The clinical spectrum and pathophysiology of neuropathic tremor

UCL Institute of Neurology

Sobell Department for Motor Neuroscience and Movement Disorders

Tabish Aziz Saifee

Supervisors:

Dr Mark J. Edwards

Professor Mary M. Reilly

Thesis submitted for the degree of Doctor of Philosophy (PhD)

UCL

Declaration that the work presented in this thesis is the candidate's own

I, Tabish Aziz Saifee, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

May 2015

Signature

Tabish Aziz Saifee

To my wife Sairah and my children, Esa and Amaya

Statement of contribution

I was involved in the study design, collation of data, experimentation, data analysis, draft writing and editing of all studies within this thesis except where indicated below. The results of chapter 8 also contributed to the MSc dissertation of Ms Cassi Fifford, whose project I supervised. I had conceptualised, designed, taught and undertook initial experiments, subsequently supervising Ms Fifford on further experiments, and guiding and supervising the analysis for this. Ms Fifford wrote the initial draft of the discussion for this study and I was involved in the revision. The experiments in chapter 6 were undertaken with Dr Petra Schwingenschuh, with whom I recruited participants, carried out experiments, helped analyse the results and prepare the manuscript. Professor Adolfo Bronstein and Dr Diego Kaski provided normative data for the comparison of eye movements in chapter 7.

Abstract

This thesis describes a series of studies involving patients with neuropathies and healthy controls. In the studies of disease, two groups were recruited: patients with inflammatory neuropathies and those with hereditary neuropathies. Each group was separated into those with and those without tremor and compared with healthy controls. Clinical assessments and neurophysiological tests were employed to correlate cerebellar function with tremor. The final study of healthy participants investigated the effect of transcranial direct current stimulation (TDCS) on the cerebellum during finger tapping.

1) Tremor was most common in IgM paraproteinaemic neuropathies, also occurring in 58% of those with chronic inflammatory demyelinating polyradiculoneuropathy and 56% of those with multifocal motor neuropathy with conduction block (MMNCB). Tremor was generally refractory to treatment and contributed to disability in some patients. Although tremor severity correlated with F wave latency, it was insufficient to distinguish those with, from those without tremor.

2) Impaired eyeblink classical conditioning and paired associative stimulation in patients with inflammatory neuropathy and tremor differentiated them from neuropathy patients without tremor and healthy controls, strongly suggesting impairment of cerebellar function is linked to the production of tremor in these patients.

3) The prevalence study in CMT1A patients revealed tremor in 21% and in 42% of those it caused impairment. Eyeblink conditioning, visuomotor adaptation and electro-oculography were no different between tremulous and non-tremulous patients and healthy controls. This argues against a prominent role for an abnormal cerebellum in tremor generation in the patients studied. Rather, they suggest an enhancement of the central neurogenic component of physiological tremor as a possible mechanism.

4) TDCS of the lateral cerebellum and its effect on paced finger tapping was examined. There was no effect on accuracy or variability of the intertap interval, providing no support for a direct role of the cerebellum in event based timing.

Acknowledgements

I would like to thank my wife Sairah for her companionship, patience, motivation and unwavering support during this fellowship. I thank my parents for their lifelong support and my children, Esa and Amaya for their welcomed distractions, providing perspective and making it all worthwhile.

For his invaluable supervision, mentoring, encouragement throughout and for making it all possible, I thank Dr Mark Edwards. I thank him for his guidance and direction, particularly during the troughs that come with research. I also thank Professor Mary Reilly for her vision, sage advice and mentorship and also to Professor John Rothwell and Professor Kailash Bhatia for their wisdom and collaboration.

Thank you to all my colleagues and collaborators in the group, too many to name all but particularly to those who inspired and taught me and will remain friends to reminisce the journey: Dr Panagiotis Kassavetis, Dr Isabel Pareés Moreno, Dr Maja Kojovic, Dr Ignacio Rubio-Agusti, Dr Anna Sadnicka, Dr Antonella Macerollo, Dr Maria Stamelou, Dr Amit Batla and Dr Christos Ganos.

The work described herein was supported by a doctoral research fellowship awarded to Dr Tabish Saifee by the National Institute for Health Research to whom I am very grateful.

Contents

Abbreviations

3-T	3 Tesla
ADM	Abductor digiti minimi
ANOVA	Analysis of variance
APB	Abductor pollicis brevis
BOLD	Blood-oxygen-level dependent
CIDP	Chronic inflammatory demyelinating polyradiculoneuropathy
CMTNS	Charcot-Marie-Tooth neuropathy score
CR	Conditioned response
CS	Conditioned stimulus
DA	Dopamine
DICS	Dynamic imaging of coherent sources
DT	Diffusion tensor
DWI	Diffusion-weighted imaging
EBCC	Eyeblink classical conditioning
EEG	Electroencephalography
ET	Essential tremor
FDIO	First dorsal interosseus
fMRI	Functional magnetic resonance imaging
FTM	Fahn-Tolosa-Marin
GPi	Internal part of the globus pallidus
GPe	External part of the globus pallidus
IgMPN	Immunoglobulin M paraproteinaemic neuropathy
ILN	Thalamic interlaminar nuclei
IN	Interpositus nucleus

ION	Inferior olivary nucleus
ISI	Inter-stimulus interval
ITI	Inter-tap interval
LC	Locus coeruleus
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MAG	Myelin-associated glycoprotein
MEG	Magnetoencephalography
MEP	Motor evoked potential
MGUS	Monoclonal gammopathy of undetermined significance
MMNCB	Multifocal motor neuropathy with conduction block
MRI	Magnetic resonance imaging
MT	Movement time
NAA	N-acetylaspartate
NE	Norepinephrine
NINDS	National Institute of Neurological Disorders and Stroke
ODSS	Overall Disability Sum Score
ONLS	Overall Neuropathy Limitations Scale
PAS	Paired associative stimulation
PC	Purkinje cell
PD	Parkinson’s disease
PET	Positron emission tomography
PFT	Paced finger tapping task
PIGD	Postural instability and gait disorder

PNS	Peripheral nervous system
PPC	Posterior parietal cortex
RaN	Raphe nuclei
RMT	Resting motor threshold
RN	Red nucleus
RRA	Retrorubral area
RT	Reaction time
S1	Primary somatosensory cortex
SAI	Short afferent inhibition
SCT	Synchronisation-continuation task
SE	Serotonin
SEP	Somatosensory evoked potential
SMA	Supplementary motor area
SNc	Substantia nigra pars compacta
STDP	Spike-timing dependent plasticity
STN	Subthalamic nucleus
TACS	Transcranial alternating current stimulation
TDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
UR	Unconditioned response
US	Unconditioned stimulus
VBM	Voxel-based morphometry
VIM	Ventrointermediate nucleus
VLa	Anterior part of the ventrolateral thalamus
VLp	Posterior part of the ventrolateral thalamus

Units

∘	Degrees
Hz	Hertz
mA	Milliamperes
mg	Milligrams
m-g	Milli-g
mm	Millimetres
ms	Milliseconds
SD	Standard deviation
SEM	Standard error of the mean

List of Tables

List of Figures

Overall Summary

The pathophysiological mechanisms underlying tremor may be investigated in a variety of ways. This thesis describes the use of a variety of electrophysiological techniques twinned with clinical assessment to determine features that may be pertinent to the occurrence of tremor in patients with neuropathies and in the final chapter aims to identify a rhythmic motor control task that can be modulated by cerebellar stimulation.

The first chapter provides a clinical introduction to tremor and its various types, leading to contemporaneous hypotheses of pathophysiological mechanisms of some of these. This is a prelude to chapter 2 where the concept and clinical spectrum of neuropathic tremor is introduced and potential hypotheses of cerebellar dysfunction described. Chapter 3 outlines the aims of the thesis. Chapter 4 provides a background and detailed description of the methods used throughout the thesis.

Chapter 5 describes the study conducted on patients with inflammatory neuropathies, comparing clinical and nerve conduction features of patients with and without tremor.

Chapter 6 develops the findings from chapter 5 and is a description of a series of experiments on patients with inflammatory neuropathies aiming to distinguish those with and those without tremor based on response to paired associative stimulation and eyeblink classical conditioning.

Chapter 7 examines patients with CMT1A using an array of electrophysiological tests investigating function of the cerebellum and correlating these with clinical features of those with and without tremor as well as healthy controls.

Chapter 8 moves beyond neuropathic tremor and aims to establish whether a paradigm of rhythmic finger tapping is amenable to modulation by non-invasive stimulation of the cerebellum.

Each of these experimental chapters includes more specific detail of methodology beyond that described in the general methods chapter 4. They outline results of experiments and lead to a discussion of methodological validity, relevance to previous work and insights into pathogenesis or motor control that they provide.

The final chapter concludes by summarising the findings and putting them in context of current literature as well as generating ideas of further work, some of which has already commenced.

Chapter 1: Tremor: an introduction

1.1 What is tremor

Tremor is characterized by a rhythmic, involuntary, oscillatory movement of one or more body parts and is perceived to be involuntary by the patient (adapted from Movement Disorder Society (MDS) consensus statement[3]). Defining tremor is fraught with difficulty given the spectrum of differing types. The components of the consensus statement are prone to difficulties in capturing all that is considered tremor and excluding those hyperkinetic movements that are not. Nevertheless, a pragmatic clinical approach often needs to be taken, excluding all those movements which in aggregate have characteristics more akin to other hyperkinetic movements such as asterixis (negative myoclonus) or clonus and inclusion of those movements where a rhythmic or semi-rhythmic movement of a body part dominates with a pattern that equates roughly to an oscillation and is of an acceptably high frequency i.e. more than 2-3 Hz or so and perhaps describable by a layperson as ‘shaking’ or ‘trembling’.

1.2 Clinical spectrum of tremor

The causes of tremor are many. It forms part of normal physiology at one end of the spectrum with so called physiological tremor, ubiquitously present when a limb is not at rest, and is of very low amplitude, relatively high frequency of oscillation and barely perceptible to the observer[4]. Tremor, when pathological can occur due to a number of hereditary or acquired

neurological and neurodegenerative conditions, systemic diseases as well as potentially arising as a side effect from a number of drugs such as sodium valproate, beta-agonists, neuroleptics, selective serotonin reuptake inhibitors, central nervous system stimulants and depressants and lithium. The diagnosis of tremor is most commonly clinical and less typically relies on imaging such as magnetic resonance imaging (MRI) or nuclear imaging of dopamine transport to positively identify an aetiology. Table 1.1 summarises a range of tremor types typically considered in a clinical setting when faced with a patient with tremor.

Essential tremor	Typically isolated action tremor of the upper limbs. Classically an autosomal dominant family history with alcohol responsiveness[5 6] but likely represents a heterogeneous group of diseases.
Parkinsonian tremor	Archetypically tremor at rest in the upper limb with asymmetry between limbs in the idiopathic form of disease. Associated with other parkinsonian features. Other typical forms of tremor exist including re-emergent postural tremor[7]. Other body parts may be affected such as the legs and jaw.
Dystonic tremor	Dystonic posturing in the tremulous

	body part although ‘tremor associated with dystonia’ where tremor occurs in a different body part to the dystonia may well represent a spectrum of the same phenomenon. Often jerky due to varying amplitude and may be relatively task or position-specific.
Drug-induced tremor	Often upper limbs, low amplitude, high frequency, symmetrical action tremor. Other forms including parkinsonian tremor may occur.
Enhanced physiological tremor	Often upper limbs, low amplitude, high frequency, symmetrical action tremor. Often identifiable precipitants.
Cerebellar tremor	Head and upper limb action tremor of low frequency and large amplitude. Other cerebellar signs. Intention component.
Oculopalatal tremor	Low frequency and sometimes associated with tremor in related body parts. May in specific types be related to olivary hypertrophy that develops subsequent to a lesion typically within the Guillain-Mollaret

	triangle, but this may be an oversimplification.
Functional (psychogenic) tremor	Behaves very similarly to volitional rhythmic oscillatory movements but perceived by patients as involuntary. Abrupt onset, distractibility, entrainment, high inter-limb coherence and response to placebo may be seen.
Neuropathic tremor	Predominantly action but can occur at rest. Infrequently in any other body part except arms. Typically found in demyelinating inflammatory neuropathies and some hereditary neuropathies.
Fragile X tremor ataxia syndrome	Associated with cognitive decline and ataxia with a family history.
Other genetic causes (e.g. spinocerebellar ataxia)	Additional features such as progressive ataxia. Often autosomal dominant family history with anticipation.
Cortical tremor	High frequency (20Hz) rhythmic cortical discharges. Family history and epilepsy may occur.
Holmes tremor	Low frequency, high amplitude

	tremor in rest, postural and kinetic conditions usually due to a lesion in the brainstem or thalamus.
Orthostatic tremor	Very low amplitude, high frequency (usually 13-18Hz) tremor of the legs on standing with high inter-limb coherence, relieved by walking, causing a latent onset unsteady feeling when standing. Isolated or in the context of other diseases such as parkinsonism.
Wilson's disease tremor	Wing-beating, rest or other action tremors seen. Usually young onset with copper metabolism abnormality due to an array of autosomal recessive mutations.

Table 1.1 List of tremor-dominant or tremor-typical conditions and their associated features.

1.3 Pathophysiological mechanisms of tremor generation

Although tremor is one of the commonest movement disorders and amongst the commonest neurologic symptoms referred to neurologists, relatively little is understood about its pathogenesis. The clinical manifestation of tremor is wide and varies according to amplitude, frequency of oscillation, body part

affected, context in which tremor occurs (e.g. rest versus action) and associated features. Parallel to this, the pathophysiological mechanisms that underpin these different tremor types also likely varies although there may be some common mechanisms, supported in part, for example, by the near ubiquitous benefit of deep brain stimulation of nuclei such as within the ventral intermediate thalamus for a variety of tremor types, irrespective of their underlying aetiology or phenotype[8].

There are pure mechanical properties of limb oscillation that relate to a mechanical object's propensity to oscillate when provided with mechanical energy. The weight, length and stiffness of the object then determine properties of the oscillation such as frequency and amplitude. Equation 1.1 below summarises this relationship between the frequency of oscillation and the mentioned variables.

$$f \propto \sqrt{(k/m)} \qquad \text{Equation 1.1}$$

f = frequency; k = stiffness; m = limb inertia

This equation demonstrates how increasing stiffness of a limb increases the natural frequency of oscillation of that limb. This natural frequency, or eigenfrequency, is the frequency at which the limb prefers to oscillate given an external mechanical perturbation such as that referred to as *cardioballistic* arising from cardiac contraction. The converse is true with limb weight; the greater the weight, the slower the natural oscillatory frequency. With enhanced physiological tremor, although these mechanics still hold,

excitation of short loop reflexes seem to enhance the oscillatory behaviour of the physiological tremor by providing EMG drive phase-locked to the tremor. Composition of the tremor may then be considered to be caused by a generator providing a perturbation, modulated by the oscillatory dynamics of the limb and the enhanced reflex circuits providing phase-locked EMG to augment the tremor. So-called *central* forms of tremor may have analogous reflex enhancement.

Control of the hand demands accurate control and thus a high gain with responsive feedback. The motor command is continually updated according to feedback received from the periphery. This feedback occurs in various forms from short latency reflex arcs involving only the spinal cord to longer latency afferents including those involving the cerebellum or motor-sensory cortices. Any excess delay in feedback may be predicted by a model of optimal feedback control to lead to instability that would be prone to oscillating. Indeed, from an engineering point of view, a propensity to oscillate is an inevitable property of a system with a controller of high gain and negative feedback. It is possible that such instability with oscillatory circuits could lead to tremor in the periphery. There is an emerging view that suggests physiological or pathological networks may exist and oscillate with varying dynamics (for review, see [9]).

An alternative pathophysiological mechanism may be that cells, presumably in syncitia or with a common ion channel mutation or abnormality, have abnormal membrane conductance that promotes spontaneous or low-

threshold recurrent depolarisation leading to a pacemaker drive modulating motor output and making it oscillatory at a tremor frequency. It is possible that abnormal local rhythmicity drives an otherwise normal circuit. The inferior olive has been proposed to be one such pacemaker locus with cerebellar modulation in oculopalatal tremor[10], with intrinsic pacemaker properties of the inferior olive relating to its syncitia and drug treatments that are in development using this rationale. However, the evidence for the inferior olive as a central node in the network generating other tremor types is less cut-and-dry[11] (see section 1.3.1 below on Essential tremor).

Nevertheless, an account of tremor pathogenesis, by necessity, requires a specific approach to differing subtypes of tremor; despite potentially common final pathways, the upstream pathogenic mechanisms may well vary between tremor types.

1.3.1 Essential tremor

Essential tremor, primarily a monosymptomatic condition, is one of the commonest types of tremor. However, an array of other milder clinical neurocognitive features have been described in association but differentiating these from likely confounds is difficult. The estimated prevalence varies markedly between studies with a study from the USA[12] suggesting an overall prevalence of 2.2% whilst an influential study from Spain, entitled NEDICES[13] suggested 8.9% although a third were not examined directly and this was a study of over 65 year-olds. Other reports

have estimated between 1% in the general population and about 5% in the population over 65 years of age[14]. ET has been reported to have a bimodal distribution of age of onset[5], including adolescence/early adulthood and after the 5th decade of life (figure 1.1). Over half have affected family members. A substantial proportion of that described in the literature as essential tremor may well represent a heterogeneous group of diseases and some have used a distinction between senile, sporadic and hereditary forms[15]. Essential tremor primarily affects the upper limbs and is an action tremor. It can also affect other body parts such as the head, jaw, tongue, voice, trunk and legs but not in isolation[3 6]. A sub-group of ET where there is a clear autosomal dominant family history and often a responsiveness of the tremor to alcohol exists that may well represent a narrower aetiology. However, even here, difficulties may arise as both GWAS studies have identified specific risk factors such as LINGO1 that seem to be predictive only in specific populations[16] and not others[17]. Other risk loci have been identified from similar GWAS studies including loci on chromosomes 2, 3 and 6 (for meta-analysis and review, see[18]). Further, specific Mendelian genetic causes (e.g. ANO3) have been described in families where an autosomal dominant family history exists with cervical dystonia as the predominant feature and isolated tremor occurring in some of the family members[19]. Clearly, the report of dystonic posturing in the family history of those with pure isolated tremor may be unreliable meaning that a small proportion of those apparent autosomal dominant ET cases may represent genotype-phenotype correlations of genes predominantly responsible for familial dystonia. Another possibly rare cause of familial ET may be the gene, FUS, fused in sarcoma. There are

further potential candidate loci[20 21] but these likely explain a very small minority of all familial ET. The surprisingly low prevalence of tremor in first-degree relatives with ET in a community-based study perhaps speaks to the possibility of alternative inheritance modes rather than autosomal dominant or otherwise poor penetrance. These have been postulated to include polygenic, mitochondrial, recessive and X-linked modes of transmission[22]. Pairwise concordance in monozygotic twins was twice that in dizygotic twins (0.60 monozygotic; 0.27 dizygotic), indicating both environmental and genetic influences on pathogenesis[23].

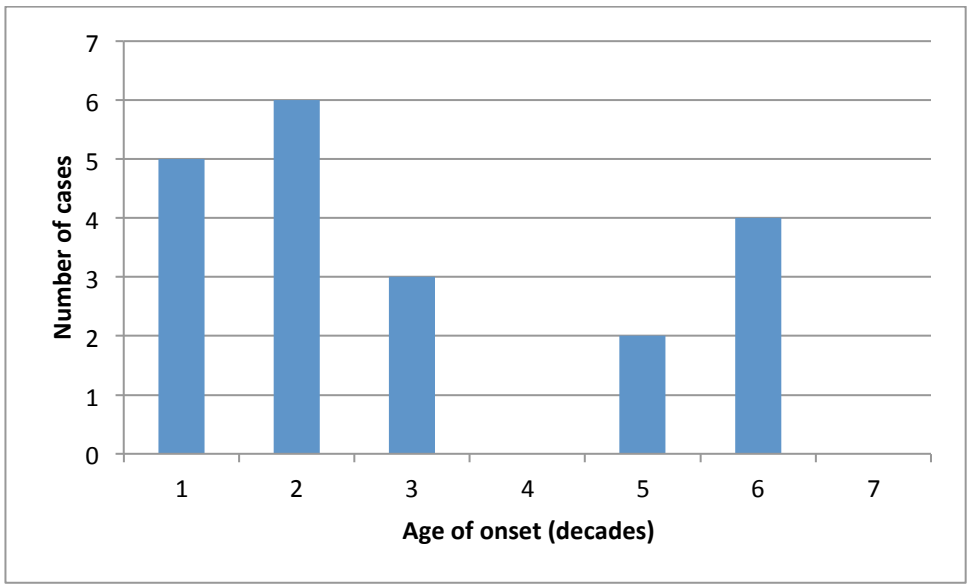


Figure 1.1 Age of essential tremor onset. Adapted from Bain et al[5]

The question of whether or not ET is a neurodegenerative disorder has been the focus of some recent debate. Louis et al have published a series of autopsies in 11 patients with ET[24], all of whom later developed dementia or progressive supranuclear palsy. Two clusters of pathology were identified, one of brainstem Lewy body disease and the other, cerebellar Purkinje cell loss. Concerns about the argument for a neurodegenerative process rest on

most of the evidence base of pathological changes in autopsy series being in patients over 70 years old where the co-existence of confounding pathologies is relatively high. Further, ET recruited in such studies does appear to be a heterogeneous group of conditions and as such, those included in autopsy series may well have heterogeneous aetiologies.

1.3.1.1 The cerebellum in essential tremor

Not entirely mutually exclusive to the neurodegenerative debate is the question of whether in ET there is evidence for a role of the cerebellum in generating the tremor. This is underpinned by functional imaging, electrophysiological and pathological studies. PET studies have demonstrated a bilateral increase in cerebellar and thalamic blood flow [25] and MR spectroscopy has suggested dysfunction in the cerebellar cortex [26]. Eye-blink conditioning is also severely impaired in essential tremor [27 28] implicating the cerebellum. A study with blood flow PET showed hyperactivity of the cerebellum at rest, and then further increase of activity in the cerebellum and red nucleus region as well[29]. The strong evidence for a role of the cerebellum in essential tremor has also been corroborated by structural imaging studies. Benito-Leon et al. [30] assessed whether white or grey matter changes occurred in 19 ET patients vs. 20 age and gender-matched controls. The authors concluded that structural white and grey abnormalities may be detected in ET patients using VBM (voxel-based morphometry) and a 3-T (3-Tesla) MRI scanner, corroborated by other groups[31]. Using lower field scanners (1.5-T) delivered contradictory results[32]

[33]. Using Diffusion-weighted imaging (DWI) to search for evidence of tissue integrity abnormalities in these areas in ET patients failed to find any significant difference from controls [34], arguing against major structural damage in the ET brain, though more subtle neurodegenerative changes could not be ruled out. However, Shin et al. [35] investigated changes in anisotropy in patients with ET by comparing fractional anisotropy (FA) images generated from diffusion tensor imaging data acquired at 1.5-T in 10 patients with ET compared with 8 control subjects using statistical parametric mapping to make voxel-by-voxel comparisons; compared with the control subjects, they found patients with ET exhibited significantly reduced FA in areas of the brainstem and bilateral cerebellum. As previously mentioned, the posterior part of the ventrolateral thalamus, a major recipient of cerebellar outflow, can be targeted with DBS for highly successful tremor suppression. Perhaps accordingly, there are also reports of damage to the cerebellum by a stroke that eradicated essential tremor on the same side[36].

With regards a neurochemical understanding of cerebellar involvement, magnetic resonance spectroscopy has shown diminished N-acetylaspartate (NAA). Moreover, decreased GABA-A and GABA-B receptors have been described in the dentate nucleus in ET[37]. However, results from other studies, although again implicating altered GABAergic transmission suggested by reduced parvalbumin staining, did so in the locus coeruleus and pons rather than the cerebellum[38]. Flumazenil PET studies have shown increased binding of radiolabelled flumazenil in the cerebellum and ventrolateral thalamus indicating increased affinity to the GABA-A receptor,

which is somewhat at odds with the pathological findings[39 40]. Increased affinity or avidity due to functional receptor changes in remaining but diminished GABA receptor numbers may resolve this apparent contradiction. CSF GABA is diminished[41], and a tremor resembling essential tremor is seen in mice with a knock-out of the alpha-1 component of the GABA-A receptor. Despite this mounting evidence implicating GABA, no GABA receptor or transporter polymorphisms associated with ET have been found in humans[42 43 44].

Pathological studies of degenerative changes in the cerebellum have been fraught with difficulty and diametrically opposed opinion. One group, as mentioned, led by Louis have provided much of the evidence purporting pathological changes consistent with neurodegeneration whilst Rajput et al[45] presented evidence in 12 ET patients and 6 controls that there was no such difference between groups. Louis et al[46] demonstrated two pathological groups of ET, one with Lewy bodies in the locus coeruleus and the other with Purkinje cell loss in the cerebellum compared with normal controls. A further study[47] reported similar findings with additional Purkinje cell axonal swelling ('Torpedoes') but this study investigated some of the same brains as the original report. A further study on 24 different ET brains compared with 21 controls reported cerebellar disease in seven of the ET patients without evidence of Lewy bodies[48]. A more recent study (discussed below in section 1.3.1.3 entitled *Inferior olive as a pacemaker in essential tremor*) found no pathological abnormalities in the inferior olive, another putative locus of tremor in ET. Overall, there are insufficient grounds for considering ET a

neurodegenerative disease on pathological evidence alone. However, in conjunction with structural imaging findings and neurochemical abnormalities described, this possibility needs further investigation, with larger corroborative autopsy studies from more than one centre. Further, distinction between aetiological pathological changes and those changes consequent of tremor or epiphenomenal must be made.

1.3.1.2 An 'oscillopathy'?

ET is thought to be a centrally generated tremor that arises due to abnormally oscillating central nervous system loops that likely involve the cerebello-thalamo-cortical pathways[49]. This seems to be congruent with the effect of deep brain stimulation on the thalamus in patients with ET[50] but appears somewhat surprisingly to be excitatory to this loop rather than inhibitory with cortical projections from the thalamus being facilitatory to cortical motor evoked potentials[51].

There may be different mechanisms underpinning the different types of tremor in ET. Pedrosa and colleagues[52] demonstrated selective deterioration in intention tremor during low frequency (10Hz) stimulation particularly of the ventral aspect of the ventrolateral thalamus and an area a little inferior to this, potentially nodal to the cerebellothalamocortical network. This was found to be relatively selective for intention components of tremor when compared to the relatively little affected postural component of tremor. However, the authors did not clearly distinguish in their results between

kinetic and purely intention tremor. Nevertheless, this selective modulation of different tremor phenotypes within the same patient has been used by Brittain and Brown[53], in their recent review, to outline the idea that tremor is not merely a fixed expression of a network or nodal pacemaker. Rather, they reinforce the idea of a dynamic network, subcircuits of which are prone to up- or down-regulation by the ambient motor state. Such dynamism is seen in coherence studies using EEG and EMG[54]. This responsiveness to the motor state could render the clinical manifestation of tremor labile and biased towards the current state of the motor system. They raise the possibility that multifocal nodes of synchronised neurones are, in fact, a physiological feature of the healthy motor network, but cause overflow to the periphery in the form of tremor when certain pathological triggers and task requirements drive supra-threshold oscillatory synchronisation[53]. This updates older views where a single focal oscillator was thought to be causal. It also fits with more contemporaneous data using coherence analysis where nodes of the network are able to become transiently phase coupled[54] in a time-variant and dynamic fashion and the finding with DBS electrodes of multiple spatially distinct clusters of cells within the posterior ventrolateral thalamus driving tremor [55]. Entrainment of typical networks such as the cerebellothalamocortical network may be better explained with such a model. For example, entrainment of the cerebellothalamocortical network can occur both in pathological tremor and voluntary rhythmic movements of the hand but only the former commands bidirectional coupling of the network[56]. Nevertheless, identifying critical nodes in such a network remains important, if only as a potential therapeutic target.

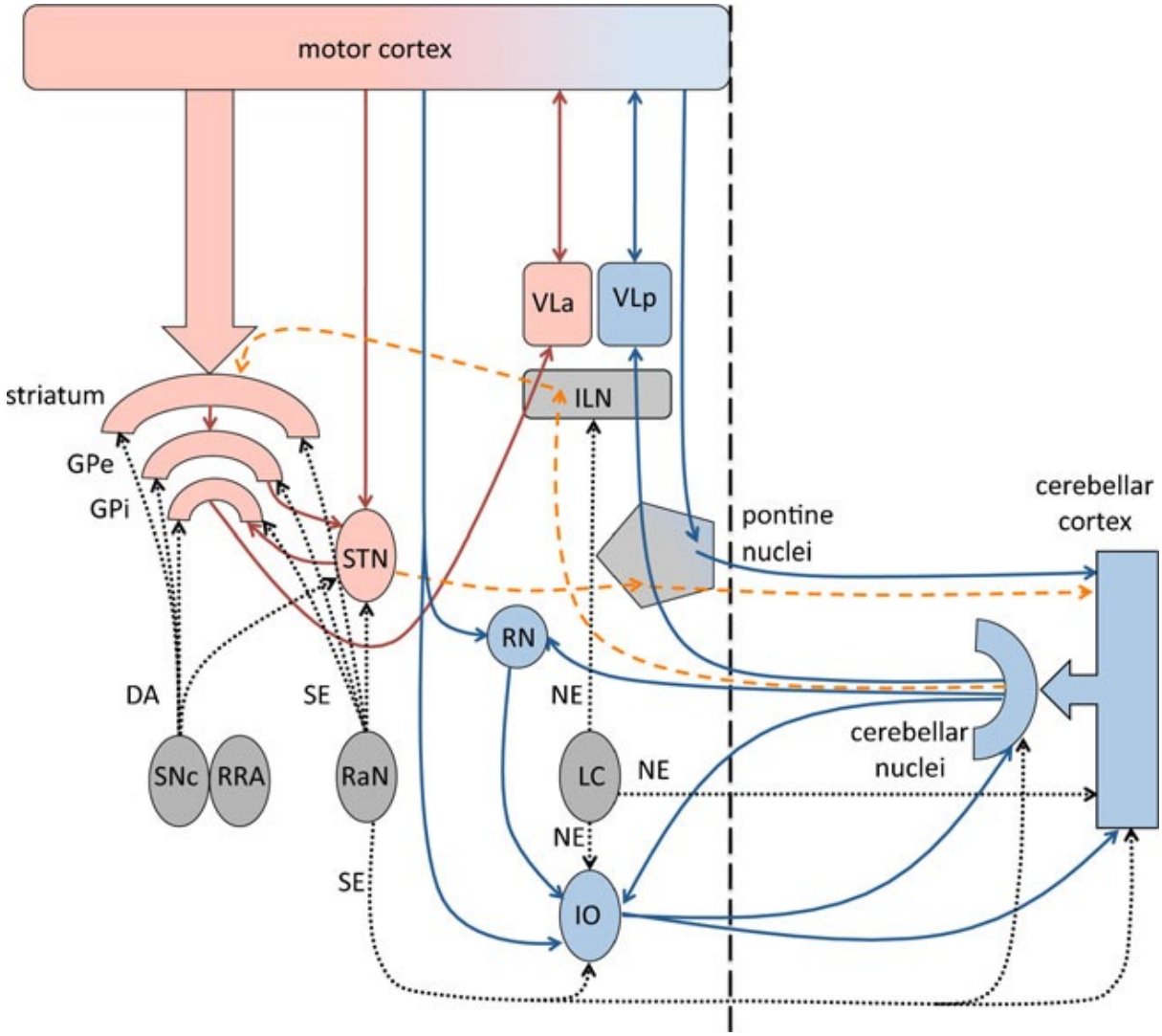


Figure 1.2 Central network in essential tremor. From Helmich et al [57]. The basal ganglia and its connections are shown in red, and the cerebellar system and its connections are shown in blue. Modulatory neurotransmitter projections are shown as dotted black lines. Anatomical connections between basal ganglia and the cerebellum are shown as dashed yellow lines. DA dopamine, GPi internal part of the globus pallidus, GPe external part of the globus pallidus, ILN thalamic interlaminar nuclei, IO inferior olive, LC locus coeruleus, NE norepinephrine, RaN raphe nuclei, RN red nucleus, RRA retrorubral area, SE serotonin, SNc substantia nigra pars compacta,

STN subthalamic nucleus, VLa anterior part of the ventrolateral thalamus, VLp posterior part of the ventrolateral thalamus.

1.3.1.3 Inferior olive as a pacemaker in essential tremor? (adapted from Saifee and Edwards, 2013[11])

On the basis of three main lines of evidence, the inferior olivary nucleus (ION) has been suggested as a node in this pathological network and even as a main player. First, the harmaline animal model of ET unequivocally represents oscillatory output from the ION. Second, there is a physiological rationale that the ION could play a role in tremor generation given the calcium-dependent neuronal synchrony of the ION sufficient to drive the cerebellothalamocortical circuit to produce tremor[58]. Third, a functional imaging study[59] has suggested abnormal activation of the ION in ET. The actual story, however, seems less clear-cut, with a lack of evidence of ION involvement in the majority of ET patients studied with functional imaging[25 60 61] and, less specifically, medullary activation in one study[62] and brainstem in another[63].

Louis and others address the important issue of whether the ION is involved in ET from a neuropathological point of view[24]. They report a case–control series of 14 ET cases and 15 controls. Of note, none of their patients had Lewy bodies in regions normally examined in their assessment, despite previous reports from the same group of a Lewy body subtype of ET. By the

authors' rationale, all the ET cases in this study would thus be expected to fall within the “cerebellar” subtype coined by the group. The bottom line of the study is that there were no identifiable pathological changes in the IONs in these patients compared with controls.

The lack of degenerative change does not exclude a role for functional disturbance within the ION in the generation of ET. However, in contrast to the key role of the ION in harmaline tremor and oculopalatal tremor, available evidence for functional disturbance is sparse. One study[59] demonstrated alcohol-associated increases of regional blood flow in the IONs of patients with ET but not controls, perhaps via cerebellar projections to the ION. In a study by Bucher et al[61] two of 12 patients with ET demonstrated ION activation. Notably, the excitatory cerebellar nuclei neurons that project to the thalamus and the inhibitory nuclei neurons that provide feedback to the olive are innervated by the same Purkinje cell axons[64] although they take part in tonic and phasic control, respectively[65]. So, different cerebellar rhythms may coexist, and their underlying networks can still, at least partly, be shared. The variable involvement of the ION in functional imaging studies in ET[59 60 61 62] could relate to the nonlinear dynamics of the network[54], the signal-to-noise ratio required for detection of activity, or the ever-present issue of case heterogeneity. We therefore still need categorical evidence for or against the functional involvement of ION in ET. If the ION is involved in the oscillatory network, this study is of importance because it would suggest that effects of abnormal synchrony are not necessarily toxic to cells leading to degenerative change as speculated[15]. Harmaline increases the oscillations in the inferior

olivary nucleus, and this might occur from one of two mechanisms (or both). The olivary neurons are spontaneously rhythmic, the rhythmicity supported by a low threshold calcium channel. The olivary neuron dendrites come together in clusters called glomeruli where they communicate with each other via gap junctions. In harmaline-induced tremor, the cells are more rhythmic and they communicate more strongly. Perhaps importantly, the gap junction communication is down-modulated by GABA, so a deficiency of GABA would increase synchronicity. The inferior olivary–cerebellar network has been long suspected as being the relevant generator, but the evidence is not strong.

1.3.2 Parkinsonian tremor

Multiple tremor types fit into this rubric. Tremor is part of the cardinal features classically used to describe the motor aspects of Parkinson's disease. Tremor can occur in multiple body parts including the jaw, lips, arms and legs in PD. The phenotype of PD tremor is relatively distinctive with three main types often seen. The first, classical type of tremor is seen predominantly at rest. This may better be described as occurring during sates of stability as it can also occur once a posture of the arms is adopted but not usually until a brief latency after adopting such a position. The term re-emergent tremor is used to encapsulate this phenomenon. Other forms of tremor in PD might include an action tremor of the arms that has a higher frequency (>1.5Hz) than the rest tremor. Dystonic tremor, enhanced physiological tremor and even functional (psychogenic) overlay tremor may additionally occur in a small minority of patients that have recently been reported[66-68]. Finally, a

pure action tremor of the limbs seems to also occur in some patients with PD. This may manifest as an action tremor of the arms without rest tremor or even as orthostatic tremor in the legs, a form of isometric tremor. The orthostatic tremor here may occur secondarily to the PD, given the higher than expected prevalence of orthostatic tremor in patients who develop PD[69]. Further, there seems to be some dopaminergic responsiveness to this type of tremor in some and also those with orthostatic tremor who also develop PD seem to be older than controls with OT alone. Recently, ocular tremor has also been described in all patients with PD in a cohort of 118 patients[70], however, there are reasonable grounds to believe that this is merely a manifestation of parkinsonian limb tremor mechanically conducted to the head tremor and resulting in a vestibulo-ocular reflex rather than primary ocular tremor, as we have recently argued[71 72].

Tremor in PD is an important part of the phenotype as some of those patients seem to have a tremor-dominant picture which when contrasted to posture and gait dominant (PIGD) subtypes, seems to predict a slower, more benign prognosis[73 74]. However, the story may not be quite as straightforward and more recently it has been suggested that this clinical distinction does not predict length of disease at death but rather the tremor-dominant group lose their slowly progressing advantage later in the disease course[75]. The hallmark pathological finding in PD of loss of dopaminergic projection cells from the substantia nigra pars compacta to the striatum seems to co-vary with the severity of bradykinesia but not so clearly with tremor[76]. Degenerative changes in other brain areas such as the retrorubral

area (A8) have instead been indicated to correlate with the severity of rest tremor rather than the substantia nigra[77]. Abnormal oscillatory activity in the basal ganglia particularly the pallidum, though intermittent, seems to relate to tremor in PD. It has been proposed that a dimmer-switch model whereby abnormal oscillatory signals in the pallidum trigger activation of the cerebellar-thalamo-cortical loop which acts as the gain for peripheral tremor and the former as the switch[7].

The tremor of PD tends to transiently improve when changing motor state, for example moving from a rest state to one of action. Tremor in PD seems somewhat divorced from other motor features of PD such as bradykinesia and rigidity in the sense that it appears to be far less dopa-responsive (certainly at conventional doses) than the aforementioned symptoms, it does not seem well correlated with diminished dopamine uptake on nuclear imaging of presynaptic dopamine transport and even on post-mortem. There may well be a dopamine connection to tremor but if so, it is likely not the nigrostriatal pathway. Although serotonin has not proven an avenue to treatment of tremor, a nuclear imaging study utilising PET imaging of a ligand that binds to 5-HT1A receptors indicates that there is more tremor associated with less binding in the raphe[78], but this may be particular to postural rather than rest tremor[79]. Differing network patterns have been revealed from an array of imaging and electrophysiological measures of tremor in PD. Using FDG-PET, the dentate nuclei of the cerebellum and rostral areas of the cerebellum, putamen and motor cortex all seem important[80]. M1 has been implicated using corticomuscular coherence in a MEG study[81]. Areas most

highly coherent with M1 on EEG seemed to be secondary somatosensory cortex, posterior parietal cortex, cingulate and supplementary motor areas, diencephalon and the cerebellum. Functional MRI on the other hand has demonstrated increased BOLD signal in the putamen and both internal and external global pallidi at the onset of tremor whilst the cerebellum, thalamus and pre-motor areas seemed to correlate with amplitude[82]. This latter finding nicely tying the cerebellothalamocortical circuit, thought to be the gain control in the dimmer switch model of PD, to amplitude modulation. This plays conveniently into the observations that targeting a node in this network, the VIM, with high frequency stimulation or destruction is a highly efficient way of ameliorating tremor. The proposed model is that of a simultaneously oscillating network in the basal ganglia and also cerebellothalamocortical loops, both coupled to produce tremor[83]. Although this hypothesis is attractive in that it integrates evidence linking both basal ganglia and thalamic networks to Parkinson's disease tremor and might explain why high frequency stimulation of both the subthalamic nucleus and of the ventrolateral thalamus can be effective in controlling Parkinson's disease tremor, Cagnan et al[84] demonstrate a role for both the subthalamic nucleus and thalamus in tremor pace-making that would question this hypothesis. Moreover, despite tremor entrainment, they did not observe significant related modulation of the amplitude of tremor from either site, arguing that any such tremor amplitude modulation might occur at remote sites. In line with this, motor cortex stimulation is known to both entrain and modulate Parkinson's disease rest tremor amplitude in a phase-dependent manner[85]. This study raised the possibility that the motor cortex is not only involved as

part of a tremor pacemaker network, but that the motor cortex and its outflow may be targets for amplitude modulation[86]. This would also be consistent with previous reports of cortico-muscular coherence at tremor frequency and its first harmonic[87]. In fact, transcranial alternating current stimulation over the motor cortex when specifically phase aligned can provide nearly 50% reduction in tremor amplitude[85].

1.3.3 Physiological tremor

Physiological tremor of the outstretched arms with a peak frequency of 7–12 Hz has been widely described[88 89 90]. A kinetic tremor of similar frequency has also been identified during slow upper limb movements[91 92]. The relative contribution of central and peripheral networks to this form of tremor remains debatable[53 93]. Considerable evidence points towards a central network underlying this type of tremor[92 94-105]. Despite this, mechanical resonance of the limb has been shown to generate a tremor with a similar spectrum[106], and some argue that the apparent motor unit synchronization and coherence between cortex and tremor EMG are an epiphenomena, reflecting peripheral resonance or reafference[107].

Lakie and colleagues[107] argue that changing mechanical properties of muscle in static versus dynamic conditions, affects how frequency and gain of tremor changes. Large amplitude movements (greater than observed in physiological tremor) of the wrist have an effect of relaxing the muscles, i.e. altering its thixotropic properties, thus modulating the resonance properties

of the hand. Lakie and colleagues showed this using a computer model of a hand with a single muscle behaving as a damped linear spring in series with a linear elastic tendon, with the exception that the muscle generated an active force. They modelled active force by passing the measured rectified EMG through a low-pass filter. They compared this scenario with the use of white noise though the low-pass filter instead of rectified EMG. Interestingly, either input (i.e. including the random perturbations from white noise) seemed to equally generate tremor-like acceleration profiles. Realistic reductions in stiffness closely reproduced the decrease in frequency and increase in amplitude observed in experiments. They argued that their simulations show that physiological tremor can be explained in entirely mechanical terms, without the need to invoke mechanisms that cause muscle force to fluctuate at tremor frequencies.

Chapter 2: Tremor in neuropathies – the clinical spectrum

Tremor seems to occur as a symptom of peripheral neuropathies including inflammatory neuropathies and hereditary neuropathies. It appears to be more prevalent in demyelinating neuropathies as opposed to pure axonal neuropathies, yet this may be specific to the type of neuropathy[1].

2.1 Tremor in inflammatory neuropathies

Immune-mediated neuropathies such as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are relatively common and disabling[108 109]. It has been recognised for many years that tremor can be an accompanying feature of peripheral neuropathy[110 111]. In immune neuropathies, tremor is found more often than not in patients with IgM paraproteinaemic neuropathy (IgMPN)[111-113], in patients with CIDP[114] and in the recovery phase of Guillain-Barré syndrome[115] although there is lack of a larger scale prospective assessment of this.

2.1.1 Tremor in IgMPN

IgMPN associated neuropathy is a primarily demyelinating neuropathy. Anti-MAG antibodies are the most common type of IgM antibody found in these acquired demyelinating peripheral neuropathies. The targets for human anti-MAG IgM in the peripheral nervous system (PNS) include MAG, a PNS glycolipid, and a PNS low-molecular-weight myelin protein, any of which (if any) may conceivably be involved in the pathogenesis of the tremor [116].

Clinically, anti-MAG neuropathy is similar to other inflammatory neuropathies such as CIDP but nerve conduction studies reveal prolonged distal motor latencies. There is a large variance in the prevalence of tremor reported in this condition. Some series do not report any tremor whilst others report up to 90% prevalence of tremor. However, more reliable reports aiming to identify this symptom report arm tremor in 40-90% of anti-MAG neuropathy patients [117-119]. Tremor was thought to be a later symptom of this condition and this was felt to explain the apparent difference in prevalence of tremor between different published cohorts [117 120-125]. Specifically, Smith [126] suggested that this variation may relate to the length of follow-up in these studies. Although anti-MAG neuropathy rarely presents with tremor, the range of time of onset between neuropathic symptoms and tremor in these studies was 1.5 to 12 years (mean 4.7 years). Another suggested reason for not reporting tremor was that it tends to be mild and thus overlooked [126], although it can be disabling at one extreme. When tremor occurs, it tends to be late-onset, male dominant, slowly progressive, symmetrical, and the neuropathy is dominated by sensory loss and is often rather refractory to treatment. Tremor has also been reported to occur in IgG and IgA paraproteinaemic neuropathies without anti-MAG activity [117 127]. The frequency of oscillation is predominantly 3-6 Hz [117 128]. There have been no previous associations with weakness, proprioceptive loss or other neurological findings predicting [111 117 128] the presence or absence of tremor in this condition. Recordings revealed a tremor frequency that seemed to co-vary between 3.3 and 10Hz with ulnar conduction velocities; higher velocities associated with higher tremor frequencies. Amplitude did not seem to share

a similar association. The pathophysiology is as yet undetermined but has been suggested to depend on central nervous system changes [128 129]. Bain et al[128] considered the alternative explanation in the neuropathy where tremor is most prevalent. They posited that in patients with IgM paraproteinaemic neuropathy, the development of a coincidental tremulous condition is unlikely on epidemiological, clinical and neurophysiological grounds. The percentage of patients with IgM paraproteinaemic neuropathy that have tremor, as mentioned up to 90% in some reports, is 45 times greater than the prevalence of essential tremor in the whole population[12]. Furthermore, the clinical features of the tremor associated with IgM paraproteinaemic neuropathy are not those of hereditary essential tremor. The male preponderance, the typically negative family history and the mean age at onset of 59 years in patients with tremor and IgM paraproteinaemic neuropathy contrast with the equal sex incidence, positive family history and a median age of onset in the second decade in patients with hereditary essential tremor, although in the latter condition the age of onset has a bimodal distribution with a second lesser peak in the fifth decade[5]. Lastly, the polymyographic findings in patients with IgM paraproteinaemic neuropathy differ from those obtained in patients with essential or parkinsonian tremor.

The largest study to address this issue to date has been a retrospective series[1]. In this cohort of patients with IgM monoclonal gammopathy of undetermined significance (IgM-MGUS), tremor occurred in 29% (60) of 207 patients compared to 9% (38) of 414 matched other-cause neuropathy controls. Of the 207 IgM patients, 70% (145) had a demyelinating neuropathy

while 30% (62) had an axonal neuropathy. Amongst the tremulous IgM patients, there was an over-representation of demyelinating neuropathies (82%; 49) compared with axonal neuropathies (18%; 11), although clearly tremor was also common in axonal neuropathies (see table 2.1). Of 414 controls, only 9.2% (38) were found to have tremor. The ratio of axonal to demyelinating neuropathies in the group was 75%:25% respectively. This would implicate the presence of an IgM paraprotein as the single strongest determinant in developing tremor in neuropathies rather than purely the presence of demyelination. Markedly absent from the report of this series, however, was the MAG antibody status in serum as this in other series has been the strongest determinant for the presence of tremor. Nevertheless, the results seem to also suggest that in the IgM group, tremor is more common and particularly in the demyelinating sub-group, whilst for non-IgM controls, tremor is less common and when it does occur, it is particularly in the axonal sub-group. This might implicate axonal neuropathies as causal or perhaps more likely, unexplored by the authors, that the axonal neuropathy group is a more heterogeneous group with a variety of confounds for tremor. Indeed, axonal neuropathies in the wider literature make up the bulk of type of neuropathies and the causes are typically more heterogeneous than demyelinating neuropathies which nearly always tend to be hereditary or inflammatory. In the demyelinating neuropathy group, all those with tremor had an inherited or inflammatory neuropathy. Interestingly, in the control group patients, age significantly correlated with tremor occurrence. Of patients with IgM neuropathies and tremor, a rest hand tremor has been reported[130 131] and comprised 13.6% of the cohort in Ahlskog et al's[1] case

series of IgM associated tremor. Other body parts may similarly but rarely be affected including chin, head or voice tremor; in aggregate comprising 5% of the Ahlskog et al[1] series, but not reported by other authors. Moreover, in the published cohort of Ahlskog et al, there was no individual case description so it is difficult to know whether this could have represented a confound such as essential or other primary tremor. These are usually patients without neuropathic symptoms in these body parts so it raises the possibility of either a confounding cause for tremor or if IgM is mechanistically related, perhaps not directly through the neuropathy, i.e. via direct central mechanisms. Also, strengthening this argument was the symmetry of tremor in the limbs, contrary to possible asymmetry of some of the neuropathies although this data was not explicitly available in the published paper and further, judgement of symmetry of tremor clinically often contrasts with potentially rather marked asymmetry on tremor quantification using accelerometry. One patient with disabling familial tremor responded well to DBS of the left VIM. The authors stated the case that this patient's tremor may have related to the neuropathy rather than being solely a familial tremor. This was argued on the grounds that his tremor was markedly worse than other family members and worsened with the course of the neuropathy. Ahlskog et al also demonstrated in their cohort, a higher prevalence of tremor in those with more severe neuropathies. However, this result needs to be taken with caution in a retrospective case series as it may well instead implicate the association of greater severity of tremor with more severe neuropathies, given the likely lack of documentation of mild tremor in a retrospective case series from a neuromuscular centre. On the other hand, some cases may

represent the concurrent diagnosis of a common condition such as essential tremor (ET). The pathophysiology of inflammatory neuropathy related tremor remains uncertain. There have not been any definitive central sensory or motor pathway abnormalities demonstrated, however, a role for the cerebellum has been postulated [128]. To add to the weight of this evidence is enhanced activation of the cerebellum in patients with neuropathy and tremor in functional imaging studies [132]. Further, there is indirect evidence of IgM binding in the cerebellum in patients with IgM paraproteinaemic neuropathies with features such as tremor [118 133].

Control group	Axonal	Demyelinating		IgM group	Axonal	Demyelinating
Tremulous	34	4		Tremulous	11	49
Non-tremulous	277	99		Non-tremulous	51	96

Table 2.1 Primary results from Ahlskog et al[1] shown as a cross-table distribution for each group.

2.1.2 Tremor in chronic inflammatory demyelinating polyradiculoneuropathy

CIDP, a chronic relapsing-remitting or progressive inflammatory neuropathy is autoimmune in nature and associated with demyelination of nerves and their roots. The proportion of patients with CIDP in whom tremor is reported varies widely from 3%[134] to 84% [135] and may well be under-recognised. In a recently discussed study reporting the prevalence of tremor in a cohort of patients with neuropathies, although the exact number of CIDP patients in the control group with tremor is not explicitly stated by the authors, by inference the figure stands at no more than 10.5%[1]. However, as mentioned,

such a retrospective series, spanning 34 years suffers from a likely under-estimation of tremor, given that this was not an intended focus when data was being documented for each patient. Dalakas et al [117] reported seven patients with CIDP and four with polyneuropathy associated with a monoclonal gammopathy who had tremor during the course of their illness. The recorded tremor frequency varied between 3.3 Hz and 6.4 Hz. Some series have shown co-variation of the presence of tremor with the severity of the neuropathy symptoms. However, there was no good correlation between the presence of tremor and the severity of muscle weakness, proprioceptive loss or motor conduction velocity. Further, motor conduction velocities seemed not to vary in those with or without a tremor[136]. Most series describe a postural and kinetic tremor of the hands, although uncommonly variants are also reported such as the existence of hand tremor at rest[130] although such individual variants are subject to potential coexistent pathology such as parkinsonism or primary tremor syndrome such as essential tremor.

2.1.3 Treatment response of tremor in neuropathies

Although tremor improves with treatment of the underlying disease in some patients[117], a significant burden of the disease may be refractory to treatment or require considerable immunosuppression[114]. The response of the neuropathy or the tremor to immunomodulatory treatment with steroids or steroid-sparing agents, intravenous immunoglobulin and plasmapharesis has been varied, improving neuropathic and tremulous symptoms in some patients but not others[117 123] but has been reported to improve in the context

of patients with ataxia[122]. The tremor is often not particularly disabling to the patient but where it is disabling, generic anti-tremor approaches to medication may be employed. Propranolol, for example, has been shown to have mixed effect[117 137 138]. Three patients with tremor were treated with steroids, one (anti-MAG positive) had no response but in two, the tremor improved as the neuropathy improved. One patient with anti-MAG neuropathy and tremor was described to have improved from the neuropathy and tremor point of view following rituximab, a monoclonal anti-CD20 treatment. This is not typical for anti-MAG neuropathy, as it can often prove fairly resistant to treatment. Deriving any certainty from current data is futile given the low numbers and heterogeneous sample reported in an array of small case series. In hereditary neuropathies, the response of treatment to propranolol is not clear but it has been suggested that some patients do respond[137]. There is accumulating evidence that VIM stimulation with DBS is an effective treatment of neuropathic tremor, as with many tremor types. A patient with a demyelinating tremor coined as hereditary Roussy-Levy syndrome but without definitive genetic confirmation[139], a patient with IgM paraproteinaemic tremor[140], another with IgM kappa associated demyelinating neuropathy but insufficient clinical detail to determine if a typical phenotype for anti-MAG (demyelinating distal sensorimotor neuropathy with widely spaced myelin and prolonged distal motor latencies)[141] and other similar cases[142 143] have all benefited from VIM stimulation.

2.2 Hereditary neuropathies

Genetically inherited neuropathies (e.g. Charcot-Marie Tooth (CMT), distal hereditary motor neuropathy (dHMN)) are an important cause of neuropathies. These disorders are estimated to affect over 24,000 people in the UK. Neuropathic tremor was described in hereditary neuropathies as part of Roussy-Levy syndrome[144], which has since been shown to be a genetically heterogeneous entity[145 146]. In some patients with CMT, tremor is a prominent symptom, and prior to advances in genetic understanding of CMT such patients were sometimes classified as *Roussy-Levy syndrome*. CMT can be divided into type 1 and type 2, amongst others. Type 1 has predominantly demyelinating damage to nerves and type 2, predominantly axonal. Tremor has been asserted to be commoner in type 1 in a comparison with type CMT2[147] but the evidence for this rests on little systematic data. Recent genetic advances have demonstrated that patients with Roussy-Levy syndrome do not have a single genetic abnormality, with the original Roussy-Levy family having been shown to have a point mutation in the myelin protein zero gene (MPZ)[146] with other families with the Roussy-Levy phenotype reported to have the chromosome 17 duplication and connexin 32 mutations[148 149]. Roussy-Levy syndrome (RLS) was originally described as a dominantly inherited early-onset syndrome with features of gait ataxia, pes cavus, areflexia, with evolution of muscle atrophy, postural limb tremor, limb ataxia, kyphoscoliosis and sensory loss. Zubair et al[150] amongst others have collectively described an array of genes associated with this phenotype. Thus, the syndrome of RLS does not provide a highly specific phenotype-genotype correlation. In their case series, one man and his daughter were

found to have a point mutation in the peripheral myelin protein (PMP)22 allele 1 with T-to-C translocation at codon position 108 (amino acid change leucine to proline). RLS has been described to be somewhat distinct from other hereditary neuropathies predominantly due to its prominent ataxia. Salisachs and colleagues[151] described seven patients with hereditary sensorimotor neuropathy and tremor, four of whom were significantly disabled by the tremor. The dominant pattern of neuropathy was distal motor weakness in the limbs with little sensory loss and markedly reduced motor conduction velocities. The tremor has been reported to be suppressible with propranolol.

2.3 Putative mechanisms of neuropathic tremor

To begin this section, it is useful to look at other types of tremor, where considerably more evidence has been published. The accumulating evidence delineating the pathological network of essential tremor comes from structural, functional and molecular imaging as well as eye movement analysis, gait analysis, electrophysiological techniques including MEG and intracranial recordings from DBS electrodes. Far fewer data exist for the rarer neuropathic tremor. Currently there are functional imaging data, electrophysiological results and immunological studies that might help shape our understanding of this symptom. These shall be appraised in relation to each other. On the basis of current evidence, neuropathic tremor appears to occur particularly in patients with demyelinating neuropathies with reduced

conduction velocities, although from the evidence presented, this appears not to be exclusive and not predictive of severity when present.

Thus, conceivably, tremor might relate to the altered timing and dispersion of combined action potentials of peripheral afferents and efferents[152], although challenging this is lack of a straightforward relationship between the development of tremor and conduction velocity. Although, Smith [153] recorded tremor frequency in patients with IgMPN and in all patients there was a direct correlation between tremor frequency and ulnar nerve motor conduction velocity (higher velocities associated with higher frequency tremor). However, no relationship seems to exist between tremor and the severity of neuropathy as assessed by proprioceptive loss, weakness or fatigue[117 136] although tremor may be commoner during a relapse [117]. There is also a lack of difference between motor nerve conduction velocities in those with versus those without tremor [136]. Nevertheless, slowing of nerve conduction may improve with improvement of the neuropathy and this has been shown in a small number of patients to be associated with an increase in the tremor frequency. Such a relationship might indicate tremor frequency is determined by conduction velocity, which if true, would likely invoke the stretch reflex mechanism as part responsible for the generation of tremor given theories of the role of the stretch reflex in so called peripheral reflex enhanced tremor. This appears not to be the only factor, given that many patients with slowed nerve conduction do not develop tremor and those with tremor may well have near-normal conduction velocities. Further, the critical finding of an association between conduction velocity and tremor frequency has not been

re-capitulated in other studies[128]. Indeed, in their study, Bain et al considered the possibility of a central nervous system integrator that may behave differently between patients and thus provide a variable determining the occurrence of tremor in this context, not provided for by parameters defining severity of the neuropathy. One would have to assume that such variance in such a putative CNS integrator would have to be particular to neuropathic tremor given the relative lack of tremor in the context of other types of neuropathy such as diabetic neuropathies which may well cause demyelination, i.e. demyelination of peripheral nerves and roots alone seems insufficient to generate tremor. This may raise the possibility of a mechanism intrinsic to the neuropathy disease that also affects the central nervous system. In inflammatory neuropathies, a direct immunogenic target, similar to the antibodies targeting peripheral nerves, to central nervous system structures, would most parsimoniously explain this. For hereditary neuropathies, the process would likely be a central degenerative process akin to occurring in the periphery. In their study, Bain et al[128] compared six patients with IgM paraproteinaemic neuropathy and tremor with healthy controls, but not with non-tremulous neuropathy patients. The tremor was of variable amplitude and frequency (not correlated with nerve conduction velocity). There was no evidence of a central delay in conduction (the central portion of somatosensory evoked potentials was of normal latency), wrist tremor could be phase reset by median nerve stimuli to an extent that would make the peripheral nervous system important in the network of tremor rather than acting as a simple final common pathway for expression of tremor. Ballistic wrist movements demonstrated a delayed second agonist

burst of the triphasic EMG and underdamped oscillations after wrist movement, seen in essential tremor[154] and cerebellar disease[155 156]. The authors suggested that temporally dispersed peripheral inputs from demyelinated nerves reach a central processor, such as the cerebellum, which is misled into producing a delayed second agonist burst and tremor. A number of aspects of motor system physiology depend on precisely timed interactions between sensory afferents from the limbs and motor output. Given the clinical finding that demyelinating neuropathies rather than axonal neuropathies most commonly give rise to tremor, again, it might suggest that afferent and or efferent delay are important pathophysiological factors in the development of neuropathic tremor. However, as discussed, delay in conduction alone cannot be the cause of neuropathic tremor given that tremor does not occur in all patients with demyelinating neuropathy and that there is no relationship between conduction velocity and presence of tremor. As such, rather than it simply being delay in conduction, it may be the degree of temporal dispersion of input and output that gives rise to tremor. Experimental paradigms that are thought to correspond to motor sensory integration are dependent on precise central nervous system conduction times. It is possible that if input and output are highly dispersed and adaptation is not possible or effective, continuous iterative errors that are not corrected may conceivably produce an oscillatory output such as tremor. Alternatively, it may be that individual differences in cerebellar function impair the ability to adapt to dispersed sensori-motor signals in the periphery.

This ties in with the proposed role of the cerebellum as an adaptive filter[157]. Damage to the cerebellum due to mechanisms innate to the neuropathic disease process such as direct cerebellar inflammation in inflammatory neuropathies or cerebellar damage in hereditary neuropathies could conceivably interfere with such sensory-motor integration. Alternatively, it may be that certain individuals do not have the capacity to adapt to mismatched motor commands and motor efference copies or mismatched sensory information with planned motor commands. This could cause problems with such integration in the cerebellum or may exist more widely affecting disparate parts of the central nervous system. In order to test such hypotheses, one could look to clinical and electrophysiological processes indicating integrity of motor control and learning in the cerebellum.

A unique insight has been gleaned from a single patient study in this regard. Weiss et al[129] reported a patient with tremor associated with IgM paraproteinaemic neuropathy after deep brain stimulation of the VIM. Coherence analysis between local field potentials (LFP) from DBS electrode leads implanted in the thalamus, M1 EEG and contralateral flexor and extensor digitorum EMG. They found significant coherence between all combinations: M1 and contralateral EMG, VIM and ipsilateral M1, VIM and contralateral EMG but not VIM and contralateral M1. Directed transfer function and phase analyses were used to demonstrate information flow unidirectionally from M1 to VIM and bi-directionally between M1 and muscle, VIM and muscle respectively. VIM stimulation caused suppression of the cortico-muscular coherence. Re-afference lagged the feedforward drive.

However, this is a single case report and as such its generalizability is limited. Activation of the VIM likely indicates cerebellar involvement in the same network but this was not explicitly measured in this study. The presence of functional involvement of the cerebellum in neuropathic tremor is also supported by positron-emission tomography (PET) findings demonstrating cerebellar hyperactivity in this condition [132]. Brooks et al[2] had presented results summarising a PET study of regional blood flow in 8 ET and 6 neuropathic tremor patients and 6 age-matched controls[2]. The results, reported in abstract form, are summarised in table 2.2. It is, however, not clear whether this finding reflects primary changes in the cerebellum or whether this is secondary to the effect of the tremor, except for the finding of bilateral cerebellar activation in limb tremor on posture in contrast to purely ipsilateral cerebellar activation on a similar task in healthy controls mimicking tremor. Also, bilateral cerebellar activation is seen at rest as well as during involuntary tremor, suggesting the possibility of a static abnormality of the cerebellum perhaps similarly reflected by the abnormal classical eyeblink conditioning. However, there remains a possibility that his could represent a function of oscillatory frequency or other confound. There have been no studies to date assessing the structural integrity of the cerebellum in patients with neuropathic tremor compared to those with neuropathy but without tremor.

	Essential tremor	**Neuropathic tremor**	**Healthy controls**
Rest state	↑cerebellar bilaterally	↑cerebellar bilaterally	Normal
Passive movement	↑some cerebellar bilaterally	↑some cerebellar bilaterally	-
Posture (extended)	-	-	↑cerebellar ipsilaterally
Involuntary tremor	↑cerebellar bilaterally ↑cingulate ↑contralateral sensorimotor cortex ↑contralateral thalamus	↑cerebellar bilaterally ↑cingulate ↑contralateral sensorimotor cortex	-
Mimicked tremor	-	-	↑ cerebellar ipsilaterally

Table 2.2 Summary results of PET imaging in neuropathic and essential tremor versus controls (Summarised from Brooks et al[2]) (↑ indicates increased PET activity).

A central nervous system component to these diseases is recognised to exist, but only in the small minority of cases. Leger et al[119], for example, investigated 13 patients with IgMPN of demyelinating type and antibodies to

glycolipids present in CNS white matter in five patients, two of whom had abnormal MRI. Three had measurably prolonged central motor conduction times, of whom two patients had anti-CNS glycoplipid antibodies. Three of those with measurable CNS anti-glycolipid antibodies also had tremor. Tremor seemed to occur only in those with anti-MAG antibodies but did not seem to correlate with paraprotein level or light chain subtype. However, the MRI changes may well be confounded by age as suggested in another study of patients selected only by the need for nerve biopsy and diagnosis of CIDP[158].

Tremor in a patient with a neuropathy could conceivably have its origin from a number of different mechanisms (which may not be mutually exclusive):

1) Concurrent central nervous system disease driven by the same process causing the neuropathy, i.e. antibody-mediated or genetic mechanism and a purely centrally driven tremor potentially akin to those seen, for example, in cerebellar, Holmes or essential tremor.
2) Coincident tremor syndrome (e.g. ET). Although this may conceivably account for a small number of cases proportional to the prevalence of ET (0.5 – 5%), the temporal onset of neuropathy and tremor and the high prevalence of tremor in patients with specific neuropathies make this unlikely an explanation for the majority of cases.
3) In the context of a hereditary neuropathy, coexistent primary tremor syndrome such as essential tremor associated by genetic linkage. Little has been established here and even where it is reported, there seems

doubt about the likelihood of this being the case. For example, Louis et al[159] presented a case series of a family with TRPV4 mutation CMT 2C presenting with typical leg and voice abnormalities. In addition, there was a possible autosomal dominant history of isolated tremor in the family but this did not seem to segregate with CMT and so it was concluded that this tremor likely represented coexistent essential tremor. However, analysis of the pedigree reveals seven patients affected with either tremor or CMT, three with both; two with tremor alone and two with CMT alone. However, there are four siblings with tremor as well as their maternal uncle. Their mother is not stated to be affected by tremor in the pedigree. If one assumes that this is autosomal dominant ET, as the authors do, then by definition, the deceased mother must have carried the gene, but not manifested either due to incomplete penetrance or being undiagnosed. The pedigree also suggests that tremor is much more likely in those with the TRPV4 gene mutation. This could lend itself to a 'two hit' hypothesis for tremor generation with a susceptibility to tremor being important and the CMT making it more likely to manifest. This is more likely than mere co-existence or co-segregation of genes as this was not the case with this case series where the tremor and TRPV4 mutation did not co-segregate. Other such susceptibility genes in GWAS studies have been reported such as LINGO1 but on repeated studies, it seems these are likely susceptibility factors in limited populations.

4) Peripheral demyelination with maladaptive central sensorimotor integration as a trigger to cerebellothalamo-cortical network driven tremor.

5) Reflex mechanical tremor (enhanced physiological).
6) An alternative hyperkinetic movement or tremor 'mimic' such as 'fasciculatory tremor', pseudoathetosis and polyminimyoclonus, all distinguishable from tremor (as defined by the MDS task force[3]) on clinical grounds.
7) Peripheral myoclonic tremor. There are case reports of tremor arising after peripheral nerve injury[160] but these likely represent myoclonus given the irregularity of tremor shown in the needle EMG and the persistence during sleep. This also raises the discussion regarding peripherally-induced movement disorders for which there is considerable controversy. Many feel that this represents a group of disorders with psychogenic/functional aetiology whilst others argue that this represents an organic group of conditions[161 162]. The conditions that are represented under this rubric are heterogeneous. Neuropathic tremor occurs in the context of damage to peripheral nerves and so is relevant in this discussion.

2.3.1 A role for the cerebellum in neuropathic tremor

Voluntary movements are based on interacting but spatially distributed neural networks. Connectivity between such disparate regions may depend upon synchronised oscillatory behaviour (for a review, see McAuley and Marsden[101]; and Schnitzler and Gross [163]). Using electroencephalography (EEG), coherent activity between the prefrontal cortex, lateral, and mesial premotor areas, the primary sensorimotor, and posterior parietal cortex

during the execution of rhythmic finger-tapping tasks[164 165 166] has been demonstrated. EEG and magnetoencephalography (MEG) have been used to demonstrate that pathological[81] as well as physiological but involuntary movements[167] are associated with oscillatory coupling in a cerebello-thalamocortical network. Pollock et al[168] (figures 2.1 and 2.2) aimed to determine in healthy controls, the oscillatory network associated with a *unimanual auditorily* paced finger-tapping task and the dynamic relationship between these structures. Subjects performed a finger tapping task with interstimulus interval (ISI) of 800 ms. Their results demonstrated that an auditory paced finger-tapping task is associated with a cerebello-thalamocortical network comprised of primary auditory cortex and cerebellum ipsilateral to the tapping hand; thalamus, SMA, PPC, and S1/M1 contralateral to the tapping hand, providing comparable agreement with previous fMRI-studies[169-173]. Within this network, the pivotal role of the cerebellum in the execution of movements has been substantiated in imaging studies[174 175]. Specifically, it has been put forth that the cerebellum is involved in monitoring and optimizing movements by processing sensory information. Indeed, data from a PET study demonstrated that the cerebellar signal is, at least partially, a result of the processing of proprioceptive information. Furthermore, the cerebellum shows increased activation during visual guidance of a movement when compared to the execution of the same movement without visual information (for a review, see Jueptner and Weiller [176]). However, it should be stressed that cerebellar activity has been observed in the absence of somatosensory or visual feedback indicating the pivotal role of the cerebellum in motor processing[177]. The cerebellum

influences the motor cortex via the cerebello-thalamocortical pathway (for a review, see Horne and Butler[178]; Middleton and Strick [179]). Evidence has been found for further connections between cerebellum and premotor and primary motor areas[179]. Although the precise functional meaning of these connections is still uncertain, it has been suggested that via connections to the primary motor cortex the cerebellum receives an efference copy, which is compared to sensory information about the outcome of the movement [178]. Connectivity between premotor areas and cerebellum may be concerned with higher order aspects of motor behaviour like the execution of movement sequences[179].

Papka et al[180] demonstrated likely overlapping cerebellar circuitry between eyeblink classical conditioning (EBCC) and tapping tasks. This was shown by providing data illustrating the effect of tapping in interfering with EBCC. Controls for hippocampal-dependent declarative memory system along with recognition and other non-cerebellar-dependent tasks were effectively used to rule out confounds.

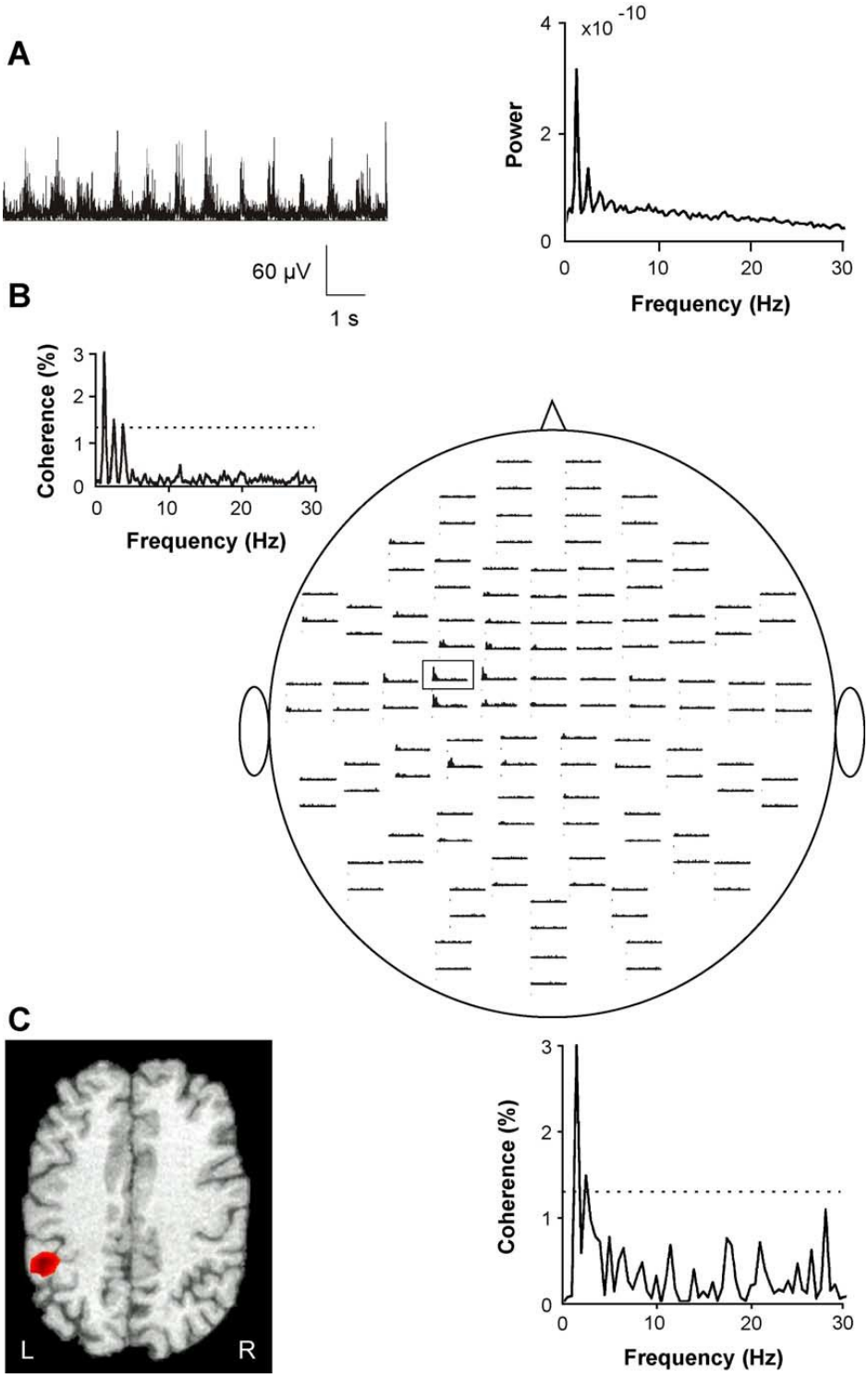


Figure 2.1 Analysis of cerebromuscular coherence of the right-hand condition in one representative subject. From Pollok et al[168]. (A) Left panel: traces of surface EMG activity of right first dorsal interosseus (FDI). EMG was high-pass filtered at 60 Hz and rectified. Regular EMG bursts occur at the frequency of the paced movement of 1.2 Hz. Right panel: FDI power

spectral activity. Distinct peaks at 1.2, 2.5, and to a lesser degree at 3.7 Hz, representing the movement frequency and its first and second harmonic. (B) Coherence between right FDI and MEG sensors. Coherent activity was restricted to the sensors covering the left parietal area. Coupling occurred at 1.2, 2.5, and 3.7 Hz representing the movement frequency and its first and second harmonic. The dashed line indicates the 95% confidence level of coherence. (C) Localization of cerebromuscular coherence at 1.2 Hz as revealed with DICS in the individual MRI scan. In all subjects, the source with the strongest coherence to right FDI was localized in the contralateral sensorimotor hand area. Coherence between EMG and the S1/M1 source was observed at 1.4 and at 2.5 Hz. The dashed line indicates the 95% confidence level of coherence.

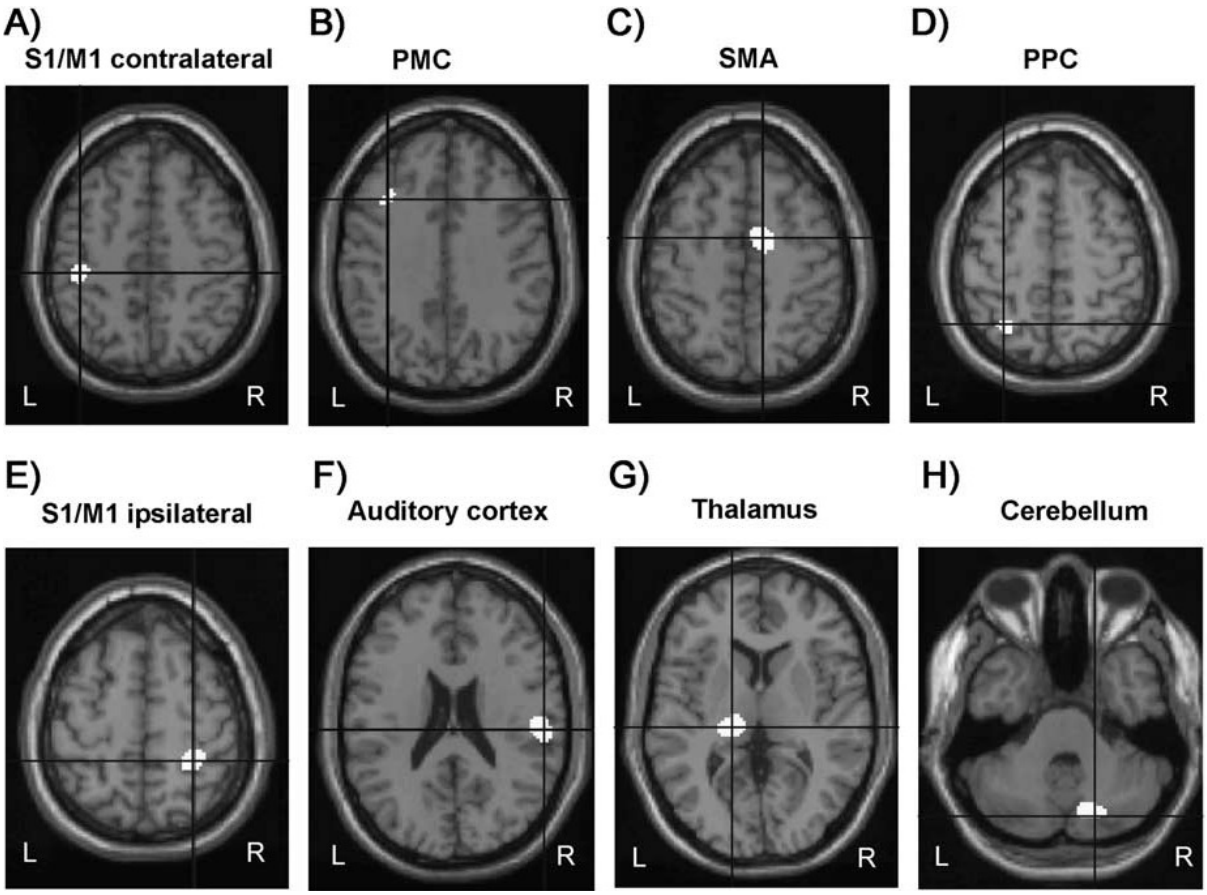


Figure 2.2 Mean localization of cerebral sources across all subjects for the right-hand condition. From Pollok et al[168]. Mean localization of cerebral sources across all subjects for the right-hand condition. Sources were localized with respect to the S1/M1 source **(A)**. Coherent activity was found in the premotor cortex **(B)**, SMA **(C)**, posterior parietal cortex **(D)**, ipsilateral S1/M1 **(E)**, and superior temporal sulcus corresponding to the primary auditory cortex **(F)**. Additionally, coherent activity was detected in the thalamus **(G)** and in the ipsilateral cerebellum **(H)**. Crossing lines indicate the local activity maximum of each source.

Chapter 3: Aims and hypotheses

We hypothesise that tremor is an occasional feature of Charcot-Marie-Tooth, common feature of inflammatory neuropathy and is a contributor to disability; that certain clinical features (e.g. more rapid progression, large fluctuations in symptom severity (immune neuropathy)) will be associated with a greater likelihood of developing tremor. There is a considerably lower prevalence of severe, disabling tremor associated with hereditary as opposed to inflammatory neuropathies. There are also fewer reports in the literature of deep brain stimulation being required to treat CMT associated tremor whilst there are emerging reports of this last resort treatment being used in tremor in inflammatory neuropathies. These features suggest that there may be different mechanisms causing tremor in hereditary versus inflammatory neuropathies. We further explore the pathophysiological features of tremor associated with both inflammatory neuropathies and CMT in this study.

We set out to explore aspects of central nervous system physiology in a group of tremulous and non-tremulous patients with inflammatory neuropathies and compared these to healthy controls. We perform a similar comparison for patients with CMT. We propose that measures of dispersion of sensory input and motor output will be different in patients with peripheral neuropathy and tremor compared to those without tremor. We hypothesise that the central compensation needed to account for (afferent and efferent) delays caused by the peripheral neuropathy would most likely depend on plastic changes within the cerebellum and connections that mediate interaction between sensory and motor systems. We hypothesise that

patients with tremor would have evidence of dysfunction in the cerebellum and interactions in sensorimotor cortex compared to non-tremulous patients and healthy controls. We tested paired associative stimulation (PAS)[181], a paradigm akin to long-term potentiation-like plasticity in the sensorimotor cortex, short-afferent inhibition (SAI), a measure of sensorimotor integration in the sensorimotor cortex and eye-blink classical conditioning (EBCC), an associative conditioning paradigm, dependent on the cerebellum for acquisition[182 183] (see Bracha[184] for review).

Our hypothesis is that normal cerebellar function is necessary to adapt to mistimed and unpredictable peripheral nerve conduction, and where this fails, it is associated with the production of tremor, perhaps due to mismatch between the efference copy and sensory feedback from the periphery. Defining the effects of cerebellar stimulation on timing modulation of rhythmic movements and the potential effect on tremor could prove a primer for further work aimed towards understanding pathogenesis but also at investigating potentially new treatment approaches. In ET, there is multimodal evidence for dysfunction in the cerebellum that includes eye movement analysis, classical associative eyeblink conditioning, structural and functional imaging and pathology. There is also evidence that rhythmic finger tapping is also abnormal and consistent with damage to the cerebellum. In fact non-invasive brain stimulation methods including rTMS to the cerebellum have probed tapping in patients with ET. Others have demonstrated that rTMS may be able to affect motor tasks attributable to the cerebellum. It has also been shown that polarity-specific stimulation can modulate motor tasks dependent

on the cerebellum[185]. In aggregate, these have demonstrated that there is an abnormality in tapping that can be temporarily ameliorated with rTMS that then reverts to the pre-stimulation state. Tapping seems to potentially use similar oscillatory networks to tremor[2 168], posing the question of whether modulating timing properties of the cerebellum may effectively alter both tapping and tremor through similar mechanisms. We aim to explore whether modulation of these putative timing properties are achievable using TDCS over the cerebellum. If timing could be enhanced (polarity specific), then a similar paradigm could be assessed (polarity specific) with NT to see if tremor modulation is achievable in correspondence with the effects on the timing properties of the cerebellum.

3.1 Aims: Chapter 5: Tremor in inflammatory neuropathies

1) Determine the prevalence of tremor in a single centre inflammatory neuropathy service.
2) Determine the clinical features and electrophysiological characteristics of those patients with tremor to determine if any variables predict occurrence or severity of tremor.
3) Determine differences in the subtypes of inflammatory neuropathies and whether these predict prevalence or characteristics of tremor.

3.2 Aims: Chapter 6: Cerebellar function in inflammatory neuropathy tremor

1) Measure and compare a group of patients with inflammatory neuropathies and tremor, those without tremor and healthy controls on the following paradigms:
    a. Tremor accelerometry and surface EMG
    b. Classical eyeblink conditioning
    c. Paired associative stimulation
    d. Short-afferent inhibition
    e. Somatosensory evoked potentials

3.3 Aims: Chapter 7: Tremor in Charcot-Marie-Tooth disease

1) Determine the prevalence of tremor in a single centre hereditary neuropathy service.
2) Determine the clinical features and electrophysiological characteristics of those patients with tremor to determine if any variables predict occurrence or severity of tremor.
3) Determine differences in the subtypes of hereditary neuropathies and whether these predict prevalence or characteristics of tremor.
4) Measure and compare a group of patients with CMT1A neuropathies and tremor, those without tremor and healthy controls on the following paradigms:
    a. Tremor accelerometry and surface EMG
    b. Classical eyeblink conditioning
    c. Eye movement analysis using electro-oculography
    d. Visuomotor adaptation

3.4 Aims: Chapter 8: Effect of TDCS of cerebellum on rhythmic finger tapping

1) Determine polarity-dependent effects of cerebellar transcranial direct current stimulation on variability and timing of rhythmic finger tapping task during synchronisation and continuation phases.

Chapter 4: Methods

Where applicable, patients were divided into tremulous and non-tremulous groups depending on whether arm tremor was clinically detectable. Healthy controls, where applicable were also considered in a separate group. This was the basis for group-wise analysis where applicable. Each group was matched for age, gender, diagnosis and severity of neuropathy. Detailed clinical assessment was performed (see section 4.2 below). Patients taking medications potentially causing tremor were excluded from electrophysiological studies. Clinical assessments included summed scores for limb strength (MRC score)[186], sensation (subset of CMT neuropathy score[187]) and deep tendon reflexes (NINDS myotactic reflex scale[188]). Tremor was assessed using the Fahn-Tolosa-Marin score[189] and the Bain and Findley spiral score depending on the study. The CMT study included saccadic and pursuit eye movements recordings as well as a positional manoeuvre for signs of cerebellar dysfunction[190]. Neuropathy severity was assessed with the CMT neuropathy score[187] (where applicable) and disability measured by the overall neuropathy limitation scale[191]. Patients were divided into two groups for subsequent analysis depending on the clinical presence of tremor (tremulous and non-tremulous groups). Patients' nerve conduction studies were reviewed.

4.1 An electrophysiological approach to studying tremor

A myriad of methodological approaches have been used to study tremor. These include direct measures of the tremor, for example, with

accelerometry, surface EMG or goniometry, measuring respectively, the acceleration of a body part, summative muscular electrical activity and angular deviation of body parts due to tremor. Other methods such as classical eyeblink conditioning and eye movement analysis or gait analysis are used to make inferences regards the pathophysiology of the tremor based on known anatomy and networks underlying such physiological tasks.

4.1.1 Non-invasive brain stimulation

Non-invasive brain stimulation includes both transcranial magnetic stimulation (TMS) and TDCS. TMS is a non-invasive focal method for modulating cortical activity. It utilizes the principle of electromagnetic induction and involves discharging a large current from a set of capacitors that flows through a circuit and copper-wire coil. This current induces a rapid time-varying magnetic field in the coil and when held over the head of a subject, the magnetic field penetrates the scalp and skull inducing a very small current parallel to the plane of the coil in the brain. With sufficient current, neuronal membranes depolarize and action potentials are generated. This ability of TMS to alter cortical activity has allowed for a wide array of uses. Multiple studies have since demonstrated that TMS can alter functional organization of the human cortex, leading to changes in both the neurophysiological properties of those brain areas and in the performance of tasks reliant on those regions. TDCS will be considered below.

4.1.1.1 Paired pulse techniques

With paired pulse techniques, two stimuli are delivered. The first is considered the conditioning stimulus followed by a test stimulus. The latter, if over motor cortex, may generate a motor evoked potential, an electrical potential measurable by EMG. The MEP amplitude generated from TMS stimulation of the contralateral motor cortex is a function of the interstimulus interval and the intensity of the stimulus. The conditioning stimulus can be TMS or electrical stimulation over brain, spinal cord or lower motor neurons. Examples of paired pulse stimulation include short latency intracortical inhibition and long latency intracortical inhibition where each stimulus is given over motor cortex with short and long interstimulus intervals respectively.

4.1.1.1.1 Short afferent inhibition

Ascending sensory information from the periphery reaches the sensory cortex and has a direct and indirect modulatory effect on motor output and motor learning. In demyelinating peripheral neuropathies, the ascending sensory input, at least at the peripheral level, is dispersed and delayed and may conceivably impact upon subsequent motor sensory integration in the cortex. It is seen to be abnormal in a number of neurological diseases including Parkinson's disease[192] and Alzheimer's disease[193].

Short afferent inhibition is a paradigm that uses peripheral nerve stimulation as the conditioning stimulus followed by a contralateral motor cortical test stimulus timed to collide with the ascending afferent volley. This phenomenon is recordable when the interstimulus interval between median nerve stimulation and motor cortex stimulation is between 2 and 8 ms longer than latency of the afferent volley from median nerve stimulation to arrive at the cortex. A ratio of the effect size is determined by measuring the MEP amplitude arising from the test stimulation as a proportion to MEP amplitudes arising from a series of test stimuli that are not preceded by conditioning stimuli. Typically, the resulting MEP amplitude from paired pulse stimulation is smaller than arising from the test stimulus alone. This is termed short-afferent inhibition and is thought to reflect sensory-motor integration in the cortex, perhaps primarily through cholinergic transmission. It is dependent on precise timing and appropriate cortical mechanisms enabling such integration. It has been demonstrated that stimulation of the median nerve at the wrist can inhibit EMG responses evoked in the FDI and APB muscles by TMS of the motor cortex[194 195]. They provided evidence to support the view that this represents a reduction of cortical excitability. Firstly, they were able to modulate the number and amplitude of descending corticospinal volleys elicited by cortical magnetic stimulation by preceding these stimuli with median nerve electrical stimuli at specific inter-stimulus intervals modified to accommodate varying muscle-cortex conduction times between patients. Secondly, comparison of TMS with Transcranial Electrical Stimulation (TES) in other studies showed that peripheral electrical pulses reduced the size of MEPs elicited with TMS but not, or much less, those following TES at

intervals between 20 and 30 ms indicating that the suppression at these intervals is intracortical in nature[195 196]. Evidence for a transcortical route of SAI comes from Tokimura et al[195] who recorded corticospinal volleys in patients with implanted electrodes in the cervical epidural space and showed that indirect waves (I2 and I3), that are of cortical origin, were smaller when the magnetic stimulus was given at appropriate ISI after the conditioning peripheral electrical pulse.

4.1.1.1.1.1 Short afferent inhibition: Methods

4.1.1.1.1.1.1 Conditioning stimuli

Conditioning stimuli were single pulses (200 μs) of electrical stimuli applied through bipolar electrodes to the median nerve at the wrist. The intensity was set at just over motor threshold for evoking a visible twitch of the thenar muscles. Somatosensory potentials were evoked on the scalp by conditioning stimuli to the left median nerve. The active electrode was attached 3 cm posterior to C3 (10–20 system) and the reference electrode was 3 cm posterior to C4. Five hundred responses were averaged to identify the latency of the N20 peak.

4.1.1.1.1.1.2 Experimental design and data analysis

Each block typically consisted of 110 trials. The motor cortex was stimulated on every trial. Ten trials included motor cortex stimulation alone and the rest of the trials were preceded by conditioning peripheral nerve stimuli at ten

different intervals in reference to subjects' N20: -18ms, -4ms, -2ms, 0ms, +2ms, +4ms, +6ms, +8ms, +10ms, +18ms. All trials were performed in randomised order. Ten trials for each state were derived. Measurements were made of MEP area, and the size of conditioned responses was expressed as a percentage of the unconditioned response. Repeated-measures ANOVA with STATE (11) as within-subjects factor and GROUP (3) as between-subjects factor. Post-hoc analysis was performed.

4.1.1.1.2 Paired associative stimulation

In the originally described form of PAS[181], a transcranial magnetic stimulus to the primary motor cortex (M1) is preceded by a single pulsed electrical stimulus applied to a peripheral nerve, such as median nerve. The inter-stimulus interval of this pairing is adjusted to ensure that the afferent volley generated by peripheral nerve stimulation arrives at the cortex at the same time as the cortex is stimulated by a TMS pulse. Repeated associative pairing of the two sources of stimuli over an extended time period increases the excitability of corticospinal neurons. Such excitability after associative pairing could be considered a neuroplastic effect. This putative effect of PAS mirrors those attributable to long-term potentiation (LTP), a long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously and reflects changes in synaptic plasticity. PAS shares similar properties, with short-latency effects that last beyond the period of stimulation but seems reversible and modifiable with the use of modulators at the NMDA receptor[197 198] and behaves in accordance with

Hebbian principles[181 199]. More specifically, since the polarity of the induced effects appears contingent upon the order of the stimulus-generated cortical events, and that effective inter-stimulus intervals are within the millisecond range, a similarity to spike-timing dependent plasticity (STDP) has been suggested[200].

An individualized approach has been employed, whereby the latency of the N20 component of an SEP, elicited in each participant by stimulating the peripheral nerve, is used as a reference. In some instances the magnetic pulse has been timed to coincide with the N20 component[201]. On the basis of the most common PAS variants alone, it is tempting to conclude not only that the order of the stimulus-generated cortical events is critical, but also that the effective inter-stimulus intervals lie within a very restricted range.

4.1.1.1.2.1 Paired associative stimulation: Methods

For the PAS protocol, subjects were seated in a comfortable reclining chair. Electromyographic (EMG) recordings were made from the abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscles with Ag-AgCl surface electrodes using a bipolar belly-tendon montage. Recordings were made from the less tremulous hand in patients. Subjects received auditory (speakers) and visual (oscilloscope) feedback of EMG activity. Traces with excessive activity were deleted online. EMG signals were amplified and filtered using a time constant of 3 ms and a lowpass filter set at 2.5 kHz

(Neurolog System, Digitimer Ltd., Welwyn Garden City, Herts, UK). EMG signals were digitized at 5 kHz using an analog to digital interface and stored on a personal computer for offline analysis (CED 1401 interface and Signal software, Cambridge Electronic Design, Cambridge, UK).

TMS was given via a figure-of-eight coil (mean loop diameter 9cm) connected to a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane and was optimally positioned to obtain motor-evoked potentials (MEPs) in the APB. TMS was used to probe corticospinal excitability before and after PAS. The coil position and orientation and the intensity of the stimulator were kept constant throughout all the experimental sessions. In each testing block, we assessed the mean MEP amplitude for the APB and FDI muscles. Thirty consecutive MEPs were recorded using an interstimulus interval of 5 s. Stimulus intensity was defined at the beginning of the experiment at a stimulator output that induced MEPs of approximately 1mV in the APB muscle. These recordings were performed before PAS (baseline) and immediately (T0), 15 min (T15) and 30 min (T30) after the end of PAS. PAS consisted of 200 electrical stimuli of median nerve at the wrist paired with TMS over the hot spot of the APB muscle area. The rate of paired stimulation was 0.25 Hz. Electrical stimulation was applied through a bipolar electrode (cathode proximal). The stimulus duration was 0.2 ms, the intensity was 300% of perceptual threshold. The intensity of the TMS pulse was adjusted to produce a MEP of ~1 mV in peak to peak amplitude in the resting APB when given without the preceding median nerve

stimulus. The conditioning median nerve electrical stimulus, given first, was given 5ms plus individual N20 (i.e. 25 ms if N20 latency was 20 ms) before the TMS pulse. Subjects were instructed to look at their stimulated hand and count the peripheral electrical stimuli they perceived in order to ensure comparable levels of attention.

Repeated measures analyses of variance (ANOVA) were performed for MEP amplitudes. For group comparisons we computed repeated measures ANOVA with TIME (baseline, T0, T15, T30) and MUSCLE (APB, FDI) as within-subjects factors and GROUP (tremulous, non-tremulous, healthy controls) as between-subjects factors). The results were corrected for multiple comparisons (Bonferroni). If the main factors showed significant main effects or interaction, the effect of time and muscle in each group was explored with separate one-way repeated measures ANOVA. The Greenhouse-Geisser method was used to correct for nonsphericity. For the ANOVA, a non-corrected p-value of < 0.05 was considered significant. Conditional on a significant F-value, post hoc t-tests were used.

4.1.1.1.3 Classical eyeblink conditioning

Eyeblink classical conditioning (EBCC) is a paradigm of associative motor learning tested in humans since the 1920s (see Freeman and Steimetz for review[202]). It involves paired presentation of a conditioned stimulus (CS), typically a tone or light, paired with an unconditioned stimulus (US). The US, often an air puff or brief electrical stimulus over the supraorbital nerve, is

chosen as a reliable method for producing eyelid closure. In humans, an unconditioned short-latency low-amplitude response is usually seen in response to an auditory stimulus, known as the auditory blink. However, the repeated pairing of the CS-US leads to the production of a conditioned eyeblink response (CR). The maximum CR occurs very close in time to the onset of the US. Animal studies reveal cerebellar circuitry underlying EBCC in which the cerebellar cortical Purkinje cell (PC) receives convergent afferent information about the CS and US via two separate pathways[203] with an additional potential convergence upon the underlying interpositus nucleus (IN)[204-206]. Initial studies demonstrated an essential role for the cerebellum in EBCC using ipsilateral lesions of the cerebellar hemisphere that abolished CRs whilst leaving the contralateral conditioning intact[207-209]. Selected lesions of lateral cell clusters of the anterior interpositus nuclei reveal that this group of cells is essential for retention of EBCC[210] and their damage could not only permanently block acquisition[211], but also abolish retention after memory consolidation[205]. Although the cerebellar cortex clearly plays an important role in EBCC, it appears that learning can still occur without its function, although progresses at a considerably slower rate in such a context[212]. EBCC, with its considerable dependence on cerebellar function, is an ideal paradigm with which to assess and potentially quantify possible abnormalities of the cerebellum and its efferent and afferent connections which by inference may be relevant in generation of tremor.

4.1.1.1.3.1 Eyeblink conditioning: Methods

The conditioning stimulus (CS) was a loud (~70 dB) pure auditory tone (2000 Hz) of 400 ms duration delivered by binaural headphones. The CS inconsistently produced an acoustic startle response (known as an alpha blink) occurring within 200 ms of the CS onset. An electrical stimulus (200 μs pulse-width at 5× sensory threshold) was given over the supraorbital nerve 400 ms after the CS, to elicit a blink reflex (US). Repeated pairs of CS and US at 400 ms intervals (i.e. delay eyeblink conditioning) yield conditioned blink responses (CRs) occurring within 200 ms before the US on subsequent trials. EMG activity was recorded from both orbicularis oculi muscles (see figure 7.1G).

Conditioning consisted of six learning blocks of 11 trials. Trials 1–9 were CS-US pairs, trial 10 was US only and trial 11 was CS only. The US only trial was to detect spontaneous blinks and the CS only trial was to confirm that CRs were being acquired independently to the US. In the inflammatory neuropathy tremor study, an eighth and ninth block were used to measure extinction. For the CMT1A tremor study, a seventh block consisted of 11 CS only trials to measure extinction. This study was designed later and fewer blocks were used to minimise the long experimentation time for each patient. To reduce habituation, the inter-trial interval was randomised between 10 and 30 s. To detect spontaneous blinks recording began 400 ms prior to the CS (800 ms before the US).

4.1.1.1.3.1.1 Data and statistical analysis

For measurement of R2, EMG data were rectified and averaged. R2 latency was measured from the first EMG deflection 30 ms after the stimulus. For measurement of eyeblink conditioning, CRs were counted manually. EMG bursts were regarded as *alpha blinks* if their amplitude exceeded 50 μV and if latency was <200 ms after the CS. EMG bursts were regarded as CRs if latency was >200 ms after the CS but before the US. CR onset was defined by an increase in EMG activity greater than 1 SD above baseline noise occurring within 200 ms before the onset of supraorbital nerve stimulation. In cases where the CR had multiple peaks, the amplitude and latency of peak amplitude were identified for the largest amplitude peak. For the CS only trials, EMG bursts occurring 200–600 ms after the CS were considered CRs.

Statistical analysis was performed using SPSS 19.0. To analyse CRs, two factorial repeated measures ANOVA was used with the 'within subject factor' block (block1, block2, block3, block4, block5, block6) and the 'between subject factor group' (tremulous, non-tremulous, healthy controls). For the extinction block, only subjects who were successfully conditioned (>40% CRs in any block) were analysed. Statistical significance was defined as $p<0.05$.

4.1.2 Eye movement analysis

A variety of different types of eye movement exist, including saccades, pursuit, vergence and reflexic movements relating to oculocephalic reflexes.

These can be measured and inferences made on integrity or disruption of well-established specific networks when these movements are abnormal. Eye movements can be measured in a number of ways. Electro-oculography exploits the permanent potential difference between the cornea and the fundus which varies with eye movements and can be recorded from the region around the eyes using skin surface electrodes (akin to EMG). Other methods including scleral search coils, video-based eye trackers and infra-red trackers are also available.

Eye movement analysis and inferences on pathophysiology in tremor has been most examined in parkinsonian tremor and ET. Gitchel and colleagues[213] used a video-based eye tracker to compare 60 ET patients with 60 age-matched controls on binocular eye position during reflexive saccades to horizontally and vertically step-displaced targets. They demonstrated the saccadic latencies in ET to be abnormally prolonged with slowed velocity profiles. Further, although ET subjects were ostensibly able to maintain stable fixation, their saccades were interrupted by increased numbers of square wave jerks, a sign suggestive of fixation instability but with little value in anatomical localisation[214] unless of large amplitude, though there was no difference in amplitude between patients and controls in their study.

Helmchen and colleagues[215] used a magnetic scleral search-coil system and direct current electro-oculography to study 17 patients with ET and compared them to 11 healthy aged-matched controls. Intention tremor was

distinguished from postural tremor. They demonstrated eye movement abnormalities that are not typically detected clinically. These included a decreased initial pursuit acceleration and also a deficient capacity to discharge the velocity storage mechanism, i.e. the impaired vestibule-cerebellar function to reduce the time constant of post-rotatory vestibular nystagmus by head tilts, both potentially implicating networks involving the cerebellum.

4.1.2.1 Eye movement study methods

5 patients and 8 age-matched healthy controls underwent electro-oculography to record horizontal saccadic and smooth pursuit eye movements. Subjects were seated with a chin support at a distance of 84cm from a target light source. For saccades, LEDs were presented at 10, 20, or 30 degrees in rightward and leftward directions in random order, with an inter-stimulus interval of 4 seconds. Smooth pursuit eye movements were assessed using 8 cycles of a sinusoidally-moving target at 4 different frequencies (0.1, 0.2, 0.3 and 0.4Hz; target displacement +/- 20º, peak velocities from 12.5, 25, 37.5, and 50º/s respectively). Subjects were instructed to keep their eyes on the laser target “as closely as possible”. Eye movements were calibrated for 30 degrees and recorded using DC electro-oculography (EOG, bandwidth 0-30Hz), and stored on a computer at a sampling rate of 250Hz for later offline analysis. Bitemporal electrodes (Viasys Healthcare GmbH, Germany) were used for conjugate horizontal eye movement recording. For saccades, we assessed the latency (time to initial

horizontal eye velocity offset from target presentation), maximum peak velocity and metrics. Velocity measures were calculated as the differential of the EOG (eye position) trace and are expressed in angular velocity units (º/s). We calculated the gain (slow phase eye velocity/stimulus velocity) for saccades and pursuit, for both right and left eye movements.

4.1.3 Transcranial direct current stimulation (tDCS)

TDCS delivered through the scalp modulates motor cortex excitability[216-218]. TDCS applied over the cerebellum has been utilised as a research tool to affect motor learning and adaptation presumably though its effects at least in part by polarising the neuronal membrane and inducing neuroplastic changes similar to those seen in LTP or long-term depression (LTD). TDCS utilises an anode and cathode connected to a direct current source. A weak direct current (0.5-2.0 mA) passes through two of these electrodes, at least one of which is attached to the scalp, with the majority passing through the brain. Anodal TDCS induces depolarization of the neural tissue immediately below the electrode, inducing a subthreshold change in membrane potential, thus increasing the tendency for neuronal firing, and therefore enhancing the overall neural activity and excitability of the stimulated cortical areas. Cathodal stimulation has the reverse effect, by hyperpolarising neuronal membranes. Beyond the acute effects, GABAergic and NMDA-glutamatergic systems may induce longer-term effects. Although the effect of TDCS is relatively non-focal compared to other brain stimulation methods, such as TMS or DBS, the accurate positioning of electrodes is still crucial. Differing

factors such as electrode positioning, size, current intensity and duration of stimulation can all affect the outcome of target stimulation.

4.1.3.1 Tapping task

Motor control and sensory perception are both dependent on precise timing mechanisms. Despite the emerging concept of a distributed network underpinning timing, the cerebellum appears to play a key role[219]. The cerebellum seems to be integral in synchronisation between a predictable timed external stimulus and a motor response, dubbed sensorimotor synchronisation (SMS)[220]. The paced finger tapping task (PFT) has been described widely in the literature as a measure of timing. Patients with unilateral cerebellar lesions performing repetitive finger tapping tasks demonstrated ipsilesional increase in the variability of the inter-tap interval[221].

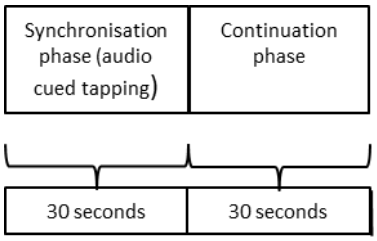
The synchronisation-continuation task (SCT) utilises the PFT, requiring participants to tap with their index finger in time to a train of auditory stimuli separated by a fixed time period, or inter-stimulus interval (ISI). In the second part of the SCT, the continuation phase, the auditory stimulus is omitted and the participant is required to continue tapping at the previous learned rate. The PFT task enables measurement of two fundamental variables, accuracy and variability of the tap interval. The accuracy of the timed response, through the framework of the tapping paradigm, demonstrates how ‘well timed’ the taps are, measurable by the mean inter-tap-interval (ITI), or alternatively the mean absolute error. Variability is a measure of the spread

of taps around the temporal target and patients with lateral cerebellar lesions demonstrate greater variability in their performance of the PFT task compared with healthy controls[222].

4.1.3.1.1 TDCS and tapping task: Methods

The main intention in the series of experiments was to examine performance of a tapping task at different frequencies with the right (dominant in all cases) index finger following application of anodal, cathodal and sham TDCS over the lateral cerebellum. In all experiments, performance was recorded before TDCS and during TDCS.

A. Trial Composition



B. Sample Participant Timetable

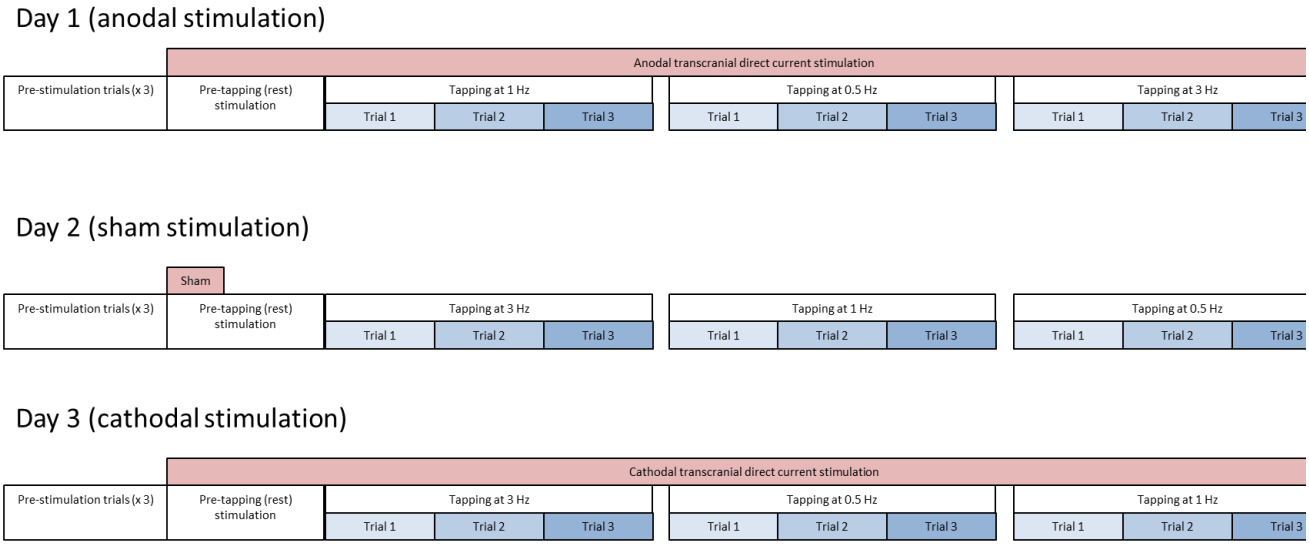


Figure 4.1 Trial design for the paced finger tapping task study. A) A trial is composed of 30s of tapping in time to a tone (synchronisation phase), and then 30s continuing tapping without the tone. B) A sample participant timetable, exhibiting the pre-stimulation, rest and stimulation stages. Note that stimulation days and order of frequency trials are randomised across participants.

4.1.3.1.1.1 Design

Experiments for all participants took place over three days with at least a week between days. On each experimental day, participants were randomly allocated to receive anodal, cathodal or sham stimulation. On each of these days, the participant would complete a pre-stimulation trial where they were required to tap in time with a fixed frequency repetitive auditory tone for a period of 30 seconds (synchronisation phase), followed by a period of tapping for 30 seconds at the same learned frequency but without the

queued tone (continuation phase). This pre-stimulation trial was repeated three times to include in randomised order, three frequencies (0.5Hz, 1Hz, 3Hz) of tapping. Participants then had a two-minute rest period during which they had stimulation with TDCS (randomised to anodal, cathodal and sham). For sham stimulation, 'real' stimulation (randomised to either cathodal or anodal) was provided for a 30 second period to improve sham credibility. For anodal and cathodal stimulation, TDCS was continued until the end of the experiment. The rest period, utilised after the pre-stimulation trials, was similarly included for the sham stimulation session. Three blocks of synchronisation-continuation trials were performed for each frequency in a randomised order for each participant. After each 3Hz trial, a small break of 90 seconds was given to allow the subject to rest their finger before the next trial, to avoid excessive fatigue from affecting performance. Participants were then required to undertake nine consecutive trials of tapping, similar to pre-stimulation trials but in blocks of three of the same tapping frequency. Order of blocks was randomised (see figure 4.1). The experiment was repeated on three days to include all three forms of stimulation (anodal, cathodal and sham) in randomised order to minimise the effect of practice.

4.1.3.1.1.1.1 Task

Participants were sat comfortably in a chair with their right arm supported by a foam pad resting on a table surface. Subjects used their right index finger to tap in the centre of a round plate. A calibrated goniometer (Biometrics) was attached at end to the proximal phalanx of the index finger and at the

other, over the dorsum of the hand to measure the angle between the two and allow measurement of tap amplitudes and timing. Participants were instructed to tap using movements at their metacarpophalangeal joints rather than using movements at the wrist or elbow. Taps were performed at a comfortable force for the participant. Participants were asked to tap up to the height of a visual target. The purpose of the target was to maintain a constant tapping height throughout trials. Prior to the beginning of the experiment, TDCS electrodes were attached to intended stimulation loci (see below). The goniometer and force plate were connected to an amplifier and data acquisition system, which relayed the information to a desktop PC. Subjects were not provided with any additional feedback regards their tapping. Information from the tapping apparatus and auditory tone was entered in to the computer and visible to the experimenter using the Spike2 program (CED, Cambridge, UK).

4.1.3.1.1.1.2 TDCS

TDCS was delivered to the right cerebellar cortex using a commercially available DC stimulator (Magstim neuroConn) as previously described[185]. The TDCS electrodes were 25cm^2 (5cm by 5cm) in surface area, encased in sponge pockets, soaked in saline solution and secured to the scalp surface with crepe bandage. To stimulate the right cerebellar cortex, one electrode was secured 3cm laterally to the inion, and the other electrode was secured over the right buccinator muscle. In the anodal condition the electrode from the negative terminal electrode was placed over the right buccinator. In the

cathodal condition the electrode positions were switched. An intensity of 2mA was used for 18 minutes for both anodal and cathodal conditions. The sham current consisted of 2mA anodal or cathodal stimulation for 30s, over the right cerebellum, enough to briefly mimic the signs of stimulation (itching, burning, metallic taste, and rarely, visual phosphenes) without allowing any known alteration in cortical activity. During the onset of stimulation the current in all conditions was increased in a ramp like fashion over a period of 15s and ramped down for the termination of stimulation over the same duration, a method shown to achieve good blinding[223]. Anodal, cathodal and sham conditions were performed on different days with at least a week's rest interval owing to the long lasting effects of TDCS. The participant but not experimenter was blinded to randomisation of stimuli settings.

4.1.3.1.1.2 Data Analysis

In the data analysis, the ITIs were determined as measurements in time between peaks of finger displacement measured by goniometry. For each frequency (0.5Hz, 1Hz, 3Hz), mean ITIs and coefficients of variation were computed for the synchronisation and continuation phase for each of the three trials, which were then averaged to gain a mean ITI for that condition. CV was used as an indicator of temporal variability where CV (%) = (SD/mean)/100. To determine effects of stimulation on variability and accuracy, a 3-way repeated measures ANOVA was planned for ITIs (accuracy) and CV ITIs (variability) with main factors of stimulation (anodal, cathodal, sham), frequency (0.5, 1, 3Hz) and block (synchronisation,

continuation). Similar 3-way repeated measures ANOVAs were planned for pre-stimulation information but with factor stimulation replaced with day (day 1, day 2, day 3 and sham). Sham was to be included in the pre-stimulation analyses to evaluate its validity as a control for the stimulation conditions. Tap amplitude data was derived from the amplitude of the movement from the goniometric data. This was to be done in the final trials of each condition, as those were most likely to show fatiguing effects. The first 3 taps and last 3 taps of each trial were removed as subjects often performed poor quality taps during these periods. After this truncation, the 4 taps at the beginning of the trial were compared to the 4 taps at the end of the trial, as these often lay outside of the 1min trial window. A three-way ANOVA was planned for tap amplitude measured by goniometry with factors of trial point (beginning, end), stimulation (anodal, cathodal, sham) and frequency (0.5, 1 and 3Hz). Post hoc *t*-test were computed using a Bonferroni correction. None of the data violated the normality assumption necessary to conduct parametric statistical tests.

4.1.4 Visuomotor adaptation

In tasks requiring a subject to reach a target, external perturbations (such as distortion of visual feedback) introduces a movement error. The difference between the sensory feedback generated after the movement from the predicted sensory movement outcome can be considered the sensory prediction error. The cerebellum seems to play a key role as an adaptive filter, updating subsequent motor performance with the sensory prediction

error. The adaptation of dynamic and kinematic errors is impaired by cerebellar lesioning[224-228].

4.1.4.1 Visuomotor adaptation: experimental method (adapted from Parees et al[229])

Participants were sat opposite a 17-inch computer screen (refresh rate: 50 Hz; distance from subject to screen: 45 cm) with a joystick secured to the table in front of them. The monitor displayed eight targets (1.5cm x 1.5cm squares) arranged in a circular array (radius 13 cm), each target equally separated from other targets by 45°. A similar square marked the centre of the circle, and a small yellow circular cursor indicated the joystick position (figure 4.2). The experiment was programmed in Matlab (version 7.0.1; MathWorks, Natick, MA) using the Cogent Toolbox (http://www.vislab.ucl.ac.uk/cogent.php).

The targets were constantly visible to participants. In the baseline condition, four blocks of 40 trials were presented. At the start of each trial, the target to be aimed for turned red, and participants were instructed to move the joystick to manipulate the yellow cursor inside the target square. Once the cursor was kept within the target square for 1 second, the target changed colour from red to green, and participants were instructed to move the joystick back to the centre square to commence the next trial (figure 4.2A and B). Targets were presented in a random sequence during each block. Participants were

instructed to react and to move to the target squares as quickly and accurately as possible upon target appearance.

Temporal and spatial variables used to characterize task performance were reaction time (RT; time in milliseconds from target presentation to movement onset), movement time (MT; time in milliseconds from movement onset to stabilisation of the cursor in the target), and displacement ratio (DR; ratio between the length—measured in pixels—of a straight line “perfect path” between the starting point and the target, and the length of the actual path taken by the participant, with higher values of DR indicating increasing deviation from the perfect path). Participants then performed a rotation learning task (ROT). With this task, implicit motor control was tested[230] by measuring the ability of participants to adapt to a visuomotor perturbation. A constant 30° anticlockwise rotation was introduced into the path of the cursor displayed on the screen but participants were not made explicitly aware. Targets were displayed in a randomized order, and participants had to move the cursor to the highlighted target as quickly as possible (figure 4.2C). Participants were not instructed how to compensate for the rotation. Improvement would be indicated by a ratio in the first 10 trials and the last 10 trials of <1 for RT, MT, and DR. The total time of the experiment was 30 minutes with opportunities for patients to rest between blocks. No patient reported problems with fatigue or concentration.

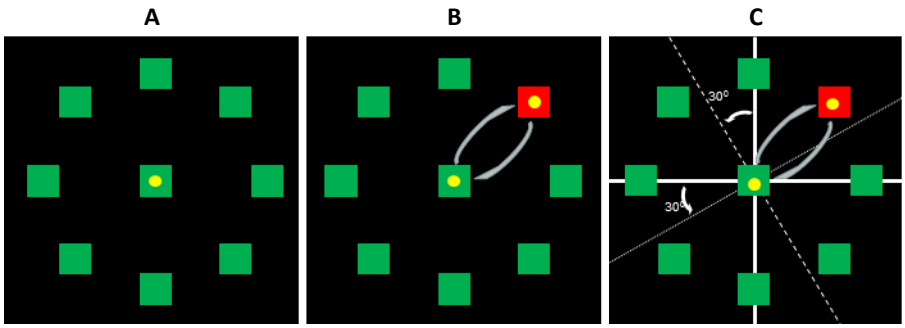


Figure 4.2 Screenshots of visuomotor adaptation experiments. Adapted from Parees et al[229]. (**A**) Baseline condition. Yellow cursor in centre square. (**B**) The target square turns red and the participant is instructed to manipulate the joystick to maneuver the yellow cursor into the target square until the target square returned to a green colour (occurred after 1 s), upon which the participant was instructed to return the cursor to the centre square. (**C**) 30-degree anticlockwise rotation is superimposed to the path of the cursor.

4.1.5 EMG and accelerometry

A variety of techniques may be used to quantify tremor. Tremor can be analysed in real time using digitisation of analogue signals at high sampling rates. The data can then be processed further off-line. Tremor is classically described electrophysiologically after tremor analysis by amplitude and frequency. The frequency, or oscillations per second, is measured in Hz. The period (or 1/frequency) and number of points sampled per second (N) then dictate the maximum recordable frequency, known as the Nyquist frequency (Nyquist frequency = N/2T Hz). Signal processing theory dictates that in order to avoid artefactual frequencies in tremor analysis due to insufficient

sampling rates (known as aliasing), the sampling rate must be kept above the Nyquist frequency.

Surface EMG is usually sufficient for tremor measurement, rather than necessitating needle EMG. It can provide useful information about the activity of muscles involved in the generation of tremor. EMG can provide useful information about motor unit synchronisation and motor unit recruitment and help clarify the relationship between muscles and the resulting limb movement, revealing information about the synchronicity or otherwise of antagonist pairs of muscles. The EMG signal is processed by rectification and smoothing to produce a tremor envelope from which tremor frequency in the tremor range can be derived.

4.1.5.1 Methods

Surface electromyographic (EMG) recordings were made with Ag-AgCl surface electrodes using a belly-tendon montage. Data were stored in a computer for display and off-line analyzed using Signal version 4.00 (and Spike version 2 for tremor analyses).

4.1.5.1.1 Accelerometry

Accelerometers measure *proper acceleration* ("g-force") rather than coordinate acceleration (rate of change of velocity). Acceleration is quantified in the SI unit metres per second per second (m/s^2), or popularly in terms of

g-force (g). Accelerometers can be considered to behave as a damped mass on a spring, such that when exposed to acceleration, the mass is displaced to the point that the spring is able to accelerate the mass at the same rate as the casing. The displacement is then measured to give the acceleration. Piezoelectric, piezoresistive and capacitive components are commonly used to convert the mechanical displacement into an electrical signal. Most micromechanical accelerometers operate in-plane, that is, they are designed to be sensitive only to a specific plane. By integrating two devices perpendicularly, a two-axis accelerometer can be made and similarly for three axes.

4.1.5.1.1.1 Accelerometry and EMG for tremor

A triaxial accelerometer transducer (sensitivity ± 100 mV/g) was attached to the dorsal surface of the middle phalanx of the index fingers. EMG recordings were made of wrist extensor muscles (WE), wrist flexors (WF), abductor pollicis brevis (APB) and biceps brachii (BB) bilaterally. Recordings were performed with arms relaxed (rest), with arms/wrists outstretched at shoulder level (posture), and a 500-g mass attached to the wrists (loading), and while performing a goal-directed task (action). Accelerometry and EMG were recorded and analyzed for 30 seconds in each condition.

EMG signals were amplified using a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK), analogue filtered (low pass at 1000 Hz and high pass with 3ms time constant and sampled at 2000 Hz, off-line digitally

full-wave rectified, smoothed (CED 1401 laboratory interface; Cambridge Electronic Design, UK) and stored on a laboratory computer for on line visual display using dedicated software (SPIKE software; Cambridge Electronic Design).

Variables measured were peak tremor frequency (PF), the total power of the spectra between 1 and 30 Hz as a measure of tremor amplitude (TP), the half-width power (HWP) given by the area under the curve between two vertical straight lines intersecting the rising and falling edge of the peak at half of the peak power (full-width at half maximum (FWHM)), the latter being a measure of frequency stability/variability[231].

4.2 Clinical assessment

4.2.1 The Fahn-Tolosa-Marin Tremor Rating Scale

The Fahn-Tolosa-Marin scale, revised in 1993[232] assesses tremor amplitude at rest, on posture and kinetic/intention components in specific body parts. Tasks such as tremor during writing, drawing, and pouring are assessed; activities of daily living; and global assessments by the patient and examiner, with each item rated on an integer scale from 0 to 4. It has been widely used in clinical trials and has favourable clinimetric properties reviewed by Elble et al[233]. The scale has a high correlation between examiners ($\rho = 0.93 – 0.99$)[232] and high test-retest reliability (intraclass correlation) of 0.859 for the total score[234] The scale has good face validity and correlates strongly with transducer measures of tremor[235 236]. It includes both clinician-based and

patient-based ratings as well as aspects of functional impairment. Its limitations include a potential ceiling effect for large amplitude tremor.

4.2.2 Bain and Findley spirography scale

Bain and Findley defined a scale of tremor severity using integer ratings from 0 to 10 to grade the tremor evident on drawing an Archimedes spiral[237 238]. Assessment of clinimetric properties of the scale reveal an inter-rater ICC was 0.93 for patients with ET[236], though somewhat lower in a cohort of patients with multiple sclerosis[239]. Bain and Findley reported 95% of normal controls had a score of 1 or lower[238]. Despite the potential ceiling effect associated with this scale[238], it correlated well with ET that was quantified with a digitizing tablet and the Fahn-Tolosa-Marin scale[236], accelerometry, an ADL questionnaire, postural tremor rating, and a handwriting scale[237]. There were also strong correlations with an ADL questionnaire, a finger-tapping task, and a pegboard task in patients with multiple sclerosis[239].

4.2.3 MRC power score

The MRC sum score is calculated by summating the total MRC score[186] for a number of muscle pairs (i.e. left and right side): upper-arm abductors, elbow flexors, elbow extensors, wrist extensors, hip flexors, knee extensors, and foot dorsiflexors. Each muscle scores from 0 (“no movement”) to 5 (“full strength”), thus generating a potential sum score ranging between 0-70.

4.2.4 Sensory score

The sensory system was graded as follows: touch and pin prick sense normal 4; distal to wrist/ankle abnormal 3; distal half forearm/leg abnormal 2; distal to elbow/knee abnormal 1; distal to axilla/groin abnormal 0. Vibration sense: tuning fork perception (128 Hz) on middle finger for 15 seconds, or on the hallux (big toe) for 10 seconds 4, decreased on middle finger/hallux 3, ulnar styloid/medial malleolus 2, elbow/knee 1, clavicula/crista or higher 0. Joint position sense of middle finger/hallux normal 2, diminished 1, absent 0. The score ranged from 0-56 (56 representing greatest sensory abnormality). This score creates a summative score for sensory abnormalities in inflammatory neuropathies[240]. A moderate to good validity was obtained for the INCAT sensory sum score, with acceptable internal consistency, inter- and intraobserver reliability as well as evidence for good responsiveness of the scale.

4.2.5 NINDS myotactic reflex score

The National Institute of Neurological Diseases and Stroke (NINDS) myotactic reflex scale provides a summation of reflex scores (0 = reflex absent, 1 = reflex less than normal; includes a trace response or a response brought out only with reinforcement, 2 = reflex in the lower half of normal range, 3 = reflex in the upper half of normal range, 4 = reflex enhanced, more than normal)[241]. A summation of scores for biceps, supinator, triceps, knee and ankle) were calculated[188].

4.2.6 Overall Neuropathy Limitations Scale

For an overall measure of disability in inflammatory neuropathies, we use the Overall Neuropathy Limitations Scale (ONLS), a modification of the pre-existing Overall Disability Sum Score (ODSS). It has been found to correlate well with its predecessor, the ODSS, as well as with the 36-item Short Form Questionnaire Physical Component Summary Score. It has a high inter-rater reliability and the responsiveness of the ONLS was considered acceptable. The ONLS has better content validity and less ceiling effect than its predecessor, the ODSS[191]. The range of possible scores is 0-12 (12 representing greatest impairment).

4.2.7 CMT sum score

The Charcot-Marie-Tooth neuropathy score (CMTNS) has been shown to be a reliable and valid composite score combining data from symptoms, signs, and neurophysiological investigations. It has been used in epidemiological studies of CMT1A and as an outcome measure in treatment trials. The CMTNS has been modified to reduce floor and ceiling effects and to standardize patient assessment with the updated scale known as CMTNS, with an aim to improve the sensitivity for detecting change over time and the effect of an intervention. It performs well clinimetrically on inter- and intra-rater reliability[242].

Chapter 5: Clinical study of inflammatory neuropathic tremor

5.1 Introduction

It has been recognised for many years that tremor can be an accompanying feature of peripheral neuropathy[110 111]. This *neuropathic tremor* was first described in hereditary neuropathies as part of Roussy-Lévy syndrome[144] which has since been shown to be a genetically heterogeneous entity[145 146]. In inflammatory neuropathies, tremor is found in up to 80% of patients with IgM paraproteinaemic neuropathy[111 120 128], in the recovery phase of Guillain-Barré syndrome[126] and is occasionally reported in patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)[114]. No relationship seems to exist between the development of tremor and the severity of the neuropathy, proprioceptive loss, weakness or fatigue[117 136] but it may relate to disease activity[117]. Thus, it is not clear why only a subset of patients develop tremor.

Tremor in neuropathies has been described as disabling but no formal study of this has been undertaken[128] except in paediatric Charcot-Marie-Tooth disease where it has been shown to be one of the strongest independent determinants of reduced quality of life (QOL)[243]; successful treatment in this context would likely have a substantial effect on QOL. Tremor in this population also predicts other disabling symptoms[244]. Currently, there is no consensus on the best way to treat neuropathic tremor, or why only some patients seem to be at risk of developing it. Certainly some cases of tremor in

inflammatory neuropathies are very disabling necessitating both medical and invasive surgical approaches to treatment[129 139 141-143].

In this case-control study we gathered consecutive and clinically well-characterised patients with inflammatory neuropathy with the main aims of 1) documenting the incidence and nature of tremor, 2) assessing the additional disability that might be present in patients with tremor and 3) determining the predictors of tremor onset and its subsequent severity.

5.2 Methods

5.2.1 Patients

All patients with a diagnosis of an inflammatory neuropathy made by a neuromuscular expert and who were receiving regular intravenous immunoglobulin treatment at the National Hospital for Neurology and Neurosurgery were approached to take part in the study. Those taking drugs known to commonly cause tremor were excluded. Written informed consent was obtained from all patients and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Variable		**Tremulous patients**	**Non-tremulous patients**
Number		27 (63%)	15 (37%)
Disease	CIDP	15 (58%)	11 (42%)
	MMNCB	5 (56%)	4 (44%)
	IgMPN	7 (88%)	1 (12%)
Sex		6 female, 21 male	7 female, 9 male
Age of patients (yrs)	Mean †	63.3 (11.0)	60.5 (12.0)
	Median	63.0 (34.0-83.0)	59.0 (44.0-85.0)
Disease duration (yrs)	Mean †	12.8 (8.9)	14.3 (9.2)
	Median	10.0 (2.0-30.0)	12.5 (2.0-33.0)

Table 5.1 Demographics of patients. Expressed as mean (SD) and median (range). †Denotes use of either mean or median for pairwise comparison. Bonferroni corrected significance value of 0.025 used and p-values where relevant are reported in the manuscript.

5.2.2 Clinical evaluation

Details regarding demographic and medical history were obtained from patients directly and corroborated with their medical notes. A standardised clinical examination was performed. In particular, careful examination for parkinsonian signs and signs of functional tremor[245] were sought. Tremor, where present, was distinguished from other spontaneous movements including fasciculations and myokymia. A modified summed Medical Research Council score (MRC score)[186] was calculated (maximum score 70)

as well as a sensory sum score[246]. Tremor was assessed using the Fahn-Tolosa-Marin scale[189 232] with higher scores attributed to worse tremor. Tremor severity in the limbs was specifically assessed with Archimedes spirals and using the Bain and Findley spiral score (rated 0-10 with 10 representing most severe tremor)[238] to rate these. Patients were divided into two groups for subsequent analysis depending on the presence, clinically, of pathological tremor (tremulous group) or absence (non-tremulous group). Disability was assessed with the Overall Neuropathy Limitation Scale (ONLS)[191]. It has separate subscales for arms and legs with higher scores indicating more functional impairment. Results of patients' most recent nerve conduction studies were obtained and correlations of clinical features of tremor to median nerve conduction alone was made to limit the number of potential comparisons. Brain imaging results, where performed, were collated.

5.2.3 Accelerometry

Nine patients with tremor (four CIDP, two MMNCB, two IgMPN) took part in this evaluation. Subjects were seated in a comfortable chair. A triaxial accelerometer transducer (sensitivity ± 100 mV/G, Biometrics ACL300; Biometrics Ltd., Cwmfelinfach, Wales, UK) was attached to the dorsal surface of the middle phalanx of the index finger bilaterally. Recordings were performed (a) with arms/wrists outstretched at shoulder level (posture), and (b) with similar posture but a 500g mass also attached to the wrists. Accelerometry was recorded and analyzed for 30 seconds. The

accelerometry traces were stored in a laboratory computer for display and off-line analysis using customized Spike version 2.

5.2.4 Statistical analysis

For accelerometry data, a Fourier analysis of the signals derived from accelerometry was performed to define peak tremor frequency (PF). Statistical analysis was performed using PASW Statistics 19 (SPSS, Inc., Chicago, IL). All results are expressed as mean (standard deviation) or median (range) where assumptions of parametric data were not met. Baseline tremor characteristics were compared between tremulous and non-tremulous controls by independent-samples *t*-test or Mann-Whitney test where data could not be transformed to fulfil assumptions for parametric distributions. For categorical data, group analysis was performed with 2 × 2 cross-tabs. We compared the frequency of abnormal test results by chi-square tests and Fisher's exact test (two-sided). Correlations were derived using Pearson’s coefficient or Kendall’s tau where data could not be transformed to fulfil assumptions for parametric distributions. *P* values below 0.05 were considered to indicate statistical significance except where indicated, in which case Bonferroni correction for multiple comparisons was employed.

5.3 Results

From a total group of 44 patients, 43 patients agreed to take part. Clinical and demographic details are summarised in Table 5.1.

5.3.1 Clinical evaluation

Twenty seven (63%) of patients had tremor. The mean age of tremor onset was 57.6 (11.6) years. The mean duration of disease before tremor onset was 5.8 (7.2) years. There were more men in the tremulous group (Table 5.1) but this did not reach statistical significance ($p = 0.14$). One patient had onset of tremor during a relapse of his MMNCB, 6 years after the clinical onset of his neuropathy. One non-tremulous patient had a previous episode of tremor during her first GBS-like presentation. A precipitating event prior to tremor onset was recalled in 10 of the 27 patients: six were at the onset of the neuropathy, two were with relapses and two were at the time of general medical complications (1 with a pulmonary embolus and ITU admission and the other with a pulmonary embolus, but neither had abnormal structural MRI brain scans to account for the onset). Of the tremulous patients, four reported tremor in one first degree relative (one of these reported it in two first degree relatives). Of those, two were in the context of a diagnosis of Parkinson's disease in old age, one in the context of substantial alcohol misuse and the other reported hand tremor in his father with no known secondary cause. Of those with tremor, seven had trialled medical treatments specifically for tremor (number of patients in brackets) (propranolol (3), atenolol (1), levodopa (1), clonazepam (2), trihexyphenidyl (benzhexol) (1), topiramate (1), gabapentin (1), pregabalin (2), primidone (1))

all without benefit. Only one reported possible benefit with alcohol. Five patients reported benefit from treatment given for their neuropathy (three with intravenous immunoglobulin, one with CHOP-R chemotherapy and one with rituximab) while the remaining 22 patients reported no benefit from treatment for their neuropathy (all these patients had only received intravenous immunoglobulin treatment apart from one who was also treated with rituximab).

Of the non-tremulous group, we could obtain neuroradiology reports of four that had previous brain imaging (sometimes for incidental purposes) with MRI and one with CT. Three of four with MRI had changes consistent with mild or moderate small vessel disease. The patient with CT had evidence suggesting small vessel disease. Of the tremulous group of patients, we were able to obtain imaging reports of eight with MRI and two with only CT. Five of those with MRI had mild or moderate changes consistent with small vessel disease, one with frontoparietal atrophy bilaterally and two reported as normal. Of those who had CT, one was reported as normal and one reported as suggesting small vessel disease. The overall proportion of those with abnormal imaging versus those with normal imaging (in any modality) was not statistically different between the two groups ($p > 0.99$).
Tremor, when present, was always seen on posture or action apart from one patient with anti-MAG associated neuropathy who had tremor solely at rest. Rest tremor in addition to postural tremor occurred in 8/15 tremulous CIDP patients, 1/5 MMNCB and 2/7 IgMPN (1 with and 1 without anti-MAG). One patient (CIDP) had mild voice tremor.

Clinical score		Tremulous patients	Non-tremulous patients	P value
MRC score	Mean †	65.7 (3.9)	56.6 (13.4),	0.017*
	Median	66.0 (58.0-70.0)	58.0 (29.0-70.0)	
Sensory score	Mean †	39.9 (14.2)	42.3 (16.2)	0.63
	Median	41.0 (8.0-56.0)	48.0 (3.0-56.0)	
ONLS score	Mean	3.7 (1.5)	4.5 (2.3)	
	Median †	4.0 (0.0-6.0)	4.5 (1.0-10.0)	0.32

Table 5.2 Clinical scores for patients comparing tremulous and non-tremulous groups. Expressed as mean (SD) and median (range). †Denotes use of either mean or median for pairwise comparison. n/a – not compared. Bonferroni corrected significance value of 0.017 used. *Denotes significance.

Nerve conduction result		Tremulous patients	Non-tremulous patients	P value
Median nerve MCV (m/s)	Mean	40.5 (19.7)	37.6 (10.1)	
	Median †	46.0 (10.0-97.0)	41.5 (21.0-56.0)	0.42
Median nerve CMAP (mV)	Mean †	5.8 (3.1)	4.0 (3.2)	0.07
	Median	5.5 (0.8-11.5)	3.3 (0.2-9.7)	
Median nerve DML (ms)	Mean	7.0 (5.0)	5.8 (2.7)	
	Median †	4.5 (3.0-18.4)	5.3 (3.3-12.4)	0.98
Median nerve SCV (m/s)	Mean	24.4 (24.8)	42.7 (19.7)	
	Median †	28.5 (0.0-59.0)	50.0 (0.0-60.0)	0.03

Median nerve F-wave latency (ms)	Mean	43.9 (24.7)	40.1 (7.8)	
	Median †	31.3 (26.3-98.0)	36.9 (31.9-51.5)	0.22
Ulnar nerve F-wave latency (ms)	Mean	43.4 (20.5)	36.3 (6.2)	
	Median †	34.4 (27.7-96.5)	35.7 (29.7-46.6)	0.82

Table 5.3 Nerve conduction study results for inflammatory neuropathy patients with tremor compared to those without tremor. Expressed as mean (SD) and median (range). MCV – motor conduction velocity; CMAP – compound motor action potential; DML – distal motor latency; SCV – sensory conduction velocity. †Denotes use of either mean or median for pairwise comparison. n/a – not compared. Bonferroni corrected significance level of 0.0083 used.

5.3.1.2 Measures of tremor severity

The median FTM score in tremulous patients was 14.0 (9.8-18.3). The FTM score includes components assessing disability and tremor in body parts other than the arms. Using spiral scores is a purer measure of tremor amplitude in the upper limb and thus of value in correlating with upper limb clinical and electrophysiological measures. Nevertheless, there was a strong correlation between both measures of tremor (Kendall's $\tau = 0.59$; $p < 0.001$). There was also a difference between the median score for spirals in those with tremor (3.0) compared to those without tremor (1.0) $U = 16$, $Z = -4.5$, $p<0.001$, $r = -0.73$. Spirals in non-tremulous patients were often abnormal but

typically lacked periodic oscillatory patterns. To investigate whether other symptoms of neuropathy were confounding the spiral score, we correlated spiral scores to strength and sensory deficit (using a Bonferroni corrected significance level of 0.0167 to adjust for 3 comparisons). In those with tremor, higher spiral scores did not correlate with worse MRC scores (see table 2) ($p = 0.45$) or worse sensory scores ($p = 0.98$). In those with tremor, there was a correlation between higher (more severe) ONLS arm scores and higher spiral scores (Kendall's $\tau = 0.44$; $p = 0.01$).

5.3.1.3 Correlation of tremor with electrophysiological markers

A number of variables measured are dependent on conduction velocity but principal component analysis was not used given the inadequately low Keiser-Meyer-Olkin measure of sampling adequacy (0.43). There was no difference in F-wave latencies between tremulous and non-tremulous groups (see table 5.3). However, with individual correlation of variables (using a Bonferroni corrected significance level of 0.025 for 2 comparisons), using spiral scores, there was a correlation between spiral scores and median nerve F-wave latency ($p = 0.02$) (see Figure 5.1). Given this, ulnar nerve F-wave latency was examined post hoc and a similar but stronger correlation was found with spiral scores ($p = 0.003$) (see figure 5.1). F-wave latency was only calculated for those in whom it could be measured. Where F-waves were not seen, the mean spiral score was 3.5 (3.0). There was no correlation between spiral scores and median nerve motor conduction velocity ($p = 0.49$) or median nerve sensory conduction velocity ($p = 0.36$).

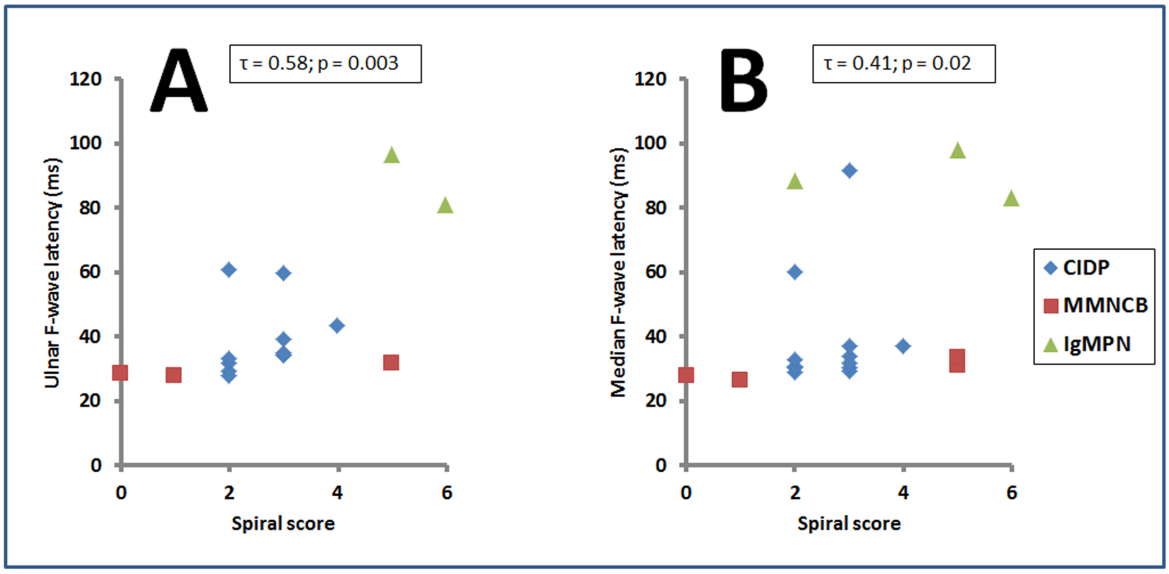


Figure 5.1 Correlations between measured F-wave latency (ms) and tremor severity as measured by spiral scores (higher scores more severe) in tremulous patients for **(A)** the ulnar nerve and **(B)** the median nerve. The correlation coefficient (Kendall's tau) and corresponding p-value for each correlation in text box.

5.3.1.4 Correlation of tremor with clinical features of neuropathy

Those with tremor had better MRC scores than those without tremor ($t(16.8)= -2.6$; $p = 0.017$), yet had similar disability scores measured by ONLS (see table 5.2). Using spiral scores as a measure of tremor severity, there was a correlation with ONLS arm sub-scores in tremulous patients (Kendall's $\tau = 0.44$; $p = 0.01$ using a Bonferroni corrected significance level of 0.01 to adjust for 5 comparisons), demonstrating an association between increasing tremor severity and increasing disability. There was no correlation of tremor severity measured by spiral scores with disease (neuropathy) duration ($p = 0.67$), MRC score ($p = 0.45$), overall sensory score in the arms

($p > 0.99$) or arm proprioception score ($p = 0.68$). In regard to hypotheses of tremor pathophysiology in these disorders, there was no difference in the proportion of tremulous patients who had a serum paraprotein (44%) from the proportion of non-tremulous subjects (31%) with a paraprotein ($p = 0.52$). There was also no difference in the proportion of tremulous patients who demonstrated pseudoathetosis (22%) compared with the proportion of non-tremulous patients with pseudoathetosis (13%) ($p = 0.69$).

5.3.2 Accelerometric measure of tremor

In all nine patients recorded with accelerometry, a bilateral rhythmic tremor was noted during posture. There was no significant difference in tremor frequency between left and right hands ($p = 0.53$). The mean frequency of tremor in both hands in all nine patients was 6.1 (1.6) Hz with the highest recorded frequency at 10.0 Hz and the lowest frequency, 3.3 Hz. There was no significant difference in tremor frequency between the three groups of patients (CIDP, MMNCB, IgMPN) ($p = 0.33$). Weight loading did not alter the mean tremor frequency ($p = 0.23$). A sample of tremor recording is demonstrated in Figure 5.2.

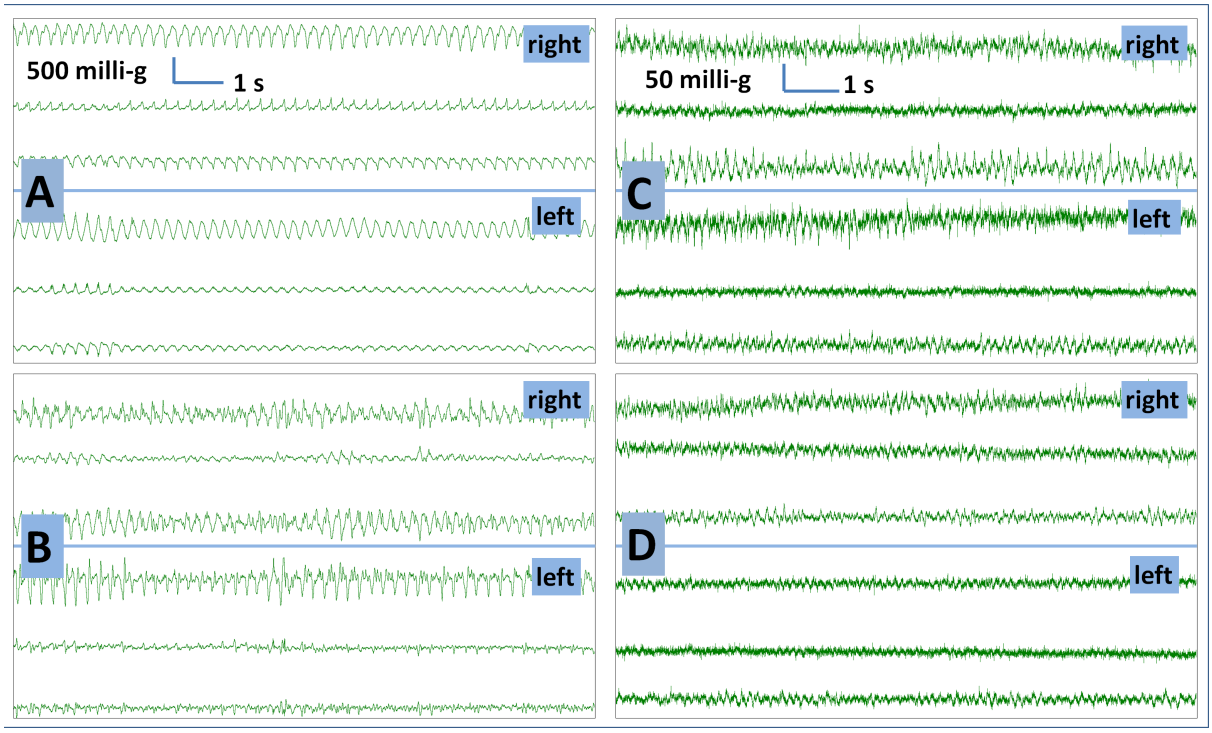


Figure 5.2 Sample of tremor recording from a patient with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) showing tremor measured by triaxial accelerometry of each hand with arms outstretched **(A)** and with 500g weight loading of limbs **(B)**. Recording of patient with IgMPN showing triaxial accelerometry in each hand with arms outstretched **(C)** and with weight loading of limbs causing tremor amplitude suppression but no modulation of frequency **(D)**.

5.4 Discussion

We describe the clinical and electrophysiological characteristics of 43 patients with inflammatory neuropathies. This is the largest prospective cohort of patients with inflammatory neuropathy reported with the primary purpose of investigating tremor. We highlight that a majority of patients with inflammatory neuropathy in our cohort had tremor (63%), particularly those

with IgMPN, but also the majority of patients with CIDP. We also describe, for the first time, tremor in the majority of patients with MMNCB. Tremor in all these patients seems not to improve with treatment of the underlying neuropathy except in a small number of cases. Specific treatments for tremor, where used in a few cases also proved to be, in the majority of cases, unsuccessful. We provide evidence that tremor may add to disability in patients with inflammatory neuropathy as has similarly been described in children with CMT[243]. We also demonstrate for the first time a correlation of tremor severity with ulnar F-wave latencies.

5.4.1 Impact of tremor on disability

Patients with tremor scored similarly to patients without tremor on the ONLS, a validated disability score. Despite this, non-tremulous patients were weaker than tremulous patients. This suggests that the presence of tremor accounts for part of the disability measured by the ONLS, similarly suggested by Ahlskog et al[1]. Interestingly, in multiple sclerosis tremor, as limb weakness progresses, tremor may improve suggesting a necessary role for limb strength in generating tremor[247]. This may similarly be true for patients with inflammatory neuropathy and tremor, however, we observe that none of our patients reported worsening of tremor associated with improvement in their neuropathy (and strength) after treatment with IVIg or other immunomodulators. All the patients examined had been given IVIg and other immunomodulatory treatments without benefit on the tremor in most of these cases. Although a few appeared to improve with treatment, this was

sometimes with substantially immunosuppressive drugs such as rituximab. Where tremor was specifically treated, in most cases, there was a poor response to usual anti-tremor drugs. The high prevalence of tremor in our cohort, the component of disability potentially attributable to tremor and the refractoriness to treatment where tried frames tremor as a potentially important symptom contributing to untreated disability in patients with inflammatory neuropathies.

5.4.2 Pathogenesis of tremor

On most demographic and clinical features, except gender, there was no difference between the tremulous and non-tremulous patient groups or any correlation within the tremulous group. There were no differences in F-wave latency between tremulous and non-tremulous patients, but there was a strong correlation between tremor severity (assessed by spiral scores) and F-wave latencies (see figure 5.1). However, this should be tempered with the fact that we, a priori, planned analysis of only median nerve data and added the ulnar F-wave correlation post-hoc. Nevertheless, the finding of similar F-wave latencies between tremulous and non-tremulous patients, yet a strong correlation between delayed F-wave latencies and tremor severity points towards F-wave delay being an important modulator of tremor in those prone to it, but in itself seems insufficient to explain the presence or absence of tremor. This might indicate the importance of an additional variable, such as the ability of a central processor (most likely the cerebellum) to adapt to such dispersion and delay, in preventing tremor from arising[128]. Recent data

demonstrating defective cerebellar associative learning in patients with inflammatory neuropathy and tremor, but not those without tremor, supports the hypothesis of cerebellar dysfunction in patients with inflammatory neuropathy and tremor[248]. Activation studies in PET demonstrating cerebellar overactivity in patients with IgM paraproteinaemic neuropathy and tremor seems concurrent with this possibility[2]. Alternative hypotheses such as that proposed by Smith et al[126] in which tremor was felt to be in part dependent on the stretch reflex given the finding of a correlation between ulnar motor conduction velocities and thumb tremor, does not seem consistent with our findings. Bain et al[128] similarly were unable to find such a correlation. Our finding of a tremor frequency that does not vary with weight loading also promotes the hypothesis of an important central mechanism in generating the tremor.

Busby and Donaghy, in their study of 102 patients with chronic dysimmune neuropathy[114] remarked upon slowed motor nerve conduction velocities and prolonged distal motor latencies in all patients with tremor, but F-waves were not commented upon. However, this was based upon conduction velocities in only four patients of their series of 102 patients all of whom had very severe tremor. This indicates a selection bias for severe tremor in their calculation and there was no comparison reported between conduction velocities in these patients compared with non-tremulous patients. Thus, there is insufficient evidence to conclude that our results differ from theirs. Indeed, other series[136] do not demonstrate a difference in motor conduction velocities in tremulous versus non-tremulous patients. A caveat is that patients here

were recruited from those attending hospital regularly for treatment with intravenous immunoglobulin which thus may have biased the sample towards one with greater severity of neuropathy. As such, conclusions drawn here can only be applied to this subgroup of patients.

Limb tremor has rarely been described in patients with MMNCB; three patients of a case series of 39 cases of MMNCB had tremor, all at rest[249]. We describe 5/9 MMNCB patients in our study to have tremor. In two of these, the tremor was jerky. In only one of these was tremor present at rest in addition to action tremor. Treatment of inflammatory tremor has previously been reported to be variable (summarised by Smith[126]) with many treatment failures but some treatment successes using immunomodulators for treating the underlying neuropathy and rare success using other medications such as pregabalin[250 251]. Here, patients with tremor were refractory to normal oral medications used for treating tremor[252] including pregabalin. There were a small number of cases that responded to treatment of the underlying inflammatory process with immunomodulators, however, the numbers were too small to draw clear conclusions.

The presence of tremor in inflammatory neuropathies seems unlikely to represent the coexistence of essential tremor (ET) on epidemiological, clinical and neurophysiological grounds[128]. In our series, the prevalence of tremor is some magnitudes higher than that reported for essential tremor in the general population[14], even for a population over the age of 40 years[253]. Further, the male predominance in tremulous patients (though not

statistically significant), similarly described previously[128] would be atypical for ET, as would the very low rate of a family history of tremor (15% in our cohort, a proportion of which may be attributable to parkinsonism rather than ET) and lack of response to alcohol as may be expected in ET. There was no evidence on careful clinical examination that these patients had an alternative tremor diagnosis such as parkinsonism or functional tremor.

5.4.3 Use of spirals for rating neuropathic tremor

The use of spirals for rating tremor and their automated analysis has attracted considerable attention in the literature[236 237 254], particularly for use in clinical trials. Our results suggesting that tremor provides a component of the disability experienced by some patients with inflammatory neuropathy, indicates that a simple measure of tremor severity would be of interest for future clinical trials in inflammatory neuropathy. However, the potential for confounding of spiral scores from factors due to the neuropathy itself (weakness, sensory impairment) has not previously been addressed. In our series, although patients without tremor made some errors in spiral drawings, these were not typical for those produced by patients with tremor, lacking periodic oscillation. We therefore suggest that this simple measure could be utilised in future clinical trials.

Tremor in inflammatory neuropathies is common, not just in IgMPN, but also in CIDP and MMNCB. Tremor appears to cause disability independent of other factors relating to the neuropathy and is therefore an important

outcome measure in future clinical trials of treatment, with simple spiral assessments offering an easy tool to do so. Outcomes for treatment of tremor are disappointing except in a small number of cases where tremor improves with immunomodulatory treatment of the neuropathy. F-wave latencies correlate strongly with the severity of tremor, but this is insufficient to fully explain the presence of tremor in only a proportion of patients with inflammatory neuropathy. Perhaps, as suggested by Bain et al[128], the presence of an additional factor such as the inability of a central processor such as the cerebellum to adapt to these mistimed signals is additionally required for patients to develop tremor.

Chapter 6: Cerebellar learning distinguishes inflammatory neuropathy with and without tremor

6.1 Abstract

6.1.1 Objectives

This study aims to investigate if patients with inflammatory neuropathies and tremor have evidence of dysfunction in the cerebellum and interactions in sensorimotor cortex compared to non-tremulous patients and healthy controls.

6.1.2 Methods

A prospective data collection study investigating patients with inflammatory neuropathy and tremor, patients with inflammatory neuropathy without tremor and healthy controls on a test of cerebellar associative learning (eyeblink classical conditioning), a test of sensorimotor integration (short afferent inhibition), and a test of associative plasticity (paired associative stimulation). We also recorded tremor in the arms using accelerometry and surface EMG.

6.1.3 Results

We found impaired responses to eyeblink classical conditioning and paired associative stimulation in patients with neuropathy and tremor compared with

both neuropathy patients without tremor and healthy controls. Short afferent inhibition was normal in all groups.

6.1.4 Conclusions

Our data strongly suggest impairment of cerebellar function is linked to the production of tremor in patients with inflammatory neuropathy.

6.2 Introduction

Inflammatory mediated neuropathies are common and potentially treatable[255]. Tremor occurs with IgM paraproteinaemic neuropathy (IgMPN)[1 111 120] and less commonly in other inflammatory neuropathies[256]. It has been suggested that temporally distorted peripheral inputs reach a normally functioning central processor, such as the cerebellum, which is misled into producing a delayed second agonist burst and tremor[128 257 258]. The involvement of the cerebellum in neuropathic tremor is supported by functional imaging abnormalities[2]. There does not seem to be a straightforward relationship between the development of tremor and conduction velocity[126]. Further, no relationship seems to exist between tremor and the severity of neuropathy as assessed by proprioceptive loss, weakness or fatigue[117 136]. However, we have shown that although conduction velocity does not predict the presence of tremor, it is correlated with its severity for those in whom tremor is present[256]. This indicates a second mechanism may be necessary to produce tremor.

Here we set out to explore aspects of central nervous system physiology in tremulous and non-tremulous patients with inflammatory neuropathies compared to healthy controls. We hypothesised that the central compensation needed to account for delays caused by the peripheral neuropathy would most likely depend on plastic changes within the cerebellum and connections that mediate interaction between sensory and motor systems and therefore that patients with tremor would have evidence

of dysfunction in the cerebellum and interactions in sensorimotor cortex compared to non-tremulous patients and controls.

6.3 Methods

6.3.1 Subjects

Eighteen out of 43 consecutive patients with a diagnosis of inflammatory neuropathy (either CIDP (chronic inflammatory demyelinating polyradiculoneuropathy), MMNCB (multifocal motor neuropathy with conduction block) or IgMPN) agreed to take part in all or just parts of the study. The latter depended on contraindications to electrical/magnetic stimulation and on the cumulative length of study sessions.

Patients were divided into tremulous and non-tremulous depending on whether arm tremor was clinically detectable. The Fahn-Tolosa-Marin score[232], a modified summed Medical Research Council score[186] (MRC score; maximum 70), a sensory score[246] (maximum 56), and the Overall Neuropathy Limitation Scale[191] (ONLS; maximum 12) were performed.

Ten tremulous patients (mean age: 60.0 (9.7) years; mean disease duration: 12.5 (8.2) years; total sensory score 41.7 (13.8); total MRC score: 65.3 (4.2); ONLS score: 3.6 (1.3)) were studied. They were compared with eight non-tremulous patients who did not differ in these characteristics (mean age: 63.3 (8.3) years ($p = 0.46$); mean disease duration: 14.1 (10.6) years ($p = 0.72$);

total sensory score: 42.0 (16.1) (p = 0.97); total MRC score: 63.2 (9.0) (p = 0.59); ONLS score: 4.2 (1.2) (p = 0.38)) (Table 1). We also recruited nine healthy age-matched controls (mean age: 59.0 (7.7) years (p = 0.54)).

Age	Sex	Disease	Duration	FTM	Group	Study
51	M	CIDP	3	9	T	T,E,S,P
66	M	CIDP	11	20	T	E,S,P
51	M	CIDP	9	13	T	T,E,S,P
63	M	CIDP	30	17	T	T,E,S,P
74	M	CIDP	7	13	T	T,E,P
70	M	CIDP	14	17	T	T,E,P
64	M	MMNCB	12	2	T	T,E
56	M	MMNCB	22	29	T	T,E
76	M	IgM (anti MAG positive)	13	37	T	T,S,P
62	M	IgM (anti MAG negative)	4	43	T	T,E,S,P
62	M	CIDP	7	-	NT	E,S,P
51	M	CIDP	28	-	NT	E,S,P
77	F	CIDP	15	-	NT	E,S,P
48	M	CIDP (IgG paraprotein)	7	-	NT	S,P
67	F	CIDP	9	-	NT	E,P
51	F	MMNCB	33	-	NT	E
61	M	IgM (anti MAG negative)	6	-	NT	E
63	F	IgM kappa (anti MAG positive) lymphoblastic lymphoma	8	-	NT	E,S

Table 6.1 Demographics, clinical characteristics and studies undertaken for patients with inflammatory neuropathies. Disease – disease duration (years), M – male, f – female. R – right, L – left. FTM – Fahn-Tolosa-Marin total score (0 (minimum) to 4 points (maximum severity) are assigned for tremor amplitude under a variety of conditions and 0 – 4 points for severity in daily activities). Group T – tremulous, NT – non-tremulous. Study T – tremor analysis, E – eyeblink classical conditioning, S – short afferent inhibition, P – paired associative stimulation.

Before inclusion in the study, written informed consent was obtained from all participants. This study was approved by the local Research Ethics Committee.

6.3.2 Electrophysiological evaluation

Surface electromyographic (EMG) recordings were made with Ag-AgCl surface electrodes using a belly-tendon montage. Data were stored in a computer for display and off-line analyzed using Signal version 4.00 (and Spike version 2 for tremor analyses).

6.3.2.1 Accelerometry and EMG for tremor

Nine patients with tremor (five CIDP, two MMNCB, two IgMPN) took part in this evaluation. A triaxial accelerometer transducer (sensitivity ± 100 mV/g) was attached to the dorsal surface of the middle phalanx of the index fingers.

EMG recordings were made of wrist extensor muscles (WE), wrist flexors (WF), abductor pollicis brevis (APB) and biceps brachii (BB) bilaterally. Recordings were performed (1) with arms relaxed (rest), (2) with arms/wrists outstretched at shoulder level (posture), and (3) a 500-g mass attached to the wrists (loading), (4) while performing a goal-directed task (action). Accelerometry and EMG were recorded and analyzed for 30 seconds in each condition.

6.3.2.2 Blink reflex and eyeblink classical conditioning (EBCC)

Three age matched groups were examined, nine healthy controls, seven non-tremulous patients (four CIDP, one MMNCB, two IgMPN), nine tremulous patients (six CIDP, two MMNCB, one IgMPN). Tremulous and non-tremulous patients did not differ regarding age ($p = 0.97$), disease duration ($p = 0.59$), total sensory score ($p = 0.72$), MRC score ($p = 0.63$) or ONLS score ($p = 0.26$).

Blink reflex and R2 blink reflex recovery cycle were assessed in all subjects according to a protocol previously described[259]. EBCC is an associative learning paradigm, dependent on the cerebellum for acquisition[205]. The conditioning stimulus (CS) was a loud (50 dB above auditory threshold) 2000 Hz tone lasting 400 ms played via binaural headphones. The CS inconsistently produced an acoustic startle response (‘‘alpha blink’’) occurring within 200 ms after the CS. An electrical stimulus (unconditioned stimulus (US); 200 μs pulse width at 5x sensory threshold) was given to the

left supraorbital nerve 400 ms after the CS, eliciting a blink reflex (unconditioned response (UR)).

Repeated pairs of CS and US at 400 ms intervals yield conditioned blink responses (CR) occurring within 200 ms before the US (see figure 6.1). EMG was recorded bilaterally from orbicularis oculi. Conditioning consisted of seven acquisition blocks (each consisting of nine CS-US pairs, one US only, one CS only trial). An eighth and ninth block consisted of eleven CS only trials to measure extinction.

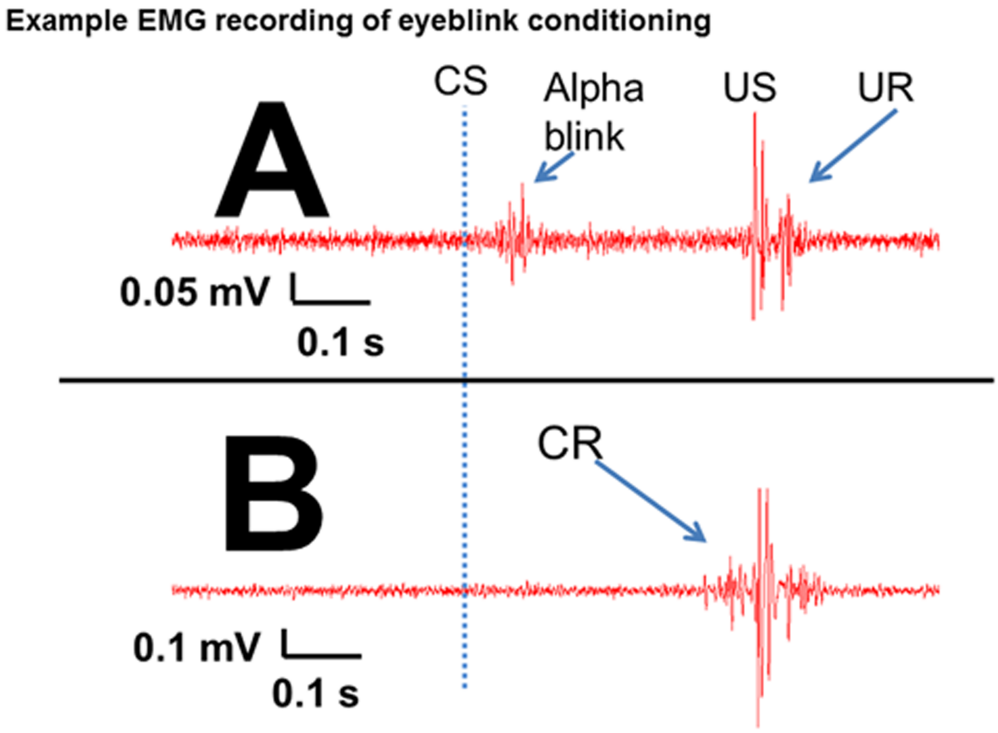


Figure 6.1 Example EMG recording of orbicularis oculi muscle during eyeblink conditioning. CS (dotted line) – onset of conditioned stimulus (auditory tone); CR – conditioned response; US – unconditioned stimulus; UR – unconditioned response. **A)** Recording demonstrating presence of an

alpha blink and absence of a CR prior to the US. **B)** Recording demonstrating presence of a CR prior to the US.

6.3.2.3 Short Afferent Inhibition (SAI) and Paired Associative stimulation (PAS)

Both SAI and PAS rely on precisely timed interactions between sensory afferents and motor cortical stimulation. In healthy subjects, these interactions occur at specific times related to the N20 response. We expected N20 responses to be delayed in our patients and therefore we evaluated N20 latency in each subject. One patient had to be excluded because N20 could not be identified. N20 could be measured in all other subjects studied (expressed as mean (SD): healthy controls 20.3(1.5); neuropathic tremor 33.8(11.5); no tremor 32.6(6.6)).

EMG recordings were made from the abductor pollicis brevis (APB), first dorsal interossei (FDI) and abductor digiti minimi (ADM) muscles of the right side. Test responses in the target muscles were evoked by transcranial magnetic stimulation (TMS) of the left primary motor cortex applied through Magstim 200 magnetic stimulators (Magstim, Whitland, Dyfed, UK) with a monophasic current waveform, connected to a figure of eight coil (mean loop diameter 9cm). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45◦ to the sagittal plane and was optimally positioned to obtain motor-evoked potentials (MEP) in the APB. TMS was used to probe corticospinal excitability before and after PAS.

The coil position and intensity were kept constant throughout the experimental sessions. Electrical stimulation was applied to the median nerve at the wrist at 300% of perceptual threshold using a constant current generator. The stimulus duration was 0.2 ms.

6.3.2.3.1 Short afferent inhibition

Three age matched groups were examined, six healthy controls, five non-tremulous patients (four CIDP, one IgMPN) and six tremulous patients (four CIDP, two IgMPN). Tremulous and non-tremulous patients did not differ regarding age ($p = 0.84$), disease duration ($p = 0.82$), total sensory score ($p = 0.63$), MRC score ($p = 0.90$) or ONLS score ($p = 0.44$).

SAI was assessed as previously described[195] (see section 4.1.1.1.1). We assessed the response to a cortical stimulus alone and when preceded by conditioning stimuli at ten interstimulus intervals in reference to subjects' N20: -18ms, -4ms, -2ms, 0ms, +2ms, +4ms, +6ms, +8ms, +10ms, +18ms. Comparison of responses between groups was based on motor evoked potential (MEP) area.

6.3.2.3.2 Paired associative stimulation

Three age-matched groups were studied, including six healthy controls, five non-tremulous patients (five CIDP) and eight tremulous patients (six CIDP, two IgMPN). Tremulous and non-tremulous patients did not differ regarding

age (p = 0.61), disease duration (p = 0.72), total sensory score (p = 0.62), MRC score (p = 0.96) or ONLS score (p = 0.83).

A conditioning median nerve electrical stimulus was given 5ms plus individual N20 (i.e. 25 ms if N20 latency was 20 ms) before a TMS pulse over the APB muscle 'hot spot' at an intensity predetermined to yield a ~1 mV resting MEP. Two hundred paired stimuli were delivered at a rate of 0.25 Hz (see section 4.1.1.1.2.1 and Stefan et al[181]). Thirty MEPs were recorded before, immediately after, 15 min and 30 min after PAS. Comparison of PAS response was based on MEP area.

6.3.3 Data Analysis and Statistics

A Fourier analysis of signals derived from accelerometry was performed to define peak tremor frequency (PF). Total power of the spectra between one and 30 Hz was used as surrogate measure of tremor amplitude (TP). All parameters were calculated for each accelerometer axis, and then averaged. For EMG, the signal was full-wave rectified and smoothed and Fourier analysis was performed to derive PF.

For measurement of eyeblink conditioning, CRs were counted manually. EMG bursts were regarded as "alpha blinks" if their amplitude exceeded 50 μV and if latency was <200 ms after the CS. EMG bursts were regarded as CRs if latency was >200 ms after the CS but before the US. For the CS only trials, EMG bursts occurring 200–600 ms after the CS were considered CRs.

Statistical analysis was performed using PASW Statistics 18. All post-hoc comparisons were corrected by the Bonferroni method. The level of statistical significance was pre-set at $P< 0.05$.

6.4 Results

6.4.1 Tremor recordings

In all nine patients a bilateral tremor was recorded during posture and action. Five patients had additional bilateral rest tremor. The power spectra of accelerometry and WE EMG showed corresponding peaks. Since there was no side-to-side difference in PF or TP in any position ($p>0.3$), we used the mean of both sides for PF and TP for further analyses. Mean PF and TP in the four recorded conditions are shown in Table 6.2.

	Accelerometry	
	PF (Hz)	**TP (milli-g)**
Rest	7.1 (1.6)	0.55 (1.65)
Posture	6.1 (1.6)	0.85 (2.41)
Weight	6.4 (1.4)	1.21 (2.48)
Action	5.5 (1.4)	7.48 (9.69)

Table 6.2 Mean peak frequency (PF) and total power (TP) (derived from accelerometry) in the four recorded conditions. Results expressed as mean (SD). Rest – rest position; posture – arms outstretched; weight – arms

outstretched with weight loading; action – repetitive finger-to-nose movements.

To compare PF and TP measured by accelerometry at rest, posture, and action we computed two repeated-measures ANOVAs. For PF, there was no effect of CONDITION [$F(2,16)=3.47$; $p=0.06$). For TP, there was an effect for CONDITION [$F(1,8)=6.76$; $p=0.03$), however post-hoc comparisons showed no differences ($p>0.09$).

A t-test for pairwise comparisons showed no difference in PF (accelerometry, WE EMG) before and after loading ($p>0.2$) indicating that loading did not decrease tremor frequency. Five out of nine patients had an increase of tremor amplitude after loading by at least 100%. However, there was no difference regarding TP before and after loading ($p=0.33$) on group level.
In three out of nine patients (two with IgMPN, one with MMNCB), PF in the APB was more than 1Hz lower compared to the biceps. However, a paired t-test comparing PF during posture in biceps and APB in the whole group of patients showed no difference ($p=0.17$).

6.4.2 Blink reflex and eyeblink classical conditioning

R2 blink reflex recovery curves, R1 and R2 latencies and latency variability did not differ between the three groups.

Repeated-measures ANOVA with BLOCK (7) as within-subject factor and GROUP (3) as between-subject factor revealed an interaction of BLOCK x GROUP ($F(12,132) = 3.34$, $p<0.001$). There were also effects of BLOCK ($F(6,132) = 12.2$, $p<0.001$) and GROUP ($F(2,22) = 16.6$, $p<0.001$) (figure 6.2). Post-hoc tests showed that tremulous patients had a lower rate of CRs as the blocks progressed compared to healthy controls and non-tremulous patients ($p<0.001$). This difference was significant in conditioning blocks three to seven (figure 6.2). Latencies of CRs, spontaneous blink rates and "alpha blinks" were not different between the groups.

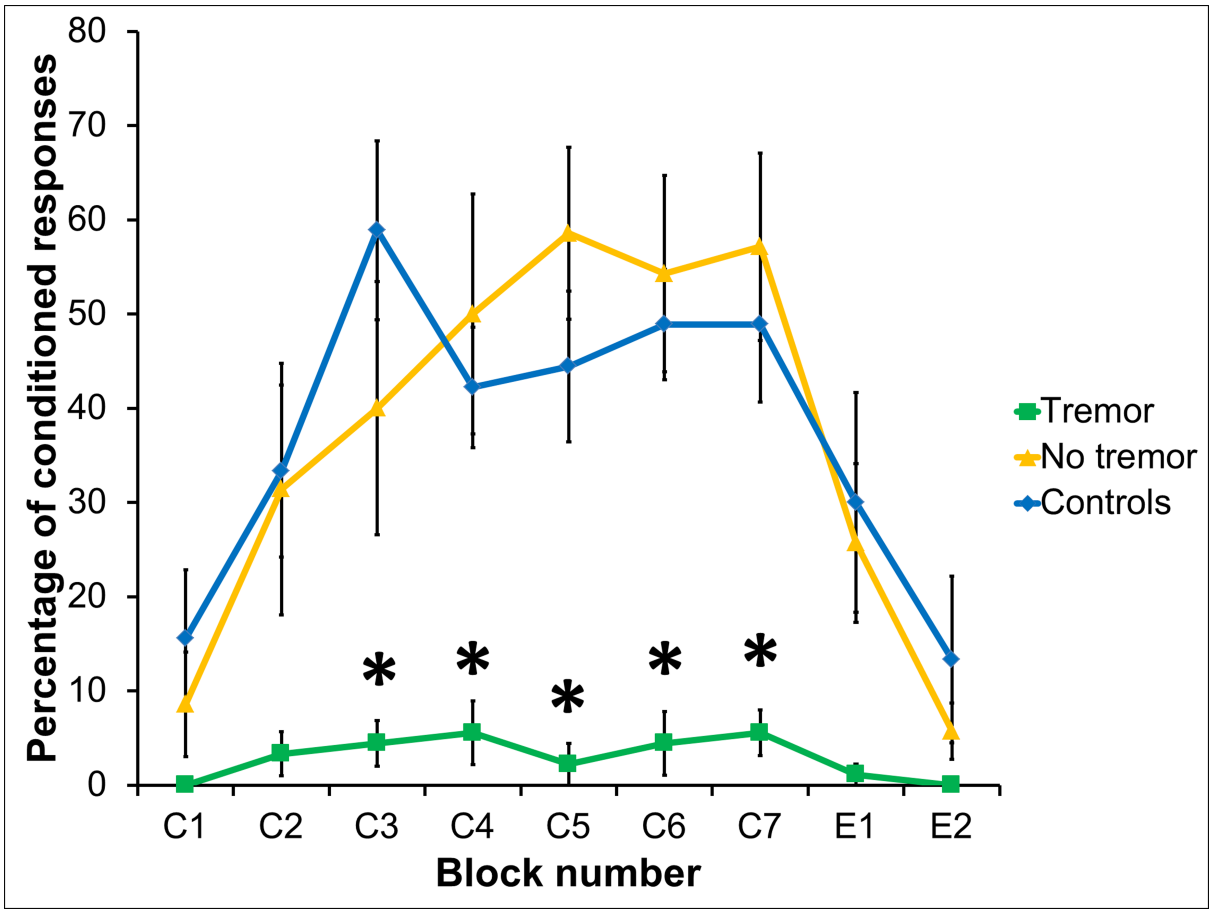


Figure 6.2 Eyeblink classical conditioning in the three groups. Mean percentage of conditioned responses of each group of subjects over the seven conditioning blocks (C1-C7). E1 and E2 represent extinction blocks.

Error bars represent standard error. * Significant lower rate of CRs in tremulous patients compared to healthy controls and to non-tremulous patients.

6.4.3 Short afferent inhibition

Repeated-measures ANOVA with STATE (11) as within-subjects factor and GROUP (3) as between-subjects factor showed an effect of STATE ($F(3,48)=6.64$; $p<0.001$). There was no effect of GROUP or the GROUP x STATE interaction. Post-hoc tests showed a reduction in MEP size occurring at ISI of N20 ($p<0.001$) and N20 minus 2 ($p=0.007$) (figure 6.3).

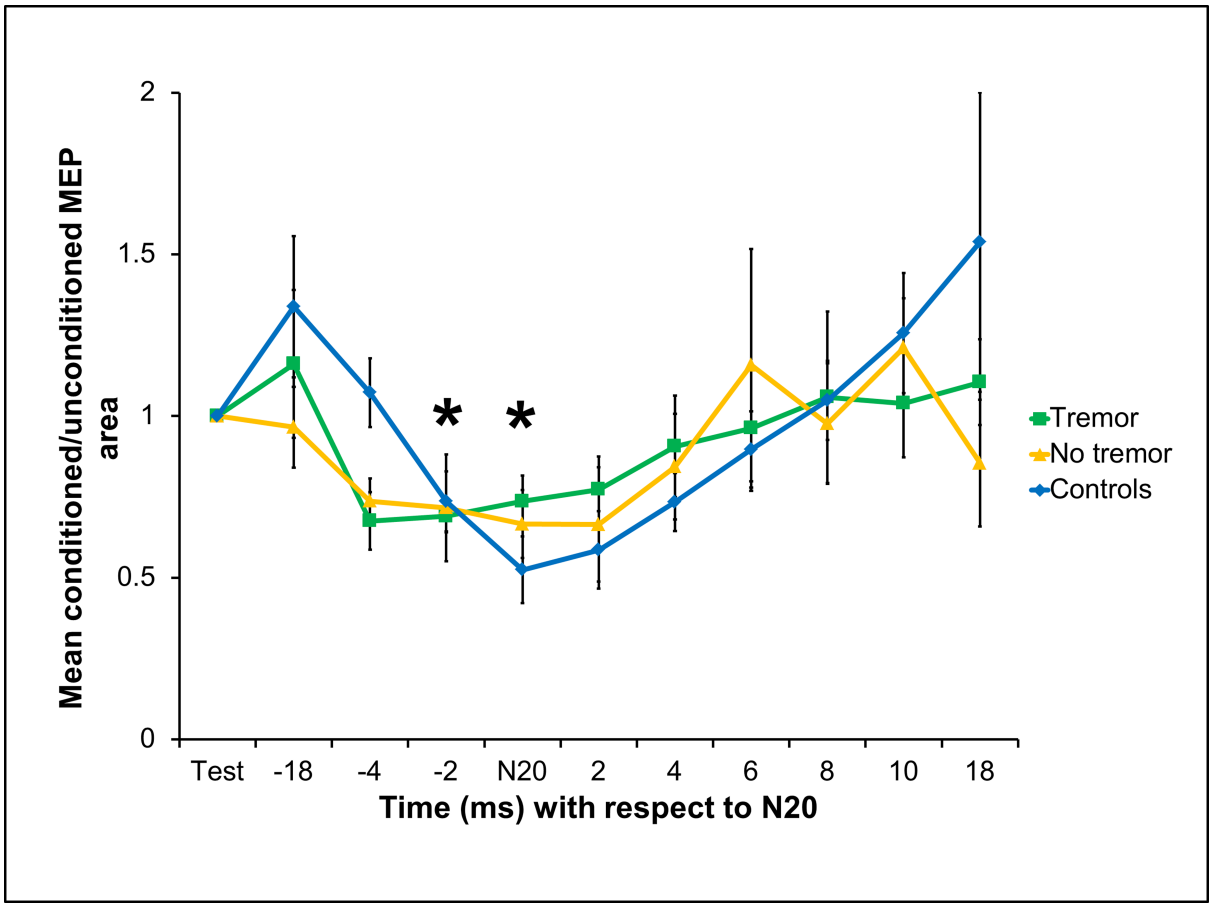


Figure 6.3 Short afferent inhibition in the three groups. Effect of short afferent inhibition on mean conditioned/unconditioned MEP area. *Significant inhibition at N20 and N20-2ms among all groups. Relative values are used for figure. Error bars represent standard error.

6.4.4 PAS

Mean intensity to produce 1mV MEPs was not different between patients (56%) and controls (61%). Mixed-factorial design ANOVA with TIME (4) and MUSCLE (3) as within-subject factors and GROUP (3) as between-subject factors revealed that PAS produced a lasting increase in mean MEP area demonstrated by an effect of TIME ($F(2,33) = 4.762$, $p = 0.014$). The size of MEP facilitation differed among groups and muscles, indicated by an effect of GROUP ($F(2,16) = 9.890$, $p = 0.002$) and an interaction of TIME x GROUP ($F(4,33) = 5.166$, $p = 0.002$). The interaction between TIME x MUSCLE x GROUP ($F(8,62) = 3.436$, $p = 0.003$) demonstrates that the effect of PAS on the homotopically (APB) and heterotopically (FDI, ADM) conditioned muscles differed time-dependently between groups (Figure 6.4).

To further explore the conditioning effects of PAS on MEP areas in each group, we computed separate repeated-measures ANOVAs with TIME and MUSCLE as within-subject factors. In controls, an effect of TIME ($F(3,15)=3.212$; $p=0.047$) was found. The facilitatory effect was stronger in the APB compared to the FDI/ADM, reflected by a strong TIME x MUSCLE interaction ($F(6,30)=7.257$; $p<0.001$). In patients without tremor, a different

pattern of PAS induced changes occurred. MEP facilitation was higher and spatial specificity was compromised as indicated by a main effect of TIME ($F(3,12)= 6.570$; $P=0.007$) without TIME x MUSCLE interaction. Patients with tremor had an effect of TIME ($F(3,21)=3.479$; $p=0.034$) due to overall MEP depression without TIME x MUSCLE interaction.

Post-hoc comparisons revealed that PAS induced an increase in MEP areas compared to baseline in the APB in controls (T15: $p=0.023$), but not in neuropathy with and without tremor. A facilitation of the MEP area in the ADM and FDI was only observed in patients without tremor (T15(FDI): $p=0.003$; T15 (ADM): $p=0.036$).

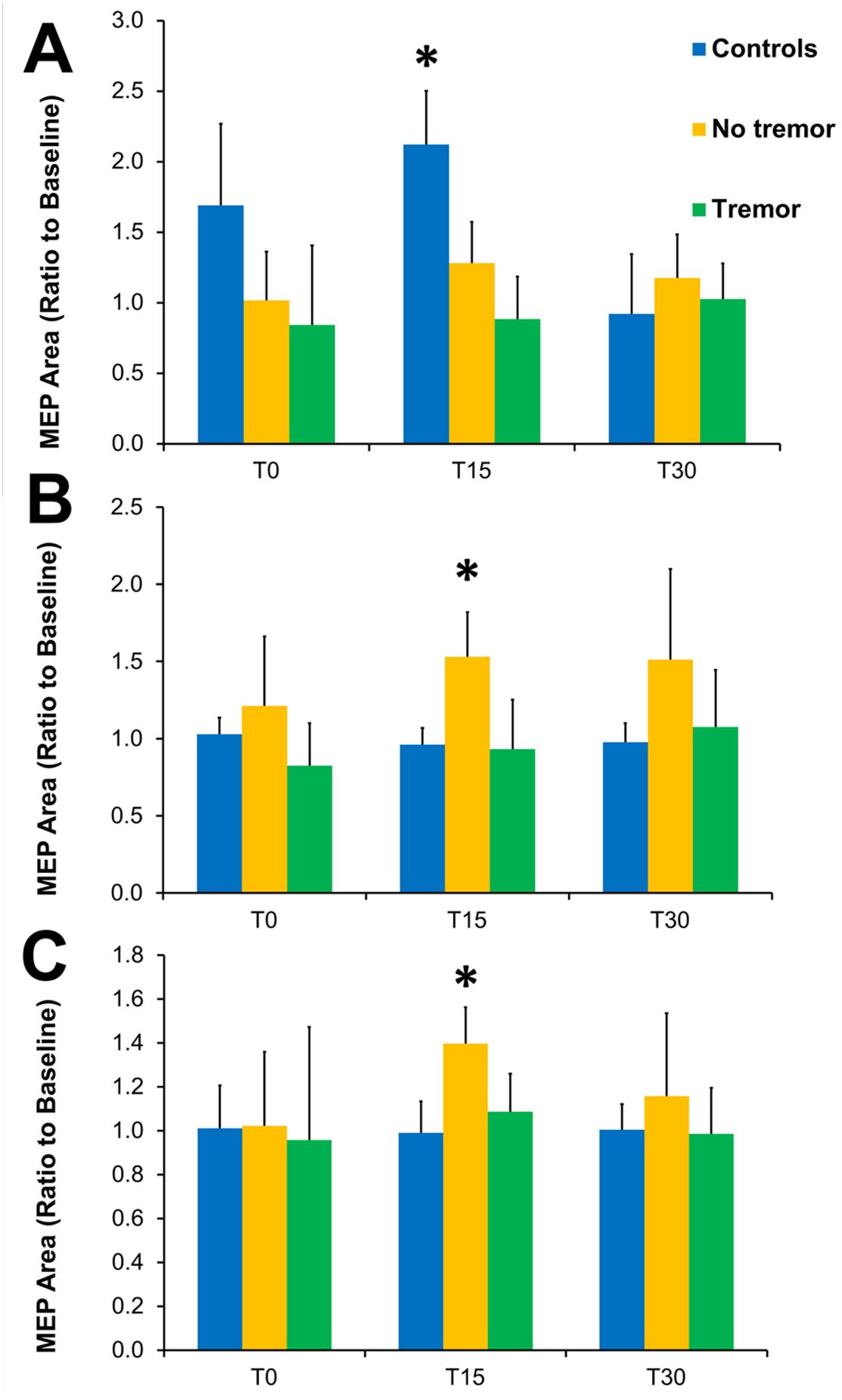

A
B
C
MEP Area (Ratio to Baseline)
MEP Area (Ratio to Baseline)
MEP Area (Ratio to Baseline)
Controls
No tremor
Tremor
*
*
*
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
T0
T15
T30

Figure 6.4 Paired associative stimulation (PAS) in the three groups. Effect of PAS on mean MEP areas in healthy controls (blue), patients with inflammatory neuropathies without tremor (yellow), and patients with inflammatory neuropathies with tremor (green). The data are plotted as a ratio to the baseline MEP area. Error bars represent standard deviation. Ratios higher than 1 indicates facilitation and ratios below 1 indicate inhibition of MEP area. The effect of PAS on MEP area for the APB (target) muscle (A), on the FDI (B), and on the ADM (C). *P < 0.05 paired t-test comparing MEP area with baseline (corrected for multiple comparisons by Bonferroni method).

6.5 Discussion

We demonstrate that patients with inflammatory neuropathy and tremor differ from patients without tremor with regard to cerebellar function and sensorimotor plasticity. We found very low rates of EBCC in patients with inflammatory neuropathy and tremor compared to non-tremulous patients and healthy controls suggesting abnormal associative learning in the cerebellum that segregates with tremor. We also describe an absence of normal facilitation in TMS-evoked EMG potentials after PAS in patients with tremor, suggesting abnormal sensorimotor cortex plasticity. In non-tremulous patients, sensorimotor plasticity, demonstrated by facilitation of TMS-evoked EMG potentials after PAS, occurred in neighbouring muscles but without a normal facilitatory response in the target muscle, suggesting a lack of topographical specificity of sensorimotor plasticity.

Tremor in our patients with inflammatory neuropathies was invariably present during posture and action. Five patients had additional rest tremor. When present in all three conditions, tremor was worst during posture or action which is in concordance with previous reports[128 260]. Previously, a lower tremor frequency in distal compared to proximal hand muscles in two out of six patients with paraproteinaemic neuropathy was described[128]. This was also observed in three of our patients. However, on a group level the peak tremor frequency did not differ between proximal and distal muscles.

EBCC is a form of simple associative learning that is well studied and for which the cerebellum is both necessary and sufficient. Structural or functional impairments of the cerebellum lead to abnormalities in acquisition of this conditioned response[182 205 259]. We demonstrate abnormal EBCC in tremulous neuropathy patients that clearly differentiates them from the normal rates of conditioning in non-tremulous neuropathy patients and controls. Mean R1 and R2 latencies and latency variability did not differ between groups making it unlikely that desynchronization of the afferent volley alone may be a factor in the lack of conditioned responses in the tremulous patients. The degree of impairment of acquisition of conditioned responses reported here is in line with the degree of impairment reported in patients with cerebellar degeneration or cerebellar lesions. A previous study showed a delayed second agonist burst[261] in patients with IgMPN and tremor suggesting that the cerebellum, although intact, would be a likely candidate for a central processor “tricked” into generating tremor in the context of

distorted mistimed peripheral signals[128]. Our data provide evidence that the cerebellum is not functioning normally in those patients who develop tremor.

We were able to record SSEPs, albeit delayed, in all CIDP or IgMPN patients with tremor. This is in line with the assertion that tremor occurs in the presence of distorted rather than absent sensory input[128]. All patients, tremulous and non-tremulous, had normal SAI as compared with normal controls. This suggests that despite the peripheral sensory-motor delay due to the demyelinating neuropathies, central processes have, remarkably, been able to adapt to such delays to reset to the new latency of the N20.

In healthy subjects PAS causes a facilitation of motor evoked potentials in the “target muscle” only, lasting for 15-30 minutes . This response shares a number of features with long-term potentiation[181]. Patients with tremor showed no response to PAS. The normal SAI in patients with tremor argues against afferent dysfunction and associated changes in the sensory motor cortex as sole explanation for the abnormal PAS response. This is supported by the findings in one tremulous CIDP patient with normal N20 and absent PAS response. In recent work, we have demonstrated that cerebellar suppression in healthy subjects by transcranial direct current stimulation impairs subsequent motor cortical facilitation by PAS[262]. We therefore speculate that the absent PAS response in tremulous neuropathy patients may reflect cerebellar dysfunction that is also responsible for their impaired EBCC.

In patients without tremor, PAS response was also abnormal. Facilitatory changes were seen but these occurred in neighbouring ulnar-innervated muscles but not in the APB. This latter finding has not, to our knowledge, previously been described in any other group of subjects. It is conceivable that altered topographical representation triggered by the neuropathy may affect sensory-motor integration required to mediate changes associated with PAS[263 264]. An additional speculation is that this unusual response to PAS may be explained by a peripheral phenomenon such as ephaptic transmission between peripheral nerve fibres.

Here we present evidence that tremor in patients with inflammatory neuropathy is associated with cerebellar dysfunction. We acknowledge that generalizability is limited by our relatively small sample size. Also, this study does not answer the question whether the cerebellar abnormalities in tremulous patients are secondary to the presence of tremor or primary. Regarding the latter, one possibility is that in those with tremor, the specific antibody involved in causing the peripheral neuropathy is capable of crossing the blood-brain barrier and binding to the cerebellum. There is indirect evidence for this in IgMPN in which tremor is typical. It would be of interest to look for evidence of antibodies that bind to cerebellum in tremulous patients with CIDP: they may share a common causative antibody for their neuropathy and the cerebellar dysfunction that drives the development of tremor.

Chapter 7: Tremor in Charcot-Marie-Tooth Disease

7.1 Abstract

7.1.1 Objectives

Tremor in Charcot-Marie-Tooth disease (CMT) can be disabling. Cerebellar abnormalities are thought to underpin neuropathic tremor. Here, we aim to clarify the potential role of the cerebellum in CMT tremor.

7.1.2 Methods

We assessed prevalence of tremor by questionnaire in 84 patients with CMT. Of those, 23 patients with CMT with and without arm tremor and healthy controls underwent a clinical assessment, classical eyeblink conditioning, electro-oculography, visuomotor adaptation test, tremor recording with surface EMG and accelerometry, and retrospective correlation with nerve conduction studies to investigate the possible mechanisms of tremor generation.

7.1.3 Results

The prevalence study revealed tremor in 21% of patients and in 42% of those it caused impairment of function. Tremor recordings revealed a mild-to-moderate amplitude tremor with a weight load-invariant 7.7Hz frequency component. Performance on classical eyeblink conditioning, visuomotor

adaptation and electro-oculography were no different between tremulous and non-tremulous patients and healthy controls.

7.1.4 Conclusions

These results argue against a prominent role for an abnormal cerebellum in tremor generation in the patients studied with CMT. Rather, our results suggest an enhancement of the central neurogenic component of physiological tremor as a possible mechanism for tremor in the patients studied.

7.2 Introduction

Tremor can occur as part of Charcot-Marie-Tooth disease (CMT) due to a variety of mutations. In paediatric CMT, tremor is one of the most disabling symptoms[243] and also predicts other disabling symptoms[244]. The mechanisms that give rise to this tremor is not known although various hypotheses have been postulated[265 266]. Cerebellar dysfunction is one hypothesis, as has been shown for tremor in inflammatory neuropathies[2 128 248 256]. Co-existence of essential tremor has also been considered, where cerebellar dysfunction would also be expected. Another possibility is fatigue causing entrainment of motor units resulting in an enhanced physiological tremor[90 267-269]. Fatigue is commonly recognised in CMT and represents an important outcome measure in clinical trials[270]. Understanding tremor mechanisms is important as little is known about treating tremor in CMT, why only some patients seem predisposed and why it predicts other disabling symptoms.

Here we investigate clinical and pathophysiological aspects of the tremor associated with CMT, with an emphasis on techniques sensitive to cerebellar dysfunction such as eyeblink classical conditioning (EBCC)[203-206], visuomotor adaptation[271] and eye movement recording[215]. We hypothesise that these should be abnormal if the cerebellum is functioning abnormally or entrained in a pathological network in tremor in CMT.

7.3 Methods

7.3.1 Screening for tremor in a large cohort of CMT

Questionnaires were sent to all patients with CMT attending a peripheral nerve outpatient clinic at the National Hospital for Neurology and Neurosurgery, London, UK (see Table 6.2 and Table 6.3) to assess clinical features of tremor. Patients completed a spiral drawing with their dominant hand to assess tremor using the Bain and Findley spiral score (0 representing no tremor; 10 representing severe tremor)[238].

7.3.2 Clinical assessment

Twenty-three patients with CMT with and without tremor were recruited. Each group was matched for age, gender, diagnosis and severity of neuropathy (see Table 6.1). Detailed clinical assessment was performed. Patients taking tremorgenic medications were excluded. Summed scores for limb strength (MRC score)[186], sensation (subset of CMT neuropathy score)[187] and deep tendon reflexes (NINDS myotactic reflex scale for biceps, supinator, triceps, knee and ankle)[188] were calculated. Tremor was assessed using the Fahn-Tolosa-Marin score[189]. Saccadic and pursuit eye movements were examined as well as a positional manoeuvre for signs of cerebellar dysfunction[190]. Neuropathy severity was assessed with the CMT neuropathy score[187] and disability measured by the overall neuropathy limitation scale[191]. Patients were divided into two groups for subsequent analysis depending on

the clinical presence of tremor (tremulous and non-tremulous groups). Patients' nerve conduction studies were reviewed.

7.3.3 Motor control studies

Differing numbers of patients were recruited for each of the following studies. The combined study duration for all experiments was not feasible for all subjects.

7.3.3.1 Accelerometry and EMG

16 patients (10 tremulous and 6 non-tremulous) participated. As detailed (see section 4.1.5), a triaxial accelerometer (Biometrics Ltd; sensitivity ±50 mV/G) was attached to the dorsal surface index finger bilaterally. Surface EMG was recorded simultaneously, from biceps brachii (BB), forearm flexors (FF), forearm extensors (FE) and abductor pollicis brevis (APB) bilaterally. Recordings were performed: a) with arms outstretched (postural condition) and (b) postural condition with 500g mass attached to the hand (weight loading).

7.3.3.2 Eye movement recordings

Five tremulous patients underwent electro-oculography (EOG) to record horizontal saccadic and pursuit eye movements. Subjects were seated 84cm from a target light source. For saccades, LEDs were randomly presented at

10, 20, or 30 degrees in rightward and leftward directions, with inter-stimulus interval of 4 seconds. Smooth pursuit eye movements were assessed using 8 cycles of a target moving horizontally about a centre point with a sinusoidal velocity curve. This was repeated for 0.1Hz, 0.2Hz, 0.3Hz and 0.4Hz (target displacement +/- 20 degrees, peak velocities from 12.5, 25, 37.5, and 50 degrees/s respectively). Subjects were instructed to maintain fixation on the laser target. Eye movements were calibrated and recorded using bitemporal DC electro-oculography.

7.3.3.3 Visuomotor adaptation

14 patients (8 tremulous and 6 non-tremulous) and nine healthy participants matched for age, sex and handedness completed this experiment, measuring visuomotor adaptation[229]. Participants used their dominant hand to manipulate a cursor (via joystick) to visual targets in a circle on a computer screen. A square marked the centre of the circle, and a white cursor indicated joystick position. The starting position was in the centre of the circle (Figure 7.1C). At the start of each trial, participants were instructed to move the joystick to place the cursor inside the (randomly determined) peripheral target square (indicated by a colour change) as quickly as possible, returning the cursor to the centre square prior to the next trial (figure 7.1D). This was repeated 40 times per block.

In the baseline condition, three sequential blocks were presented. In the fourth block, the rotation condition, a constant 30-degree anticlockwise

angular displacement was introduced into the path of the cursor displayed on the screen without participants' prior explicit knowledge (Figure 7.1E).

7.3.3.4 Eyeblink conditioning

14 patients (7 tremulous and 7 non-tremulous groups) and 12 healthy participants matched for age, sex and handedness completed this experiment. Methods are described in full in chapter 4 (Figure 7.1G).

7.3.4 Data analysis and statistics

7.3.4.1 Tremor analysis

Fast Fourier transform of the accelerometry and envelope EMG signal was performed to define the peak tremor frequency and total power of the spectra between 1 and 30 Hz. All parameters were calculated for each accelerometer axis. The square root of the sum of the squares of each axis[272] was derived for each measure of total power. For frequency comparisons, the highest total amplitude channel was chosen.

7.3.4.2 Eye movement recordings

For saccades, latency (time to initial horizontal eye velocity offset from target presentation), maximum peak velocity and metrics were assessed. Velocity measures were calculated as the differential of the EOG trace and are

expressed in angular velocity units (degrees/s). Gain (slow phase eye velocity/stimulus velocity) for saccades and pursuit, for both rightward and leftward eye movements were calculated. Patient data was compared to that of eight healthy age and sex-matched controls.

7.3.4.3 Visuomotor adaptation

Temporal and spatial variables used to characterize task performance were *reaction time* (from target presentation to movement onset; RT), *movement time* (from movement onset to stabilisation of the cursor in the target; MT), and *displacement ratio* (ratio between the length, measured in pixels, of a straight line “perfect path” between starting point and target, and actual path length taken by participant; DR). Improvement was indicated by a ratio in the first 10 trials and the last 10 trials below 1 for RT, MT, and DR.

7.3.4.4 Eyeblink conditioning

For EBCC, the percentage of conditioned responses (CR), the onset and peak latency of the CR, the amplitudes of the CR and unconditioned responses (UR) were used as dependent variables. Latencies to onset and peak of conditioned eyeblink responses were visually identified. CR onset was defined as an increase in EMG activity greater than 1 SD above baseline noise occurring within 200 ms before the onset of supraorbital nerve stimulation.

7.3.4.5 Statistical analysis

Statistical analysis was performed using PASW Statistics 19. ANOVA was used to compare baseline characteristics between groups. For two-way comparisons of tremulous versus non-tremulous patients in the questionnaire, independent T-tests were used. Spearman's rank correlation coefficient was used for correlation between EMG/accelerometry and clinical features. Frequency of abnormal test results between groups was detected using Chi-Square Tests, Fisher's exact (2-sided). Non-parametric tests (Mann-Whitney U test, Friedman ANOVA completed by post hoc analysis with Wilcoxon test) were used to compare groups where Kolmogorov-Smirnov test indicated non-normally distributed data. Post-hoc comparisons were corrected by the Bonferroni method. Results are expressed by mean and SD unless otherwise specified. P-values below 0.05 were considered significant.

7.4 Results

7.4.1 Clinical assessment

Clinical characteristics are described in table 7.1. Tremor, where present, occurred on posture in both arms, with little detectable clinically on movement and no rest component. Minor postural leg tremor was noted in two patients with arm tremor. One tremulous patient (1/13) reported benefit with alcohol; eight did not report benefit and four do not drink alcohol. There

were no differences between the tremulous and non-tremulous group in nerve conduction measures of median or ulnar nerve.

Variable	**Tremulous patients**	**Non-tremulous patients**	**p-value**
Number of patients	13	10	n/a
Age (years)	54.7 (12.6)	48.5(13.4)	0.41
Sex	31% female	33% female	1.00
Diagnosis	CMT1A (92%) CMT1B (8%)	CMT1A (80%) CMT1B (10%) CMT2 mitofusin (10%)	
MRC score (arm)	35.9 (5.1)	34.1 (9.7)	0.34
NINDS reflex score (arm)	1.3 (3.6)	3.8 (5.3)	0.11
Sensory score (arm)	22.8 (7.5)	23.3 (4.5)	0.83
CMT neuropathy score	17.5 (7.2)	16.5 (8.6)	0.82
ONLS score (arm)	1.9 (1.1)	2.0 (1.4)	0.60
Median nerve conduction velocity (wrist to elbow) (m/s)	21.2 (9.0)	21.7 (12.5)	0.92
Median nerve CMAP (wrist) (mV)	4.2 (3.4)	2.8 (2.1)	0.29
Median nerve F-wave latency (wrist) (ms)	56.9 (18.4)	42.9 (16.0)	0.32

Table 7.1 Clinical features comparing CMT1A patients with and without tremor. Results reported as mean (standard deviation); n/a – not applicable.

Genetic diagnoses of responders to survey	**Percentage of all responders (n=84)**
CMT1A	49
CMT1B	4
CMT1 (other)	10
CMT intermediate	1
CMT2	34
CMT4C	1

Table 7.2 Genetic diagnoses of responders to postal survey.

Genetic diagnoses of non-responders to survey	**Percentage of all non-responders (n=89)**
CMT1A	31
CMT1B	7
CMTX	9
CMT1 (other)	10
CMT2	43

Table 7.3 Genetic diagnoses of non-responders to postal survey.

7.4.2 EMG/accelerometry

Table 7.4 summarises the results. For tremulous subjects, there was no correlation between age and frequency of postural arm tremor in contrast to reports in ET[273]. There was no difference in postural tremor frequency between males, 7.6 (0.61) Hz and females, 8.3 (2.4) Hz with tremor. There was no difference in EMG peak frequency between proximal (BB) and distal muscles (APB). Six of ten tremulous patients had an EMG peak at the same frequency as the main accelerometer peak. Weight loading and alteration of posture had no effect on the postural tremor frequency in tremulous patients as a group. However, with weight loading, four patients demonstrated a secondary accelerometry peak with lower frequency.

Accelerometry	**Tremulous patients**		**Non-tremulous patients**	
	Value	**p-value**	**Value**	**p-value**
Accel freq (Hz) on posture	7.7 (1.7)	0.90	8.8 (3.4)	0.08
Accel freq (Hz) with weight	7.8 (2.3)		7.6 (2.8)	
Accel power on posture (mG)	1.0 (3.1)	0.34	0.02 (0.02)	0.22
Accel power with weight (mG)	2.0 (6.8)		0.03 (0.02)	

Table 7.4 Accelerometry measured tremor peak frequency and power between tremulous and non-tremulous patients.

7.4.3 Eye movement recordings

Clinically, there was no spontaneous or gaze-evoked nystagmus in any patient. Head impulse test was normal bilaterally, with normal pursuit and saccadic eye movements. There was no difference in horizontal saccadic velocity, metric gain or latency between patients and controls. Pursuit gain was similar between groups, for all target frequencies (figure 7.1A). There were no within-subject effects of direction or saccadic amplitude (figure 7.1B). For saccadic velocity there was, as expected, an effect of amplitude ($F(1,11)=74.9$, $p<0.001$) but not direction.

7.4.4 Visuomotor adaptation

All three groups performed the task similarly without differences. All groups demonstrated improvement after the first two blocks in all variables, without change between Block 2 and 3 suggesting a ceiling effect of learning. In the rotation block, all groups demonstrated improved performance in the last 10 trials compared with the first 10 of the block in variables RT ($F(1,21)=13.4$; $p=0.001$), MT ($F(1,21)=17.6$; $p<0.0005$) and DR ($F(1,21)=17.3$; $p<0.0005$). There was no Group x Block interaction for RT ($F(2,21)=1.07$; $p=0.36$), MT ($F(2,21)$-1.15; $p=0.34$) or DR ($F(2,21)=1.34$; $p=0.28$) (figure 7.1F).

7.4.5 Eyeblink conditioning

There were no differences between the three groups in acquisition or timing of CRs. All three groups had an increased proportion of CRs as the blocks

progressed F(2.7,63.0)=10.0, p<0.0005 (between block factor). There was no difference in acquisition of CRs between groups (figure 7.1H). Total number of CRs over all blocks was similar between groups (healthy controls 38 (7.1)%, non-tremulous patients 28.8 (8.4)% and tremulous patients 30.7 (8.8)%).

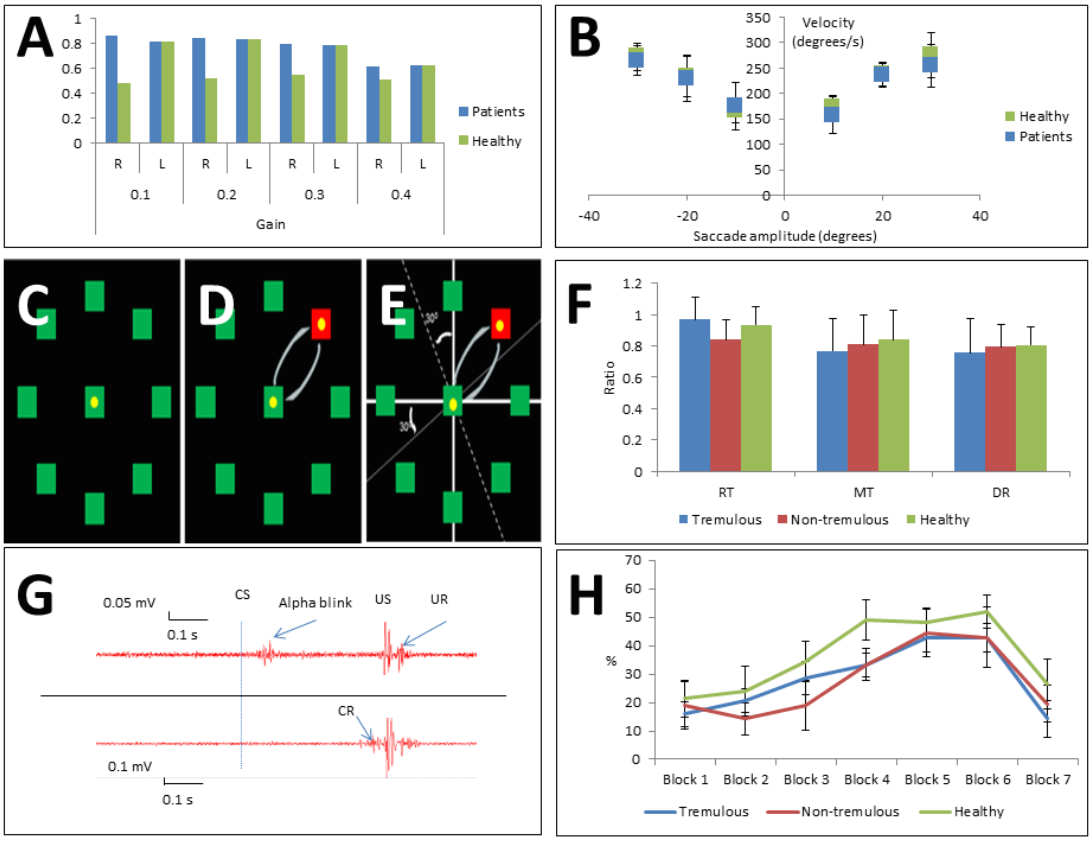


Figure 7.1 Summary results for electrophysiology and eye movement studies. **(A)** Gain (y-axis) of pursuit eye movements. Gain illustrated according to angular velocity (degrees/second) and side (R – right; L – left). **(B)** Butterfly plot of saccadic eye movement velocity. Standard error bars point upwards for healthy controls, downwards for patients. **(C)** Test of visuomotor adaptation. Screenshot of monitor display at the start of each trial. **(D)** Colour change (to dark grey) indicated cue to move cursor to

peripheral target. Arrows demonstrate direction of movement to and from peripheral target. **(E)** Rotation task testing visuomotor adaptation with 30 degree anti-clockwise perturbation covertly applied to path of cursor. **(F)** Results of visuomotor adaption task showing three groups' performance on RT (reaction time), MT (movement time) and DR (displacement ratio). **(G)** Representation of recording from eyeblink conditioning. Upper trace reveals CS (conditioning stimulus) succeeded by an alpha blink and an US (unconditioned stimulus) followed by an UR (unconditioned response). The lower trace represents acquisition of the CR (conditioned response) after the CS and prior to the forthcoming US. **(H)** Results of eyeblink conditioning.

7.4.6 Screening for tremor in a large cohort of CMT

Eighty-four of 157 questionnaires sent were returned (54%). Fifty-six percent were female. Forty-eight percent reported to have upper limb tremor. Mean duration of tremor was 11.3 (8.5) years. Of those with tremor, 32% had a positive family history of tremor, 7% reported their tremor to improve transiently with alcohol, 66% had tremor when drinking or pouring fluids and 42% had tremor when fastening buttons. Thirty-nine percent reported that other people had commented on their tremor. Twenty-two percent with tremor were on medication that could potentially cause tremor. None of them had been diagnosed with medication-induced tremor. Only 18 of those who reported tremor had a formal diagnosis of tremor by a doctor (21% of total respondents). Of those, only three had specific causes cited for their tremor, namely neuropathic tremor (2) and subarachnoid haemorrhage related

tremor (1). Those diagnosed with tremor by a doctor did not have worse performance on spiral scores (2.4(1.3)) than those self-reporting tremor (2.5(1.3)), t(37) = 0.097, p = 0.92. Tremor caused some impairment of daily activities. Those who reported difficulty buttoning clothes due to tremor scored significantly worse on the Bain Findley spiral score (3.1(1.4)) than those with tremor who did not report such difficulties (2.0(1.0)), t(36)=-2.9, p = 0.007.

7.5 Discussion

7.5.1 Overview

In this study, we report the most detailed physiological study of tremor in CMT to date. The tremor in CMT has been varyingly reported as indistinguishable from 6-8 Hz essential tremor[266 110], similar to enhanced physiological or cerebellar tremor[274] or resulting explicitly from weakness and abnormal stretch reflexes mediating central drive to enhanced physiological tremor[275]. Tremor is one of the strongest independent determinants of reduced QOL in children with CMT1A[243] and multivariate modelling suggests that interventions designed to improve tremor might have a beneficial effect on QOL.

Essential tremor[5] involves the cerebellum but whether or not this is due to degenerative change seems questionable[11]. We demonstrate lack of evidence for functional abnormalities in the cerebellum associated with

tremor in CMT exemplified by normal visuomotor adaptation, control of eye movements and EBCC. Instead, EMG and accelerometry data demonstrate a tremor consistent with enhancement of central mechanisms underlying enhanced physiological tremor. Our survey of tremor in CMT suggests tremor is often under-diagnosed and may be contributing to disability in some patients.

7.5.2 Lack of evidence for cerebellar dysfunction underlying tremor in CMT

Cerebellar dysfunction has been proposed as a potential mechanism for tremor in CMT based on an apparent clinical overlap between the tremor of CMT with ET, and the results of experimental studies in patients with tremor associated with inflammatory neuropathies which have suggested cerebellar involvement[248]. In this study, we examined a range of physiological and behavioural markers of cerebellar dysfunction. We found no differences between tremulous CMT patients, non-tremulous CMT patients and healthy controls in cerebellar dependent associative conditioning, adaptation to a visuomotor perturbation and assessment of eye movements. Our results suggest that the tremor seen in patients with CMT is unlikely to be related to cerebellar dysfunction, in contrast to patients with ET or cerebellar pathology[28 215 271 276]. This, along with the relative lack of alcohol responsiveness found in our series and the high prevalence of tremor compared to that expected in the general population[14] also suggests that tremor in patients with CMT is unlikely to be due to chance co-occurrence of

ET. Although a family history of tremor was commonly reported by some patients, we believe that it may represent tremor present in other family members with CMT, rather than ET. Our results imply that the association between ET and CMT seems improbable and would thus not support pursuing genetic linkage analysis of ET and CMT as has been suggested in the past[266].

7.5.3 Tremor in CMT is consistent with enhancement of the central component of physiological tremor

Two distinct components are thought to exist in enhanced physiological tremor. The first is a peripheral *mechanical-reflex* component mediated by the natural oscillation properties of the limb and the properties of reflex arcs. The second is a central neuronal component. Our results demonstrate the tremor in CMT to be a postural tremor of 7.7 Hz. The lack of dependence of EMG tremor frequency on weight loading, the proximity of muscles to spinal cord, or peripheral nerve conduction velocity argues against a dominant mechanical-reflex component[117 274 275]. Further, the mechanical component of physiological tremor is thought to arise in part due to irregularities in motor unit firing providing a broad-spectrum signal that drives oscillations in the limb at eigenfrequency. In the finger this would be expected to be a higher frequency (20-25Hz)[277] than observed in our patients. The tremor seen in patients studied here most likely reflects enhancement of the central component of physiological tremor as previously described[278]. This is invariably associated with a driving modulation of motor-unit activity at 7-

13Hz regardless of their mean discharge frequency[278], not simply a passive response to sensory feedback; being present in deafferented patients[279]. The central-neurogenic component of physiological tremor may originate from an oscillating network within the central nervous system[97 101]. This has been postulated to include direct central feedback loop from the motor neuron pool to Renshaw cells[278] or the spinal interneuronal system[280]. As seen here, this central component of enhanced physiological tremor is independent of reflex arc length and the frequency is not modulated by increased limb inertia[281] or stiffness[90 278 282 283]. We found that tremulous patients had a weight-invariant EMG spectral peak at the frequency of the dominant accelerometry peak consistent with enhancement of the central component of physiological tremor[284]. More recently, modulation of physiological tremor phase has been possible with cerebellar stimulation without effect on amplitude[285], in line with the contemporaneous idea that although physiological tremor is driven centrally, there may be gain modulation more distally, perhaps modulated by the neuropathy in the case of tremor in CMT.

Here, neither clinical nor electrophysiological markers of severity differentiated between those with or without tremor. Our data suggesting the tremor of CMT reflects an enhanced central component of physiological tremor provides a basis for speculation regarding mechanism. First, while the 10-Hz component of physiological tremor may be explained in part by unit firing rates, external synchronization may account for increased tremor amplitudes observed on strong or fatigued contraction. Fatigued finger movements are associated with increased tremor and this is not confined to

the fatigued finger, as there is fatigue-related enhancement of a common drive at the supraspinal level. Fatigue in neuromuscular disorders is pervasive[286]. Fatigue has been suggested to be an important symptom linking tremor and cramp in paediatric CMT1A[244]. Indeed, our results show that for some patients, limb weight loading worsened tremor, although this was not significant on a group level. Fatigue could therefore be one factor behind the augmentation of physiological tremor in CMT, warranting further investigation. An important proposed mechanism for preventing 10Hz central oscillations being transmitted to motoneurones resulting in tremor is a “phase cancellation” system mediated by spinal interneurons[287]. There is evidence that pathology in peripheral nerves may extend to involve spinal interneurons[288 289] and spinal cord motoneurone[290], and it is possible that individual differences in the extent of this pathology is a factor driving the manifestation of tremor in a proportion of patients with CMT.

7.5.4 Tremor in CMT may be under-reported

Tremor contributes to disability in CMT and may need assessment in future clinical trials. The results of our questionnaire corroborates reports that tremor is common in hereditary neuropathies[266]. Our results indicate that the tremor is typically mild to moderate. However, self-report of tremor was commoner than doctors’ diagnosis of tremor, so this symptom seemed under-reported.

7.5.5 Limitations

There are a number of limitations to our study including sample sizes. However, the relative rarity of the disease and the requirement for study participants to undergo a long duration of experimentation given the multiple methods, means that these numbers compare favourably to previous studies. Nevertheless, generalisation of the results found here need to be tempered until this is replicated by others. Our postal survey had a response rate of only 54%. However, there were no obvious differences in the genetic subtypes of respondents versus non-respondents in our survey. Nevertheless, multiple variables determine response rates to surveys and this may include the ability of participants to easily complete a survey by hand, thus potentially introducing a bias of lower severity of neuropathy or tremor in the responder group.

In summary, tremor occurs in some patients with CMT, is often mild but under-diagnosed and may contribute to disability in some patients. We demonstrate lack of cerebellar dysfunction and therefore it distinguishes itself from tremor in inflammatory neuropathies and essential tremor. Given the relatively small sample size, these findings would require validation in a larger sample. Nevertheless, our data suggest that tremor in at least some patients with CMT represents an enhancement of the central component of physiological tremor which may have implications for future studies on the role of fatigue and consequence of disability in hereditary neuropathies.

Chapter 8: The effect of transcranial direct current stimulation of the cerebellum on voluntary rhythmic finger movements

8.1 Summary

Previous chapters have demonstrated evidence of how the cerebellum appears to be implicated in tremor generation in a sub-set of patients with neuropathic tremor, namely those with an inflammatory cause. In other tremor disorders, such as ET, non-invasive brain stimulation has been used to investigate potential treatment approaches to tremor. Gironel has used 1Hz rTMS and found tremor amplitude suppression when applied over the cerebellum. This may have multiple potential effects, some undesirable. In terms of non-invasive approaches to treatment, transcranial direct current stimulation (TDCS) has favourable cost implications, ease of use and potential portability and translational value. TDCS over the cerebellum is a natural potential interest in terms of its possible effects on tremor in ET but also in NT, given our results and those of others suggesting an aberrant role of the cerebellum. Here, we turn attention to the normal function of the cerebellum and how this might be adversely affected by TDCS. We investigate a well-established function of the cerebellum, timing of rhythmic finger movements and aim to determine qualitatively and quantitatively changes in this function induced by cerebellar stimulation.

8.2 Abstract

Mounting evidence indicates that the cerebellum plays a pivotal role in the timing of rhythmic movements. Previous studies using patients with cerebellar lesions have provided evidence for the cerebellum underlying sub-second movements, but imaging studies and TMS experiments have been less consistent. In this study, polarity-specific TDCS was used to modulate the activity of the lateral cerebellum. During modulation with TDCS, any effect on timing mechanisms was assessed through performance on a rhythmic tapping task. A trial comprised of healthy participants tapping in time with an auditory cue for 30s (synchronisation phase) and then continuing to tap at the same rate without a cue (continuation phase) for a further 30 seconds. Trials were completed with tapping frequencies of 0.5, 1 and 3Hz. Each trial was completed three consecutive times for each of the respective frequencies, the latter randomised. Different stimulation modes were used on different days, the order randomised across participants. TDCS to the right lateral cerebellum was shown to have no effect on accuracy or variability of the intertap interval. Whilst it may be possible that TDCS does affect cerebellar timing networks, our results failed to reject the null hypothesis that the cerebellum does not have a critical role in event based timing.

8.3 Introduction

Sensory perception and effective motor control are both dependent on precise timing mechanisms. Despite the emerging concept of a distributed network underpinning timing, the cerebellum appears to play a key role, given in part, its ability to respond to inputs with a variety of oscillatory outputs[220]. Disrupting cerebellar circuits can manifest as problems with timing[156] including the ability to perform specifically timed rhythmic movements. Multiple patient studies with cerebellar insults purporting timing deficits support the role of this area as a central clock. Supporting the role of the cerebellum in timing are neuroimaging studies that demonstrate increased cerebellar activation during imagined timing tasks[291]. As part of its putative role in timing, the cerebellum appears crucial for sensorimotor synchronisation (SMS), whereby motor responses are synchronised with predictably-timed external stimuli[220]. In this context, the role of the cerebellum is likely confined to discontinuous movements that require event-based timing for explicit representation of a temporal goal. Such a goal can be evaluated for accuracy and any error in timing used to inform the next movement in a longer sequence as opposed to continuous tasks where emergent timing seems to dominate. The paced finger tapping task (PFT) has been used widely in the literature as a measure of timing. Spencer et al[221] investigated individuals with unilateral cerebellar lesions performing repetitive finger tapping tasks and demonstrated ipsilesional increase in the coefficient of variability of the inter-tap interval (ITI).

The synchronisation-continuation task (SCT) also utilises the PFT and requires participants to tap with their index finger in time to a train of auditory tones separated by a fixed time interval, or inter-stimulus interval (ISI). In the second stage of this test, the continuation phase, the stimulus is discontinued but the participant is instructed to continue tapping at the previously learned rhythm. The PFT task enables measure of two baseline variables, accuracy and inter-tap variability variability. The accuracy of the timed response, through the framework of the tapping paradigm, demonstrates how 'well timed' the taps are, measurable by the mean ITI, or alternatively the mean absolute error. Variability is a measure of the spread of taps around the temporal target[292]. Patients with lateral cerebellar lesions seem to have greater variability in their performance of the PFT task compared with healthy controls[222].

The Wing-Kristofferson model suggests that variability be divided into central (the internal clock within the brain) and motor variance (execution of the movement). In the continuation phase of the PFT task (i.e. tapping without the tone) the variability can be associated with the variability of the central timekeeper, as the motor variance theoretically remains constant[293]. Motor variance is consistently small except at very short ITI durations, where faster tapping may incur fatigue and larger variability (for review, see[294]). The model implies that central and motor variance are processed independently of one another, and thus may have separate neural correlates[293]. Since the Wing-Kristofferson model is specific to the continuation phase of the PFT task, it is also relevant to ask whether synchronisation and continuation

phases of the PFT task have separate neural correlates and whether the cerebellum is key to either.

Synchronising finger tapping to an external auditory cue requires formation of an internal temporal representation of the stimulus. Representation of this stimulus to timed motor outputs requires feed-forward mechanisms rather than solely feedback systems given the observation that deafferented individuals are able to tap in phase with a metronome despite being unable to see or hear their taps[294]. The cerebellum may play such a feedforward role (for review see[295]), perhaps by modulating efference copy output from other areas such as M1[296]. Despite the importance of feedforward mechanisms on tap timing, proprioceptive, visual and auditory feedback are also required to update the planned motor actions for subsequent taps, perhaps via the cerebellum given its prominence in error correction.

Theoret et al[297] applied rTMS over the medial cerebellum demonstrating increased variability of tapping in the PFT task. Whilst Del Olmo et al[298] also found an increase in variability of the PFT task at 2Hz when rTMS was applied to the lateral cerebellum, Jancke et al[299] found no effect on timing when rTMS was given over the the same area. However, Rao et al also found that during a temporal task activations of the cerebellum arise later than one might expect if it were to be involved in explicit timing[300]. Although cerebellar TDCS has previously been used successfully to modulate cerebellar function in a polarity specific manner[185 262 271], it has not yet been shown in relation to timing.

The cerebellum has a number of roles that relate to repetitive movement control. Cerebellar abnormalities are known to affect rhythmic hand movement control. Indeed in tremor disorders where the cerebellum is rather clearly linked to pathophysiology, such as ET, a timing function thought to be reliant on the cerebellum is affected as demonstrated by an increased variability of rhythmic hand movements when compared with healthy controls[301]. Similarly, it has been shown that inhibitory rTMS, potentially by reducing neuronal excitability, can disrupt cerebellar activity and interfere with the execution of rhythmic movements in healthy participants. Perhaps surprisingly then, in patients with ET, transient improvement in arm tremor can be achieved using 1Hz rTMS over the lateral cerebellum on the ipsilateral side[40]. This was postulated by the authors to be due to an interference with ongoing oscillatory loops involving the cerebellum. Del Olmo et al[298] confirmed and expanded the idea that the cerebellum plays a main role in the selection of motor strategy of rhythmic finger movements, particularly in terms of temporal organization of movement. In particular, they showed that there is an evident correlation between the pacing variability (ITICV) and the motor strategy adopted by patients with ET. Motor impairments observed in these patients can transiently be modified by 1 Hz-rTMS over the lateral cerebellum, supporting the role of cerebellum in the pathogenesis of ET.

8.4 Aims

The aims of the present study are to investigate the effect of TDCS over the cerebellum on timing accuracy and variability in the PFT task. We hypothesise that anodal stimulation will modulate a central timing mechanism, measurable by increased tapping accuracy or decreased tapping variability compared with control. Given the polarity dependent effects of TDCS, cathodal stimulation is thus predicted to cause a significant deficit in timing parameters, demonstrable by greater inaccuracy in tapping with/or increased variability[302 297 298].

8.5 Methods

8.5.1 Subjects

Fourteen healthy right-handed volunteers (6 female; mean age 22.5 ± 3.6 (SD) yrs; range 20-34) participated in the study. None of the subjects had a past history of seizures or hearing disorders and none had an implantable pacemaker nor were any taking medications or illicit drugs during the study. Participants were naive to the aims of the study. Subjects gave informed written consent and the study was approved by the local ethics committee, and conformed to the *declaration of Helsinki.*

8.5.2 TDCS and tapping task

The main intention in the series of experiments was to examine performance of a tapping task at different frequencies with the right (dominant in all cases)

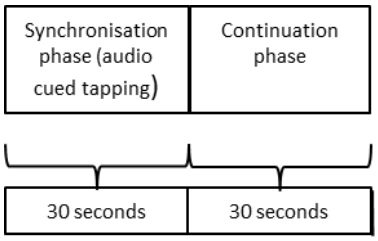
index finger comparing the effect of anodal, cathodal and sham TDCS over the lateral cerebellum. In all experiments, tapping performance was recorded before TDCS and during TDCS.

8.5.2.1 Design

Experiments for all participants took place over three days with at least a week between days. On each experimental day, participants were randomly allocated to receive anodal, cathodal or sham stimulation. On each of these days, the participant would complete a pre-stimulation trial where they were required to tap in time with a fixed frequency repetitive auditory tone for a period of 30 seconds (synchronisation phase), followed by a period of tapping for 30 seconds at the same learned frequency but without the queued tone (continuation phase). This pre-stimulation trial was repeated three times to include in randomised order, three frequencies (0.5Hz, 1Hz, 3Hz) of tapping. Participants then had a two minute rest period during which they had stimulation with TDCS (randomised to anodal, cathodal and sham). For sham stimulation, ‘real’ stimulation (randomised to either cathodal or anodal) was provided for a 30 second period to improve sham credibility. For anodal and cathodal stimulation, TDCS was continued until the end of the experiment. After the pre-stimulation trials, TDCS was initiated and the subject rested for two minutes to allow time for the stimulation to begin to take effect. A similar rest period was included for sham stimulation. Participants were then required to undertake nine consecutive trials of tapping, similar to pre-stimulation trials but in blocks of three of the same

tapping frequency. After each 3Hz trial, a small break of 90 seconds was given to allow the subject to rest their finger before the next trial, to avoid excessive fatigue from affecting performance. Order of blocks was randomised (see figure 8.1). The experiment was repeated on three days to include all three forms of stimulation (anodal, cathodal and sham) in randomised order to minimise the effect of practice.

A. Trial Composition



B. Sample Participant Timetable

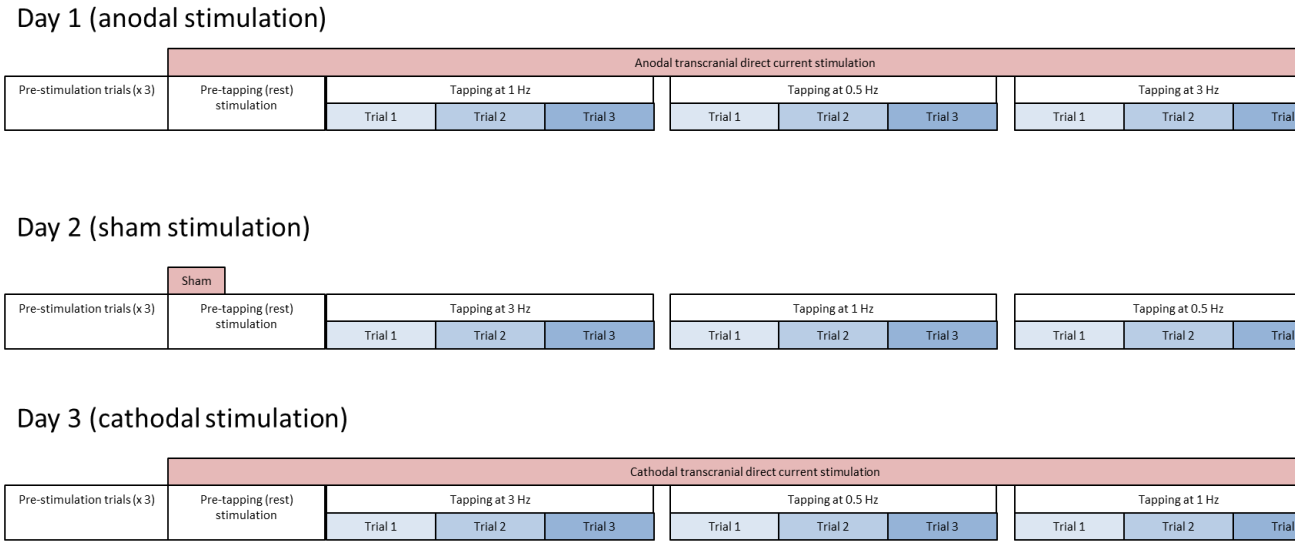


Figure 8.1 Tapping experiment – study design **A)** A trial is composed of 30s of tapping in time to a tone (synchronisation phase), and then 30s continuing tapping without the tone. **B)** A sample participant timetable, exhibiting the

prestimulation, rest and stimulation stages. Note that stimulation days and order of frequency trials are randomised across participants.

8.5.2.2 Task

Participants were sat comfortably in a chair with their right arm supported by a foam pad resting on a table surface. Subjects used their right index finger to tap in the centre of a round plate. A calibrated goniometer (Biometrics) was attached at one end to the proximal phalanx of the index finger and at the other, over the dorsum of the hand to allow measurement of the angle between the two, tap amplitudes and timing. Participants were thus instructed to tap using movements at their metacarpophalangeal joints rather than using movements at the wrist or elbow. Taps were performed at a comfortable force for the participant. Participants were asked to tap up to the height of a visual target. The purpose of the target was to maintain a constant tapping height throughout trials. Prior to the beginning of the experiment, TDCS electrodes were attached to intended stimulation loci (see below). The goniometer was connected to an amplifier and data acquisition system which relayed the information to a desktop PC. Subjects were not provided with any additional feedback regards their tapping. Information from the tapping apparatus and auditory tone was entered in to the computer and visible to the experimenter using Spike2 software (CED, Cambridge, UK).

8.5.2.3 TDCS

TDCS was delivered to the right cerebellar cortex using a commercially available DC stimulator (magstim neuroConn) (see chapter 4). The TDCS electrodes were 25cm^2 (5cm width square) in surface area, encased in sponge pockets, soaked in saline solution and secured to the scalp surface with crepe bandage. To stimulate the right cerebellar cortex, one electrode was secured 3cm lateral to the inion, and the other electrode was secured over the right buccinator muscle. In the anodal condition the electrode from the negative terminal electrode was placed over the right buccinator. In the cathodal condition the electrode positions were reversed. An intensity of 2mA was used for 18 minutes for both anodal and cathodal conditions. The sham current consisted of 2mA anodal or cathodal stimulation for 30s, over the right cerebellum, enough to briefly mimic the signs of stimulation (itching, burning, metallic taste, and rarely, visual phosphenes) without allowing any known alteration in cortical activity. During the onset of stimulation the current in all conditions was increased in a ramp like fashion over a period of 15s and ramped down for the termination of stimulation over the same duration, a method shown to achieve good blinding[223]. Anodal, cathodal and sham conditions were performed on different days with at least a week's rest interval owing to the long lasting effects of TDCS. The participant but not experimenter was blinded to randomisation of stimuli settings.

8.5.3 Data Analysis

In the data analysis, the intertap intervals (ITIs) were determined as measurements in time between peaks of finger displacement measured by goniometry. For each frequency (0.5Hz, 1Hz, 3Hz), mean ITIs and coefficients of variation were computed for the synchronisation and continuation phase for each of the three trials which were then averaged to determine a mean ITI for that condition. CV was used as an indicator of temporal variability where CV (%) = (SD/mean)/100. To determine effects of stimulation on variability and accuracy, a 3-way repeated measures ANOVA was planned for ITIs (accuracy) and CV ITIs (variability) with main factors of stimulation (anodal, cathodal, sham), frequency (0.5, 1, 3Hz) and block (synchronisation, continuation). Similar 3-way repeated measures ANOVAs were planned for pre-stimulation information but with factor stimulation replaced with day (day 1, day 2, day 3 and sham). Sham was to be included in the pre-stimulation analyses to evaluate its validity as a control for the stimulation conditions. Tap amplitude data was extracted from the tapping data by taking the area under the tapping curve generated from the force plate. This was to be done in the final trials of each condition, as those were most likely to show fatiguing effects. The first 3 taps and last 3 taps of each trial were removed as subjects often performed poor quality taps during these periods. After this truncation, the 4 taps at the beginning of the trial were compared to the 4 taps at the end of the trial, as these often lay outside of the 1min trial window. A three-way ANOVA was planned for tap amplitude measured by goniometry with factors of trial point (beginning, end),

stimulation (anodal, cathodal, sham) and frequency (0.5, 1 and 3Hz). Post hoc *t*-test were computed using a Bonferroni correction. None of the data violated the normality assumption necessary to conduct parametric statistical tests.

8.6 Results

All participants completed all three stimulation sessions. There were no complications. No significant main effects were found between TDCS stimulation and the accuracy of ITIs in either the synchronisation or continuation phases of the PFT task. Neither were there any significant main or interaction effects on accuracy between days in the pre-stimulation conditions.

The variability of tapping did not significantly differ between TDCS stimulation conditions. However, a significant interaction was shown between frequency and cue type (synchronisation or continuation), indicating that frequency type affected variability differently according to the cue type. Analysis of pre-stimulation variability also showed this FREQ*CUE interaction. In the stimulation condition, t-tests confirmed that this FREQ*CUE interaction was due to differences in CV between 0.5Hz synchronisation and continuation phases and 3Hz synchronisation and continuation phases. This was also the case for pre-stimulation variability with 0.5Hz and 3Hz underlying the FREQ*CUE interaction. Interestingly, in both stimulation and pre-stimulation trials, variability of 0.5Hz decreased from

synchronisation to continuation phases, whereas for 3Hz variability it increased in the continuation phases.

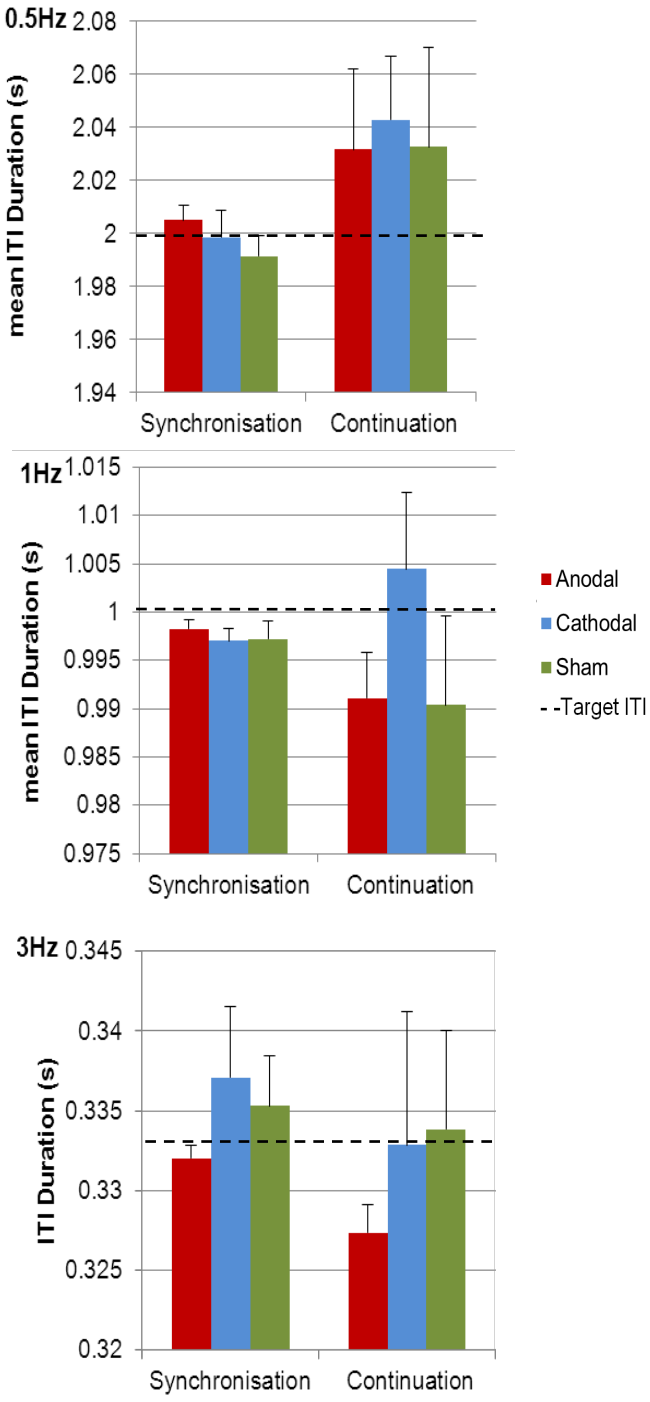


Figure 8.2 Mean ITIs of participants tapping to a target ITI of a specified frequency during anodal, cathodal or sham cerebellar TDCS stimulation. The target ITIs are denoted by the dotted line. No significant changes can be

seen in mean ITI of the response in the different stimulation groups across the frequencies.

Analysis revealed that the size of participants' taps were significantly different from the beginning of the trial to the end of the trial. Further comparisons showed that this was almost entirely due to the 3Hz condition, whereby participants began with taps much larger than those from the 1 and 0.5Hz conditions, and ended with taps of similar height as those from other conditions.

8.6.1 Effect of Stimulation on Accuracy

A 3-way repeated measures ANOVA with main factors of STIMULATION (anodal, cathodal, sham), FREQ (0.5, 1, 3Hz) and CUE (synchronisation, continuation) revealed no significant main effects or interactions of ITIs, apart from a borderline FREQ*CUE interaction ($F=2.62$, $df=2, 26$, $P = 0.09$). This indicates that change in accuracy in each cue condition varied with tapping frequency. Figure 8.2 shows the mean ITI in the stimulation and continuation phases of the PFT task across the 3 stimulation conditions.

Accuracy can be determined by how close the group mean ITI matches the target ITI, denoted by the dotted line in each frequency condition; 2s for 0.5Hz, 1s for 1Hz and 0.333s for 3Hz. The results show no trends in stimulation effect on accuracy of performance during synchronisation or continuation phases, as shown by inconsistent deviations from the target ITI

in each frequency. During 1Hz synchronisation, participants in all stimulation conditions were noted to tap slightly prior to the metronome tone, previously coined negative mean asynchrony, though this was not observed at other frequencies or cues.

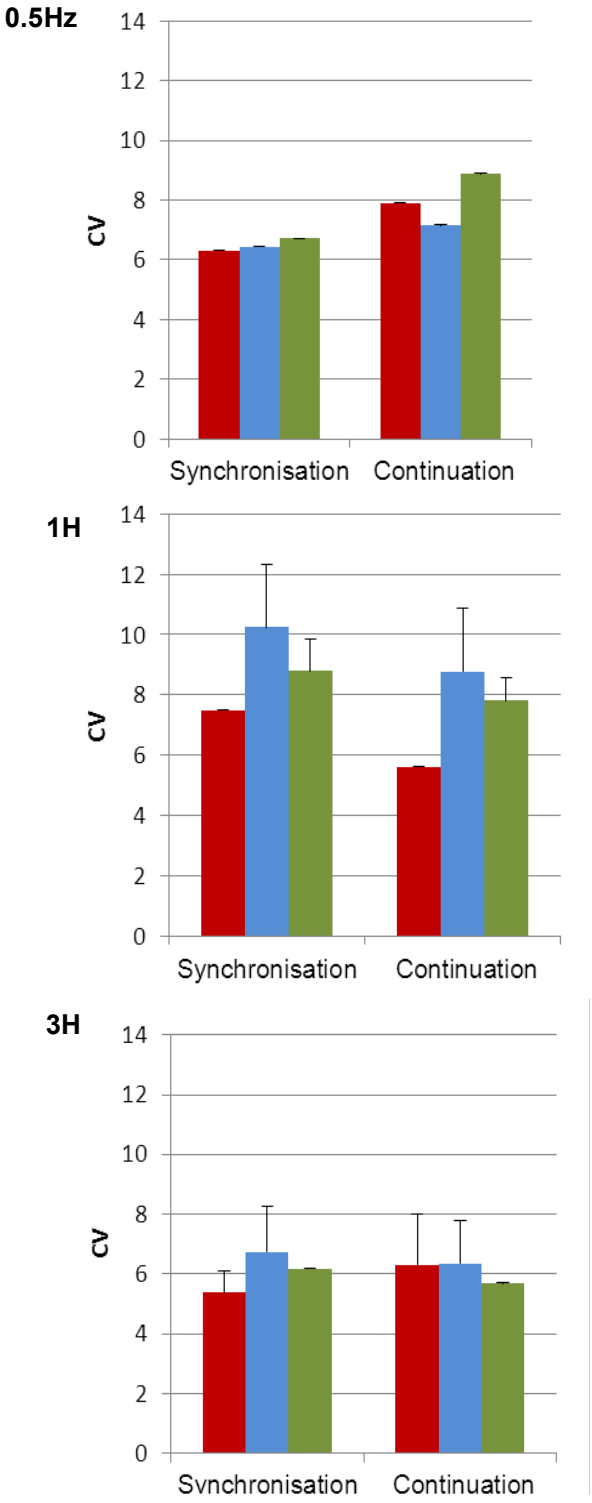


Figure 8.3 CV ((SD/mean)/100) of finger tapping in the synchronization and continuation phase of the PFT task, during anodal, cathodal and sham cerebellar TDCS. There are no significant differences in the variability of tapping in any frequency under different stimulation modalities.

8.6.2 Effect of Stimulation on Variability

A 3-way repeated measures ANOVA with main factors of STIMULATION (anodal, cathodal, sham), FREQ (0.5, 1, 3Hz) and CUE (synchronisation, continuation) revealed no significant main effects of coefficient of variability (CV) of ITI (see fig. 8.3). A significant FREQ*CUE interaction was found ($F= 10.01$, $df= 2, 26$, $p=0.001$). This indicates that the effect of frequency on CV is different between the cue conditions (see Figure 8.4). Figure 8.4 shows that cue type affects CV ITI in different ways depending on frequency; in the 0.5Hz condition mean CV decreases from synchronisation to continuation whereas CV stays the same in 1Hz condition, and increases from synchronisation to continuation phase in the 3Hz condition. This was confirmed by paired t-tests which showed a significant difference between synchronisation and continuation CVs for 0.5Hz ($t(41)=3.47$ and $p=0.001$) and for 3Hz ($t(41)= -2.18$, $p= 0.035$), the negative t value for the 3Hz condition denoting the reversal in directionality of the effect, observable from fig. 8.4. There was no significant difference in CV ITI between 1Hz synchronisation and continuation.

8.6.3 Effect of Day on Pre-stimulation Variability and Sham Validity

A 3-way repeated measures ANOVA with main factors of DAY (day 1, day 2, day 3 or SHAM stimulation), FREQ (0.5, 1 or 3Hz) and CUE (synchronisation, continuation) revealed no significant differences in accuracy on different days or during the Sham stimulation. Figure 8.5 shows the mean ITI in the stimulation and continuation phases on different days and during sham stimulation. The results show no effect of the day of performance or of sham stimulation on accuracy of ITIs. This means that participants did not improve their timing accuracy with practise over the three experimentation days. Additionally, the comparison confirms that the use of 30s of anodal stimulation in the sham condition to improve participant blinding did not have any significant effect on accuracy or CV in comparison to the pre-stimulation condition, validating the control. Negative mean asynchrony was again observed in the 1Hz condition only, with tapping slightly preceeding the tone during the synchronisation phase.

A 3-way repeated measures ANOVA with main factors of DAY (day 1, day 2, day 3 or SHAM stimulation), FREQ (0.5, 1 or 3Hz) and CUE (synchronisation, continuation) revealed a significant main effect of FREQ ($F=3.56$, $df= 2, 26$, $p=0.04$), and a significant interaction of FREQ*CUE on CV ITI ($F=10.29$, $df=1,13$, $p=0.01$). Figure 8.6 shows the differences in CV ITI on different days and during sham stimulation in the different frequencies, showing CV ITI is consistently lower in 1Hz condition than 0.5 and 3Hz conditions. Paired t-tests revealed that the main FREQ effect was due to a

significant difference between 1Hz CV and 0.5Hz CV (t(111)=3.68, p=0.00), and also a significant difference between 1Hz and 3Hz CV (t (111)=-2.15, p=0.03). There was no significant difference between 3Hz and 0.5Hz CV ITIs.

The FREQ*CUE interaction revealed by the 3-way repeated measures ANOVA implies that the effect of frequency on CV is different between the cue conditions. Figure 8.4 illustrates this FREQ*CUE interaction, with CV ITI decreasing from the synchronisation to the continuation phase for 0.5Hz and increasing for 3Hz. A paired t-test confirmed these observations showing a significant difference between 3Hz synchronisation and continuation phases (t(56)=-2.11, p=0.04) and a borderline non-significant difference between 0.5Hz synchronisation and continuation (t(56)=1.75, p=0.09) which was of a different directionality. There was no difference in 1Hz synchronisation and continuation CV ITIs. Additionally, this comparison proves that the sham stimulation is a valid control for the anodal and cathodal conditions, as there is no significant change in CV in the sham condition compared to the pre-stimulation condition.

8.6.4 Tap Amplitude

A 3-way repeated measures ANOVA with main factors of STIMULATION (anodal, cathodal and sham), FREQ (0.5, 1 and 3Hz) and TRIAL POINT (beginning, end) was used to determine whether the amplitude of participants taps varied from the beginning to the end of each 1min trial, with

stimulation and/or during different frequencies. A significant main effect of TRIAL POINT was revealed ($F=5.78$, $df= 1,13$ $p=0.03$), implying that there was a difference in size of taps at the beginning and end of each trial, see Figure 8.8. Figure 8.8 demonstrates the difference in mean tap amplitude from beginning to end in each of the frequencies, much of which appears to be due to a large change in 3Hz tap amplitude. Paired t-tests between tap amplitude at the beginning and end of each frequency explored this observation, and showed the main effect to be almost completely accounted for by difference in tap amplitude from the beginning to the end of 3Hz condition ($t(41)=2.24$, $p=0.03$) with only an additional borderline (non-significant) change in tap amplitude in the 1Hz condition ($t(41)=1.80$, $p=0.08$). There was no significant difference in tap amplitude in 0.5Hz condition. The large difference in tap amplitude from the beginning to the end of 3Hz condition may be explained by fatiguing of the finger during this period of fast tapping. However, the 3Hz end taps are a similar size to the end taps of 0.5 and 1Hz, indicating a fatigue effect may not have occurred. There were no other main effects or interactions.

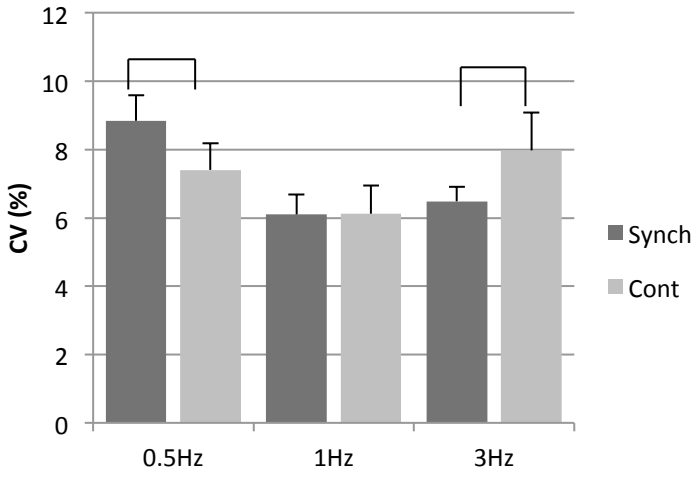


Figure 8.4 CV interaction of frequency and cue type. This graph shows that the effect of frequency on CV is different between the cue types (synchronization vs. continuation). Paired t tests revealed a significant difference between 0.5Hz synchronization and continuation phases ($t(41)=3.47$ and $p=0.00$) and 3Hz synchronization and continuation ($t(41)= -2.18$, $p= 0.04$). CV ITI for 0.5Hz decreases from synchronization to continuation phases, whereas it increases in the 3Hz condition.

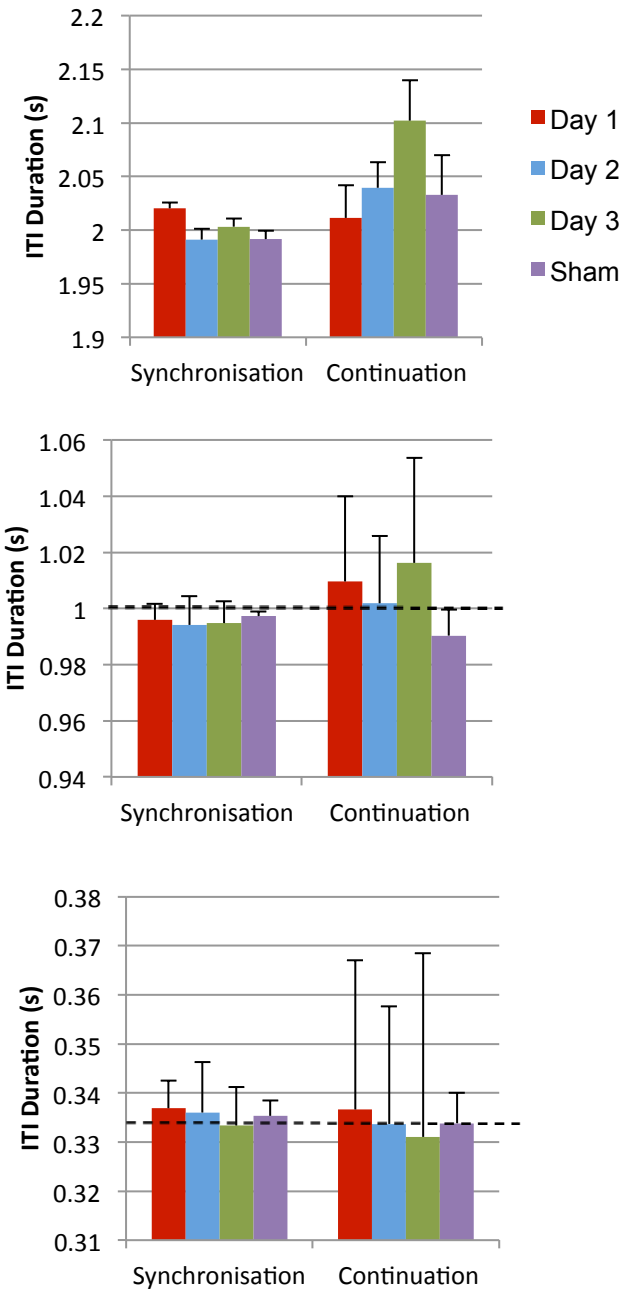


Figure 8.5 Mean ITIs of participants tapping to a target ITI of a specified frequency during day 1, 2, 3 of experimentation and sham stimulation. The target inter-tap-intervals are denoted by the dotted line. No significant changes can be seen in mean ITI of the response on the different days, or during sham stimulation across the frequencies.

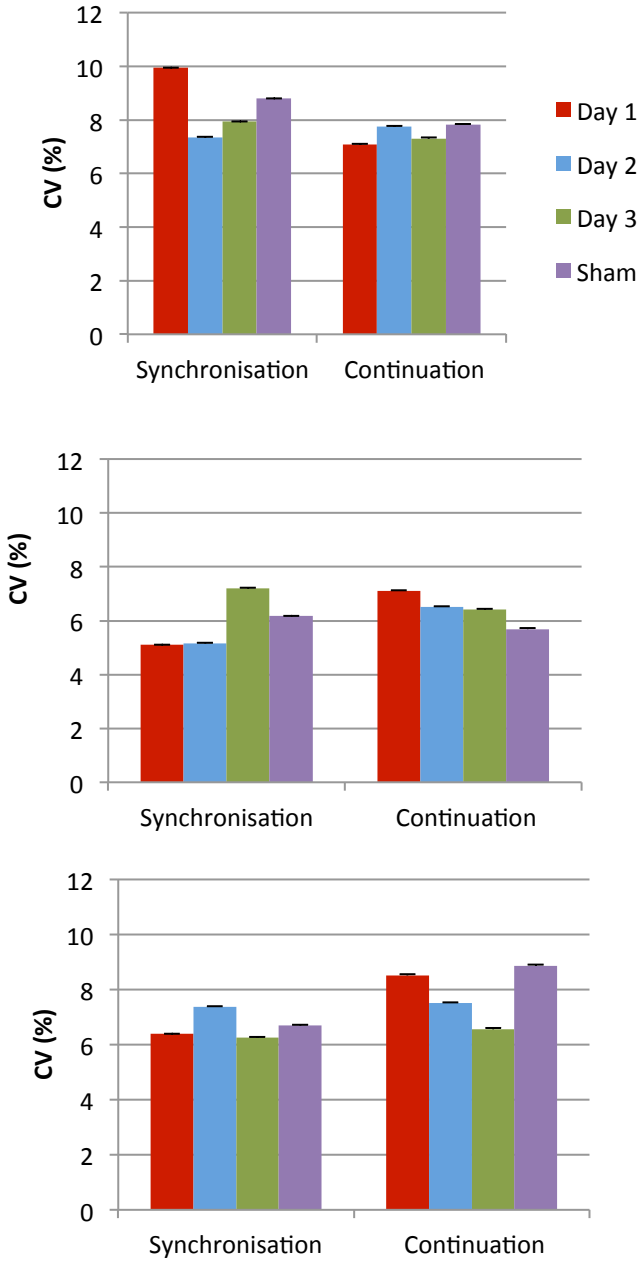


Figure 8.6 CV of finger tapping in the synchronization and continuation phase of the PFT task, during day 1, day 2 and day 3 pre-stimulation and during sham TDCS. A main effect of Frequency was found from a 3 way repeated measures ANOVA ($F=3.56$, $df= 2, 26$, $p=0.04$) which is accounted for by significant differences in overall CV of 1Hz in comparison to 0.5Hz and 3Hz CVs ($t(111)=3.68$, $p=0.00$) and ($t (111)=-2.15$, $p=0.03$) respectively.

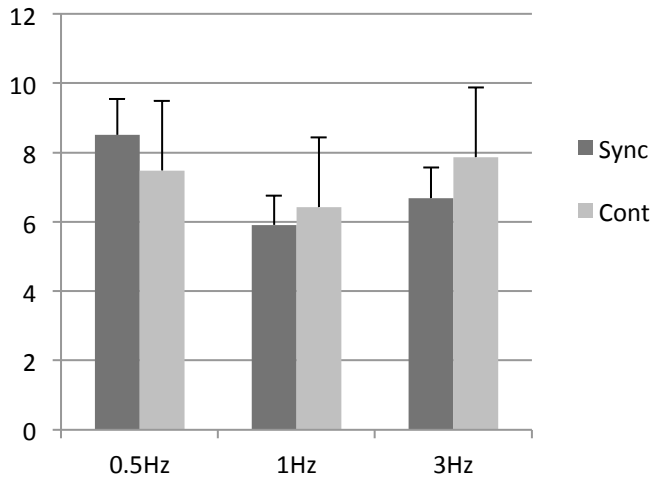


Figure 8.7 CV (%) of ITI and cue type interaction for prestimulation sessions. This graph shows the effect of frequency on CV varies in the different cue types (synchronization, continuation). Paired t tests showed a significant difference between 3Hz synchronization and continuation ($t(56)=-2.11$, $p=0.04$), and a borderline significant difference between 0.5Hz synchronization and continuation phases ($t(56)=1.75$, $p=0.09$).

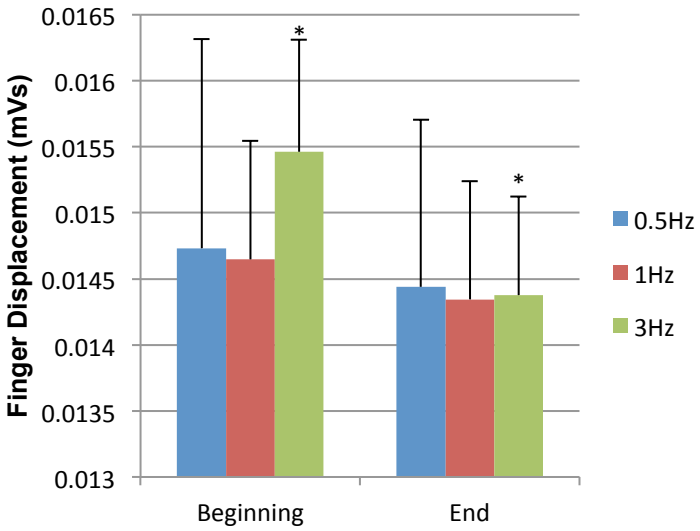


Figure 8.8 Mean tap amplitudes at the beginning and end of each finger tapping trial at different frequencies (0.5, 1 and 3Hz). A significant main effect of TRIAL POINT ($F=5.78$, df= 1,13 $p=0.03$) indicates a significant difference in tap amplitude from beginning to the end of the trial. 3Hz undergoes a substantial decrease in tap amplitude from the beginning to the end of the trial ($t(41)=2.24$, $p=0.03$) whilst there are no other significant differences in tap amplitude.

8.7 Discussion

In this study, TDCS over the lateral cerebellum did not appear to have any effect on the variability or accuracy of ipsilateral paced finger-tapping movements. This data suggests that increasing or decreasing the excitability of cerebellar neuronal circuits has no significant effect on timing ability, although it may alternatively question the effect of our stimulation paradigm on the cerebellum.

In both the stimulation and pre-stimulation conditions, analysis of variability revealed significant interactions between frequency and cue type. There were similarities in this FREQ*CUE interaction in the stimulation and pre-stimulation conditions: analysis of both showed differences between 3Hz and 0.5Hz cue types of different directionalities. Regardless of whether the participant received stimulation or not, tapping in time to the tone during the 0.5Hz synchronisation stage was associated with a greater variability than tapping without a tone (continuation phase). In contrast, in the faster 3Hz condition, variability increased only when subjects had to tap without a tone. This suggests that internal timing mechanisms without sensory cues are more consistent at slower rates of tapping, of those frequencies studied.

8.7.1 Stimulation of the cerebellum

Given the negative results, i.e. the apparent lack of effect of cerebellar TDCS does not have an effect on rhythmic tapping, it is necessary to consider whether the experimental paradigm was able to successfully stimulate the cerebellum. A TDCS intensity of 2mA was employed, a standard intensity used in numerous studies of TDCS to the cerebellum at which modulation in cerebellar activity has been observed[185 271]. This intensity is significantly higher than TDCS stimulation applied to non-cerebellar cortical areas, such as M1 where 1mA is commonly used successfully[303 304], or slightly higher for prefrontal areas[305]. This increased intensity for cerebellar TDCS is necessary, given the greater distance of the cerebellum from the scalp

surface than M1. Nevertheless it has proven sufficient in other studies aiming to influence cerebellar circuits[271].

Other studies applying TDCS to the cerebellum have investigated cerebellar circuitry underlying other proposed functions such as adaptive learning[271], eyeblink conditioning[259] and cerebellar influence on M1 plasticity[262]. It is conceivable that the lack of a clear stimulation effect could be because cerebellar circuitry involving timing may not be susceptible to TDCS modulation. Del Olmo et al suggest that circuits in the cerebellum are differentially sensitive to external disruption, and that timing circuits for synchronisation taps to fast cues (2Hz) may be more sensitive to stimulation than other frequencies[298], a frequency not tested here. Given the uncertainty, it cannot be ruled out that these timing circuits, which appear to have been influenced by alternative stimulation paradigms, such as repetitive TMS[298], may not be sensitive to TDCS.

Nearby circuits in non-cerebellar brain areas may also have impacted on our results due to inadvertent stimulation, for example brainstem pathways and their connections to the sensorimotor cortex and the thalamus. There is a possibility that brainstem pathways such as the medial leminiscus and spinothalamic tract are affected during tDCS, impacting on sensory transmission[262]. However, Galea et al found numerous sensory transmission dependent variables were not affected by TDCS, such as brainstem MEP threshold and size, and blink reflex[185], refuting this possibility. Further, our negative results do not provide specific evidence for this possibility.

The choice of lateral cerebellum as a target for stimulation in this study can be justified on the grounds of considerable imaging and lesion data that indicates the lateral cerebellum as a locus for temporal processing[298 306 307]. However, a few studies also implicate medial and vermal areas as involved in timing[170 308 309 297]. Despite this, there are also TMS-induced virtual lesion studies and imaging evidence to support damage to lateral cerebellum, rather than medial areas, is accompanied by timing abnormalities[298 307]. However, the distinction between damage to the dorsal and ventral portions of the dentate which have different projections has been insufficiently explored[310]. The dorsal dentate exerts its effects on M1 and ventral PM areas that have sensorimotor roles, whilst the ventral dentate projects to areas with cognitive roles such as dorsolateral prefrontal areas. This provides two competing ideas to explain deficits in timing following cerebellar damage; one favouring sensorimotor disruption[311 312], and an opposing cognitive viewpoint[313].

The use of sham stimulation with participant blinding could potentially be inadequate and thus bias results although this might be expected to lead to a type 1 rather than type 2 statistical error in contrast to the nature of our results. However, our use of real anodal stimulation for the initial 30s of sham stimulation provided adequate blinding to the stimulation type as this initial phase was when most participants were aware of any side effects of stimulation. Further, there was no difference found between the sham stimulation and the pre-stimulation phase in terms of performance accuracy and variability.

8.7.2 Aspects of the PFT task

We demonstrated a difference between tap amplitude at the beginning and end of the trial, attributable to a large reduction in tapping size at the 3Hz frequency. A possibility could be that participants experienced fatigue particularly in the 3Hz condition. However, measures were taken to ensure excessive fatigue was avoided. Ninety second breaks between each of the 3Hz trials was introduced for this reason. Additionally, the change in tapping size did not necessarily suggest fatigue (see Figure 8.8). The mean amplitude of the 3Hz taps in the early part of the trials were larger than initial taps at 0.5 and 1Hz frequencies and mean final taps of 3Hz were of a size very similar to 0.5 and 1Hz. If fatiguing had occurred one might expect the final mean taps to be smaller in amplitude than other frequency trials. Excessive fatigue in a single condition may be expected to generate greater variability, as suggested previously[294]. Variability deteriorated from synchronisation to continuation phase (i.e. from beginning to end) in the 3Hz category (see Figure 8.7) and was similar to variability in other frequency conditions.

One explanation for the negative results in this study may simply suggest that our method investigated timing intervals irrelevant to cerebellar timing. Much work has focused on the cerebellum as a controller of timing in the millisecond to second time scale, consistent with temporal aspects of muscle control that are also under cerebellar control[302 314]. Yet the specialised sub-second timing role of the cerebellum has been the subject of much debate[221]

[309]. Lewis and Miall found distinct brain activation patterns for temporal discrimination tasks of 0.6s and 3s, and greater cerebellar activation in the 0.6s group[315]. Repetitive TMS over the lateral cerebellum disturbed timing in the millisecond range, at 2Hz[298]. Similarly, in an additional cerebellar rTMS study Oliveiri et al. found that in a visual time reproduction task deficits in timekeeping were seen in millisecond time interval targets[316]. However, cerebellar patients with focal lesions in the lateral cerebellum showed deficits in remembering durations of second timescales[307]. The current study investigated a range of timing intervals to cover proposed cerebellar millisecond and second timescales; with intervals of 0.33, 1 and 2s. Therefore it seems unlikely that an insignificant result was obtained simply due to exploration of inappropriate tapping frequencies.

The PFT task is well established in the timing literature[317]. However data exists to suggest preferable cerebellar activation in more demanding tasks such as highly complex rhythms[318]. Our study relied upon a simple tapping paradigm that conversely benefits from ease of control of variables.

In concordance with the novelty of the task, cerebellar rTMS disruptions are seen only in the synchronisation part of the PFT task[298] and not the continuation phase. In the novel synchronisation phase the participant must prepare and execute motor outputs in time to an external stimulus, during which an internal representation of the interval may be formed. It has been suggested that the continuation phase may not be cerebellar dependent[298] but rather rely on other brain areas. One study demonstrated that

sensorimotor cortex, cerebellum and superior temporal gyrus were active during synchronisation, but during the continuation phase the supplementary motor area, putamen, inferior frontal gyrus, and ventrolateral thalamus were additionally activated[172]. The prefrontal cortex may play an important role in the continuation phase, or when time intervals must be kept in memory[314 319], supported by evidence from patients with prefrontal lesions demonstrating deficits in millisecond time processing.

Given the likely importance of prefrontal brain regions in the continuation phase, it poses the question of whether cerebellar stimulation was thus unlikely to disrupt this ability to maintain consistent timing. However, this seems unlikely as studies disrupting time estimation tasks using rTMS over the right dorsolateral prefrontal cortex (DLPFC), have only interfered with estimations in the second interval, (2s average) rather than millisecond intervals (0.5ms average)[320].

8.7.3 Cerebellum and Timing

It is conceivable, given timing ranges, that the cerebellum is not influencing timing *per se* in SMS, but is rather performing its motor coordination roles of starting and stopping fast accurate movements[315]. Motor coordination can be considered a state-dependent process whereby motor commands to one effector are dependent on predictions on the state of another at that moment in time, whereas timing is a process independent of external influence. In a series of experiments by Diedrichsen et al[312], the timing and coordination

roles of the cerebellum were dissociated. The cerebellum was found to be active during state-dependent control tasks, representing the predictive motor abilities of the cerebellum, but it was not seen to be active during time dependent control. As such, the single finger movement in our task would not require state-dependent control and according to the proposed dissociation, may not require the cerebellum for predictive timing.

8.8 Conclusion

Our experiments suggest that TDCS applied to the lateral cerebellum does not interfere with the control of hand movements as measured by rhythmic timing and variability. Limitations of the study include the possibility that the TDCS dose and position was insufficient for successful cerebellar stimulation, or rather induced stimulation in other nearby brain areas. The emerging view is that timing implicates wide brain networks. Further, timing mechanisms may be split into different categories depending on their temporal niche (sub-second, or suprasecond) and also depending on the context of the timing (state dependent or time dependent). This study found significant interactions between frequency and cue type that suggest timing mechanisms greater than 1s work most efficiently independent of timed sensory information, as demonstrated in the 0.5Hz category where there was a (non-significant) trend for lower variability in the continuation phase. Conversely we found that sub-second timing mechanisms function better with a timed sensory stimulus, as seen in the synchronisation phase for 3Hz where there was less variability than in the continuation phase.

The cerebellum may be involved in the synchronisation of subsecond movements to timing intervals, as suggested by Del Olmo et al[298], though this may not involve a ‘pure’ timing mechanism like that suggested as the central timekeeper in the Wing and Kristofferson model. Through this framework the cerebellum may be involved in the sensorimotor coordination required to predict accurately timed movements in relation to other muscle states (state dependent control). To further investigate this, it would be useful to compare the effects of cerebellar TDCS on finger tapping as compared to another movement such as an arm movement that would require predictive timing mechanisms about muscle states to be employed.

Chapter 9: General discussion, conclusions and further work

Attention is increasingly being turned to the pathophysiological mechanisms of differing types of tremor. For example, a proposition for the mechanisms underlying Parkinson's disease tremor has been outlined by Helmich and colleagues[7]. For some commoner tremor types, such as ET and Parkinson's disease tremor, the added insight given by in vivo recording during deep brain stimulation implantation has added a unique insight into these commoner tremors. Even so, the fundamental causes and the dynamical systems involved remain somewhat opaque. For rarer tremors such as neuropathic tremor, such insight has not been feasible except on a case report basis and from a single neurophysiologial study[128]. Newer approaches to treatment of movement disorders such as tremor is partly dependent on an understanding of pathophysiological mechanisms, such as the trials of new drugs (e.g. Octanol in ET) or the development of novel uses for deep brain stimulation.

This thesis presents the results of a series of experiments exploring aspects of sensorimotor physiology associated with patients who have neuropathic tremor with the aim of furthering the understanding of the pathophysiological basis behind why this symptom arises. The studies were split into those investigating patients with inflammatory neuropathies and secondly, those with hereditary neuropathies, namely CMT1A. The a priori hypothesis was that the cerebellum was functioning abnormally in these patients, likely either as a trait or due to a direct targeting of the cerebellum through the immunological or hereditary process causing the neuropathy. Tremor

occurrence seems requisite in those with inflammatory neuropathies on an abnormally functioning cerebellum. Aspects of the neuropathy, in the presence of tremor then seem to influence severity lending support to a two hit hypothesis for the generation of tremor. Results from patients with inflammatory neuropathies supported this hypothesis yet those with hereditary neuropathies did not. Those patients with CMT1A and tremor seemed rather to demonstrate tremor consistent with an enhanced central or physiological tremor. We postulated that this may relate to fatigue. Differing tremor types have been shown to involve cerebellothalamocortical circuits and on this basis, a number of groups have looked at stimulation of the cerebellum as a potential modulator of tremor. With this in mind, the final experiment presented in this thesis explores the effects of cerebellar stimulation with TDCS on timing of rhythmic movements of the finger in healthy controls but with results showing no demonstrable effect.

The work described here generates a number of further questions that will be outlined and contextualised in the light of other contemporaneous work. This will be presented in the form of planned further work.

9.1 Further work

9.1.1 Cortico-muscular coherence in neuropathic tremor

The proposition that neuropathic tremor in inflammatory neuropathies is contingent predominantly on abnormal function of the cerebellum would be

analogous to the role it has in some other tremor types such as essential tremor. As such, the cerebellothalamocortical network, likely active in tremor propogation in ET, may be expected to have activity coherent with the tremor phase measured peripherally. It would be helpful to decipher whether there is a cortical (motor cortical) signal coherent to peripherally measured tremor in NT as there are some reports suggesting targeting relevant motor areas with repetitive TMS may prove helpful for abating symptoms in tremor of cortical origin[321]. Different cortical areas may be temporarily entrained in essential tremor[54]. Determining brain areas coherent with tremor peripherally may prove useful in coordinated resetting of tremor[322].

9.1.1.1 MEG-EMG correlation

The correlation between cortical activity and final motor output remains a central question in motor physiology. This has been probed using methods to record electrical brain activity using electroencephalography (EEG) or magnetoencephalography (MEG) and correlating it with EMG. For example, cortical myoclonus has been investigated using such approaches, where jerk-locked back averaging allows subtle cortical activity to be recorded using EMG movement-related activity onset as the fiducial point for averaging preceeding EEG signal. Similarly, premovement cortical potentials such as the Bereitschaftspotential and the contingent negative variation can be measured by correlating EEG activity with the onset of EMG activity and averaging multiple trials to derive common cortical signals.

In addition to time-based approaches to correlation as described, frequency-based techniques may be employed. There are a number of statistical and methodological approaches to determining correlation between two linear signals. Such approaches estimate functional connectivity of two regions. Coherence analysis, an example of this, enables calculation of a normalised magnitude of correlation as a function of frequency. Cortico-muscular interaction was shown indirectly in studies correlating single motor unit activity in different muscles[323 324]. MEG was first utilised to demonstrate cortico-muscular coherence (above significance levels by revealing coherence at beta band frequencies (15-35Hz))[94]. Other such measures of functional connectivity include dynamic imaging of coherent sources (DICS).

Given our findings of an abnormal EBCC in patients with inflammatory NT, it would be useful to know if there is coherence between MEG-EMG in these patients. This would be compared with other areas of the brain such as motor cortex to determine if networks similar to ET may be active. This could provide a picture of tremor that is similar between the two conditions and potentially speak to the cerebellothalamocortical circuit as a common final end point of tremor and therefore explain the ubiquitous benefit of VIM stimulation for most tremor types, including provisional reports for NT.

9.1.2 Structural imaging study

The presence of functional involvement of the cerebellum in neuropathic tremor is supported by positron-emission tomography (PET) and functional

magnetic resonance imaging (fMRI) [132], but again, it is not clear whether this finding reflects primary or secondary changes in the cerebellum. Critically, there have been no studies to date assessing the structural integrity of the cerebellum in patients with neuropathic tremor compared to those with neuropathy but without tremor.

The results here in patients with inflammatory neuropathies, provide a new perspective of evidence supporting the role of an abnormally functioning cerebellum on tremor occurrence. Similar findings in ET exist, with functional imaging, electrophysiological and pathological studies. PET studies have demonstrated a bilateral increase in cerebellar and thalamic blood flow [25] and MR spectroscopy has suggested dysfunction in the cerebellar cortex [26]. Eye-blink conditioning is also severely impaired in essential tremor [27 28] suggesting abnormal plasticity in the cerebellum. The strong evidence for a role of the cerebellum in essential tremor has also been corroborated by structural imaging studies. Benito-Leon et al. [30] demonstrated structural abnormalities in ET patients using VBM and a 3-T MRI scanner. Using lower field scanners (1.5-T) contradictory results have been reported [32 33]. Using Diffusion-weighted imaging to search for evidence of tissue integrity abnormalities in these areas in ET patients failed to find any significant abnormalities [34], arguing against major structural damage in the ET brain, though more subtle neurodegenerative changes could not be ruled out. However, Shin et al. [35] investigated changes in anisotropy in patients with ET by comparing fractional anisotropy (FA) images generated from diffusion tensor imaging data acquired at 1.5T; compared with the control subjects,

they found patients with ET exhibited significantly reduced FA in areas of the brainstem and bilateral cerebellum.

Neurodegenerative changes in the cerebellum have been found on pathological specimens of essential tremor [46 48] confirming this suggestion. Essential tremor has also in turn been associated with structural abnormalities of the cerebellum in the form of atrophy. In a similar way, it would be important to determine whether there are any structural changes in the cerebellum in neuropathic tremor, as this will provide significant evidence of the underlying aetiological factors and help guide the approach to treatment. Similar methods of structural MR imaging used in essential tremor might be used in studying cerebellar atrophy in neuropathic tremor. There are potential benefits of identifying the role of the cerebellum in treatment of neuropathic tremor. For example, activation of the cerebellathalamocortical circuit is essential to the presence of ET; such reports have led to lesional therapies of the ventral intermediate (VIM) nucleus of the thalamus with marked benefit. Effective treatment of neuropathic tremor, though, is less clear. However, DBS of the VIM has been reported with sustained benefit in a case of hereditary motor sensory neuropathy type 1 [139] and a small number of cases of anti-MAG neuropathy [141-143] less clear [129 139 141-143].

A sufficiently sized group of patients with inflammatory neuropathies with and without tremor as well as healthy controls would need to be recruited. By using high field (3-T magnet) imaging of the brain in each of these participants, one could assess the extent of structural change or atrophy of

the cerebellum, comparing each group. Clinical assessment of the tremor would need to be undertaken.

Subjects would undergo 3D dimensional isotropic structural imaging of the whole brain at 3T (Siemens TIM Trio). A standard T1-weighted MPRAGE sequence, optimised for volumetric imaging would be used with an acquisition time of approximately 10 minutes. (Typical parameters: 1.1x1.1x1.1mm isotropic voxels, 200 sagittal slices, TR/TE 2200/3ms, Inversion time 900ms, 256x256 acquisition matrix, flip angle 10 degrees, 1 signal average). The signal would be received with a 12 channel head matrix coil without parallel imaging acceleration with the patient supine and positioned to minimise the effect of any tremor on the acquisition.

In addition to the 3D volumetric acquisition it is proposed that each subject would also receive standard T1 and T2–weighted diagnostic turbo spin echo volumes to allow radiological evaluation of any prominent structural or pathological features of the cerebellum (acquisition time ~10 minutes).

The 3D MPRAGE volumes would be analysed using voxel based morphometric methods [325], including appropriate steps to smooth the data, transform the volumes into a standard anatomical space, segment into grey matter, white matter and CSF and perform statistical parametric mapping by application of a general linear model. It is anticipated that the SPM5 software would be used for VBM analysis (Wellcome Trust Imaging Laboratory, UCL).

In addition to the VBM analysis, it is feasible that a region of interest based analysis focussing on certain areas of the cerebellum (using methods similar to those used by Quattrone and Cerasa [32]) might also be performed, providing an independent verification of any apparent features in the cerebellum using the 3D structural data.

9.1.3 CSF antibody study

Since our work, results of an interesting immunological study of a sub-group of patients with CIDP associated with anti-neurofascin 155 antibodies of the IgG4 subclass. All patients had a severe, predominantly distal, motor demyelinating (but not axonal) neuropathy (as per their initial nerve conduction study) with ataxia. Three of these four patients showed a striking low-frequency postural and intention tremor that was not present in any other patient with CIDP of their series. None of the four patients responded to IVIg, perhaps due to a relatively different mechanism of action of the antibody in relation to complement binding and binding to Ig Fc domain receptors[29]. When an IVIg-resistant cohort was selected, a higher proportion were positive for the antibodies compared with the unselected group of their CIDP patients. These antibodies may thus have a therapeutic and prognostic implication.

Neurofascin 155 has a key role in myelination functional organisation at the node of Ranvier. Selective knockout of neurofascin in mice causes a severe demyelinating neuropathy with marked drop in conduction velocities and

degeneration of cerebellar Purkinje neurons, precipitating considerable tremor and ataxia.[25] The authors make this case asserting the likely pathogenic nature of these anti-neurofascin 155 antibodies.

No other patient from their cohort showed such a disabling tremor. The features of the tremor in their patients suggested a cerebellar origin although other signs of cerebellar impairment (nystagmus, oculomotor disturbances) were not present. They demonstrate that the serum of anti-NF155+ patients react intensely with the neuropil of rat brain, with a pattern of immunostaining of hippocampus and cerebellum that was identical in all cases, suggesting that neurofascin 155 is one of the target antigens in patients with CIDP who have low-frequency, high amplitude tremor. One of their patients did not show a prominent tremor but showed a rat brain staining pattern similar to the other three. This patient had lower anti-neurofascin155 titres than the other three and this could influence the intrathecal levels of anti-neurofascin155 antibodies and, thus, the presence of tremor. Nevertheless, further studies are needed to clarify whether anti-neurofascin155 antibodies cause cerebellar pathology and whether these antibodies distinguish between CIDP patients with and without overt tremor of cerebellar origin.

In their series, anti-neurofascin155 antibodies were not present in 204 controls, including 51 patients with GBS. Central demyelination was not found on MRI in any of the patients with the antibodies. It would be valuable to determine from the sera of our patients with tremor whether or not these antibodies are present when controlled against those without tremor.

9.1.4 Phase dependent stimulation

Given the lack of effect of TDCS of cerebellum on rhythmic finger tapping, a few conclusions could be considered. These have been dealt with in chapter 8, but essentially include the possibility that the methods used failed to provide sufficient stimulation to the cerebellum or that stimulation was achieved, but with no effect on the variables measured.

Alternative experiments utilising cerebellar stimulation may require a more refined approach. Using transcranial alternating current stimulation (TACS), we have used such an approach on patients with differing tremor types to determine whether such cerebellar and separately motor cortical stimulation might be able to modulate tremor in a phase specific manner when analysed retrospectively.

Closed-loop stimulation may allow stimulation in real-time in reaction to a measurable biomarker. Phase dependent approaches to closing this loop may provide greater understanding of how oscillatory activity within the nervous system is generated, propagated and potentially suppressed. In the physical sciences, a broad range of biological oscillators, ranging from circadian rhythms to the cardiac pacemaker rhythm, have been characterised using a mathematical approach known as dynamical systems theory. More specifically, the phase response curve, which characterises an oscillator's response to perturbation as a function of its phase, provides an intuitive insight into how the oscillation is generated, how it becomes entrained by other oscillators, and how it may be desynchronised.

In contrast to the single neuron PRC where estimation is straightforward, estimates of PRC of an oscillatory neuronal network (i.e a population of synchronised neurons with a “cumulative” PRC) has only recently been characterised in vitro, where the timing of neuronal firing is known. In attempting to estimate the PRC from a neuronal oscillation (such as tremor or local field potential from a DBS electrode) both the relationship between the underlying single neuronal firing to the population neuronal oscillation and the precise timing of the perturbing pulse are unknown. Using single neuron simulations we aim to demonstrate how these unknowns invalidate the traditional approach to PRC estimation. We have gone on to develop a novel iterative method, which overcomes these limitations of the traditional PRC estimation. We have begun to apply this method to patients with tremulous Parkinson’s disease with preliminary results suggesting the ability to recover the tremor PRC derived from TMS applied to primary motor cortex.

References

1. Ahlskog MC, Kumar N, Mauermann ML, Klein CJ. IgM-monoclonal gammopathy neuropathy and tremor: a first epidemiologic case control study. *Parkinsonism Relat Disord* 2012;18(6):748-52.
2. Brooks D, Jenkins IH, Bain P, Colebatch JG, Thompson PD, findley LJ, Marsden CD. A comparison of the abnormal patterns of cerebral activation associated with neuropathic and essential tremor. *Neurology* 1992;42(Suppl 3):423.
3. Deuschl G, Bain P, Brin M. Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. *Mov Disord* 1998;13 Suppl 3:2-23.
4. Schafer EA. On the Rhythm of Muscular Response to Volitional Impulses in Man. *J Physiol* 1886;7(2):111-7.
5. Bain PG, Findley LJ, Thompson PD, Gresty MA, Rothwell JC, Harding AE, et al. A study of hereditary essential tremor. *Brain* 1994;117 (Pt 4):805-24.
6. Bain P, Brin M, Deuschl G, Elble R, Jankovic J, Findley L, et al. Criteria for the diagnosis of essential tremor. *Neurology* 2000;54(11 Suppl 4):S7.
7. Helmich RC, Hallett M, Deuschl G, Toni I, Bloem BR. Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits? *Brain* 2012.
8. Ondo W, Jankovic J, Schwartz K, Almaguer M, Simpson RK. Unilateral thalamic deep brain stimulation for refractory essential tremor and Parkinson's disease tremor. *Neurology* 1998;51(4):1063-9.

9. Hallett M. Tremor: pathophysiology. *Parkinsonism Relat Disord* 2014;20 Suppl 1:S118-22.

10. Shaikh AG, Hong S, Liao K, Tian J, Solomon D, Zee DS, et al. Oculopalatal tremor explained by a model of inferior olivary hypertrophy and cerebellar plasticity. *Brain* 2010;133(Pt 3):923-40.

11. Saifee TA, Edwards MJ. A normal inferior olive in essential tremor-peacemaker or pacemaker? *Mov Disord* 2013.

12. Louis ED, Ottman R. How many people in the USA have essential tremor? Deriving a population estimate based on epidemiological data. *Tremor Other Hyperkinet Mov (N Y)* 2014;4:259.

13. Romero JP, Benito-Leon J, Bermejo-Pareja F. The NEDICES Study: Recent Advances in the Understanding of the Epidemiology of Essential Tremor. *Tremor Other Hyperkinet Mov (N Y)* 2012;2.

14. Louis ED, Ferreira JJ. How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor. *Mov Disord* 2010;25(5):534-41.

15. Deuschl G, Elble R. Essential tremor--neurodegenerative or nondegenerative disease towards a working definition of ET. *Mov Disord* 2009;24(14):2033-41.

16. Stefansson H, Steinberg S, Petursson H, Gustafsson O, Gudjonsdottir IH, Jonsdottir GA, et al. Variant in the sequence of the LINGO1 gene confers risk of essential tremor. *Nat Genet* 2009;41(3):277-9.

17. Liang H, Song Z, Deng X, Xu H, Zhu A, Zheng W, et al. Genetic analysis of the leucine-rich repeat and Ig domain containing Nogo receptor-

interacting protein 1 gene in essential tremor. *J Mol Neurosci* 2013;51(2):403-7.

18. Kuhlenbaumer G, Hopfner F, Deuschl G. Genetics of essential tremor: meta-analysis and review. *Neurology* 2014;82(11):1000-7.
19. Stamelou M, Charlesworth G, Cordivari C, Schneider SA, Kagi G, Sheerin UM, et al. The phenotypic spectrum of DYT24 due to ANO3 mutations. *Mov Disord* 2014;29(7):928-34.
20. Pasini E, Busolin G, Nobile C, Michelucci R. Autosomal dominant essential tremor: a novel family with anticipation. *Neurol Sci* 2013;34(5):761-3.
21. Deng H, Le W, Jankovic J. Genetics of essential tremor. *Brain* 2007;130(Pt 6):1456-64.
22. Louis ED. Etiology of essential tremor: should we be searching for environmental causes? *Mov Disord* 2001;16(5):822-9.
23. Tanner CM, Goldman SM, Lyons KE, Aston DA, Tetrud JW, Welsh MD, et al. Essential tremor in twins: an assessment of genetic vs environmental determinants of etiology. *Neurology* 2001;57(8):1389-91.
24. Louis ED, Babij R, Ma K, Cortes E, Vonsattel JP. Essential tremor followed by progressive supranuclear palsy: postmortem reports of 11 patients. *J Neuropathol Exp Neurol* 2013;72(1):8-17.
25. Jenkins IH, Bain PG, Colebatch JG, Thompson PD, Findley LJ, Frackowiak RS, et al. A positron emission tomography study of essential tremor: evidence for overactivity of cerebellar connections. *Ann Neurol* 1993;34(1):82-90.

26. Pagan FL, Butman JA, Dambrosia JM, Hallett M. Evaluation of essential tremor with multi-voxel magnetic resonance spectroscopy. *Neurology* 2003;60(8):1344-7.

27. Shill HA, De La Vega FJ, Samanta J, Stacy M. Motor learning in essential tremor. *Mov Disord* 2009;24(6):926-8.

28. Kronenbuerger M, Gerwig M, Brol B, Block F, Timmann D. Eyeblink conditioning is impaired in subjects with essential tremor. *Brain* 2007;130(Pt 6):1538-51.

29. Wills AJ, Jenkins IH, Thompson PD, Findley LJ, Brooks DJ. Red nuclear and cerebellar but no olivary activation associated with essential tremor: a positron emission tomographic study. *Ann Neurol* 1994;36(4):636-42.

30. Benito-Leon J, Alvarez-Linera J, Hernandez-Tamames JA, Alonso-Navarro H, Jimenez-Jimenez FJ, Louis ED. Brain structural changes in essential tremor: voxel-based morphometry at 3-Tesla. *J Neurol Sci* 2009;287(1-2):138-42.

31. Bagepally BS, Bhatt MD, Chandran V, Saini J, Bharath RD, Vasudev MK, et al. Decrease in cerebral and cerebellar gray matter in essential tremor: a voxel-based morphometric analysis under 3T MRI. *J Neuroimaging* 2012;22(3):275-8.

32. Quattrone A, Cerasa A, Messina D, Nicoletti G, Hagberg GE, Lemieux L, et al. Essential head tremor is associated with cerebellar vermis atrophy: a volumetric and voxel-based morphometry MR imaging study. *AJNR Am J Neuroradiol* 2008;29(9):1692-7.

33. Daniels C, Peller M, Wolff S, Alfke K, Witt K, Gaser C, et al. Voxel-based morphometry shows no decreases in cerebellar gray matter volume in essential tremor. *Neurology* 2006;67(8):1452-6.

34. Martinelli P, Rizzo G, Manners D, Tonon C, Pizza F, Testa C, et al. Diffusion-weighted imaging study of patients with essential tremor. *Mov Disord* 2007;22(8):1182-5.

35. Shin DH, Han BS, Kim HS, Lee PH. Diffusion tensor imaging in patients with essential tremor. *AJNR Am J Neuroradiol* 2008;29(1):151-3.

36. Dupuis MJ, Evrard FL, Jacquerye PG, Picard GR, Lermen OG. Disappearance of essential tremor after stroke. *Mov Disord* 2010;25(16):2884-7.

37. Paris-Robidas S, Brochu E, Sintes M, Emond V, Bousquet M, Vandal M, et al. Defective dentate nucleus GABA receptors in essential tremor. *Brain* 2012;135(Pt 1):105-16.

38. Shill HA, Adler CH, Beach TG, Lue LF, Caviness JN, Sabbagh MN, et al. Brain biochemistry in autopsied patients with essential tremor. *Mov Disord* 2012;27(1):113-7.

39. Boecker H, Weindl A, Brooks DJ, Ceballos-Baumann AO, Liedtke C, Miederer M, et al. GABAergic dysfunction in essential tremor: an 11C-flumazenil PET study. *J Nucl Med* 2010;51(7):1030-5.

40. Gironell A, Figueiras FP, Pagonabarraga J, Herance JR, Pascual-Sedano B, Trampal C, et al. Gaba and serotonin molecular neuroimaging in essential tremor: a clinical correlation study. *Parkinsonism Relat Disord* 2012;18(7):876-80.

41. Mally J, Baranyi M, Vizi ES. Change in the concentrations of amino acids in CSF and serum of patients with essential tremor. *J Neural Transm* 1996;103(5):555-60.

42. Thier S, Kuhlenbaumer G, Lorenz D, Nothnagel M, Nebel A, Christensen K, et al. GABA(A) receptor- and GABA transporter polymorphisms and risk for essential tremor. *Eur J Neurol* 2011;18(8):1098-100.

43. Garcia-Martin E, Martinez C, Alonso-Navarro H, Benito-Leon J, Lorenzo-Betancor O, Pastor P, et al. Gamma-aminobutyric acid (GABA) receptor rho (GABRR) polymorphisms and risk for essential tremor. *J Neurol* 2011;258(2):203-11.

44. Garcia-Martin E, Martinez C, Alonso-Navarro H, Benito-Leon J, Lorenzo-Betancor O, Pastor P, et al. Gamma-aminobutyric acid GABRA4, GABRE, and GABRQ receptor polymorphisms and risk for essential tremor. *Pharmacogenet Genomics* 2011;21(7):436-9.

45. Rajput AH, Robinson CA, Rajput ML, Robinson SL, Rajput A. Essential tremor is not dependent upon cerebellar Purkinje cell loss. *Parkinsonism Relat Disord* 2012;18(5):626-8.

46. Louis ED, Faust PL, Vonsattel JP, Honig LS, Rajput A, Robinson CA, et al. Neuropathological changes in essential tremor: 33 cases compared with 21 controls. *Brain* 2007;130(Pt 12):3297-307.

47. Louis ED, Faust PL, Ma KJ, Yu M, Cortes E, Vonsattel JP. Torpedoes in the cerebellar vermis in essential tremor cases vs. controls. *Cerebellum* 2011;10(4):812-9.

48. Shill HA, Adler CH, Sabbagh MN, Connor DJ, Caviness JN, Hentz JG, et al. Pathologic findings in prospectively ascertained essential tremor subjects. *Neurology* 2008;70(16 Pt 2):1452-5.

49. Pinto AD, Lang AE, Chen R. The cerebellothalamocortical pathway in essential tremor. *Neurology* 2003;60(12):1985-7.

50. Molnar GF, Sailer A, Gunraj CA, Lang AE, Lozano AM, Chen R. Thalamic deep brain stimulation activates the cerebellothalamocortical pathway. *Neurology* 2004;63(5):907-9.

51. Molnar GF, Sailer A, Gunraj CA, Cunic DI, Lang AE, Lozano AM, et al. Changes in cortical excitability with thalamic deep brain stimulation. *Neurology* 2005;64(11):1913-9.

52. Pedrosa DJ, Auth M, Eggers C, Timmermann L. Effects of low-frequency thalamic deep brain stimulation in essential tremor patients. *Exp Neurol* 2013;248:205-12.

53. Brittain JS, Brown P. The many roads to tremor. *Exp Neurol* 2013;250:104-7.

54. Raethjen J, Govindan RB, Kopper F, Muthuraman M, Deuschl G. Cortical involvement in the generation of essential tremor. *J Neurophysiol* 2007;97(5):3219-28.

55. Pedrosa DJ, Reck C, Florin E, Pauls KA, Maarouf M, Wojtecki L, et al. Essential tremor and tremor in Parkinson's disease are associated with distinct 'tremor clusters' in the ventral thalamus. *Exp Neurol* 2012;237(2):435-43.

56. Muthuraman M, Heute U, Arning K, Anwar AR, Elble R, Deuschl G, et al. Oscillating central motor networks in pathological tremors and voluntary movements. What makes the difference? *Neuroimage* 2012.

57. Helmich RC, Toni I, Deuschl G, Bloem BR. The pathophysiology of essential tremor and Parkinson's tremor. *Curr Neurol Neurosci Rep* 2013;13(9):378.

58. Park YG, Park HY, Lee CJ, Choi S, Jo S, Choi H, et al. Ca(V)3.1 is a tremor rhythm pacemaker in the inferior olive. *Proc Natl Acad Sci U S A* 2010;107(23):10731-6.

59. Boecker H, Wills AJ, Ceballos-Baumann A, Samuel M, Thompson PD, Findley LJ, et al. The effect of ethanol on alcohol-responsive essential tremor: a positron emission tomography study. *Ann Neurol* 1996;39(5):650-8.

60. Colebatch JG, Findley LJ, Frackowiak RS, Marsden CD, Brooks DJ. Preliminary report: activation of the cerebellum in essential tremor. *Lancet* 1990;336(8722):1028-30.

61. Bucher SF, Seelos KC, Dodel RC, Reiser M, Oertel WH. Activation mapping in essential tremor with functional magnetic resonance imaging. *Ann Neurol* 1997;41(1):32-40.

62. Hallett M, Dubinsky RM. Glucose metabolism in the brain of patients with essential tremor. *J Neurol Sci* 1993;114(1):45-8.

63. Schnitzler A, Munks C, Butz M, Timmermann L, Gross J. Synchronized brain network associated with essential tremor as revealed by magnetoencephalography. *Mov Disord* 2009;24(11):1629-35.

64. De Zeeuw CI, Hoebeek FE, Schonewille M. Causes and consequences of oscillations in the cerebellar cortex. *Neuron* 2008;58(5):655-8.

65. Uusisaari M, Obata K, Knopfel T. Morphological and electrophysiological properties of GABAergic and non-GABAergic cells in the deep cerebellar nuclei. *J Neurophysiol* 2007;97(1):901-11.

66. Parees I, Saifee TA, Kojovic M, Kassavetis P, Rubio-Agusti I, Sadnicka A, et al. Functional (psychogenic) symptoms in Parkinson's disease. *Mov Disord* 2013.

67. Onofrj M, Bonanni L, Manzoli L, Thomas A. Cohort study on somatoform disorders in Parkinson disease and dementia with Lewy bodies. *Neurology* 2010;74(20):1598-606.

68. Onofrj M, Thomas A, Tiraboschi P, Wenning G, Gambi F, Sepede G, et al. Updates on Somatoform Disorders (SFMD) in Parkinson's Disease and Dementia with Lewy Bodies and discussion of phenomenology. *J Neurol Sci* 2011;310(1-2):166-71.

69. Gerschlager W, Munchau A, Katzenschlager R, Brown P, Rothwell JC, Quinn N, et al. Natural history and syndromic associations of orthostatic tremor: a review of 41 patients. *Mov Disord* 2004;19(7):788-95.

70. Gitchel GT, Wetzel PA, Baron MS. Pervasive ocular tremor in patients with Parkinson disease. *Arch Neurol* 2012;69(8):1011-7.

71. Saifee TA, Kaski D, Buckwell D, Bronstein AM. Tremor of the eyes in Parkinson's disease: Merely a measure of the head movement. *Parkinsonism Relat Disord* 2014.

72. Kaski D, Saifee TA, Buckwell D, Bronstein AM. Ocular tremor in Parkinson's disease is due to head oscillation. *Mov Disord* 2013;28(4):534-7.

73. Rajput AH, Pahwa R, Pahwa P, Rajput A. Prognostic significance of the onset mode in parkinsonism. *Neurology* 1993;43(4):829-30.

74. Schiess MC, Zheng H, Soukup VM, Bonnen JG, Nauta HJ. Parkinson's disease subtypes: clinical classification and ventricular cerebrospinal fluid analysis. *Parkinsonism Relat Disord* 2000;6(2):69-76.

75. Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ. A clinico-pathological study of subtypes in Parkinson's disease. *Brain* 2009;132(Pt 11):2947-57.

76. Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, et al. Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. *Lancet Neurol* 2009;8(12):1128-39.

77. Jellinger KA. Post mortem studies in Parkinson's disease--is it possible to detect brain areas for specific symptoms? *J Neural Transm Suppl* 1999;56:1-29.

78. Doder M, Rabiner EA, Turjanski N, Lees AJ, Brooks DJ. Tremor in Parkinson's disease and serotonergic dysfunction: an 11C-WAY 100635 PET study. *Neurology* 2003;60(4):601-5.

79. Loane C, Wu K, Bain P, Brooks DJ, Piccini P, Politis M. Serotonergic loss in motor circuitries correlates with severity of action-postural tremor in PD. *Neurology* 2013;80(20):1850-5.

80. Mure H, Hirano S, Tang CC, Isaias IU, Antonini A, Ma Y, et al. Parkinson's disease tremor-related metabolic network: characterization, progression, and treatment effects. *Neuroimage* 2011;54(2):1244-53.

81. Timmermann L, Gross J, Dirks M, Volkmann J, Freund HJ, Schnitzler A. The cerebral oscillatory network of parkinsonian resting tremor. *Brain* 2003;126(Pt 1):199-212.

82. Helmich RC, Janssen MJ, Oyen WJ, Bloem BR, Toni I. Pallidal dysfunction drives a cerebellothalamic circuit into Parkinson tremor. *Ann Neurol* 2011;69(2):269-81.

83. Helmich RC, Hallett M, Deuschl G, Toni I, Bloem BR. Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits? *Brain* 2012;135(Pt 11):3206-26.

84. Cagnan H, Little S, Foltynie T, Limousin P, Zrinzo L, Hariz M, et al. The nature of tremor circuits in parkinsonian and essential tremor. *Brain* 2014.

85. Brittain JS, Probert-Smith P, Aziz TZ, Brown P. Tremor suppression by rhythmic transcranial current stimulation. *Curr Biol* 2013;23(5):436-40.

86. Hirschmann J, Hartmann CJ, Butz M, Hoogenboom N, Ozkurt TE, Elben S, et al. A direct relationship between oscillatory subthalamic nucleus-cortex coupling and rest tremor in Parkinson's disease. *Brain* 2013;136(Pt 12):3659-70.

87. Volkmann J, Joliot M, Mogilner A, Ioannides AA, Lado F, Fazzini E, et al. Central motor loop oscillations in parkinsonian resting tremor revealed by magnetoencephalography. *Neurology* 1996;46(5):1359-70.

88. Stiles RN, Randall JE. Mechanical factors in human tremor frequency. *J Appl Physiol* 1967;23(3):324-30.

89. Marsden CD, Meadows JC, Lange GW, Watson RS. The relation between physiological tremor of the two hands in healthy subjects. *Electroencephalogr Clin Neurophysiol* 1969;27(2):179-85.

90. Elble RJ, Randall JE. Mechanistic components of normal hand tremor. *Electroencephalogr Clin Neurophysiol* 1978;44(1):72-82.

91. Vallbo AB, Wessberg J. Organization of motor output in slow finger movements in man. *J Physiol* 1993;469:673-91.

92. Wessberg J, Vallbo AB. Human muscle spindle afferent activity in relation to visual control in precision finger movements. *J Physiol* 1995;482 (Pt 1):225-33.

93. Herbert R. Shaking when stirred: mechanisms of physiological tremor. *J Physiol* 2012;590(Pt 11):2549.

94. Conway BA, Halliday DM, Farmer SF, Shahani U, Maas P, Weir AI, et al. Synchronization between motor cortex and spinal motoneuronal pool during the performance of a maintained motor task in man. *J Physiol* 1995;489 (Pt 3):917-24.

95. Wessberg J, Vallbo AB. Coding of pulsatile motor output by human muscle afferents during slow finger movements. *J Physiol* 1995;485 (Pt 1):271-82.

96. Wessberg J, Vallbo AB. Pulsatile motor output in human finger movements is not dependent on the stretch reflex. *J Physiol* 1996;493 (Pt 3):895-908.

97. Elble RJ. Central mechanisms of tremor. *J Clin Neurophysiol* 1996;13(2):133-44.

98. Kakuda N, Nagaoka M, Wessberg J. Common modulation of motor unit pairs during slow wrist movement in man. *J Physiol* 1999;520 Pt 3:929-40.

99. Wessberg J, Kakuda N. Single motor unit activity in relation to pulsatile motor output in human finger movements. *J Physiol* 1999;517 (Pt 1):273-85.

100. Duval C, Panisset M, Bertrand G, Sadikot AF. Evidence that ventrolateral thalamotomy may eliminate the supraspinal component of both pathological and physiological tremors. *Exp Brain Res* 2000;132(2):216-22.

101. McAuley JH, Marsden CD. Physiological and pathological tremors and rhythmic central motor control. *Brain* 2000;123 (Pt 8):1545-67.

102. Gross J, Timmermann L, Kujala J, Dirks M, Schmitz F, Salmelin R, et al. The neural basis of intermittent motor control in humans. *Proc Natl Acad Sci U S A* 2002;99(4):2299-302.

103. Evans CM, Baker SN. Task-dependent intermanual coupling of 8-Hz discontinuities during slow finger movements. *Eur J Neurosci* 2003;18(2):453-6.

104. Proudlock FA, Scott J. Tremor in the human hand following peripheral nerve transection and reinnervation. *Brain Res* 2003;989(2):238-45.

105. Bye RT, Neilson PD. The BUMP model of response planning: intermittent predictive control accounts for 10 Hz physiological tremor. *Hum Mov Sci* 2010;29(5):713-36.

106. Vernooij CA, Reynolds RF, Lakie M. A dominant role for mechanical resonance in physiological finger tremor revealed by selective minimization of voluntary drive and movement. *J Neurophysiol* 2013;109(9):2317-26.

107. Lakie M, Vernooij CA, Osborne TM, Reynolds RF. The resonant component of human physiological hand tremor is altered by slow voluntary movements. *J Physiol* 2012;590(Pt 10):2471-83.

108. Lunn MP, Willison HJ. Diagnosis and treatment in inflammatory neuropathies. *Postgrad Med J* 2009;85(1006):437-46.

109. Merkies IS, Hughes RA, Donofrio P, Bril V, Dalakas MC, Hanna K, et al. Understanding the consequences of chronic inflammatory demyelinating polyradiculoneuropathy from impairments to activity and participation restrictions and reduced quality of life: the ICE study. *J Peripher Nerv Syst* 2010;15(3):208-15.

110. Shahani B. Tremor associated with peripheral neuropathy. In: Findley L, Capildeo, R, editor. *Movement Disorders: Tremor*. London: Macmillan, 1984.

111. Smith IS. The natural history of chronic demyelinating neuropathy associated with benign IgM paraproteinaemia. A clinical and neurophysiological study. *Brain* 1994;117 (Pt 5):949-57.

112. Sha XY, Xiong ZF, Liu HS, Di XD, Ma TH. Maternal-fetal fluid balance and aquaporins: from molecule to physiology. *Acta Pharmacol Sin* 2011;32(6):716-20.

113. Howden R, Lightfoot JT, Brown SJ, Swaine IL. The effects of isometric exercise training on resting blood pressure and orthostatic tolerance in humans. *Exp Physiol* 2002;87(4):507-15.

114. Busby M, Donaghy M. Chronic dysimmune neuropathy. A subclassification based upon the clinical features of 102 patients. *J Neurol* 2003;250(6):714-24.

115. Shen XY, Dong Q, Ma YL, Cui W, Yang GH, Xie LQ. [Preliminary study on protecting effect of two kinds of Chinese medical herbs on changes of three physiology systems in suspended rats]. *Space Med Med Eng (Beijing)* 2002;15(4):250-4.

116. Murray N, Page N, Steck AJ. The human anti-myelin-associated glycoprotein IgM system. *Ann Neurol* 1986;19(5):473-8.

117. Dalakas MC, Teravainen H, Engel WK. Tremor as a feature of chronic relapsing and dysgammaglobulinemic polyneuropathies. Incidence and management. *Arch Neurol* 1984;41(7):711-4.

118. Lehmann HC, Hoffmann FR, Meyer Zu Horste G, Hartung HP, Kieseier BC. Central nervous system involvement in patients with monoclonal gammopathy and polyneuropathy. *Eur J Neurol* 2010;17(8):1075-81.

119. Leger JM, Younes-Chennoufi AB, Zuber M, Bouche P, Jauberteau MO, Dormont D, et al. Frequency of central lesions in polyneuropathy associated with IgM monoclonal gammopathy: an MRI, neurophysiological and immunochemical study. *J Neurol Neurosurg Psychiatry* 1992;55(2):112-5.

120. Smith IS, Kahn SN, Lacey BW, King RH, Eames RA, Whybrew DJ, et al. Chronic demyelinating neuropathy associated with benign IgM paraproteinaemia. *Brain* 1983;106 (Pt 1):169-95.

121. Fitting JW, Bischoff A, Regli F, De Crousaz G. Neuropathy, amyloidosis, and monoclonal gammopathy. *J Neurol Neurosurg Psychiatry* 1979;42(3):193-202.

122. Spencer SS, Moench JC. Progressive and treatable cerebellar ataxia in macroglobulinemia. *Neurology* 1980;30(5):536-8.

123. Melmed C, Frail D, Duncan I, Braun P, Danoff D, Finlayson M, et al. Peripheral neuropathy with IgM kappa monoclonal immunoglobulin directed against myelin-associated glycoprotein. *Neurology* 1983;33(11):1397-405.

124. Julien J, Vital C, Vallat JM, Lagueny A, Ferrer X, Leboutet MJ. Chronic demyelinating neuropathy with IgM-producing lymphocytes in peripheral nerve and delayed appearance of "benign" monoclonal gammopathy. *Neurology* 1984;34(10):1387-9.

125. Meier C, Vandevelde M, Steck A, Zurbriggen A. Demyelinating polyneuropathy associated with monoclonal IgM-paraproteinaemia. Histological, ultrastructural and immunocytochemical studies. *J Neurol Sci* 1984;63(3):353-67.

126. Smith IS. Tremor in Peripheral Neuropathy. In: Findley LJ, Koller, W. C., editor. *Handbook of Tremor Disorders*. New York: Marcel Dekker, Inc., 1995:12.

127. McLeod JG, Pollard, J. D. Peripheral neuropathy associated with paraproteinaemia. In: Matthews WB, editor. *Handbook of Clinical Neurology*. Amsterdam: Elselvier, 1987:429-44.

128. Bain PG, Britton TC, Jenkins IH, Thompson PD, Rothwell JC, Thomas PK, et al. Tremor associated with benign IgM paraproteinaemic neuropathy. *Brain* 1996;119 (Pt 3):789-99.

129. Weiss D, Govindan RB, Rilk A, Wachter T, Breit S, Zizlsperger L, et al. Central oscillators in a patient with neuropathic tremor: Evidence from intraoperative local field potential recordings. *Mov Disord* 2010.

130. Matthews WB, Howell DA, Hughes RD. Relapsing corticosteroid-dependent polyneuritis. *J Neurol Neurosurg Psychiatry* 1970;33(3):330-7.

131. Yeung KB, Thomas PK, King RH, Waddy H, Will RG, Hughes RA, et al. The clinical spectrum of peripheral neuropathies associated with benign monoclonal IgM, IgG and IgA paraproteinaemia. Comparative clinical, immunological and nerve biopsy findings. *J Neurol* 1991;238(7):383-91.

132. Boecker H, Brooks DJ. Functional imaging of tremor. *Mov Disord* 1998;13 Suppl 3:64-72.

133. Querol L, Nogales-Gadea G, Rojas-Garcia R, Diaz-Manera J, Pardo J, Ortega-Moreno A, et al. Neurofascin IgG4 antibodies in CIDP associate with disabling tremor and poor response to IVIg. *Neurology* 2014;82(10):879-86.

134. McCombe PA, Pollard JD, McLeod JG. Chronic inflammatory demyelinating polyradiculoneuropathy. A clinical and

electrophysiological study of 92 cases. *Brain* 1987;110 (Pt 6):1617-30.

135. Feasby TE, Hahn AF, Koopman WJ, Lee DH. Central lesions in chronic inflammatory demyelinating polyneuropathy: an MRI study. *Neurology* 1990;40(3 Pt 1):476-8.

136. Dalakas MC, Engel WK. Chronic relapsing (dysimmune) polyneuropathy: pathogenesis and treatment. *Ann Neurol* 1981;9 Suppl:134-45.

137. Shahani BT, Young, R. R. Action tremors: a clinical neurophysiological review. In: Desmedt JE, editor. *Physiological Tremor, Pathological Tremors and Clonus*, 1978:129-37.

138. Dyck PJ. Intravenous immunoglobulin in chronic inflammatory demyelinating polyradiculoneuropathy and in neuropathy associated with IgM monoclonal gammopathy of unknown significance. *Neurology* 1990;40(2):327-8.

139. Breit S, Wachter T, Schols L, Gasser T, Nagele T, Freudenstein D, et al. Effective thalamic deep brain stimulation for neuropathic tremor in a patient with severe demyelinating neuropathy. *J Neurol Neurosurg Psychiatry* 2009;80(2):235-6.

140. Weiss D, Govindan RB, Rilk A, Wachter T, Breit S, Zizlsperger L, et al. Central oscillators in a patient with neuropathic tremor: evidence from intraoperative local field potential recordings. *Mov Disord* 2011;26(2):323-7.

141. Ruzicka E, Jech R, Zarubova K, Roth J, Urgosik D. VIM thalamic stimulation for tremor in a patient with IgM paraproteinaemic demyelinating neuropathy. *Mov Disord* 2003;18(10):1192-5.

142. McMaster J, Gibson G, Castro-Prado F, Vitali A, Honey CR. Neurosurgical treatment of tremor in anti-myelin-associated glycoprotein neuropathy. *Neurology* 2009;73(20):1707-8.

143. Bayreuther C, Delmont E, Borg M, Fontaine D. Deep brain stimulation of the ventral intermediate thalamic nucleus for severe tremor in anti-MAG neuropathy. *Mov Disord* 2009;24(14):2157-8.

144. Roussy G, Levy, G. Sept cas d'une maladie familiale particuliere. Troubles de la marche, pieds bots et arreflexie tendineuse generalisee avec accesoirement legere maladresse des mains. *Rev Neurol (Paris)* 1926;12:427-50.

145. Thomas PK, Marques W, Jr., Davis MB, Sweeney MG, King RH, Bradley JL, et al. The phenotypic manifestations of chromosome 17p11.2 duplication. *Brain* 1997;120 (Pt 3):465-78.

146. Plante-Bordeneuve V, Guiochon-Mantel A, Lacroix C, Lapresle J, Said G. The Roussy-Levy family: from the original description to the gene. *Ann Neurol* 1999;46(5):770-3.

147. Harding AE, Thomas PK. The clinical features of hereditary motor and sensory neuropathy types I and II. *Brain* 1980;103(2):259-80.

148. Auer-Grumbach M, Strasser-Fuchs S, Wagner K, Korner E, Fazekas F. Roussy-Levy syndrome is a phenotypic variant of Charcot-Marie-Tooth syndrome IA associated with a duplication on chromosome 17p11.2. *J Neurol Sci* 1998;154(1):72-5.

149. Karadima G, Koutsis G, Raftopoulou M, Floroskufi P, Karletidi KM, Panas M. Four novel connexin 32 mutations in X-linked Charcot-Marie-Tooth disease. Phenotypic variability and central nervous system involvement. *J Neurol Sci* 2014;341(1-2):158-61.

150. Zubair S, Holland NR, Beson B, Parke JT, Prodan CI. A novel point mutation in the PMP22 gene in a family with Roussy-Levy syndrome. *J Neurol* 2008;255(9):1417-8.

151. Salisachs P. Charcot-Marie-Tooth disease associated with "essential tremor": Report of 7 cases and a review of the literature. *J Neurol Sci* 1976;28(1):17-40.

152. Adams RD, Shahani, B.T., Young, R. R. Tremor associated with polyneuropathy. *Trans Am Neurol Assoc.*, 1972:44-8.

153. Tremor associated with peripheral neuropathy. Electroencephalography and Clinical Neurophysiology; 1989.

154. Britton TC, Thompson PD, Day BL, Rothwell JC, Findley LJ, Marsden CD. Rapid wrist movements in patients with essential tremor. The critical role of the second agonist burst. *Brain* 1994;117 (Pt 1):39-47.

155. Hallett M, Berardelli A, Matheson J, Rothwell J, Marsden CD. Physiological analysis of simple rapid movements in patients with cerebellar deficits. *J Neurol Neurosurg Psychiatry* 1991;54(2):124-33.

156. Hore J, Wild B, Diener HC. Cerebellar dysmetria at the elbow, wrist, and fingers. *J Neurophysiol* 1991;65(3):563-71.

157. Dean P, Porrill J, Ekerot CF, Jorntell H. The cerebellar microcircuit as an adaptive filter: experimental and computational evidence. *Nat Rev Neurosci* 2010;11(1):30-43.

158. Hawke SH, Hallinan JM, McLeod JG. Cranial magnetic resonance imaging in chronic demyelinating polyneuropathy. *J Neurol Neurosurg Psychiatry* 1990;53(9):794-6.

159. Louis ED, Hernandez N, Ottman R, Ionita-Laza I, Clark LN. Essential Tremor in a Charcot-Marie-Tooth Type 2C Kindred Does Not Segregate with the TRPV4 R269H Mutation. *Case Rep Neurol* 2014;6(1):1-6.

160. Costa J, Henriques R, Barroso C, Ferreira J, Atalaia A, de Carvalho M. Upper limb tremor induced by peripheral nerve injury. *Neurology* 2006;67(10):1884-6.

161. Jankovic J. Post-traumatic movement disorders: central and peripheral mechanisms. *Neurology* 1994;44(11):2006-14.

162. Koller WC, Wong GF, Lang A. Posttraumatic movement disorders: a review. *Mov Disord* 1989;4(1):20-36.

163. Schnitzler A, Gross J. Normal and pathological oscillatory communication in the brain. *Nat Rev Neurosci* 2005;6(4):285-96.

164. Gerloff C, Richard J, Hadley J, Schulman AE, Honda M, Hallett M. Functional coupling and regional activation of human cortical motor areas during simple, internally paced and externally paced finger movements. *Brain* 1998;121 (Pt 8):1513-31.

165. Knyazeva MG, Kurganskaya ME, Kurgansky AV, Njiokiktjien CJ, Vildavsky VJ. Interhemispheric interaction in children of 7-8: analysis of EEG coherence and finger tapping parameters. *Behav Brain Res* 1994;61(1):47-58.

166. Svoboda J, Sovka P, Stancak A. Intra- and inter-hemispheric coupling of electroencephalographic 8-13 Hz rhythm in humans and force of static finger extension. *Neurosci Lett* 2002;334(3):191-5.

167. Gross J, Tass PA, Salenius S, Hari R, Freund HJ, Schnitzler A. Cortico-muscular synchronization during isometric muscle contraction in humans as revealed by magnetoencephalography. *J Physiol* 2000;527 Pt 3:623-31.

168. Pollok B, Gross J, Muller K, Aschersleben G, Schnitzler A. The cerebral oscillatory network associated with auditorily paced finger movements. *Neuroimage* 2005;24(3):646-55.

169. Moritz CH, Haughton VM, Cordes D, Quigley M, Meyerand ME. Whole-brain functional MR imaging activation from a finger-tapping task examined with independent component analysis. *AJNR Am J Neuroradiol* 2000;21(9):1629-35.

170. Jancke L, Loose R, Lutz K, Specht K, Shah NJ. Cortical activations during paced finger-tapping applying visual and auditory pacing stimuli. *Brain Res Cogn Brain Res* 2000;10(1-2):51-66.

171. Lutz K, Specht K, Shah NJ, Jancke L. Tapping movements according to regular and irregular visual timing signals investigated with fMRI. *Neuroreport* 2000;11(6):1301-6.

172. Rao SM, Harrington DL, Haaland KY, Bobholz JA, Cox RW, Binder JR. Distributed neural systems underlying the timing of movements. *J Neurosci* 1997;17(14):5528-35.

173. Sadato N, Campbell G, Ibanez V, Deiber M, Hallett M. Complexity affects regional cerebral blood flow change during sequential finger movements. *J Neurosci* 1996;16(8):2691-700.

174. Seitz RJ, Canavan AG, Yaguez L, Herzog H, Tellmann L, Knorr U, et al. Successive roles of the cerebellum and premotor cortices in trajectorial learning. *Neuroreport* 1994;5(18):2541-4.

175. Jueptner M, Ottinger S, Fellows SJ, Adamschewski J, Flerich L, Muller SP, et al. The relevance of sensory input for the cerebellar control of movements. *Neuroimage* 1997;5(1):41-8.

176. Jueptner M, Weiller C. A review of differences between basal ganglia and cerebellar control of movements as revealed by functional imaging studies. *Brain* 1998;121 (Pt 8):1437-49.

177. Weeks RA, Gerloff C, Honda M, Dalakas MC, Hallett M. Movement-related cerebellar activation in the absence of sensory input. *J Neurophysiol* 1999;82(1):484-8.

178. Horne MK, Butler EG. The role of the cerebello-thalamo-cortical pathway in skilled movement. *Prog Neurobiol* 1995;46(2-3):199-213.

179. Middleton FA, Strick PL. Cerebellar output channels. *Int Rev Neurobiol* 1997;41:61-82.

180. Papka M, Ivry RB, Woodruff-Pak DS. Selective disruption of eyeblink classical conditioning by concurrent tapping. *Neuroreport* 1995;6(11):1493-7.

181. Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* 2000;123 Pt 3:572-84.

182. Bracha V, Zhao L, Wunderlich DA, Morrissy SJ, Bloedel JR. Patients with cerebellar lesions cannot acquire but are able to retain conditioned eyeblink reflexes. *Brain* 1997;120 (Pt 8):1401-13.

183. Topka H, Valls-Sole J, Massaquoi SG, Hallett M. Deficit in classical conditioning in patients with cerebellar degeneration. *Brain* 1993;116 (Pt 4):961-9.

184. Bracha V. Role of the cerebellum in eyeblink conditioning. *Prog Brain Res* 2004;143:331-9.

185. Galea JM, Jayaram G, Ajagbe L, Celnik P. Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. *J Neurosci* 2009;29(28):9115-22.

186. *Aids to the investigation of the peripheral nervous system*. London: Her Majesty's Stationary Office, 1943.

187. Shy ME, Blake J, Krajewski K, Fuerst DR, Laura M, Hahn AF, et al. Reliability and validity of the CMT neuropathy score as a measure of disability. *Neurology* 2005;64(7):1209-14.

188. Litvan I, Mangone CA, Werden W, Bueri JA, Estol CJ, Garcea DO, et al. Reliability of the NINDS Myotatic Reflex Scale. *Neurology* 1996;47(4):969-72.

189. Stacy MA, Elble RJ, Ondo WG, Wu SC, Hulihan J. Assessment of interrater and intrarater reliability of the Fahn-Tolosa-Marin Tremor Rating Scale in essential tremor. *Mov Disord* 2007;22(6):833-8.

190. Bertholon P, Bronstein AM, Davies RA, Rudge P, Thilo KV. Positional down beating nystagmus in 50 patients: cerebellar disorders and

possible anterior semicircular canalithiasis. *J Neurol Neurosurg Psychiatry* 2002;72(3):366-72.

191. Graham RC, Hughes RA. A modified peripheral neuropathy scale: the Overall Neuropathy Limitations Scale. *J Neurol Neurosurg Psychiatry* 2006;77(8):973-6.

192. Yarnall AJ, Rochester L, Baker MR, David R, Khoo TK, Duncan GW, et al. Short latency afferent inhibition: a biomarker for mild cognitive impairment in Parkinson's disease? *Mov Disord* 2013;28(9):1285-8.

193. Di Lazzaro V, Oliviero A, Tonali PA, Marra C, Daniele A, Profice P, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology* 2002;59(3):392-7.

194. Delwaide PJ, Olivier E. Conditioning transcranial cortical stimulation (TCCS) by exteroceptive stimulation in parkinsonian patients. *Adv Neurol* 1990;53:175-81.

195. Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol* 2000;523 Pt 2:503-13.

196. Maertens de Noordhout A, Rothwell JC, Day BL, Dressler D, Nakashima K, Thompson PD, et al. Effect of digital nerve stimuli on responses to electrical or magnetic stimulation of the human brain. *J Physiol* 1992;447:535-48.

197. McKay BE, Lado WE, Martin LJ, Galic MA, Fournier NM. Learning and memory in agmatine-treated rats. *Pharmacol Biochem Behav* 2002;72(3):551-7.

198. Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J Physiol* 2002;543(Pt 2):699-708.

199. Quartarone A, Bagnato S, Rizzo V, Siebner HR, Dattola V, Scalfari A, et al. Abnormal associative plasticity of the human motor cortex in writer's cramp. *Brain* 2003;126(Pt 12):2586-96.

200. Muller-Dahlhaus F, Ziemann U, Classen J. Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. *Front Synaptic Neurosci* 2010;2:34.

201. Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci* 2004;24(7):1666-72.

202. Freeman JH, Steinmetz AB. Neural circuitry and plasticity mechanisms underlying delay eyeblink conditioning. *Learn Mem* 2011;18(10):666-77.

203. Yeo CH, Hesslow G. Cerebellum and conditioned reflexes. *Trends Cogn Sci* 1998;2(9):322-30.

204. Linden DJ. Neuroscience. From molecules to memory in the cerebellum. *Science* 2003;301(5640):1682-5.

205. Christian KM, Thompson RF. Neural substrates of eyeblink conditioning: acquisition and retention. *Learn Mem* 2003;10(6):427-55.

206. De Zeeuw CI, Yeo CH. Time and tide in cerebellar memory formation. *Curr Opin Neurobiol* 2005;15(6):667-74.

207. McCormick DA, Lavond DG, Thompson RF. Concomitant classical conditioning of the rabbit nictitating membrane and eyelid responses: correlations and implications. *Physiol Behav* 1982;28(5):769-75.

208. McCormick DA, Clark GA, Lavond DG, Thompson RF. Initial localization of the memory trace for a basic form of learning. *Proc Natl Acad Sci U S A* 1982;79(8):2731-5.

209. Lincoln JS, McCormick DA, Thompson RF. Ipsilateral cerebellar lesions prevent learning of the classically conditioned nictitating membrane/eyelid response. *Brain Res* 1982;242(1):190-3.

210. Lavond DG, Hembree TL, Thompson RF. Effect of kainic acid lesions of the cerebellar interpositus nucleus on eyelid conditioning in the rabbit. *Brain Res* 1985;326(1):179-82.

211. Steinmetz JE, Logue SF, Steinmetz SS. Rabbit classically conditioned eyelid responses do not reappear after interpositus nucleus lesion and extensive post-lesion training. *Behav Brain Res* 1992;51(1):103-14.

212. Nolan BC, Freeman JH. Purkinje cell loss by OX7-saporin impairs acquisition and extinction of eyeblink conditioning. *Learn Mem* 2006;13(3):359-65.

213. Gitchel GT, Wetzel PA, Baron MS. Slowed saccades and increased square wave jerks in essential tremor. *Tremor Other Hyperkinet Mov (N Y)* 2013;3.

214. Sharpe JA, Herishanu YO, White OB. Cerebral square wave jerks. *Neurology* 1982;32(1):57-62.

215. Helmchen C, Hagenow A, Miesner J, Sprenger A, Rambold H, Wenzelburger R, et al. Eye movement abnormalities in essential

tremor may indicate cerebellar dysfunction. *Brain* 2003;126(Pt 6):1319-32.

216. Priori A, Berardelli A, Rona S, Accornero N, Manfredi M. Polarization of the human motor cortex through the scalp. *Neuroreport* 1998;9(10):2257-60.

217. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol* 2000;527 Pt 3:633-9.

218. Priori A. Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clin Neurophysiol* 2003;114(4):589-95.

219. Molinari M, Leggio MG, Solida A, Ciorra R, Misciagna S, Silveri MC, et al. Cerebellum and procedural learning: evidence from focal cerebellar lesions. *Brain* 1997;120 (Pt 10):1753-62.

220. Molinari M, Leggio MG, Thaut MH. The cerebellum and neural networks for rhythmic sensorimotor synchronization in the human brain. *Cerebellum* 2007;6(1):18-23.

221. Spencer RM, Zelaznik HN, Diedrichsen J, Ivry RB. Disrupted timing of discontinuous but not continuous movements by cerebellar lesions. *Science* 2003;300(5624):1437-9.

222. Ivry RB, Keele SW. Timing functions of the cerebellum. *J Cogn Neurosci* 1989;1(2):136-52.

223. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin Neurophysiol* 2006;117(4):845-50.

224. Martin TA, Keating JG, Goodkin HP, Bastian AJ, Thach WT. Throwing while looking through prisms. I. Focal olivocerebellar lesions impair adaptation. *Brain* 1996;119 (Pt 4):1183-98.

225. Baizer JS, Kralj-Hans I, Glickstein M. Cerebellar lesions and prism adaptation in macaque monkeys. *J Neurophysiol* 1999;81(4):1960-5.

226. Maschke M, Gomez CM, Ebner TJ, Konczak J. Hereditary cerebellar ataxia progressively impairs force adaptation during goal-directed arm movements. *J Neurophysiol* 2004;91(1):230-8.

227. Diedrichsen J, Verstynen T, Lehman SL, Ivry RB. Cerebellar involvement in anticipating the consequences of self-produced actions during bimanual movements. *J Neurophysiol* 2005;93(2):801-12.

228. Smith MA, Shadmehr R. Intact ability to learn internal models of arm dynamics in Huntington's disease but not cerebellar degeneration. *J Neurophysiol* 2005;93(5):2809-21.

229. Parees I, Kassavetis P, Saifee TA, Sadnicka A, Davare M, Bhatia KP, et al. Failure of explicit movement control in patients with functional motor symptoms. *Mov Disord* 2013;28(4):517-23.

230. Mazzoni P, Wexler NS. Parallel explicit and implicit control of reaching. *PLoS One* 2009;4(10):e7557.

231. Raethjen J, Lauk M, Koster B, Fietzek U, Friege L, Timmer J, et al. Tremor analysis in two normal cohorts. *Clin Neurophysiol* 2004;115(9):2151-6.

232. Fahn S, Tolosa, E., Marin, C. Clinical rating scale for tremor. In: Jankovic J, Tolosa, E., editor. *Parkinson's Disease and Movement Disorders*. Baltimore, MD: Williams & Wilkins, 1993:225-34.

233. Elble R, Bain P, Forjaz MJ, Haubenberger D, Testa C, Goetz CG, et al. Task force report: scales for screening and evaluating tremor: critique and recommendations. *Mov Disord* 2013;28(13):1793-800.

234. Elble RJ, Lyons KE, Pahwa R. Levetiracetam is not effective for essential tremor. *Clin Neuropharmacol* 2007;30(6):350-6.

235. Elble RJ, Pullman SL, Matsumoto JY, Raethjen J, Deuschl G, Tintner R. Tremor amplitude is logarithmically related to 4- and 5-point tremor rating scales. *Brain* 2006;129(Pt 10):2660-6.

236. Haubenberger D, Kalowitz D, Nahab FB, Toro C, Ippolito D, Luckenbaugh DA, et al. Validation of digital spiral analysis as outcome parameter for clinical trials in essential tremor. *Mov Disord* 2011;26(11):2073-80.

237. Bain PG, Findley LJ, Atchison P, Behari M, Vidailhet M, Gresty M, et al. Assessing tremor severity. *J Neurol Neurosurg Psychiatry* 1993;56(8):868-73.

238. Bain PG, Findley, L. J. *Assessing tremor severity*. London: Smith-Gordon and Company Limited, 1993.

239. Alusi SH, Worthington J, Glickman S, Findley LJ, Bain PG. Evaluation of three different ways of assessing tremor in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2000;68(6):756-60.

240. Merkies IS, Schmitz PI, van der Meche FG, van Doorn PA. Psychometric evaluation of a new sensory scale in immune-mediated polyneuropathies. Inflammatory Neuropathy Cause and Treatment (INCAT) Group. *Neurology* 2000;54(4):943-9.

241. Hallett M. NINDS myotatic reflex scale. *Neurology* 1993;43(12):2723.

242. Murphy SM, Herrmann DN, McDermott MP, Scherer SS, Shy ME, Reilly MM, et al. Reliability of the CMT neuropathy score (second version) in Charcot-Marie-Tooth disease. *J Peripher Nerv Syst* 2011;16(3):191-8.

243. Burns J, Ramchandren S, Ryan MM, Shy M, Ouvrier RA. Determinants of reduced health-related quality of life in pediatric inherited neuropathies. *Neurology* 2010;75(8):726-31.

244. Blyton F, Ryan MM, Ouvrier RA, Burns J. Muscle cramp in pediatric Charcot-Marie-Tooth disease type 1A: Prevalence and predictors. *Neurology* 2011.

245. Schwingenschuh P, Katschnig P, Seiler S, Saifee TA, Aguirregomozcorta M, Cordivari C, et al. Moving toward "laboratory-supported" criteria for psychogenic tremor. *Mov Disord* 2011;26(14):2509-15.

246. Teunissen LL, Notermans NC, Franssen H, van der Graaf Y, Oey PL, Linssen WH, et al. Differences between hereditary motor and sensory neuropathy type 2 and chronic idiopathic axonal neuropathy. A clinical and electrophysiological study. *Brain* 1997;120 (Pt 6):955-62.

247. Hyam JA, Aziz TZ, Bain PG. Post-deep brain stimulation--gradual non-stimulation dependent decrease in strength with attenuation of multiple sclerosis tremor. *J Neurol* 2007;254(7):854-60.

248. Schwingenschuh P, Saifee TA, Katschnig-Winter P, Reilly MM, Lunn MP, Manji H, et al. Cerebellar learning distinguishes inflammatory neuropathy with and without tremor. *Neurology* 2013;80(20):1867-73.

249. Le Forestier N, Chassande B, Moulonguet A, Maisonobe T, Schaeffer S, Birouk N, et al. [Multifocal motor neuropathies with conduction blocks. 39 cases]. *Rev Neurol (Paris)* 1997;153(10):579-86.

250. Coltamai L, Magezi DA, Croquelois A. Pregabalin in the treatment of neuropathic tremor following a motor axonal form of Guillain-Barre syndrome. *Mov Disord* 2010.

251. Alonso-Navarro H, Fernandez-Diaz A, Martin-Prieto M, Ruiz-Ezquerro JJ, Lopez-Alburquerque T, Jimenez-Jimenez FJ. Tremor associated with chronic inflammatory demyelinating peripheral neuropathy: treatment with pregabalin. *Clin Neuropharmacol* 2008;31(4):241-4.

252. Deuschl G, Raethjen J, Hellriegel H, Elble R. Treatment of patients with essential tremor. *Lancet Neurol* 2011;10(2):148-61.

253. Rautakorpi I, Takala J, Marttila RJ, Sievers K, Rinne UK. Essential tremor in a Finnish population. *Acta Neurol Scand* 1982;66(1):58-67.

254. Miralles F, Tarongi S, Espino A. Quantification of the drawing of an Archimedes spiral through the analysis of its digitized picture. *J Neurosci Methods* 2006;152(1-2):18-31.

255. Lunn MP, Willison HJ. Diagnosis and treatment in inflammatory neuropathies. *J Neurol Neurosurg Psychiatry* 2009;80(3):249-58.

256. Saifee TA, Schwingenschuh P, Reilly MM, Lunn MP, Katschnig P, Kassavetis P, et al. Tremor in inflammatory neuropathies. *J Neurol Neurosurg Psychiatry* 2012.

257. Stanton M, Pannoni V, Lewis RA, Logigian EL, Naguib D, Shy ME, et al. Dispersion of compound muscle action potential in hereditary

neuropathies and chronic inflammatory demyelinating polyneuropathy. *Muscle Nerve* 2006;34(4):417-22.

258. Kiers L, Clouston P, Zuniga G, Cros D. Quantitative studies of F responses in Guillain-Barre syndrome and chronic inflammatory demyelinating polyneuropathy. *Electroencephalogr Clin Neurophysiol* 1994;93(4):255-64.

259. Hoffland BS, Bologna M, Kassavetis P, Teo JT, Rothwell JC, Yeo CH, et al. Cerebellar Theta Burst Stimulation Impairs Eye-Blink Classical Conditioning. *J Physiol* 2011.

260. Pedersen SF, Pullman SL, Latov N, Brannagan TH, 3rd. Physiological tremor analysis of patients with anti-myelin-associated glycoprotein associated neuropathy and tremor. *Muscle Nerve* 1997;20(1):38-44.

261. Berardelli A, Hallett M, Rothwell JC, Agostino R, Manfredi M, Thompson PD, et al. Single-joint rapid arm movements in normal subjects and in patients with motor disorders. *Brain* 1996;119 (Pt 2):661-74.

262. Hamada M, Strigaro G, Murase N, Sadnicka A, Galea JM, Edwards MJ, et al. Cerebellar modulation of human associative plasticity. *J Physiol* 2012.

263. Freund P, Weiskopf N, Ward NS, Hutton C, Gall A, Ciccarelli O, et al. Disability, atrophy and cortical reorganization following spinal cord injury. *Brain* 2011;134(Pt 6):1610-22.

264. Freund P, Rothwell J, Craggs M, Thompson AJ, Bestmann S. Corticomotor representation to a human forearm muscle changes following cervical spinal cord injury. *Eur J Neurosci* 2011;34(11):1839-46.

265. Barbieri F, Filla A, Ragno M, Crisci C, Santoro L, Corona M, et al. Evidence that Charcot-Marie-tooth disease with tremor coincides with the Roussy-Levy syndrome. *Can J Neurol Sci* 1984;11(4 Suppl):534-40.

266. Cardoso FE, Jankovic J. Hereditary motor-sensory neuropathy and movement disorders. *Muscle Nerve* 1993;16(9):904-10.

267. Stiles RN. Frequency and displacement amplitude relations for normal hand tremor. *J Appl Physiol* 1976;40(1):44-54.

268. Stiles RN. Mechanical and neural feedback factors in postural hand tremor of normal subjects. *J Neurophysiol* 1980;44(1):40-59.

269. Young RR, Hagbarth KE. Physiological tremor enhanced by manoeuvres affecting the segmental stretch reflex. *J Neurol Neurosurg Psychiatry* 1980;43(3):248-56.

270. Pareyson D, Reilly MM, Schenone A, Fabrizi GM, Cavallaro T, Santoro L, et al. Ascorbic acid in Charcot-Marie-Tooth disease type 1A (CMT-TRIAAL and CMT-TRAUK): a double-blind randomised trial. *Lancet Neurol* 2011;10(4):320-8.

271. Galea JM, Vazquez A, Pasricha N, de Xivry JJ, Celnik P. Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. *Cereb Cortex* 2011;21(8):1761-70.

272. Elble RJ, Brilliant M, Leffler K, Higgins C. Quantification of essential tremor in writing and drawing. *Mov Disord* 1996;11(1):70-8.

273. Elble RJ. Essential tremor frequency decreases with time. *Neurology* 2000;55(10):1547-51.

274. Smith IS, Furness, P., Thomas, P.K. Tremor in Peripheral Neuropathy. In: Findley L.J. C, R., editor. *Movement Disorders: Tremor*. London: Macmillan, 1984.

275. Said G, Bathien N, Cesaro P. Peripheral neuropathies and tremor. *Neurology* 1982;32(5):480-5.

276. Deuschl G, Toro C, Zeffiro T, Massaquoi S, Hallett M. Adaptation motor learning of arm movements in patients with cerebellar disease. *J Neurol Neurosurg Psychiatry* 1996;60(5):515-9.

277. Abbott BP, Abbott R, Acernese F, Adhikari R, Ajith P, Allen B, et al. An upper limit on the stochastic gravitational-wave background of cosmological origin. *Nature* 2009;460(7258):990-4.

278. Elble RJ, Randall JE. Motor-unit activity responsible for 8- to 12-Hz component of human physiological finger tremor. *J Neurophysiol* 1976;39(2):370-83.

279. Marsden CD, Meadows JC, Lange GW, Watson RS. Effect of deafferentation on human physiological tremor. *Lancet* 1967;2(7518):700-2.

280. Allum JH, Dietz V, Freund HJ. Neuronal mechanisms underlying physiological tremor. *J Neurophysiol* 1978;41(3):557-71.

281. Homberg V, Hefter H, Reiners K, Freund HJ. Differential effects of changes in mechanical limb properties on physiological and pathological tremor. *J Neurol Neurosurg Psychiatry* 1987;50(5):568-79.

282. Fox JR, Randall JE. Relationship between forearm tremor and the biceps electromyogram. *J Appl Physiol* 1970;29(1):103-8.

283. Stephens JA, Taylor A. The effect of visual feedback on physiological muscle tremor. *Electroencephalogr Clin Neurophysiol* 1974;36(5):457-64.

284. Raethjen J, Pawlas F, Lindemann M, Wenzelburger R, Deuschl G. Determinants of physiologic tremor in a large normal population. *Clin Neurophysiol* 2000;111(10):1825-37.

285. Mehta AR, Brittain JS, Brown P. The selective influence of rhythmic cortical versus cerebellar transcranial stimulation on human physiological tremor. *J Neurosci* 2014;34(22):7501-8.

286. Kalkman JS, Schillings ML, van der Werf SP, Padberg GW, Zwarts MJ, van Engelen BG, et al. Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and HMSN-I. *J Neurol Neurosurg Psychiatry* 2005;76(10):1406-9.

287. Williams ER, Soteropoulos DS, Baker SN. Spinal interneuron circuits reduce approximately 10-Hz movement discontinuities by phase cancellation. *Proc Natl Acad Sci U S A* 2010;107(24):11098-103.

288. Di Giulio AM, Tenconi B, Mannavola A, Mantegazza P, Schiavinato A, Gorio A. Spinal cord interneuron degenerative atrophy caused by peripheral nerve lesions is prevented by serotonin depletion. *J Neurosci Res* 1987;18(3):443-8.

289. Oliveira AL, Risling M, Negro A, Langone F, Cullheim S. Apoptosis of spinal interneurons induced by sciatic nerve axotomy in the neonatal rat is counteracted by nerve growth factor and ciliary neurotrophic factor. *J Comp Neurol* 2002;447(4):381-93.

290. Wasserschaff M. Coordination of reinnervated muscle and reorganization of spinal cord motoneurons after nerve transection in mice. *Brain Res* 1990;515(1-2):241-6.

291. Thach WT. A role for the cerebellum in learning movement coordination. *Neurobiol Learn Mem* 1998;70(1-2):177-88.

292. Claassen DO, Jones CR, Yu M, Dirnberger G, Malone T, Parkinson M, et al. Deciphering the impact of cerebellar and basal ganglia dysfunction in accuracy and variability of motor timing. *Neuropsychologia* 2013;51(2):267-74.

293. Joundi RA, Brittain JS, Green AL, Aziz TZ, Jenkinson N. High-frequency stimulation of the subthalamic nucleus selectively decreases central variance of rhythmic finger tapping in Parkinson's disease. *Neuropsychologia* 2012;50(10):2460-6.

294. Repp BH. Sensorimotor synchronization: a review of the tapping literature. *Psychon Bull Rev* 2005;12(6):969-92.

295. Ohyama T, Nores WL, Murphy M, Mauk MD. What the cerebellum computes. *Trends Neurosci* 2003;26(4):222-7.

296. Glickstein M, Doron K. Cerebellum: connections and functions. *Cerebellum* 2008;7(4):589-94.

297. Theoret H, Haque J, Pascual-Leone A. Increased variability of paced finger tapping accuracy following repetitive magnetic stimulation of the cerebellum in humans. *Neurosci Lett* 2001;306(1-2):29-32.

298. Del Olmo MF, Cheeran B, Koch G, Rothwell JC. Role of the cerebellum in externally paced rhythmic finger movements. *J Neurophysiol* 2007;98(1):145-52.

299. Jancke L, Steinmetz H, Benilow S, Ziemann U. Slowing fastest finger movements of the dominant hand with low-frequency rTMS of the hand area of the primary motor cortex. *Exp Brain Res* 2004;155(2):196-203.

300. Rao SM, Mayer AR, Harrington DL. The evolution of brain activation during temporal processing. *Nat Neurosci* 2001;4(3):317-23.

301. Farkas Z, Szirmai I, Kamondi A. Impaired rhythm generation in essential tremor. *Mov Disord* 2006;21(8):1196-9.

302. Ivry RB. The representation of temporal information in perception and motor control. *Curr Opin Neurobiol* 1996;6(6):851-7.

303. Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Exp Brain Res* 2004;156(4):439-43.

304. Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci* 2004;24(13):3379-85.

305. Javadi AH, Cheng P, Walsh V. Short duration transcranial direct current stimulation (tDCS) modulates verbal memory. *Brain Stimul* 2012;5(4):468-74.

306. Schubotz RI, Friederici AD, von Cramon DY. Time perception and motor timing: a common cortical and subcortical basis revealed by fMRI. *Neuroimage* 2000;11(1):1-12.

307. Malapani C, Dubois B, Rancurel G, Gibbon J. Cerebellar dysfunctions of temporal processing in the seconds range in humans. *Neuroreport* 1998;9(17):3907-12.

308. Penhune VB, Zattore RJ, Evans AC. Cerebellar contributions to motor timing: a PET study of auditory and visual rhythm reproduction. *J Cogn Neurosci* 1998;10(6):752-65.

309. Harrington DL, Boyd LA, Mayer AR, Sheltraw DM, Lee RR, Huang M, et al. Neural representation of interval encoding and decision making. *Brain Res Cogn Brain Res* 2004;21(2):193-205.

310. Ivry RB, Keele SW, Diener HC. Dissociation of the lateral and medial cerebellum in movement timing and movement execution. *Exp Brain Res* 1988;73(1):167-80.

311. Bo J, Block HJ, Clark JE, Bastian AJ. A cerebellar deficit in sensorimotor prediction explains movement timing variability. *J Neurophysiol* 2008;100(5):2825-32.

312. Diedrichsen J, Criscimagna-Hemminger SE, Shadmehr R. Dissociating timing and coordination as functions of the cerebellum. *J Neurosci* 2007;27(23):6291-301.

313. Ivry RB, Spencer RM. Evaluating the role of the cerebellum in temporal processing: beware of the null hypothesis. *Brain* 2004;127(Pt 8):E13; author reply E14.

314. Koch G, Oliveri M, Caltagirone C. Neural networks engaged in milliseconds and seconds time processing: evidence from transcranial magnetic stimulation and patients with cortical or subcortical

dysfunction. *Philos Trans R Soc Lond B Biol Sci* 2009;364(1525):1907-18.

315. Lewis PA, Miall RC. Brain activation patterns during measurement of sub- and supra-second intervals. *Neuropsychologia* 2003;41(12):1583-92.

316. Koch G, Oliveri M, Torriero S, Salerno S, Lo Gerfo E, Caltagirone C. Repetitive TMS of cerebellum interferes with millisecond time processing. *Exp Brain Res* 2007;179(2):291-9.

317. Repp BH, Su YH. Sensorimotor synchronization: a review of recent research (2006-2012). *Psychon Bull Rev* 2013;20(3):403-52.

318. Thaut MH, Stephan KM, Wunderlich G, Schicks W, Tellmann L, Herzog H, et al. Distinct cortico-cerebellar activations in rhythmic auditory motor synchronization. *Cortex* 2009;45(1):44-53.

319. Harrington DL, Haaland KY, Knight RT. Cortical networks underlying mechanisms of time perception. *J Neurosci* 1998;18(3):1085-95.

320. Jones CR, Rosenkranz K, Rothwell JC, Jahanshahi M. The right dorsolateral prefrontal cortex is essential in time reproduction: an investigation with repetitive transcranial magnetic stimulation. *Exp Brain Res* 2004;158(3):366-72.

321. Houdayer E, Devanne H, Tyvaert L, Defebvre L, Derambure P, Cassim F. Low frequency repetitive transcranial magnetic stimulation over premotor cortex can improve cortical tremor. *Clin Neurophysiol* 2007;118(7):1557-62.

322. Tass PA. Desynchronization of brain rhythms with soft phase-resetting techniques. *Biol Cybern* 2002;87(2):102-15.

323. Farmer SF, Swash M, Ingram DA, Stephens JA. Changes in motor unit synchronization following central nervous lesions in man. *J Physiol* 1993;463:83-105.

324. Farmer SF, Bremner FD, Halliday DM, Rosenberg JR, Stephens JA. The frequency content of common synaptic inputs to motoneurones studied during voluntary isometric contraction in man. *J Physiol* 1993;470:127-55.

325. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage* 2000;11(6 Pt 1):805-21.