHRA.

Patients were not asked to withdraw any regular medication as we intended to investigate the use of LEV as an adjunctive therapy. Clinical data from patients completing the study is shown in table 4-1. Mean patient age was 40.7 years and disease duration 7.3 years.

The study design is illustrated in Fig 4-2; we chose a double blind, placebo controlled, crossover approach. Following recruitment, patients were randomized to one of two treatment groups.

Each patient received a six week course of both LEV and placebo tablets, separated by a washout phase of 2 weeks. Dose was titrated up from 250mg BD to 1500mg BD or the maximum tolerated dose. Of the 10 patients who completed this schedule, only one (patient 10) reported side effects, principally dizziness, limb weakness and exacerbation of fatigue. This resolved after a few days and the patient elected to continue with the trial. Consequently, all patients reported here achieved the full dose of 3000mg/day.

A total of 10 patients were withdrawn from the trial (4 x adverse effects, 2 x inadequate 3-8Hz tremor on first assessment, 2 x non compliance, 1 x infection, 1 x rapid progression of MS). This is consistent with previous findings showing that patients with MS are more

Patient	Age	Sex	MS type	Yrs since Δ	Tremor type	Medication
1	41	F	RR	13	Postural & Intention	Rebif 22, Gabapentin
2	50	F	SP	13	Intention	Baclofen, Amantidine
3	33	F	RR	1	Postural	Copaxone
4	41	M	SP	3	Intention	Avonex
5	24	F	PP	9	Postural & Intention	Mitoxantrone, Solifenacin, Gabapentin, Tizanidine Co-codamol, Warfarin
6	26	F	RR	2	Postural & Intention	Gabapentin, Amitriptyline
7	60	M	PP	0.5	Postural & Intention	Etoricoxib, Amlodipine, Fluoxetine
8	38	F	RR	11	Postural, Intention & Orthostatic	Natalizumab, Gabapentin, Baclofen, Citalopram
9	45	F	PP	1.5	Intention	Baclofen, Citalopram, Codeine, Gabapentin
10	49	M	SP	19	Postural & Intention	None

RR, Relapsing Remitting; PP, Primary Progressive; SP, Secondary Progressive

Table 4-1: Patient demographics are shown for the participants who completed the study.

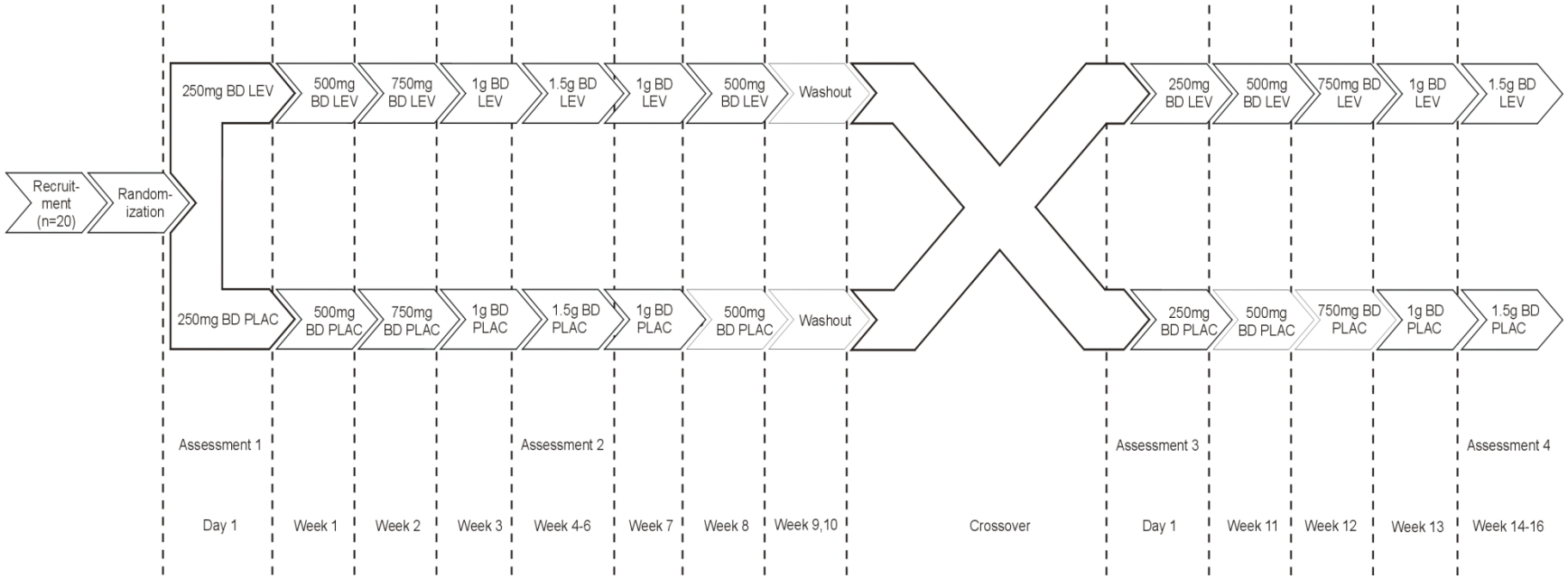


Figure 4-2: Flow chart showing crossover trial design.

likely to experience adverse effects with antiepileptic medication (Solaro et al., 2005). Since the study design was placebo-controlled, there was no need to perform a balanced randomisation process; consequently following dropouts, data was analysed from 7 patients who initially received LEV and the remaining 3 commenced the trial with placebo treatment.

Motion recordings

Tremor is usually measured using an accelerometer; this measures the magnitude and direction of acceleration of an object which is particularly useful in the assessment of movement disorders since it is an objective measurement rather than the traditional subjective clinical scoring methods. Advancement in technology has led to very small and sensitive accelerometers that lend themselves well to measurement of human movements such as tremor and myoclonus. Triaxial piezoelectric accelerometers (Model 35A Isotron® accelerometer, Endevco) were attached to the finger and elbow of the limb under observation. Signals were amplified using an Endevco Isotron signal conditioner (Model 2792B) to produce an overall sensitivity of $5mV/ms^{-2}$ and digitised using a Power1401 (CED Ltd, Cambridge, UK) before being stored on a PC using Spike 2 software (sampling rate 1kHz).

Arm movements were recorded using a 3D motion tracking system (Liberty, Polhemus Inc); four sensors were positioned on the arm to record movements. A teardrop sensor was placed on the index finger and three standard sensors were attached to the wrist, elbow and shoulder. The source was located in a fixed position on the task board; all experiments were performed within a 1 metre locus of this point. A custom written software interface (programmed in Borland Delphi 7) was used to collect data from the Polhemus system which was sampled at 240Hz.

Surface EMGs were also recorded from an intrinsic hand muscle (FDI), 2 forearm muscles (FDS and EDC) and 2 upper arm muscles (triceps and biceps).

Behavioural tasks

To investigate postural tremor, patients were asked to hold a specific posture for a total of a

minute. This required arm extension in front of the body with the arm held at 90 degrees to the trunk. Patients were also required to perform whole arm reaching movements to assess kinetic tremor. Two small posts were mounted on a table at a distance of 30cm apart in the sagittal plane. On the top of each post was a light emitting diode (LED, 3mm diameter); these were illuminated alternately every 3 seconds. Patients were required to point at the lit LED. They were instructed not to touch the posts, support the limb with their other arm or rest their arm between trials. Approximately 30 trials (defined as a movement from the closest LED to the furthest, and back again) were completed at each assessment. Patient 5 was not able to complete this task due to the severity of her kinetic tremor.

Clinical assessments

As a standard clinical assessment of tremor, patients were asked to complete a 9-hole peg test. Two of these pegboard tasks were used, one with small 6mm diameter pegs and one with larger 12mm pegs. The time taken for completion of the task was measured. Population data was averaged to test for significant changes in performance.

A video recording was also taken during each session; this included footage of finger to nose pointing, rapidly alternating movements, walking and the pegboard tests. From these videos, an independent clinician scored the various elements of cerebellar tremor using a previously published 10 point visual analogue scale (Bain et al., 1993). Patients were also required to perform a self-assessment of their tremor using the same scale.

Analysis

Data analysis was performed using custom written programs in the MATLAB environment (Mathworks Inc). For postural tremor, the power spectrum was calculated from each axis of the elbow accelerometer recording. Spectral estimation used the whole 60 s recording, divided into 29 non-overlapping 2-second windows for Fourier analysis; this provided a frequency resolution of 0.5 Hz. The three spectra were summed, providing an estimate of the total acceleration power. Confidence limits on the spectral estimates were determined as in Press et al (1989).

The tremor band of interest was selected as 2.5-10Hz, encompassing the known

frequencies of cerebellar tremor (Elble and Koller, 1990). The area under the curve for each subject and visit was measured for this region; values were normalised as a percentage of the baseline assessment.

The therapeutic effect of LEV, or the *efficacy*, was calculated across the population using the mean area under curve values from the data as:

$$Efficacy = \frac{LEV/Baseline}{PLC/Baseline}$$

An efficacy value less than 1 would therefore indicate that LEV tended to reduce MS tremor. The significance of this difference between the effect of LEV and placebo was determined using a Monte Carlo method, involving randomly shuffling the data between drug and placebo classes and recomputing the difference. The rank of the experimentally observed difference amongst a large number of shuffles (10,000) was used to assign an approximate significance level.

To determine whether there was a significant effect of LEV on MS tremor within individual subjects, Z-scores were calculated according to:

$$Z = \frac{[(AUC^{LEV} - AUC^{BL}) - (AUC^{PLC} - AUC^{BL})]}{\sqrt{SD_{LEV}^2 + SD_{BL}^2 + SD_{PLC}^2 + SD_{BL}^2}}$$

where AUC is the area under curve for the tremor frequency band and SD are the corresponding standard deviations. Z-scores less than -1.96 lie outside the 95% prediction interval and are therefore considered to reflect a significant effect of LEV on MS tremor.

Deficits during movement were assessed using 3D motion tracking data from the wrist during pointing movements. The recording was first divided into individual pointing movements, using the times of illumination of each LED target as the onset and offset time of the movement segment. The three axes of finger position were low-pass filtered (20Hz cut-off), to remove measurement noise. The path length of the trajectory taken by the finger in travelling from the initial to final movement was then measured as:

$$T = \sum_{i=1}^{N-1} |\vec{x}_{i+1} - \vec{x}_i|$$

where $\vec{x}_i$ represents the three-dimensional vector of position measured at sample point *i* (*i*=1..*N*), and $|\vec{x}_{i+1} - \vec{x}_i|$ represents the distance between positions measured at sample points *i* and *i+1*. The total distance moved by the finger was calculated as:

$$D = |\vec{x}_N - \vec{x}_1|$$

The values of T and D were averaged across the approximately 60 pointing movements available, yielding average values $\bar{T}$ and $\bar{D}$. An index of movement impairment was calculated as $I = \bar{T}/\bar{D} - 1$. If the finger trajectory followed a perfect straight line between the start and end points of each movement, T would equal D, and hence the index I would equal 0. Larger values of I indicate increasing deviation from a straight line path.

The index I is affected by increases in finger path length due both to tremor, and also to hypermetria. Therefore the analysis was repeated, with the initial low-pass filter configured to remove all frequencies above 2 Hz. This smoothed out tremulous movements; the value of *I* was then sensitive mainly to dysmetria (I_{dys}). The difference between this measurement and that made with the low-pass filter set to 20 Hz (I_{all}) was taken as an assessment of the impact of action tremor (I_{tremor}). For each patient, measurements were normalised to those made during the baseline visit.

LEV efficacy for MS action tremor was calculated as described previously. Again, significance was assigned using a Monte Carlo method.

Results

To determine which patients had significant postural tremor, clinical tremor scores were plotted against the sum tremor power between 3-7Hz (Fig. 4-3A). Patients who fell below the dotted line were considered not to have postural tremor and were therefore removed from the population analysis. This left 6 remaining patients. Figure 4-3B shows representative power spectra for one patient with severe MS tremor. In each plot, the dark grey line represents represents baseline data and the light grey corresponds to maximum treatment dose; 95% confidence limits are also shown. A clear peak in tremor is evident at approximately 3Hz, which is reduced following LEV (Fig. 4-3B: left) and returned to baseline level with placebo (Fig. 4-3B: right). In this patient there appears to be a

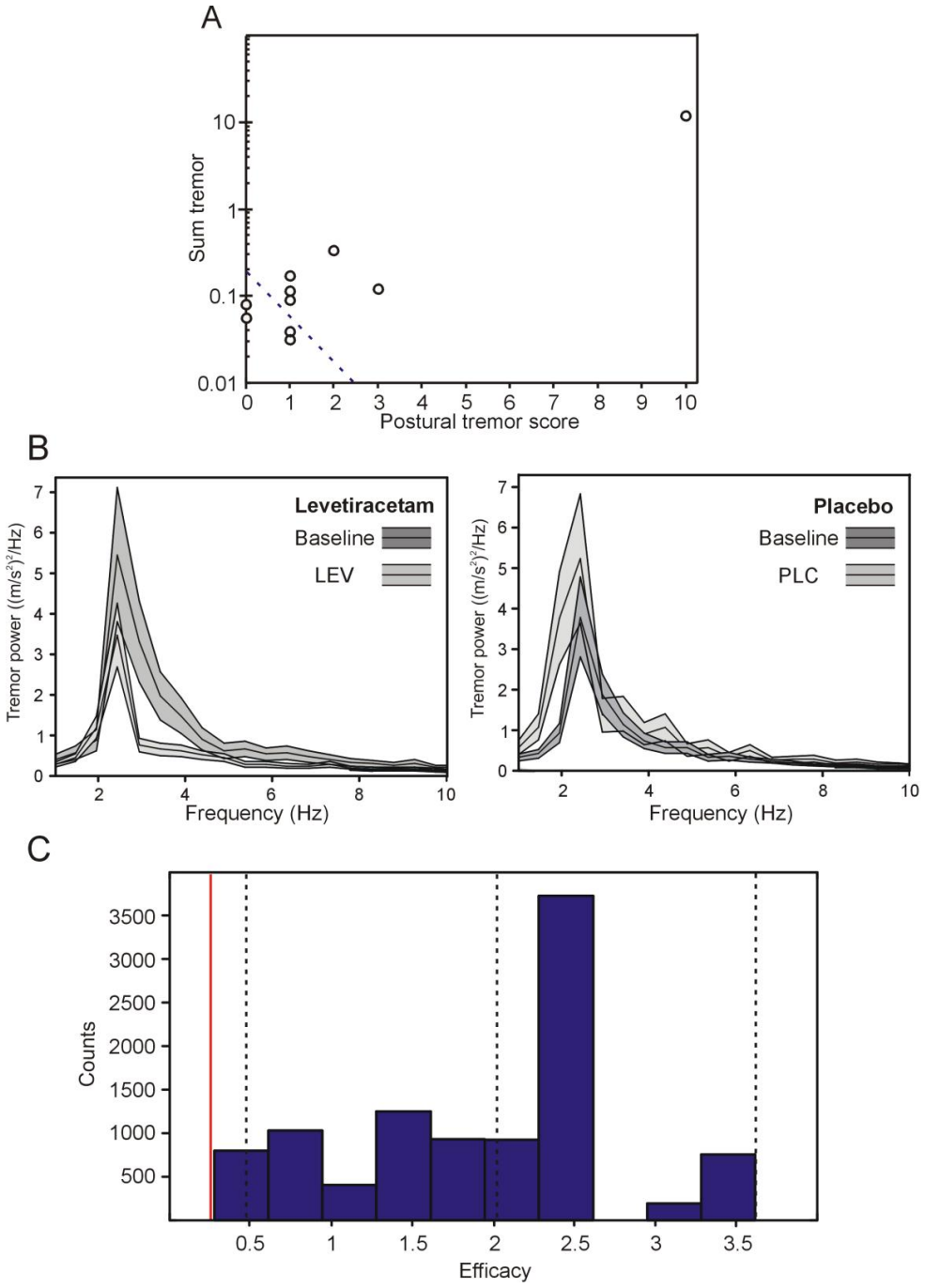


Figure 4-3: A, Postural tremor scores for all 10 patients plotted against the sum tremor power over the selected frequency range. Dotted line indicates the cut-off point below which patients were not deemed to have significant postural tremor. B, Postural tremor power spectra from patient 5. C: Efficacy histogram from Monte Carlo analysis. Study population efficacy is denoted by the red line and dotted lines represent the median and upper and lower quartile values.

measurable improvement in tremor following treatment with LEV, however this did not reach statistical significance (Z=-1.53). Taken individually, none of the six patients with postural tremor showed a significantly greater reduction in tremor with LEV than placebo (Z< -1.96). Population analysis focussed on the 6/10 patients with postural tremor. On average there was a significant reduction in postural tremor in these patients following LEV (Monte Carlo test, P=0.0251; Fig. 4-3 C).

Example reaching movements re-constructed from Polhemus motion data (wrist sensor) are shown in Fig. 4-4B along with simultaneously recorded raw EMG and accelerometry data (Fig. 4-4 A). It is easy to see the complexity of these aberrant movements which comprise not only a rhythmic tremor, but also a profound ataxia. The high amplitude oscillations close to the targets result in a movement path which is significantly larger than the actual distance between targets. Consequently our analysis probed the length of the path travelled during each trial; depending on filter properties, this correlates to either a measure of tremor or dysmetria alone. Across the patient cohort, this method revealed no significant change in either dysmetria or cerebellar tremor (Fig 4-4 C); this was confirmed by a Monte Carlo analysis in each case (Fig 4-4D; Dysmetria: P=0.524; Tremor: P=0.580).

Figure 4-5 shows the change in tremor scores as self-reported by patients (Fig. 4-5 A, B) and an independent clinician (Fig. 4-5 C, D). Neither shows any significant trend though it is perhaps worth noting that patients generally scored tremor as being more severe than an experienced clinician. In addition, the change in time taken to complete the 9-hole peg test (Fig. 4-5 E: large pegboard; Fig. 4-5 F: small pegboard) did not show any significant change over the course of the study.

Discussion

LEV is a good candidate for anti-tremor medication as it has a good safety profile, few side effects and does not interact with other commonly prescribed drugs. In general, LEV was well tolerated during our study, though where adverse effects were reported, they tended to involve exacerbation of MS symptoms.

Postural tremor did not appear to be a significant problem in our patient cohort. Only 6

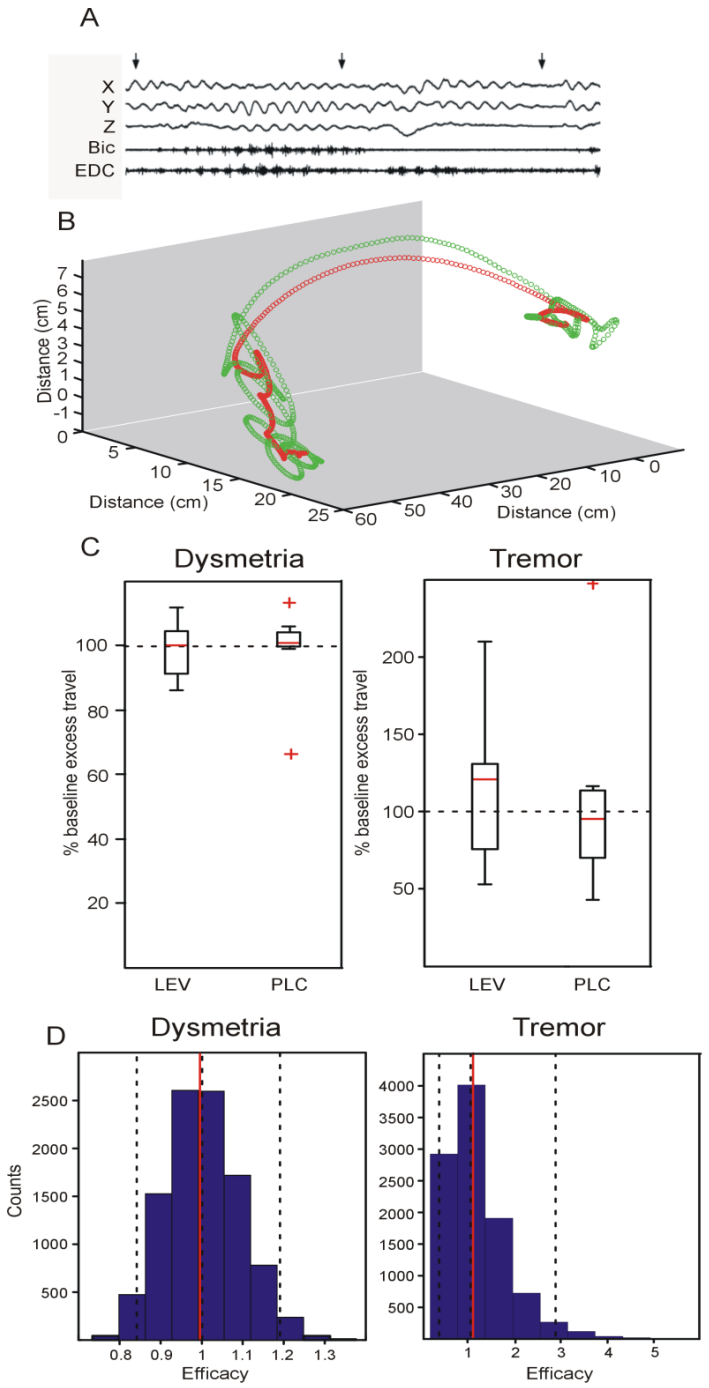


Figure 4-4: A, Raw EMG and accelerometer data. Arrows denote LED illumination which cues movement; this occurred every 3 seconds. B, 3-D plot of one reaching movement; each colour denotes a different reaching direction. C, Boxplots of population data for treatment efficacy on tremor and dysmetria. D, Histograms of efficacy probability from Monte Carlo analysis carried out on both filtered datasets. Study population efficacy is denoted by the red line and dotted lines represent the median and upper and lower quartile values.

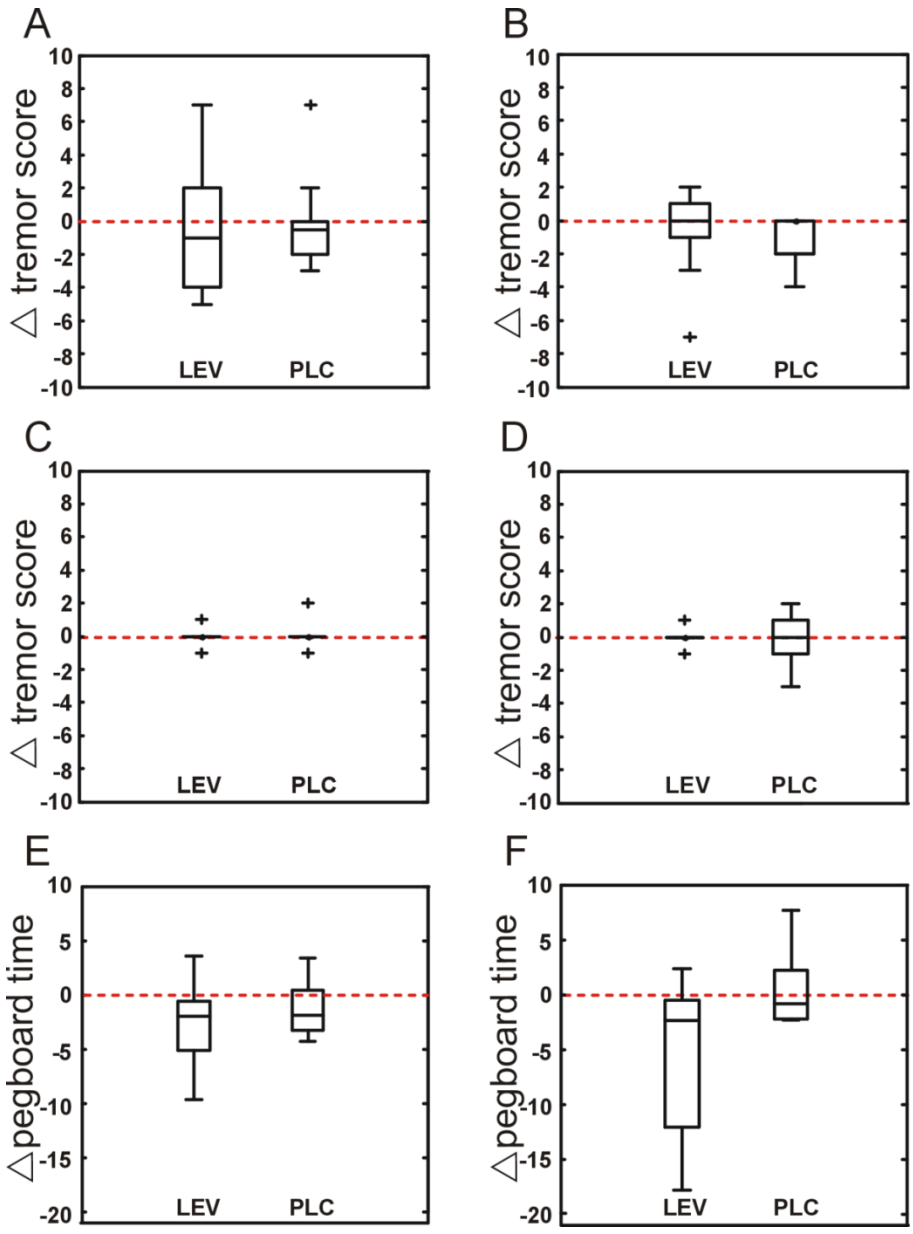


Figure 4-5: Change in patient self-reported scores for postural tremor (A) and intention tremor (B) of the limb under observation, averaged across all 10 patients. Tremor assessment taken from Bain et al., 1993; 0: no tremor, 1-3: mild, 4-6: moderate, 7-9: severe, 10: extremely severe. Clinician reported scores for postural (C) and intention (D) tremor. Pegboard completion times are shown in E (large pegboard) and F (small pegboard). Error bars represent standard error of the mean.

patients with postural tremor were identified and five of these were considered to be very mild. In this small group, Z-scores revealed no significant effect within individual patients however the effect of LEV on postural tremor was significant with the increased statistical power of averaging across the population. The clinical relevance of this is unclear however since there was no corresponding improvement in the tremor scores reported by either patients or the independent clinician.

In contrast, this study was not able to demonstrate any significant effect on MS cerebellar intention tremor or dysmetria when averaged across the population. This is echoed in the subjective measures, judged by both patients and a clinical assessment, suggesting that this drug has, on average, no clinically significant effect on intention tremor or dysmetria across the whole patient cohort.

One advantage of the use of quantitative measures such as accelerometry is that significant changes can be detected in tremor amplitude in a single patient, since power spectral estimates can be computed with their own confidence limits (e.g. Fig. 4-3B). In this study however, we did not find any significant benefit from LEV treatment within any individual which was surprising given positive findings from other reports in the literature (Mandler, 2003; Striano et al., 2006).

It may be that the effect of LEV is only very weak or perhaps only a subset of patients with MS tremor are likely to benefit from LEV. By its very nature, multiple sclerosis is a highly variable disease; in many cases, the complicated underlying pathology may confound any potentially beneficial effects of the drug.

Previous studies have universally focussed on the population effect of LEV on MS tremor. Results have been conflicting; Striano et al (2006) found a positive effect in contrast to Solaro et al (2008) and Feys et al (2009). There are a number of potential reasons for these differences. Firstly, a wide variety of LEV doses were administered (between 1000-4000mg/day). Low doses of the drug might explain the negative results found in two of the reports. In addition, there were differences in study design; a placebo control was not employed by Striano et al (2006) therefore a placebo effect cannot be ruled out in their case. Furthermore, the intrinsic variability of MS means that we cannot ever have a

uniform population of patients; as such it is difficult to compare results between studies.

In addition to the variability between studies, there are also many within-trial problems. During this experiment, there was a high dropout rate, in part due to adverse effects known to have an increased incidence in patients with MS (Solaro et al., 2005). There was also an issue of compliance; some patients failed to take the medication correctly or at all. It was also noted that factors other than tremor could affect patient performance, such as fatigue and depression. In my experience, patient self-reported scores were an unreliable measure of disability. Disease burden and in particular cognitive impairment often precludes the ability to attribute realistic, comparable scores on self-assessment. Anecdotally, scores were found to be sometimes a reflection of mood; often reflecting the severity of the MS in general, not specifically the tremor. Pegboard times could be similarly affected; in some cases, patients experiencing low mood were found to be perceptibly less motivated.

Where subjective measures have obvious limitations, quantitative measures of movement should allow us to gauge precisely any changes in tremor over the course of a trial. However, during the course of this study some potential confounds were identified which could affect the sensitivity of this technique. One of the major problems is the natural variation of MS tremor. This fluctuated on a daily basis and could be affected by factors such as temperature, emotional states, fatigue and stress. Although acceleromtery is the conventional technique used for tremor measurement, these piezoelectric units are extremely sensitive and probably therefore inappropriate for this type of fluctuating tremor unless incorporated into a wearable device which could be used to monitor tremor over much longer periods of time.

Similarly, the laboratory environment can initially be intimidating to many patients; the anxiety associated with this could easily result in transiently increased tremor on the baseline visit. It is difficult to control for this variation during an extended study and therefore important to consider that it may cause a false positive result. The recent introduction of an intravenous preparation of LEV might help reduce the variability in this type of study. However, any positive effect of LEV might be due to a cumulative effect which would only be visible in an extended trial.

Despite the difficulties experienced during this trial, 3/10 patients reported a subjective improvement in their tremor and requested to continue taking LEV once the trial was completed. The results presented here suggest that LEV may have a weak beneficial effect for the alleviation of MS tremor however there are many caveats to be taken into consideration. Further extended studies with tremor quantification are necessary to elucidate the full therapeutic profile of LEV in relation to MS tremor.

CHAPTER V: TRANSCRANIAL MAGNETIC STIMULATION ACTIVATES NEURONS IN THE RETICULAR FORMATION AT SURPRISINGLY LONG LATENCIES.

Transcranial magnetic stimulation (TMS) is a powerful tool for the non-invasive investigation of brain function. This technique has been popular in the neuroscience community since it's advent in 1985, superceding transcranial electrical stimulation (TES) as a painless method of stimulating brain tissue. It was originally designed with the motor system in mind, but has since been applied to countless cortical areas for a variety of purposes.

Over recent years, *in vivo* studies in macaques (Edgley et al., 1990; Edgley et al., 1997) and humans (Di Lazzaro et al., 1998; Houlden et al., 1999) have given us insight into the physiological mechanisms underlying the responses we see to TMS. Stimulation over the motor cortex gives rise to a series of repetitive discharges which can be observed from single cells within the corticospinal tract; these are considered either direct (D) or indirect (I) in nature. D-waves are characterized by their large amplitude and short, fixed latency. They arise from direct stimulation of corticospinal axons close to the cell body where action potentials are initiated. The same neurons are also excited trans-synaptically, generating much later I-waves. These tend to be smaller in amplitude and are typically much more variable temporally.

In addition to the responses observed centrally, stimulation over the motor cortex also gives rise to peripheral motor evoked potentials (MEPs) which can be measured in the muscles. A rectified MEP is characterized by a bifid peak occurring at a fixed latency relative to the distance from the CNS.

TMS is used extensively in basic science research, but it also has a role to play as an aid to clinical diagnosis and is implicated as a therapy in a range of neurological conditions (Chen et al., 2008). Twenty five years after its introduction, TMS equipment is now a common

finding in neuroscience laboratories worldwide. As a consequence, there are now multiple companies competing in the market and pushing the limits of the technique.

Despite the widespread use of TMS, its underlying effects on the brain are poorly understood. Most of our knowledge results from modelling studies and indirect measures in human subjects. Very few studies have directly examined neuronal responses to TMS and so the fundamental mechanism of how TMS works at a cellular level remains to be elucidated.

The mechanism of TMS is based on Faraday's principle of electromagnetic induction whereby a rapidly changing magnetic field induces an electrical current in a conductor. In this case, the brain acts as the conductor and current flow in cortical cells can cause local depolarisation. Optimal activation occurs when neurones are oriented parallel to the direction of the current. This makes pyramidal neurones originating in the primary motor cortex particularly susceptible to activation by TMS.

The induced magnetic field falls off rapidly with distance from the coil (Pascual-Leone et al., 2002); this has traditionally restricted the technique to investigation of very superficial cortical structures. Conventional TMS coils are only able to induce an electric field up to an estimated 1.5cm beneath the skull surface (Bohning et al., 1997; Roth et al., 2007). However, deeper structures can be activated through a transynaptic pathway.

Neuronal recordings during TMS

Effects occurring local to the site of stimulation have historically been very difficult to record for technical reasons. In fact, there are very few reports at all which show either single-unit or LFP recordings from neurones anywhere in the brain during TMS. One series of studies has looked at the effects of TMS on single unit activity and LFPs in visual cortex in anaesthetised cats (Aydin-Abidin et al., 2006; Moliadze et al., 2003; Moliadze et al., 2005). However, they were unable to look at the first 10ms following each stimulus because of the blanking effect of the artefact. Another group recently performed TMS in patients undergoing surgery to implant DBS electrodes for the treatment of severe Parkinson's disease (Strafella et al., 2004). Again, this was not ideal because the tissue undergoing investigation could not be considered healthy.

A recent paper by Tischler and colleagues (2011) unveiled a new mini TMS coil (outside diameter: 26.5mm). The authors demonstrated in monkeys that this coil could provide focal stimulation with the same characteristics as the conventional 'human' TMS coils whilst also permitting simultaneous recording of underlying neural activity. This is an exciting development in the field which will open up the opportunity for directly investigating the underlying mechanism of TMS at a cellular level in healthy brains.

TMS and the motor system

TMS is frequently used to investigate the motor system both in healthy subjects and for diagnostic and therapeutic purposes in patients. The superficial location of the motor cortex on the anterior bank of the central sulcus lends itself to the technique. In general, the application is to investigate the function of the corticospinal tract (CST). TMS over the primary motor cortex (M1) elicits multiple volleys in the descending pyramidal tract (Di Lazzaro et al., 1998; Edgley et al., 1990; Edgley et al., 1997). Direct activation of corticospinal axons close to the cell bodies and indirect activation on the same neurons give rise to D and I-waves respectively. The D-wave is very precisely timed since the cell is excited by current directly, but I-waves are temporally dispersed and have a variable onset latency which depends largely on the location of activation.

In the periphery the summation of these responses can be recorded as biphasic muscle potentials called motor evoked potentials (MEPs). These are usually recorded in the contralateral musculature since the corticospinal tract is predominantly a crossed pathway. Although MEPs are an indirect measure of CST function, they are largely consistent amongst individuals; this has helped establish their use for a variety of experimental and diagnostic purposes.

The last decade has seen an explosion of interest in the field of repetitive TMS (rTMS). This involves rapid, repetitive discharge of the coil in place of the conventional single pulse stimulation. rTMS is proposed to either increase or decrease the excitability of cortical areas depending on the frequency of stimulation. Particular focus has been on using rTMS as a therapeutic intervention for patients with neurological disease. In general, results have been mixed but beneficial effects have been reported in stroke (Khedr et al.,

2005), tinnitus (Rossi et al., 2007), depression (Berman et al., 2000) and Parkinson's disease (Hamada et al., 2008).

Ipsilateral motor evoked potentials (iMEPs)

Ipsilateral EMG responses (iMEPs) to M1 stimulation have also been reported. These are easiest to observe in proximal and trunk muscles but some reports also show the presence of distal iMEPs (Wassermann et al., 1994; Ziemann et al., 1999). For most people, iMEPs tend to be small and highly variable; they also have a higher threshold than those for contralateral MEPs. Amplitude depends roughly linearly on size of contraction; indeed iMEPs are rarely seen at rest. Responses occur at a long latency; this can be 5-13 ms longer than for contralateral MEPs for distal muscles (Ziemann et al., 1999).

The presence of iMEPs is not entirely surprising considering that anatomical studies have demonstrated that approximately 10% of CST neurons are uncrossed (Armand and Kuypers, 1980). However, the long response latency means iMEPs are unlikely to be due to stimulation of the slow uncrossed corticospinal tract fibres. This is also too long for simple spread of current to the opposite hemisphere. The prevailing view is that iMEPs arise via a corticobulbar projection. This notion is supported by evidence suggesting that finger & wrist extensors and also elbow flexors receive greater input from the reticulospinal tract (Peterson et al., 1979); these particular muscle groups have better, more consistent iMEPs. In addition, head rotation towards the side of stimulation is known to facilitate iMEPs. This is proposed to occur via neck muscle afferents which project to reticulospinal neurons in the brainstem (Ziemann et al., 1999).

The proposed pathway underlying iMEPs is largely supported by patient data. Subjects who acquire brain damage such as stroke or MND commonly display iMEPs with a similar prolonged latency relative to contralateral MEPs (Benecke et al., 1991; Krampfl et al., 2004; Netz et al., 1997; Turton et al., 1996). Ipsilateral MEPs are also more common in children under the age of 10 years (Muller et al., 1997) which corresponds with maturation of the CST as assessed by TMS (Koh and Eyre, 1988).

Interestingly, there may also be a reticulospinal component to contralateral MEPs. A recent study has shown that two distinct responses to TMS can be observed in single unit

recordings from pectoralis muscles (Unpublished results; personal communication with Prof. J. Rothwell). An early response occurs contralateral to the stimulus at a latency of approximately 9ms. This is followed by a bilateral response around 10ms later, a delay which could correspond to corticoreticular activation of the reticulospinal tract.

The Reticulospinal tract

The reticulospinal tract (RST) is one of the major descending pathways through which the brain influences the spinal cord. It is present in all vertebrates yet it has largely been overlooked in primates in favour of the phylogenetically younger corticospinal tract (CST). Here, strong monosynaptic connections with spinal motoneurones control complicated, dextrous movements.

Diffuse and widespread connections in the RST led to the thinking that it was responsible for co-ordination of gross proximal movements and posture and was not capable of selective movement of individual limbs or muscles. Experiments in lamprey (Deliagina et al., 2000), and cats (Drew et al., 1986; Perreault et al., 1993; Stapley and Drew, 2009) have demonstrated a role for RST in locomotion, postural control and gait adjustments; all can be considered global processes involving action across many muscles.

More recently, Drew and Rossignol (Drew and Rossignol, 1990a; Drew and Rossignol, 1990b) demonstrated that a wide range of movements, often bilateral, could be elicited by PMRF microstimulation including reciprocal movements of the forelimbs. This suggested that the RST was capable of more selective movements and was not limited to global changes in muscle activity.

In primates RST function is poorly understood. However, the importance of the brainstem motor pathways was highlighted in detail in the seminal work of Lawrence and Kuypers. Following complete bilateral lesion to the CST at the level of the medullary pyramids, monkeys recovered most motor function quickly with the exception of fractionated finger movements (Lawrence and Kuypers, 1965). This demonstrated that there was substantial redundancy in the motor system but suggested that brainstem pathways may not have the specificity and selectivity of connections to allow very fine, distal movements.

Recent work has challenged the dogmatic theory that the RST is solely responsible for the co-ordination of global movements for posture and locomotion. Modulation of reticulospinal activity during reaching movements has been demonstrated in cats (Schepens and Drew, 2004; Schepens and Drew, 2006) and primates (Buford and Davidson, 2004; Davidson and Buford, 2006; Davidson et al., 2007) although the cells encoded movement direction poorly.

Riddle et al (2009) carried out intracellular recordings from motoneurones in macaque monkeys and found both monosynaptic and disynaptic connections from the medial longitudinal fasciculus (MLF) projecting to hand muscles. In their yield of cells, they observed just as many projections to distal muscles as to proximal. Moreover in a second paper, Riddle et al (2010) showed that there was convergence of CST and RST axons onto the same spinal interneurones. Monosynaptic RST projections to distal muscles were certainly weaker than those from the CST but nevertheless are present. This lends weight to the argument that the RST forms a parallel system of descending control alongside the CST.

A further study from our laboratory has demonstrated that there are functional changes in the RST following unilateral lesion of the pyramidal tract (Zaaimi et al., Submitted). Stimulation of the MLF during intracellular recording of cervical motoneurones revealed strengthening of both monosynaptic and disynaptic RST connections, specifically to neurons supplying the forearm flexors and intrinsic hand muscles. This finding demonstrates that the connections are in place so that plasticity in the parallel system is capable of restoring motor function.

Anatomy of the RST

The origin of the reticulospinal tract is the pontomedullary reticular formation (PMRF) which constitutes the medial brainstem region. Anatomical and electrophysiological evidence has shown that there are two distinct arms to the RST which originate in the PMRF. The lateral portion arises mostly from the gigantocellular reticular nucleus (Gi) (Peterson et al., 1975) and projects bilaterally in the ventrolateral funiculus. Although projections extend the length of the spinal cord, terminations are predominantly in cervical

regions within laminae V-IX.

The medial RST arises mainly in the caudal pontine reticular nucleus (PnC) or oral pontine reticular nucleus (PnO; and to a lesser extent from the dorsal region of Gi). This descends ipsilaterally in the medial longitudinal fasciculus (MLF) in the caudal brainstem, continuing through the ventromedial funiculus in the spinal cord. Termination of medial RST fibres occurs in laminae VI-IX at all levels of the spinal cord (Nyberg-Hansen, 1965).

Pontomedullary reticular formation (PMRF)

At first glance, the PMRF is a relatively diffuse and unorganised collection of neurons in the medial brainstem. On the basis of this, the RST was long thought to be incapable of control of individual muscles or limbs; instead the anatomy suggested it could only subserve global control of relatively gross movements. However anatomical and electrophysiological evidence across species has subsequently shown the PMRF to consist of a number of interconnected yet highly distinct nuclei (Newman, 1985; Paxinos and Huang, 2000; Sakai et al., 2009; Valverde, 1962).

A parasagittal cross-section through PMRF is shown in Fig. 5-1B; this illustrates the elaborately complex network of nuclei present within primate PMRF. Distinct boundaries between nuclei can be difficult to identify both on the basis of cytoarchitecture and electrophysiology and it is clear that the areas overlap to some extent. There is also a high level of interconnectivity between nuclei; reticular neurons are highly collateralised and project to neighbouring areas (Ito and McCarley, 1987; Matsuyama et al., 1993; McCarley et al., 1987; Shammah-Lagnado et al., 1987).

There is a wide distribution of cell types across PMRF but most distinct are the giant cells which are primarily localised within Gi. This is the largest nucleus in the medial brainstem region occupying two thirds of the space. Aside from the giant cells, there are also a range of smaller cell types, both multipolar and fusiform in shape which become increasingly common in rostral areas. Rostral to Gi are two other major nuclei: PnC and PnO. Small and medium sized cells dominate these regions although a sparse population of giant cells are also found. Reticulospinal cells have been shown to be widely distributed throughout Gi,

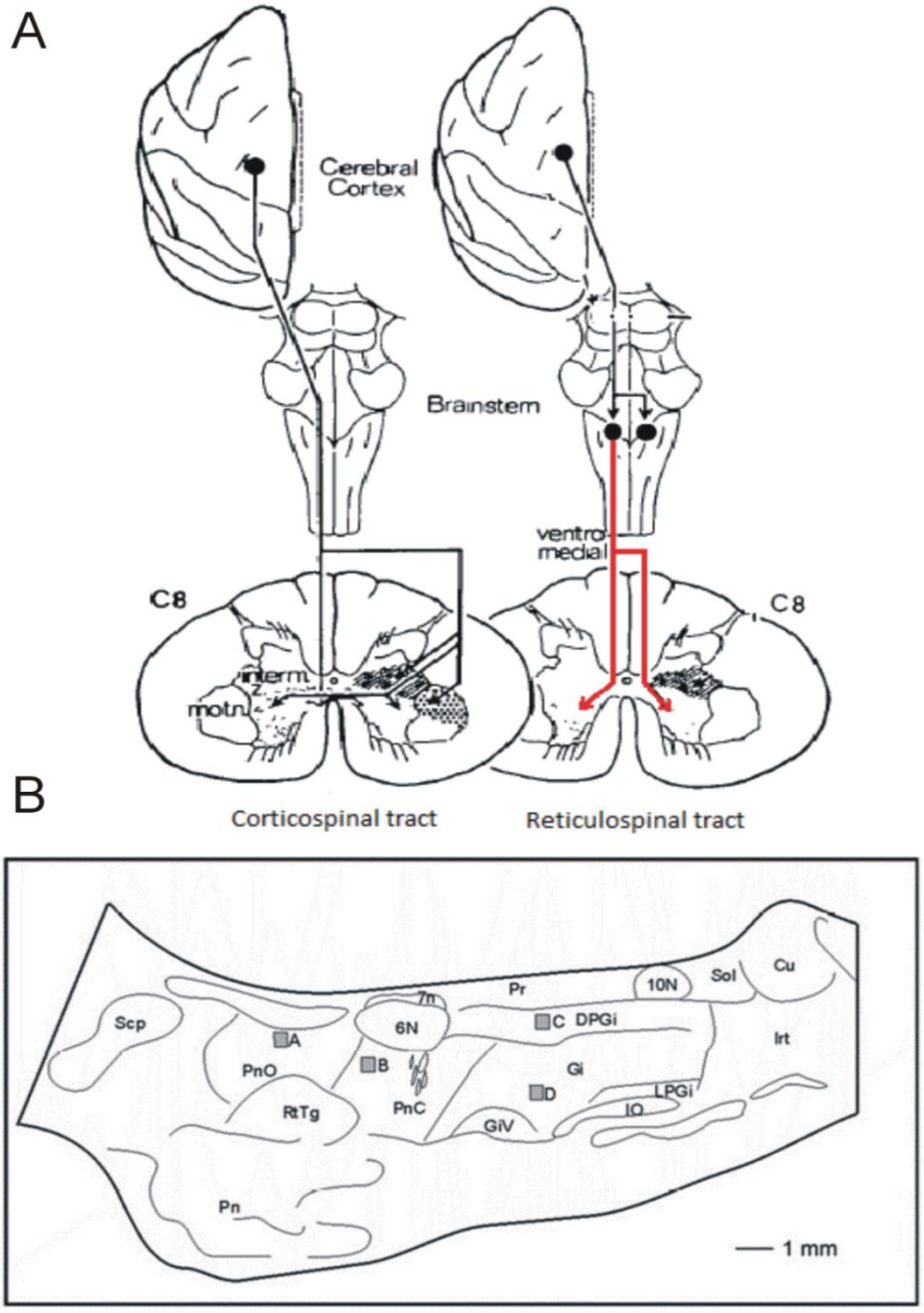


Figure 5-1: A: Schematic representation of the the corticospinal and reticulospinal descending motor pathways in primates. Note the direct, bilateral input from primary motor areas to the PMRF. Adapted from Brinkman and Kuypers (1973). B: Parasagittal section through PMRF in the macaque showing the major nuclei. From Sakai and Buford (2009).

PnC and PnO although there is a caudo-rostral hierarchy of frequency in primates; Sakai and Buford (2009) found there to be a ten-fold decrease in labelling of cells projecting to the RST from Gi to PnC.

The PMRF is a phylogenetically old part of the brain which is crucial for many lower species. As a consequence the reticular formation has a myriad of functions, only one of which is sensorimotor control. In fact, most useful information about the body is relayed via the PMRF. This coupled with the high interconnectivity both within the brainstem and with cortex and spinal cord means that the PMRF has a role in many basic and high level physiological functions including control of autonomic functions, consciousness and gating of sensory information.

Inputs to PMRF

PMRF receives an assortment of input from many cortical and subcortical areas as well as the spinal cord; in fact on the basis of its high interconnectivity, the PMRF is ideally placed simultaneously to filter ascending sensory information and review descending motor information.

Sensory inputs arrive from both central and peripheral sources. Ascending information carried in the spinal cord is relayed to PMRF via spinoreticular afferents (Rossi and Brodal, 1957) along with collaterals from other ascending sensory pathways. In addition, PMRF also receives input from central relays concerning all of our senses; considerable input comes from areas including the frontal eye fields (Stanton et al., 1988), superior colliculus (May, 2006), the cochlea (Irvine and Jackson, 1983; Lingenhohl and Friauf, 1992) and the vestibular system (Carleton and Carpenter, 1983; Matesz et al., 2002; Peterson and Abzug, 1975).

Corticoreticular fibres arising from pericruciate cortex relay descending motor programs to the brainstem. These fibres largely originate in areas 4 and 6, and traverse the internal capsule to reach the brainstem (Jinnai, 1984; Kably and Drew, 1998a; Matsuyama and Drew, 1997; Pilyavsky and Gokin, 1978; Rho et al., 1997). Corticoreticular neurons terminate bilaterally in PMRF (Berrevoets and Kuypers, 1975; Matsuyama et al., 2004;

Rossi and Brodal, 1956). Some are collaterals of CST neurons and have been proposed to modify posture during movement (Kably and Drew, 1998b).

In addition to the sensorimotor inputs to PMRF, afferent connections also include the cerebellum, inferior olive, frontal cortex and the limbic system.

Projections from PMRF

Ascending fibres from the PMRF project to multiple areas including the thalamus, hypothalamus, the preoptic area, basal ganglia and cortex. These projections usually arise from the most caudal regions of Gi, overlapping with the origin of reticulospinal neurones. The most recognised efferent projection comprises the reticular activating system which is critical for the maintenance and regulation of consciousness and attention.

Descending fibres from the PMRF form the reticulospinal tract. In broad terms, the RST projects bilaterally, sending axons out from the PMRF to motoneurones innervating both ipsilateral and contralateral musculature. RST axons are highly collateralised (Matsuyama et al., 1993; Matsuyama et al., 1999), much more so than those seen in the CST. Individual neurons therefore have the ability to activate motoneurones at multiple levels of the spinal cord. This widespread recruitment of motoneurones allows the RST a greater range of muscles over which it has influence at one time.

Both ascending and descending fibres are highly collateralised and there are widespread reticulo-reticular connections between neighbouring PMRF nuclei allowing for intra-reticular communication (Kably and Drew, 1998).

Aims/outline

In light of the recent surge of interest in the role of the reticulospinal tract in the control of hand movements in humans, it is pertinent to determine whether the reticular formation can be activated using a non-invasive method. Therefore during this series of experiments, the aim is to understand the effects of TMS over M1 on the PMRF and elucidate the pathways involved.

It is well known that TMS has transynaptic effects that are distant to the site of stimulation;

an MEP for example is a potential recorded in muscle which arises from activation of motor cortical cells. It is also known that primary motor cortex has very strong connections with the pontomedullary reticular formation and so it is reasonable to expect to see responses to TMS at a short latency because of the fast conduction speeds of these fibres.

Methods

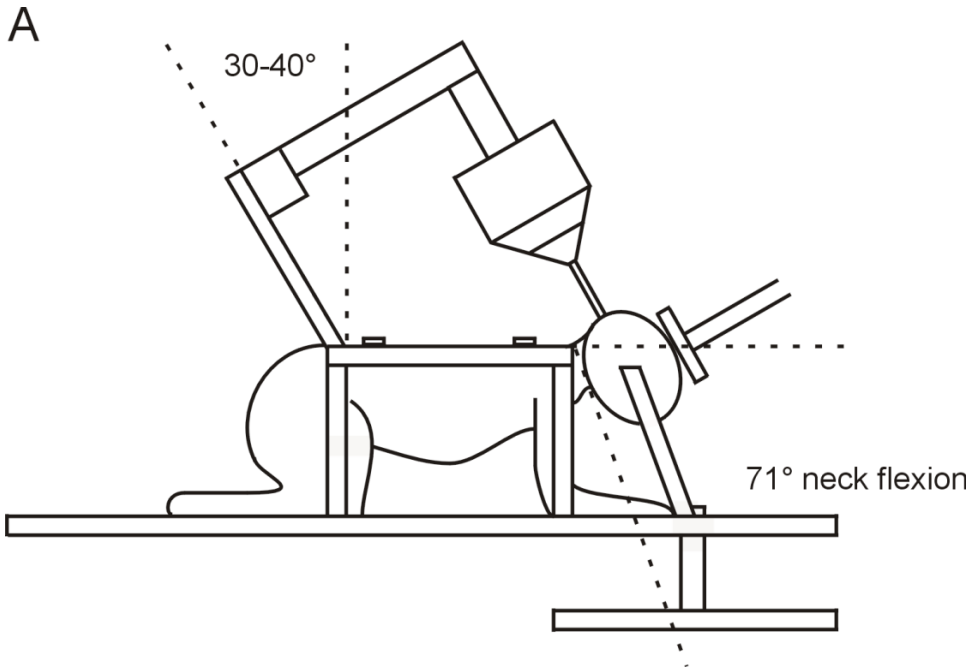
All animal procedures were carried out in accordance with UK Home Office regulations (Animals in Scientific Procedures Act, 1986) and approved by the local research ethics committee of Newcastle University.

Experiments were performed in 3 adult female terminally anaesthetised rhesus macaques: P (14years, 9.47kg), U (15 years, 10.52kg), V (15 years, 8.43kg).

Surgical preparation

Initial surgical procedures were performed under deep general anaesthesia using sevoflurane (3-5% in 100% O_2) and alfentanil (7-23µg/kg/hr). Methylprednisolone (5.4mg/kg/hr) was also infused throughout to reduce intracranial swelling. All animals underwent a laminectomy which exposed spinal segments C5-T1. A craniotomy window was also created extending 5-8mm bilaterally from midline over the occiput. The dura was removed and a pool was created around the recording site and filled with paraffin oil to prevent drying of the exposed tissue. A tracheotomy was performed to allow artificial ventilation. Body temperature was maintained with an underbody heating blanket and an intraoperative heating blanket. Urinary catheterisation allowed drainage of fluids.

Following surgery, the anaesthesia schedule was switched to a Propofol (5-14mg/ kg/ hr) and alfentanil (7-23µg/kg/hr) infusion. The vertebral column was clamped at the high thoracic and midlumbar levels and the head was fixed in a stereotaxic frame, angled to give a 71 degree flexion of the neck. To prevent the metal ear and eye bars interfering with the TMS, we substituted them with plastic rods anchored to the head with dental acrylic; this was done once all measurements had been made just prior to recording. Neuromuscular blockade was achieved with atracuronium (0.7mg/kg/hr). Bilateral pneumothorax minimized respiratory movements. Continuous monitoring of physiological measures was



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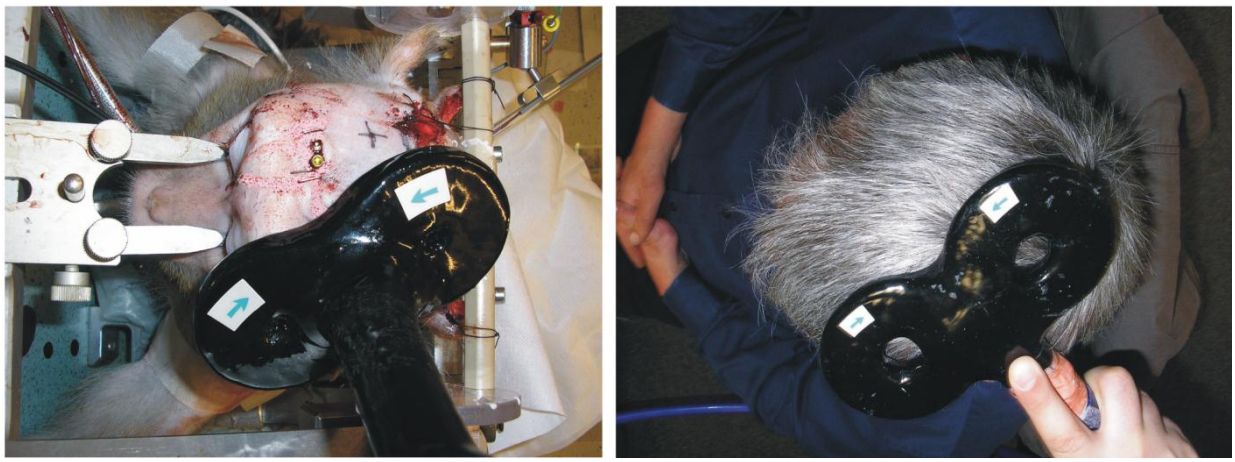

Figure 5-2: A: Schematic of the experimental set-up showing the position of the monkey in the spinal and stereotaxic frames as well as the drive penetration angle. B: Relative size of the TMS coils to monkey and human heads.

performed throughout the experiment to facilitate the maintenance of a deep, stable anaesthesia (arterial and venous blood pressure, oxygen saturation, heart rate, end-tidal CO_2 as well as core and peripheral temperature).

TMS target sites were marked on the scalp using stereotaxic measurements (before removal of ear bars). The main targets were left and right primary motor cortex (M1) which are located at A13, L/R 18.

Reticular formation recordings

Recordings were made using a 16 channel Eckhorn microdrive (Thomas Recording GmbH) (Eckhorn and Thomas, 1993) which was mounted on an arch angled between 30-40° (Fig. 5-2A). Guide tubes were organised in a 4x4 array with 500µm spacing. The total span of the array was therefore 1.5mm square. Electrodes within the array could be moved independently. Recording from neurons in the PMRF utilised tetrodes in 2 of the 3 monkeys in order to optimise the recording area and signal to noise ratio.

A double small 25mm TMS coil was mounted on a cross-bar of the stereotaxic frame using a Kopf manipulator for accurate positioning (see Fig. 5-2B: relative size of coil to monkey and human head). Due to the length of the experiment, the coil was prone to overheating and so was cooled using ice packs.

Stimulating electrodes were placed in the medial and lateral spinal cord between the C4/C5 vertebrae to target the reticulospinal tract. Stimulation was performed pair-wise between spinal electrodes and was triggered at specific intervals following a spontaneous spike in the PMRF. Cells were classified simply as either ‘reticulospinal’ (those that responded antidromically to spinal stimulation) or ‘unidentified’ (those that either were not spontaneously active or did not respond to spinal stimulation). In addition, there was also a silver ball electrode placed on the cord dorsum to record surface volleys of the spinal cord.

Spikes were continuously sampled at 25k via a National Instruments data acquisition card. Filters were 300Hz-10kHz. Gains were set at 10k for most recordings but were reduced where appropriate to avoid signal clipping. Stimulus markers were captured along with the data.

Protocol

A target site within PMRF was identified and the guide tubes were inserted a few millimetres into the tissue overlying this region. Electrodes were then individually driven into the brain. A 1Hz spinal cord stimulus was applied during this stage to check for antidromic field potentials which would indicate a region projecting to the reticulospinal tract. Once in the right area, the stimulus was changed to TMS in order to find cells which responded.

Following identification of a set of cells, TMS was delivered at 0.2Hz over primary motor cortex at a range of intensities. If the cells were stable, the coil was carefully moved to the opposite hemisphere since we know that reticular formation receives bilateral corticoreticular projections. The coil was positioned tangentially to the scalp with an orientation 45 degrees to the midsagittal axis. Current therefore flowed in an anteromedial direction in the brain; this is the conventional orientation used in human studies since it is optimal to elicit contralateral MEPs.

To investigate the origin of responses to TMS, a series of control experiments were performed. In all cases, an initial intensity series dataset of responses to TMS over M1 were collected. Then, the coil was gradually and precisely lifted off the head a millimetre at a time using the manipulator; responses to TMS were recorded throughout. When the response disappeared, a 6.35mm thick plastic disc was placed between the coil and the head to allow the click elicited when the stimulator discharged to be more effectively transmitted to the skull. Finally, a bone vibrator was used (B71, Radioear) over the M1 site as well as each mastoid process. The stimulus consisted of a 0.1ms square wave click with variable gain. Intensity was determined via a power amplifier (TPA 50, HH Electronics) which generated pulses of up to 50V.

Following successful penetrations, marker lesions were made by passing current at 100μA for 20 seconds (electrode negative) through relevant electrodes in the Eckhorn drive.

At the end of the experiment, anaesthesia was increased to a lethal level and the animals were perfused through the heart with a phosphate buffered saline solution followed by 4% paraformaldehyde. Relevant brain and spinal cord areas were removed and placed in a

series of ascending concentrations of sucrose (10% ,20%, 30%) solution for cryoprotection. The brainstem and spinal cord were subsequently sectioned at 50 µm using a freezing microtome. Sections were then mounted and stained with cresyl violet to enable reconstruction of penetrations.

Analysis

Spike discrimination was performed using custom written clustering software (GetSpike; Prof. SN Baker). Prior to this, the stimulus artefact was removed by fitting response sweeps to a double exponential function and subtracting this from the recording; this allowed visualisation of the first 2ms post-stimulus. Any mains noise on the recordings was also removed at this stage using an algorithm which removed phase-locked 50Hz. Only single units which had consistent waveforms and no interspike intervals <1ms were used.
Matlab programs provided the detailed analysis. Spikes were first aligned to stimulus onset and raster plots and peri stimulus time histograms (PSTH) were generated.

A population response probability plot was generated to allow us to determine the different responses to TMS. Time windows were identified for 3 different responses: 1-3ms, 3-7ms and 7-25ms. To confirm that the correct response groups had been selected, response jitter in each window was calculated. This was done by collating the time to first spike for all responding cells tested with 100% TMS intensity. Jitter was then calculated as the standard deviation of these values.

The cell population was classified according to whether significant responses were present in each time window. Significance was calculated by comparing the number of spikes occurring in the response window to an estimation of the count expected to occur in that window. This was done based on a Poisson process by calculating the mean number of spikes occurring at baseline to find the probability distribution of spikes occurring in each time window.

We chose to apply a Bonferroni correction to the statistical analysis to give a conservative measure of significance. This was performed to correct for 8 comparisons, since usually ~10 stimulus intensities were tested. Reduction of the significance level to $P<0.005$ was

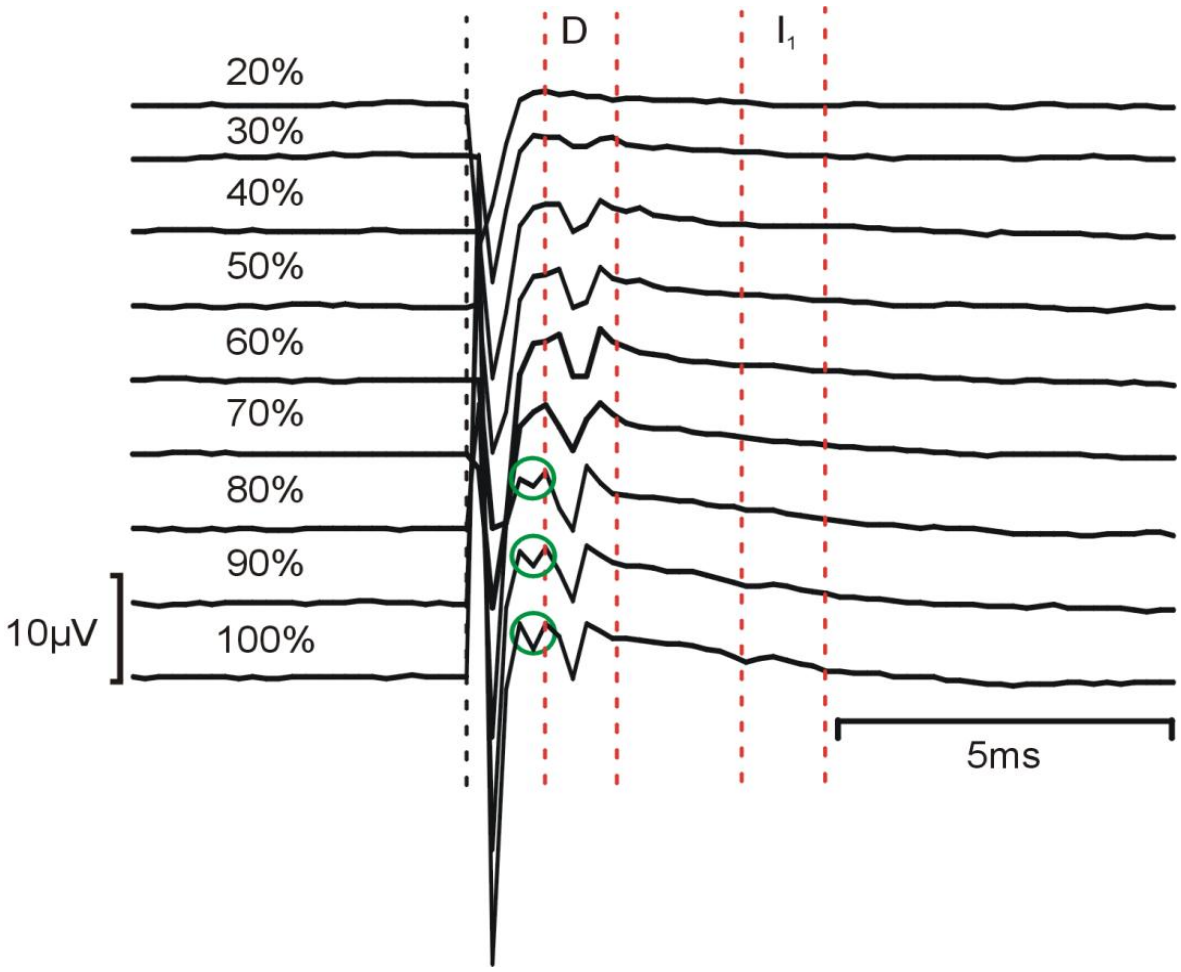

Figure 5-3: Epidural responses to ascending intensities of TMS are shown from monkey P. These were recorded using a ball electrode on the surface of the spinal cord between the C4 and C5 vertebrae. Stimulus onset is marked with a dashed black line whilst the direct and indirect responses are shown within the dashed red lines. Green circles denote probable white matter activation.

required to minimise the chance occurrence of false positives.

Unpaired t-tests were used for comparison of threshold values between the different response groups.

Results

Epidural spinal volleys were recorded through two ball electrodes placed between the C4/C5 vertebrae. An intensity series revealed the threshold for a spinal volley in each animal (Fig. 5-3; P=30%, U=35%, V=30%). D-waves were observed at ~1.5ms and small I_1 waves at ~4ms could be seen at high intensities. There was also an early deflection at the highest intensities which probably reflects white matter activation; this was reported by Edgely et al. (1990) following high intensity electrical stimulation.

Recordings were made from 210 cells in the macaque PMRF; all cells were within the gigantocellular reticular nucleus (Gi). An anatomical reconstruction is shown for the total cell population across all animals in figure 5-6. Previous studies have shown multiple subdivisions within Gi but we were not able to achieve this level of detail.

Example raster plots showing individual cell responses to TMS are shown in figure 5-4. Most of these comprise an increase in firing rate but there was also a group of cells that responded to TMS with suppression (example raster shown in Fig. 5-4D). Here, responses can be seen occurring very late (after 25ms); this may represent the rebound of activity following cessation of firing. Given the difficulty in accurately measuring suppression (Aertsen and Gerstein, 1985), this particular group have not been included in the analysis.

Powerful responses to TMS over M1 were observed in many cells within PMRF; these are summarised in figure 5-5. Responses fell into 3 main categories according to latency (Fig. 5-8); these were either early (1-3ms or 3-7ms onset) or late (7-25ms). Sometimes these responses were observed exclusively but often they could be seen in combination.

The number of cells showing a significant increase in firing ($P<0.005$) during these windows was 51 (24%), 58 (28%) and 84 (40%) respectively. Cells ipsilateral and contralateral to the stimulation site were initially pooled as there were no differences in response characteristics; moreover we know that PMRF receives bilateral input from motor

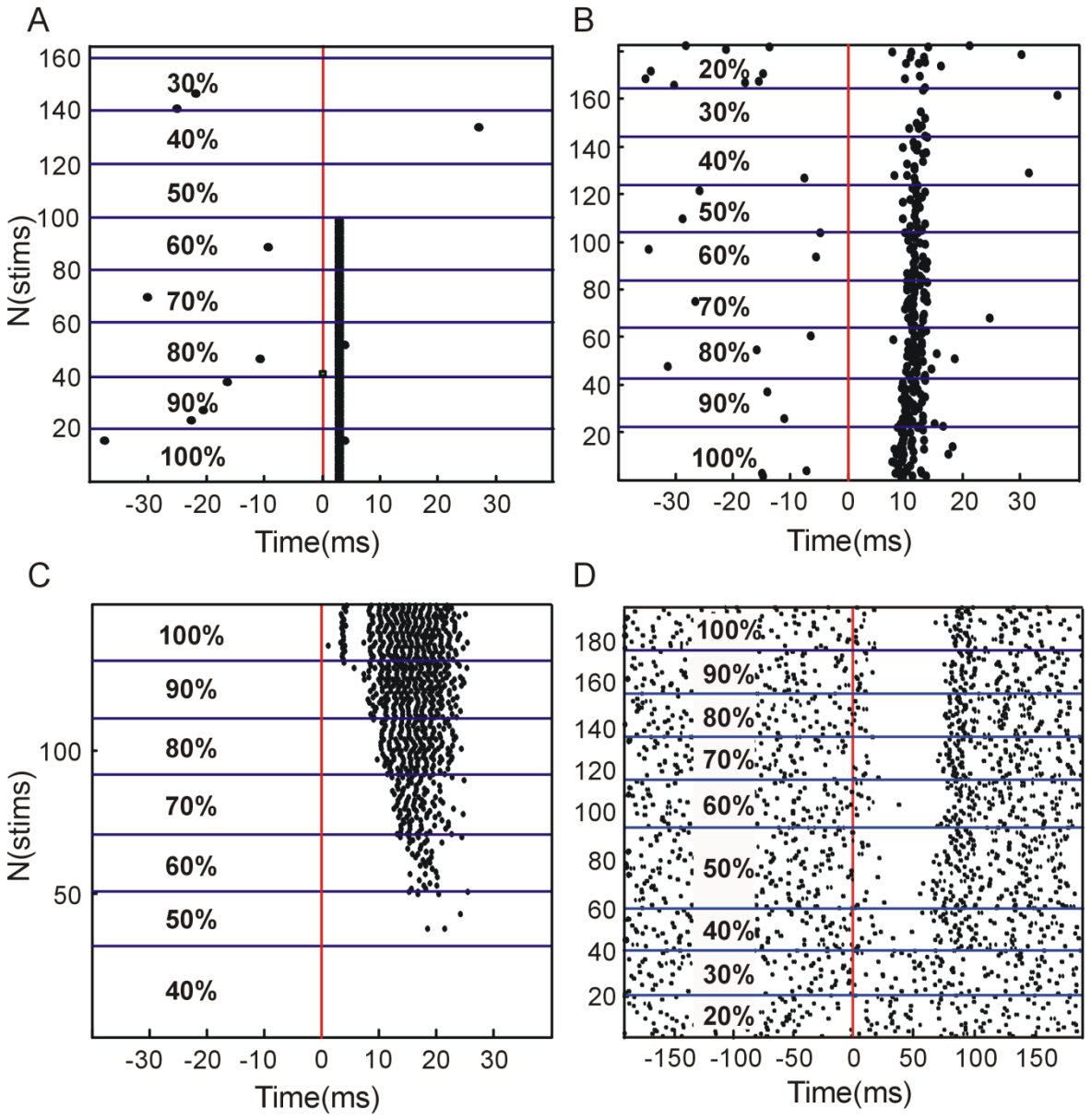

Figure 5-4: Example raster plots of cells responding early (A), late (B), both early and late (C) and a cell that shows suppression of firing following TMS (D). Red line denotes stimulus onset.

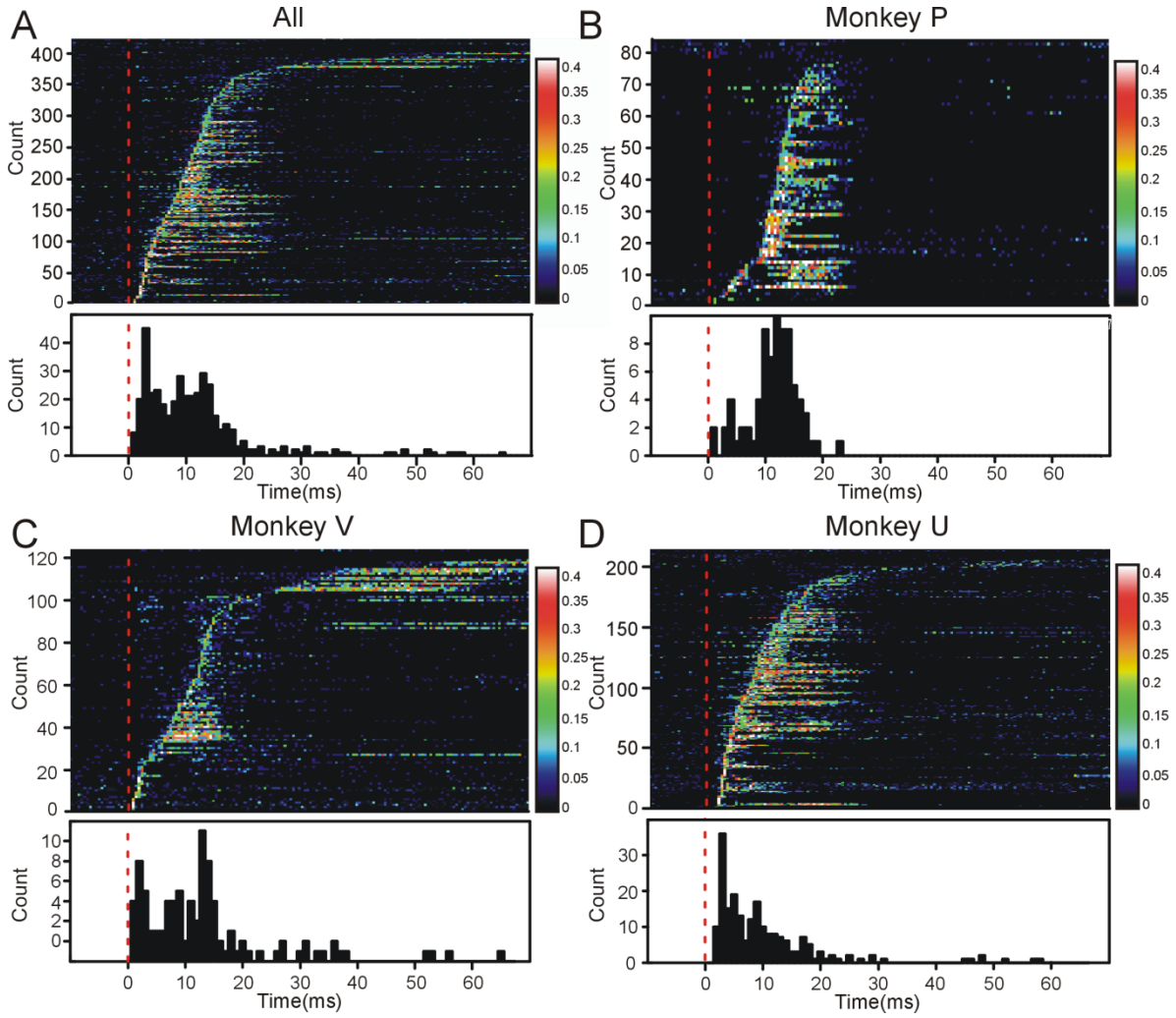

Figure 5-5: A: Plot of response probability for all cells across the population which respond to TMS. Each horizontal bar represents a stimulus intensity at which there is a response. The dotted red line denotes stimulus onset and the calibration bar indicates the probability per 1ms bin. The corresponding latency histogram is also shown. Individual response probability plots and latency histograms are also shown for each animal (B: monkey P, C: monkey V, D: monkey U).

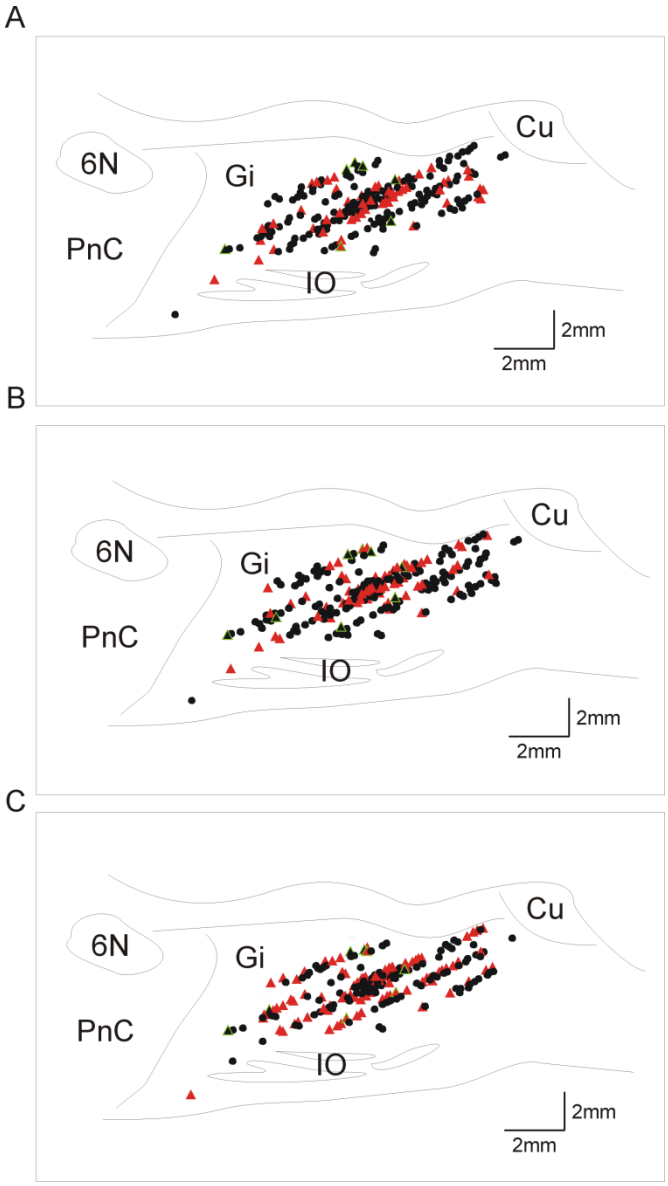

Figure 5-6: Parasagittal sections through the brainstem showing the recording sites and the cells responding between 1-3ms (A), 3-7ms (B) and 7-26ms (C). Red triangles are cells that showed a late response to TMS; those outlined in green indicate identified RST cells. Black circles indicate cells that did not show a late response to TMS at the intensities tested; those outlined in green are RST identified cells. Some cells were moved to enable all recording sites to be seen; movement was always less than 0.5mm. Gi=gigantocellular reticular nucleus, Cu=cuneate, IO=inferior olive, PnC=caudal pontine reticular nucleus, 6N=abducens nucleus.

cortex. Collision of spontaneous spikes with antidromic spikes elicited by spinal cord stimulation (pair-wise stimulation through electrodes in the medial and lateral spinal cord) revealed that 8 cells out of the total population could be identified as reticulospinal (example collision shown in figure 5-7). Of these, 2 cells showed an early response and 3 showed responses within the later window (one cell responded with both).

Response jitter versus mean onset latency is shown in figure 5-8. There is a very strong correlation between these two variables. Jitter increased markedly with later responses; there were 3 clear groups within the data which confirms the classification of responses.

Mean thresholds were 71%MSO for the earliest response, 68%MSO for the second response and 55%MSO for the late response (Fig. 5-9). In each case there was no significant difference between contralateral and ipsilateral TMS. The two earliest responses show no significant difference between their thresholds but the late response was significantly different from both ($P<10^{-4}$).

The mean number of late spikes evoked per stimulus is plotted for all responding cells versus TMS intensity in figure 5-10. For the majority of cells, the response to TMS was by nature all or nothing; the number of spikes did not change with increasing stimulus intensity. However, in some cases, more spikes were recruited with increasing stimulus intensity which leads to strong bursts of spikes at the highest levels.

Sound appears to be important in the generation of the late response to TMS since it often persisted when the coil was lifted off the head (Fig. 5-11B). Since the magnetic field is known to fall off very rapidly with distance (Pascual-Leone et al., 2002), such a strong response to TMS would not be expected at low output intensities. Moreover, there were sometimes late responses in PMRF at intensities below the threshold for a spinal volley (Fig 5-9).

Where the response disappeared as the coil was lifted from the head, a piece of plastic (thickness=6.35mm) was sometimes inserted underneath the coil. This renewed contact between the head and coil; as a result the response often returned with the same features (Fig. 5-12). In this instance, simple activation of cutaneous receptors could be sufficient to activate the PMRF which is known to receive substantial sensory input. However, the

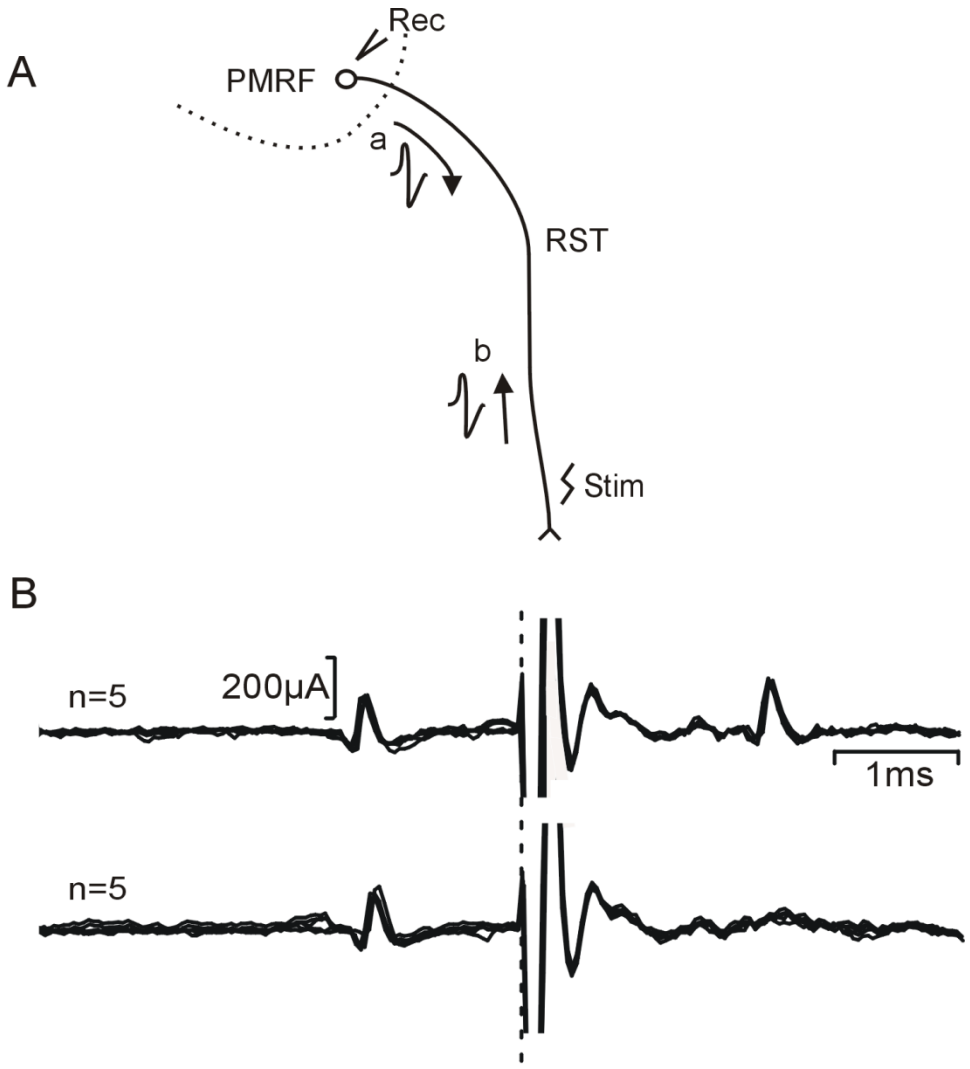

Figure 5-7: A: Schematic showing the principle of antidromic identification of a reticulospinal neuron. A spontaneous spike (a) generated within the PMRF triggers stimulation of the spinal cord (b) within a given interval. This antidromic spike travels up the axon and will collide out the spontaneous orthodromic spike when appropriately timed so that no response is observed at the recording site. Failure to collide would lead to the presence of a spike within the PMRF. B: Successful collision of a spontaneous spike from a neuron within PMRF with an antidromic volley from the spinal cord at an interval of 1.3ms (bottom) but not at 1.4ms (top).

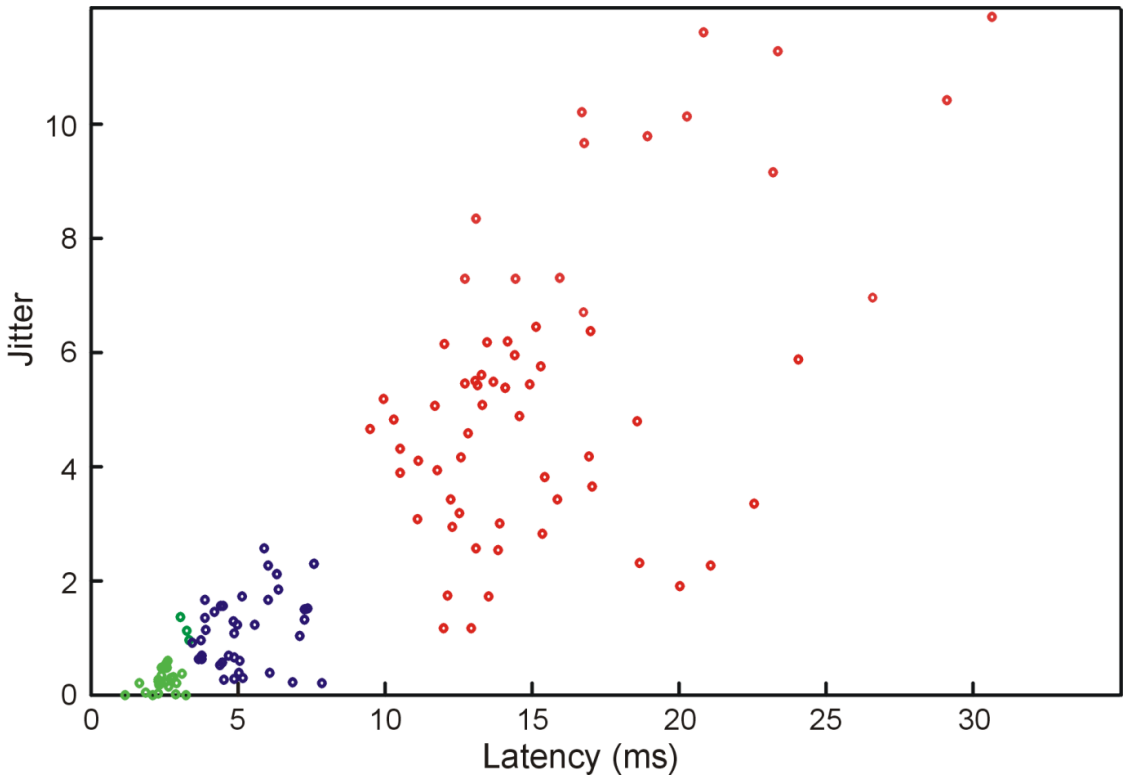

Figure 5-8: Scatter plot of response jitter against latency. Each circle describes the response observed in one cell. The response windows are separated according to colour: green=early (n=25); blue=second response (n=42); red=late response (n=64).

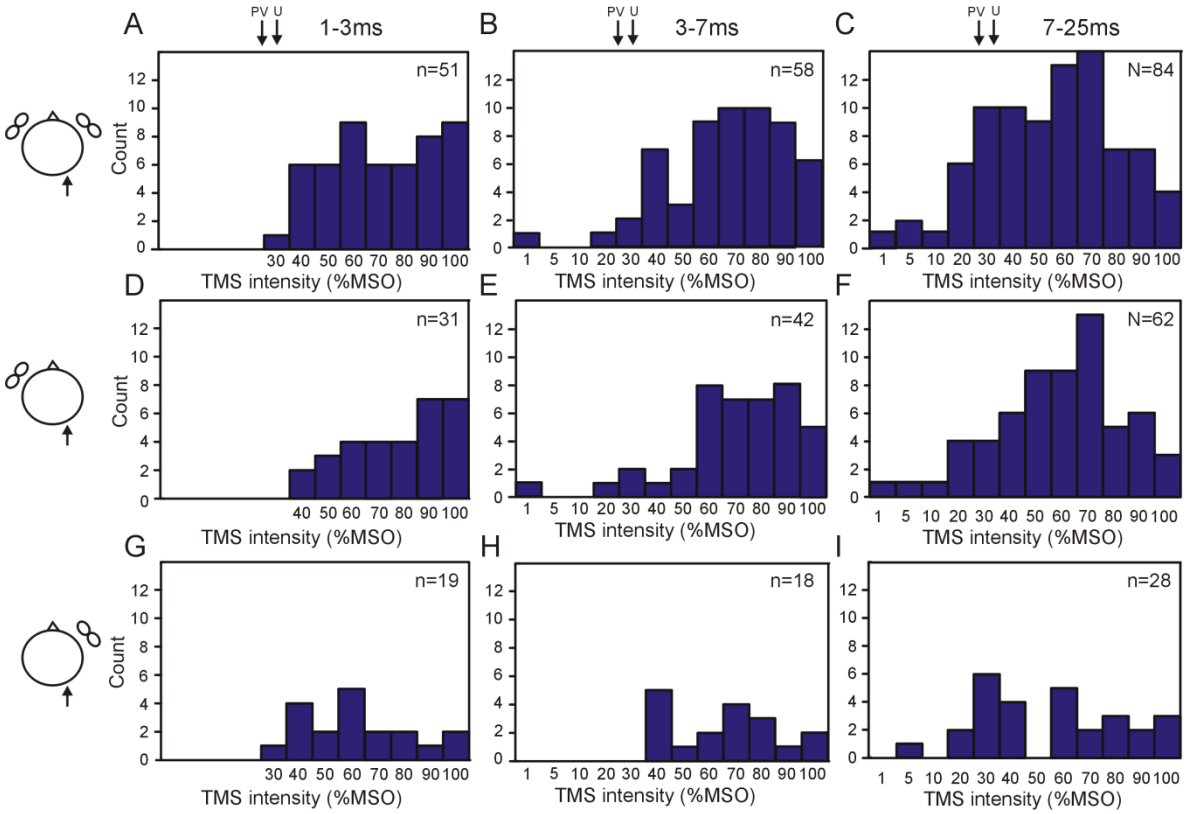

Figure 5-9: Histograms of response thresholds of cells with a response onset between 1-3ms (A), 3-7ms (B) and 7-25ms (C). Responses in each latency window are divided into cells contralateral (D, E, F) and ipsilateral (G, H, I) to the stimulus. In each plot, 'n' indicates the number of cells responding. Arrows indicate the threshold for a spinal volley in each of the 3 animals (P=30%, V=30%, U=35%).

responses observed did not appear to arise from activation of sensory pathways since electrical stimulation of the scalp in a small number of cells (n=3) did not have the same effect.

PMRF responses to TMS often persisted when the coil was not in contact with the head and in many cases could be enhanced when contact was renewed with a piece of plastic. This does not appear to simply be a result of sensory input and therefore it is likely that sound plays an important role. Similar responses to air and bone conducted sound have been shown in the vestibular system (Colebatch et al., 1994; Curthoys and Vulovic, 2010; McCue and Guinan, 1997) which is known to project strongly to the PMRF (Peterson and Abzug, 1975). A common non-invasive method to probe the integrity of this system in humans is to use a click stimulus. Brief pulses of sound delivered through headphones or via bone tappers are capable of activating the vestibular nerve and nucleus and consequently the vestibulospinal tract. This results in myogenic potentials which can be recorded in neck muscles.

Twelve cells responding late to TMS were therefore tested with a click stimulus as described by Colebatch et al (1994). Seven of these cells responded to the stimulus within the same latency range as they did to TMS. An example cell which responded to both TMS and bone vibration is shown in figure 5-13. The response latency and number of spikes elicited per stimulus were strikingly similar. Moreover, the cell was extremely sensitive to bone vibration, responding down to the minimum intensity tested. It is clear that both stimuli are capable of strongly activating cells within the PMRF. Moreover, the similar response latency suggests that there may be an overlap in the underlying pathway.

Discussion

The observation of strong responses to TMS in the PMRF is a novel one. It is clear that the early and late responses are mediated by different pathways. Not only is there a clear divide in the onset latency, response thresholds were also significantly different; thresholds were 71%, 68% and 55% for the three responses respectively ($P<0.005$; see Fig. 5-9). In addition, whereas early responses tended to be a tightly time-locked single spike, later responses were more variable. Often these consisted of bursts of spikes and sometimes the

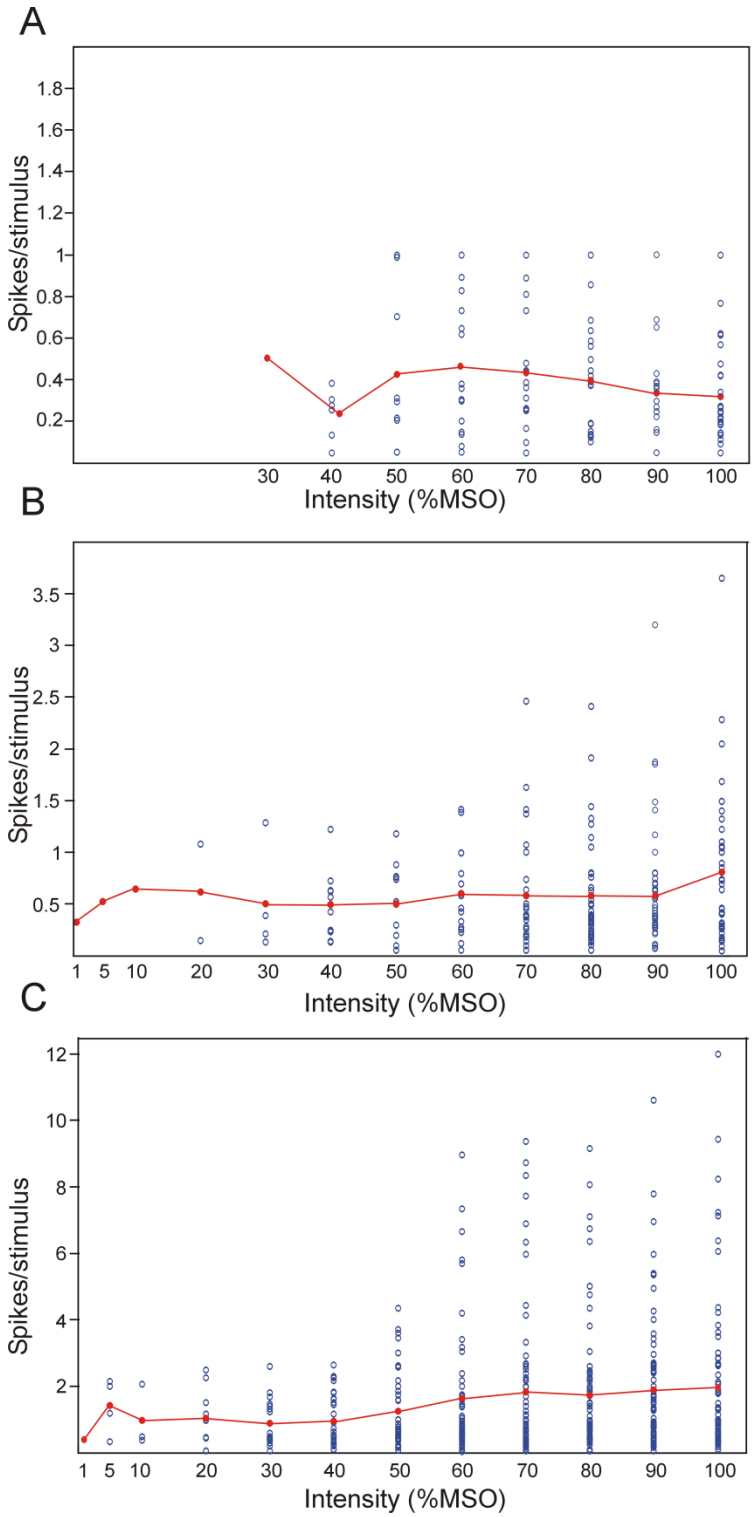

Figure 5-10: Scatter plots showing the number of spikes per stimulus for each cell that responds at a given intensity. Each plot represents a different response group: A: 1-3ms; B: 3-7ms; C: 7-25ms. Red lines show the mean values.

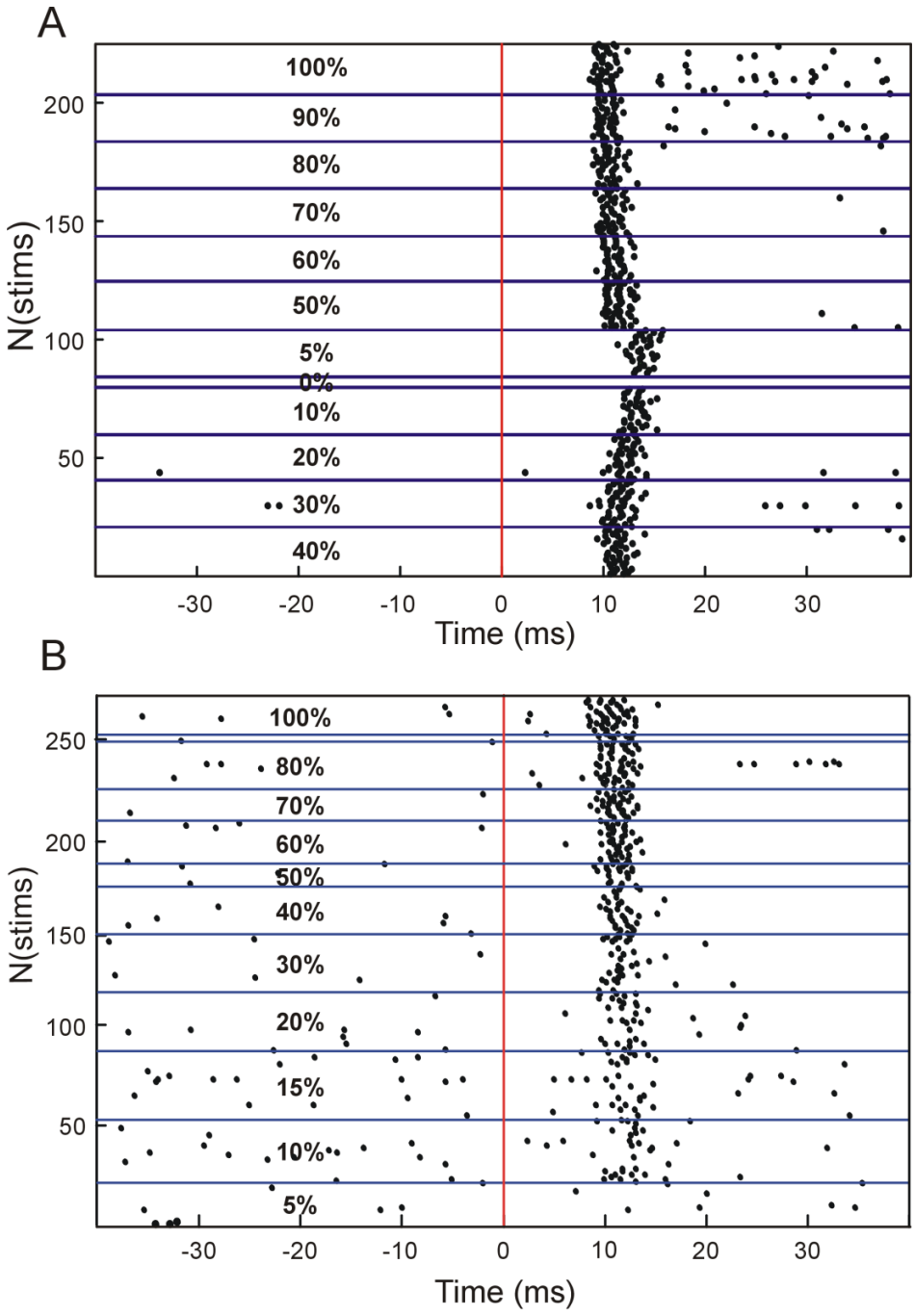

Figure 5-11: A: Raster plot showing responses from a cell in the right PMRF during TMS over contralateral motor cortex. Note that the responses persist even at the lowest intensities. B: Raster plot showing responses from a different cell (left RF; TMS to contralateral M1) during a sham stimulation protocol during which the coil was positioned 1cm above the head.

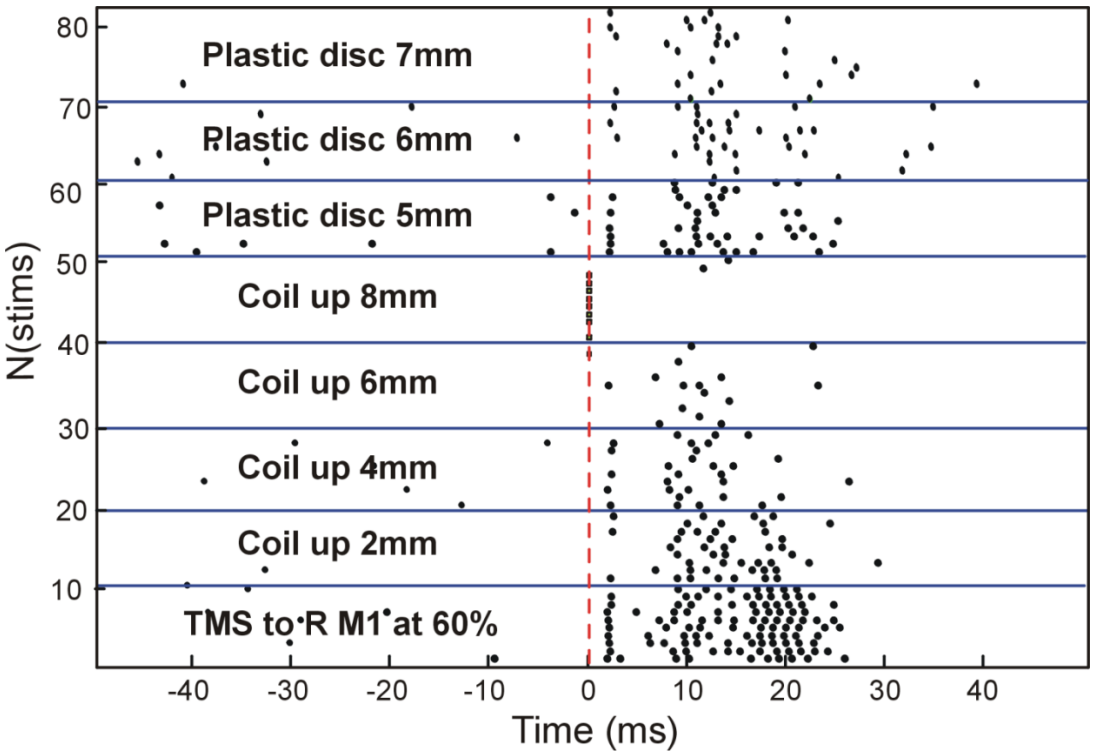

Figure 5-12: Raster plot showing spikes occurring in response to TMS over right motor cortex from a cell recorded within the contralateral PMRF. The coil was raised off the head by the designated amounts to test whether sound was important for the generation of the response. At the end, a plastic disc (6.35mm thickness) was placed between the coil and the head.

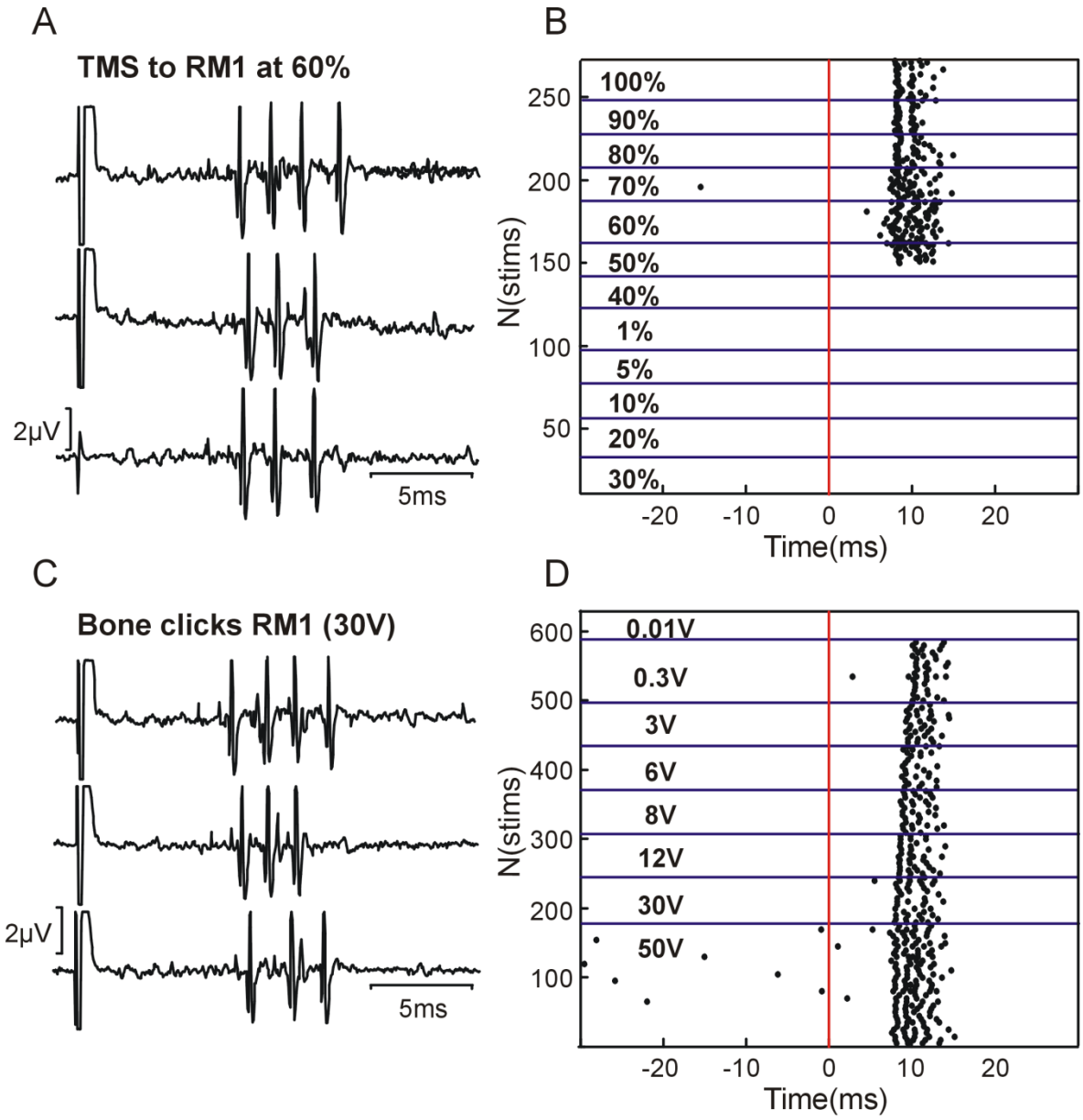

Figure 5-13: Data are shown from a single cell which was tested with both TMS and bone vibration. A: Spike responses to individual TMS stimuli over right motor cortex at 60%MSO. B: Raster plot showing the full intensity profile of responses to TMS. C: Spike responses from the same cell to bone vibration stimuli under the coil. D: Raster plot showing response profile to bone vibration stimuli over the intensity series.

onset latency changed with increasing intensity (see Fig. 5-4C). In line with this, jitter increased with response latency.

During this experiment, only a small number of reticulospinal cells were identified due to the lack of spontaneous firing under anaesthesia (and the intraspinal electrodes were not available for technical reasons during monkey V's experiment). However, the anatomical reconstructions suggest that recordings were from a broad area of the medial brainstem region, mostly in the gigantocellular reticular nucleus. Anatomical tracer and electrophysiological studies in cats and monkeys have shown that the majority of descending fibres to both the medial and lateral RSTs originate here (Holstege and Kuypers, 1982; Peterson et al., 1975; Sakai et al., 2009; Torvik and Brodal, 1957).

Early response 1: a corticoreticular projection

The earliest response observed within PMRF was always a single spike response which was very tightly time locked to the stimulus. There was almost no jitter (0.32ms) indicating a fast conducting, direct pathway. The most likely candidate for this pathway is the corticoreticular projection. This system is very well understood in the cat where there are two separate groups of pericruciate neurons which project to the medial brainstem and are classified by conduction velocity. The fastest fibres relay M1 activity to cat reticulospinal neurones between 1.2-2.7ms. For slower corticoreticular neurones, this drops to 2.9-6.8ms (Pilyavsky and Gokin, 1978). During the experiment reported here, the earliest response onset latencies were 1.2-3.24ms with a mean of 2.49ms for the population; this is therefore consistent with a fast corticoreticular pathway.

Corticoreticular fibres terminate widely throughout the PMRF (Kably and Drew, 1998a; Matsuyama and Drew, 1997). Although there is a weak ipsilateral preference, these projections are frequently bilateral (Kably and Drew, 1998a). Responses to TMS were found throughout the gigantocellular reticular nucleus both ipsilateral and contralateral to the site of stimulation. Although there was a contralateral bias, this was only because many more contralateral cells were tested.

Early response 2

The second response which occurred at short latency typically comprised 1-2 spikes and showed much more variability than the earliest response (Fig 5-8, 5-10). This suggests an indirect pathway which occurs via more than one synapse. There are a number of different possibilities for the pathway mediating this response.

The mean onset latency for the second response was 4.65ms, roughly 2ms after the early corticoreticular response. It is entirely possible therefore that this response could also be mediated by input from pericruciate fibres since the latencies fall within the range shown by slow corticoreticular neurones. Perhaps therefore this second response could just be a slower, second corticoreticular response. This certainly could be the case in cells which have very low jitter in terms of the response spike distribution. The observation of higher jitter for this response could also simply result from dispersion in the slow corticoreticular pathway.

Another obvious candidate would be local reticulo-reticular activation of PMRF cells. Electrophysiological studies have repeatedly demonstrated that nuclei within the PMRF are highly interconnected both within hemisphere and between hemispheres (Ito and McCarley, 1987; Matsuyama et al., 1993; McCarley et al., 1987; Shammah-Lagnado et al., 1987). This observation of high interconnectivity and heavily collateralised neurones has been ascribed to facilitate a role for PMRF in the control of co-ordinated movements. Monosynaptic postsynaptic potentials were found in almost all cells recorded in PMRF during one study of microstimulation within cat brainstem (Ito and McCarley, 1987; McCarley et al., 1987). Moreover, the response latencies were 0.6-2ms which is consistent with the second response occurring just a few milliseconds after the first. The addition of an extra synapse with a reticulo-reticular projection would account for the small variability in onset latency and jitter.

An alternative and attractive hypothesis would be collateral input relaying late CST activation to the brainstem. Epidural recordings in both animals and humans have demonstrated clearly that TMS elicits multiple descending volleys in the spinal cord (Di Lazzaro et al., 1998; Edgley et al., 1997). Indirect activation of CST neurons in particular causes a dispersed succession of late volleys or I-waves. In primates, the first I-wave

occurs approximately 2.5ms after the D-wave (Edgley et al., 1997). It is reasonable to believe therefore that collaterals of descending CST axons could relay this information to PMRF within the time window of the second response.

Pathway for long latency response to TMS

The origin of the late response is much more difficult to determine. The mean onset latency was 15ms although a very broad range were observed (minimum: 5.9ms; maximum: 30.7ms). PMRF receives projections from a broad range of cortical and subcortical areas and the timing is such that multiple synapses could be involved.

Given the strong sensory input to the reticular formation via cutaneous afferents, simple contact of the coil with the scalp could be sufficient to activate cells in the PMRF. The observation of strong responses which persisted when the coil was lifted above the head would not support this notion (Fig. 5-11, 5-12). Moreover, electrical stimulation of the scalp did not generate any responses in a subset of cells tested (n=3).

Many different pathways were considered on the basis of all the different inputs to PMRF. Firstly, the possibility of a relay via dentate nucleus was investigated since we know there is a strong projection from here to the PMRF (Bantli and Bloedel, 1975). However, substantial mapping of the deep cerebellar nuclei in one monkey revealed no responses to TMS in this region.

Secondly, the role of sound in the generation of the late response was investigated. This appears to be important and it would therefore be careless to discount the auditory system as a candidate pathway. Indeed this would seem to be the simplest and most obvious choice. Interestingly, PMRF responses to acoustic stimuli have previously been shown to occur at latencies between 10-30ms (Irvine and Jackson, 1983). Moreover, sound has also been shown to be very important in the PMRF which is a critical relay in the acoustic startle circuit (Davis et al., 1982a; Leitner et al., 1980). Damage to the caudal pontine reticular nucleus (PnC) has been shown to abolish the acoustic startle reflex (Davis et al., 1982a) and Kuhn et al., (2004) showed that contralateral biceps MEPs were inhibited when TMS was delivered 30-60ms after a startling stimulus. The inhibitory pathway is unclear but these findings demonstrate a strong influence of PMRF on motor output.

The sound generated by discharge of the TMS coil could influence PMRF cells in one of two main ways; it could either be transmitted to the brainstem via the cochlea or via the vestibular system. If the cochlea was implicated in the pathway, obstructing the ear canal should prevent or reduce the sound waves being transmitted to the inner ear. This was attempted by recording from a small number of responding cells whilst ear bars were inserted or the pinna were covered, however there was not sufficient data to make any inferences as to whether transmission of sound via the cochlea is of primary importance. It is worth noting however that the acoustic startle reflex mediated via the cochlea habituates very rapidly (Brown et al., 1991; Davis et al., 1982b) whereas we were able to record PMRF responses to TMS consistently for the duration of our recordings (up to 30 minutes).

The vestibular system is also capable of transmitting information to the brainstem about sound. Both air and bone conducted sounds are known to activate vestibular afferents (Curthoys and Vulovic, 2010; McCue and Guinan, 1997). Activation of the vestibulospinal system in this way produces a short latency myogenic response which can be measured in neck muscles – the vestibular evoked myogenic potential (VEMP). VEMPs can be observed even in the presence of complete sensorineural deafness confirming that the response pathway is completely independent of the cochlea (Colebatch et al., 1994). The stimulus conventionally used to elicit VEMPs is a short duration click which is highly reminiscent of that generated by discharge of the TMS coil. Given this striking similarity, the vestibular system is an interesting candidate in the pathway underlying PMRF responses to TMS.

VEMPs arise from activation of the otoliths of the inner ear (in particular the saccule) and subsequent activity in the vestibular nerve and nuclei. The PMRF is known to receive strong input from the vestibular apparatus (Carleton and Carpenter, 1984; Peterson and Abzug, 1975). Moreover, electrophysiological evidence in cats shows that the connection, although direct is slow and variable at between 0.6-3ms (Peterson and Abzug, 1975). Combined with the sound conduction time and a synapse in the vestibular nuclei, the latency would be appropriate for the late response.

A recent report suggests that the 0.1ms click might not be the optimal stimulus to elicit VEMPs; Rosengren et al., (2009) showed that a 500Hz 2ms sine wave was optimal in

healthy control subjects. Moreover, the best stimulation site was found to be mastoid; midline skull was shown to be the least effective (Stenfelt and Goode, 2005). A further consideration is that the presence of VEMPs has been shown to decrease with age (Brantberg et al., 2007; Su et al., 2004; Welgampola and Colebatch, 2001). The monkeys used for this particular experiment were all 14-15 years of age; average lifespans for macaques in the wild is 15 years and in captivity 15-20 years. This may account for the cells observed that did not respond to bone conducted clicks.

In this experiment, a number of pieces of evidence have been presented which support a role for the vestibular system in the generation of the late PMRF response to TMS. A clear limitation of the study is the small population of cells which were tested with the bone vibration stimulus. However, the finding of responses to bone vibration coupled with the observation of low threshold responses to TMS and air and plastic-conducted responses to TMS strongly support this claim.

Implications for future use of TMS

This experiment has revealed a strong indirect pathway projecting to the brainstem which can be accessed by TMS over primary motor cortex. This demonstrates that TMS can have potent effects downstream of the stimulation site. It has long been known that this could be the case but in the investigation of descending motor pathways, motor cortex stimulation is generally considered to specifically and exclusively activate the CST (for contralateral MEPs). However, given recent findings regarding the largely parallel reticulospinal system, it should no longer be assumed that this is the case.

Similar modulation of brainstem activity by TMS has been demonstrated by another group who reported disruption of saccades following appropriately timed stimuli (Xu-Wilson et al., 2011). Interestingly, this was shown to be the case regardless of stimulation site. If this effect is a result of the click stimulus, there are serious implications for future placebo controlled TMS studies. Placebo coils have become the accepted form of sham stimulation in the field. These coils elicit the same click sound as the regular TMS coils (along with some sensory stimulation) but without the magnetic field. Since the transmission of the click is important in the generation of late PMRF responses to TMS and is independent of

stimulation site, we may be forced to readdress the use of placebo coils in research. The observations here might also go some way to explain some of the beneficial 'placebo' effects shown in several clinical trials.

Implications for future use of click stimuli

The most exciting finding of this study is that a simple click stimulus could allow a non-invasive window to access the reticulospinal system. This area of the brain is very deep and normally inaccessible to direct stimulation by conventional electrophysiological techniques. There are already a number of other paradigms which can indirectly access the PMRF for example the acoustic startle (Davis et al., 1982a), startReact paradigm (Valldeoriola et al., 1998) and prepulse inhibition of the blink reflex (Valls-Sole et al., 1999). However, the observation of powerful, bilateral and widespread activation of PMRF to the click would suggest this could also be an interesting and straightforward way to study the reticulospinal tract in healthy subjects.

The most useful application for the click however would be in rehabilitation of patients following injury to the CST. Damage to this pathway occurs in many conditions but stroke is by far the most common; in the UK alone, this is the leading cause of disability in adults affecting ~150,000 people every year. For chronic patients, damage to the CST is critical to this disability. Animal studies demonstrate a plastic reorganisation of medial brainstem motor pathways following CST insult which contributes to functional recovery (Lawrence and Kuypers, 1965; Lawrence and Kuypers, 1968a; Zaaimi et al., Submitted). Rehabilitative interventions seek to expedite and enhance this process but are fundamentally indirect by nature.

Recent work from our laboratory (Zaaimi et al., Submitted) has shown electrophysiologically that there are functional changes in the RST following pyramidal lesions. There is a strengthening of RST projections to motoneurones, particularly to those controlling flexor and intrinsic hand muscles. This mirrors the pattern of recovery of stroke patients whereby overactive flexor muscles can often result in spasticity. An exciting prospect would be the pairing of appropriately timed click stimuli with muscle activation to induce plasticity. This could involve either facilitation of weak extensor muscles or

suppression of the counterproductive flexor activity.

Future directions

This experiment has revealed a strong late response to TMS in the brainstem reticular formation. Although a role for the vestibular system has been suggested in the generation of this response, this is speculation and the underlying pathway is far from determined. To confirm whether the vestibular system is critical to the response, other stimulus frequencies and intensities which are known to elicit VEMPs would have to be tested. In particular, frequency tuning has been described for both air and bone conducted sound; although this varies with stimulus intensity, it typically occurs at rates of around 500-1000Hz (McCue and Guinan, 1995; Rauch et al., 2004; Rosengren et al., 2009). Investigation of the tuning profile of PMRF responses to bone vibration and TMS should enable us to determine whether the vestibular apparatus is critical for their generation.

Anaesthesia is also an important consideration when interpreting the results described here. The PMRF is particularly susceptible to anaesthetic drugs and as a consequence it may be that such strong responses are observed as a side effect of the anaesthesia. Many cells are quiescent during this time when the animal is still and there are few external stimuli. In this situation therefore, perhaps only a very small input to cells in the PMRF would be sufficient to produce a strong and widespread response. Conversely, in the awake animal the PMRF is under constant bombardment with information from ascending sensory tracts and a whole host of cortical and subcortical areas in which case the input may have less importance and therefore not produce the same modulation in activity. Alternatively, perhaps the suppressive effect of anaesthesia would reduce the occurrence of late responses since it is relatively difficult to activate cells. In favour of this argument is the recent observation of reticulospinally mediated contralateral MEPs in healthy human subjects (Prof J. Rothwell; personal communication); this would suggest that the reticular formation is powerfully activated by TMS in the awake state.

Investigation in the awake animal would further enable us to test whether the response could be enhanced or modified with muscle contraction. VEMPs in human subjects are only elicited when the target muscle is active. This is also the case for ipsilateral MEPs.

Again, this would help us to deduce whether there is a vestibular-reticular pathway in the generation of these responses.

CHAPTER VI: GENERAL DISCUSSION

There is no doubt that non-invasive methods of investigating brain function are of huge value. The benefit for diagnostic purposes is clear to see; the introduction of MRI for example revolutionised brain imaging and substantially improved the identification and localisation of brain tumours and stroke lesions. Moreover, the ability specifically to access particular brain regions or pathways has proved invaluable in learning about human motor function. There are numerous such techniques which have each pushed back the boundaries and enabled us to progress our knowledge or improve our diagnostic capabilities.

From the results described in this thesis, intermuscular coherence is suggested to be a promising new non-invasive biomarker of upper motor neuron dysfunction which could be applied in neurological conditions such as MND. This technique remains to be proven in larger and more heterogeneous patient populations, however, the initial results are promising. If the clinical utility is confirmed, this technique could offer clinicians a window into an otherwise inaccessible part of the motor system; an area which is affected in many conditions but which is currently extremely difficult to assess.

Despite the benefits associated with non-invasive techniques like intermuscular coherence, it must be understood that they cannot answer all questions about the brain; animal studies still have a critical role to play in many ways. Indeed, we need these basic studies to validate the non-invasive methods in the first place. Basic physiological investigations underpin all of the methods we use whether invasive or non-invasive. They help us to understand the mechanisms of action as well as to define safety parameters. Without this fundamental and invaluable knowledge, non-invasive methods would be useless since there would be no context within which to assess any differences.

This thesis confirms the importance of non-invasive methods but also provides a number of cautionary tales for those using these techniques. The first lesson learned is that careful experimental design is paramount. Retrospectively, it is easy to see that the trial design for the LEV study was not optimal and there were too many factors beyond our control. In particular, the nature of the disease means that tremor, the parameter we were measuring,

was highly variable. Conventionally tremor is measured using accelerometers but perhaps this highly quantitative technique was not appropriate to account for the day-to-day variability in MS tremor. It is easy to see how false positives could arise when measurements were made within a very small time window. This problem may have been difficult to avoid, however different experimental approaches could have perhaps provided a better platform to address the problem. The recent introduction of an IV drug preparation could be more suited to this kind of clinical trial, permitting all data to be collected under the same conditions. Alternatively, a wearable device could have been used to measure tremor over longer periods of time; this would have had the added benefit of use within the 'safe' environment of the patient's home.

A second warning concerns the assumptions made when using non-invasive methods. Non-invasive stimulation techniques by nature can be very non-specific due to current spread or indirect downstream effects. When using techniques such as TMS it is often very difficult therefore to isolate a causal pathway for a specific phenomenon. However, often this fact can be overlooked if there is an obvious or attractive pathway to explain the observation. Care must always be taken to exclude all other possibilities before we ascribe a particular effect on one brain area. This is certainly the case for 'cerebellar' TMS; this study has demonstrated that the technique is not selective since there is concomitant activation of other structures at the intensities routinely used.

The key message throughout this thesis is the importance of understanding the basic physiology underpinning the methods being utilised. There is a rather frustrating tendency for new techniques to become fashionable long before this basic physiology is understood. rTMS is a classic example of a technique that has fallen foul of this trend. Over the last 20 years rTMS has been applied to a whole host of neurological conditions for therapeutic purposes. Yet, the underlying processes are still completely unknown. We can often see the effects, for example the induction of speech arrest with stimulation over Broca's area. However, we have no comprehension of the complex processes taking place or even how many different pathways contribute to these effects. In addition, we must be concerned about safety aspects of the technique since there are at least 16 documented cases of seizure following application of this method. Safety recommendations are now in place but these

were only implemented following multiple incidents (Rossi et al., 2009a; Wassermann, 1998) .

We should also be careful to thoroughly evaluate the techniques which are already in common use. Within this thesis, I have demonstrated that even commonplace methods such as TMS require careful evaluation. Everything known to date about this technique has resulted from a combination of indirect measurements and modelling studies. There is no direct evidence showing specificity or selectivity which is critical. The studies described here have shown that there is potentially confounding activation of brainstem pathways not only whilst stimulating locally over the base of the skull but also whilst stimulating primary motor cortex; a method which is conventionally thought to selectively measure corticospinal tract function.

The results of the TMS studies demonstrate that non-invasive methods must be appropriately validated before they are accepted into mainstream use. We must understand the basic physiology. It is often assumed that techniques are appropriate and fit for purpose just because they are considered to be 'standard'. For example it is generally accepted that accelerometry is the best way to measure tremor when it may not always be optimal. In addition, questions about the precise timing of certain processes are often addressed using functional MRI which has a very poor temporal resolution.

Current research trends mean that this is an appropriate time to review non-invasive methods to investigate brain function. The interest in non-invasive brain-machine interfaces and neuroprosthetics is continuing to grow and technology is progressing at a rapid rate. With this constant stream of new developments and the race to develop the first commercially viable and effective non-invasive device, the basics can often be overlooked. However, in order to make a useful contribution with our research we need to be sure we understand at a fundamental level what is happening in the brain. Only then can the knowledge be applied in the wider neuroscience community

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