University of Strathclyde

Department of Bioengineering

Inter-muscular coherence and neurogenic tremor as tools to evaluate the effects of motor training, transcranial stimulation and peripheral stimulation on cortical activity

by

Pauline Axford

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Abstract

The primary aims of this thesis were to develop and use inter-muscular coherence (IMC) and physiological neurogenic tremor (PNT) analysis as investigatory tools of cortico-spinal activity and to investigate the effects, on these outcomes, of transcranial direct current stimulation (tDCS), motor training, and peripheral nerve stimulation. To improve the coherence investigations baseline stability analysis and a robust analysis technique were incorporated and it was demonstrated that, under certain task conditions, IMC and PNT are appropriate investigation tools. The effects of anodal and cathodal tDCS on cortical excitability, IMC and PNT were tested. There was a reduction in cortical excitability after only cathodal tDCS; both polarities caused similar changes to IMC that may be suggestive of an opposing effect on a homeostatic response induced by the motor task; only cathodal tDCS interacted with PNT causing both decreases and increases that may be suggestive of changes to multiple inputs of PNT. The secondary aims of this thesis were to investigate whether any effects could be enhanced through the incorporation of a sinusoidal waveform onto the DC stimulation signal, or through the inclusion of peripheral nerve stimulation. Small sinuosoidal modulations of 5 Hz, 10 Hz and 20 Hz were imposed onto anodal tDCS. The effects on IMC and PNT were different from tDCS alone, but were not larger. Both tDCS and the sinusoidal variants were combined with peripheral nerve stimulation. Again, while there were some similarities, each stimulation protocol caused a different result on IMC and PNT from any other, but none exceeded the magnitude or duration of the effects caused by tDCS alone. It was concluded that IMC and PNT are appropriate tools for the investigation of cortical stimulation and that alterations to the tDCS protocol in the form of sinusoidal varying signals and peripheral nerve stimulation did not enhance the after effects of the stimulation.

Copyright Statement

Signed: Date:

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Abbreviations

1DI First Doral Interosseous.

1DI-A First Dorsal Interosseous-Accelerometer.

ADM Abductor Digiti Minimi.

APB Abductor Policis Brevis.

BCM Bienstock-Cooper-Munro.

BDNF Brain-Derived Neurotrophic Factor.

BOLD Blood Oxygen Dependent Level.

CMC Cortico-Muscular Coherence.

CST Cortico-Spinal Tract.

ED Extensor Digitorum.

EEG Electroencephalography.

EMG Electromyography.

fMRI Functional Magnetic Resonance Imaging.

GABA γ-Aminobutyric Acid.

ICF Intracortical Facilitation.

IMC Inter-Muscular Coherence.

JT Jebson-Taylor Hand Test.

LTD Long Term Depression.

LTP Long Term Potentiation.

M1 Primary Motor Cortex.

MEG Magnetoencephalography.

MEP Motor Evoked Potential.

NIBS Non-Invasive Brain Stimulation.

NMDA N-Methyl-D Asparate.

PAS Paired Associative Stimulation.

PET Positron Emission Tomography.

PNS Pheripheral Nerve Stimulation.

PNT Physiological Neurogenic Tremor.

rCBF Regional Cerebral Blood Flow.

SICI Short Intracortical Inhibition.

tACS Transcranial Alternating Current Stimulation.

tDCS Transcranial Direct Current Stimulation.

TES Transcranial Electric Stimulation.

TMS Transcranial Magnetic Stimulation.

tRNS Transcranial Random Noise Stimulation.

tSCS Transcranial Sinusoidal Current Stimulation.

Chapter 1

Research Statement

Reports from the literature suggest that low level polarisation of the motor cortex may occur with transcranial direct current stimulation (tDCS). The stimulation is reported to plastically alter cortical motor functions in a polarity dependent manner (Nitsche and Paulus (2000); Baudewig et al. (2001); Nitsche et al. (2003c); Power et al. (2006)) which may prove useful in the motor rehabilitation of brain or spinal cord injured patients if it can be demonstrated to be a reliable intervention.

Single pulse transcranial magnetic stimulation (TMS) is a tool that is widely used for both altering and investigating cortical activity, cortical excitability in particular. Within the literature the extent of the tDCS induced alterations to cortical excitability, as measured via TMS, vary substantially. These variations may be caused by the inherent variability of the TMS investigation technique, variability in the tDCS intervention to induce consistent changes, or priming effects caused by the combination of TMS and tDCS.

In order to evaluate the effectiveness of tDCS as a clinical tool it is important to explore both variability and possible priming effects caused by the traditional testing regime and to this end new investigation techniques must be developed.

Since neurones communicate in a frequency dependent manner and synchronised rhythmic oscillations are frequently observed between different regions of the nervous systems then appropriate investigation techniques may include inter-muscular coherence (IMC) and monitoring changes in neurogenic components of physiological tremor (PNT) as these processes are sensitive to changes in common synaptic drive. The initial aim of this thesis was to develop these tools through studies conducted on healthy adult volunteers, and to improve and extend the current understanding of the effects of tDCS on cortico-spinal activity. In view of these considerations the initial aim of the thesis was broken down as follows:

1. Assess reports from the literature that anodal tDCS plastically facilitates cortical excitability and cathodal tDCS depresses it.

2. Examine reports from the literature that tDCS plastically alters inter-muscular coherence in a polarity dependent way and explore methods to extend IMC as an investigatory tool.

3. Investigate the effects of tDCS on PNT and explore its potential as an investigatory tool of cortico-spinal activity.

The oscillatory nature of sub-components within the nervous system suggests that a DC signal may not be the most appropriate form of electrical intervention. It is hypothesized that external stimulation of the cortex at physiologically relevant frequencies may promote or suppress intrinsic oscillations and potentially alter cortical activity. This concept was investigated in the second stage of the project through the delivery of transcranial sinusoidal current stimulation (tSCS):

4. Assess conflicting reports of plastic changes to cortical excitability and rhyth-

micity caused by tSCS using the IMC and PNT tools developed in the previous section.

Peripheral stimulation delivered concomitantly with cortical stimulation in a paired associative stimulation (PAS) paradigm, has reportedly enhanced plasticity in the motor cortex compared to each stimulation delivered alone. The effects of tDCS and tSCS paired with peripheral nerve stimulation (PNS) were investigated here:

5. Investigate the effects of tDCS and tSCS when paired with peripheral nerve stimulation on inter-muscular coherence and neurogenic tremor.

Chapter 2

Introduction

Interruption or damage to motor pathways can be devastating to an individual, and, depending on the severity and location, can cause the loss or degradation of motor functions. New neurones cannot grow and scar tissue is often a contributor to mechanisms that inhibit the reconnection of broken pathways. Rehabilitation after injury is therefore dependent on the existing neurones'and pathway's abilities to alter their functionality and connectivity to compensate for the lost functions of the dead or damaged cells. This ability is known as neuroplasticity, and is a process associated with normal brain behaviour and learning that is not just a response to damage. 'Use it or lose it' and 'neurones that fire together wire together' (Doidge (2007)) are popular phrases that, while crude, are used to summarise decades of research that suggests that neuroplasticity, and potentially rehabilitation, may be enhanced by altering neuronal environments and communications between neurones. Electromagnetism is a tool that can be used to non-invasively influence cellular environments and may ultimately cause the affected neurones to alter their function. Non-invasive stimulation of the brain and peripheral nerves may then have therapeutic potential for the motor rehabilitation of those with brain or spinal injury, or other kinds of neurological disorder.

This thesis will explore the effects of low current electric brain stimulation and peripheral nerve stimulation on a variety of cortical functions. An improved understanding of these techniques will ultimately be of use in optimising stimulation protocols and inferring the clinical effectiveness of non-invasive stimulation for motor disorders.

2.1 Voluntary Movement

Voluntary motor control requires the coordination of signals between many neural structures in both the central and peripheral nervous systems. Some of the functionality of these structures can be extrapolated by observing how damage to them changes patients' abilities to learn, plan and execute movement.

2.1.1 Anatomical and Functional Organisation

Decision making, interpretation of sensory information, proprioception and voluntary motor control are generally associated with the cortical regions of the brain. A major class of cortical cells are the pyramidal neurones, these cells have well defined axons and apical dendrites, in contrast to stellate cells which have many dendrites that extend in all directions around the cell body, Figure 2.1. The cortical cells are organised in six layers, Figure 2.2:

(I)) Molecular layer: the outermost layer, it consists mostly of pyramidal neurones dendrites.
(II) External granular layer: small pyramidal neurones and stellate cells.
(III) External pyramidal layer: consists mostly of pyramidal cells.
(IV) Internal granular layer: both stellate and pyramidal cells.
(V) Ganglionic layer: large pyramidal neurones, Betz cells.
(VI) Multiform layer: lots of different types of neurones.

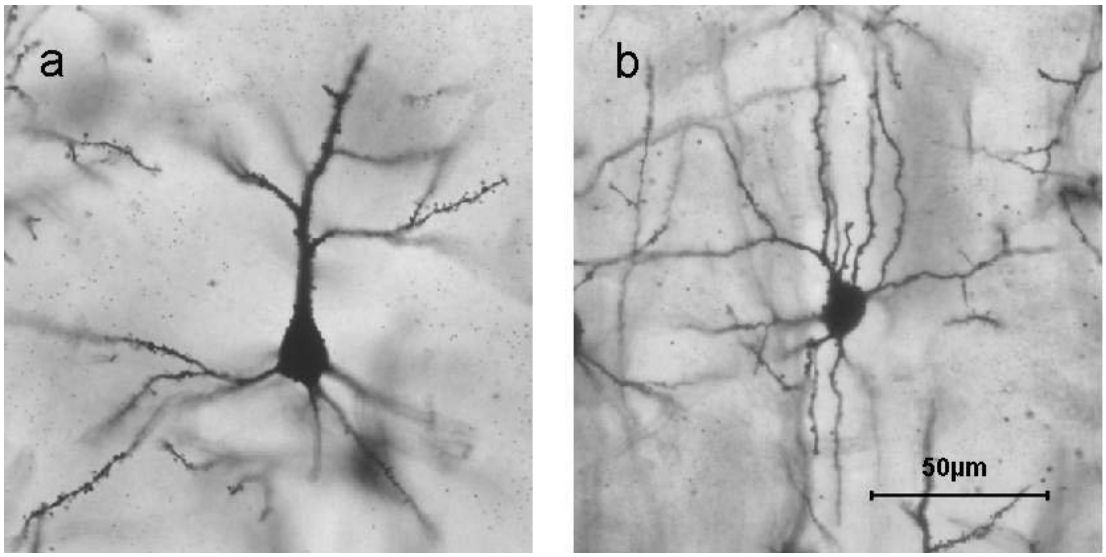


Figure 2.1: Golgi stained cortical neurones: a) pyramidal neurone. b) stellate cell. Churchill et al. (2004)

The primary motor cortex (M1) is often considered the hub of neural activity that is associated with the execution of voluntary movement; paralysis of body regions can occur if it is damaged. M1 is arranged somewhat somatotopically, Figure 2.3; however, the mapping is not always one to one and there is considerable overlap in the neuronal representation of the different muscle and body regions. Much of the information that is passed to the motor cortex arrives in other brain structures: the pre-motor cortex and the supplementary motor area are particularly important in constructing a plan for movement and in initiating movement. Damage to the pre-motor cortex impairs planning that is based on sensory information; damage to the supplementary motor area impairs abilities to reconstruct learned movements from memory. Communications generally arrive at the stellate cells in M1, these cells function as inhibitory interneurones primarily interacting through the release and uptake of GABAergic neurotransmitters. The motor command signals from M1 are sent to the muscles via the pyramidal neurones that form the corticospinal pathways, Figure 2.3. The pyramidal axons

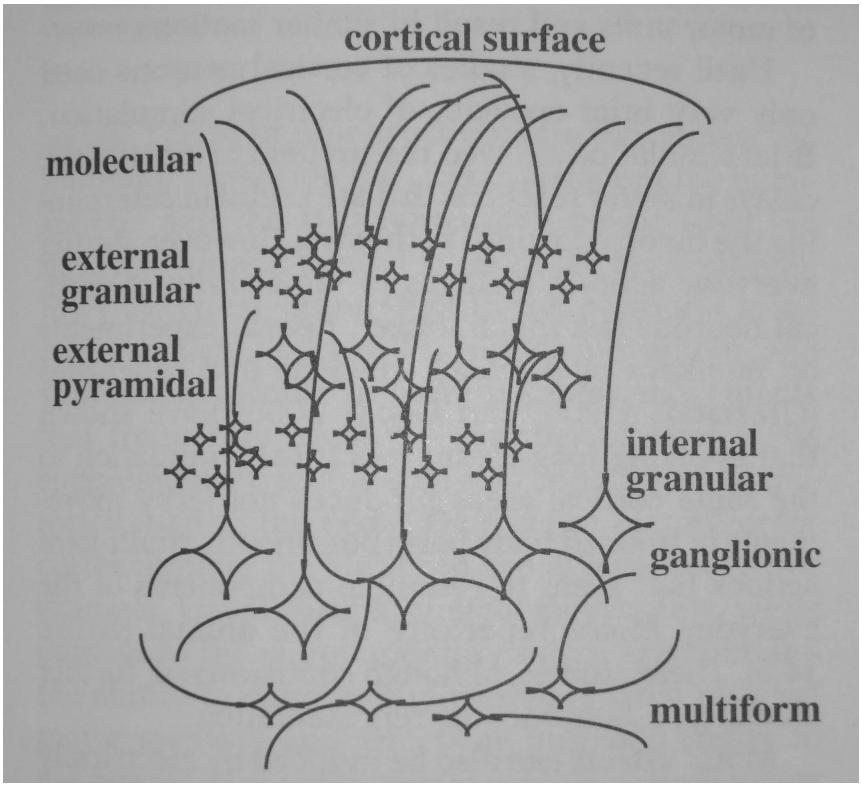


Figure 2.2: Simplified representation of the neuronal organisation in the six cortical layers. Latash (2008)

synapse onto the lower motor neurones at their target segment in the spinal cord. Most of the axons cross to the contralateral side at the level of the brain stem; however, some descend down a different, ipsilateral pathway and cross at the level of the target motor pools. This organisation of the descending motor pathways is the reason some function can be retained after an incomplete spinal cord injury. Sensory information from the periphery is sent back to the brain via the ascending sensory pathways and can modulate the motor output at various levels resulting in a continuous loop of nervous activity for every voluntary movement we execute.

2.1.2 Rhythmicity, Synchronicity and Motor Control

Neurones exhibit a tendency towards rhythmic firing and there are a number of neuronal properties that can lead to the initiation and propagation of oscillations.

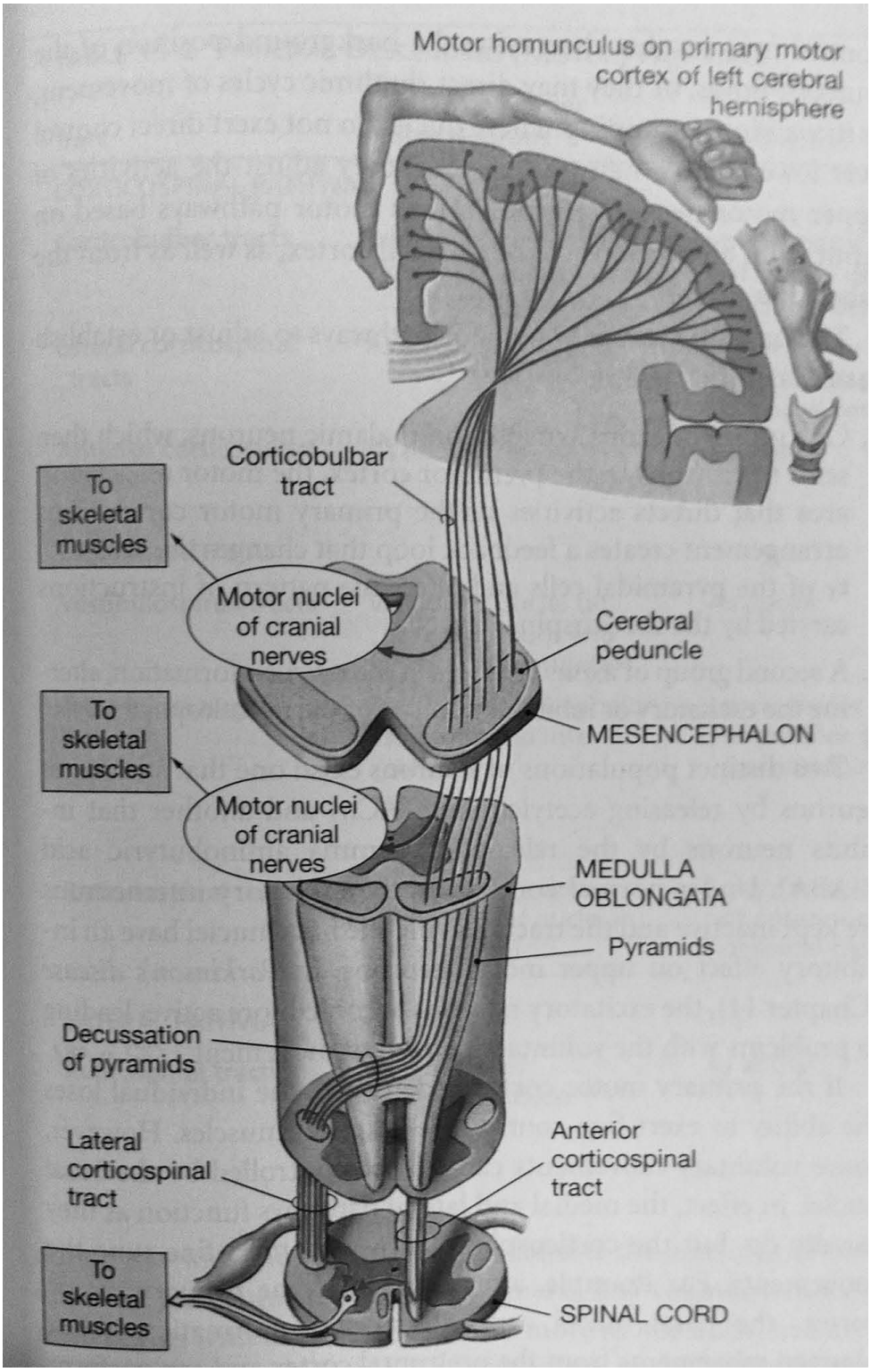


Figure 2.3: The somatotopical organisation of the cortex and the corticospinal pathways. Martini (2001)

The simplest mechanistic example is that of the refractory period which will impose a time delay on the transmission of continuous excitatory inputs to a cell resulting in a defined firing frequency. Again, at a single cell level the drift in membrane potential in pacemaker cells can also impose a tendency to rhythmic firing. On a larger network scale interactions between excitatory and inhibitory cells within neural circuits can impose time delays on the firing of the cells, which if repeated can result in reverberating oscillations with frequencies that are defined by the period of inhibition (Baker (2007)).

In the sensori-motor system independent rhythmic oscillations have been observed from the cortex down to the motor units, generally occurring in discrete frequency bands. Some motor cortical neurones exhibit a peaked post spike membrane potential trajectory, this peak in the membrane potential following hyperpolarisation promotes repetitive firing in particular frequency ranges (Baker (2007)).

Oscillations often occur independently of other sensori-motor structures, but under certain circumstances synchronisation between signals in different structures occurs. This coupling was first observed between the motor cortex and the contralateral muscles (Conway et al. (1995)) and is known as cortico-muscular coherence (CMC). Cortico-muscular coherence occurs within discrete frequency ranges and the frequencies tend to be characteristic of certain motor tasks: the α band ((6 - 12) Hz) is associated with the initiation of new movement. Cortico-muscular coupling in the β band ((15 - 30) Hz) does not occur during a movement, or even imagined movement, but is present during weak, maintained contractions. Since 1995 oscillations in the deep cerebellar nuclei, basal ganglia, somatosensory and posterior pariatal cortex have also been shown to synchronise with motor cortex oscillations (Soteropoulus and Baker (2006); Witham and Baker (2007)); coupling has also been observed between muscle activity and peripheral afferents, dorsal

ganglia and Ia muscle spindle afferents, and muscle activity and accelerometers (Baker (2007); Elble and Koller (1990))

2.2 Neuroplasticity and Rehabilitation

Neuroplasticity is the intrinsic ability of the nervous system to adapt its functional and, to some extent, its anatomical organisation in response to external stimuli. In healthy individuals plastic events are driven by learning and training, particularly training that involves sensory feedback and skill acquisition (Nudo (2011)). Plasticity is a consequence of injury but is not always beneficial for recovery. Motor rehabilitation after injury is dependent on the ability to learn, or relearn, movement functionality and this could be enhanced by encouraging plasticity in the undamaged neurones. Interventions that induce, shape or enhance plasticity may therefore prove useful in the motor rehabilitation of patients with neurological disorders, or those who have suffered damage to their motor pathways through brain or spinal cord injury. In recent years evidence has been presented that suggests that plastic events can also be artificially induced with electromagnetic stimulation of the nervous system.

The exact mechanisms involved in inducing plasticity are still unknown but have been linked, in the short term, to the unmasking of silent pathways and changes in synaptic efficacy. These, in the longer term, may lead to anatomical changes such as synaptogenesis and even dendritic growth (Nudo (2006)). These processes have been associated with the release of N-methyl-D aspartate (NMDA) and γ-aminobutyric acid (GABA) neurotransmitters.

Some clinical success in rehabilitation has been achieved through physiotherapy techniques which are founded in the Hebbian theory of plasticity that repeti-

tive stimulation of neurones, achieved through repetitive movement, sufficiently increases synaptic efficacy to promote recovery. Unfortunately intensive physiotherapy, in which a patient repeats a movement many times, has had limited success. The Hebbian model of plasticity is demonstrably over simplified: the system will quickly become destabilised if repetitive inputs drive enhanced, or reduced, synaptic strengthening. Empirical research has shown that sensory feedback and skill driven acquisition are also important in the recovery process and these are not represented in this over simplified model. These considerations have led to more sophisticated models of plasticity such as the Bienenstock-Cooper-Munro (BCM) model. Here more complex neuronal interactions are accounted for, such as such as firing frequencies and spike timing which as discussed above are also representative of synchronization between groups of cells. More sophisticated physiotherapy regimes have also evolved, these include constraint induced movement therapy, in which the healthy limb is constrained forcing the use of the impaired limb; virtual reality and robotic therapies. Robotic rehabilitation is particularly interesting since it can deliver high dose and high intensity training to patients in a safe environment. Depending on the severity of the motor impairment practice can be undertaken in either assistive or impeditive force fields providing sensory feedback or strength training respectively. Impeditive fields carry the additional advantage of being able to guide a patient's movements along a trajectory; under these conditions the patient can make mistakes. This is important as errors are thought to be integral for skill based motor learning.

Long term potentiation (LTP) and long term depression (LTD) are important forms of synaptic plasticity that are strongly linked to learning (Cooke and Bliss (2006)), they are characterised by the long duration of the induced change to synaptic efficacy (greater than 30 minutes) and an NMDA dependency. A form of LTP known as associative LTP has been artificially induced in postsynaptic

cells when a strong, depolarising stimulus is preceded by a weak one; similarly associative LTD is induced when the strong stimulus causes the postsynaptic neurone to fire before the weak stimulus is delivered (Stefan et al. (2000); Wolters et al. (2003)).

2.3 Non-Invasive Brain Stimulation

Electricity and neurology have a long and graphic history; at the end of the 18th century Luigi Galvani demonstrated, for the first time, that an electric current could induce movement in animal tissue by making the leg of a dead frog twitch (Piccolino (1998)). He wrongly concluded that he had discovered the force that distinguished life from the inanimate and a number of experiments were conducted to discover if electricity could revive dead tissue and reinstate the soul back into the recently deceased. The experiments were unsuccessful, but the concept was immortalised in Mary Shelley's gothic horror novel Frankenstein.

> "I collected the instruments of life around me, that I might infuse a spark of being into the lifeless thing that lay at my feet." Shelley (1818)

Galvani had misunderstood the role of electricity in organisms but he clearly demonstrated that it plays a vital part in motor control. His work, and the work of those that followed, revealed that electricity is not only created in chemical batteries and conducted through metal wires, but is a fundamental force that innervates life and flows through biological tissue. More recent research has demonstrated that the body's electric signals are not a binary stop and go system, but a complex communication network capable of producing and integrating a range of electrical signals of different intensities and frequencies.

The electric signals in the human body can be altered by inducing electromagnetic fields around the cells that produce them. This can be achieved non-invasively

by inducing an electric or a magnetic field near the nerve or skull. In the brain the tools and techniques used to do this are known collectively as non-invasive brain stimulation (NIBS). Transcranial electric stimulation (TES) and transcranial magnetic stimulation (TMS) are relatively high intensity techniques which produce large electric or magnetic pulses that directly stimulate cortical pyramidal neurones, causing them to fire (Rothwell (1997)). Transcranial direct current stimulation is a low intensity technique in which a small electric field is reported to alter the firing frequency of tonically discharging neurones by influencing the cellular environment and membrane potentials of the network (Ziemann et al. (2008)).

2.3.1 Electric Stimulation

For transcranial electrical stimulation, in the form of TES, a large voltage is discharged into the brain through the scalp inducing a current that spreads radially through the brain and produces a twitch in peripheral muscles. This is not a comfortable procedure for a conscious subject, and, as such, this type of stimulation is usually only used in unconscious patients during surgery (Rothwell (1997)).

Recordings of the descending volleys from the spinal epidural space indicate that TES directly stimulates the large pyramidal neurones resulting in a descending volley known as a D-wave. These D-waves are not changed by altering the excitability of the cortex and so the stimulation of the neurones is thought to occur several nodes away from the cell body in the white matter, a region which is relatively insensitive to changes in cortical excitability (DiLazzaro et al. (2004)).

Until quite recently lower currents were thought to produce little or no effect; however, a series of experiments conducted by Nitsche and Paulus (2000) suggested that low current tDCS is capable of plastically altering cortical excitability.

Since cortical excitability relates to the ease with which a group of cortical neurones can fire plastic changes to excitability may cause long term alterations to neuronal communication, potentially leading to improvements in motor control, or motor rehabilitation.

Nitsche and Paulus (2000) delivered low currents to healthy individuals via two electrodes positioned on the head, one over the motor cortex and the other over the contralateral orbital, Figure 2.4. They demonstrated that excitability was facilitated or depressed depending on the polarity of the electrode over the motor cortex. Anodal stimulation, where the electrode was positive, increased excitability while cathodal stimulation depressed it. The magnitude and duration of the changes were dependent on the stimulation intensity and duration. Prompted by this evidence, and by the ease and low cost of the technique, tDCS has re-emerged in NIBS research; however there remains a large degree of variability in reports of its effectiveness and therefore controversy into its effectiveness as a clinical tool for motor rehabilitation.

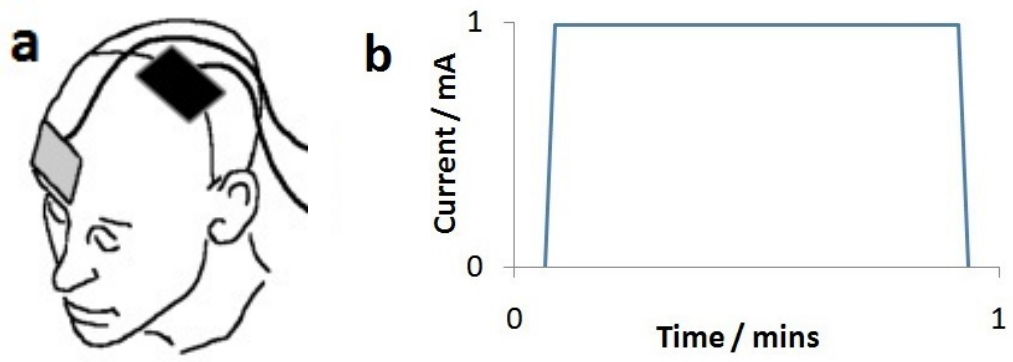


Figure 2.4: a) Electrode configuration for tDCS: one electrode is placed over the motor cortex and the other is placed over the contralateral orbital. b) Example waveform for tDCS: the current ramps up to 1 mA and stays constant for the duration of the intervention.

The effects caused by modifying the traditional DC signal are now also being investigated. These modifications include transcranial alternating current stimula-

tion (tACS), transcranial sinusoidal current stimulation and transcranial random noise stimulation (tRNS). tACS is simply a sinusoidally alternating current with no DC offset (Antal et al. (2008)), Figure 2.5a. tSCS is the alteration of the DC signal through the incorporation of oscillatory modulations; it can therefore be applied for both polarities, anodal and cathodal (Marshall et al. (2006)), Figure 2.5b. For tRNS the signal is made up of a range of frequency components: all the components have the same coefficient in the frequency spectrum and the random numbers are normally distributed; tRNS is therefore another signal that has no DC offset (Terney et al. (2008)). There is evidence to suggest that these modulated stimulation signals are capable of altering cortical function by interfering with rhythmicity in the brain (Marshall et al. (2006); Antal et al. (2008); Terney et al. (2008)).

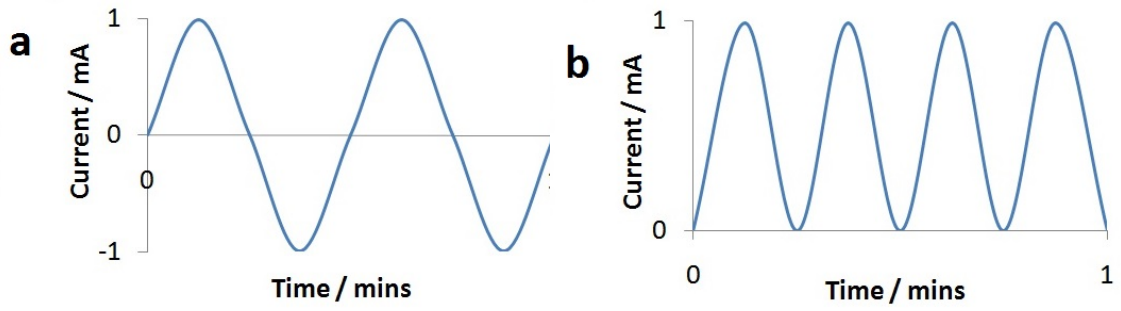


Figure 2.5: a) Example waveform for tACS: the current oscillates sinusoidally about zero. b) Example waveform for tSCS: the current increases and decreases sinusoidally, but does not change polarity.

2.3.2 Magnetic Stimulation

TMS is a well documented and widely used technique in NIBS and can be employed to investigate, inhibit or facilitate cortical excitability. The technique is based on Faraday's law of electromagnetic induction; a current in a coil induces a magnetic field, and the magnetic field in turn induces a secondary electric field in the brain, Figure 4.3. The induced electric field can cause changes in ionic

gradients in the cell or the cellular environment, leading to changes in cellular discharge characteristics.

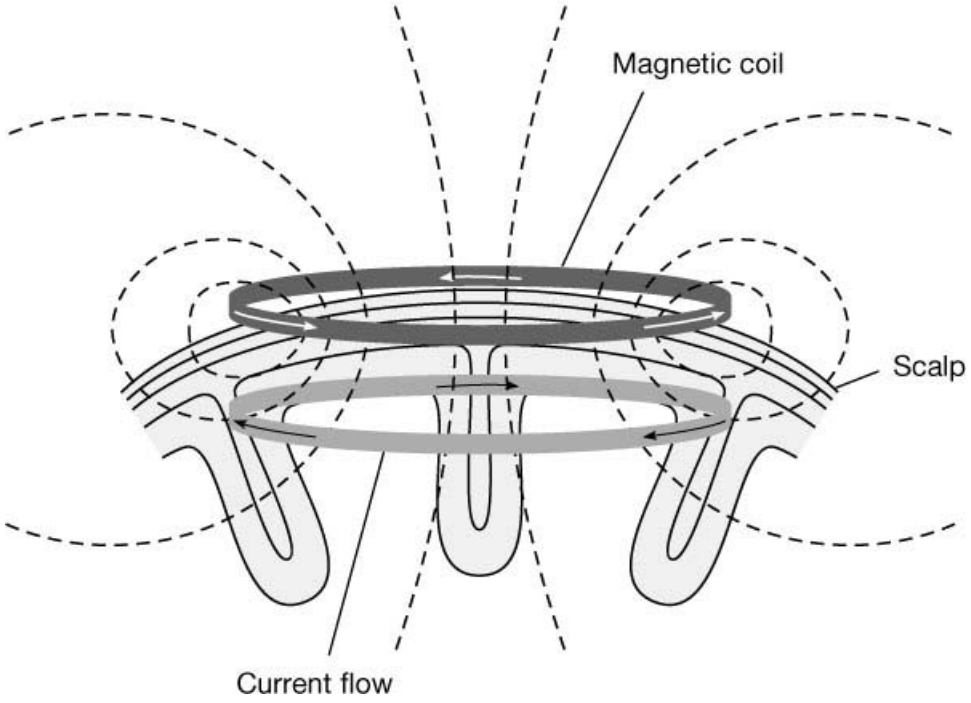


Figure 2.6: TMS: A circular magnetic coil produces a magnetic field that induces an electric current in the brain.

The response of the neurones to the magnetically induced electric field is dependent on the shape of the coil, the intensity of the stimulation, the direction of the induced current and the topography of the underlying cortex. Figure of eight coils are most commonly used as they provide more focal stimulation and was the coil type employed in this study. Figure 2.7 shows the current density induced in the cortex following stimulation with this kind of coil; unlike TES the induced current flows parallel to the skull instead of radially.

For stimulation of the hand area spinal epidural space recordings show that at threshold intensity a postero-anterior coil orientation causes a descending volley that occurs at a greater latency than would be expected if causing a direct stim-

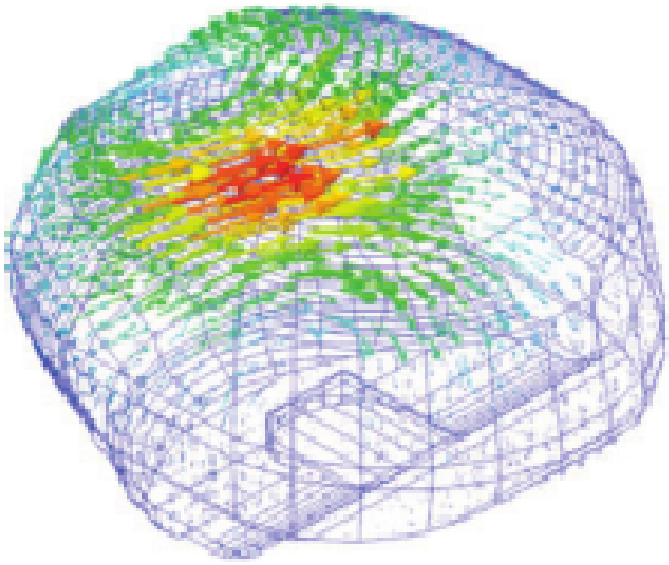


Figure 2.7: Distribution of magnetically induced current in the cortex with a figure of eight coil. (Wagner et al. (2007)).

ulation of the neurones (D-wave). This descending volley is known as an indirect wave, or I-wave, and its presence suggests that TMS, unlike TES, does not stimulate the pyramidal cells directly but trans-synaptically (DiLazzaro et al. (2004)).

Like TES the D-wave is largely insensitive to changes in cortical excitability; however the later I-waves do change proportionally in both amplitude and number as cortical excitability changes. The neural elements and mechanisms involved in the production of I waves are still not clear, however it has been shown that during changes in cortical excitability caused by voluntary contraction the threshold for activation is not altered. This indicates that the site of the pre-synaptic TMS activation is not located near the cortico-cortical neurones cell bodies. The excitability of the pyramidal neurones are reflected in the first I wave and changes to subsequent I waves are reflective of changes to the cortico-cortical elements that have themselves been induced trans-synaptically.

When applied repetitively there is evidence to show that TMS, or repetitive TMS (rTMS), can plastically alter cortical excitability (Ziemann et al. (2008)). A train of consecutive magnetic pulses are delivered to the patients at frequencies between (0.9 - 25) Hz. Low frequency rTMS ((0.9 - 1) Hz) is reported to plastically decrease cortical excitability, as measured through TMS induced motor evoked potentials (MEPs) ((Frequency $<$ 0.9) Hz), while high frequency rTMS (Frequency $>$ 1) Hz increases it (Ziemann et al. (2008)). Theta burst stimulation is a second generation form of rTMS and is modelled on a physiological signal (Huang et al. (2005)). Interestingly, the facilitatory and inhibitory effects are not produced through altering the frequency of the pulses but by changing how the pattern of bursts is repeated. Continuous bursts of stimulation depress excitability and intermittent bursts promote it and these effects are produced at lower intensities and after shorter application times compared to traditional rTMS. As yet the reason for these distinctions remains unclear; however, they do highlight the complexity of neuronal signalling, and suggest that improved results may be achieved when the intervention signal is based on a physiological meaningful one.

While rTMS has been shown to influence neuroplasticty there are downsides to the technique: the equipment used is very bulky and relatively expensive to purchase (compared to tDCS); it can be difficult to accurately deliver repeatable stimulation to specific targets without additional brain mapping technologies, and optimisation has been difficult due to the large number of parameters: duration, frequency, intensity, pulse width and pulse number to name a few. For a patient the stimulation, while not painful, can be an uncomfortable experience at high intensities.

2.4 Paired Associative Stimulation

Stimulation of the peripheral nerves has also been shown to induce plasticity in the associated region of the cortex (Riding et al. (2000)). Electrodes are positioned over one, or a group of peripheral nerves and an electric current is delivered. The current can be a single pulse of varying duration, or a train of repetitive pulses and the stimulation intensity can also be varied for different effects (Chipchase et al. (2011)). This afferent stimulation can cause depolarisation of the nerve axon consequently sending signals both to the muscles that are innervated by that nerve and to CNS structures at spinal and cortical levels (Riding et al. (2000)).

PNS has also been used in combination with direct cortical stimulation; mostly in conjunction with TMS, but sometimes with tDCS. The effects on cortical function are reportedly enhanced further when compared to each stimulation delivered alone. For TMS-PNS the timing between the peripheral and magnetic stimuli is extremely important and is configured such that they reach the cortex at the same time. This combination of carefully timed stimuli is known as paired associative stimulation. In TMS paradigms both potentiation and depression of the cortex can be achieved through altering the interval between the TMS and PNS pulses and the order that they arrive at the cortex.

The first paper to describe this in healthy individuals (Stefan et al. (2000)) reported increases in cortical excitability that persisted for more than 30 minutes; in later papers these after effects have also been shown to be NMDA dependent (Stefan et al. (2002)). The characteristics of PAS induced plasticity are similar to those of physiologic forms of synaptic plasticity: associative LTP and LTD. As such the PAS induced associative LTP/D is considered to be caused by the simultaneous input of the two separate signals on a post synaptic cell.

Direct current stimulation of the motor cortex has not been as well researched as TMS-PAS. In the tDCS-PAS paradigm the brain stimulation is constant while the PNS is pulsed. Rizzo et al. (2014) reported changes in cortical excitability following only tDCS stimulation that were similar to those reported by Nitsche and Paulus (2000). The effects only lasted for ten minutes; however when tDCS was combined with PNS the effects on cortical excitability reportedly persisted for at least thirty minutes. Celnik et al. (2009) also delivered a tDCS-PAS protocol to chronic stroke patients and reported greater improvements in motor function compared to PNS or tDCS only.

2.5 Investigating Neural Activity

Neurophysiologic investigation techniques, such as electromyography (EMG), TMS, electroencephalography (EEG), functional magnetic resonance imaging (fMRI) and positron emission topography scans (PET), have allowed researchers to map cortical regions, investigate neurophysiologic functions and diagnose a variety of neurological disorders. These techniques are also of use in investigating if and how NIBS plastically alters neural activity. EMG, TMS and frequency analysis were used extensively throughout this project. EEG, MRI and PET were not used, but will be discussed in relation to other studies.

2.5.1 Surface Electromyography

Surface electromyographic recording is an important technique for investigating motor control. It does not detect the electrical activity of the neurones as they synapse onto the muscle, instead it detects the activation of skeletal muscle as the electric pulse that sweeps across the muscle membrane fills the T tubules with ion rich extracellular fluid. Despite this it offers a simple method to infer neural

drive and is, therefore, an important tool in human studies. It is used extensively, often in combination with other testing techniques, to investigate many neurophysiologic events in both normal and abnormal motor function.

Surface EMG is non-invasive and does not, in itself, interact with the neural signals that it records. It does carry the disadvantage that it requires muscular contraction in order to produce a trace. Voluntary contraction is capable of altering neuroplasticity in its own right and this must be considered when utilising EMG in the study of plasticity.

2.5.2 Time and Frequency analysis

As mentioned previously the motor system produces task dependent rhythmic oscillations that can be observed in recordings of brain and muscle activity. The similarity and synchrony of pairs of these signals can be analysed and quantified using cross covariance techniques in both the time and frequency domain. In the time domain cross correlation analysis, known as the cumulant density, illustrates temporal association in the signals: these are depicted by a series of peaks centred around a central maxima, an example data set is shown in Figure 2.8. The result has no upper boundary but the relative synchronisation can be observed by comparing the sizes of the central peaks and the time interval separating the peaks which can be used to provide an estimate of the synchronised frequency (Halliday et al. (1995)).

Within the frequency domain the frequency power spectra of the two signals can be calculated using Fourier transforms. Example data are shown in Figure 2.9a. The cross correlations in frequency and phase of the signals is described by the coherence, Figure 2.9b; no correlation at a given frequency (that is, no linear phase relation at that frequency) is represented by a coherence value of zero and

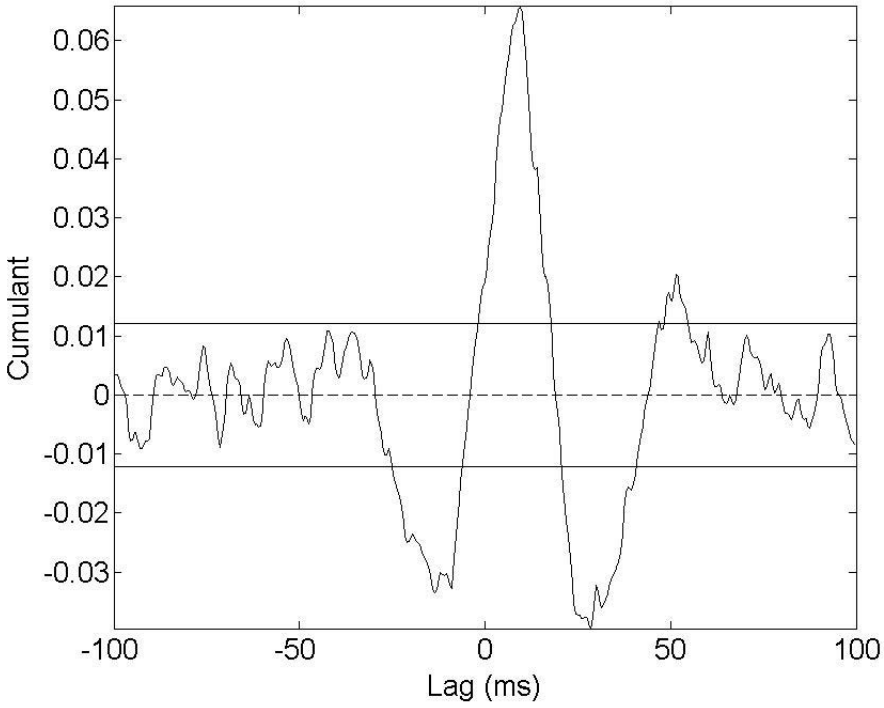


Figure 2.8: Cumulant density of two signals exhibiting synchronisation as shown by series of peaks centred around a lag of 0 ms.

full correlation, one.

Electroencephalography and magnetoencephalography (MEG) are non-invasive investigative techniques used to record the extracellular electrical and magnetic fields that are generated by networks of neurones in the brain. These signals may not in themselves be functionally significant, however, their frequency content does reflect the functionality and synchronization of the neurones and their synaptic inputs that produced them. For motor control the main frequency bands of interest are the α ((6 - 12) Hz) and β ((15 - 30) Hz) bands; these can be observed in both the EEG, MEG and EMG signals during muscle contraction.

In this thesis frequency domain analysis was used to estimate the coherence between EMG signals from co-contracting muscles, and also coherence between an

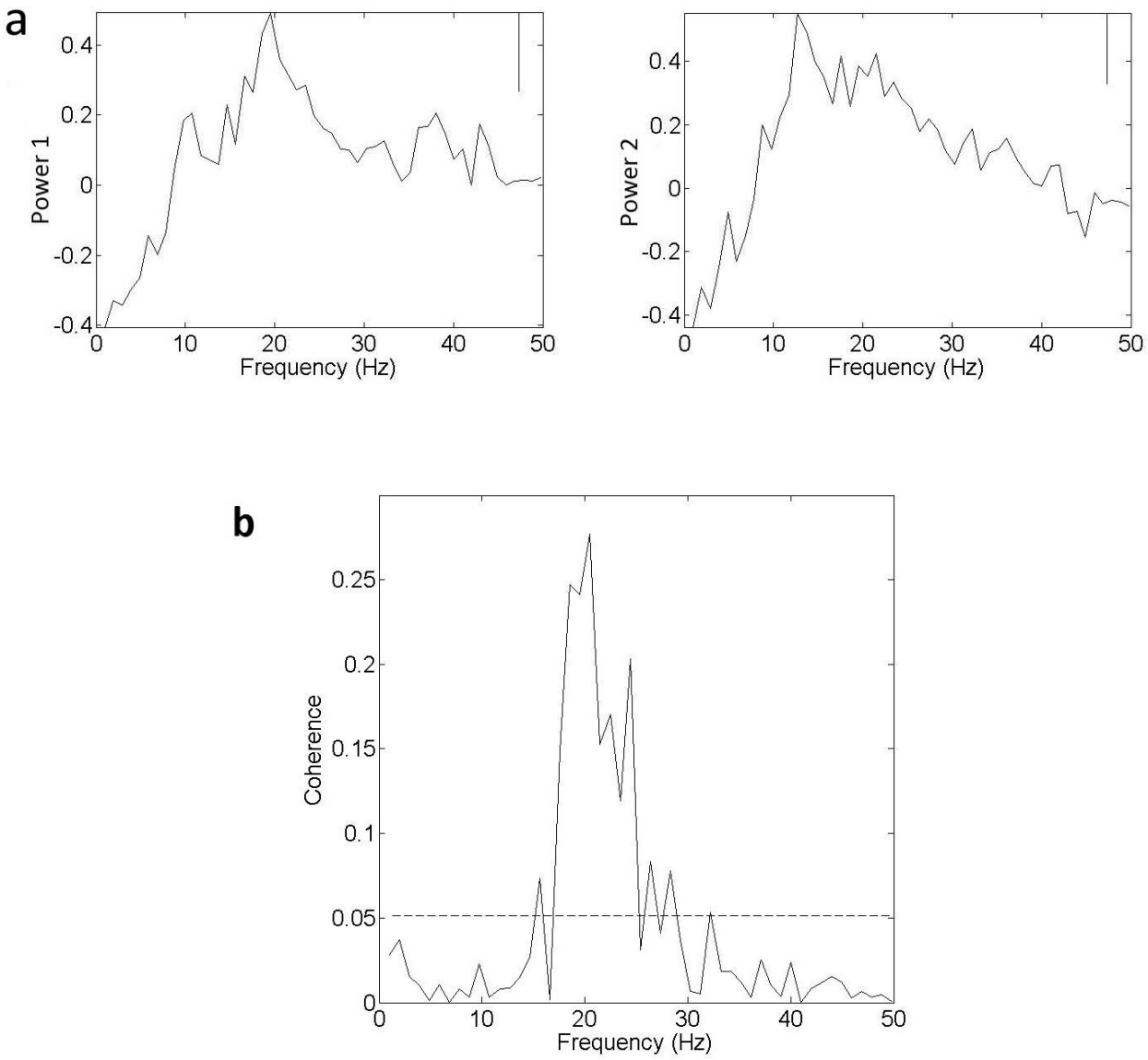


Figure 2.9: a) Example of frequency power spectra from two independent signals. b) Example cross correlation of the spectra in the frequency domain, or the coherence.

accelerometer trace and an EMG trace. These will be discussed in more detail later.

2.5.3 Transcranial Magnetic Stimulation

Single and paired pulse magnetic stimulation protocols are important for investigating changes in corticospinal pathways; they can be used to produce cortical representations, or cortical maps, and information on cortical excitability, motor thresholds, intracortical inhibition and facilitation, and interhemispheric inhibition. In its simplest form a single magnetic pulse, of sufficient intensity and directed over the motor cortex, will produce a motor evoked potential in the associated muscle. A lot of information about the state of the cortico-spinal tract (CST) can be obtained from this simple procedure. The onset latency of an MEP can be indicative of the health of the pathway, and it can be used in the diagnosis of brain or spinal cord injury and in evaluating the effectiveness of treatments. A representational map of the motor cortex can be built by stimulating different regions of the cortex and observing which muscles respond; neuroplastic events can then be assessed by observing changes in the topography of these maps.

More complex paired pulse protocols can reveal other aspects of cortical organisation as different combinations of inter-pulse intervals (IPI) and pulse intensities provide information on inhibition and facilitation within the cortical circuitry. Short intracortical inhibition (SICI), short intracortical facilitation (SICF), long interval intracortical inhibition (LICI) and intracortical facilitation (ICF) can all be investigated. Both SICI ($1 < \text{IPI} < 5$) ms and ICF ($6 < \text{IPI} < 15$) ms can be explored using a small conditioning stimulus followed by a suprathreshold test stimulus (Kujirai et al. (1993)). Short intracortical facilitation can be observed when both the conditioning and test stimuli are supra-threshold, the time between the two pulses must be quite accurate (1.3 ms, 2.5 ms and 4.3 ms) since the second stimulus is thought to interact with the residual effects of the first pulse (Hallett and Chokroverty (2005)). Long intracortical inhibition is also investigated through two supra-threshold stimuli ($50 < \text{IPI} < 200$) ms the large

conditioning stimulus inhibits the response to a test stimulus of the same size (Hallett and Chokroverty (2005)).

As noted above TMS equipment is bulky, uncomfortable to the participant/patient and difficult to accurately deliver without additional expensive neuro-navigation equipment. It has one other disadvantage as an investigatory tool: it is capable of plastically altering cortical excitability; this has made it useful as an intervention but must be kept in mind when employing it to investigate plasticity.

2.5.4 Magnetic Resonance Imaging

Nuclear magnetic resonance imaging (MRI) uses the intrinsic nuclear property of spin to, non invasively, distinguish between different tissue types in the human body. When the tissue is exposed to a magnetic field the protons, or hydrogen nuclei, present in the tissue align with the magnetic field; the field is then pulsed to force the nuclei to resonate about their equilibrium position. It takes time for the nuclei to return to their normal position and when they do they release the energy that they received from the magnetic field. Different tissue types can be distinguished by the amount of energy that is released and the time it takes to release that energy; these factors are dependent on the amount of hydrogen within the tissue and its configuration (Aine (1995)).

Function magnetic resonance imaging uses MRI to investigate task dependent brain activities occurring in different brain structures. It identifies changes in metabolic activity by quantifying the changes in oxygenated and deoxygenated blood levels (BOLD). Like EMG fMRI investigations in the motor areas require contraction and the same considerations of the effects of voluntary movement on cortical activity must be accounted for (Aine (1995)).

2.5.5 Positron Emission Tomography

Positron emission tomography is another imaging technique that can be used to identify metabolic brain activity. A radioactive isotope is attached to a metabolite and injected into the circulatory system. The isotope will emit radiation as the metabolite is processed in the body and the source of the radiation can be identified with external receptors. Regions of increased metabolism can indicate regions of increased activity (Aine (1995)).

Chapter 3

Literature Review

3.1 Transcranial Direct Current Stimulation

Cortical recordings in animal studies, conducted in the 1960s, suggested that the weak electric fields produced by tDCS alter local ionic concentrations thus modifying the affected neurones' membrane potentials (Bindman et al. (1964); Purpura and McMurtry (1965)). The weak stimulus did not cause the neurone to spontaneously fire, as is the case for TMS or TES, but rather reduced or enhanced the probability that an action potential would be produced: anodal tDCS making it more likely and cathodal, less.

More recent human studies suggest that tDCS can alter a range of physiological characteristics including cortical excitability, common cortical drive and regional cerebral blood flow (Nitsche and Paulus (2000); Power et al. (2006); Lang et al. (2005)). The effects have been reported to persist after the stimulation has ended and have therefore been linked to neuroplasticity. The reports suggest that the strength, duration and direction of the after effects are dependent on the polarity, duration and amplitude of the applied current(Nitsche and Paulus (2000)). While the mechanisms by which these changes occur are not yet clearly understood it

is often asserted that the prolonged change in neuronal firing rates induce plastic changes to synaptic efficacy and there are reports that the after effects are linked to NMDA dependent types of plasticity such as LTD and LTP (Liebetanz et al. (2002)). This presents the exciting possibility that tDCS may be beneficial for motor rehabilitation.

Unfortunately, however, conflicting reports and a substantial degree of variability in the strength and duration of the reported after effects remains amongst different studies. This makes it exceedingly difficult to evaluate the effects of tDCS and ultimately identify its effectiveness as a clinical intervention for motor rehabilitation.

3.2 Transcranial Direct Current Stimulation and Cortical Excitability

In the first paper to suggest a physiological effect of tDCS in man Nitsche and Paulus (2000) showed a significant, polarity dependent effect on cortical excitability. Following five minutes of 1 mA tDCS to the primary motor cortex anodal stimulation increased MEP amplitudes in the abductor digiti mimimi (ADM) by 40% and cathodal stimulation decreased them by 50%. Importantly, from a neuroplasticity and rehabilitation perspective, the changes persisted and remained significant for four minutes after anodal stimulation and three minutes after cathodal. The magnitude and duration of the changes to cortical excitability were dependent on the polarity, duration and intensity of the electrical stimulation as well as the electrode configuration. Cortical excitability is linked to synaptic efficacy which is an important component in neuroplasticity.

3.2.1 Variability in Motor Evoked Potentials

The polarity dependent effects of tDCS on cortical excitability observed by Nitsche and Paulus (2000) have been replicated by a number of other studies (Nitsche and Paulus (2001); Nitsche et al. (2003b); Lang et al. (2004a); Nitsche et al. (2005); Furubayashi et al. (2008); Stagg et al. (2009)); however, the magnitude and duration of the changes in MEP amplitude vary considerably across groups and studies. Direct comparison between different studies is often difficult since different intervention and investigation parameters are usually employed, however, in those papers that do conform in their methodologies there is still considerable variation in results. This is illustrated in Figure 3.1, which shows the results obtained from two studies which used the same stimulation parameters (Ntische et al. (2007) and Furubayashi et al. (2008)), clearly there are discrepancies in the magnitude and duration of significant changes and also in the variability of the MEP amplitudes within each time interval. Figure 3.2 further illustrates the variability in results of different groups: anodal tDCS has been reported to increase MEP amplitude by 16% - 50% (Stagg et al. (2009); Nitsche and Paulus (2001))and cathodal tDCS to decrease MEP amplitude by 20% - 40% (Stagg et al. (2009); Nitsche et al. (2003b).

There are a number of possible reasons for the variability in the reported results. Variance amongst MEP amplitudes is a well known and major drawback of single pulse TMS investigations that probably accounts for a large proportion of these discrepancies (Amassian et al. (1989); Kiers et al. (1993); Van; Ellaway et al. (1998); Mitchell et al. (2007)). Fluctuations in the responses of subjects in a group, and even amongst sessions with one participant, can be caused by changes in the coil position or the relaxation state of the individual. The angles of the coil in relation to the hot spot and the skull are particularly vital components in the reproducibility of the technique that can easily be altered by subject movement,

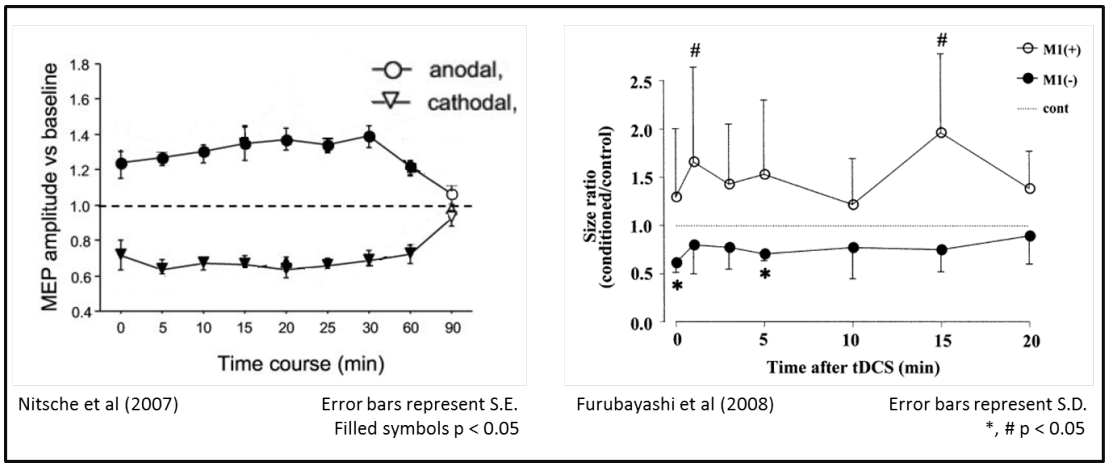
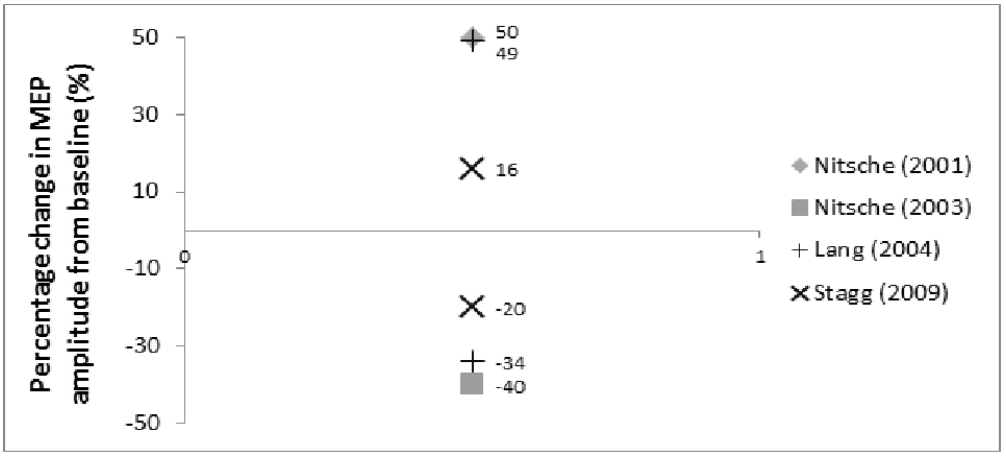


Figure 3.1: The results obtained from Ntische et al. (2007) and Furubayashi et al. (2008). Illustrates the variability in both MEP amplitudes and the duration of the after effects from different studies which used the same stimulation parameters.

or can be managed differently amongst different groups. It is important that these variables are highlighted as they represent a problem with the reproducibility and robustness of the testing regime. They are particularly important when the small subject populations in these trials (n = 5 to 19) are also taken into consideration. The inherent variability in the testing regime combined with small populations make it difficult to estimate an accurate mean change in tDCS induced cortical excitability in healthy populations and ultimately identify its effectiveness as a clinical intervention for brain or spinal cord injured patients.

There is also a possibility of a responder/non-responder effect based on brain-derived neurotrophic factor (BDNF) secretion. BDNF has been implicated in synaptic plasticity and its levels have been reported to be dependent on voluntary activity (Gomez-Pinilla et al. (2002)). Lamy and Boakye (2013) demonstrated that individuals carrying particular BDNF single nucleotide polymorphisms (BDNF Val66MET and Val66Val) secreted different amounts of the neurotrophin and responded differently to spinal DC stimulation. The variability in the MEP amplitude amongst different participants may then reflect differences

(a) Five minutes of tDCS



(b) Ten minutes of tDCS

Figure 3.2: Variability in MEP amplitudes amongst comparable studies for a) five minutes and b) ten minutes of anodal and cathodal tDCS.

in BDNF secretion caused either by genotype or by previous voluntary activity.

The delivery of TMS with the tDCS electrodes in-situ may also account for some of the variation amongst different study results. Most of the papers in the literature are not explicit about whether the stimulating electrodes are removed for TMS testing and for some studies it seems unlikely that there would be time to do so before testing began. The most commonly used electrodes are carbon rubber, encased in saline soaked sponges. Magnetically stimulating the brain through these may cause issues for two reasons: they are bulky and it can be difficult

to ensure that the magnetic coil is appropriately positioned on the scalp if the electrode is in the way, and they are conductive so will respond to the magnetic field produced by the coil, potentially warping it and thus affecting the resultant data. Whether either of these affects would be large enough to alter the data has not been tested, however the only paper in the literature that clearly states that the electrodes were removed prior to any magnetic stimulation did report much smaller tDCS after effects compared to other studies (Stagg et al. (2009)) (Figure 3.2).

Because postero-anterior TMS produces I waves and not D waves (at threshold) and I waves are sensitive to cortical excitability then postero-anterior TMS is generally considered to be a good test for cortical excitability. However Nitsche and Paulus (2000) did not provide any direct justification that it is a good tool for investigating the tDCS induced changes to cortical excitability. The distinction is important because TMS can be used as an interventional technique as well as an investigatory one and can alter cortical excitability in its own right. They did conduct tests in which very low tDCS durations and intensities were shown to have no significant effect on MEP amplitudes and they concluded that single pulse TMS was not inducing the changes in excitability that were observed at higher intensities and durations of tDCS. This is not a very robust test and the evidence from it is further weakened in the light of more recent evidence that investigatory TMS could be priming the cortex (Siebner (2010); Delvendahl et al. (2010)). Delvendahl et al. (2010) demonstrated that single pulse TMS delivered at 0.1 Hz altered the outcome of a paired associative stimulation protocol. TMS at 0.1 Hz is usually considered as non-functional, that is, it does not alter cortical excitability when applied alone; however, Delvendahl et al. (2010) indicated that TMS at 0.1 Hz primed the cortex by altering the result of the subsequent intervention from what it would have been without TMS. Priming will be discussed in

more detail below but to date these effects have not been accounted for in any of the studies investigating tDCS through TMS induced MEPs. There is therefore no way to know if the observed alterations in cortical excitability are caused by tDCS, or a combination of the two stimulation procedures. Many of the studies discussed above used different numbers of pulses and inter-pulse intervals in their TMS paradigms. They may have, in fact, been priming the cortex to different extents, thus producing different results.

Of course another reason for the variability in results may be grounded in the inability of tDCS to induce meaningful changes in cortical excitability and the observed variability may simply be representative of a low effect size.

The analysis undertaken by Nitsche and Paulus (2000) and many of the other groups is also weak in some areas. Nitsche and Paulus (2000) clearly state that following their ANOVA they did not correct for multiple t-tests in their post-hoc analysis. This would result in them reporting statistically significant results where there were none. Another problem with this, and many others', analysis is the manual removal of MEPs in which voluntary muscle activity was supposed to have occurred. While this sounds reasonable, since voluntary contraction is known to have an effect on MEP amplitude, there is no definition of what activity actually is. This makes it impossible to evaluate or repeat their analysis in its entirety. Hallett and Chokroverty (2005) have previously presented this argument and have suggested using a defined, acceptable muscle activation in TMS studies.

In order to better understand the effects of tDCS on cortical activity other tools must be developed and employed. These would enable researchers to understand more of the uncertainty inherent in the variability of MEP amplitude and to exclude or quantify combinatory effects of the two stimulation techniques.

3.3 Mechanistic Insights

Pharmacological studies have found that delivery of flunarizine and carbamazepine, Ca^{2+} and Na^{+} channel antagonists, respectively diminished and abolished the persistent changes to cortical excitability that were induced by anodal tDCS (Nitsche et al. (2003a)). These results suggest that the after effects of anodal tDCS are initially induced by shifts in membrane polarization. For cathodal tDCS these same ion channel blockers had no effect (Nitsche et al. (2003a)). While this initially seems to suggest that membrane potential shifts do not induce the after effects of cathodal tDCS a membrane shift towards hyper-polarization would cause the same result. The plastic after effects on cortical excitability were also suppressed with the administration of NMDA antagonist, dextrometorphan (Liebetanz et al. (2002); Nitsche et al. (2003a)), a neurotransmitter that has been associated with LTP/LTD like plasticity. Together these data suggest, in accordance with the initial theories, that tDCS initially induces polarity dependent shifts in membrane polarization which in turn induce plastic shifts in synaptic efficacy.

3.3.1 Electrode Configuration

Nitsche and Paulus (2000) assert that their results are in accordance with basic neurophysiology concepts; however, a small set of tests, conducted to identify the optimal electrode configuration, cast further implications on the mechanisms of tDCS. In their study MEP amplitudes were investigated under six different electrode configurations: motor cortex-contralateral forehead, occipital-contralateral forehead, area posterior to motor cortex-contralateral forehead, motor cortex-occipital, motor cortex-contralateral motor cortex and occipital-area anterior to motor cortex. Of these configurations the only one to result in significant changes in MEP amplitude was motor cortex-contralateral forehead. This is now the most common configuration for stimulation of the primary motor cortex; however, other

configurations in which an electrode was placed over M1 (motor cortex-occiptal and motor cortex-contralateral motor cortex) did not cause changes in MEP amplitude. If, in accordance with basic neurophysiological concepts, it's a simple case of de/hyperpolarizing the region under the electrode then these results are surprising and the implication is that the specific path of the current is what drives the motor cortical excitability changes.

There are some concerns with the experimental method for this past study. The results were obtained after only 4 s of stimulation, however, in further tests on the duration of stimulation required to produce significant after effects Nitsche and Paulus (2000) demonstrated that stimulation for one minute did not cause significant changes in cortical excitability. In both of these tests (electrode configuration and duration of stimulation) cortical excitability was evaluated using single pulse TMS, however, for the electrode configuration tests TMS pulses began 0.05 s before the end of the stimulation, and for the duration of stimulation tests pulses began immediately after stimulation ended. If there are no significant after effects after one minute then it is unlikely that there would be significant after effects after 4 s of stimulation, and is possible that the results for electrode configuration hinge on the single TMS pulse that occurred before the DC stimulation ended. There are two problems with this protocol: the first is the issue of magnetic stimulation delivered through a conductive electrode, particularly one that is discharging a current, discussed above; the second is that to reduce variability the majority of the literature agrees that results on MEP amplitudes should be obtained from the averages of blocks of pulses. Basing results on a single TMS pulse is not considered to be a robust testing method, and yet it would appear that the traditional electrode montage used throughout the literature is based on just that.

After asserting the importance of their electrode configuration Nitsche and Paulus (2000) did not explore whether it is the orientation of the electric field that is important in altering cortical excitability or the co-activation of M1 and the frontal cortex, this question has yet to be fully explored in the literature. Indeed there is a lot of doubt that the low current even penetrates the skull to the underlying cortex and a recent (as yet unpublished) cadaver study (Underwood (2016)) demonstrated that 90% of the delivered current was shunted across the scalp. While the electrical properties of dead tissue are different from living there is little doubt that much of the 1 mA of current does not get to the brain tissue. Proponents for tDCS maintain that enough current does get through though, as evidenced by their reports on the impacts of the stimulation on cortical activity.

Neuronal orientation is important in TMS and it is possible that there is a parallel for tDCS. For TMS the order of the I-wave recruitment is different for postero-anterior and latero-medial orientations (DiLazzaro et al. (2004)); this is supposed to be caused by the relative axonal alignment of the pyramidal neurones, generally perpendicular to the skull, and the cortico-cortical neurones, parallel to the skull. For investigation of hand area cortical excitability the optimum coil orientation for a figure of eight coil is facing forward and angled 50° to the sagittal plane. This orientation corresponds to a current flowing perpendicular to the central sulcus and is thought to be in alignment with the coritico-cortical elements involved in I wave production. This induced current flow is also very similar to that caused by tDCS in a cortex-contralateral forehead montage, and if the Nitsche and Paulus (2000) data are correct it suggests that for the best results the orientation of tDCS induced current flow should be in alignment with the cortico-cortical interneurones.

Due to the difference in cortical orientation of the hand and leg area stimula-

tion of the leg region may provide insight into this issue. Jeffery et al. (2007) found that 2 mA anodal tDCS enhanced excitability, but cathodal had no effect. They suggest that the result for cathodal stimulation was caused either by the difference in orientation between the hand and leg areas or a difference in the inhibitory and excitatory circuitry. Cathodal stimulation may not be the main concern here though; if path is important then the results for anodal stimulation are contradictory since the current path is unlikely to be flowing in the same direction with respect to the cortico-cortico neurones as it did in the hand area and therefore the achievement of the same result is surprising. As yet these issues have not been resolved and the majority of the literature employs the motor cortex-cotralateral orbit as the main electrode configuration. An understanding of the implication of electrode position is vital in extending the technique into other regions of the body.

3.3.2 Other Transcranial Magnetic Stimulation Investigations

Other TMS investigations have provided additional insight into tDCS induced effects on cortical activity. While they did not tackle the question of combinatory effects or current path they have provided information suggestive of the mechanisms involved in tDCS. A detailed study by Nitsche et al. (2005) investigated motor thresholds (MTs), input/output curves, and intracortical inhibition and facilitation. Each of these parameters was investigated after 4 s, 7 minutes and 13 minutes stimulation and were supposed to be representative of during, short and long term effects.

The amount of stimulation required to produce an MEP is known as the motor threshold. Motor thresholds are increased with Na^+ and Ca^{2+} channel blockers (carbamazepine and lamotrigine), but are insensitive to drugs that are involved

in synaptic transmission (the GABA analogue, vigabatrin, and agonist, baclofen, and the NMDA agonist, dextromethorphan) (Ziemann et al. (1996, 1998)). MTs are also insensitive to cortical excitability increases induced by voluntary contractions (DiLazzaro et al. (2004)). It is suggested, therefore, that MTs are indicitive of axonal membrane excitability and not synaptic excitability. Nitsche et al. (2005) found that MTs were not altered after any stimulation duration. This result is fine for the longer duration stimuli, which are linked to changes in synaptic potentiation and not membrane polarisation; however, these changes are supposed to only be induced after the initial membrane alterations. The 4 s stimulation was supposed to represent 'during' tDCS affects and so one would have expected to see changes to membrane dependent outcomes. This result may have been caused by problems with the methodology of the test, discussed below.

The recruitment of neural networks can also be investigated through single pulse TMS by increasing the stimulator intensity and observing how the MEP amplitude increases. A larger TMS intensity will induce an electric current in a larger area of the brain, induce activity in less excitable neurones and induce D-waves as well as I waves thus producing a larger MEP (DiLazzaro et al. (2004)). These MEPs are therefore reflective of both axonal and synaptic excitability, and the slope of the curve is reflective of neuronal recruitment. Nitsche et al. (2005) found that the slope did respond in a polarity dependent manner compared to sham: increasing and decreasing following anodal and cathodal stimulation respectively and for all durations of tDCS (4 s, 7 minutes and 13 minutes). They reported significant changes in MEP amplitudes compared to sham tDCS, but unfortunately undermined the power of this tool in doing so. Numerous cortical excitability studies have already demonstrated that populations of neurones are significantly more or less excitable following tDCS and this data only reiterates this finding. What would have been of more interest is an investigation into any

significant changes in the slope of the recruitment curve by instead looking at the differences between each intervention's data points. A study conducted by Hummel et al. (2005) did test the change in the slope of the recruitment curve, but only following 20 minutes of anodal tDCS and in a stroke population of only five participants. They did report a significant increase in the slope of the curve suggesting a significant increase in neuronal recruitment.

Nitsche et al. (2005) also reported that after 4 s cathodal tDCS there was a significant difference in MEPs at 130% of the resting motor threshold. The authors suggest that their data demonstrate that plastic effects of tDCS are induced by alterations to membrane polarization. This result is surprising as such low durations of tDCS have never been shown to interact significantly with cortical excitability. Suggestions for this result are discussed below.

Paired pulse TMS protocols can provide further insight into the characteristics of tDCS: a small, sub-threshold conditioning stimulus is too small to produce a response in the muscle, however it does suppress or facilitate the normal response to a later, supra-threshold test stimulus. DiLazzaro et al. (2004) demonstrated NMDA and GABAergic dependency and as such the SICI and ICF are suggested to be representative of synaptic excitation in the interneurones. Nitsche et al. (2005) again found a response after 4 s cathodal stimulation: a significant reduction to ICF. For the longer duration tests a polarity dependent effect was reported: that is, anodal tDS reduced SICI while increasing ICF and cathodal did the opposite.

The after effects caused by the longer stimulation durations are in line with theorised mechanisms of tDCS. The results for only 4 s cathodal stimulation are more surprising if it does represent 'during' tDCS effects. Once the stimulation is

removed the induced changes to membrane polarization would end and it is probable that they were only testing weak after effects in which case motor thresholds would not be expected to be different from baseline. For both neuronal recruitment and ICF the significant results suggest the possibility of a relatively fast acting but weak change to synaptic potentiation. It may also represent variability in MEP data and again highlights the need for a clearer understanding of MEP variability and a defined exclusion criteria for data. There are some problems with the design of the 'during' tDCS test however. Like the previous electrode configuration tests it consisted of a 4 s long stimulation where the 0.1 Hz TMS testing procedure began 'immediately before' the end of the stimulation. There is no information on how many pulses were delivered before tDCS ended, but as discussed above any pulses administered before the end may have affected the tDCS intervention by stimulating through conductive electrodes and over an existing electric field.

3.4 Inter-Muscular Coherence and Physiological Neurogenic Tremor as Investigatory Tools

As noted in the Introduction, oscillatory physiological signals are often observed in the both the central and peripheral nervous systems. Generators for these oscillations are not clearly defined. For the β band the common view was that it originated in the motor cortex, and was propagated down the cortico-spinal tract to the periphery where it drove the muscle activity (Grosse et al. (2002)). Mounting evidence, however, suggests that oscillations also travel the ascending pathway to the somatosensory cortex where they can modulate descending motor cortical rhythmicity (Baker and Baker (2003); Kilner et al. (2004); Riddle and Baker (2005); Witham et al. (2011)). The structure that generates the oscillations, if indeed there is only one, is therefore difficult to identify in this looped

circuit.

In the same way that the function of anatomical regions of the brain can be inferred from changes after injury so too can the role of oscillations, and abnormal synchronous oscillations are implicated in a number of movement disorders. Patients with cortical myoclonus have amplified coherence between ipsilateral muscles (Brown et al. (1999)). Parkinson's disease is characterised by increases in β synchronisation between the cortex and the basal ganglia resulting in the slowing down of voluntary movement (Grosse et al. (2002)). In primary lateral sclerosis, characterised by the degradation of the layer V Betz cells, a significant reduction in β inter-muscular coherence is observed (Fisher et al. (2008)). In various kinds of tremor exaggerated synchronisation between the cortex and the muscles, and between the muscles themselves, is also observed (Elble and Koller (1990)).

The precise functional role for these oscillations in motor control is still unclear though. For β oscillations, for example, there have been a number of suggestions and there are now two main schools of thought. The first is that they drive motor tasks, perhaps at the most efficient frequency for muscle contraction, (Baker et al. (1999)) or that they act as a carrying frequency upon which modulations in the signal can be more easily detected (Baker (2007)). The second hypothesis is that they cause recalibration of the motor system after movement; here a modulated rebound signal from a known test signal provides information on the state of the periphery (Jenkinson and Brown (2011)). There is evidence to support all of these roles and it may be possible that some, or all are implicated during motor tasks; a final opinion must be deferred until more evidence is presented.

3.4.1 Inter-Muscular Coherence

Task dependent coherence between synergystic cortical and EMG recordings is observed within distinct frequency ranges. Coherence is also observed between co-contracting muscle pairs and is known as inter-muscular coherence. Farmer et al. (1993) first observed peaks in the cumumlant density between pairs of motor unit spike trains recorded from co-contracting muscles in normal subjects. Further analysis in the frequency domain showed that the signals were coherent in both the α and β frequency ranges. They argued that excitatory post synaptic potentials do not contribute discrete frequency components to motor unit coherence and as such it is probable that the observed synchronicity originates as a common branched presynaptic input. In stroke and a de-afferented patient the synchronicity of the β signals was reduced while α was unaffected. Based on this it has been suggested that during constant contractions the β components of IMC originate in the brain and the α components originate in the peripheries. As mentioned above discussions on the origins of oscillations should proceed cautiously due to the looped circuits of the nervous system. For the α band in particular there is controversy about the role of the cortex. Conway et al. (1995) did not find α synchronisation between MEG and EMG traces during a constant contraction of first dorsal interosseous (1DI), but a later study by Marsden et al. (2001) did, as did an electrocorticogram (ECoG) study (Raethjen et al. (2002)).

Kilner et al. (1999) studied β band IMC and CMC in healthy subjects during a motor task and showed that the two investigatory tools exhibit the same task dependent modulations, further evidence that at least some of the synchrony between co-contracting muscles observed in IMC originates in the cortex. Brown et al. (1999) also investigated CMC and IMC in cortical myoclonus and found the same relationship between task dependency and frequency content. It is reasonable to conclude that some of the IMC frequency content reflects the common

pre-synaptic drive to the two co-contracting muscles. Under the correct conditions, therefore, IMC has the advantage over CMC, which involves the additional use of awkward EEG or MEG equipment.

3.4.2 Physiological Neurogenic Tremor

Tremor is an involuntary and relatively rhythmic movement in the limbs. Some tremors are caused by neurological conditions and diseases, but a degree of tremor occurs in everyone and is known as physiological tremor. Accelerometer recordings during steady postural contractions highlight tremor rhythms and when recorded with EMG activity reveal coupling at ranges below 30 Hz. It has been shown that there are two components involved in this coupling: the mechanical-reflex component, and a central neurogenic drive. The mechanical-reflex tremor measured at the fingers accounts for much of the oscillations in the (15 - 30) Hz frequency range (Stiles and Randall (1967)). It comes about through a complex interaction between the mechanical properties of the limb, ie limb stiffness, mass and loading, and the stretch reflex (Elble and Koller (1990)). Neurogenic features have been described within α, β and γ frequency ranges. The β and γ components tend to be smaller than the main α features. This α neurogenic component is manifested as a tremor in all the limbs in the (8 - 12) Hz frequency range and is insensitive to limb mechanics (Elble and Koller (1990); Raethjen et al. (2002)), and while neurogenic features have been described within the β and γ frequency ranges they are smaller than the main α features.

Clearly the frequency range of interest for (8 - 12) Hz PNT is encompassed by the α band and as noted above there is some evidence of coherence between cortical and EMG recordings in this frequency range (Marsden et al. (2001); Raethjen et al. (2002)). Both of these studies conclude that this result reflects a level of cortical drive to PNT. While Marsden et al. (2001) did not attempt to demon-

strate a link between (8 - 12) Hz CMC and (8 - 12) Hz tremor in the limb; Raethjen et al. (2002) reported that when CMC was present in this range so to was coherence between the ECoG-Accelerometer traces. Unfortunately, Raethjen et al. (2002) only studied these phenomena in a very small epileptic population in which each participant was taking the perscribed anti-convulsant, carbamazepine, and not all patients exhibited this phenomenon. A study by Riddle et al. (2004) delivered carbamazepine to a normal population and reported no change in 10 Hz oscillations. They did, however, report enhanced β IMC, a finding in direct contradiction with Raethjen et al. (2002) who observed very little and poorly reproducible β CMC. It is possible that carbamazepine did not affect 10 Hz oscillations but, given that it affected β oscillations differently in a patient group, this should be tested more thoroughly in a normal population. McAuley and Marsden (2000) reviewed a number of early and modern studies investigating the role of central control in PNT. They concluded that the evidence suggests that PNT is controlled by a range of inputs from the brain to the peripheries rather than being directly driven by the cortex.

Some of the literature therefore suggest an association between PNT and the motor cortex; however the links between (8 - 12) Hz CMC, IMC and PNT are still tenous and warrant further investigation. There is the possibility that PNT may be a useful investigative tool of plastic changes in cortical activity which it would be worthwhile to explore. Additionally the effects of tDCS on PNT have yet to be tested and the effects of cortical stimulation may suggest whether or not PNT is modulated by the cortex.

3.4.3 Inter-Muscular Coherence and Transcranial Direct Current Stimulation

IMC can be quantified using frequency domain analysis, and components of the IMC frequency content reflect the presynaptic, common drive to the muscles. While it is still unclear how common cortical drive affects motor control there have been reports indicating that it can be plastically altered (Power et al. (2006); Norton and Gorassini (2006); Pogosyan et al. (2009)). It is of interest to see if this common cortical drive can be altered with tDCS, and whether it is observed as changes in IMC.

Tests of inter-muscular coherence were used in combination with TMS tests in a trial conducted by Power et al. (2006) which was the first study to use inter-muscular coherence analysis to investigate the effects of anodal and cathodal tDCS. The stimulation was delivered for 10 minutes at 1 mA. Tests of MEP amplitude and IMC were conducted every 5 minutes for 10 minutes; the tests involved blocks of 15 single magnetic pulses targeted at the hot spot for the first dorsal interosseous muscle followed immediately by a simple 30 s constant co-contraction of 1DI with the extensor digitorum (ED) muscle.

The authors reported that their TMS results were 'in keeping with previous studies' and that there were increases in MEP amplitudes after anodal tDCS and reductions after cathodal tDCS; however, the only significant data point that they report was immediately after cathodal stimulation ended: MEP amplitudes were reduced by 30% in 1DI and 26% in ED ($p < 0.05$). Like Nitsche and Paulus (2000) they did not use post hoc adjustments for their t-tests. A Bonferonni adjustment would change their significance level to $p < 0.017$ potentially making this data point non significant. They reported no significant changes after anodal tDCS.

Despite the claims that the results for cortical excitability are in line with those previously reported the data were not significant. The authors offered two suggestions for these discrepancies: the investigation of different muscle groups, and the differences in subjects' relaxation state. It is impossible to quantify and compare the relaxation states of subjects across past studies. Power et al. (2006) delivered tDCS to the area of the motor cortex that controlled 1DI instead of ADM which previous studies had targeted, it may be possible that changes in the topography of the cortex alter the applied electric field and cause different results in different muscle groups, this would have to be tested.

Additionally the incorporation of the contraction task (discussed below) means that direct comparison with other TMS studies may be inappropriate and the difference from the literature may be caused by priming of the cortex as most are performed with the limbs in a relaxed state. Of course, the discrepancies may also have been caused by the variation of MEP amplitudes which has been discussed in detail above, or the inability of tDCS to induce consistent effects.

Inter-muscular coherence analysis was performed over two frequency ranges, α (defined as (5 - 15) Hz) and β (defined as (15 - 35) Hz). There were no significant changes to coherence found in the α band. Coherence in the β band was significantly altered from baseline in a polarity dependent way with an 18% increase following anodal tDCS and a 17% decrease following cathodal tDCS. The plots associated with these data sets however indicate a 75% increase after anodal tDCS and a 75% decrease after cathodal tDCS. Discrepancies like these in the data make it difficult to evaluate their reports. For anodal stimulation the significant result persisted for five minutes and for cathodal, reportedly persisted to the end of the ten minute testing time. Sham tDCS did not induce any signif-

icant changes in IMC. These results support the TMS studies discussed above by suggesting that tDCS is capable of penetrating the skull and influencing cortical activity; in conjunction with this they suggest that inter-muscular coherence, in the β band at least, can reflect tDCS induced changes to activity.

tDCS is suggested to alter cortical excitability by enhancing or suppressing transynaptic activation of the large pyramidal cells and it has been postulated that increased cortical excitability will lead to increased motor performance. These IMC results may imply that altered synaptic activation promotes/depresses the propagation down the CST of an already present oscillation in a polarity dependent manner. That is, here, the β oscillations were induced in the cortex and reinforced by sensory feedback during the constant contraction task, and since the cortex is in a state of altered excitability its presence in the IMC is enhanced or diminished. There are some matters for query with this proposal though: The TMS data did not show any significant change to cortical excitability and yet there are significant changes to IMC making the mechanistic link between the two tenuous. One could postulate that IMC is a more sensitive investigatory tool for the tDCS induced changes to cortical activity compared to TMS MEPs which, as noted above, are compromised by the inherent variability in the data. This would have to be tested more rigorously.

Mathematical studies have also suggested that increased excitation of an oscillatory neural network is damped by inhibitory interneurones, and it is these inhibitory interneurones that set the oscillatory frequency (Pauluis et al. (1999)). In line with this model Baker and Baker (2003) pharmacologically promoted cortical inhibition by delivering diazepam, which increases GABAergic activity, and reported enhanced cortical β oscillations. It is possible that anodal tDCS enhances activity in the inhibitory interneurones causing the increase in β IMC

reported by Power et al. (2006). Unfortunately a number of GABAergic agonist studies complicate this suggestion. Baker and Baker (2003) reported that the enhanced β oscillations were not reflected as enhanced β CMC and Raethjen et al. (2002) report that the GABAergic agonist carbamazepine reduced β CMC in an epiplectic population; however Riddle et al. (2004) delivered carbamazepine to healthy individuals and reported increased β CMC. The results of these studies suggest that enhancing oscillations in the cortex may not always mean they will be propagated in the CST although they may be representative of other effects caused by the drugs.

Additionally, if, as these pharmacological papers suggest, increasing cortical activity increases inhibition then an increase in SICI should be seen, but Nitsche et al. (2005) report that anodal tDCS decreased SICI.

It is also possible that the analysis used by Power et al. (2006) resulted in inaccurate significant values. Their analysis technique averages over a large range of frequencies and over all the subjects, Figure 4.10. Averaging over many frequencies will cause the spread of the data to influence the result and will obscure identification of more subtle changes in IMC. Their statistical analysis looks to observe if there are significant changes in significant coherence, but causes piling of statistical tests that may invalidate the results and requires further investigation.

The enhancement of β IMC following anodal tDCS, while neurophysiologically interesting, may not be beneficial from a motor rehabilitation perspective. Enhanced oscillation in this band have been linked with the slowing of voluntary movement (Pogosyan et al. (2009)) which is unlikely to lead to better motor outcomes. If enhanced cortical excitability is desirable for motor rehabilitation then

this study may suggest that care should be taken in the promotion of certain oscillations. It would be of interest to investigate whether it is possible to promote other, perhaps more beneficial oscillations by employing a different movement task.

A major inadequacy of this study was that the TMS test and the contraction task were combined in the experimental protocol. Repetitive contractions are a form of training and may be causing their own affect on the brain. The combination of magnetic stimulation, contraction task and electric stimulation may have caused complex priming of the cortex which is impossible to untangle since the effects of tDCS on inter-muscular coherence were not tested independently. In order to establish if, and by how much, each test is affecting the results the tests must be separated. This has yet to be explored in the literature.

3.5 Transcranial Direct Current Stimulation and Brain Imaging

Given the observations of tDCS induced, polarity dependent changes to cortical excitability it was proposed that these changes may be represented as reduced or enhanced blood oxygenation levels and regional cerebral blood flow (rCBF) in the region below the electrode, M1. Early studies (Baudewig et al. (2001) and Lang et al. (2005)) found that neither polarity of tDCS produced significant changes in blood oxygenation levels or rCBF from baseline when compared to changes induced by a finger movement task. Baudewig et al. (2001) suggested that the lack of alteration may represent a weakness in the investigation methods, that of a ceiling effect caused by the necessary inclusion of a movement task. An inadequacy with this study was the lack of a sham procedure to compare the effects to the task alone. Lang et al. (2005) did include a sham study in their

trials and showed that both anodal and cathodal stimulation caused increases in rCBF when compared to sham. Another, similar, task dependent BOLD study by Jang et al. (2009) saw a significant decrease in M1 activity compared to baseline following sham stimulation. The authors suggest that the task related decreases were caused by subject relaxation and habituation.

The time dependent relationship of BOLD activity during a motor task was explored further by Stagg et al. (2009); they also observed a task related decrease in blood oxygenation levels when no tDCS had been delivered and quantified it as a linear decrease in activation over time. The results from the tDCS intervention were therefore compared to sham rather than to baseline. They found, like Lang et al. (2005) that both anodal and cathodal tDCS resulted in increases to BOLD activity in M1; this appears to contradict the observation of Baudewig et al. (2001) that anodal tDCS caused no change in M1 BOLD activity compared to baseline. Further analysis, however found that not only did anodal tDCS increase BOLD activity compared to sham, but that it actually abolished the linearly decreasing 'habituation' effect observed without stimulation. A proposal for the observation of Baudewig et al. (2001), that no change occurred in M1 after anodal tDCS, can now be presented: by removing the habitation trend, but by only stimulating for five minutes it is possible there would be no observable increases or decreases in M1 BOLD. Jang et al. (2009) delivered anodal tDCS for 20 minutes and observed an increase in BOLD activation suggesting that increased durations of stimulation may result in the looked for increased activity in M1.

Interestingly these imaging studies also observed tDCS induced changes to regions in the cortex other than M1. Lang et al. (2005) and Stagg et al. (2009) reported that compared to sham anodal tDCS caused increases in both the BOLD MRI

representation and the rCBF of the ipsilateral supplementary motor area and dorsal pre-motor cortex. Following cathodal stimulation both Stagg et al. (2009) and Lang et al. (2005) reported increases in blood oxygenation and rCBF in the both the ipsilateral and contrateral M1, and posterior parietal cortex. These PET and BOLD MRI studies highlight the importance that other regions of the brain play in the overall effects of tDCS.

3.6 Transcranial Direct Current Stimulation and Rehabilitation

The primary drive for investigating tDCS is to explore its potential use for motor rehabilitation of patients who suffer a neurological disorder or brain or spinal cord damage. The studies discussed above, while neurophysiologicaly interesting, do not provide evidence that motor function or rehabilitation will be improved with tDCS.

3.6.1 Motor Learning

Brain imaging and TMS studies have demonstrated an increase in motor cortical activity and cortical excitability during an implicit motor learning task (Honda et al. (1998); Pascual-Leone et al. (1994)). As discussed above anodal tDCS, reportedly, increases cortical excitability and so Nitsche et al. (2003c) postulated that anodal tDCS would enhance implicit motor learning. Both polarities of tDCS were delivered to the motor cortex during a serial reaction time test. This test comprises of blocks of random and non-random sequential finger pushing tasks; a significant increase in error, or an increase in reaction times, between the sequential and random blocks is considered to be representative of implicit motor learning. Nitsche et al. (2003c) observed a significantly larger reaction time following anodal tDCS compared to sham, cathodal did not cause any changes.

The serial reaction time test may be indicative of task learning, but is, arguably, not motor learning. We certainly see improved learning of a sequence but not an improvement in how to move, that is, the participant doesn't get better at pressing the button, but instead gets better at knowing which button to press.

A similar study design was implemented by Hunter et al. (2009); however, instead of observing changes in reactions times in a sequential task they observed the changes in movement error between movements executed with and without a resistive robotic force field. Motor adaptation occurs while the force field is active, when it is removed participants experience an overshoot error in the reach task; the error is largest when adaptation to the force field was higher. They found that in a population of normal individuals anodal tDCS increased the overshoot error when compared to sham tDCS. The effects of cathodal tDCS were not tested.

In these studies tDCS was delivered in tandem with a motor task. This raises the question of whether there is a combinatory effect of tDCS and training that contributes to the positive outcome. This is slightly different from the usual discussions of tDCS induced after effects, in which stimulation is delivered alone, and doesn't provide information on the effects of tDCS preceding training. The combination of training and stimulation may be more beneficial to rehabilitation but this hypothesis should be tested through a proper comparison.

3.6.2 Stroke

A range of clinical studies have been undertaken to investigate the effects of tDCS in depression, migraine, chronic pain, stroke and Parkinson's disease (Nitsche et al. (2008)). There are studies on the affects of tDCS for pain in spinal injury, but none on its effects on motor recovery. Of particular interest, from a motor

rehabilitation perspective, are the investigations into stroke. For stroke both anodal and cathodal stimulation have been reported to produce positive effects.

Anodal tDCS, delivered to the lesioned hemisphere of patients with chronic stroke, was reported to significantly improve their performance in the Jebson Taylor Hand Function test (JT) (Hummel et al. (2005); Fregni et al. (2005); Boggio et al. (2007)). The JT test consists of a group of functionally relevant hand and arm movements and the time taken to competently complete the task is indicative of motor improvement. The groups reported between 6.8% and 8.9% improvements in the time taken to complete the task compared to baseline values. Neither Hummel et al. (2006) or Fregni et al. (2005) compared the interventions to a control. A comparison to sham would have been informative in putting the tDCS induced improvements into context. This point is highlighted by the results of Fregni et al. (2005) who did not find any significant interaction beween anodal and sham tDCS in their ANOVA. This result may suggest that there is little to no improvement in movement with tDCS. The same study was undertaken by Boggio et al. (2006) in a healthy population. They did compare to sham and reported similar results as the stroke populations with a 9% increase in JT test time.

Fregni et al. (2005) suggested that tissue damage in the lesioned hemisphere could cause its electrical properties to vary, therefore causing unpredictable results. This is not unreasonable as scar tissue will alter the electric field and impede augmented neuronal communication. This study included one patient with a motor cortical lesion, and following anodal tDCS to the lesioned hemisphere this patient was the only one who had a negative change from baseline. Interestingly all the patients included in the trials by Boggio et al. (2006) and Hummel et al. (2005) had sub-cortical lesions. To overcome the problems posed by scar tissue Fregni et al. (2005) hypothesized that cathodal stimulation to the

un-lesioned hemisphere would increase excitation in the lesioned thus leading to better motor recovery. They reported improved motor outcomes with an average decrease of 12% in the patient groups' JT test performance. The patient with the motor cortical lesion improved in line with the other patients; however, amongst all the participants there was no significant difference between anodal tDCS of the lesioned hemisphere and cathodal tDCS of the unlesioned. The study was later repeated by Boggio et al. (2007) in a sub-cortical lesion population and resulted in a more modest improvement of 9.5%. The location of the lesion site may then be important in defining the correct form of tDCS delivery, and where there is a motor cortical lesion in particular cathodal stimulation of the contralateral hemisphere may be of more benefit, but this should be tested more thoroughly.

The above tests were all carried out in subjects who had gained enough upper limb movement through conventional rehabilitation therapy to complete the JT test. Hummel et al. (2006) delivered anodal stimulation to more severe stroke patients, all with subcortical lesions. Anodal tDCS, delivered to the lesioned hemisphere, resulted in a small but significant improvement in reaction time compared to baseline, but no significant change compared to sham, and no alteration to pinch force. This study suggests that tDCS may have little beneficial value for severe stroke patients; however Fregni et al. (2005); Boggio et al. (2006) and Boggio et al. (2007) delivered tDCS while the patients were doing the JT tests. Hummel et al. (2005) and Hummel et al. (2006) did not. As discussed above there may be additional effects caused by combining the stimuli that should be researched more thoroughly.

While these results are generally positive the test groups have all been very small ((6 - 11) patients) and transient improvements in function have not been linked to improvements in overall rehabilitation or quality of life. The longest post

simulation follow up was conducted by Hummel et al. (2005) who found that 10 days after stimulation the positive improvements has vanished and patient's scores had returned to baseline. This is not particularly surprising as the after effects of one session of tDCS do to not persist for very long, but it is also not very promising for rehabilitation. These improvements in motor function after one session are very modest and their magnitude is certainly not reflective of the reported alterations to cortical excitability or IMC (Nitsche et al. (2003b); Power et al. (2006)), 40% and 18% respectively. The TMS and IMC studies were not conducted in a stroke population but this may suggest a weakness in these investigation techniques in that they do not translate into functionally relevant outcomes. Of course there is the possibility that the low magnitude reflects the inability of tDCS to induce functionally relevant changes.

3.6.3 Enhancing the Effects of Transcranial Direct Current Stimulation

Reports on the duration of the plastic, after effects of tDCS vary from (1 to 60) minutes (Power et al. (2006); Nitsche and Paulus (2001); Nitsche et al. (2003b)). Despite the claims in the literature that there is an LTP/LTD like mechanism the after effects do not usually persist long enough to meet this criteria, which by definition is plastic changes that persist for 30 minutes and longer. The alterations to cortical function are physiologically interesting but the magnitude and durations are not sufficient to induce large and long term improvements in motor function or rehabilitation. It is hypothesized, however, that alterations to the parameters, or delivering tDCS in combination with training or other stimuli will enhance the effects into a beneficial range.

Boggio et al. (2007) investigated the cumulative effects of cathodal tDCS delivered each day for five days to the un-lesioned cortex in chronic stroke patients.

There was a significant, average improvement of 16.7% compared to baseline and the effects persisted for the duration of the two week test period. There was no comparison to a control and so it is difficult to say if the benefits of the stimulation were larger than normal rehabilitation. This result was mirrored in a healthy population when Reiss et al. (2009) demonstrated anodal tDCS delivered every five days during a skill acquisition task significantly improved task speed and accuracy compared to sham 11 weeks post intervention. Note that unlike Boggio et al. (2007) Reiss et al. (2009) found that memory retention was not constant; there was a roughly linear decrease over time that was not altered by tDCS.

In these studies tDCS was given at the same time as the motor task and as mentioned above it is possible that there are combinatory effects of the two techniques. For the purposes of comparison it would be nice to observe the differences between repetitive tDCS alone and repetitive tDCS in combination with training.

3.7 Sinusoidal Stimulation

As discussed in the introduction different frequencies of rTMS cause different effects on cortical excitability, and imitating physiologically relevant frequencies has reportedly enhanced the effects of rTMS even further (Huang et al. (2005)). It has been hypothesised that sinusoidally altering the tDCS signal would produce similar effects. Studies into cortical rythmicity also suggest that oscillations may play a functional role in motor control and the enhancement or inhibition of certain frequencies may improve motor function. These hypotheses have been tested in a few studies and the results suggest that sinusoidal changes to tDCS alter some kinds of cortical function. It is, however, difficult to analyse the effects as few of the reports have properly detailed the stimulation waveform and those who did have not used the same stimulation parameters.

Marshall et al. (2006) were the first to investigate the effects of a sinusoidally varying form of tDCS. The electrodes were positioned bilaterally at fronto-lateral locations and at the mastoids and stimulation was delivered at 0.75 Hz and 5 Hz during slow wave sleep. Rhythmcity at 0.75 Hz is associated with slow wave sleep and hippocampal declarative learning; 5 Hz osscilations are associated with REM sleep. After 0.75 Hz stimulation they reported an increase in EEG slow wave oscillations as well as increased declarative memory upon waking; 5 Hz stimulation reduced the amount of time spent in slow wave sleep promoting REM instead. There were inadequacies in how they reported their study: there is no example waveform, only the description that it is sinusoidal, and so there is no way to infer whether this was tACS or tSCS. There was also no information on the current intensity.

Kanai et al. (2008) tested the effects of 10 Hz and 20 Hz tACS (see Figure 2.5 for an example waveform) on the visual cortex during both light and dark conditions. In the light β frequency oscillations dominate EEG activity in the visual cortex and α dominates in the dark. They reported increased perception of phosphenes during physiologically relevant stimulations, that is 20 Hz tACS in the light and 10 Hz in the dark.

These studies are clearly not motor studies, but they do suggest that an external stimulation of a physiologically meaningful frequency can interact with ongoing cortical oscillations. Any persistent, plastic effects caused by modulating the frequency are not, however, expressed. The differences in the electrode montages are also of note and they bring the interesting question of optimum electrode configuration back to the surface: Are these effects a result of current path or stimulation of the underlying cortex?

3.7.1 Transcranial Sinusoidal Current Stimulation and Motor Cortical Function

The effects of sinusoidally altering the tDCS signal on cortical oscillations have been tested in the motor cortex by Antal et al. (2008) and Pogosyan et al. (2009).

A very detailed study (Antal et al. (2008)) delivered tSCS and tACS over a large range of frequencies ((1 - 45) Hz) and tested both MEP amplitudes and EEG activity but found no significant alterations post stimulation. Prompted by safety concerns, of flickering sensations reported by some subjects, the authors chose to maintain the maximum current for tSCS at 0.25 mA and tACS, 0.4 mA and the stimulation times to between two minutes and four minutes for tSCS and five minutes for tACS. Nitsche and Paulus (2000) demonstrated that current intensities below 1 mA did not induce after effects on MEP amplitudes. The low current here probably accounts for the lack of any observable changes following the stimulation.

Motor learning in a serial reaction time task was also investigated. Here the stimulation was delivered for longer, seven minutes, (current intensities were unchanged) during four blocks of a sequential button pressing task. Compared to sham there was a significant increase in reaction times between the final sequential block and the subsequent random button pressing block for 10 Hz tACS only. The authors postulated that this result was suggestive of motor learning, but, as noted above, 10 Hz oscillations are also associated with PNT and the initiation of new movement. It is possible that tACS improved aspects of new movement and led to decreased reaction times during the stimulation. When the stimulation was removed the reaction times may have increased and the effect would be compounded by the change from a sequential task to a random one. The authors did investigate this further by delivering the stimulation throughout the entirety of

the serial reaction time test, including the random task and they, again, observed the increase in reaction times. They therefore asserted that 10 Hz tACS had improved motor learning and the effects were not caused by improving the initiation of movement. As previously noted Nitsche et al. (2003c) also implied facilitated implicit motor learning with 10 minutes of 1 mA anodal tDCS; here Antal et al. (2008) demonstrated that a smaller intensity and duration (0.4 mA for 7 minutes) of tACS (which has no DC offset) interacted with the same motor function. EEG studies conducted during SRTTs have suggested a relationship between 10 Hz oscillations in M1 during implicit and explicit motor learning Zhuang et al. (1998, 1997). If 10 Hz oscillations are present in the cortex and lead to the promotion of implicit learning then it is possible that they are facilitated by both anodal tDCS and 10 Hz tACS. Unfortunately the study by Antal et al. (2008) offered no way to compare the effectiveness of these two techniques and determine whether tACS has enhanced the effect of tDCS.

It is unfortunate that the smaller duration of stimulation in their EEG study meant that there was no additional insight into how the stimulation actually interacted with brain's rhythmicity. It would also have been interesting to see how EEG data changed under the different forms of 10 Hz tACS and tSCS. Unfortunately the low current and durations also deprived the study of information on any persistent effects of sinusoidal stimulation on motor cortical activity that may have been suggested in both the MEP and EEG tests.

Pogosyan et al. (2009) also investigated the effects of 5 Hz and 20 Hz tACS on the motor cortex. They aimed to ascertain whether imposed stimuli could entrain oscillations that were already present in the nervous system and whether this would translate as changes in motor output. 20 Hz tACS increased 20 Hz CMC when the stimulation was concurrent with a constant contraction and disappeared once

the participants began the a movement. There was also a small but significant slowing of initial and peak movement velocities, compared to sham. tACS of 5 Hz did not interact with CMC. These results may suggest that sinusoidal stimulation is not capable of inducing oscillations but instead facilitates what is already present. This finding was implicated but not demonstrated in Antal et al. (2008). These results are the first to suggest that external stimuli can interact with motor cortical oscillations and lead to alterations in motor function; however the effects were very small and while physiologically interesting they may not be relevant for motor rehabilitation.

The stimulation protocol was different from previous studies in that tACS was delivered at a low current of 0.6 mA and for a very small duration of only 10 s during the contraction. As discussed previously these kinds of low intensities and durations are not usually associated with persistent after effects. This study reported that entrainment of the brain's 20 Hz oscillations, observed as increased CMC, occurred after only (1.12 ± 0.23) s of stimulation and that 10 s of stimulation were sufficient to induce changes to motor function. These results hint that inducing long term plasticity may not actually be necessary in improving motor function.

This study would have been improved by investigating the effects of the longer durations and intensities of tDCS so that a comparison between the two techniques could be achieved. Longer stimulation duration and higher intensities may have induced persistent plastic changes that were not observed here.

These tSCS and tACS studies suggest that it is possible to interact with oscillations that are present in the brain. They do not show that the induced changes persist once the stimulation has been removed. Almost all of the literature fo-

cuses on producing persistent, plastic after effects. These studies imply that long lasting plastic changes to cortical excitability or even CMC may not be necessary to promote motor learning or function. Instead well timed stimuli during a learning or training task may be sufficient to improve motor outcomes by enhancing the brain's intrinsic systems for learning and movement rather than inducing a persistent heightened or inhibited state of excitation or rhythmicity.

3.8 Paired Associative Stimulation

3.8.1 Peripheral Nerve Stimulation

The first studies to investigate effects of PNS on cortical excitability delivered the stimulation to the median nerve at 10 Hz with a 50% duty cycle (over 1 s) for 2 hours (36000 pulses) (Riding et al. (2000); McKay et al. (2002); Charlton et al. (2003)). Riding et al. (2000) demonstrated cortical excitability was increased post stimulation and a follow up in three participants suggested that the effects persisted for at least 15 minutes. Charlton et al. (2003) attempted a more robust study of the persistence of the cortical excitability increases but was restricted by the variation in MEP amplitudes that was observed across their subject group. Variability in MEP data was discussed above, but it is interesting that here is another example of it obscuring the ability to assess the effects of an intervention. Rizzo et al. (2014) and Uy and Ridding (2003) showed that 5 minutes and 10 minutes (1500 pulses and 300 pulses) respectively of PNS were not sufficient to induce significant changes to cortical excitability. An imaging study (Wu et al. (2005)) also delivered PNS for two hours and found increases in BOLD fMRI in M1, sensory motor cortex and pre-motor cortex that persisted for one hour post stimulation. These studies suggest that PNS is capable of plastically altering cortical activity.

To date there are no reports of the effects of PNS on IMC or PNT.

3.8.2 Paired Associative Stimulation: Transcranial Magnetic Stimulation and Peripheral Nerve Stimulation

Associative LTP occurs when the inputs to a postsynaptic neurone are synchronous and occur at the same time, or when the input is synchronised and concommitant with depolarisation of the postsynaptic cell (Buonomano and Merenich (1998)). Stefan et al. (2000) demonstrated that carefully timed electrical stimulation to peripheral nerves and magnetic stimulation to the motor cortex resulted in long lasting ($>$ 30 minutes) changes to MEP characteristics, including increased cortical excitability. They postulated that these alterations were representative of associative LTP like plasticity. To achieve these results ninety pairs of PNS and TMS were delivered to 22 healthy participants at an inter-pulse interval of 25 ms. The was chosen on the premise that the signal from the afferent stimulation of the peripheral nerve takes approximately 20 ms to reach the cortex and the magnetic pulse takes about 3 ms. The peripheral stimulation consisted of short pulses (200 μs) at 300% of sensory perception and delivered to the right median nerve at the wrist. TMS was delivered in the traditional manner to an area activating the right abductor policis brevis (APB). Stefan et al. (2000) tested the effects of different inter-pulse intervals ((100, 525, 500) ms) and only 25 ms caused any changes suggesting that it was indeed the synchronous and concommitant activation of the postsynaptic cell that caused the observed results. Another study (Wolters et al. (2003)) demonstrated that a shorter inter-pusle interval, 10 ms, resulted in the magnetic stimulus arriving before the electric and caused LTD like plasticity in cortical excitability; this is line with models of associative LTD. Both Stefan et al. (2002); Wolters et al. (2003) demonstrated that the NMDA blocking drug, dextromethorphan, abolished the effects of these PAS induced changes.

3.8.3 Paired Associative Stimulation: Transcranial Direct Current Stimulation and Peripheral Nerve Stimulation

In TMS-PAS paradigms the LTP and LTD like plasticity are induced by carefully timed delivery of two discrete stimuli; however, for tDCS-PAS the tDCS stimuli is held constant. This kind of protocol does not necessarily resemble associative LTP/D; however, when the afferent stimulation arrives at the cortex it is, arguably, still a concommittant pair of stimuli.

The effects of tDCS in combination with peripheral stimulation have been reported in two papers (Celnik et al. (2009); Rizzo et al. (2014)). The first, Celnik et al. (2009), investigated the effects of the PNS and anodal tDCS both alone and together in 9 chronic stroke patients. PNS was delivered to the paretic hand at 10 Hz with a 50% duty cycle (over 1 s) for 2 hours (36000 pulses); in the final 20 minutes of the PNS anodal tDCS was also delivered to the lesioned motor cortex. Motor function was tested by quantifying the number of correct entries in a button pressing task. All interventions improved motor function compared to both baseline and sham stimulation. The combination of PNS/tDCS significantly improved correct buttons presses by 41.3% while PNS and tDCS alone improved it by 15.4% and 22.7% respectively. These results suggest that the pairing of tDCS and PNS, like the pairing of TMS and PNS, improve motor output beyond that achieved with each stimulation delivered alone. These data are particularly positive from a motor rehabilitation perspective, suggesting, in this small group at least, that motor outcomes for chronic stroke patients can be improved with non invasive stimulation.

Rizzo et al. (2014) conducted a study into the neuro-physiological effects of anodal

and cathodal tDCS/PAS compared to the effects of PNS, anodal, and cathodal tDCS alone in healthy participants. The tDCS was delivered to APB representation of the motor cortex for five minutes. Peripheral stimulation was delivered for five minutes at a rate of 5 Hz to the median nerve (1500 pulses) and with an intensity of two times sensory perception. Resting motor threshold, active motor threshold and cortical excitability were measured in the same testing period using 0.1 Hz TMS, but, necessarily, different stimulation intensities.

There were no significant changes from baseline following five minutes of PNS alone; this result is in line with previous studies suggesting that much longer stimulation durations are required to induce lasting changes to cortical excitability. The results for anodal and cathodal tDCS alone were also in line with the literature in that cortical excitability was affected in a polarity dependent manner (Nitsche and Paulus (2000)). Five minutes of cathodal tDCS significantly reduced MEP amplitudes immediately after the end of the intervention and anodal tDCS enhanced MEPs for 10 minutes post stimulation. The significant result following anodal stimulation was not, however, consistent with the literature since Nitsche and Paulus (2000) reported a significant result that only persisted for four minutes and had dissipated entirely by ten minutes. This discrepancy may have been caused by a difference in the removal of MEP data points. The authors state that MEPs in which the muscle was not relaxed were discarded but neglected to identify a criteria for this important data exclusion protocol. The baseline for anodal tDCS was lower than any of the other baseline data points; this may have been caused by the removal of 'unsatisfactory' MEPs, which ultimately resulted in the appearance of a later, significant change from baseline. This, again, highlights the necessity of defining a criteria for eliminating MEP data. They also neglected to apply the post hoc adjustments which resulted in the report of a significant increase from baseline at 10 minutes after anodal tDCS which may not have been

significant; they reported $p < 0.02$ which, with a Bonferoni Adjustment would be $p < 0.0125$. There are also, again, the issues with stimulating through the electrodes which were discussed above.

Another weakness with this study design was that three TMS investigations were combined during the course of the experiment. Resting motor threshold was tested first, this technique employs a low TMS intensity, it was followed by active motor threshold tests, which require the participant to voluntarily contract a muscle whilst the TMS pulse is delivered, and finally cortical excitability was tested, here the stimulation intensity must be increased in order to produce a larger MEP. As discussed above it is not known to what extent investigatory TMS interacts with tDCS or PNS and the concomitant contraction and magnetic stimulation employed for active motor threshold tests is another example of combining investigatory tests that are capable of causing changes to cortical activity in themselves; it is possible that following this stimulation immediately with yet another and stronger stimuli may have affected the final results for cortical excitability.

The pairing of tDCS and PNS in a five minute stimulation paradigm also resulted in polarity dependent changes to cortical excitability. Here anodal tDCS enhanced and cathodal tDCS reduced MEP amplitudes, but the significant effects persisted for the duration of the 60 minute testing period. These persistent plastic changes are similar to those observed in TMS-PAS and have been likened to associative LTP/D. While it certainly appears that tDCS and PNS combine to enhance the effects of either stimulation alone the points expressed above make it difficult to ascertain the extent to which this occurs.

3.9 Priming the Cortex

A simple thought experiment demonstrates that the induction of persistent enhanced or reduced cortical excitability will not be conducive to a stable state. Mechanisms exist in the brain to limit rampant positive feedback and this is accounted for in the BCM model which describes the homeostatic response to changes in synaptic activity: where previous stimulation has increased synaptic activity then the response to subsequent stimulation will be to reduce synaptic strengthening perhaps to the point of synaptic depression and vice versa for decreased synaptic activity. Many NIBS investigations neglect to account for the effects of this homeostatic response but evidence for its presence has been reported in a group of studies (Iyer et al. (2003); Uy and Ridding (2003); Lang et al. (2004b); Siebner et al. (2004); Delvendahl et al. (2010)).

Lang et al. (2004b) delivered anodal and cathodal tDCS to the motor cortex and reported the same, previously documented polarity dependent shifts in cortical excitability. tDCS was then followed 10 minutes later with excitatory rTMS (stimulus frequency 5 Hz); instead of eliciting larger MEPs cortical excitability was reduced when the rTMS was preceded by anodal tDCS and enhanced when preceded by cathodal tDCS. Siebner et al. (2004) reported similar results, however in this study the secondary stimulus was inhibitory rTMS (stimulus frequency 1 Hz). Here enhanced MEPs from anodal tDCS were subsequently reduced, and the opposite occurred for cathodal tDCS. tDCS followed by inhibitory rTMS resulted in a larger final increase or decrease in cortical excitability than would have been achieved with tDCS alone. The same priming results have been reported from other NIBS protocols, Iyer et al. (2003) demonstrated the same result by priming with excitatory rTMS (6 Hz) followed with inhibitory rTMS (0.1 Hz) and Delvendahl et al. (2010) demonstrated that 0.1 Hz rTMS altered the outcome of a PAS protocol, discussed above.

Priming does not only occur when two different NIBS protocols are combined. Fricke et al. (2011) primed the cortex with seven minutes of tDCS and followed it with five minutes of tDCS with an interval of (1 - 30) minutes between the two stimuli. When the time between them was less than 10 minutes the priming responses observed above were seen here. Not only were the data in accordance with previous reports and the BCM model, but they also provide an indication of the time course involved in this kind of homeostatic response.

For both Lang et al. (2004b) and Siebner et al. (2004) both the rTMS paradigms used were non functional; that is, when delivered alone they generally do not produce significant changes to excitability. In addition Lang et al. (2004b) reported that the tDCS induced changes to cortical excitability were non significant (yet another example of variability in the reported effects of tDCS); interestingly however, though both of these interventions alone caused weak after affects they produced a large change form baseline when combined in this priming protocol. This may suggest that the thresholds for activation were altered by the priming intervention and may have important ramifications for other studies on cortical excitability in which a 'non functional' TMS pulse train is delivered for investigation purposes. It is possible that the post stimulation investigation may be of sufficient strength to behave as a conditioning stimulus thus altering the outcome that it is attempting to investigate in a classic analogue of the Heisenberg uncertainty principle.

These priming studies impose additional complications on the use of NIBS for motor rehabilitation by highlighting the potential ceiling effects that homeostatic plasticity can impose on rehabilitative interventions. This may go some way to explaining the relatively modest improvements that have been reported in clinical

trials. More positively they demonstrate that it may be possible to exploit the homeostatic response in order to leave the brain in a more susceptible learning state by combining NIBS interventions, although a more detailed understanding of the response in both healthy and injured people would be required. Motor training is also capable of altering cortical activity and so the use of training either before or after a stimulation may have to be carefully designed so as to deliver the best outcome. The state of the cortex before an intervention may also shape the outcome from a single stimulation session and this may account for some of the variation that has been observed amongst different studies.

In order to explore the effects of priming new techniques must be developed to probe the brain.

3.10 Summary

A key issue in the tDCS literature that has been highlighted here is the variability in reported effects of tDCS on TMS induced MEPs. The polarity dependent effects of tDCS on MEPs will be assessed in the present study and MEP variability will be explored. Changes to inter-muscular coherence and physiological neurogenic tremor will be tested under the same tDCS parameters as the MEPs in order to develop these as measures of tDCS induced changes to cortical activity.

As noted in this literature review the effects of tDCS in the clinic have been poor, but there are suggestions in the literature that sinusoidal alterations to the DC signal may enhance the effects of the intervention. The incorporation of peripheral nerve stimulation to the tDCS paradigm, as a paired associative stimulation protocol, may also enhance the effects of tDCS alone. The effects of

both of these interventions on IMC and PNT were studied here.

Chapter 4

Methods

4.1 Summary of Experiments

A number of experiments were conducted to test the effects of tDCS and tSCS both alone and in combination with PNS and are listed below.

1. The effects of tDCS on cortical excitability.
2. Development of inter-muscular coherence contraction task.
3. The effects of tDCS on inter-muscular coherence and physiological neurogenic tremor.
4. The effects of paired associative stimulation (tDCS and peripheral nerve stimulation) on inter-muscular coherence and physiological neurogenic tremor.
5. The effects of tSCS on inter-muscular coherence and physiological neurogenic tremor.
6. The effects of paired associative stimulation (tSCS and peripheral nerve stimulation) on inter-muscular coherence and physiological neurogenic tremor.

4.2 Ethics

All subjects who participated in these tests provided informed written consent in accordance with the Declaration of Helsinki and approved by the University of Strathclyde Ethics Committee. The participants were all in good general health, between the ages of 18 and 60 and capable of producing voluntary movements of the hand and wrist without pain or excessive fatigue. The exclusion criteria is set out below.

4.2.1 Exclusion Criteria

Subjects with any of the following CANNOT participate in this study:

- Are receiving medical treatment for any neurological (including all forms of epilepsy), musculoskeletal, respiratory, cardiovascular disease or condition.
- Are receiving physical therapy for any neurological, musculoskeletal, respiratory or cardiovascular disease.
- Have a history of cognitive, motor or sensory dysfunction.
- Have a history of arthritis or repetitive strain injury.
- Are currently taking a prescribed medicine or have completed a course of prescribed medication within the last 30 days.
- Have a history of migraine.
- Are unable to provide informed consent.
- Are pregnant.
- Are known to be diabetic.
- Have a known or suspected skin allergy to adhesives used in skin dressings (e.g. Elastoplast, Bandaid etc.).

- Have an implanted electronic medical device (eg cochlea implant, pacemaker, brain stimulator etc.)
- Have a history of autonomic dysreflexia or syncope (fainting).
- Have a visual impairment that has not been corrected to normal.

4.3 1. Transcranial Direct Current Stimulation and Cortical Excitability

Nitsche and Paulus (2000) reported that anodal and cathodal tDCS significantly increased and decreased MEPs respectively. The aim of this study was to implement tDCS protocols and establish repeatability with respect to the published work.

- Number of subjects: 8
- Stimulation type: Anodal and Cathodal tDCS
- Current: 1 mA
- Duration: 10 minutes
- tDCS Device: Magstim DC Stimulator Plus
- TMS Device: Magstim 200 - Figure eight coil
- Target Muscle: 1DI

4.3.1 Electromyography

Disposable, bipolar, self adhesive electrodes [Blue Sensor N, Ambu, Denmark] were used to record surface EMG from 1DI. The muscle was identified according to techniques discussed in Chu-Andrews and Johnson (1986) the location of 1DI

is shown in Figure 4.1 and Figure 4.2 illustrates the electrode positioning. To ensure that a good EMG signal was achieved the skin was gently abraded using an abrasive gel [Nuprep, D.O. Weaver & Co., USA] before the electrodes were positioned. The ground wire was fixed to the protrusion of the pisiform bone in the wrist. Motion artefact caused at the skin-electrode interface were minimised by securing the electrodes in place with medical tape; motion artefact in the EMG wires were minimised by strapping them to the subject's arm.

The EMG signals were amplified by a gain of 2000, band-pass filtered at (5 - 500) kHz using the Neurolog System [Digitimer Ltd, Hertfordshire, England], sampled at 2 kHz by a CED 1401 analogue to digital converter [Cambridge Electronic Design, Cambridge, England] and displayed on screen through a CED compatible program, Spike 2 [Cambridge Electronic Design, Cambridge, England].

4.3.2 Transcranial Direct Current Stimulation

Transcranial direct current stimulation was delivered through two conductive rubber-carbon electrodes encased in saline soaked sponges. The electrodes were positioned with the reference electrode placed above the contralateral right orbital and the active electrode above the target area of the left motor cortex; they were held in place with fabric straps, see Figure 4.3. This is the traditional montage, as defined by Nitsche and Paulus (2000) and is the most commonly used configuration in the literature.

To achieve maximum focality at the target site, while staying within the recommended maximum current density (Ntische et al. (2007); Ziemann et al. (2008)), the electrode above M1, or the active electrode, was 6 cm x 4 cm and the reference electrode was 7 cm x 6 cm. Again these are the most common electrode dimensions found in the literature. Reports suggest that a current amplitude of 1

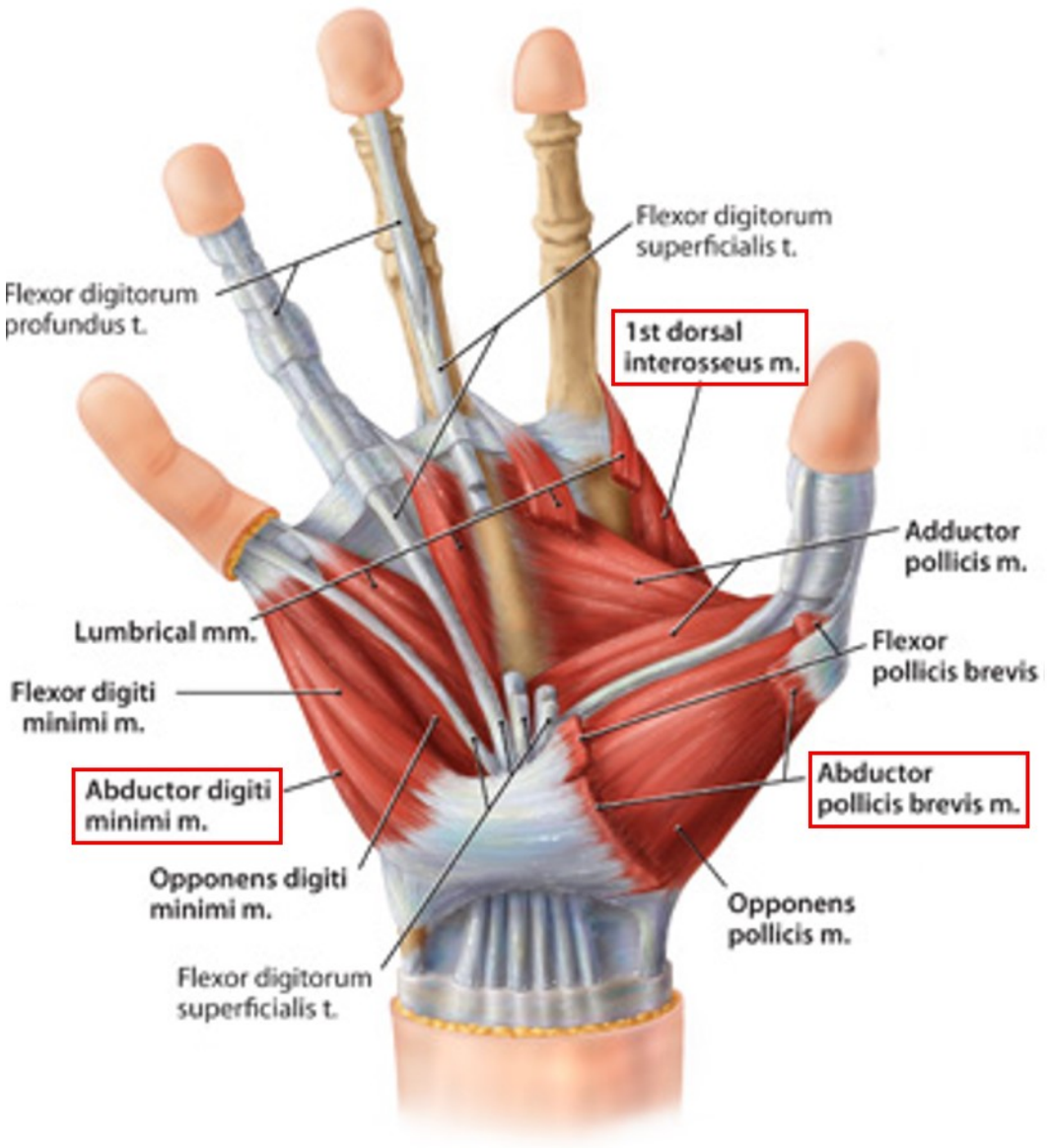


Figure 4.1: Muscles of the hand.

mA is necessary to produce persistent after effects (Nitsche and Paulus (2000)). Some groups are beginning to increase this to 1.5 mA and 2 mA; however, there have been reports of adverse events at these higher currents (Bikson et al. (2009); Palm et al. (2008)). Until further evidence is presented safety concerns would indicate that it is prudent to maintain stimulation at a maximum amplitude of 1 mA. This also enables better comparability with the majority of the literature.

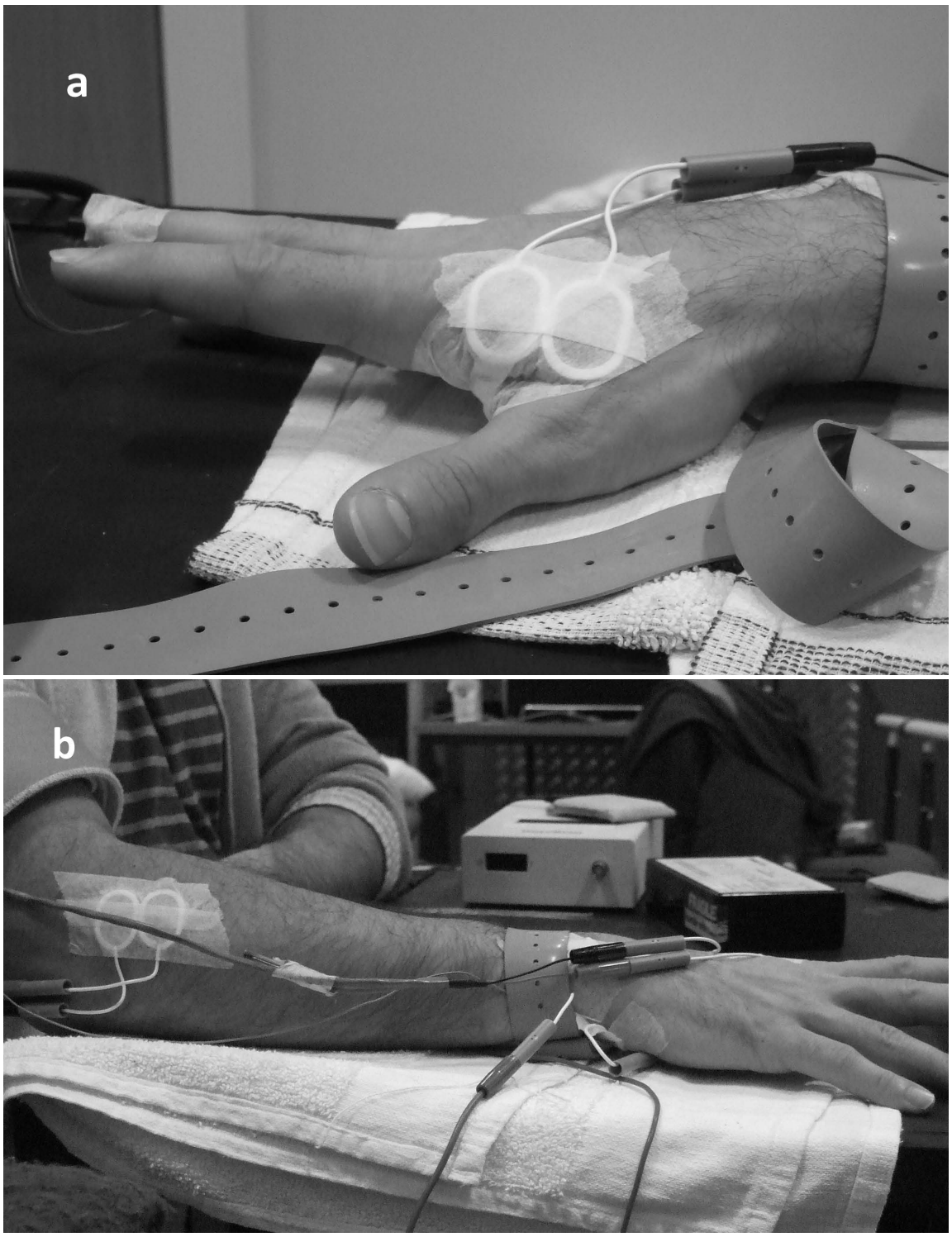


Figure 4.2: Electrode placement for EMG recording from a) 1DI and b) ED.

The current was ramped up and down over 10 s at the beginning and end of 10 minute stimulation epochs. This ramp up/down reduces the effects of a very quick change in the electric field which can be uncomfortable to the participant.

Figure 4.3: Electrode configuration used for delivery of transcranial direct current electric and transcranial sinusoidal current stimulation. The rubber-carbon electrodes are placed in saline soaked sponge pockets and held in place with fabric straps. The 'active electrode' is positioned over the left M1 and the 'reference electrode' over the right contralateral orbital.

A minimum of one week passed before a subject underwent further stimulation in order to minimise cumulative effects caused by repetitive stimulation. Anodal,

cathodal and sham stimuli were randomly mixed and participants were not informed of which stimulus they would receive.

The sham stimulation here consisted of 10 s ramp up/down and 30 s at 1 mA (Figure 4.4). This is in line with protocols described in the literature which suggest that any perception of active tDCS is lost after the first 30 s of stimulation (Nitsche et al, 2008).

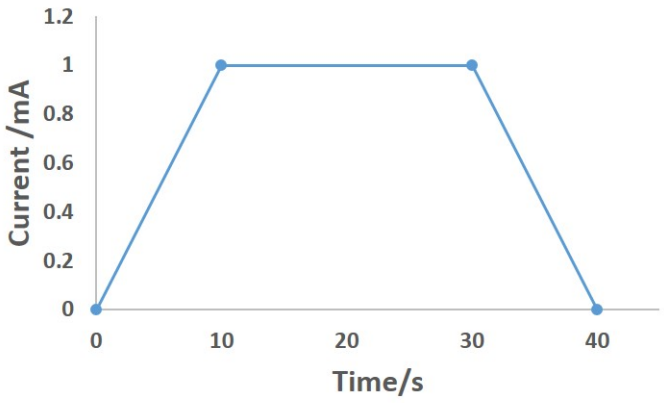


Figure 4.4: Sham waveform: 10 second ramp up to 1 mA for 30 seconds followed by 10 second ramp down.

4.3.3 Transcranial Magnetic Stimulation

Hot-Spotting

TMS was used to identify the target muscle hot-spot, that is the area of the motor cortex that has the lowest threshold to stimulus in the target muscle. The target muscle was 1DI. A figure of eight magnetic coil [Magstim 200, Magstim, Carmanthenshire, Wales] was placed postero-anteriorly and tangentially to the skull (Fig 4.5), and over the approximate region of the motor cortex for activation of 1DI. A single magnetic pulse was delivered to the brain every 10 s (0.1 Hz). The coil was moved around the scalp in a methodical, grid-like fashion in order to identify the site which elicited the largest and most consistent MEP, with

the smallest intensity of stimulation. This site was the optimum position for the electrical stimulation electrode.

Figure 4.5: Postero-anterior orientation of figure of eight TMS coil for stimulation of 1DI

Cortical Excitability

When investigating cortical excitability using TMS it is important that the coil is positioned at the correct angle and over the hot spot throughout the test. To minimise movement of the subject and coil the coil was mounted on a spring loaded stand and the subject rested their chin on a metal support rig. The rig was positioned so that the subject could remain comfortable and stationary for the test duration. The hot spot was identified using the technique described above and three additional reference marks were used to ensure that the coil alignment and position did not move relative to the participant's head throughout the ex-

periment.

The magnetic stimulator output was set to consistently elicit an MEP with a peak to peak value of 1 mV, it remained at this output throughout the entirety of the test. MEPs were elicited by delivering blocks of 10 single TMS pulses at a rate of 0.1 Hz. These blocks were delivered before stimulation, and one, five and ten minutes after ten minutes of tDCS had ended.

4.3.4 Analysis of Motor Evoked Potentials

MEPs from these tests were extracted and epoched to the 0.5 s before the TMS onset by a custom built Matlab script and stored as a raw data file (Figure 5.1). Each MEP could then be individually evaluated or grouped as required. The peak to peak amplitude of each MEP was determined and logged for later analysis in SPSS.

Data Quality

Data quality checks were conducted on the MEPs and cross referenced with the notes from the experiment. Where the subject had moved and the coil was subsequently incorrectly positioned the MEP was removed from further analysis. The data was also checked to ensure that noise did not adversely affect any of the MEPs.

Statistical Tests

All statistical tests were conducted in SPSS [IBM SPSS Statistics 21] . Box plots were produced to show the distribution of each individual's peak to peak MEP amplitudes for each of the four time intervals (baseline, one, five and ten minutes post intervention). Extreme outliers, as identified by SPSS, were removed.

For each time interval the distribution of the data was tested for normality using the Shapiro-Wilk test and the results informed the type of inferential tests that were applied. As most of the data was not normally distributed the Friedman Test for non parametric data was used instead of an ANOVA. A Wilcoxon Signed Rank test with a Bonferoni adjustment was then used to determine at which time points there had been a significant change from baseline.

4.4 2. Development of Contraction Task for Inter-muscular Coherence

A maintained contraction in the muscles of interest is integral to the investigation of inter-muscular coherence and a good contraction task is vital in achieving good quality data. Three different contraction tasks were explored in a group of eight participants and a robust analysis technique was implemented.

4.4.1 First Contraction Task

EMG was recorded from two muscles in the forearm: flexor carpi radialus (FCR) and extensor digitorum, and two muscles in the hand: first dorsal interosseous and abductor digiti minimi using the techniques described in section 4.3.1. Figures 4.1 and 4.6 illustrate the location of these muscles. Four of the participants executed this task. The participants sat with their right arm supported by an arm rest and their hand holding a joystick, Figure 4.7. A two minute co-contraction of the muscles was achieved by asking the subject to maintain the joystick at the eleven o'clock position, this activated both forearm and hand muscles. A screen was located in front of the subject and a custom built graphic display was used to provide cues (Figure 4.8). The appearance of a box at the 11 o'clock position on the screen was the cue to execute the contraction. The box remained in position for two minutes before disappearing, this was the cue to relax. Each participant

repeated the task six times.

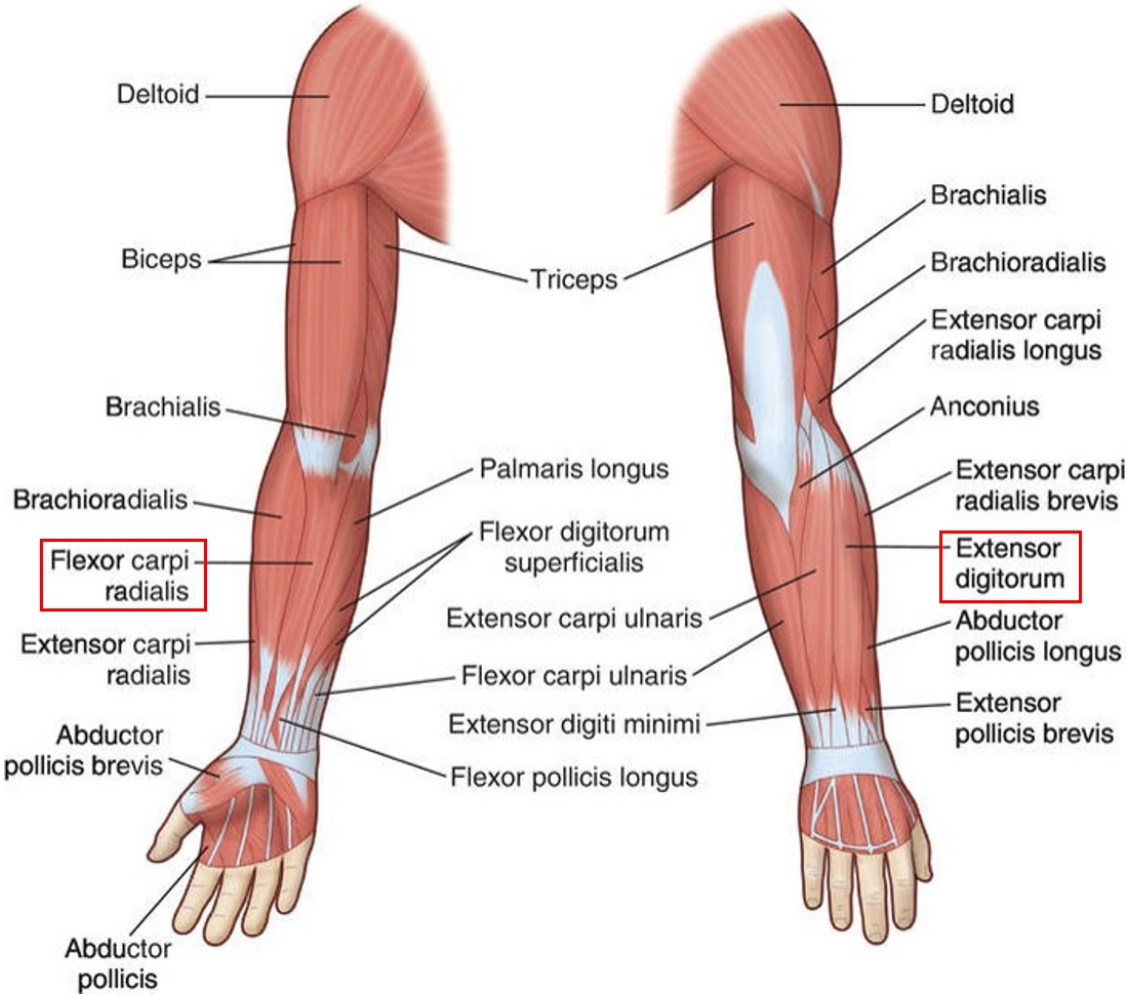


Figure 4.6: Muscles of the arm.

4.4.2 Spectral and Coherence Analysis

Analysis of EMG data was undertaken in the Matlab toolbox Neurospec [Neurospec 2.0](Halliday et al. (1995)). This program performs spectral analysis between pairs of single or pooled time series data sets. Good quality data were read into Neurospec where linear de-trending, unit variance normalisation, rectification and mains frequency suppression were performed on the EMG recordings (Grosse et al. (2002)). Neurospec calculates the frequency power spectra of the recordings and can implement cross spectral analysis between pairs of recordings

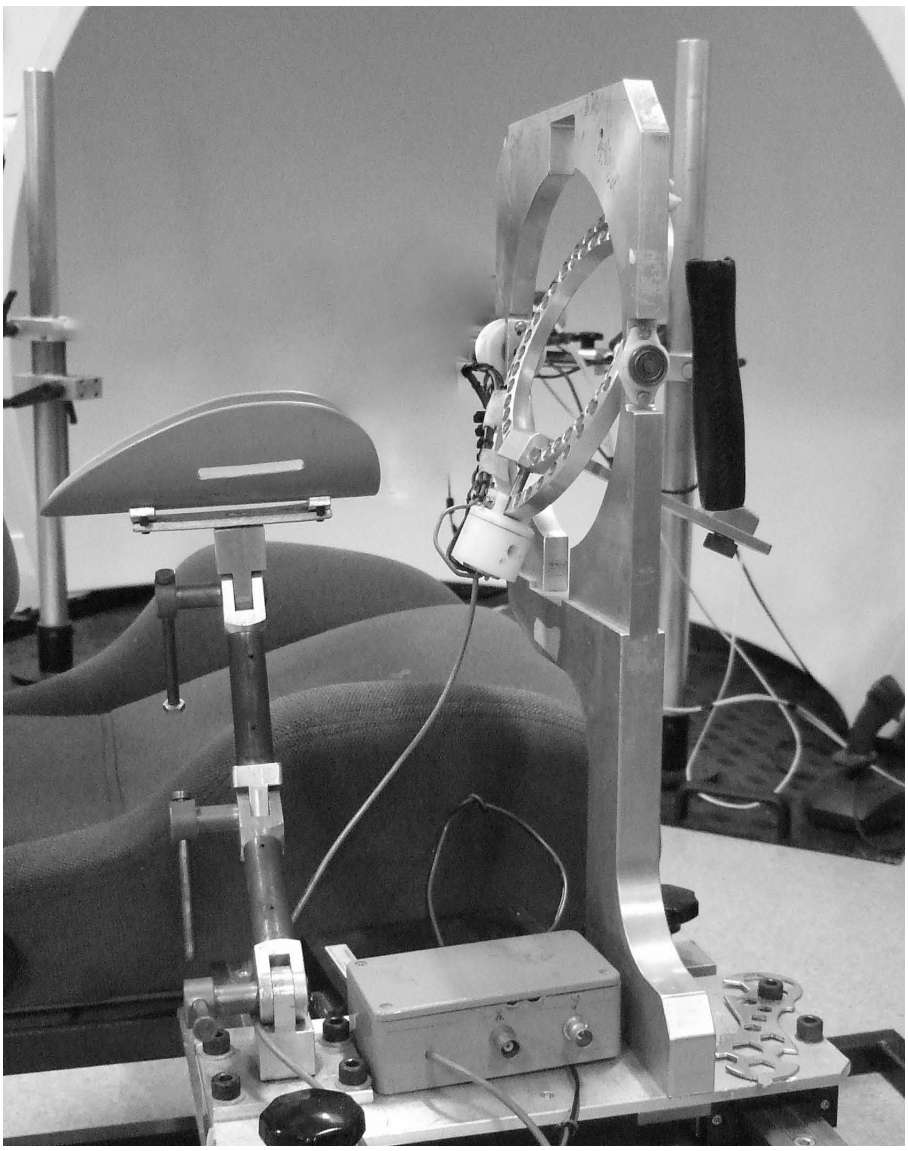


Figure 4.7: Manipulandum device used in the first and second contraction task to encourage participants to co-activate all the muscles of interest.

to produce coherence estimates for the two signals (Figure 2.9).

Evaluating Data Quality

Each raw EMG trace was first visually inspected to ensure only good quality data was analysed; if task compliance was poor, or the signal was dominated with noise then the record was removed from the analysis protocol.Further inspection of the data quality was carried out on the frequency power spectra plots

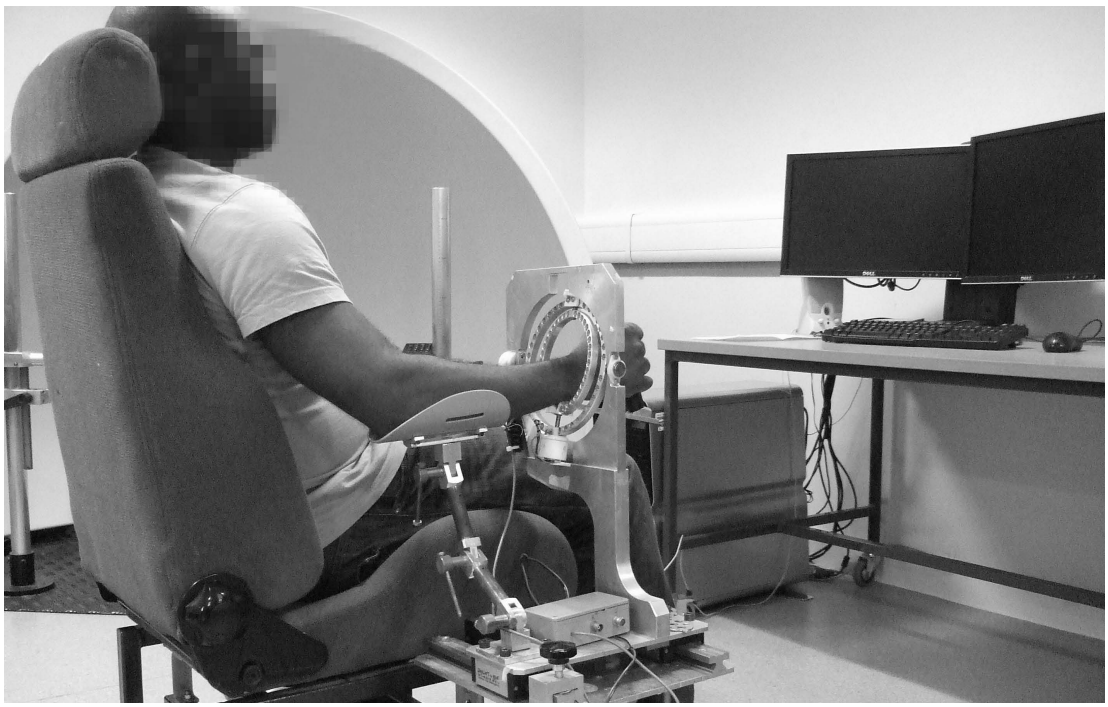


Figure 4.8: Participant's position during the fist and second contraction tasks. Participant is relaxed, the elbow is supported and the hand grips the joystick to activate all the muscles of interest.

since poor quality data often exhibit low power output and a poorly defined frequency profile (Figure 5.6). Poor quality data were removed from further analysis.

Baseline Stability Analysis

Due to the problems of variability in the effects of tDCS reported in the literature it was deemed important in this study to ensure that sources of variability in the experimental data sets were accounted for and minimized. For this reason great care was taken to test that the data were free from artefacts or other factors that could influence the fidelity of the results.

Before IMC can be considered a reliable tool for investigating cortical activity it is vital to demonstrate that it stays stable before an intervention is delivered. For each individual participant all the repetitions of the trial were pooled together in Neurospec and χ^2 extended difference of spectra and coherence tests were

conducted. The null hypothesis is that the input data sets to the pooling regime are the same. These tests produce plots that identify any frequencies at which the input spectra or coherence data are significantly different from one another (Figure 5.14).

4.4.3 Second Contraction Task

The EMG data and the results from the χ^2 extended difference of spectra and coherence tests from the first task suggested that participants found it difficult to maintain activity in all the muscles simultaneously for the two minute task duration. To make the data more useful the 1DI EMG was changed to APB, all other muscles stayed the same. In addition the duration of the trial was reduced to one minute. Three participants executed this task.

4.4.4 Third Contraction Task

Again the EMG data and the results from χ^2 extended difference of spectra and coherence tests from the second task suggested that task compliance was poor and insufficient quality of data was being generated to allow for meaningful analysis. A simpler contraction task was therefore implemented: the fore-finger and middle finger were extended for one minute while the rest of the hand rested on the table (see Figure 4.9), five participants executed this task. Participants were cautioned against fatigue and informed that it was important not to over exert their fingers too much, if a stronger contraction was required the participants were asked to imagine their fore-finger floating closer to their thumb and their middle finger floating up. The EMG trace was displayed on a computer screen that was positioned in front of the participant, movement was prompted by the display turning from red (don't go/relax) to orange (get ready) to green (go). Data quality from this task was good and it was implemented in all the follwing IMC trials.

Figure 4.9: Illustration of the third contraction task; extension of the fore-finger and middle finger while the rest of the hand is supported on the table. Note the accelerometer positioned on the end of the middle finger.

4.5 3. Transcranial Direct Current Stimulation, Inter-Muscular Coherence and Physiological Neurogenic Tremor

Power et al. (2006) reported that 10 minutes of anodal and cathodal tDCS significantly increased and decreased β IMC respectively. The aim of this study was to investigate this report and to examine if an effect on physiological neurogenic tremor could also be observed. An accelerometer was therefore attached to the middle finger, as shown in Figure 4.9.

- Number of subjects: 10

- Stimulation type: Anodal, Cathodal DC and Sham
- Current: 1 mA
- Duration: 10 minutes
- Device: Magstim DC Stimulator Plus
- Muscles: ED & 1DI
- Duration of contraction: 1 minute

EMG was recorded from 1DI and ED in the same way as described above (section 4.3.1), tDCS was delivered in the same way as for the cortical excitability trial above (section 4.3.2) and recordings for IMC were obtained using the third contraction task described in section 4.4.4. Baseline IMC was established before ten minutes of anodal, cathodal or sham tDCS was delivered; IMC was tested again at 1, 5, 10, 15, 20, 25 and 30 minutes after the stimulation had ended. Data quality and baseline stability analysis of the EMG data was undertaken as described above in section 4.4.2.

4.5.1 Tremor

To achieve a tremor recording a miniature Entran Egax-5 accelerometer [Entran Devices Inc, Fairfield USA] was attached to the tip of the middle finger. Tremor of the digit was recorded while the fore finger and middle finger were extended during the postural contraction task, Figure 4.9. The accelerometer had a flat frequency response from DC to 200 Hz and was orientated with its sensitive axis aligned vertically. The signal was amplified using a standard DC bridge amplifier and digitised at 2 kHz by a CED 1401 analogue to digital converter [Cambridge Electronic Design, Cambridge, England].

Accelerometer Recordings

Data from the accelerometer were treated in much the same way as the EMG records: the recordings were visually inspected to identify if it had been overcome with noise and good quality data was read into Neurospec where linear de-trending, unit variance normalisation and mains frequency suppression were performed. The frequency power spectra were produced and coherence estimates undertaken between the accelerometer frequency spectra and the EMG frequency spectra. This tremor-muscle coherence reflects components of physiological neurogenic tremor and will be referred to as PNT. Baseline stability of each individual participant's accelerometer spectra and tremor-muscle coherence estimates was investigated using the χ^2 extended difference of spectra and coherence tests.

4.5.2 Comparison of Coherence Analysis

The aim of this study was to investigate the effects of each intervention (anodal cathodal and sham tDCS) on IMC and PNT. To this end IMC and PNT data sets were created for each intervention by, separately, pooling the IMC and PNT data from all participants with stable baselines. This regime produces pooled frequency power spectra and coherence estimates that can be treated in the same way as the individual ones.

To determine if there were statistically significant differences in the IMC or PNT following an intervention comparison of coherence tests were undertaken in Neurospec. First the coherence estimates from each time interval were compared to the baseline. This analysis compares the coherence estimates of two signals at each frequency and determines if they are significantly different on a 95% significance level, it also determines whether one is significantly larger than the other. The plots produced illustrate the frequencies in which the two coherence esti-

mates are significantly different from one another and show whether signal two is larger than signal one (Figure 5.17).

To exclude effects from training (caused by repetitively contracting ED and 1DI in the contraction task) the active intervention data sets were also compared against the sham data for each time interval in another comparison of coherence test.

4.5.3 Alternative Analysis: Average Significant Coherence

For the purposes of comparison with Power et al. (2006) their analysis procedure was also replicated here; this included investigating the same time tests and employing the same definitions for the α and β frequency bands. The IMC of each participant was calculated in Neurospec for each time interval: baseline (before stimulation), one minute, five minutes and ten minutes after the stimulation had ended. The coherence was split into the frequency ranges of interest α ((5 - 15) Hz) and β ((16 - 35)Hz), as defined by Power et al. (2006). The total significant coherence for each band was found by summing all the elements within that frequency range that were above a 95 % confidence interval. Figure 4.10 highlights the area of the curve that was used for this analysis. The results were averaged over all the participants. Power et al. (2006) tested for significant changes from baseline using paired t-tests, here this analysis varies from theirs as Shapiro-Wilk tests of normality were conducted first. The results from the Shapiro-Wilk test informed the type of inferential test that was used. For non-parametric data the Friedman's test was implemented and for normally distributed data a one way ANOVA was used.

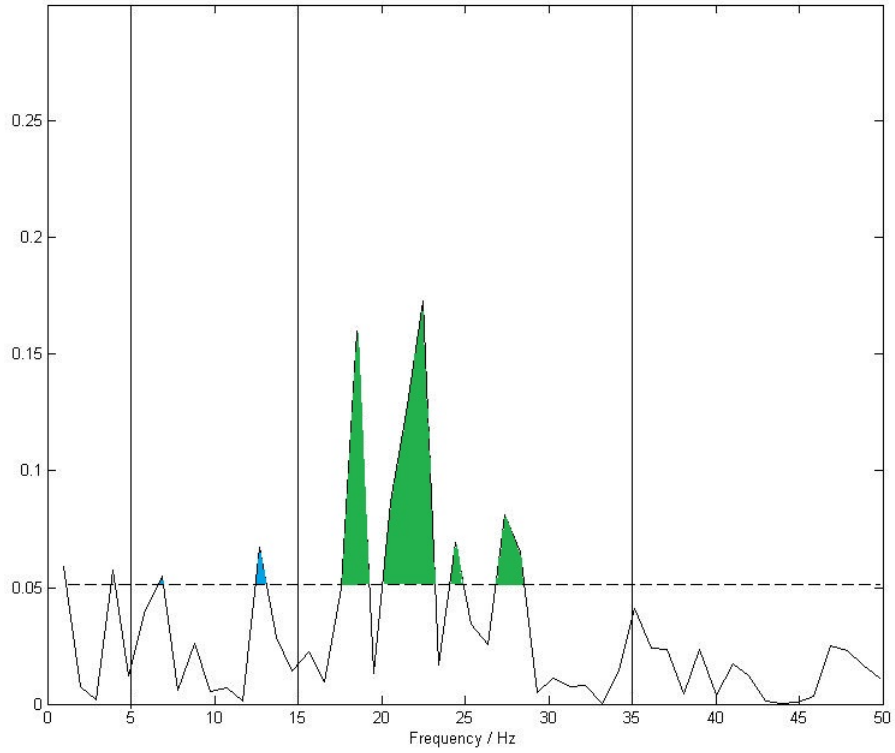


Figure 4.10: Example of the area under the curve used for the alternative analysis of average inter-muscular coherence.

4.6 4. Paired Associative Stimulation: Transcranial Direct Current Stimulation and Peripheral Nerve Stimulation

The literature suggests that pairing cortical stimulation, such as TMS, with peripheral stimulation enhances the plastic effects of either stimulation alone. This study aimed to test the effects of tDCS paired with PNS on IMC and PNT.

- Number of subjects: 10
- Stimulation type: Anodal, Cathodal DC and Sham
- Current: 1 mA

- Duration: 10 minutes
- Device: Magstim DC Stimulator Plus
- Muscles: ED & 1DI
- Duration of contraction: 1 minute

With the exception of the peripheral nerve stimulation, described below, each component of the trial was carried out as has already been described above: EMG recording (section 4.3.1), contraction task (section 4.4.4), tremor recording (section 4.5.1), TMS hot-spotting (section 4.3.3), tDCS delivery (section 4.3.2) and IMC and PNT analysis (section 4.4.2, 4.5.1 and 4.5.2).

4.6.1 Peripheral Nerve Stimulation

Disposable, bipolar, self adhesive electrodes [Blue Sensor N, Ambu, Denmark] were used to deliver peripheral stimulation. The nerve of interest was the ulnar nerve and its approximate location was identified using the techniques described in Chu-Andrews and Johnson (1986) for peripheral stimulation of the ulnar nerve at the wrist. To minimise resistance at the skin electrode interface the skin was gently abraded using an abrasive gel [Nuprep, D.O. Weaver & Co., USA] before the electrodes were positioned over the nerve. The anode and cathode were placed side by side with the anode more distal. Pressure was applied to the cathodal electrode using additional padding and a strap, this reduced the stimulation intensity needed to elicit a response and made the experience more comfortable for the participant, Figure 4.11.

The stimulation began at its lowest setting and was slowly increased until the participant could perceive it, this level was the sensory perception level for that individual. Maximum stimulation that would be delivered to each individual was

calculated as three times their sensory perception level (Stefan et al. (2000); Ridding and Nordsrtom (2007)). Stimulation was slowly ramped up to this maximum level and the EMG response in 1DI was observed. If no M-wave was induced at maximum stimulation then the cathode was repositioned down the nerve (proximally) to find the branching location of the nerve for that individual and hence provoke an M-wave.

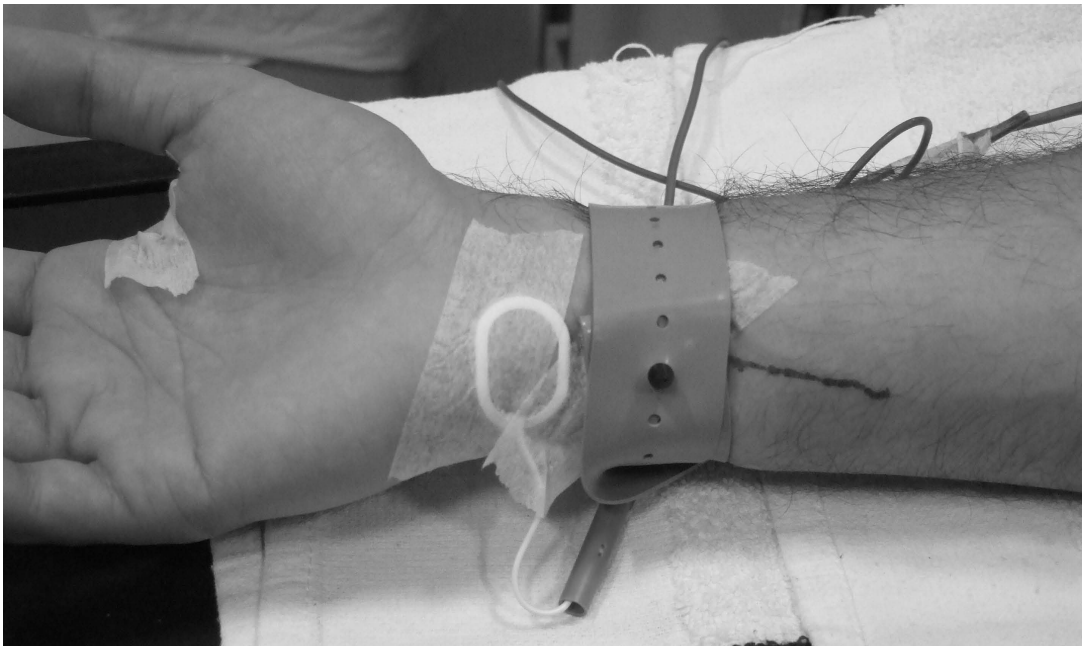


Figure 4.11: Positioning of the electrodes for peripheral stimulation of the ulnar nerve at the wrist.

The duration of each peripheral stimulation pulse was 1 ms. A train of five pulses was delivered at 10 Hz every 10 seconds for 10 minutes, making a total of 300 pulses. Peripheral nerve stimulation was delivered during the 10 minutes that tDCS was delivered. Again IMC and PNT tests were conducted before stimulation began and 1, 5, 10, 15, 20, 25 and 30 minutes post intervention. Each particpant also received PNS alone, with no tDCS stimulation in order to test the effects of PNS on IMC and PNT.

4.7 5. Transcranial Sinusoidal Current Stimulation

The aim of this study was to investigate whether any effects could be enhanced by employing a sinusoidally varying signal. The effects of three frequencies (5 Hz, 10 Hz & 20 Hz) of tSCS on IMC and PNT were investigated.

- Number of subjects: 10
- Stimulation type: 5 Hz, 10 Hz and 20 Hz tSCS
- Maximum Current: 1 mA
- Minimum Current: 0.95 mA
- Duration: 10 minutes
- Device: Magstim DC Stimulator Plus
- Muscles: ED & 1DI
- Duration of contraction: 1 minute

EMG and accelerometer recordings were treated in the same way as for the previous trials, section 4.3.1 and section 4.5.1 using the contraction task described in section 4.4.4.

4.7.1 Transcranial Sinusoidal Current Stimulation

For transcranial sinusoidal current stimulation the DC signal was modulated by a small sinusoidally varying signal of 5 Hz, 10 Hz or 20 Hz. The current amplitude oscillated between 0.95 mA and 1 mA following ramp up (Figure 4.12). The optimum location for the electrodes was identified using TMS hot spotting as described in section 4.3.3

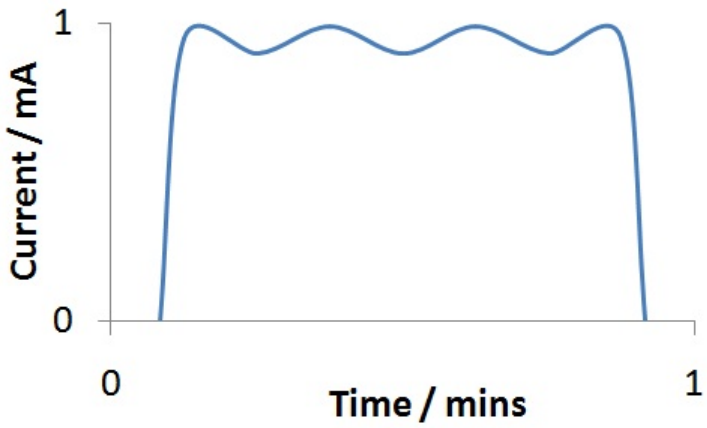


Figure 4.12: Example waveform for tSCS

Analysis of both IMC and PNT was the same as the previous trial, sections 4.4.2), 4.5.1 and 4.5.2.

4.8 6. Paired Associative Stimulation:Transcranial Sinusoidal Current Stimulation and Peripheral Nerve Stimulation

The effects of tSCS and peripheral stimulation on IMC and PNT were tested here.

- Number of subjects: 10
- Stimulation type: 5 Hz, 10 Hz and 20 Hz tSCS
- Maximum Current: 1 mA
- Minimum Current: 0.95 mA
- Duration: 10 minutes
- Device: Magstim DC Stimulator Plus
- Muscles: ED & 1DI

- Duration of contraction: 1 minute

All of the components of this trial were the same as those described above: EMG recording (section 4.3.1), contraction task (section 4.4.4), tremor recording (section 4.5.1), TMS hot-spotting (section 4.3.3), tSCS delivery (section 4.12) and IMC and PNT analysis (section 4.4.2, 4.5.1 and 4.5.2).

Chapter 5

Results

In order to meet the aims described in the Research Statement (section 1) the following experiments were conducted:

1. The effects of tDCS on cortical excitability.
2. Development of inter-muscular coherence contraction task.
3. The effects of tDCS on inter-muscular coherence and physiological neurogenic tremor.
4. The effects of paired associative stimulation (tDCS and peripheral nerve stimulation) on inter-muscular coherence and physiological neurogenic tremor.
5. The effects of tSCS on inter-muscular coherence and physiological neurogenic tremor.
6. The effects of paired associative stimulation (tSCS and peripheral nerve stimulation) on inter-muscular coherence and physiological neurogenic tremor.

No participants reported any adverse effects either during or after the interventions or investigations. Accordingly, at the currents tested, tDCS appears to be safe.

5.1 1. Transcranial Direct Current Stimulation and Cortical Excitability

In order to assess reports from the literature the effects of anodal and cathodal tDCS on cortical excitability were tested using single pulse TMS. The methods are detailed in section 4.3 and the results are detailed below.

- Number of Subjects: 8 (22-35)
- Stimulation type: Anodal and Cathodal tDCS
- Target muscle: 1DI

5.1.1 Data Quality

Data quality was generally very good and no data had to be removed because of noise. An example of an individual MEP collected from the target muscle (1DI) is shown in Figure 5.1; there is a low noise to signal ratio and the MEP has a peak to peak amplitude of 1mV.

For each time interval test (baseline, 1, 5 and 10 minutes post stimulation) 10 MEPs were collected and the amplitudes were measured. Box plots were produced in SPSS for each of these data sets to identify outliers and illustrate the distribution of each participant's MEP amplitudes. The data are shown in Figure 5.2. Extreme outliers, as identified by the SPSS software, were removed from further analysis.

5.1.2 Results from Statistical Tests

Shapiro-Wilk Tests were used to evaluate the distribution of the raw MEP amplitudes for each intervention (anodal and cathodal tDCS) and the results are

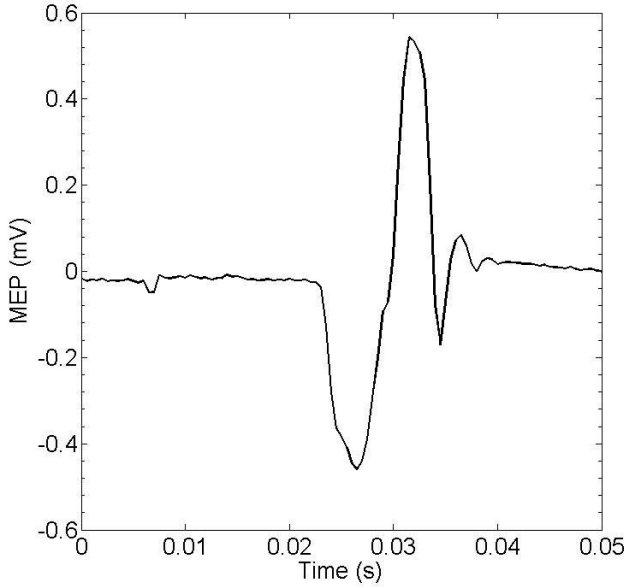


Figure 5.1: Example of an individual MEP recorded from the target muscle (1DI) in this trial

illustrated in Figures 5.3 and 5.4 respectively. For the baseline data of both interventions the test showed a significance value of $p > 0.05$ suggesting a normal distribution. For all other time intervals $p < 0.05$ suggesting non-parametric data. Since the data were not normally distributed Figures 5.3 and 5.4 show the median value and the interquartile ranges for each time interval data set.

A Friedmans Test for non-parametric data was used in place of an ANOVA to compare the post intervention data to its baseline. There were statistically significant differences in MEP amplitudes in both anodal, $\chi^2(3) = 14.678$, $p = 0.04$, and cathodal tDCS, $\chi^2(3) = 18.287$, $p = 0.011$, data sets. Post hoc analysis was conducted with Wilcoxon signed-rank tests and a Bonferroni correction; the significance level was $p < 0.017$. There was a statistically significant 24% reduction from baseline one minute after cathodal stimulation ($Z = -2.312$, $p = 0.005$) and a 29% reduction five minutes after stimulation ($Z = -2.323$, $p = 0.005$). There were no significant differences between baseline and any time intervals for anodal stimulation.

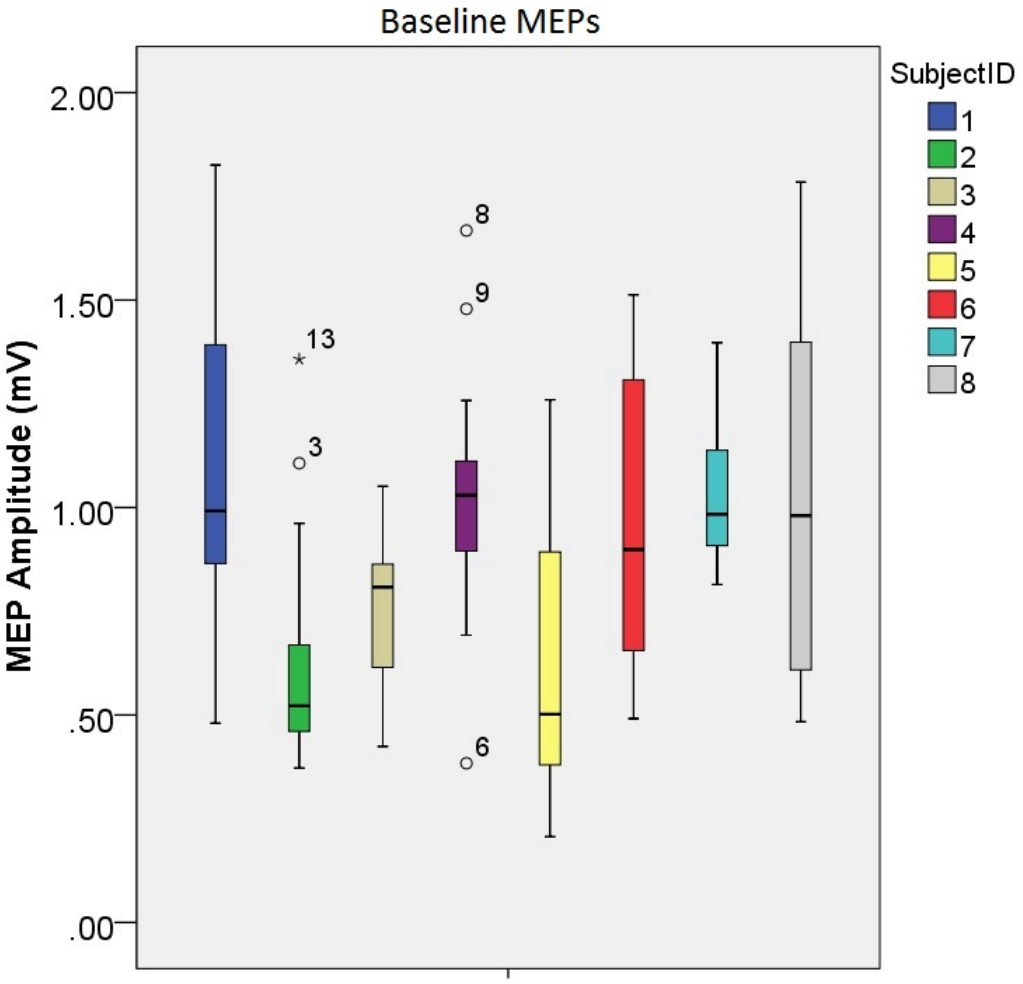


Figure 5.2: Example of the distribution of participants baseline 1DI MEP amplitudes at constant stimulator output for each subject. Outliers (*) and suspected outliers (°) are labelled by pulse number.

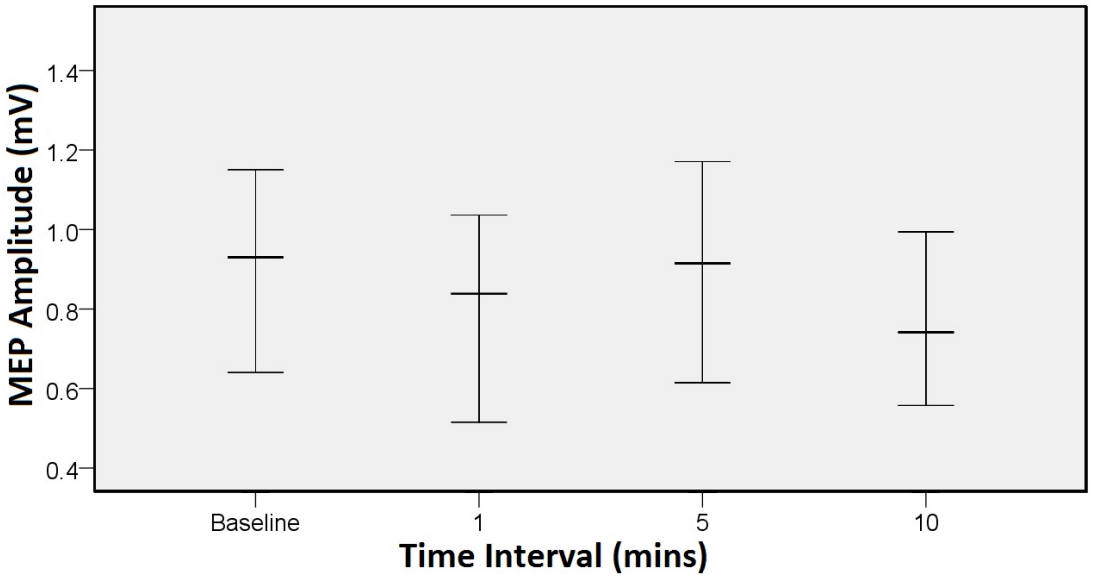


Figure 5.3: MEP amplitudes before and after anodal tDCS. The median and interquartile ranges are displayed. There were no significant differences in MEP amplitudes from baseline following anodal tDCS.

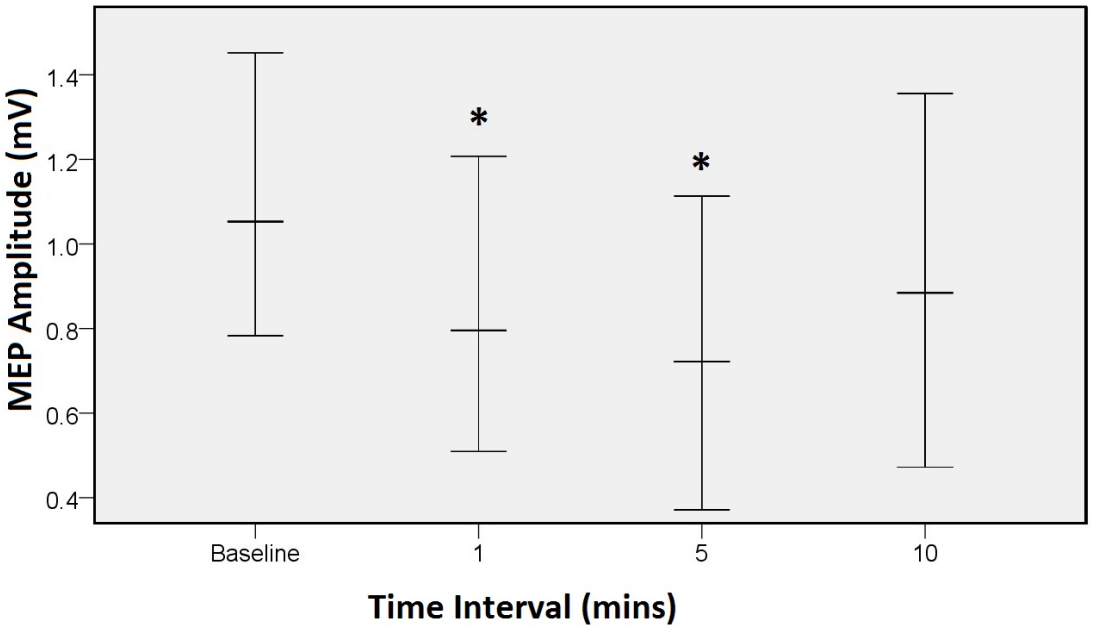


Figure 5.4: MEP amplitudes before and after cathodal tDCS. The median and interquartile ranges are displayed. There was a significant 24% reduction from baseline one minute post stimulation ($p = 0.005$) and a 29% reduction five minutes post stimulation ($p = 0.005$). Significant values are indicated by *.

5.2 2. Development of the Contraction Task

These experiments were carried out with the intention of assessing the suitability of different contraction tasks in an IMC trail. The tasks and the analysis used to assess them are detailed in section 4.4 of the Experimental Methods chapter.

5.2.1 First Contraction Task

Data Quality

In the first task EMG was recorded from four muscles (FCR, ED, 1DI and ADM) and examples of both good and poor raw EMG recordings are shown in Figure 5.5. The recording from 1DI is of good quality, demonstrating a clear EMG signal with low noise to signal ratio; the ADM recording is of poor quality, characterized by weak contraction strength and a low level of EMG pick up and is contaminated with background noise.

The frequency spectra from the two minute EMG recordings are shown in Figure 5.6 to highlight the differences in the signal content for recordings of different quality. Good quality data is characterised by a higher power output over the EMG band width and a clear frequency profile that illustrates peaks in both the α and β frequency ranges; poor quality data has low power output and a poorly defined frequency profile. In the top right of each graph a 95% confidence limit is shown and this can also be used to demonstrate the difference in the features of the spectra: the peaks in Figure 5.6a. are larger than this confidence level and therefore likely reflect separable EMG signal components. Such features are absent in Figure 5.6b.

EMG data quality, for each muscle, was evaluated by observing both raw EMG and frequency power spectra. In general EMG recordings from ADM for all par-

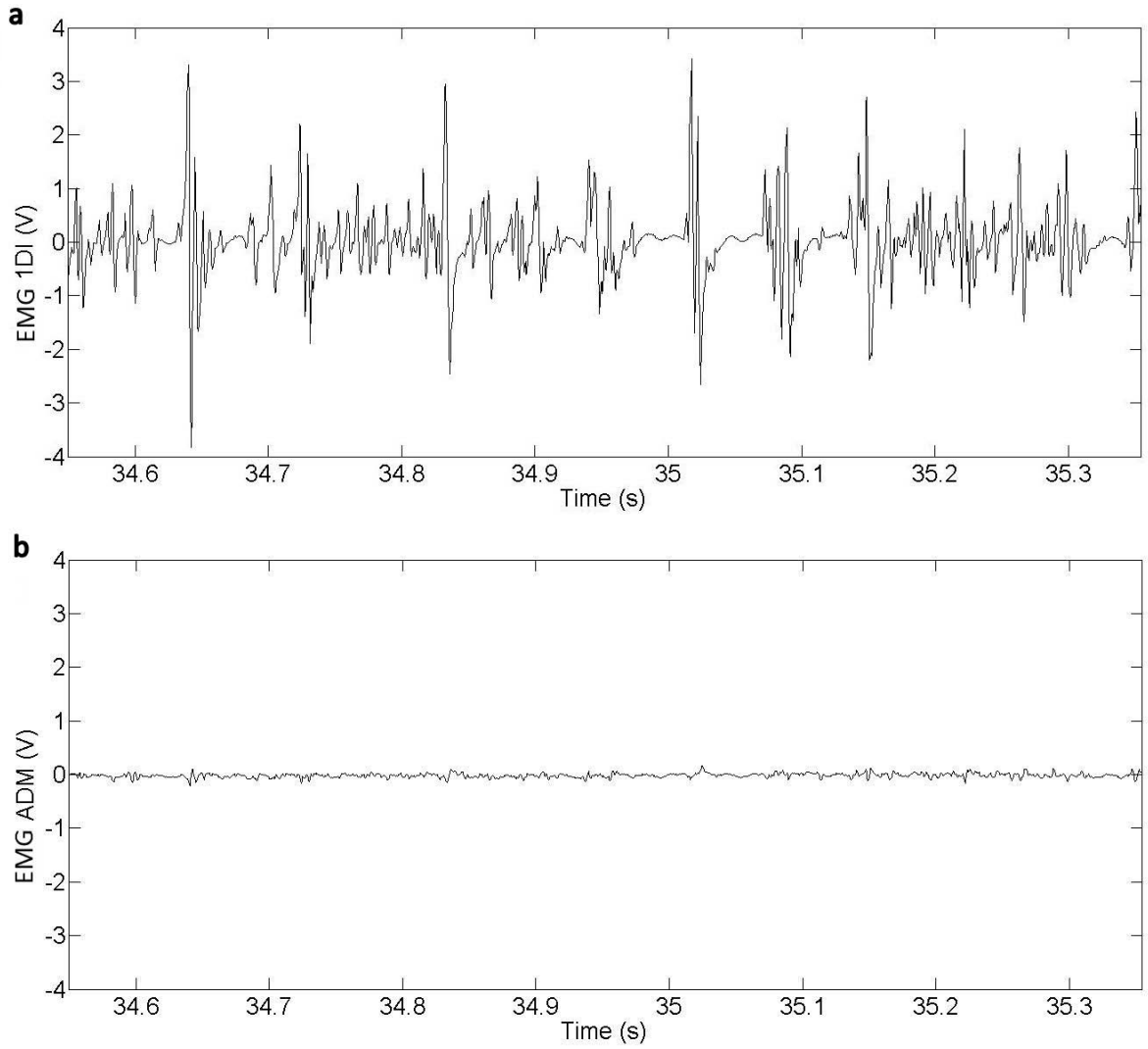


Figure 5.5: Examples of raw EMG data recorded in the first contraction task. a) Good quality data exhibits a strong contraction with low noise to signal ratio. b) Poor quality data exhibits a weak contraction and a high noise to signal ratio.

ticipants were consistently deemed of poor quality and were removed from further analysis.

Baseline Stability

For this analysis each participant's EMG data from the six trials was pooled. Figure 5.7 shows examples of the spectral outputs for ED and 1DI, note that the pooled data sets have the same characteristics as an individual one and are representative of good quality EMG recordings. A typical individual's pooled IMC for

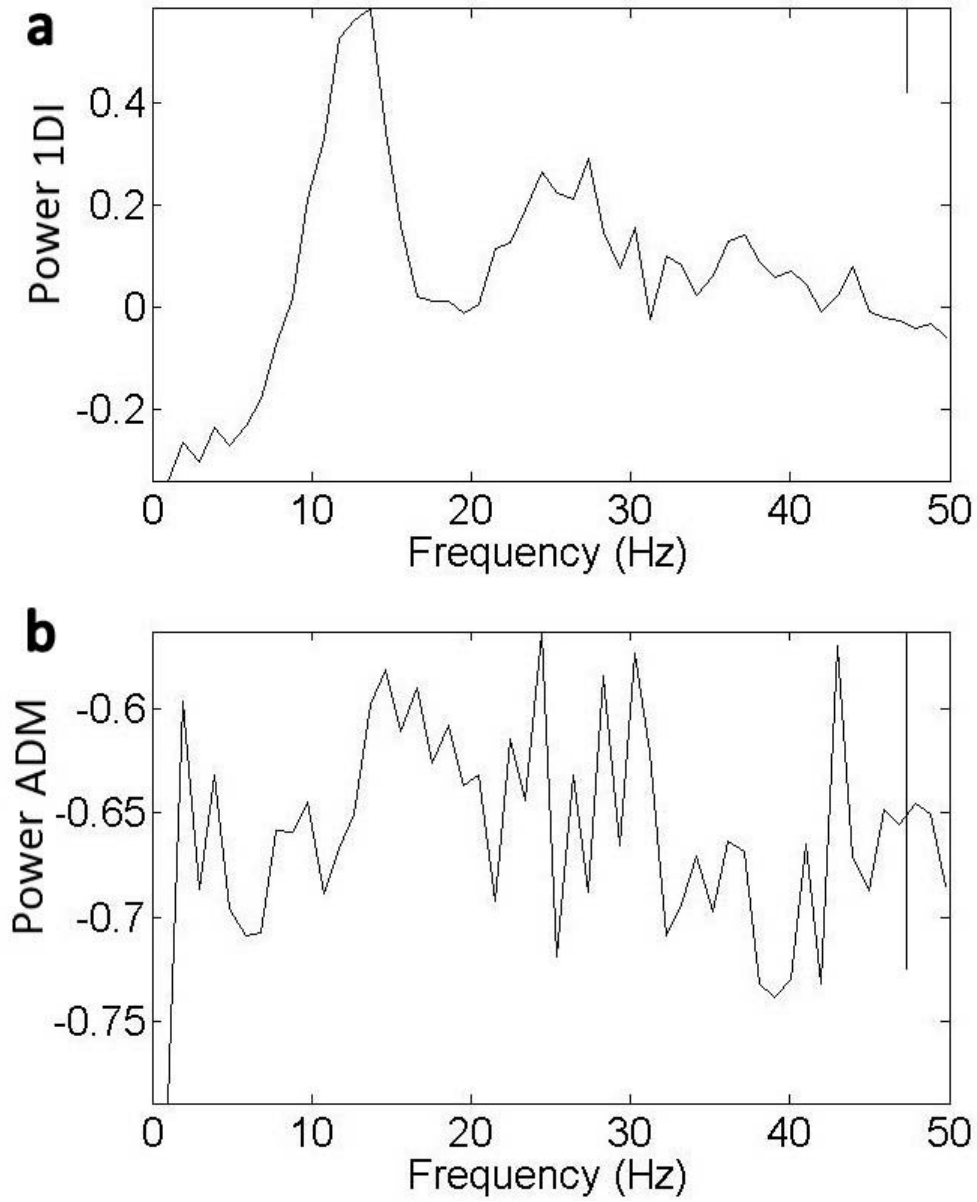


Figure 5.6: Example results of spectral analysis carried out on good and poor quality EMG data collected in the first contraction task. a) Good quality EMG exhibits a high power output and clear frequency profile with peaks in the α and β bands. b) Poor quality EMG has a low power output and a poorly defined frequency profile.

ED-1DI is shown in Figure 5.8. Coherence that is above the 95% confidence level (denoted by the dashed line) is considered significant. These data demonstrate that significant IMC exists in both the α and β bands.

To be used in an investigatory tool it is of vital importance that the task produce

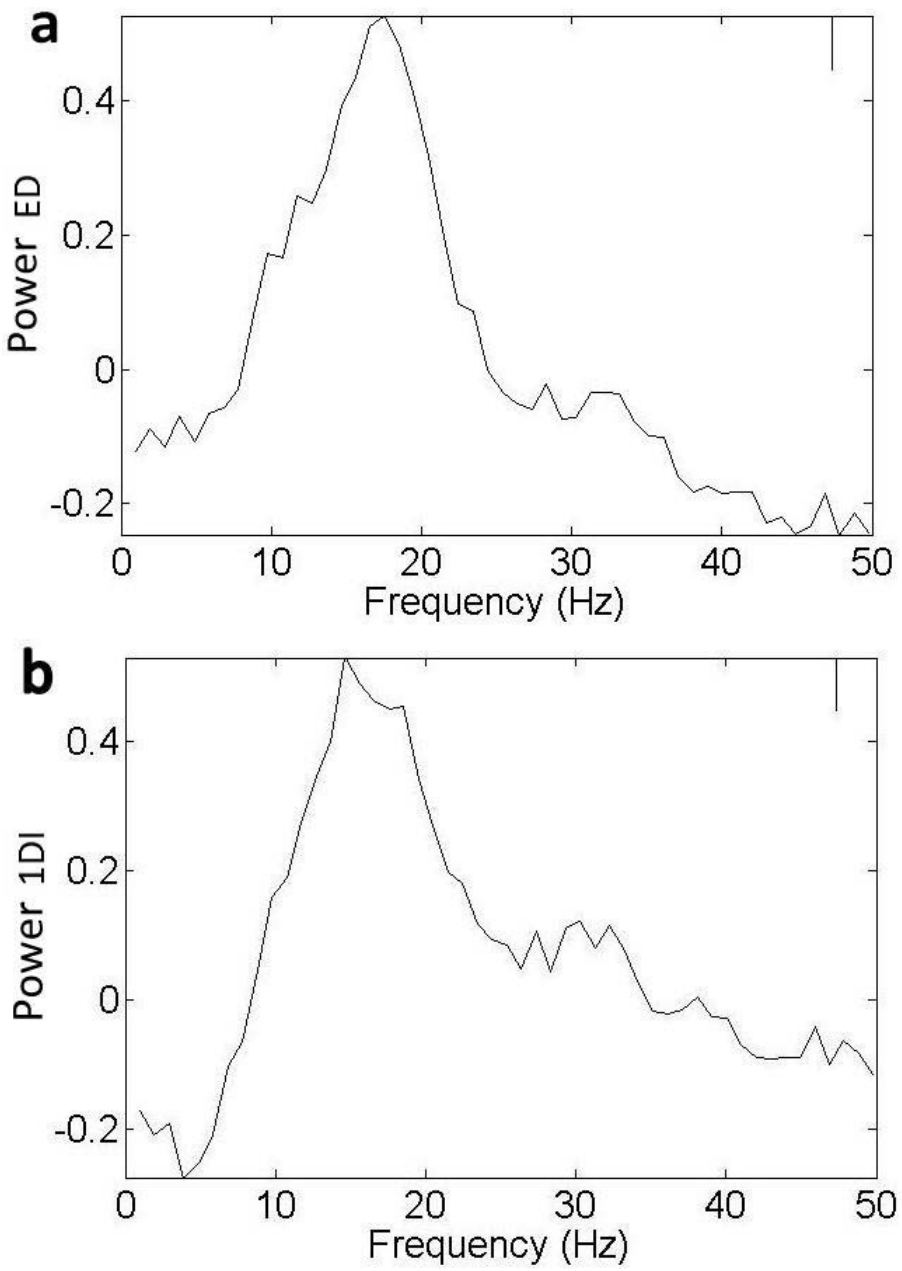


Figure 5.7: Example of an individual's pooled baseline spectra for muscles: a) ED and b) 1DI.

spectral or IMC data that exhibit baseline stability; that is, for each individual participant the spectra or IMC data do not significantly change when there is no intervention. The stability of each participants pooled spectra and coherence was evaluated using Neurospec's χ^2 extended difference of spectra and coherence tests. These tests analyse the six input data sets to identify frequencies where there were significant differences in either the spectra or coherence estimates for

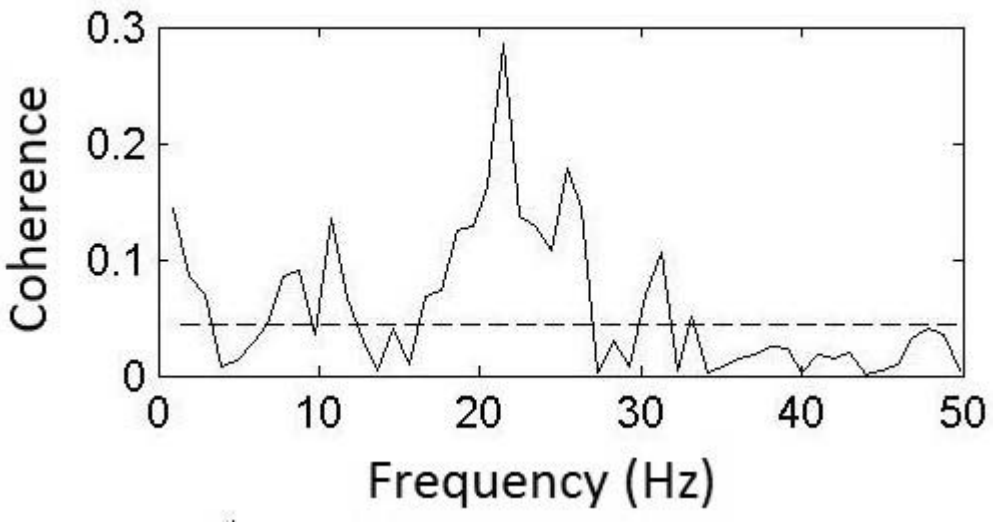


Figure 5.8: Example of an individual's pooled, baseline IMC between ED-1DI illustrating significant coherence in the α and β frequency ranges.

that individual's muscle or muscle pairs.

The χ^2 extended difference of spectra tests showed that for each participant there were significant differences in the input spectra for all of the muscles of interest. Example data from a participant's FCR and 1DI are shown in Figure 5.9; there were significant differences in both the α and β bands as demonstrated by power spectra levels above the 95% confidence level.

The χ^2 extended difference of coherence tests showed that for most individual's there were significant differences in the input IMC in both the α and β frequency ranges for almost all the muscle pairs. Figure 5.10 highlights the difference between a participant who demonstrates stability in their FCR-ED IMC and one who does not. For participant 1 (Figure 5.10a.) the difference in IMC does not exceed the 95% confidence interval and as such this participant was deemed to have stable IMC. Conversely for participant 2 the difference in IMC does exceed the confidence limit in both the α and β bands(Figure 5.10b.) . This participant was classified as exhibiting non stable IMC. Instability was very common amongst

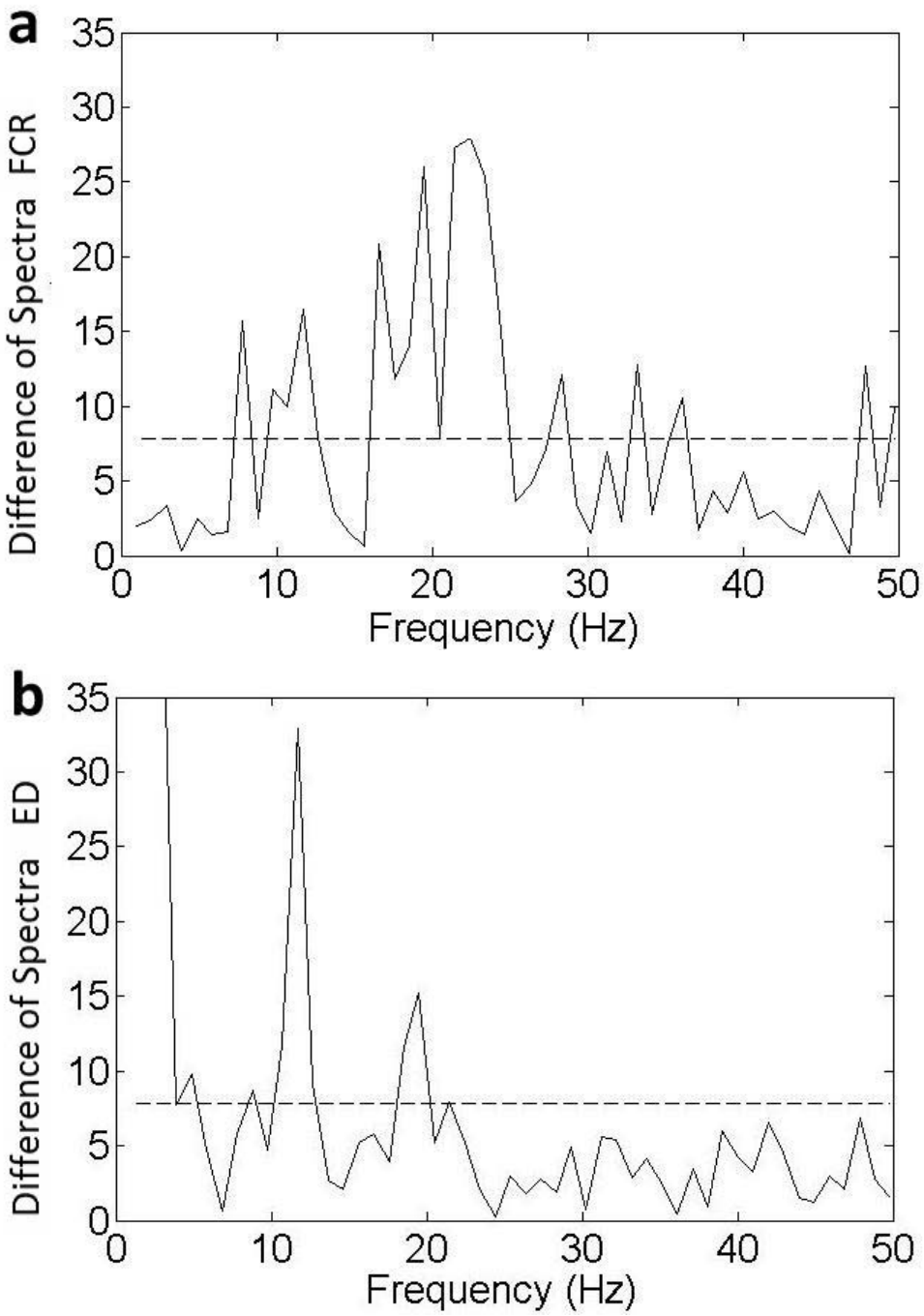


Figure 5.9: Typical results for the χ^2 extended difference of spectra test show that there are significant differences in the input spectra at a number of different frequencies. a)FCR, b) ED

the participants for this contraction task and Table 5.1 summarizes these results.

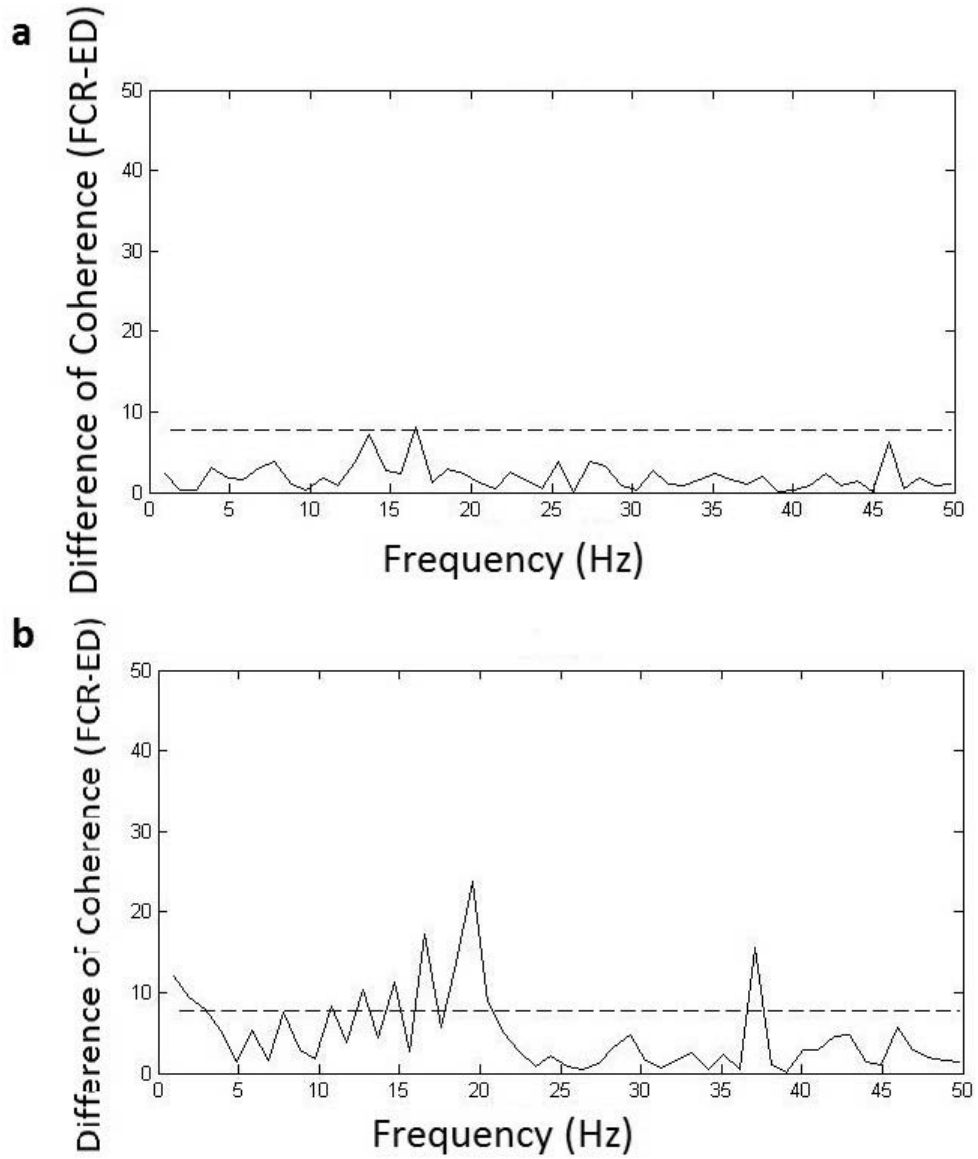


Figure 5.10: Example results for the χ^2 extended difference of coherence tests for FCR-ED. The dashed line represents the 95% confidence level, frequency components above this line represents frequencies in which the input data to the pooled set were significantly different. a) A participant demonstrating stability in their IMC: the χ^2 test shows no significant differences in the input IMC. b) A participant demonstrating instability in their IMC: the χ^2 test shows significant differences in both the α and β frequency ranges of the input IMC.

5.2.2 Second Contraction Task

The results from the first contraction task suggested that satisfactory compliance in completing the task was not achieved. To improve compliance the target muscle 1DI was changed to APB and the duration of the contraction was reduced

Muscle Pair	Participant 1	Participant 2	Participant 3	Participant 4
FCR-ED	✓	*	*	*
FCR-1DI	*	*	*	*
ED-1DI	*	*	*	*

Table 5.1: χ^2 extended difference of coherence results for each participant's muscle pairs for the first contraction task. Significantly different input to the χ^2 test is represented with * and non-significant data denoted with ✓. The table shows that all but one of the participant's data set resulted in significantly different input IMC.

from two minutes to one in order to reduce potential fatigue and to improve data quality.

Data Quality and Baseline Stability

EMG data quality was assessed as described above by observing both the raw EMG and the spectral plots. Further to the reduction in time to 1 minute it was also decided to discard ADM recordings as these proved difficult to obtain over the full time course of the test.

Each individual's pooled spectra and IMC data were evaluated for stability using χ^2 extended difference of spectra and coherence tests, as discussed above. Again there was no stability exhibited in the spectral data. There was some improvement in IMC stability, but the majority of participants did not exhibit it in their muscle pairs. The results are summarized in Table 5.2.

5.2.3 Third Contraction Task

The results from the first two contraction tests suggested that the task should be revised; here a simpler one minute co-contraction of 1DI and ED was implemented.

Muscle Pair	Participant 1	Participant 2	Participant 3
FCR-ED	*	✓	*
FCR-1DI	*	*	✓
ED-1DI	✓	*	*

Table 5.2: Summary of results for χ^2 extended difference of coherence tests for the second contraction task. This table illustrates that the majority of individuals exhibited significantly different inputs to their pooled IMC for the muscle pairs of interest.

Data Quality

Data quality was very good for these EMG recordings and a typical EMG trace for ED is shown in Figure 5.11; there is a clear EMG from the muscle and a low noise to signal ratio. The good quality of the data was also reflected in the muscle spectra (Figure 5.12) which shows data from both 1DI and ED. Inter-muscular coherence was present between the signals and clear peaks are displayed in the β band Figure 5.13.

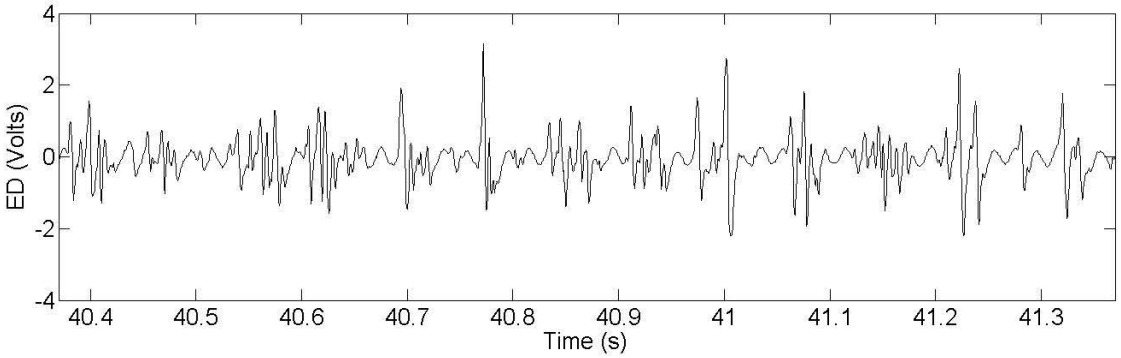


Figure 5.11: Typical example of raw EMG collected in the third contraction task. This data is of good quality with a strong contraction and a low noise to signal ratio.

Baseline Stability

The χ^2 extended difference in spectra tests showed that, again, individuals did not demonstrate stability in their input baseline spectra across the frequencies of interest. Figure 5.14 shows an example of this data recorded from both ED and 1DI. Figure 5.15 shows a typical result for the χ^2 extended difference of coherence

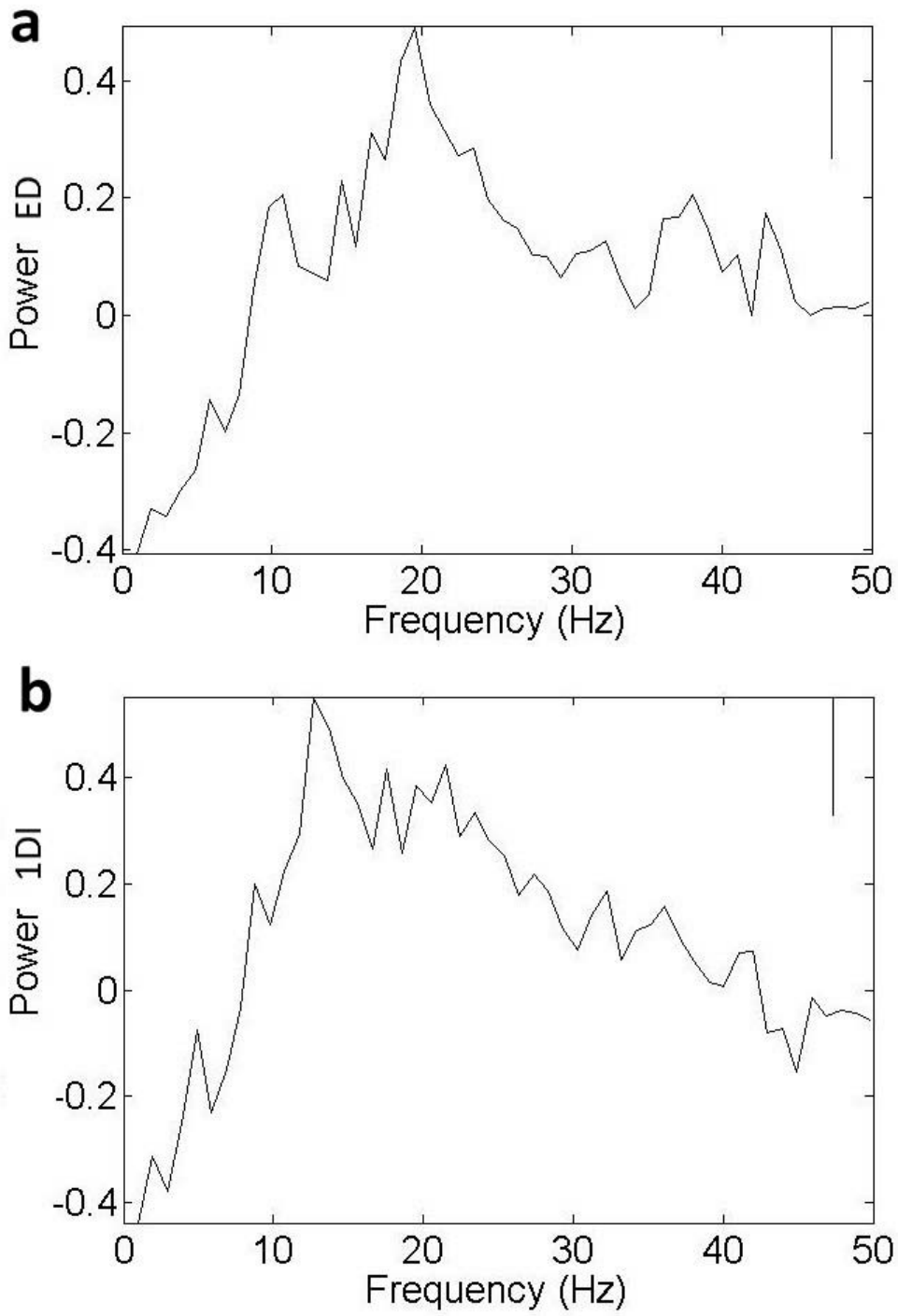


Figure 5.12: Typical example of muscle spectra from a) ED and b) 1DI EMG recordings for the third contraction task.

test; for this task there are no IMC components above the 95% confidence interval indicating that the input data were not significantly different and that IMC was stable. This was true for all participants.

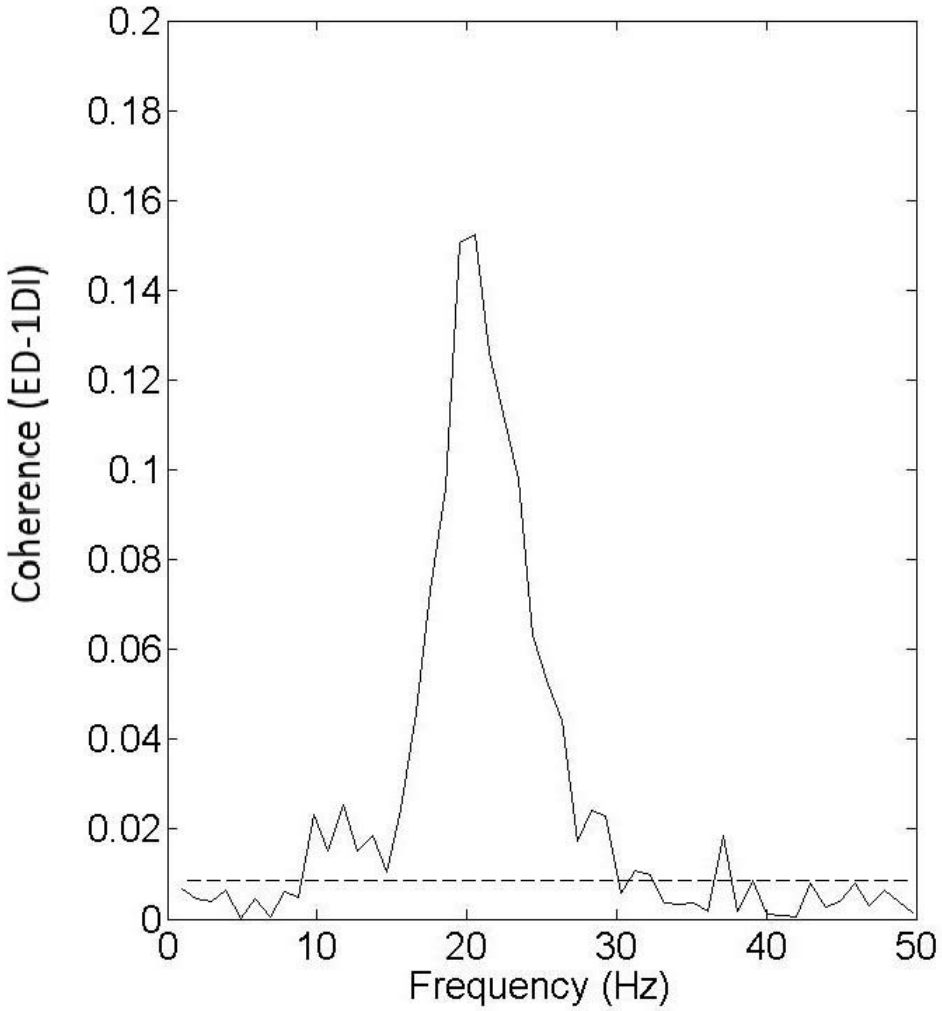


Figure 5.13: Typical example of IMC between ED and 1DI for the third contraction task.

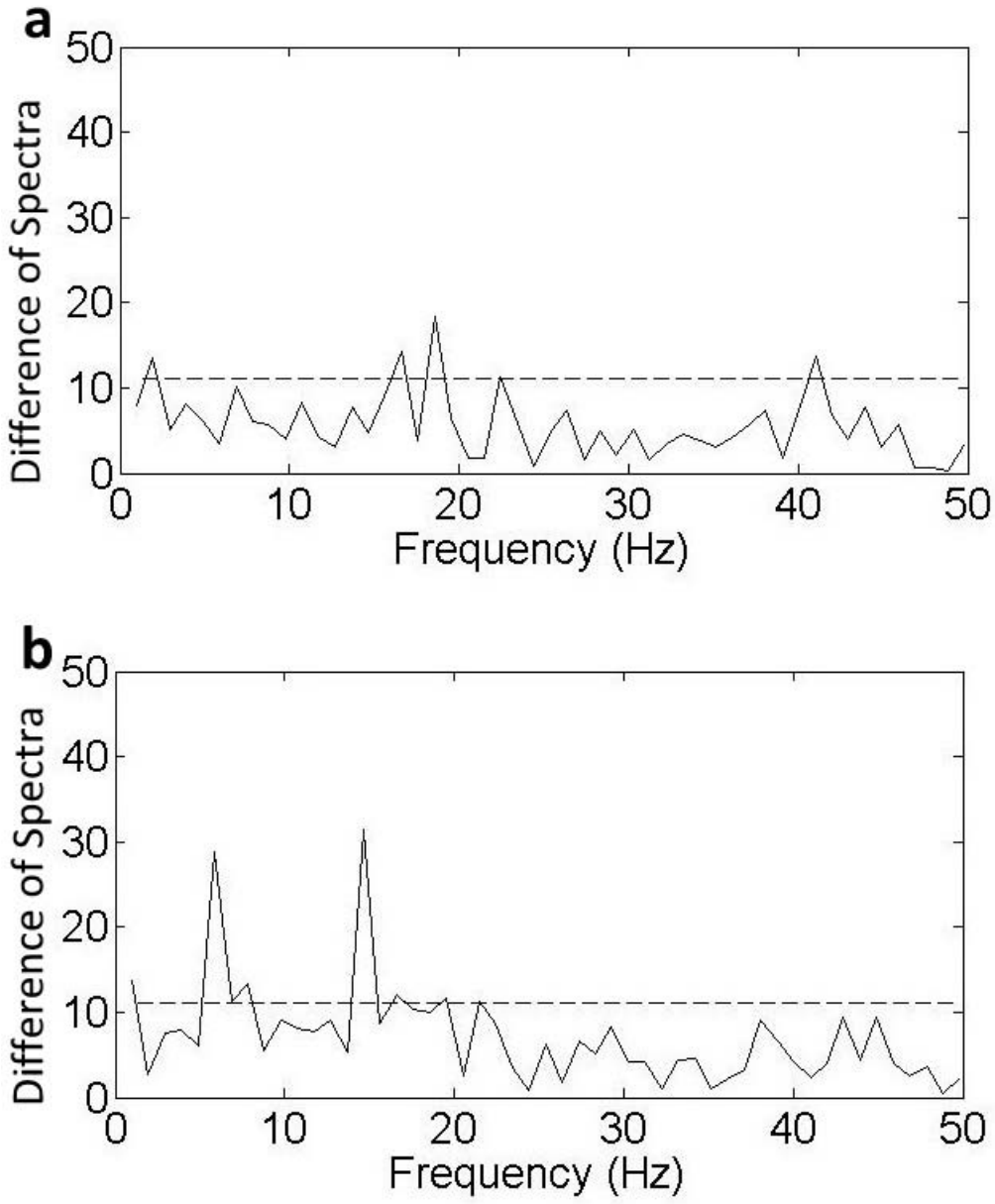


Figure 5.14: Typical result for the χ^2 extended difference of spectra test conducted on an individual's pooled spectra. Here it is seen that for both ED and 1DI there are significant differences in the input spectra at a number of frequencies

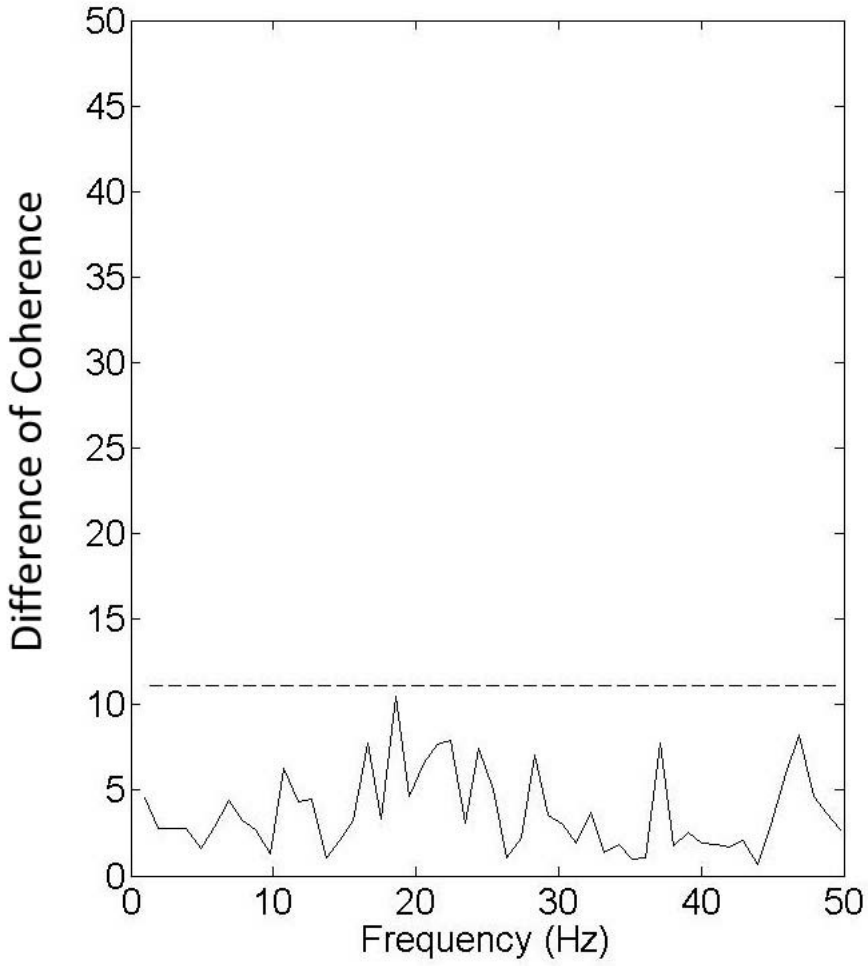


Figure 5.15: Typical result for the χ^2 extended difference of coherence test for an individual's pooled ED-1DI coherence. There are no significant differences in the input coherences.

5.3 3. Transcranial Direct Current Stimulation, Inter-Muscular Coherence and Physiological Neurogenic Tremor

In this test the aim was to assess the claim that anodal and cathodal tDCS affect IMC in a polarity dependent way. The effects of anodal and cathodal tDCS on neurogenic tremor were also investigated. The methods are detailed in section 4.5 and the results are detailed below.

- Number of subjects: 10
- Stimulation type: Anodal, Cathodal DC and Sham
- Muscles: ED & 1DI

5.3.1 Inter-Muscular Coherence

Data Quality and Baseline Stability

All data sets were deemed of good quality. The stability of each participants baseline coherence was investigated using the χ^2 extended difference of coherence test. Two of the participants did not demonstrate stability in their IMC baseline and a third began taking prescribed muscle relaxant medication partway through the testing. The data from these three participants were removed from further analysis leaving n = 7.

Comparison of Coherence Analysis

The data from the seven remaining subjects were pooled together to make data sets for each intervention (anodal, cathodal and sham). Figure 5.16 shows a typical example of the pooled ED-1DI coherence. This data set is of baseline IMC before anodal stimulation was delivered and shows that coherence between ED

and 1DI spanned both the α and β frequency bands.

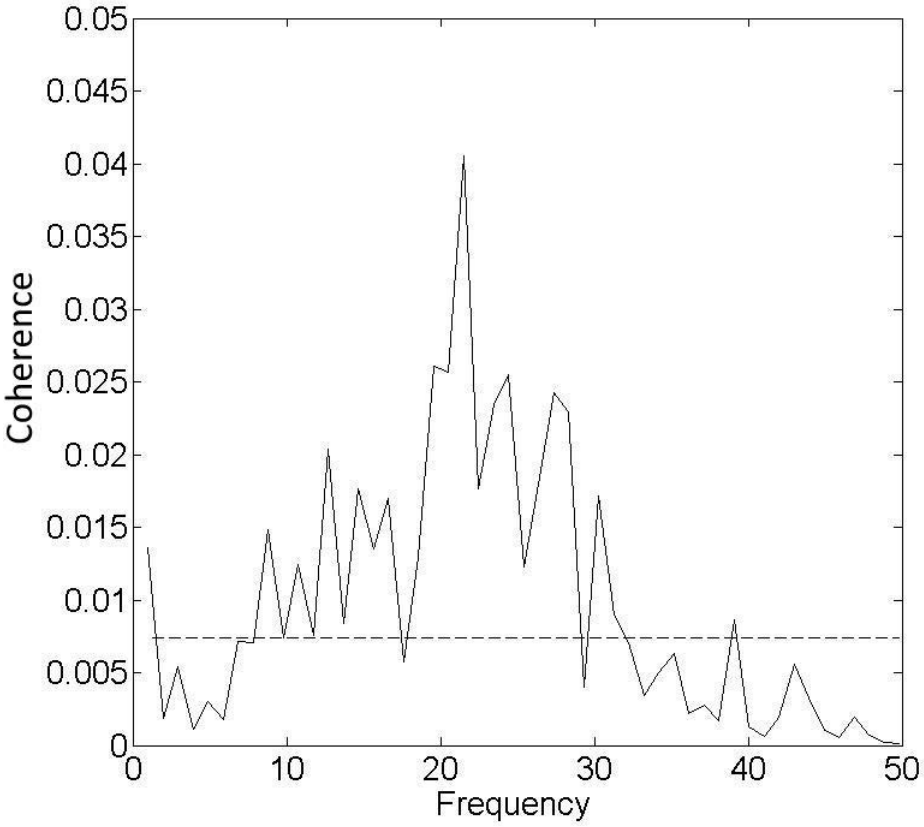


Figure 5.16: Typical example of pooled baseline IMC. There is coherence in both the α and β bands.

Comparison to Baseline

Comparison of coherence tests were used to assess if IMC at each post stimulation time interval (1, 5, 10, 15, 20, 25 and 30 minutes) had changed relative to the pre-stimulation baseline measure. The comparison of coherence test is described in section 4.5.2. The output plots illustrate where the frequency of one of the data sets is significantly larger or smaller than the other. Example output from the comparison of coherence test is shown in Figure 5.17 which is a comparison of coherence between baseline and sham tDCS five minutes post stimulation. The solid lines represent the 95% confidence intervals; at (8 - 10) Hz the post stimulation data is larger than baseline and at approximately 20 Hz its lower than

baseline. For all other frequencies there had been no significant difference.

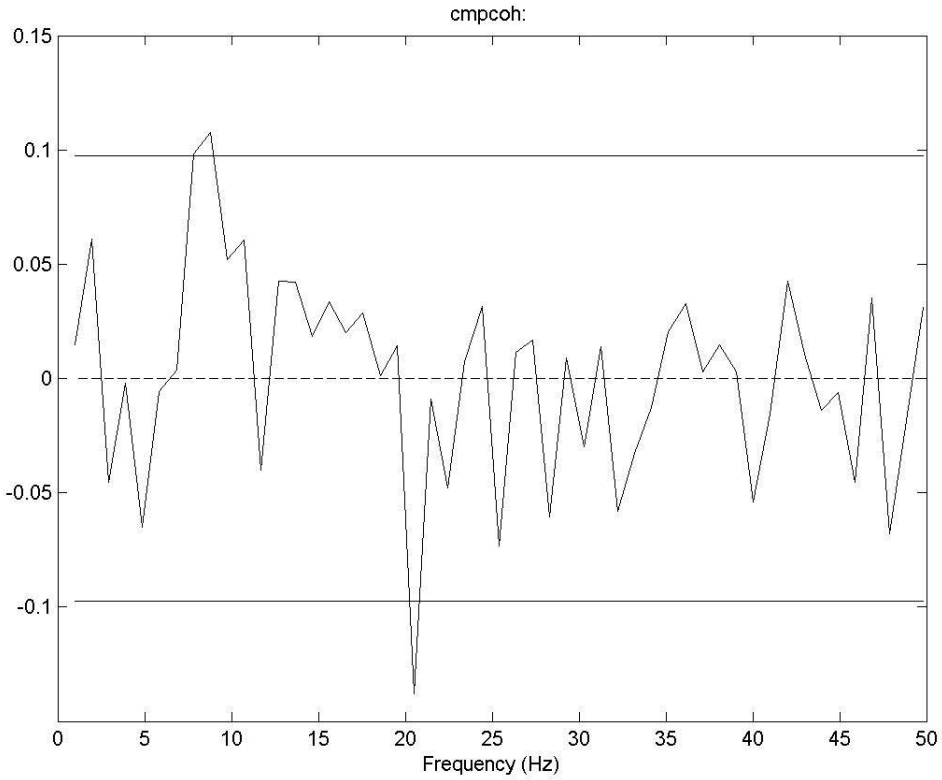


Figure 5.17: Example comparison of coherence analysis output shows the coherence comparison for baseline and five minutes post sham tDCS. The solid lines represent the 95% confidence intervals. There was a significant increase compared to baseline at (8 - 10) Hz and a simultaneous reduction around 20 Hz.

Comparison of coherence tests between baseline and each time interval were conducted for each of the three interventions (anodal, cathodal and sham tDCS). The data were amalgamated in filled contour time-frequency plots in order to illustrate post stimulation changes in IMC over the entirety of the test time intervals. Caution should be employed when viewing these plots as they do not represent a continuous change in the post intervention coherence, but rather the coherence at each of the time points of interest. The significant differences that were described in Figure 5.17 are also represented in Figure 5.19 at the five minute time interval. The (8 - 10) Hz significant increase is shown in hot colours, the 20 Hz significant

decrease in cold colours and all non-significant changes remain white.

There were small but significant changes from baseline for both anodal (Figure 5.18a) and cathodal tDCS (Figure 5.18b). Anodal stimulation resulted in small increases in β band IMC, but only for the 5, 25 and 30 minute time intervals. There were no changes to α IMC. Cathodal stimulation caused small reductions in β IMC at 5, 10 and 25 minutes and an increase at 20 minutes. There were also increases in α IMC at 20 and 30 minutes.

Of particular interest there were significant changes from baseline when compared with the sham intervention (Figure 5.19). The results were dominated by increases in the α band at 5 and 10 minutes and again at 20 minutes. In the β band there were reductions at 1 and 5 minutes followed by increases at 15 and 25 minutes. This would suggest that a simple comparison against baseline is not capturing the true effect and that comparison to sham is a more appropriate test.

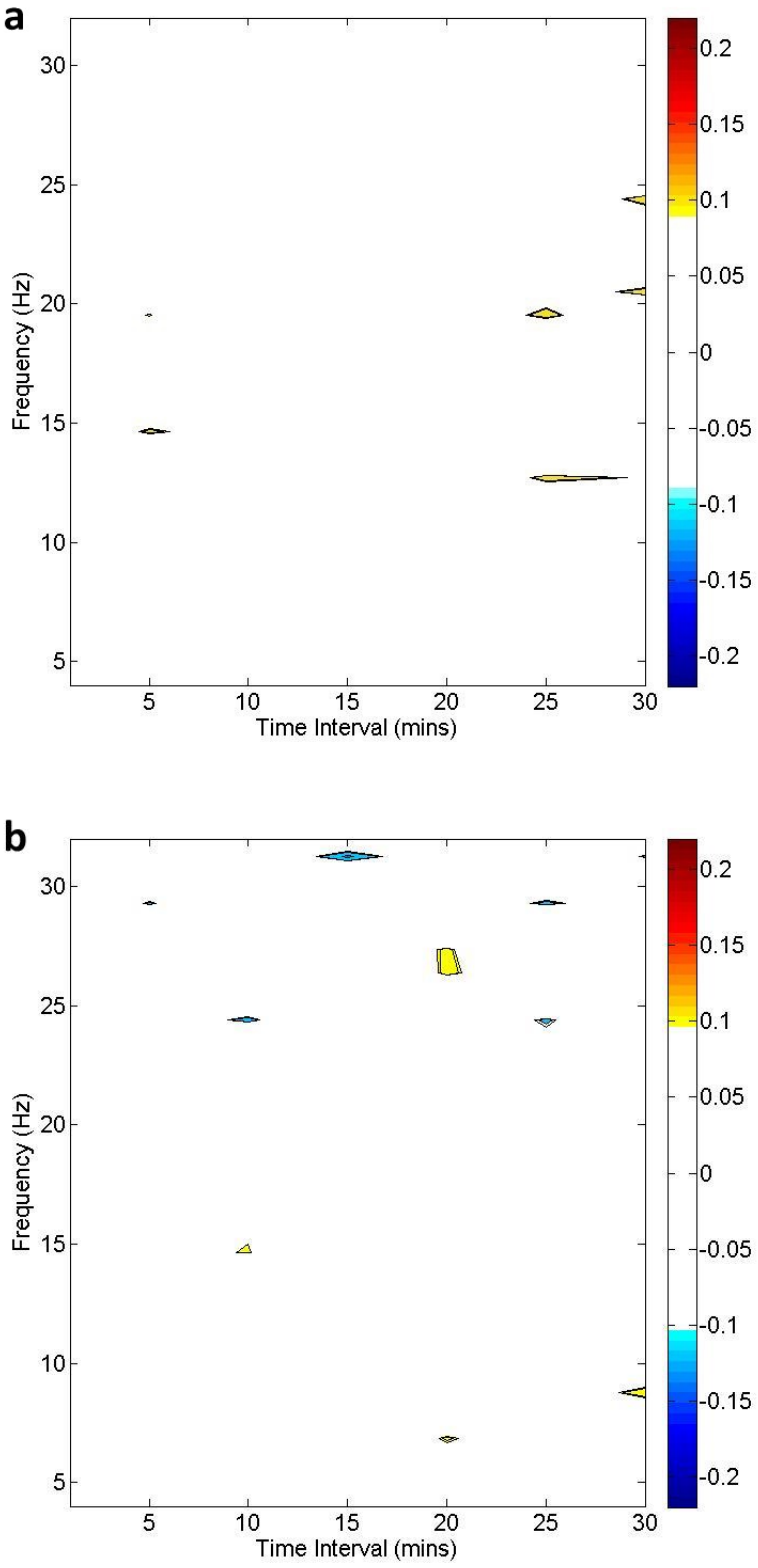


Figure 5.18: Comparison to baseline IMC following tDCS. a) Compared to baseline anodal tDCS caused significant increases in the β band. b) Compared to baseline IMC cathodal tDCS caused increases in the α and mostly decreases in the β band.

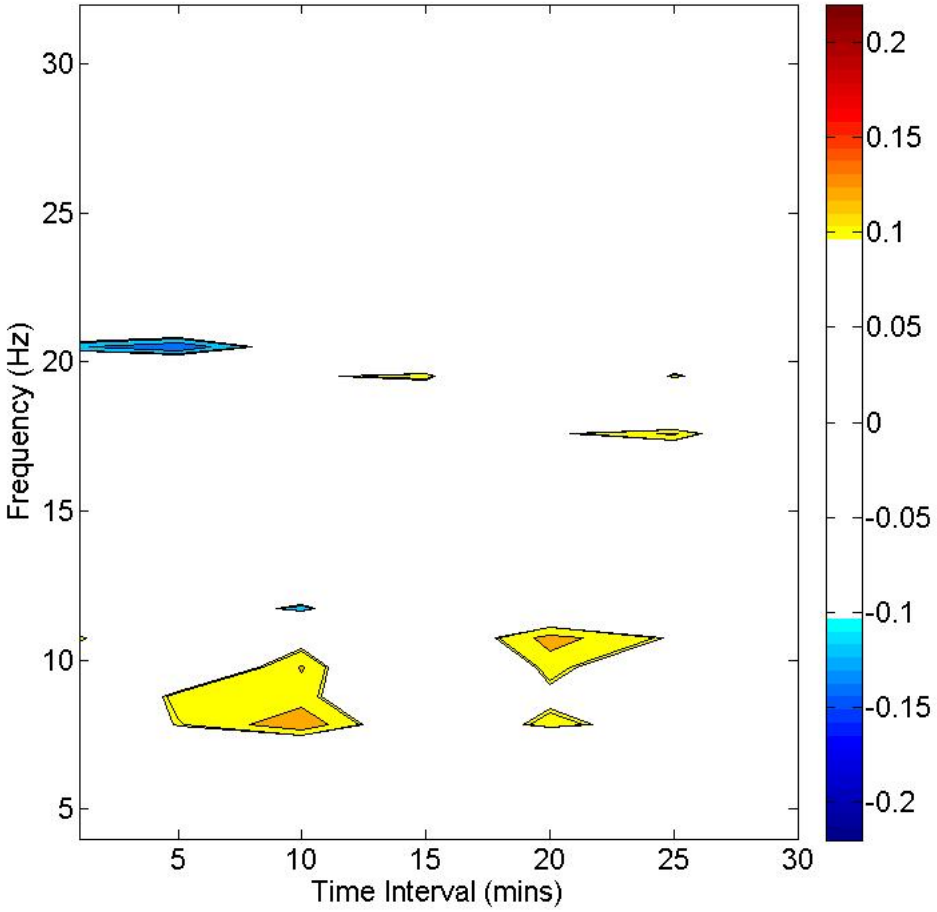


Figure 5.19: Comparison to baseline IMC following sham tDCS. There are significant increases in the α and both increases and decreases in the β band.

Comparison to Sham tDCS

A comparison of coherence between the sham and the intervention at each time point was undertaken. The results, shown in Figure 5.20 are similar for anodal and cathodal tDCS and appear to be split into significant changes that occurred in the first ten minutes and those that occurred in the final ten minute testing periods. The β band was dominated with increases in IMC following both polarities: anodal tDCS caused increases at 1 and 5 minutes which recurred between 15 and 30 minutes post intervention; cathodal tDCS caused changes in the first ten minutes after stimulation which recurred at the 25 and 30 minute test intervals. In the α band both interventions resulted in increased IMC being observed at 10 minutes and a reduced IMC at 5 minutes. For cathodal tDCS this reduction was stronger and persisted to the 10 minute test interval.

The effects on the β band appear to have been stronger after anodal stimulation and alternatively changes that occurred to the α band appear to be stronger following cathodal stimulation.

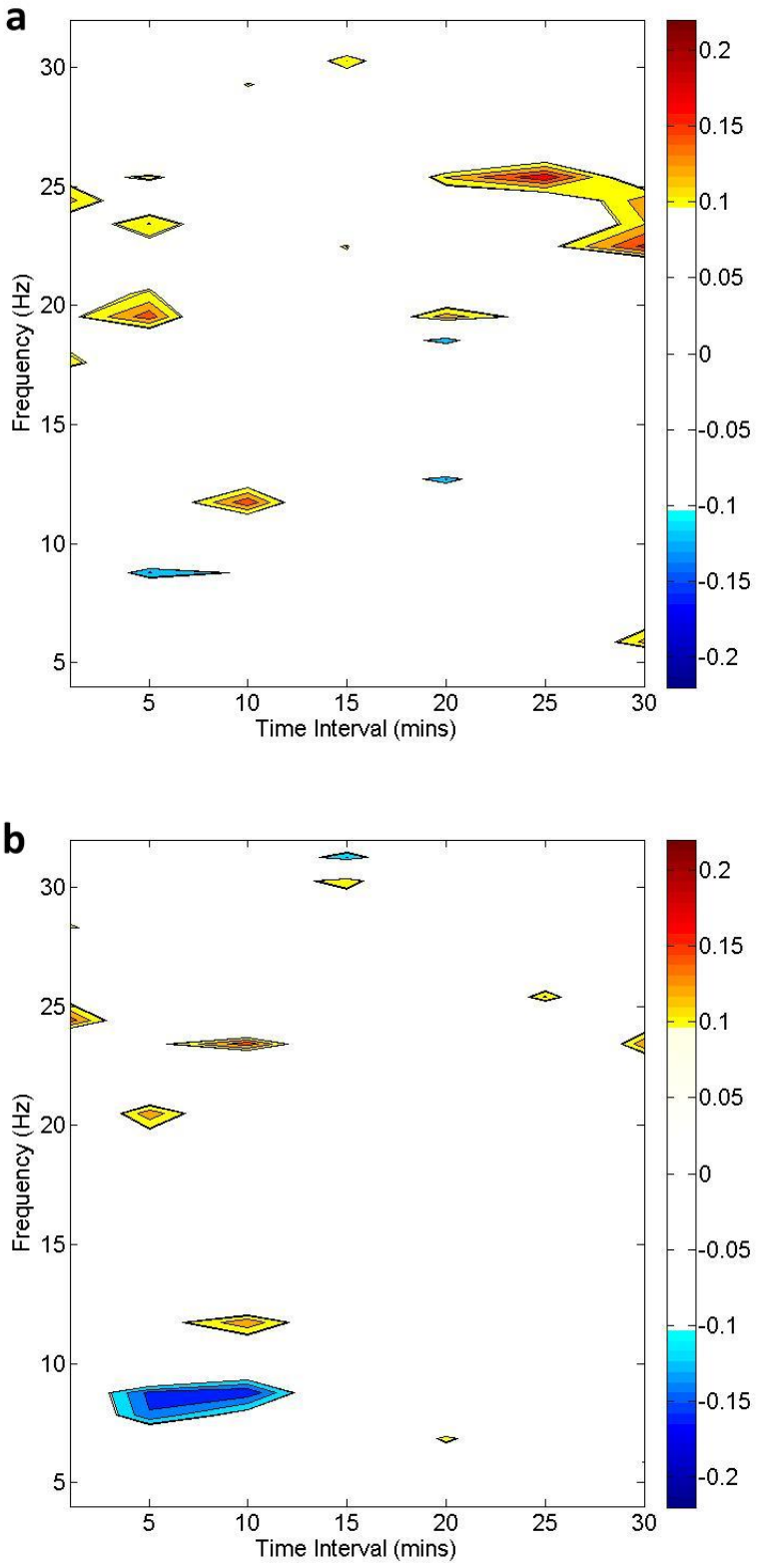


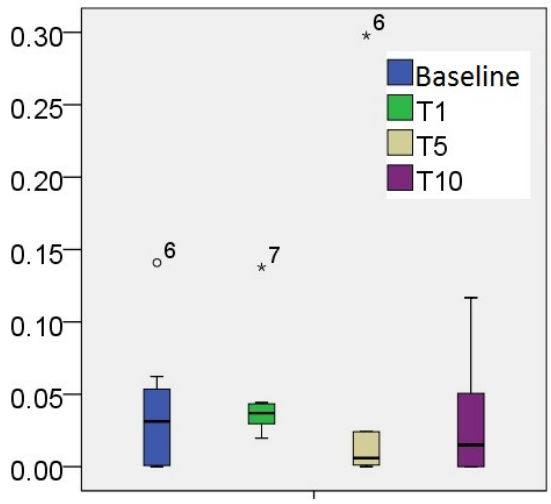
Figure 5.20: Comparison to sham tDCS following tDCS. a) Anodal tDCS caused both significant increases and decreases in the α band and increases in the β band. b)Cathodal tDCS caused significant increases and decreases in the α band and increases in the β band.

Alternative Analysis: Average Significant Coherence

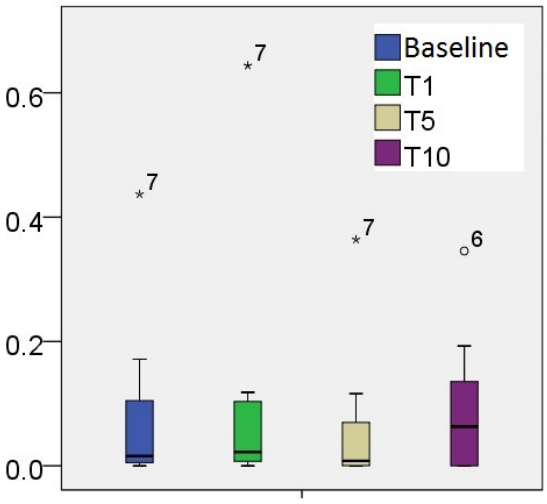
For the purposes of comparing this trial with the results of Power et al. (2006) their analysis was reproduced here. A description of this analysis is given in the Methods, section 4.5.3. For the α and β frequency bands the average sum of the significant area under the coherence curve and above the 95% confidence level was calculated. Figure 4.10 illustrates the data that were included in this analysis. In direct comparison with Power et al. (2006) this protocol was only conducted on the baseline and 1, 5 and 10 minute post stimulation tests.

These data sets were tested for normality using the Shapiro-Wilk test. The α frequency band IMC data was not normally distributed for any time intervals in the three interventions ($p < 0.05$). Figure 5.21 is a box plot which illustrates the non-normal distribution of the participants' data. For the β frequency range the participants' IMC data were normally distributed ($p > 0.05$) and this is illustrated in Figure 5.22.

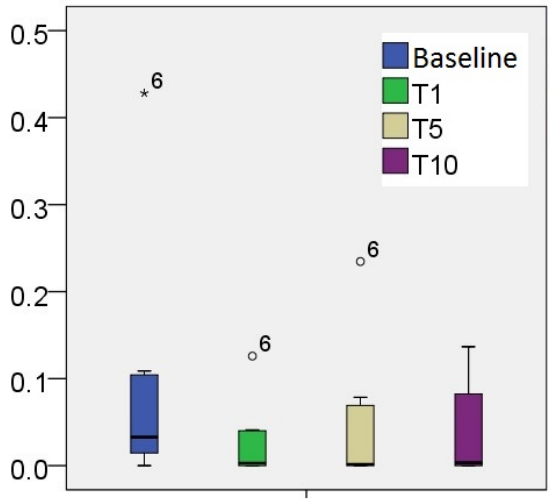
Due to the non-parametric distribution of the α data set a Friedman's test was implemented to test for significant differences between the baseline and each of the three post stimulation time intervals. There were no significant differences from baseline for any tDCS intervention: anodal $p = 0.398$, cathodal $p = 0.768$ and sham $p = 0.461$. For the normally distributed β band data a one way ANOVA was implemented. There were no significant differences from baseline for any tDCS intervention: anodal $p = 0.891$, cathodal $p = 0.478$ or sham $p = 0.650$.



(a) α band, anodal tDCS

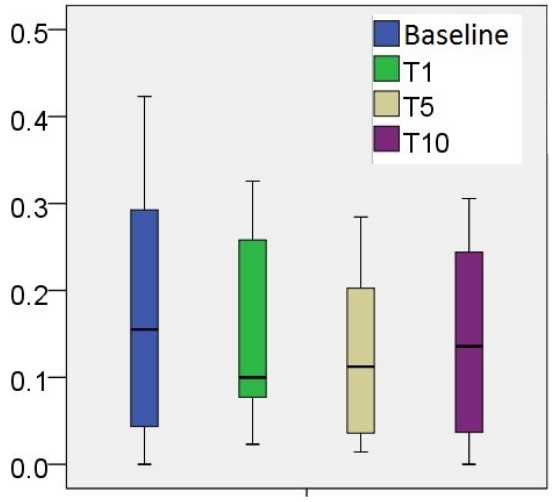


(b) α band, cathodal tDCS

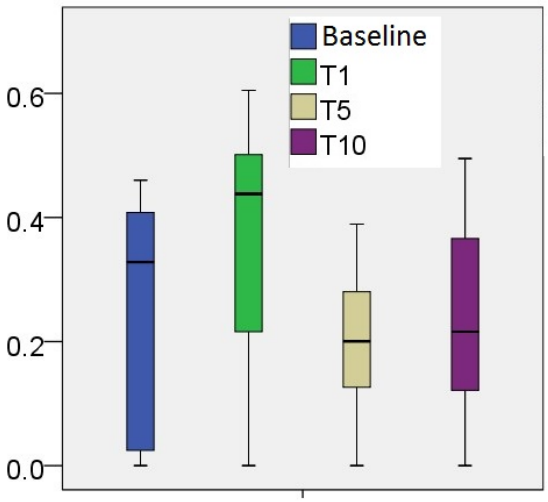


(c) α band, sham tDCS

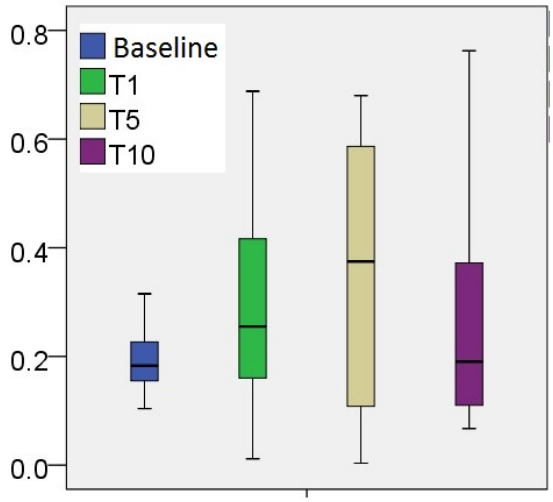
Figure 5.21: Box plots illustrating the distribution of the average IMC in the α band. Data in the α band were not normally distributed. Suspected outliers (°) and extreme outliers (*) are labelled by subject number.



(a) β band, anodal tDCS



(b) β band, cathodal tDCS



(c) β band, sham tDCS

Figure 5.22: Box plots illustrating the distribution of the average IMC in the β band. Data in the β band were normally distributed.

5.3.2 Phsyiological Neurogenic Tremor

An investigation into any effects of tDCS on PNT was also carried out. The results for the coherence between the accelerometer recording and each of the muscles are presented here.

Data Quality and Baseline Stability

Accelerometer record quality was evaluated in the same way as EMG, described above; all data sets were deemed acceptable. A raw accelerometer trace is shown in Figure 5.23 and illustrates the repetitive bursts of activity characteristic of tremor. Spectral analysis describes the frequency content of the signal and is shown in Figure 5.24. There is a peak between (8 - 12) Hz representative of the neurogenic component of tremor, and there are peaks between (15 - 30) Hz that are representative of higher frequency neurogenic components in the β band and the load sensitive mechanical reflex components of tremor.

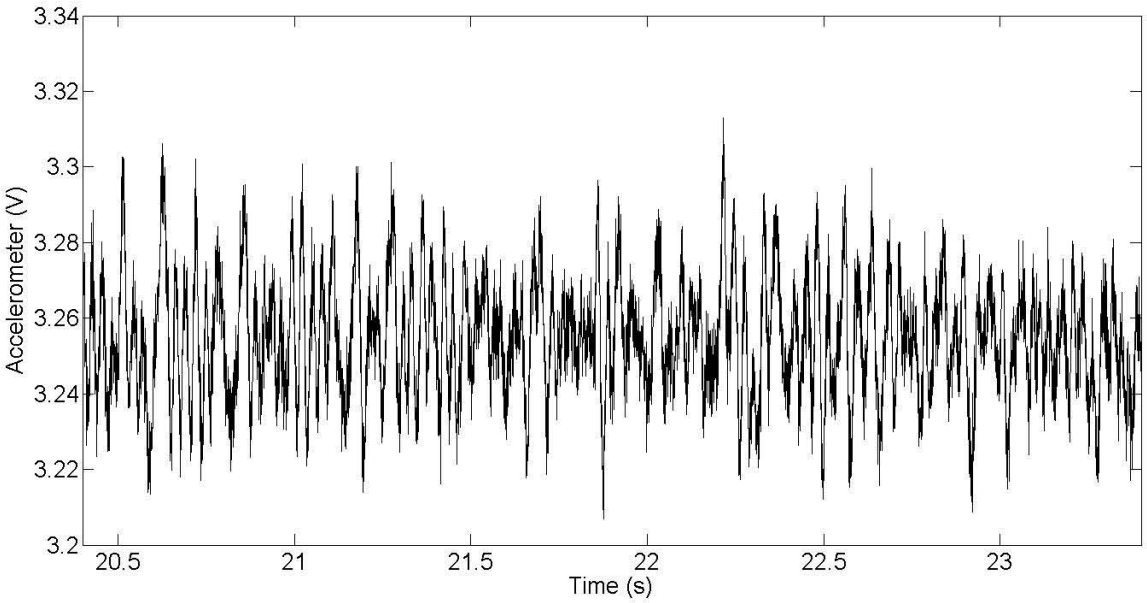


Figure 5.23: Example raw accelerometer trace.

Baseline spectra and coherence stability of PNT was investigated using χ^2 differ-

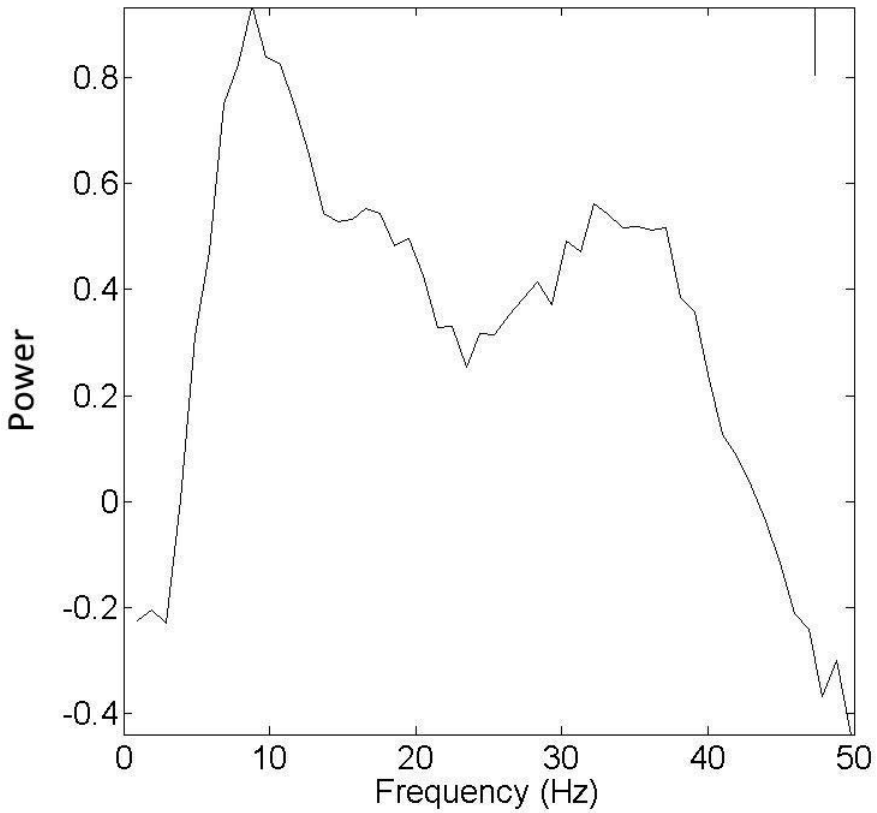


Figure 5.24: Typical accelerometer spectral data showing the main neurogenic component of tremor as a peak between (8 - 12) Hz as well as the β neurogenic component.

ence of spectra and χ^2 difference of coherence tests. This is the same analysis that was used to evaluate the EMG data which has already been described above. χ^2 extended difference of spectra tests showed that for each participant there were significant differences in the input accelerometer spectra. These differences were observed across a range of frequencies and were always present in the (8 - 12) Hz PNT range. Figure 5.25 shows a typical participant's data set, as for EMG all components above the dashed 95% confidence level represent frequencies that are significantly different amongst the input spectra to the pooled data set for that individual. Note that in the (8 - 12) Hz region there are significant differences in the input spectral data. As for EMG spectral analysis PNT spectral analysis was deemed an inappropriate tool due to this instability in the absence of an

intervention.

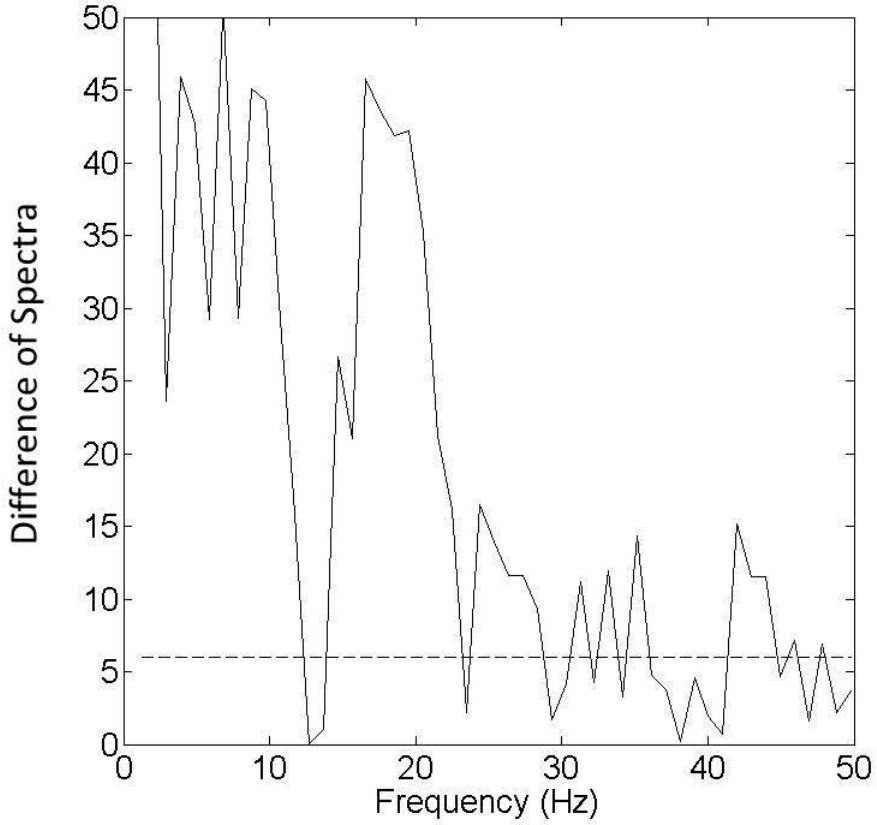


Figure 5.25: Typical result for the χ^2 extended difference of spectra test in which the input data sets are significantly different over a range of frequencies.

Coherence between the EMG trace and the accelerometer was observed for both ED-accelerometer (ED-A) and 1DI-accelerometer (1DI-A). Figure 5.26 is a typical example and shows coherence in the neurogenic α and β ranges reflecting muscle input into these tremor components.

As for IMC the baseline stability for ED-A and 1DI-A was evaluated using χ^2 extended difference of coherence tests. For every participant there were significant differences in the input ED-A coherence data sets across a range of frequencies and in particular in the (8 - 12) Hz range. Conversely for most participants there

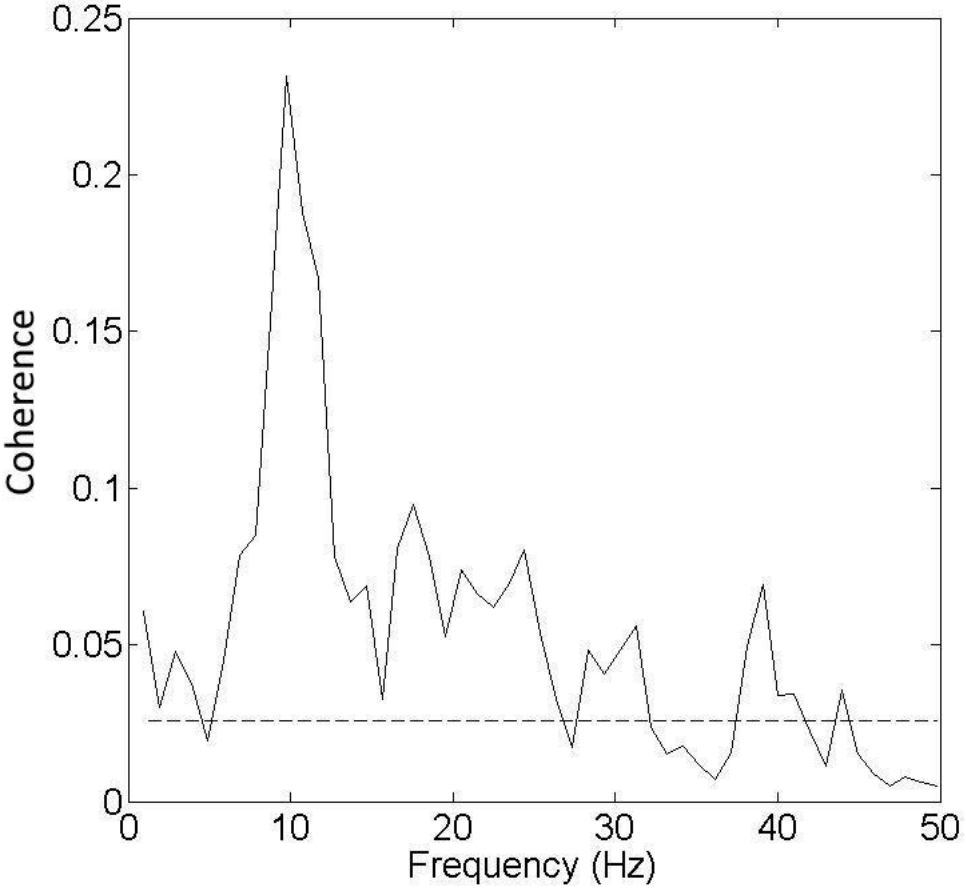


Figure 5.26: Typical example of coherence between the EMG recording and the accelerometer.

were no significant difference in the input 1DI-A data in the (8 - 12) Hz range, although there were at other frequencies. Examples of these results are shown in Figure 5.27: Figure 5.27.a. illustrates the significant differences observed for ED-A, Figure 5.27.b. shows that for 1DI-A there were no significant differences in the input coherence in the (8 - 12) Hz range. Since stability in 1DI-A was not always observed at other frequencies only the (8 - 12) Hz range is shown in the following time frequency plots. This is also the main frequency range of interest for PNT.

Interestingly the same three participants who had been removed from IMC analysis due to their unstable baselines did not demonstrate stability in 1DI-A either and were removed from the following analysis too; n = 7.

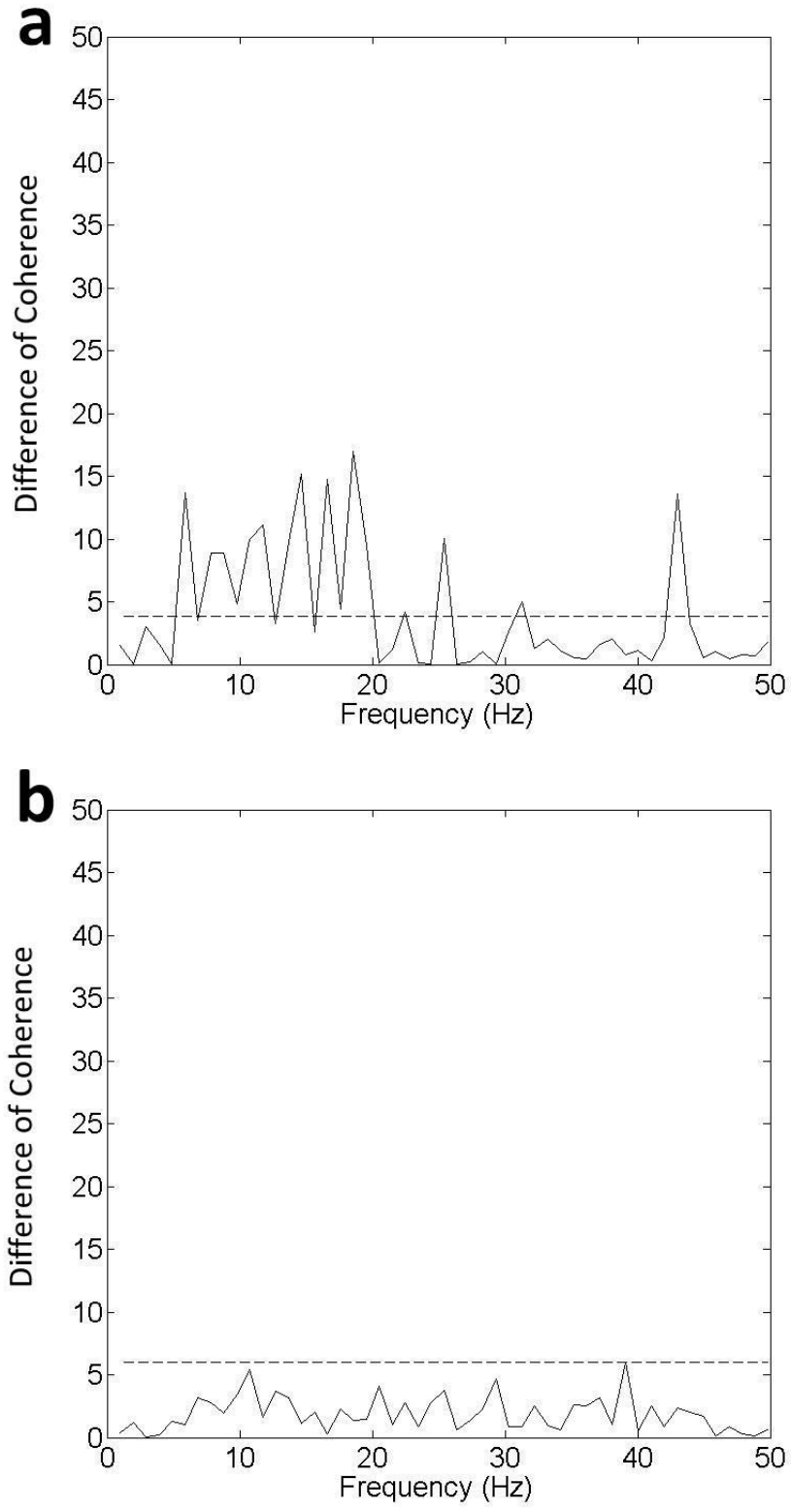


Figure 5.27: Typical examples of individual's χ^2 extended differences of coherence results. a) ED-A: There are significant differences in the input baseline coherence data. b) 1DI-A: There were no significant differences the input baseline coherence data.

Comparison of Coherence Analysis

As for IMC the 1DI-A data for the remaining participants was pooled together to make data sets for each intervention (anodal, cathodal and sham).

Comparison to Baseline

Comparison of coherence tests were used to assess post stimulation 1DI-A coupling compared to baseline, and as for IMC above the data were amalgamated in a time-frequency plot. Figure 5.28a shows the effects of anodal stimulation compared to baseline: the (8 - 9) Hz range was dominated by increases in 1DI-A coupling at all but the 10 minute test time. Figure 5.28b shows that there was also an interaction in 1DI-A coupling following cathodal tDCS, but this was to a much lesser and more sporadic extent occurring around 11 Hz only at 1 and 20 minutes post simulation. When sham stimulation was compared to baseline there were increases in 1DI-A coherence at 8 Hz and 11 Hz at 25 and 30 minutes respectively (Figure 5.29).

Comparison to Sham tDCS

A comparison to sham at each time interval was also conducted. Figure 5.30a shows that for anodal tDCS there were no significant differences between the intervention and sham. For cathodal stimulation, Figure 5.30b the (10 - 12) Hz band saw mostly increases in 1DI-A coupling throughout the test period and occurring at 5, 15, 25 and 30 minutes, there was also decrease in coherence around 9 Hz at the 5 minute test.

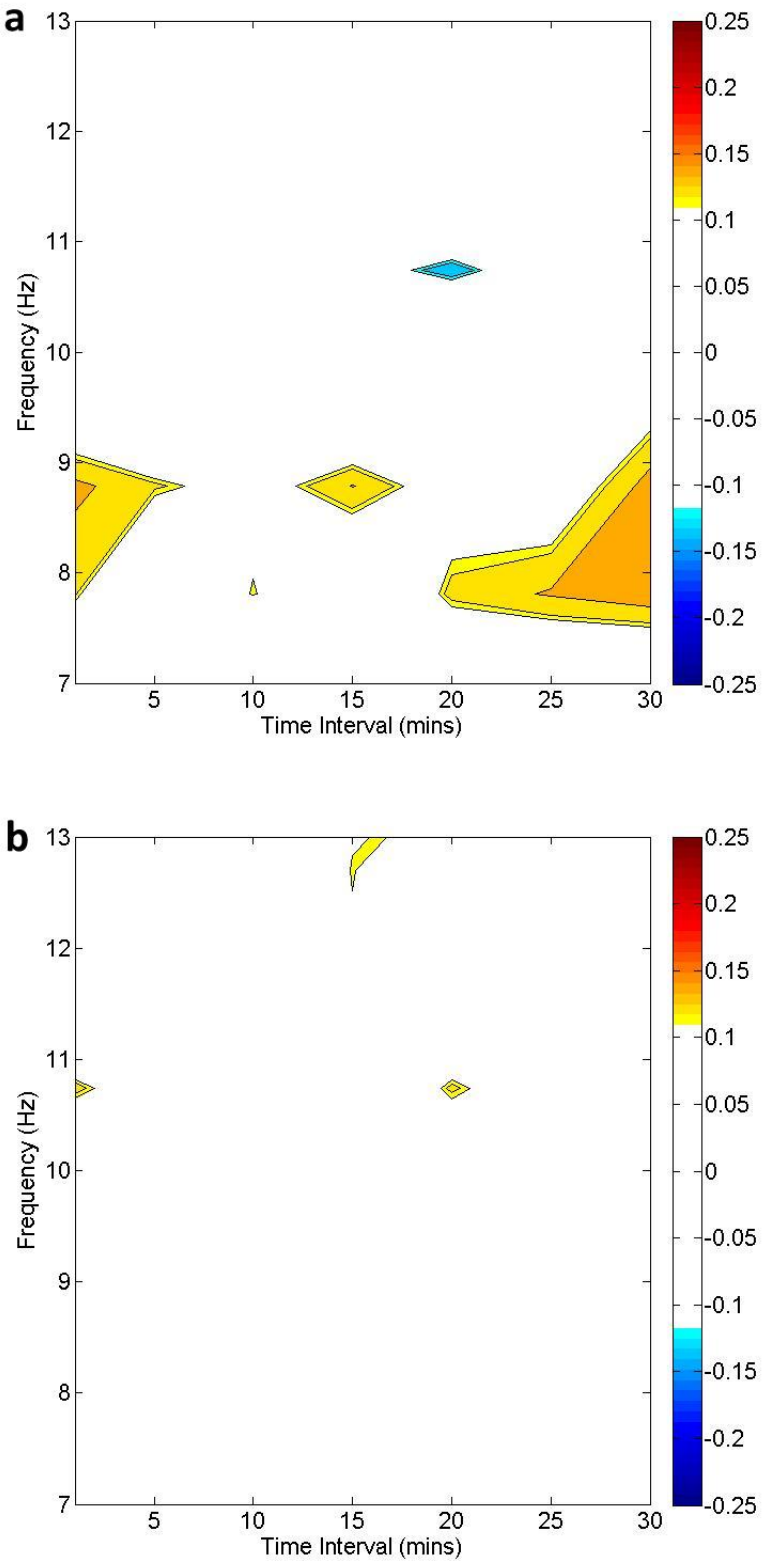


Figure 5.28: Comparison to baseline 1DI-A coherence. a) Anodal tDCS caused significant increases in the (8 - 12) Hz frequency range. b) Cathodal tDCS caused some small, but significant increases around 11 Hz.

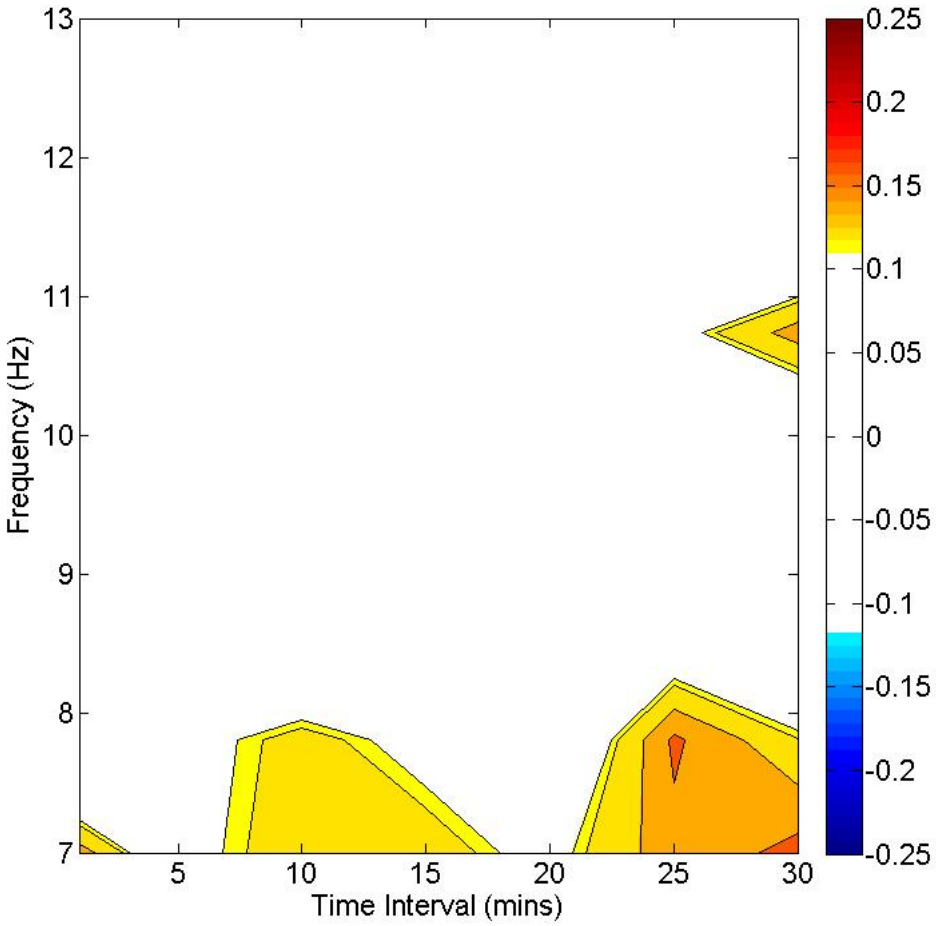


Figure 5.29: Comparison to baseline 1DI-A coherence following sham tDCS. There are some small, but significant increases.

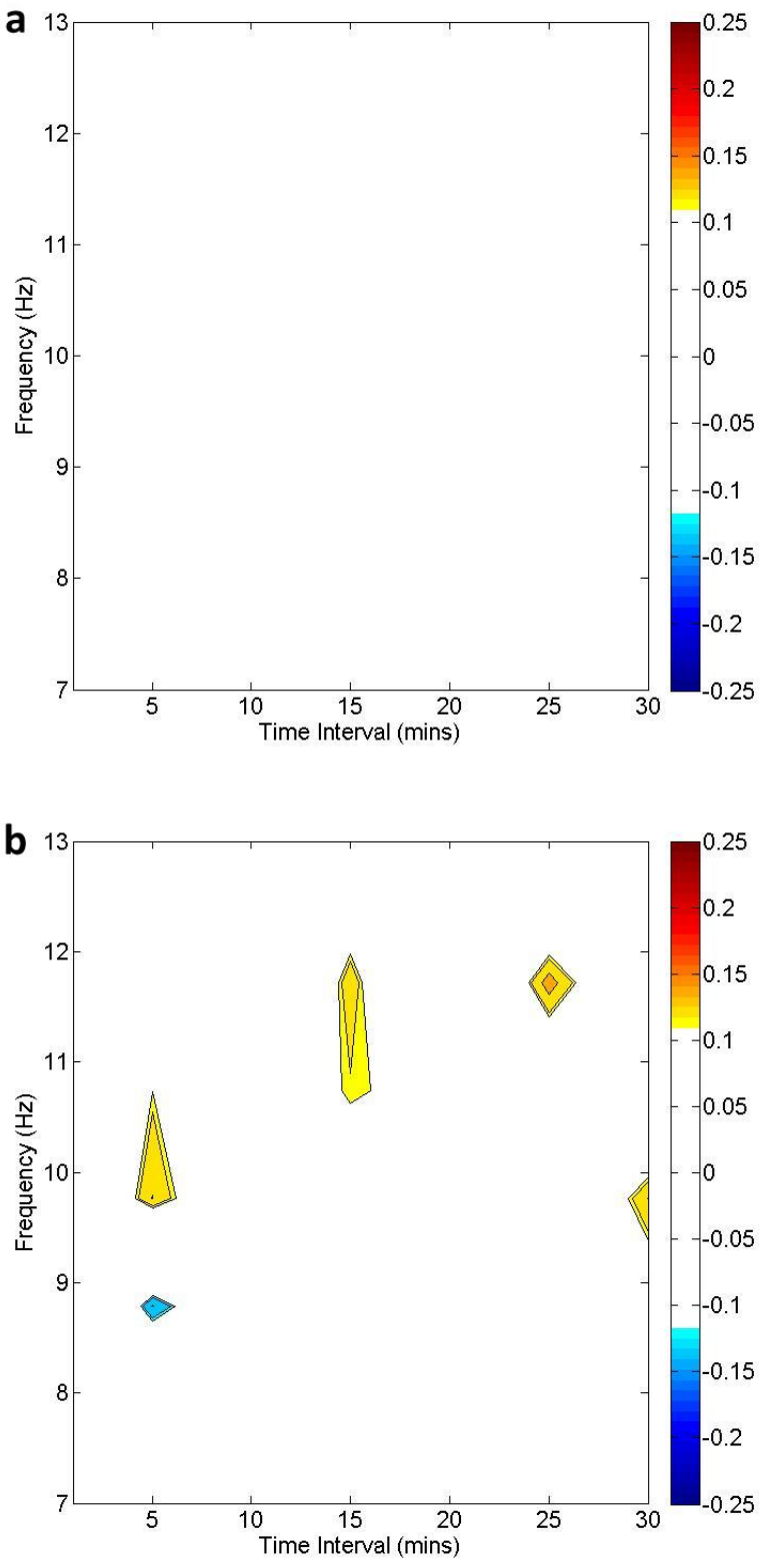


Figure 5.30: Comparison of 1DI-A coherence for sham tDCS and active tDCS. a) There are no significant differences following anodal tDCS. b) Cathodal tDCS caused significant increases over the (10 - 12) Hz range.

5.4 4. Paired Associative Stimulation: Transcranial Direct Current Stimulation and Peripheral Nerve Stimulation

In this section of the thesis the effects of peripheral nerve stimulation, alone and in combination with tDCS, on IMC and PNT were investigated.

- Number of subjects: 10
- Stimulation type: Anodal, Cathodal DC and Sham
- Muscles: ED & 1DI

5.4.1 Inter-Muscular Coherence

Baseline Stability

All participants demonstrated stability in their baseline IMC data; n = 10.

As for the tDCS analysis above the data from the subjects were pooled to make data sets for each intervention and comparison of coherence tests at each time interval were used to produce time-frequency plots.

Comparison of Coherence

The effects of the motor task compared to baseline IMC were tested again for this different population. The results were similar to the sham tDCS that were described above, that is there were large increases in α IMC and some small increases in β, Figure 5.31. For this trial the enhancements in α IMC were stronger than previously observed and the changes to β were smaller.

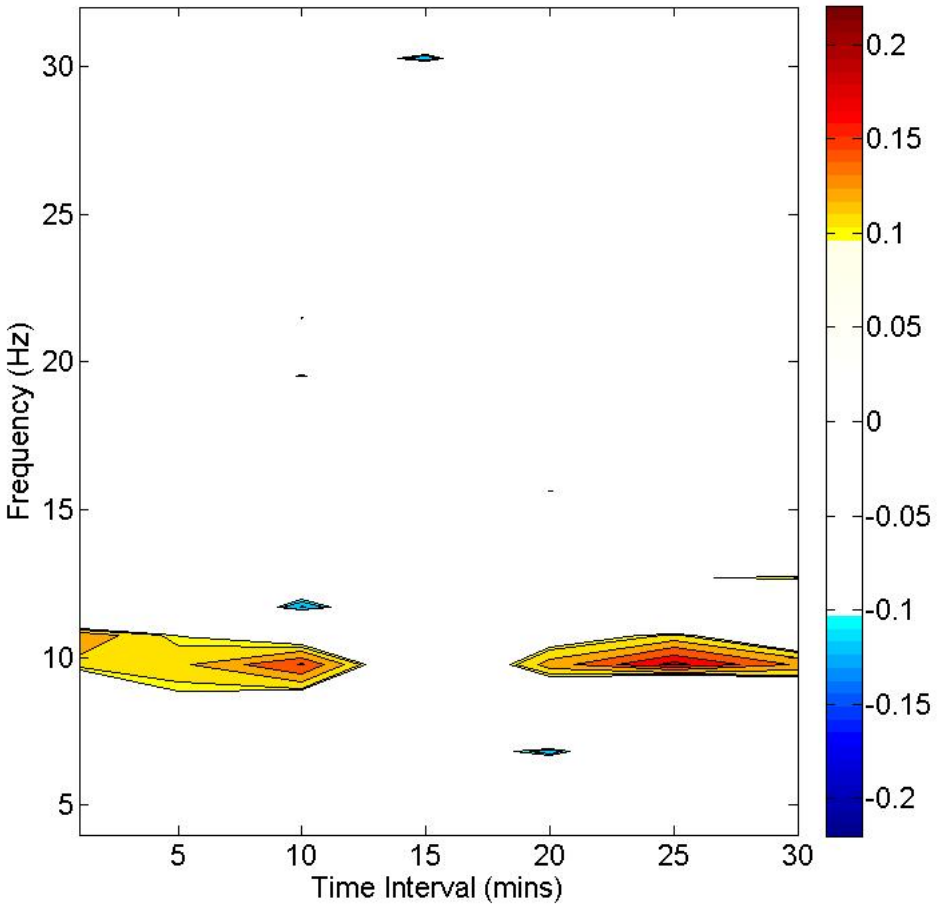


Figure 5.31: Comparison to baseline IMC following repetition of the contraction task. The result is dominated by significant increases in the α frequency range.

A comparison to baseline was conducted first and Figure 5.32a shows that sham tDCS, or peripheral stimulation alone, caused enhanced β band IMC between (20 - 25) Hz 1 minute post intervention and then again between 10 and 25 minutes.

Compared to the effects of the motor task peripheral nerve stimulation by itself caused increased β IMC between (20 - 25) Hz in the first 10 minutes post stimulation. There were also later increases in α IMC, between 10 and 25 minutes, at a range of frequencies, Figure 5.32b.

Compared to baseline anodal tDCS/PNS caused the α band to be dominated with wide spread increases in IMC that persisted throughout the test period, (Figure 5.33a). There were mostly reductions to β band IMC across a range of frequencies between 1 and 20 minutes post stimulation, some of these recurred at the 30 minute test interval.

Compared to the motor task anodal tDCS/PNS reduced β IMC at 5, 10, 15, 20 and 30 minutes post stimulation, Figure 5.33b. In the α band there was an increase in IMC at 25 minutes.

For cathodal tDCS/PNS Figure 5.34a shows that there were a range of increases in α IMC that begin at the first test interval and persisted until the end. The β band saw a mix of both increases and decreases; the reductions were centered between (20 - 25) Hz in the first 10 minutes and recurred in the last 10 minutes of the test period. The increases in β IMC occurred at 5 and 10 minutes between 25 Hz and 30 Hz and again between (20 - 25) Hz at 25 minutes.

The results for cathodal tDCS/PNS when compared to the effects of the motor task (shown in Figure 5.34b) illustrate that in the first 10 minutes there were increases in β IMC. There was also an increase in α IMC at 10 minutes. Later, at 15, 20 and 30 minutes there were reductions in β IMC between 15 and 20 Hz; however there was also a small increase in β IMC at 25 minutes. There was also a small increase in α at 20 minutes.

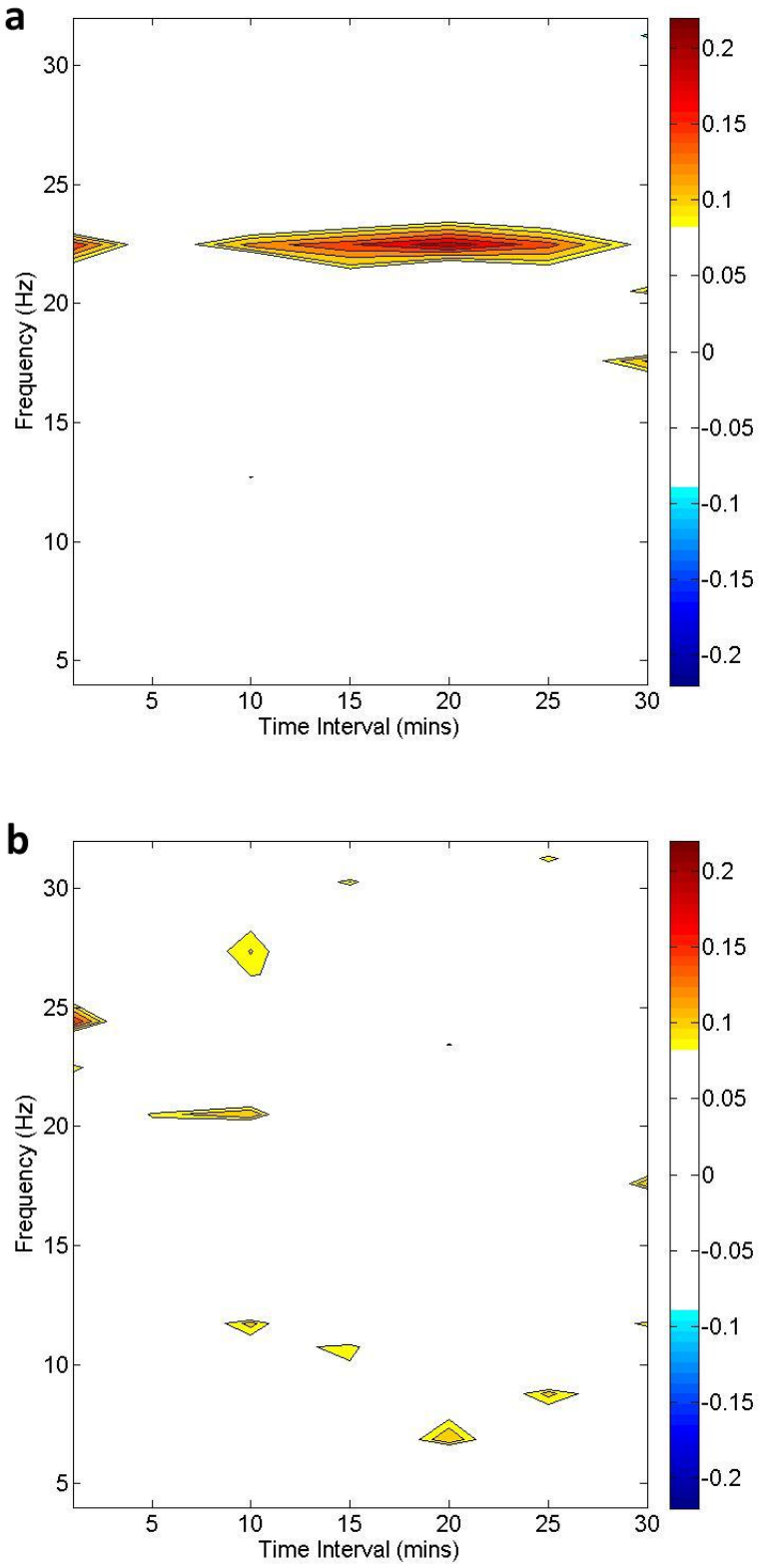


Figure 5.32: Comparison to baseline IMC and the motor task following PNS alone. a) Compared to baseline there is a significant increase in β IMC. b) For comparison to the motor task there were both significant increases in both the α and β bands.

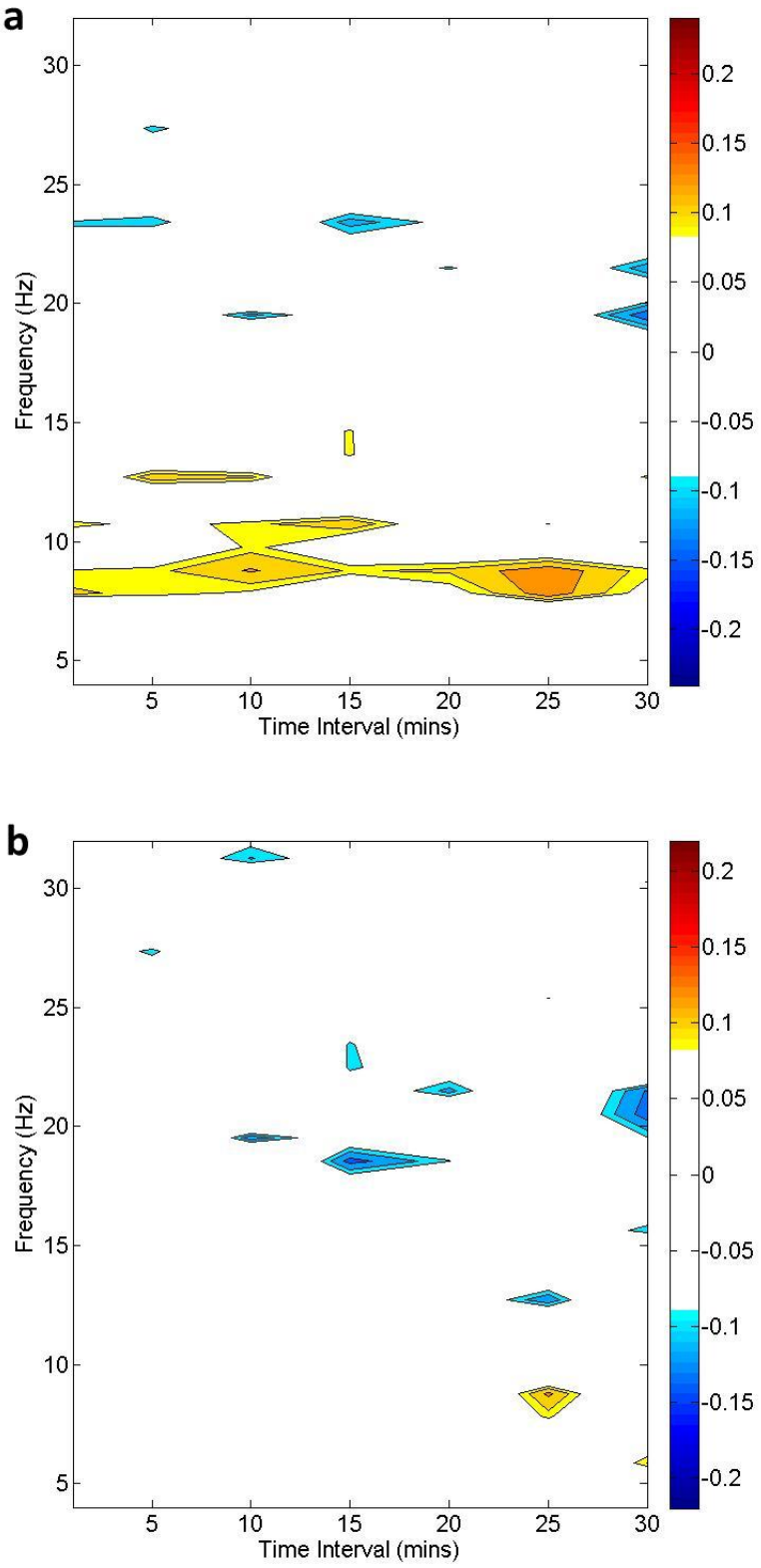


Figure 5.33: Comparison to baseline IMC and motor task following anodal tDCS/PNS. a) Compared to baseline there are significant decreases in β IMC and increases in α. b) Compared to the effects of the motor task there are significant decreases in the β band and a significant increase in the α.

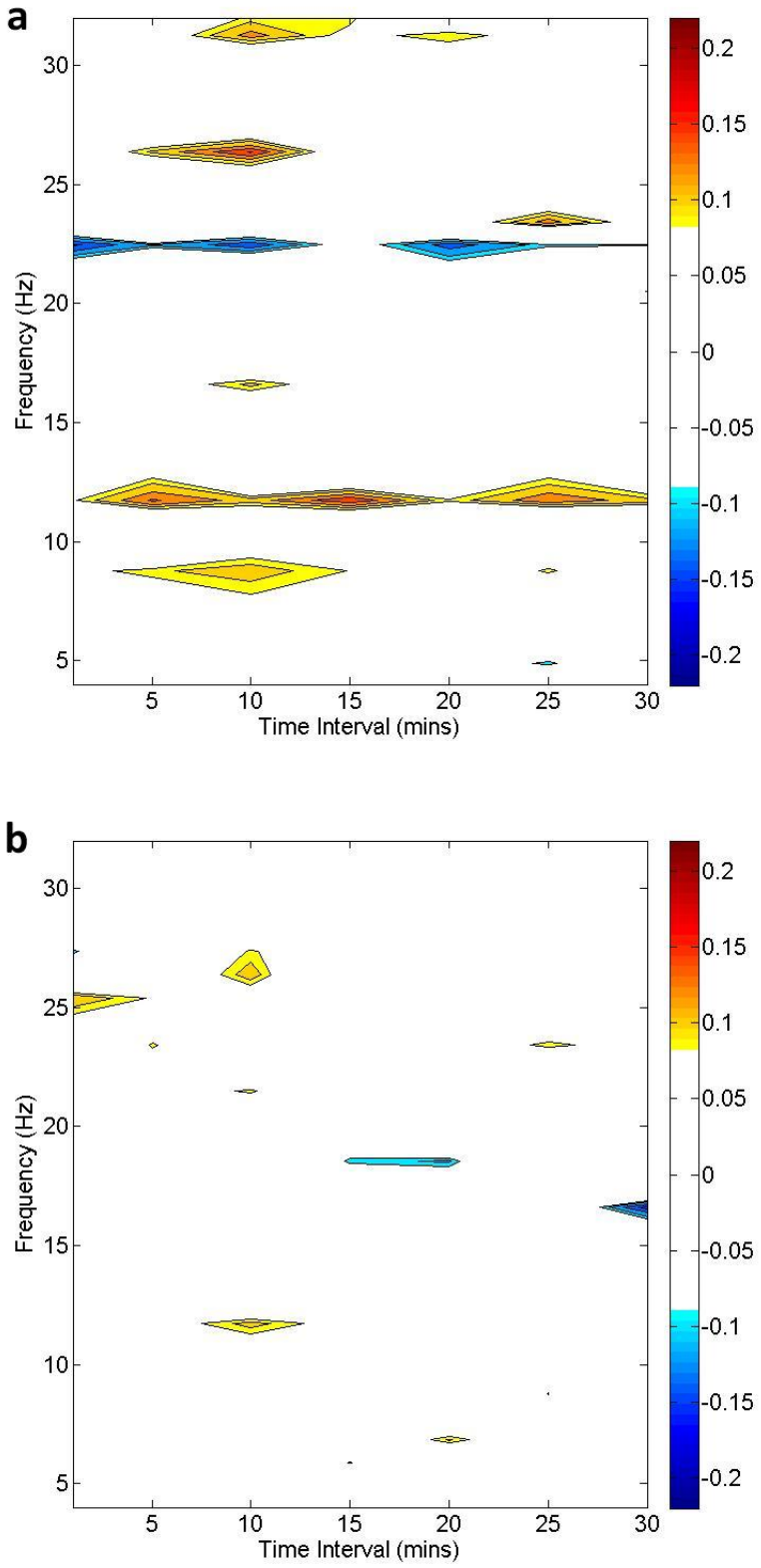


Figure 5.34: Comparison to baseline and motor task for cathodal tDCS/PNS. a) Compared to baseline there are significant increases and decreases in the β band and increases in the α. b) Compared to the motor task there are both significant increases and decreases in the β band and increases in the α.

5.4.2 Physiological Neurogenic Tremor

Here the effects of peripheral nerve stimulation, both alone and in combination with tDCS, were tested on muscle-accelerometer coherence for both ED and 1DI.

Baseline Stability

Baseline stability analysis revealed that, once again, there were significant differences in the input ED-A coherence to individual's pooled baseline data set. For 1DI-A only two participants did not demonstrate stability in their baseline; the data from these participants were discarded from the analysis (n = 8). The data from the remaining eight participant's were pooled to create data sets for each intervention and comparison of coherence analysis was employed to create time frequency plots.

Comparison of Coherence

Comparison of coherence tests were first used to compare the post intervention IMC to baseline. Comparisons between the motor task and baseline in this new population show a small increase in 1DI-A coherence around 11 Hz at the 20 minute test (Figure 5.35).

The time frequency plot shown in Figure 5.36a shows that peripheral nerve stimulation alone increased 1DI-A around 9 Hz in the first 10 minutes after the intervention and again at 20 and 30 minutes.

Comparison of coherence between PNS and the motor task (Figure 5.36) shows that for peripheral stimulation alone there were no changes to 1DI-A coherence in the first 10 minutes when compared to the motor task. Later increases between (9 - 12) Hz were observed at 15, 20 and 25 minutes post stimulation.

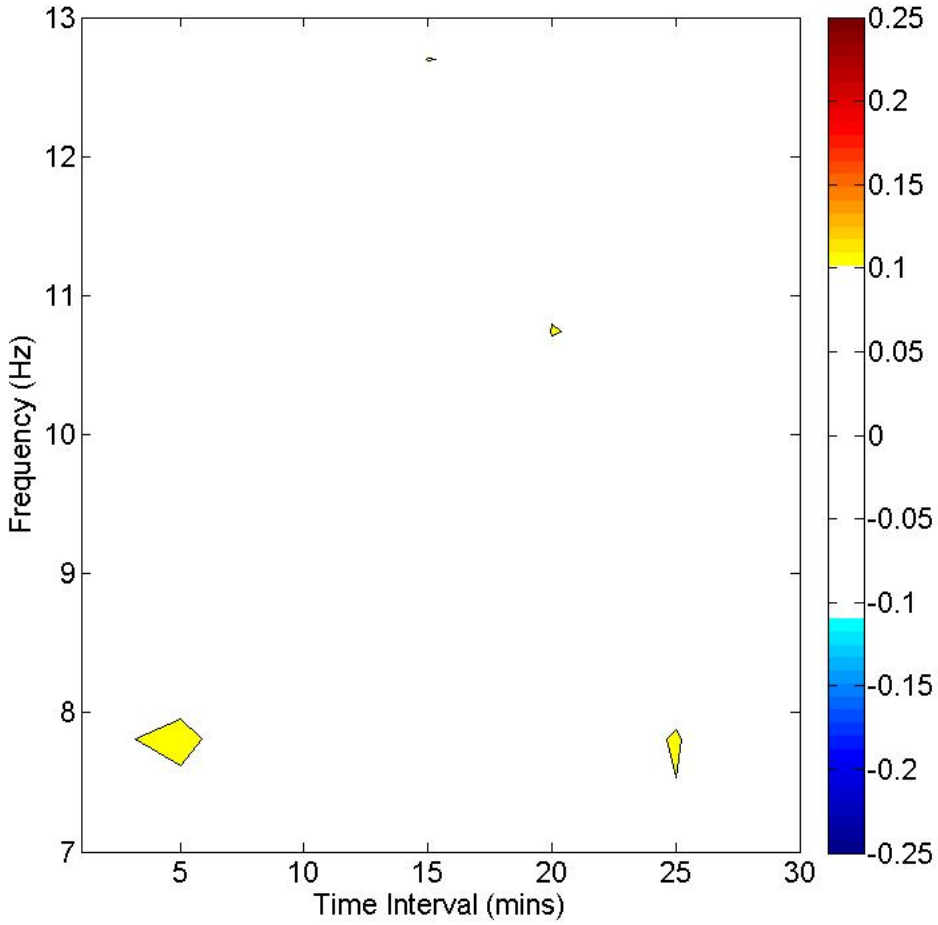


Figure 5.35: Comparison to baseline 1DI-A coherence following sham tSCS. There is a very small decrease 25 minutes post stimulation.

Anodal tDCS/PNS caused similar changes to neurogenic tremor as peripheral stimulation (Figure 5.37a). There were increases around 9 Hz 1DI-A coherence at 5 and 10 minutes that occurred again at 25 and 30 minutes post intervention.

Cathodal tDCS/PNS did not cause any changes in neurogenic tremor when compared to baseline (Figure 5.38a).

Both anodal (Figure 5.37b) and cathodal (Figure 5.38b) tDCS/PNS caused increases in coupling around 11 Hz at 15 minutes when compared to sham. The

effect was largest for cathodal tDCS/PNS. There was also a small decrease between (11 - 12) Hz after anodal tDCS/PNS.

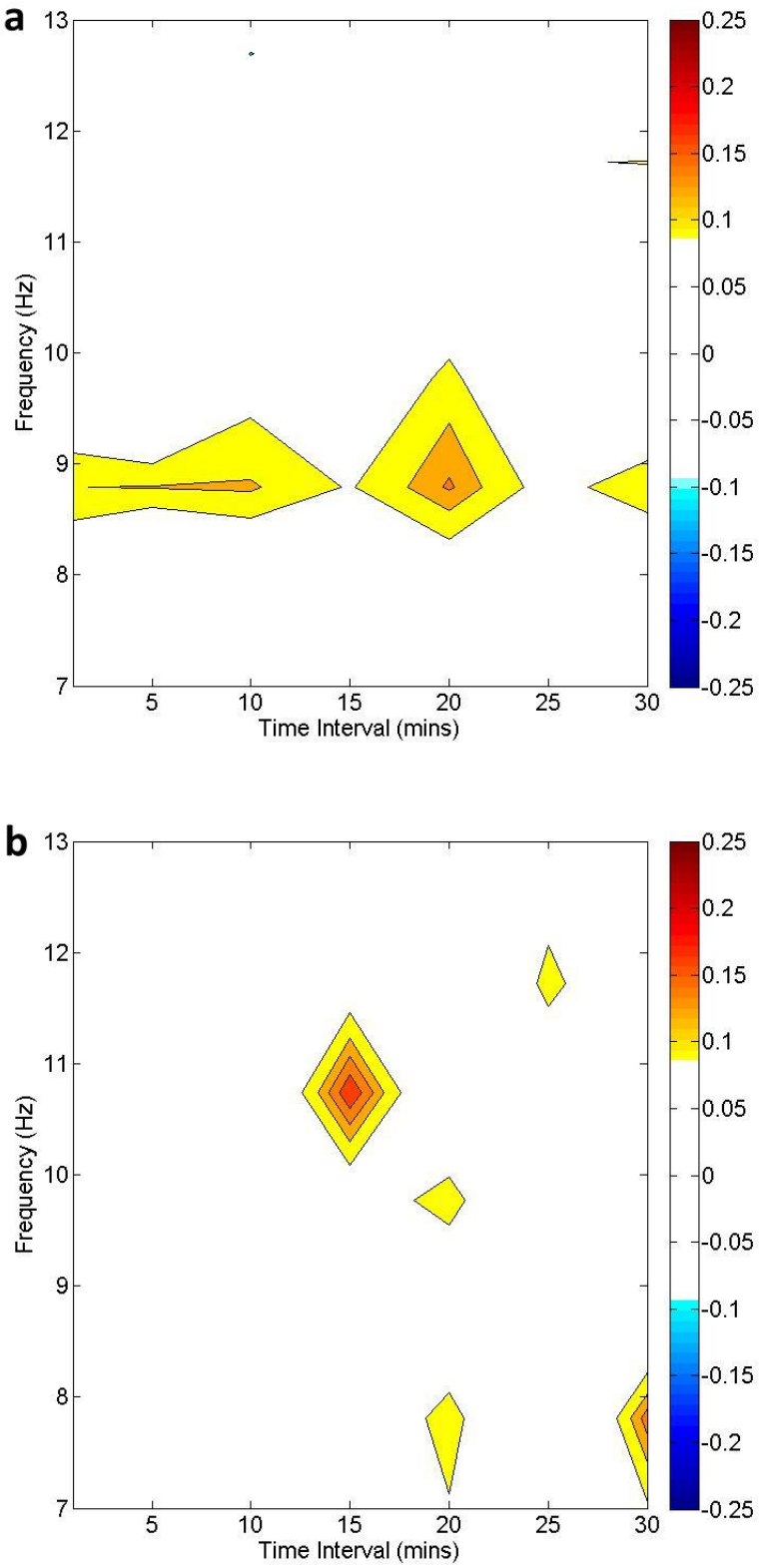


Figure 5.36: Comparison to baseline and the motor task 1DI-A following PNS alone. a) Compared to baseline there is a significant increase in coherence. b) Compared to the motor task there are increases in coherence.

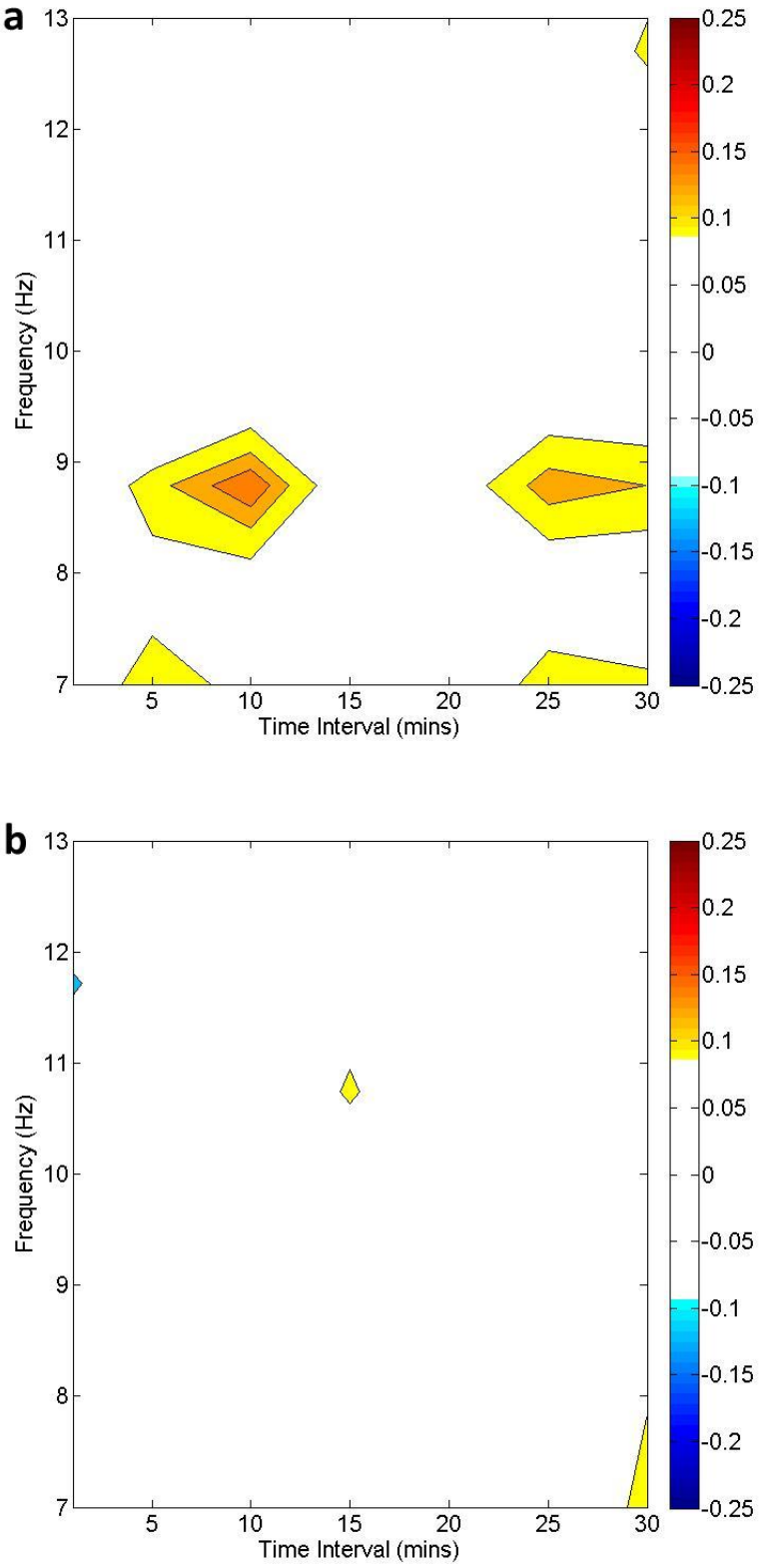


Figure 5.37: Comparison to baseline and motor task 1DI-A coherence following anodal tDCS/PNS. a) Compared to baseline there is a significant increase in coherence. b) Compared to the motor task there is a small increase in coherence at 15 minutes post intervention.

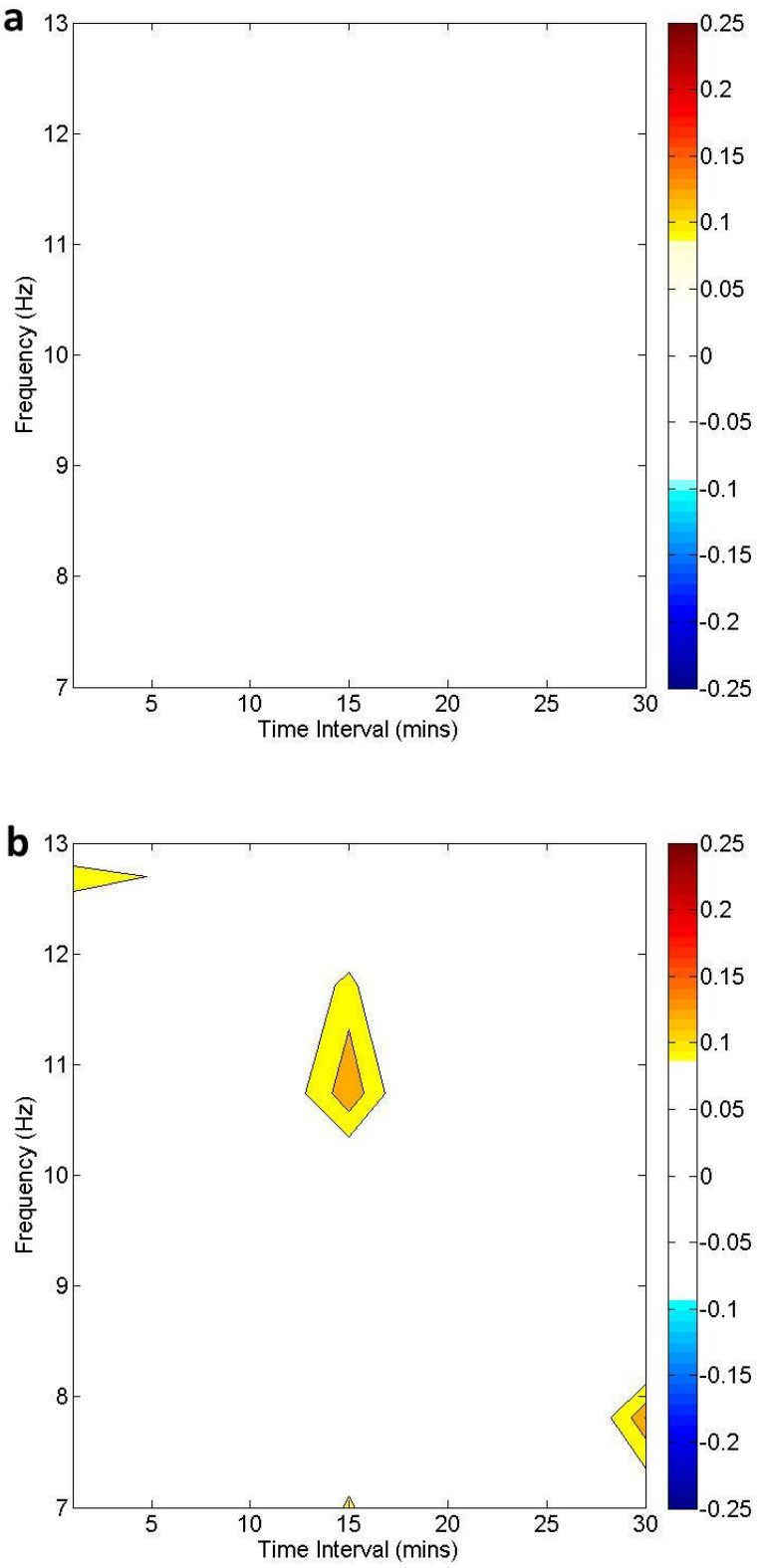


Figure 5.38: Comparison to baseline and motor task 1DI-A coherence following cathodal tDCS/PNS. a) Compared to baseline there are no significant changes. b) Compared to the motor task there is an increase in coherence at 15 minutes post intervention.

5.5 5. Transcranial Sinusoidal Current Stimulation

In this part of thesis the effects of sinusoidally altering tDCS were tested on IMC and PNT. Frequencies of 5 Hz, 10 Hz and 20 Hz were imposed on the anodal DC signal.

5.5.1 Inter-Muscular Coherence

- Number of subjects: 10
- Stimulation type: 5 Hz, 10 Hz and 20 Hz tSCS
- Maximum Current: 1 mA
- Minimum Current: 0.95 mA
- Muscles: ED & 1DI

Baseline Stability

Data quality for this trial was evaluated as very good and ED-DI IMC baseline stability was acceptable for all but three participants; the data for these subjects were not included in further analysis. The data from the remaining participants' were pooled to make data sets for each intervention and comparison of coherence tests were used to make time-frequency plots.

Comparison of Coherence

As for the tDCS trial above a comparison to baseline for each time interval was carried out first. The time-frequency plots show that tSCS of 5 Hz (Figure 5.39a) did not cause any changes to α and caused an increase in β IMC centered around (15 - 20) Hz and occurring 10, 15 and 20 minutes post stimulation. There was

also a small increase increase around 25 Hz at 25 minutes.

Comparison of coherence tests were also carried out to test the effects of the tSCS intervention compared to the motor task. The time-frequency plot in Figure 5.39b shows the results following 5 Hz tSCS. In the β band there was an increase at 5 minutes that recurred between 20 and 30 minutes post intervention. There was also a decrease in the β band at the 20 minute test time. There were no changes to α IMC and no changes around 5 Hz.

Compared to baseline 10 Hz tSCS resulted in a reduction in α IMC 1 minute after stimulation; there were then increases around 11 Hz at 5 minutes that recurred at 20 minutes. In the β band there were widespread increases that began 5 minutes post intervention and persisted until the end of the test time, see Figure 5.40.

When the effects of 10 Hz tSCS were compared to the effects of the motor task (Figure 5.40) there were increases in β IMC at one and five minutes. These changes to β IMC recurred at the 20 and 25 minute test times. There was also a small decrease in β IMC at one minute. In the α band there were increases at 15 and 25 minutes, of particular interest is the increase in 10 Hz IMC at 15 minutes. There was also a decrease in the α band at the 10 minute test point.

Compared to baseline 20 Hz tSCS, Figure 5.41a, caused similar, wide spread, increases in β IMC as 10 Hz tSCS. The changes began one minute post intervention and persisted until the end of the test interval. There was also a very small increase in α IMC at 25 minutes.

Figure 5.41 shows that, when compared to the motor task, 20 Hz tSCS caused a later reduction to the β band at 20 minutes that was similar to that caused by

5 Hz tSCS. Like 10 Hz tSCS α IMC was enhanced at 10 and 15 minutes. There were no changes to 20 Hz IMC.

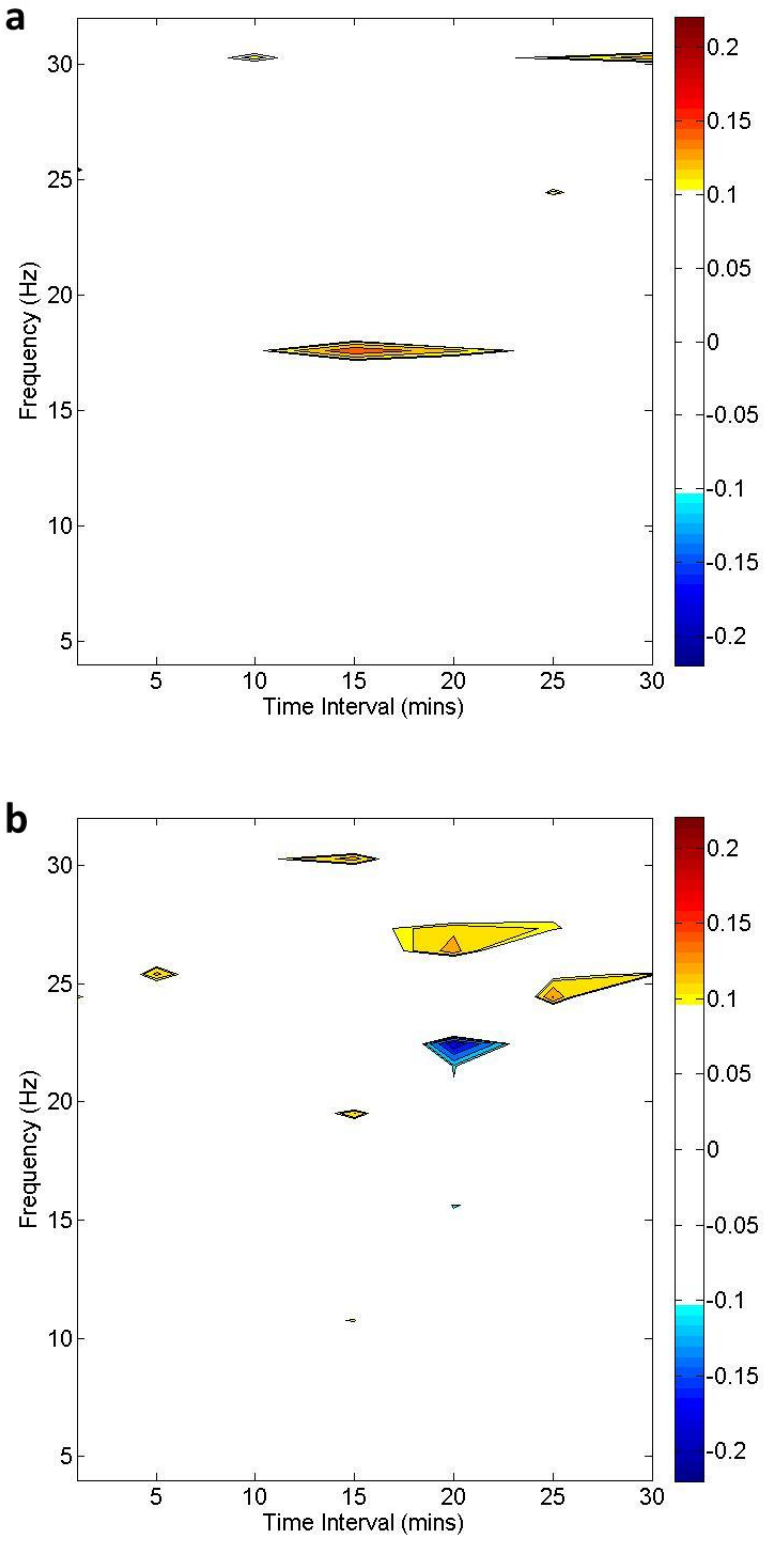


Figure 5.39: Comparison to baseline and motor task IMC following 5 Hz tSCS. a) Compared to baseline there was a significant increase in the β frequency range. b) Compared to the motor task there were both significant increases and decreases in the β band.

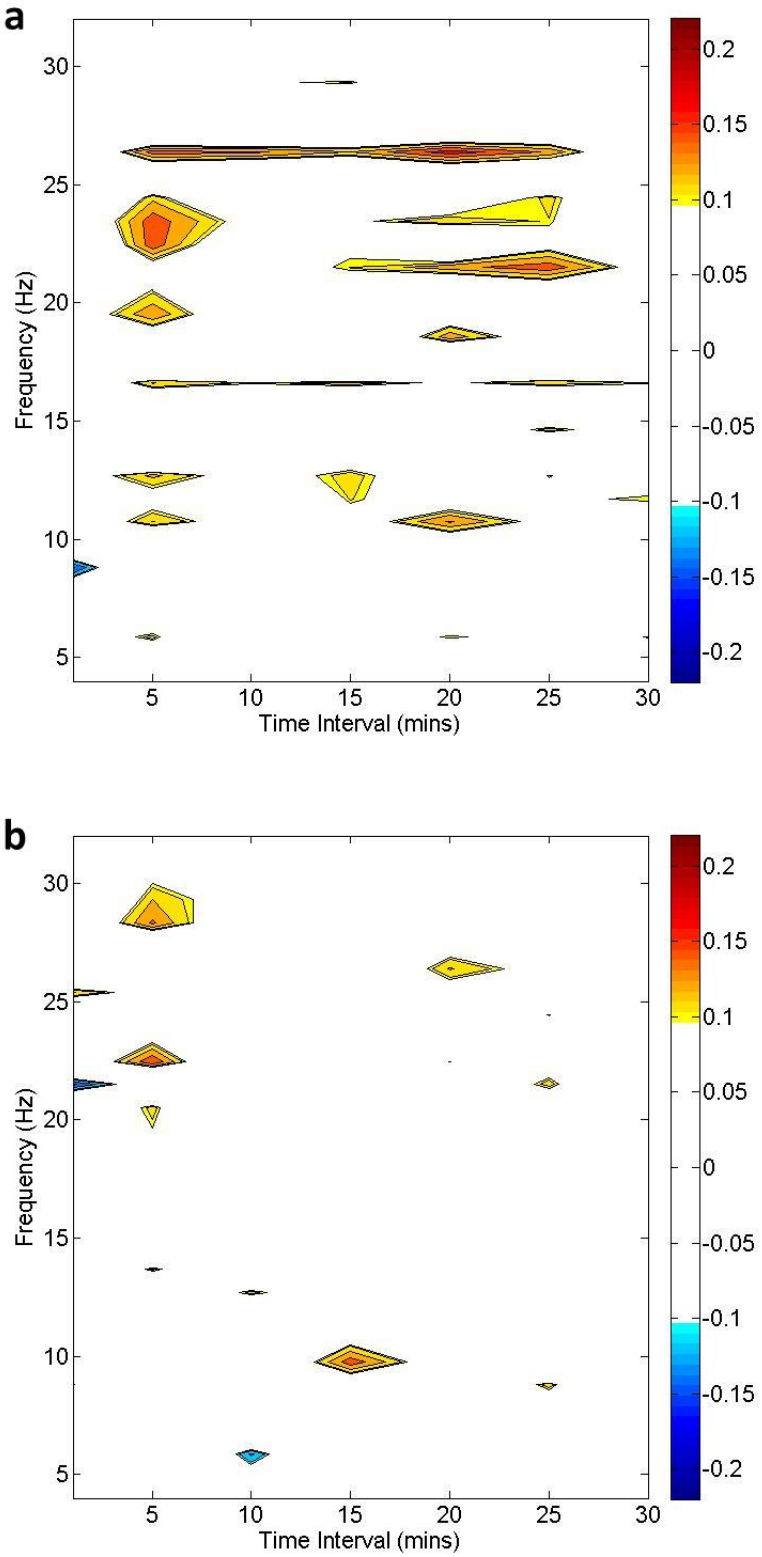


Figure 5.40: Comparison to baseline and motor task IMC following 10 Hz tSCS. a) Compared to baseline there is a significant increase in both the α and β frequency ranges. b) Compared to the motor task there are both significant increases and decreases in the β band and increases in the α band.

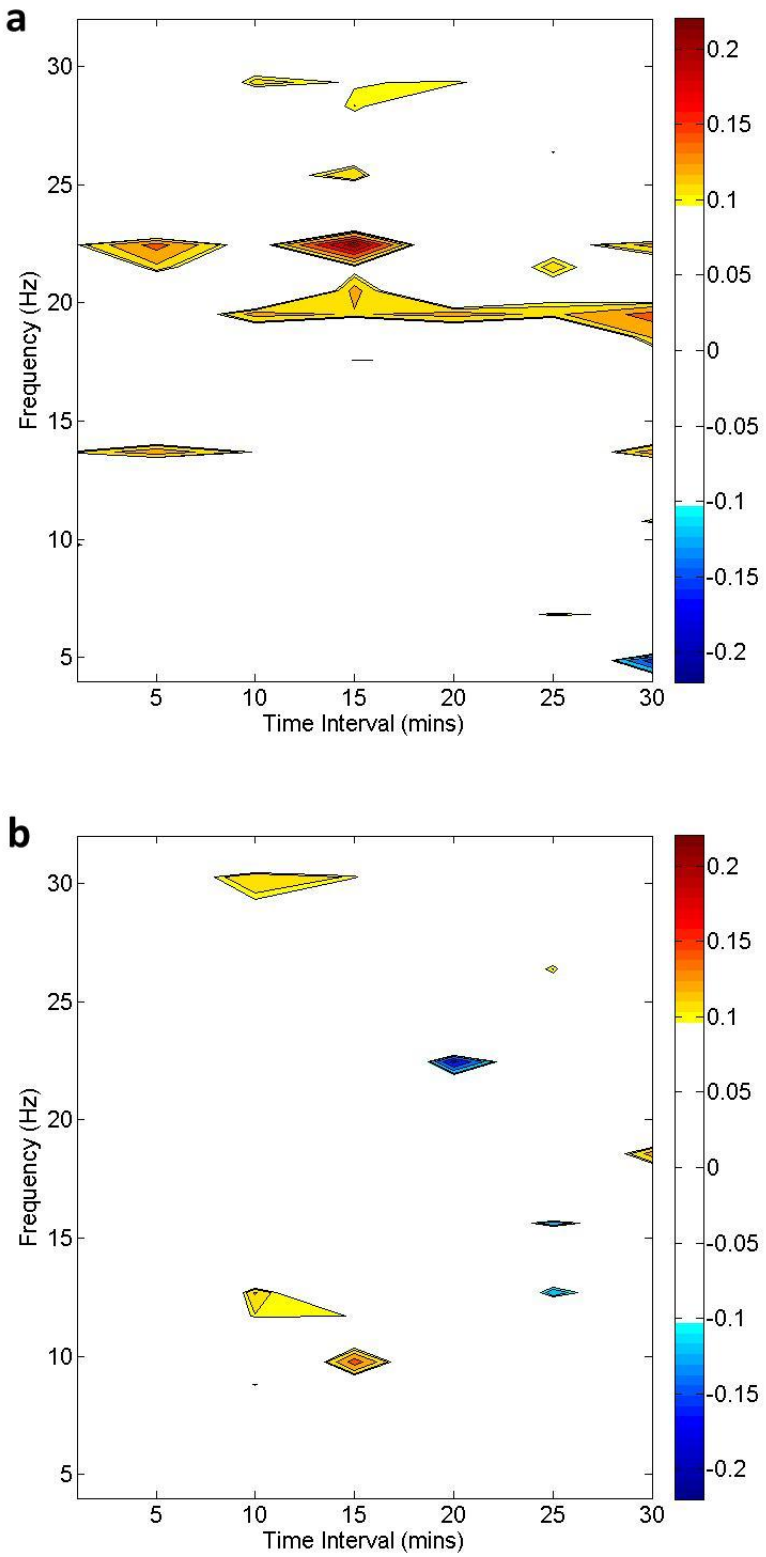


Figure 5.41: Comparison to baseline and motor task IMC following 20 Hz tSCS. a) Compared to baseline there are significant increases in the β frequency range. b) Compared to the motor task there are both significant increases and decreases in the β band and significant increases in α.

5.5.2 Physiological Neurogenic Tremor

Here the effects of 5 Hz, 10 Hz and 20 Hz tSCS on muscle-accelerometer coherence (ED-A and 1DI-A) were investigated and the results are presented.

Baseline Stability

Once again there was no stability in any of the ED-A data. Also three participants did not exhibit baseline stability in their 1DI-A data and as such were not included in the following pooled analysis where n = 7.

Comparison of Coherence

Comparison of coherence tests were used to compare the pooled post intervention data to the pooled baseline data for each intervention. Figure 5.42 shows that for 5 Hz tSCS there was a small increase in 1DI-A coupling at (11 - 12) Hz and five minutes post intervention. Compared to baseline 10 Hz tSCS (Figure 5.43a) caused larger changes to 1DI-A with a decrease in (8 - 9) Hz coherence one minute after stimulation and increases in the (11 - 12) Hz range at 5 and 25 minutes post stimulation. For 20 Hz tSCS (Figure 5.44a) there was a very small increase in (10 - 11) Hz 1DI-A coherence at 10 minutes.

When comparison of coherence tests were employed to control for the effects of the motor task the time-frequency plot showed that tSCS at 5 Hz (Figures 5.42b) caused a small increase in 1DI-A coherence around 11 Hz at the 10 minute test interval, but a large decrease between (10 - 12) Hz at 20 minutes. Following 10 Hz tSCS (Figures 5.43b) there was a reduction in (8 - 9) Hz coupling at 1 and 10 minutes post stimulation as well as at 12 Hz and 20 minutes. There was also a small increase around 11 Hz at the 15 minute test. 20 Hz tSCS resulted in an increase in 1DI-A around 11 Hz at 15 minutes followed by a decrease at the same frequencies five minutes later at the 20 minute test (Figure 5.44).

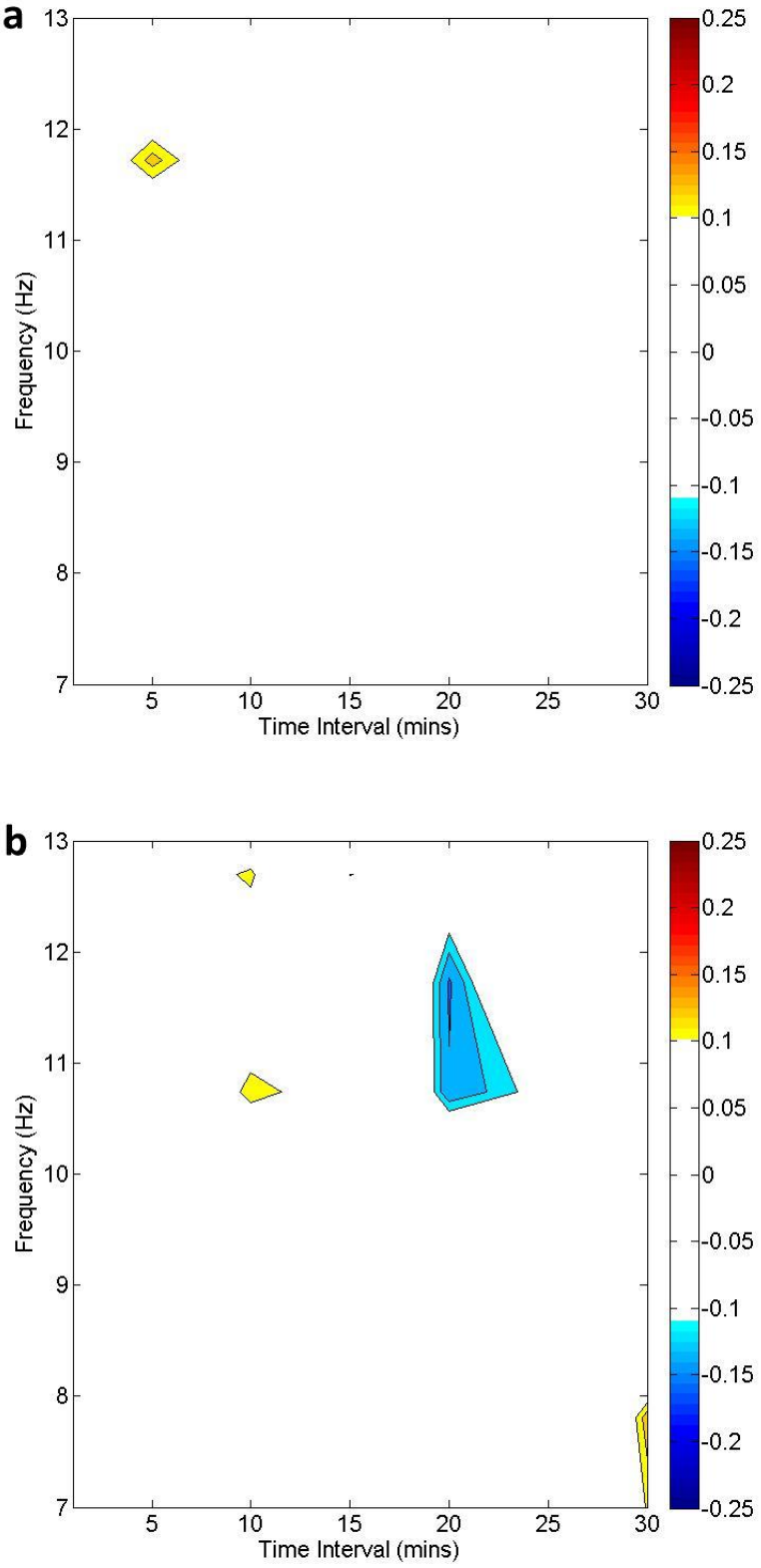


Figure 5.42: Comparison to baseline and motor task 1DI-A coherence following 5 Hz tSCS. a) Compared to baseline there is a significant increase at 5 minutes post stimulation. b) Compared to the motor task there are both significant increases and decreases.

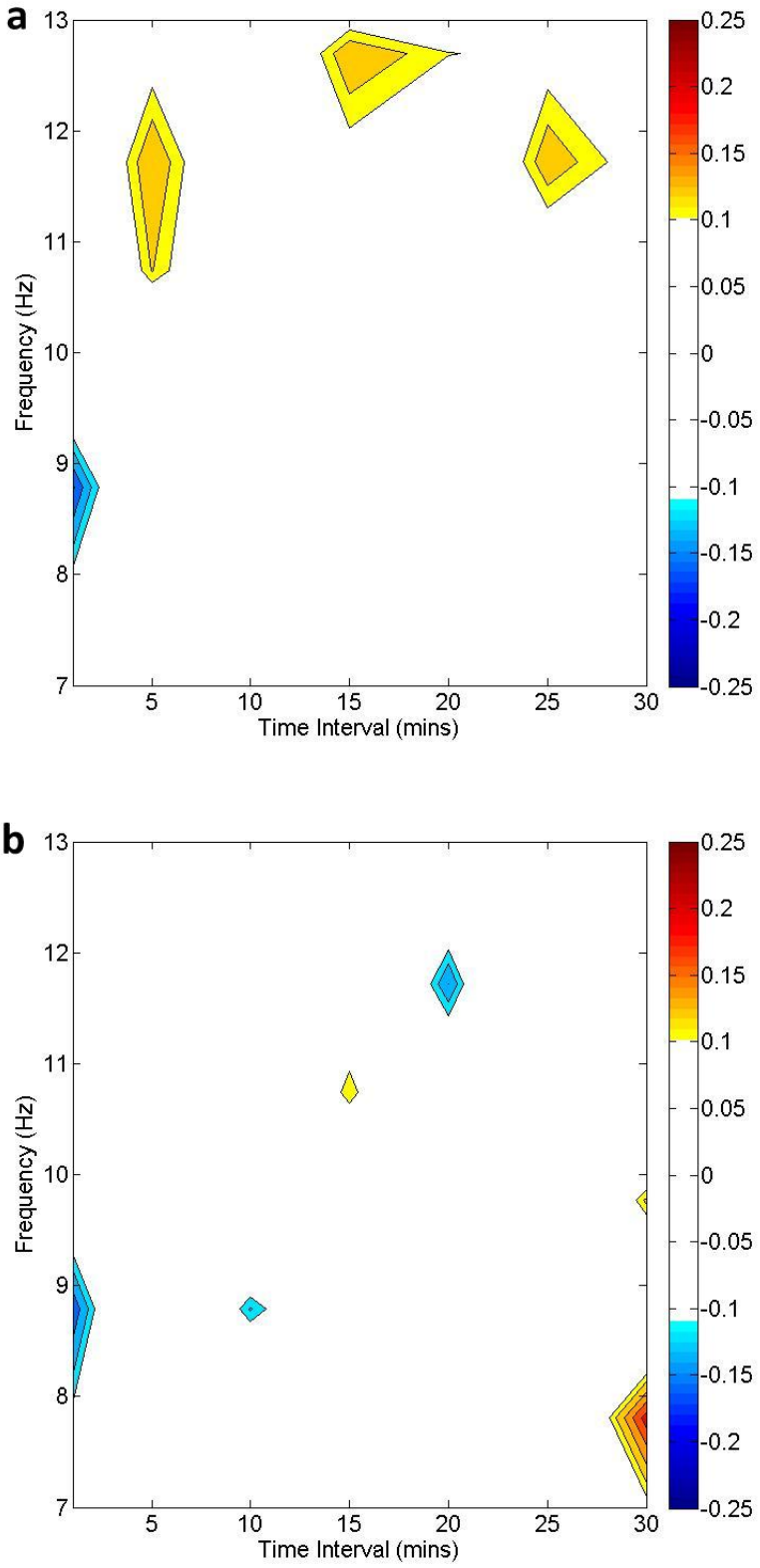


Figure 5.43: Comparison to baseline and motor task 1DI-A coherence following 10 Hz tSCS. a) Compared to baseline the result is dominated by significant increases. b) Compared to the motor task the results are dominated by decreases in coherence.

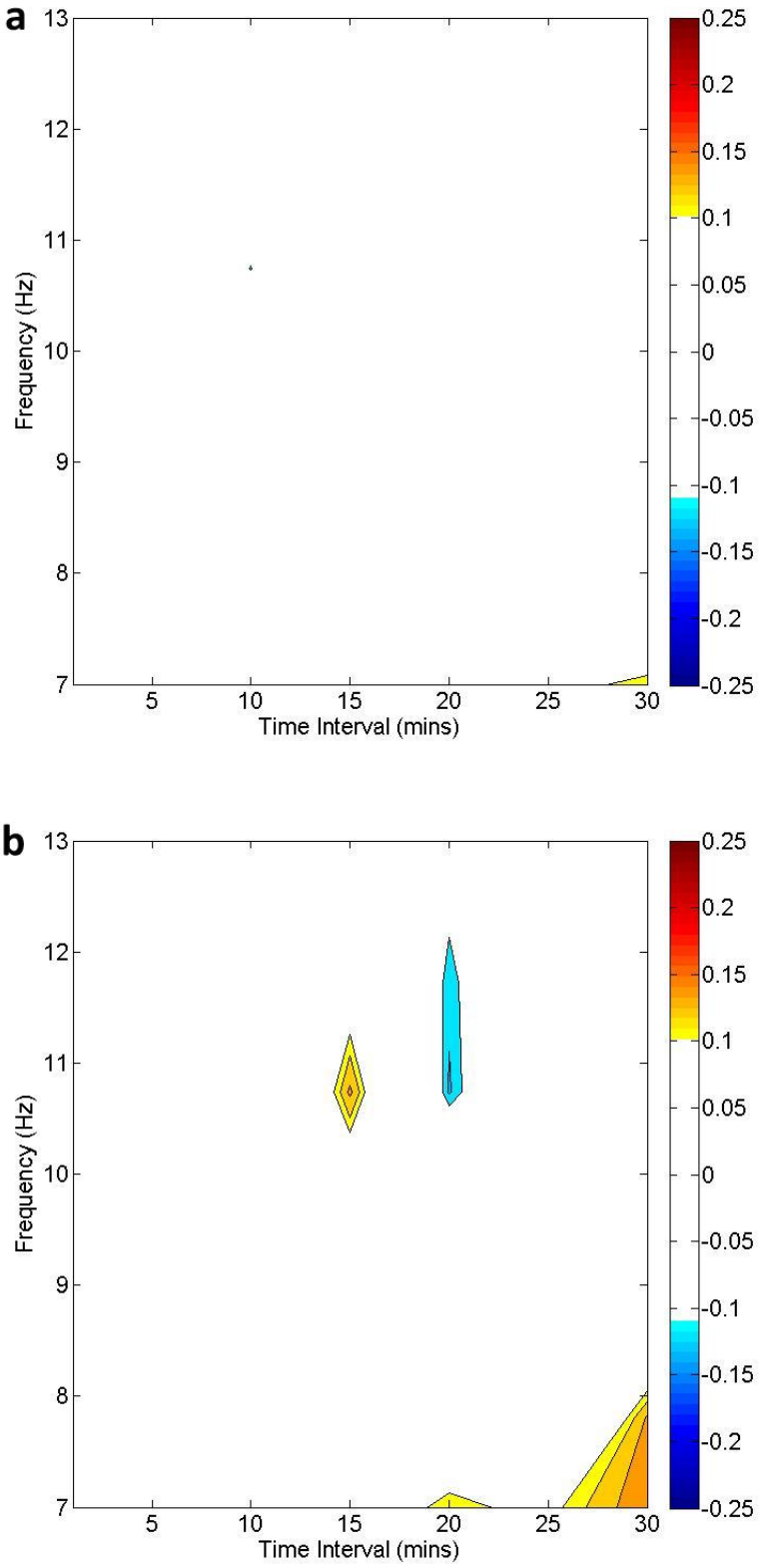


Figure 5.44: Comparison to baseline and motor task 1DI-A coherence following 20 Hz tSCS. a) Compared to baseline there is a very small decrease 10 minutes post stimulation. b) Compared to the motor task there are both significant increases and decreases.

5.6 6. Paired Associative Stimulation: Transcranial Sinusoidal Current Stimulation and Peripheral Nerve Stimulation

The final part of this thesis involved pairing tSCS of 5 Hz, 10 Hz or 20 Hz with peripheral nerve stimulation. The results are reported here.

- Number of subjects: 10
- Stimulation type: 5 Hz, 10 Hz and 20 Hz tSCS
- Maximum Current: 1 mA
- Minimum Current: 0.95 mA

5.6.1 Inter-Muscular Coherence

Baseline Stability

Four participants did not demonstrate stability in their IMC baselines and so were discarded from for the pooled IMC analysis, leaving n = 6.

Comparison of Coherence

Comparison of post stimulation coherence to baseline showed that after 5 Hz tSCS/PNS there was a decrease in α IMC around 6 Hz at 10 minutes that persisted in the 15 minute test; at 15 minutes there was also an concomitant increase at 10 Hz that recurred at 30 minutes (Figure 5.45a). In the β band there were widespread increases between 5 and 30 minutes post stimulation that got stronger as the test period progressed. The β band also saw decreases around 25 Hz 1 minute, 5 minutes and 15 minutes post stimulation.

When the effects of the motor task were controlled for 5 Hz tSCS/PNS increased IMC in the β band across a range of frequencies within the first ten minutes and then again at 25 minutes post stimulation. There was a small reduction in α IMC one minute after stimulation followed by increases at 15 and 30 minutes (Figure 5.45b).

Compared to baseline 10 Hz tSCS/PNS caused strong increases in IMC between (10 - 11) Hz at 1, 5 and 15 minutes as well as a reduction in the first 5 minutes at 12 Hz. The β band was dominated by wide spread increases in IMC in the 25 minutes post stimulation period (Figure 5.46).

Compared to the motor task 10 Hz tSCS/PNS caused a small increase in α IMC 15 minutes post stimulation. In the β band there were increases in the first five minutes; at 15 minutes there was a small decrease which became an increase at 25 minutes. At 30 minutes there were both increases and decreases in β IMC at about 18 and 23 Hz respectively (Figure 5.46b).

For 20 Hz tSCS/PNS compared to baseline there was a very small increase in α IMC at the end of the test period (30 minutes post stimulation). In the β band there were small decreases at 5 minutes and a small increase at 25 and 30 minutes post stimulation (Figure 5.47a) and an increase between (20 - 25) Hz at 25 minutes post intervention.

For 20Hz tSCS/PNS compared to the motor task there were alterations to β band IMC in the form of enhanced IMC 1 minute, 10, 20, 25 and 30 minutes post stimulation. There was also a small decrease around 25 Hz during the final test interval. In the α band the increase at 10 Hz at 15 minutes was again observed and recurred at 30 minutes post stimulation, Figure 5.47.

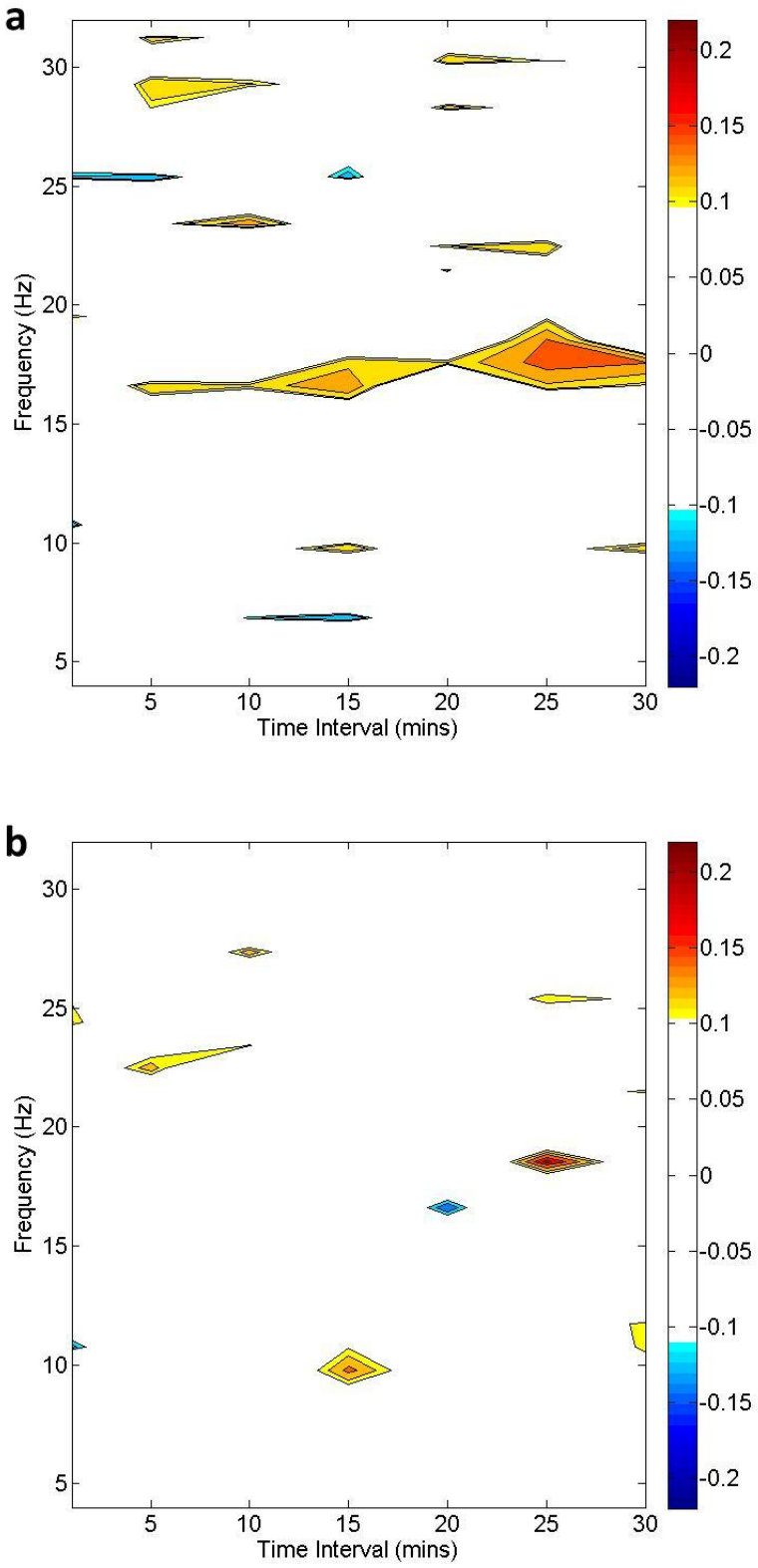


Figure 5.45: Comparison of baseline and motor task IMC following 5 Hz tSCS/PNS. a) Compared to baseline both the α and β bands have significant increases and decreases in IMC. b) Compared to the motor task there are both significant increases and decreases in both the α and β bands.

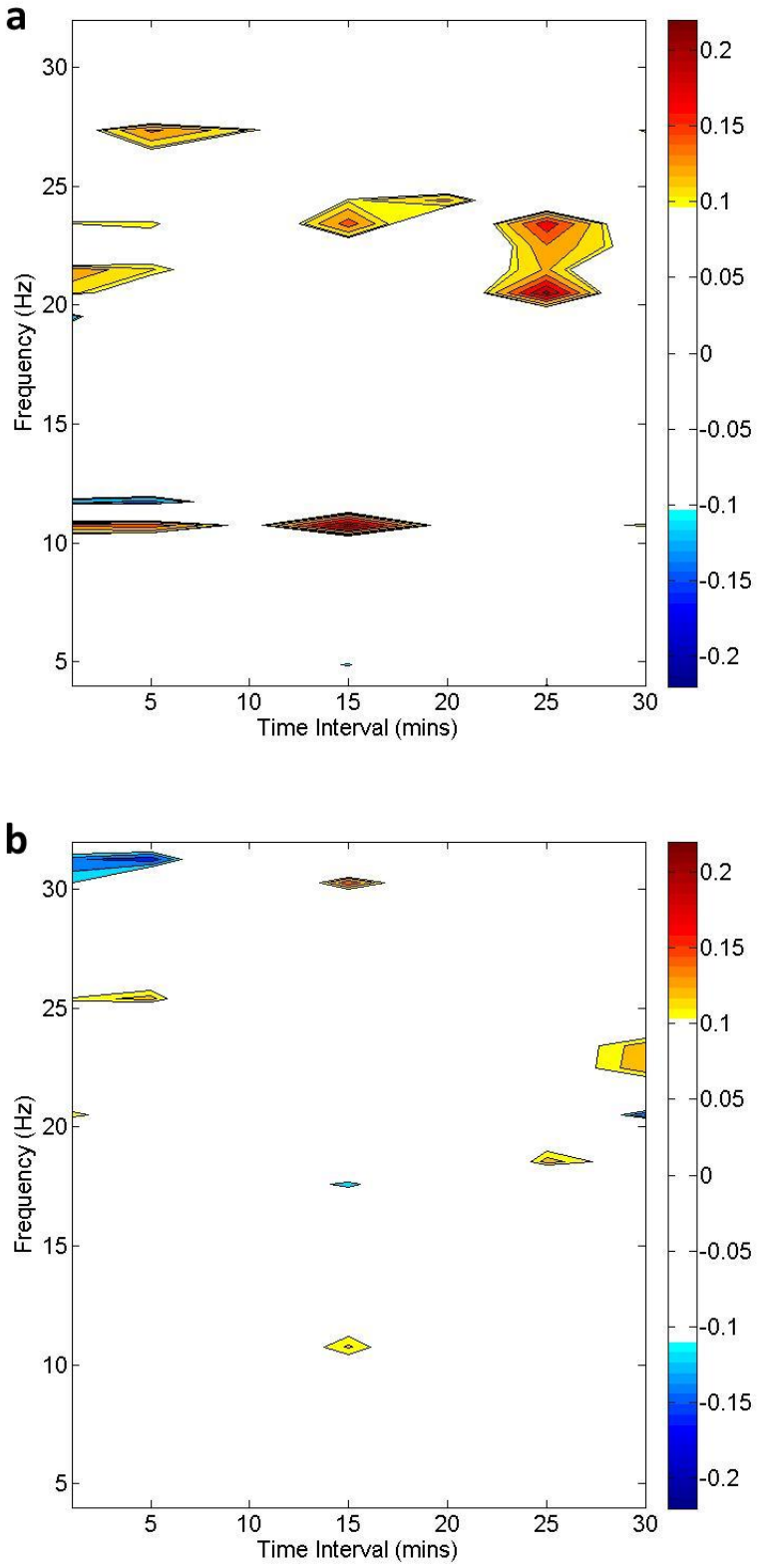


Figure 5.46: Comparison to baseline and motor task IMC following 10 Hz tSCS/PNS. a) Compared to basline both the α and β bands are dominated by significant increases in IMC. b) Compared to the motor task there are mostly significant increases both the α and β bands.

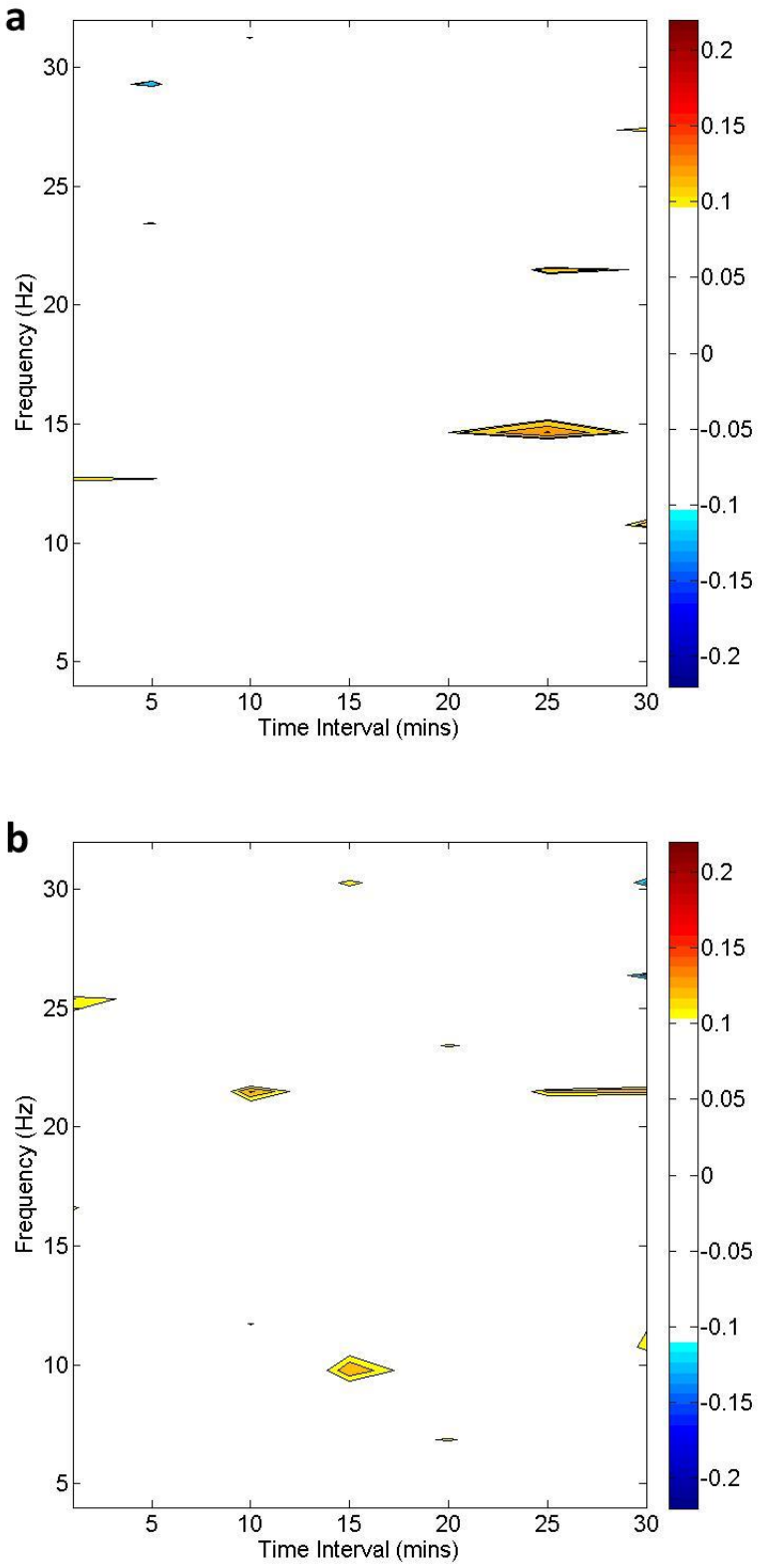


Figure 5.47: Comparison to baseline and motor task IMC following 20Hz tSCS/PNS. a) Compared to baseline both the α and β bands have significant increases and decreases in IMC. b) Compared to the motor task there are significant increases in both the α and β bands.

5.6.2 Physiological Neurogenic Tremor

The effects on phsiological neruogenic tremor of 5 Hz, 10 Hz, and 20 Hz tSCS paired with peripheral nerve stimulation were also investigated. The results are presented below.

Baseline Stability

As expected baseline stability was not exhibited in the ED-A coherence data. Additionally three participants did not have stable 1DI-A baselines in the (8 - 12) Hz range and so were not included in the following analysis. The data from the remaining seven participants were therefore used in the pooled analysis protocol.

Comparison of Coherence

Comparison of coherence to baseline showed that 5 Hz tSCS/PNS enhanced the lower range ((8 - 10) Hz)of the frequency band from 15 minutes until the end of the stimulation period, Figure 5.48a.

When the effects of the contraction task were controlled for it was seen that following 5 Hz tSCS/PNS the low range increases were no longer present, instead there was a large, wide spread increase in (8 - 12) Hz 1DI-A coherence only at 15 minutes post stimulation (Figure 5.48b).

The combination of 10 Hz tSCS/PNS increased 1DI-A at around 8 Hz and 11 Hz at 5 minutes and again at 15 minutes. At 20 and 25 minutes only the 8 Hz component was increased, but at 30 minutes post stimulation both 8 Hz and 11 Hz enhancements are seen Figure 5.49a.

Compared to the motor task stimulation 10 Hz tSCS/PNS increased 1DI-A cou-

pling between (10 - 12) Hz at 5, 15 and 25 minutes post stimulation. There was also a decrease at 12 Hz and 20 minutes (Figure 5.49b).

PNS and 20 Hz tSCS caused effects only at 5 minutes compared to baseline, where there was a decrease in around 9 Hz and a concomittant increase around 11 Hz (Figure 5.50a).

When the effects of 20 Hz tSCS/PNS were compared to the effects of the motor task there were increases in (10 - 12) Hz 1DI-A coherence evident at 5 and 15 minutes post stimulation. There was also a small decrease in coupling around 12 Hz occurring at 20 minutes, Figure 5.50b.

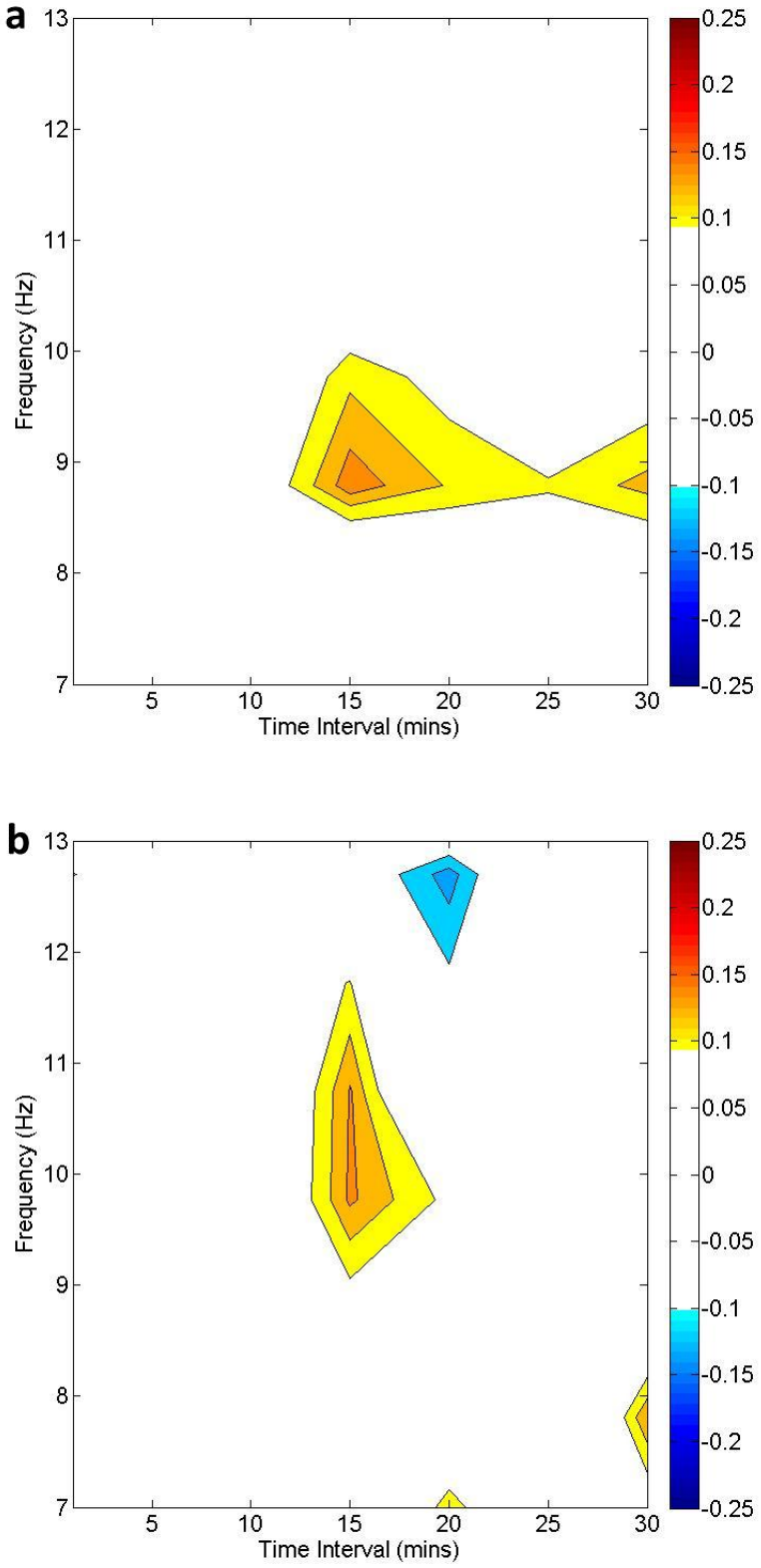


Figure 5.48: Comparison to baseline and motor task 1DI-A coherence following 5 Hz tSCS/PNS. a) Compared to baseline there are significant increases in coherence. b)Comparison to the motor task caused the result to be dominated by an increase in coherence at 15 minutes.

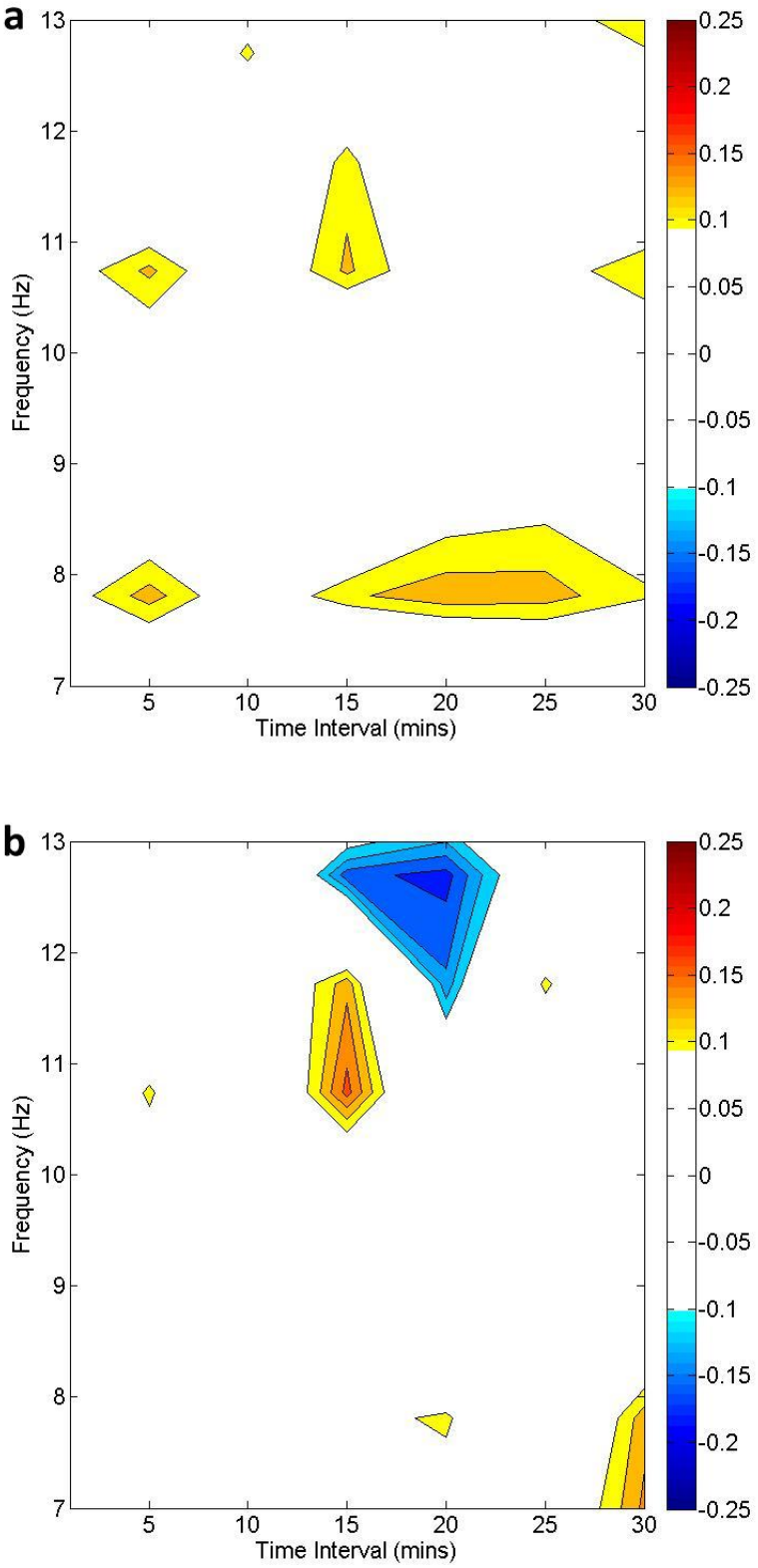


Figure 5.49: Comparison to baseline and motor task 1DI-A coherence following 10 Hz tSCS/PNS. a) Compared to baseline there are significant increases in coherence. Compared to the motor task there are mostly increases in coherence.

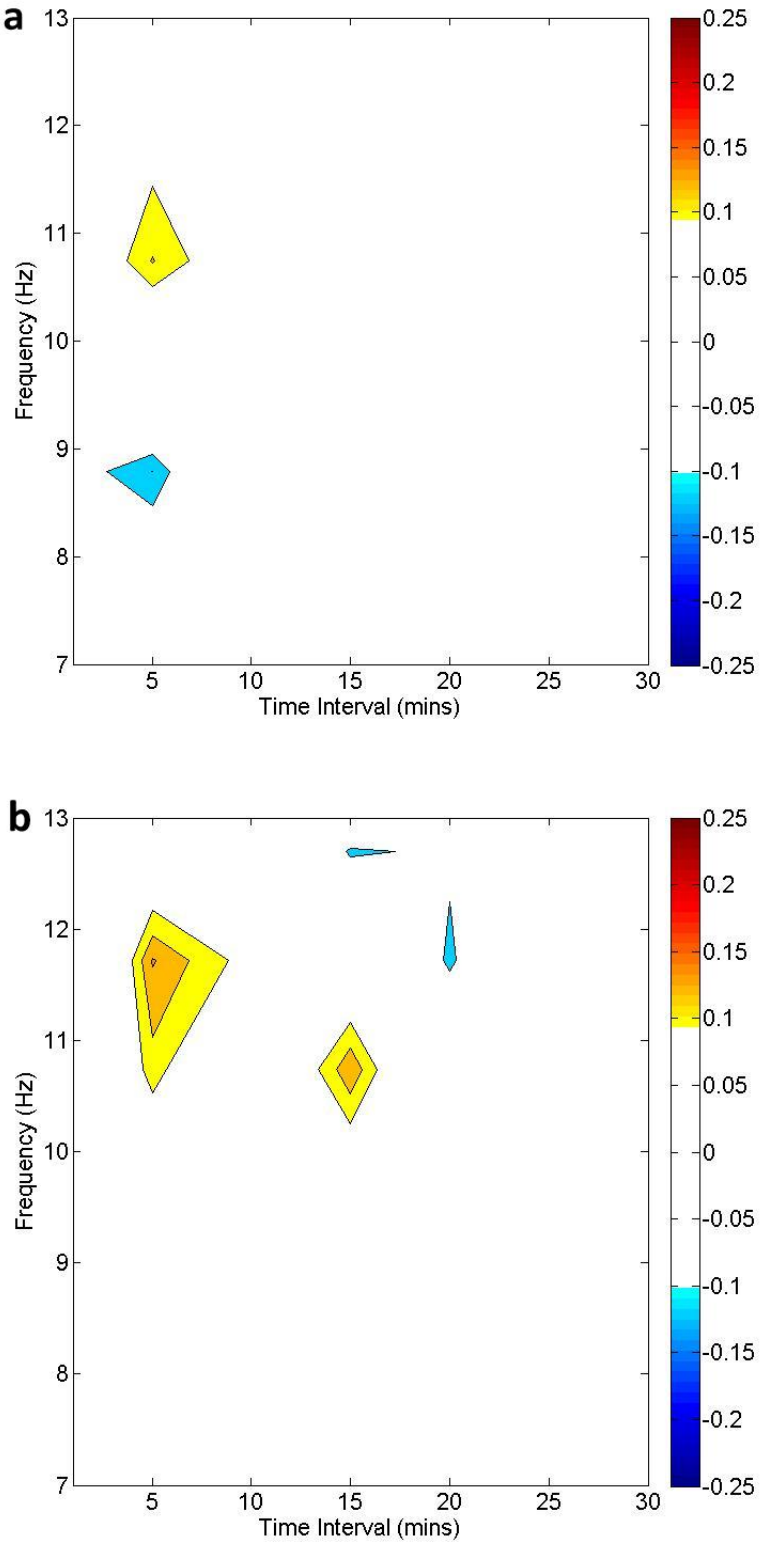


Figure 5.50: Comparison to baseline and motor task 1DI-A coherence following 20Hz tSCS/PNS. a) Compared to baseline there are both significant increases and decreases in coherence. b) Compared to the motor task the result is dominated by and increases in coherence.

Chapter 6

Discussion

6.1 Transcranial Direct Current Stimulation and Cortical Excitability

In the resting cell the characteristics of the cell membrane, its permeability to ions and the difference in ionic concentrations across it (intracellular fluid is more negative with respect to extracellular), result in its characteristic negative membrane potential difference. An action potential is initiated when voltage gated Na^+ channels open in response to depolarisation of the membrane and is therefore facilitated when the membrane potential is reduced; that is either the intracellular fluid becomes more positive or the extracellular fluid becomes more negative. Likewise an action potential is inhibited when the membrane potential is increased, that is when the intracellular environment is more negative and the extracellular is more positive. In theory an electric field of sufficient magnitude would interact with these ionic concentrations, most likely in the extracellular fluid. A positive electrode will produce an electric field that will repel positive ions and attract negative ones making the region under the electrode more negative, reducing membrane potentials and promoting depolarization; conversely a negative electrode will repel negative ions and attract positive causing the region

under the electrode to be more positive thus increasing membrane potential and promoting hyperpolarization. This effect on cortical excitability was reported in the early animal studies of Bindman et al. (1964) and Purpura and McMurtry (1965), but was dismissed for many years as clinically irrelevant.

More recently a number of studies have reported the same polarity dependent effect of tDCS on cortical excitability in humans and have suggested that the effects persist after the stimulation has ended (Nitsche and Paulus (2000, 2001); Nitsche et al. (2003b); Power et al. (2006)). Pharmacological studies have demonstrated that Ca^{2+} and Na^{+} channel antagonists reduce or abolish the after effects of the stimulation. This suggests that in addition to altering the neuronal environment during stimulation tDCS also interacts with neuroplastic mechanisms and that the technique may be relevant in rehabilitation after all. Unfortunately, however, there remains a considerable degree of variability in the results associated with those reports (section 3.2.1). This variability makes it difficult to evaluate the effects of tDCS on cortical activity and assess its usefulness as a clinical technique and possible aid for rehabilitation. As such the effects of anodal and cathodal tDCS on TMS induced MEPs were assessed here.

The results for cathodal tDCS were somewhat in accordance with the literature: statistically significant reductions in baseline MEP amplitudes were observed one and five minutes post stimulation (24% and 29% respectively) (Figure 5.4). Unlike many reports, however, there were no significant changes at any other time intervals. The magnitude of this result was more modest than that stated by Nitsche et al. (2003b) who reported a 40% decrease in baseline MEP amplitude after cathodal stimulation. The reductions observed in the present study was closer in magnitude to the small 20% reduction reported by Stagg et al. (2009).

For anodal tDCS the results from this study showed no significant change to baseline MEP amplitudes at any time interval after the stimulation had ended (Figure 5.3). Studies by other groups that have used similar stimulation and testing parameters have reported increases in MEP amplitudes of 16% - 50%.

Taken with the results from the literature the small but significant change to MEP amplitudes seen here suggests that tDCS is capable of penetrating into the brain and, that cathodal at least, is capable of plastically altering cortical excitability. The extent to which it can do this is still unclear, but the results from this study suggests that stimulation causes only a small change to cortical excitability that does not persist long after stimulation ends.

6.1.1 Variability in Motor Evoked Potentials

Variability in MEP amplitudes within a trial is an acknowledged problem with TMS. Groups that have achieved low variance tend to report larger means and longer durations of significant changes in cortical excitability. It is possible that the large variance in the data from this study have made it difficult to estimate accurate means and have obscured statistically significant results at the individual and group level. Here a number of systems were implemented in an attempt to reduce the variability that is caused through subject or TMS coil movement. Participants sat comfortably in a chair and rested their heads on a rig that supported their chins and forehead. The TMS coil was held on a spring mounted stand that allowed some subject movement to be corrected for. The hot spot for the target muscle was identified and three marks forming a triangle on the head were used to allow the coil position to be monitored and corrected if required. The coil position was observed throughout the entirety of the testing period. The data shown in Figures 5.3 and 5.4 clearly show that MEP variability was still very high, particularly in comparison with results reported by Nitsche

and Paulus (2000) (Figure 3.1). It is known that MEPs are often very variable in size, but it is possible that these measures were not sufficient in minimizing coil-subject movement. There are mapping technologies available to ensure that the coil position is maintained; but this equipment was not available and it is worth noting that Nitsche and Paulus (2000) and Nitsche and Paulus (2001) do not report using this equipment. While every care was taken to ensure the accuracy of the data, subject-coil movement cannot be ruled out as a component in the high variability seen here.

Interestingly in this study the baseline MEPs were normally distributed, while the post intervention were not. This may be suggestive of a difference amongst how individuals respond to tDCS. Unfortunately the population size of this study, n = 8 was too low to investigate this further. A responder/non-responder effect has not been directly suggested in the tDCS literature although Stagg et al. (2009) also reported non-normally distributed data. According to neuroplasticity models intervention induced synaptic strengthening or depression are dependent on previous activity in the same subset of neurones and have also been linked to secretion of BDNF (Gomez-Pinilla et al. (2002); Lamy and Boakye (2013)). Differences amongst participants' activity may therefore alter how each one responds to the stimulation and may account for the variance and non-normal distributions seen in these data. It would be worthwhile to explore this in a larger population.

The low variance reported by other groups may also have been caused by differences in their analysis. Analysis of MEP amplitudes in this study was different from Nitsche and Paulus (2000) in that here only extreme outliers were removed. The removal of extreme outliers is undertaken by other groups; the justification being that the inherent variability of TMS causes rogue MEPs. In addition to this Nitsche and Paulus (2000) also removed MEPs where muscle activity was

deemed to have occurred too close to the pulse onset. They did not detail their discrimination criteria and so it was impossible to repeat here. Close inspection of the data from the present study did not show any link between muscle activity and suspected outliers and so these data was retained in the analysis. This is not the first time that this has been highlighted as a problem (Hallett and Chokroverty (2005); Rothwell (1997)). There have been calls to introduce a defined protocol for MEP data collection but issues over whether to employ resting or active muscles have yet to be resolved and as yet there is no clear definition of what activity in the muscle is acceptable in a resting paradigm.

The magnitude of induced changes and the non-normal distribution of data from this trial were most similar to those reported by Stagg et al. (2009). Another similarity between the studies was the removal of the rubber-carbon tDCS electrodes prior to TMS testing. Delivering a magnetic stimulation through the conductive electrodes is likely to cause unknown warping effects on the electro-magnetic field. The impact of this on the resultant data is not known and certainly worthy of investigation. It is interesting to note that studies that removed the electrodes report much lower changes to MEP amplitudes than those which appear to magnetically stimulate the brain with the electrodes in situ. This may even be suggestive of novel techniques to shape the induced electric fields without the complicated and expensive alterations to a magnetic coil.

In the Literature Review the possibility of priming effects caused by the investigatory TMS pulses was discussed as a potential reason for the variability in reported results. An investigation into this was out with the scope of this study; however the same TMS paradigm as Nitsche and Paulus (2000) was employed here and yet caused very different results. This may suggest that priming of the cortex is not a leading cause of variability, but the reports of Siebner (2010) and

Delvendahl et al. (2010) mean that it cannot yet be ruled out as a component.

6.1.2 Mechanistic Insight

A final observation of this study worth noting was the difference in the cortical excitability response to anodal and cathodal tDCS. Other groups have also reported differences between the strength of the effects of the two polarities. Nitsche and Paulus (2001) and Nitsche et al. (2003b) demonstrated that a shorter duration of cathodal stimulation was required to cause cortical excitability changes of equal magnitude, but opposite direction, to anodal tDCS. If the same intensity and duration of stimuli are delivered the after effects of cathodal tDCS persist for longer durations than anodal. Many groups have questioned the reason for these findings since anodal stimulation is the literal polar opposite of cathodal and it is tempting, but ultimately too simplistic, to assume that the effects would be equal and opposite. Pharmacological studies have also found differences between the effects of the two polarities: anodal after effects are reduced with lorazapam (a GABAergic agonist) but the drug has no affect on cathodal after effects (Nitsche et al. (2004)). For both anodal and cathodal stimulation acetylcholine and serotonin both modulate the after effects but catecholomines only modulate anodal after effects and dopamine only cathodal (Stagg and Nitsche (2011)). These reports remind us that neurones do not work in a binary on off manner but rather as an analogue spectrum of response and neurotransmitter release based on changing thresholds that are dependent on previous activity and it would be foolish to expect anodal and cathodal tDCS to turn activity up and down to equal and opposite extents.

In conclusion this study has demonstrated that tDCS is capable of penetrating the skull and interacting with cortical excitability levels. The extent to which it can do this, however, is obscured due to critical issues with MEP variance, when used

as an excitability test, which may be reflective of differences in methodologies, variance in MEP amplitudes, responder/non-responder effects or a low effect size for the intervention. The question of whether tDCS can make a clinically relevant impact on cortical excitability remains. As discussed previously these issues prompt the development of new investigatory tools to extend the current understanding of how tDCS interacts with cortical activity and to put the variance of MEPs into context.

6.2 Inter-Muscular Coherence as an Investigatory Tool

The alterations to MEP amplitudes caused by tDCS suggest that an individual group of neurones have changed their output. The twitch effect that an MEP represents, however, is not reflective or natural movement. Movement is not created by binary on or off signals occurring muscle by muscle, but through the synchronisation of neuronal activity to form diverse and distinct patterns. Changes to cortical excitability levels are supposed to reflect changes in synaptic activity and firing frequencies and if synaptic activity is altered then neuronal communication between different networks will likely be altered as well (neurones that fire together, wire together). Coherence tools can probe the synchronization, or communication, between different neural networks. The work of Farmer et al. (1993), Brown et al. (1999) and Kilner et al. (1999) has suggested that IMC can provide insight into components of the common presynaptic inputs involved in motor output and is suggestive of how semi-distinct groups of neurones are behaving during movement instead of in a relaxed participant or during a twitch. This makes IMC a particularly attractive tool for investigating more functionally relevant tDCS induced changes for motor rehabilitation.

Baker and Baker (2003) demonstrated that promoting inhibitory intra-cortical interneurones caused enhanced cortical β oscillations during a hold task. The study by Nitsche et al. (2005), discussed in the literature review, suggested that interneurone dependent measures of short intracortical inhibition and intracortical facilitation were affected by tDCS: anodal tDCS decreased SICI and increased ICF, and cathodal tDCS increased SICI and decreased ICF. It is possible that these alterations to interneurone activity may be represented by changes in cortical drive to the muscles and Power et al. (2006) reported results to this effect: anodal tDCS enhanced β IMC and cathodal reduced it. As discussed in the Literature Review (section 3.4.3) however there are problems with the Power et al. (2006) study and what appears to be some contradictory results amongst the literature.

To explore these results and to use IMC to evaluate the effects of tDCS IMC must be a demonstrably robust tool. A group of preliminary trials were carried out here with the intention of developing such a tool by adapting both the contraction task and analysis regime used to collect and quantify IMC data.

6.2.1 First Contraction Task

In this first preliminary experiment the contraction task was designed to replicate a grip motion since complex and challenging tasks tend to improve motor outcomes. To maximize the quantity of data available EMG was recorded from four muscles (FCR, ED, 1DI and ADM).

Data Quality

When the data quality of the recordings was evaluated it was clear that even in this simple maintained grip task there were differences in the EMG patterns amongst the muscles. Figure 5.5 depicts typical 1DI and ADM EMG recordings

collected for this task; the ADM recordings were consistently poor across all participants, the signal was weak and often contaminated by noise.

Further evaluation of the data was undertaken using spectral analysis of the EMG traces in Neurospec. This is an effective mechanism for assessing data as there are clear differences in the spectral profile of good and poor quality recordings (Figure 5.6). Like the raw EMG recordings ADM data produced consistently poor spectral plots with a low power output and poor frequency profile.

Almost all the participants had difficulty in maintaining a constant contraction in the ADM muscle despite continual verbal encouragement to do so. ADM is a superficial muscle and so highly accessible for surface EMG recording but was ultimately not a good target muscle for this study and the task subjects were asked to perform. This was reflected in the consistently poor ADM EMG recordings and as a result they were deemed unsuitable for further analysis.

Baseline Stability

As previously discussed a prerequisite for any investigatory measure is that it stay stable and not change significantly out with the delivery of an intervention. To assess the stability of each individual's data χ^2 extended difference of spectra and coherence tests were implemented (described in Methods section 4.4.2). For each individual the data from each of the repetitions of the task were pooled; the χ^2 extended difference of spectra and coherence tests determine if and at what frequencies there are any significant differences between the input data sets to the pooling regime.

The χ^2 extended difference of spectra test showed that the baseline data sets were significantly different in their frequency content for both the α and β bands (ex-

ample data set in Figure 5.9). This was not entirely unexpected, as it is unlikely that participants would produce the same EMG recordings each time they repeat the contraction task.

The coherence between the pooled spectra is illustrated in the pooled inter-muscular coherence plot (example shown in Figure 5.8). They showed that the signals were synchronised in both the α and β frequency bands. This was expected for a constant contraction task and these data were similar to others reported in the literature. Like the χ^2 difference of spectra test mentioned above individuals exhibited significant differences in their input baseline coherence data (Figure 5.10 shows the result for a typical participant). This was true for the muscle pairs of all but one participant (Table 5.1 for a review). Since IMC is the parameter that will be used to compare and evaluate the intervention it is important that included participants have stable IMC baselines. If the baselines are unstable then intervention induced changes will be indistinguishable in post intervention tests; as such this contraction task was deemed unsuitable for future IMC tests.

IMC baseline stability does not appear to have been investigated to a large extent in the literature, but for the reasons stated above should be incorporated into future coherence studies. In this study it highlighted a major problem with the experimental design that would not have been observed otherwise.

Taken with the results for the raw EMG data quality and the fact that many of the subjects found difficulty in maintaining the contraction, particularly in ADM, it suggested that the problem was being caused by the inability to co-activate ADM with the other muscles. To improve data quality and baseline stability the study design was therefore changed. In an attempt to make it easier for participants

to co-activate a set of muscles capable of giving high quality EMG records the target muscle 1DI was changed to APB and the contraction time was reduced to one minute.

6.2.2 Second Contraction Task

EMG quality was analysed as discussed above, once again recordings from ADM were uniformly poor and data from this muscle were not included in the baseline stability tests. The χ^2 extended difference of spectra tests showed that, again, there were significant differences in each individual's spectra. The χ^2 extended difference of coherence tests showed that the stability of each participant's IMC recordings had improved compared to the first contraction task. Each participant exhibited stability in at least one muscle pair, but, again most data sets were not stable and there was no trend seen for a particular muscle pair (summarised in Table 5.2). Subject compliance was still poor and many participants reported difficulties in trying to activate all the target muscles.

These results demonstrated that the changes made to the contraction task did not improve subject compliance, data quality or the baseline stability. The contraction task was again redesigned with the aim of finding a task that would produce stability in baseline IMC.

6.2.3 Third Contraction Task

The results from the two grip tasks suggested that the contraction was physically difficult for participants to execute for the necessary time. The task was therefore simplified to a two digit extension and only two muscles, ED and 1DI, were investigated.

The results from this trial immediately showed an improvement in raw EMG

data quality (example EMG traces in Figures 5.11); there was a strong contraction and a very low noise to signal ratio in both the investigated muscles. This was reflected in the EMG power spectra for both muscles (Figure 5.12) with clear peaks in the α and β bands and high power amplitude observed throughout the data sets. Coherence between the two muscles was also present (Figure 5.13) with peaks in both the α and β bands were visible.

The χ^2 extended difference of spectra tests (Figure 5.14) mirrored the results from the first two contraction tasks, that is, there was a significant difference in the input spectra for the muscles. The baseline stability test explicitly showed that evaluating intervention induced changes to spectra would not be useful and on this basis it was not included in any further analysis. Spectral analysis is employed in the literature and these results suggest that it should be used with caution.

The results from the χ^2 extended difference in coherence tests were more interesting; there were no significant differences in the input data for each individual (Figure 5.15). This result demonstrated that for this contraction task the participant's baseline coherence remained stable over different days and different times of day, a pre-requisite for establishing intervention induced changes to IMC.

This contraction task was used in all of the following trials to investigate the effects of the interventions on IMC. These were the same muscles used by Power et al. (2006) and the task resembled theirs. This meant that there would be a reduction in the information that could be extracted from our test due to the fewer number of target muscles, but there would be increased comparability between these tests and those conducted by Power et al. (2006).

6.3 Transcranial Direct Current Stimulation, Inter-Muscular Coherence and Physiological Neurogenic Tremor

The aim of this study, as discussed in the Research Statement, was to assess the reports from the literature that tDCS alters IMC in a polarity dependent way. Power et al. (2006) reported that 10 minutes of anodal and cathodal stimulation caused increases and decreases in IMC respectively. The data sets were also extended to include a tremor measurement and any effects of tDCS on PNT were investigated.

6.3.1 Inter-Muscular Coherence

Baseline Stability

IMC baseline stability was evaluated for each participant. Two did not demonstrate stability; interestingly these two both found the contraction task difficult to maintain. It is possible that subject compliance and task difficulty are related to baseline IMC stability or this may suggest a limitation of the coherence test in that it requires a certain amount of stationary data before it is reliable. This is something to consider for future study designs.

Comparison of Coherence Analysis

For the assessment of any tDCS induced changes to IMC the pooling regime in Neurospec was employed. This protocol was chosen because it provides a robust analysis technique: that of combining the individuals' data into a pooled data set thus allowing observations of significant changes to the group. This technique applies the proper weighting of the individual data sets and the correct combinations of statistical tests.

The IMC plots that are produced represent the group's data and Figure 5.16 demonstrates that the pooled data exhibit the same coherence profile as individuals, with significant coherence in both the α and β frequency ranges present.

Neurospec also offers comparison of coherence tests which allow for the comparison of two coherence data sets. The protocol outputs plots which visually represent the frequencies in which the second data set has significantly more or less coherence (example shown in Figure 5.17).

Comparison to Baseline

A comparison of each post intervention time point to baseline IMC was first undertaken and the information from each of the comparison of coherence tests was collected and displayed in time-frequency plots, see Methods section 4.5.2.

Figure 5.18 showed that both anodal and cathodal tDCS caused small changes to IMC when compared to baseline. The results were similar to Power et al. (2006) in that anodal tDCS increased β IMC at five minutes post stimulation, cathodal decreased it at five and ten minutes and there were no changes to α IMC.

Interestingly, unlike Power et al. (2006) the largest effects in this study were caused by the sham stimulation (Figure 5.19). There were significant, wide spread increases from baseline in the α band (around 10 Hz) and a reduction in β IMC one and five minutes post stimulation followed by small increases later in the testing period. As discussed above there was a concern that each investigatory tool is capable of altering cortical function within its own right, and when combined with other intervention techniques could cause priming effects on the brain. The strong affects of the contraction task compared to baseline suggests that repeti-

tive contraction does alter IMC. In their trial Power et al. (2006) did not observe any significant changes from baseline in the first 10 minutes after sham stimulation. A second sham test, undertaken here on a separate group of participants (for the PNS and tSCS trials below) showed similar results to the present study (Figure 5.31). Again there was a large increase in the α band, particularly around 10 Hz although the changes to the β band were less marked.

While this result is different from Power et al. (2006) the affect of the contraction task may be comparable with the results of the imaging studies in which the motor task reduced rCBF and BOLD MRI in M1 (Jang et al. (2009); Stagg et al. (2009)). These reductions were suggested to represent habituation/adaptation to the motor task. Since the tasks were different caution must be used when comparing these results (the imaging studies used repetitive finger opposition movements or button presses whereas a constant contraction was employed here), but it is possible that the changes in some of the components of IMC may also be representative of habituation. Habituation to sensory stimulation and motor learning have been associated with cortical rhythmicity around 10 Hz (Endroczi et al. (1970); Zhuang et al. (1997, 1998); Antal et al. (2008)), one of the frequencies that was altered in these IMC results. It is impossible to deduce from the present data whether the increase in α IMC is representative of a cortical or sub-cortical interaction, but there is a possibility that it is represents habituation/adaptation to the motor task.

Frequencies of (8 - 10) Hz have also been associated with descending muscular output during slow movements (Vallbo and Wessberg (1993)). Since this was a constant contraction task and not a slow movement it seems unlikely to be linked with that pulsatile (8 - 10) Hz output although the data is tightly spread over those frequencies. One might have expected repetitive constant contraction to

promote 20 Hz IMC and inhibit movement by reducing any (8 - 10) Hz components. Instead repetition of the task caused the opposite effect and is reminiscent of Newton's Third Law: 'every action causes an equal and opposite reaction'. Instead of habituation then these results may be suggestive of homeostatic plasticity working to inhibit unstable positive feedback.

As noted in the Literature Review (section 3.4.2) cortical oscillations and CMC in the α freqeuncy range have also been tenuously related to (8 - 12) Hz PNT (Marsden et al. (2001); Raethjen et al. (2002)). Unfortunately an association between IMC and PNT cannot be deduced from these data, it was postulated that the incorporation of the accelerometer recording into this trial (discussed below) would provide insight into this issue.

These results for anodal, cathodal and sham tDCS suggest that the cortical stimuli altered IMC in a small but polarity dependent way when compared to baseline. The strong affect of the contraction task however, suggests, like the imaging studies, that in order to control for this a comparison between sham and active tDCS would be appropriate.

Comparison to Sham tDCS

To control for the effects of the motor task comparison of coherence tests between the sham IMC and each of the post-intervention time interval data sets were implemented. Interestingly, the significant affects of tDCS on IMC were quite similar for both stimulation polarities (Figure 5.20): both enhanced β and reduced α band IMC. Again, like the motor task result, this is comparable to the imaging studies in which both polarities of tDCS caused increases in rCBF and BOLD MRI in M1 compared to the motor task alone (Lang et al. (2005); Stagg et al. (2009); Jang et al. (2009)). Inspection of these data sets also revealed that

for both anodal and cathodal tDCS there was a gap around 15 minutes where there was no, or very little IMC present. This may suggest that the data can be split into two parts: primary events that occurred in the first 10 minutes and secondary events that occurred in the final 10. Most of the literature suggest that 10 minutes of 1 mA tDCS produces effects which persist for about 10 minutes. It is possible that the primary effects observed here were caused directly by the de/hyper-polarisation of the underlying cortex. It is also possible that there was a rebound, or feedback into the motor cortex and it is proposed that these secondary effects are represented in the later changes to IMC. There are other possible reasons: the primary changes may reflect an effect associated with the removal of the tDCS, that is an off response and the secondary could be a consequence of plasticity since it may take time for connectivity to establish.

Compared to the effects of the motor task anodal tDCS enhanced β band IMC (Figure 5.20a). At first sight this result appears to be in accordance with most of the tDCS literature and with the suggested mechanisms of action: anodal tDCS reduces membrane potentials, promotes neuronal firing and causes increases in rCBF, blood oxygen levels and cortical excitability. As noted above β oscillations are strongly associated with the cortex during constant contraction. It is possible that tDCS induced increases to cortical excitability and enhanced the activity of the neural networks that are involved in sustaining this rhythm. Enhanced cortical oscillations may then result in enhanced synchronous activity to the common pre-synaptic pool resulting in the increased β IMC that was observed here and for Power et al. (2006). As noted previously, however, Power et al. (2006) did not find significantly enhanced cortical excitability following anodal tDCS and neither did the present study.

The pharmacology studies also complicate this hypothesis: as noted in the Litera-

ture Review (section 3.4.3) Baker and Baker (2003) reported that the GABAergic agonist diazepam enhanced cortical β oscillations by promoting cortical inhibition. It is possible that anodal stimulation promotes cortical excitability in these inhibitory circuits thus resulting in enhanced β rhythms, but this contradicts the results of Nitsche et al. (2005) who report that anodal tDCS reduced SICI and promoted ICF. This is further complicated by the report, of Baker and Baker (2003), that the enhanced β rhythm was not propagated down the CST, indeed CMC was reduced. A similar result was observed when Raethjen et al. (2002) delivered carbamazpine (another GABAergic agonist) to an epileptic population; although the same drug delivered to a healthy population caused enhanced β CMC (Riddle et al. (2004)). These contradictory results may have been caused by other affects of the drugs or may be suggestive of more complex interactions in the cortex. Differences in the oscillatory characteristics between different regions have previously been observed: the oscillatory power in the somato-sensory cortex is often larger than M1 (Witham and Baker (2007)) but the neurones in the motor cortex have a tendency to follow a post spike peak membrane potential trajectory not mirrored by the neurones in the sensory cortex. Baker (2007) suggests that peaked membrane potentials in M1 are a way for circuits to couple more effectively and for the signal to be propagated down the corticospinal tract more reliably (Witham and Baker (2007)). The propagation down the CST is therefore likely to be controlled by different mechanisms that may also have been inhibited by diazepam whereas anodal tDCS may have enhanced all of them leading to increased rhythmicity and increased propagation of MEPs and oscillations.

As noted above cathodal stimulation also enhanced β band IMC. The similarity in effects between the two stimulation polarities is akin to that reported by the imaging studies, but is difficult to reconcile with the TMS studies that report opposing effects. Cathodal tDCS purportedly suppresses cortical activity, a re-

sult that was also found in the present study. One way of reconciling this may be found in a report by Lee et al. (2003) who observed enhanced PET activity following inhibitory rTMS (Lee et al. (2003)). It is not suggested that rTMS and cathodal tDCS alter the cortex in the same way, but the similarity between the results cannot be ignored and may suggest mechanistic insight. Lee et al. (2003) suggested that inhibitory rTMS may result in decreased MEPs and increased rCBF for two possible reasons. The first is that the inhibitory stimulation increases the activity of inhibitory neurones; the second is that it reduces synaptic efficacy, here the pre-synaptic input would stay the same, seen as task related increase in rCBF, but the output would be reduced, observed as a decrease in MEP amplitude and an increase in SICI. As discussed above Baker and Baker (2003) demonstrated that β oscillations in the cortex are promoted by inhibition and Nitsche et al. (2005) demonstrated that cathodal tDCS facilitated SICI. Like the rTMS study one could postulate that cathodal tDCS enhances inhibition in the cortex thus leading to increases in rCBF, blood oxygen levels and β oscillations.

Unlike the current study Power et al. (2006) did not find enhanced β IMC following cathodal tDCS. They theorized that as cathodal tDCS reduces cortical excitability then it suppresses activity in the circuits that are associated with this rhythm. A small, but significant reduction in cortical excitability was observed in both theirs and the present trials; however they did not take into account that β oscillations are related to inhibitory circuits and inhibition of inhibitory circuits will lead to facilitation.

There were some differences in the present study's data that may be suggestive of the polar opposing effects on MEP amplitudes. Anodal tDCS enhanced β IMC more than cathodal and cathodal enhanced α more than anodal. The imaging studies demonstrated that cathodal stimulation interacts more with other regions

of the brain than anodal does (Lang et al. (2005); Stagg et al. (2009)) and so the stronger effects of cathodal on α IMC may be representative of a stronger interaction with the peripheries; however this is conjecture as the relation between the effects of tDCS and the BOLD signal are not clearly defined.

In the α frequency range both interventions reduced (8 - 10) Hz IMC at the five minute test point which was concomitant with an increase at 20 Hz. That is, the effects at five minutes were directly opposite to the motor task alone which had increased (8 - 10) Hz IMC and reduced 20 Hz (Figure 5.19). It was hypothesized above that the effects of the motor task on IMC may be representative of habituation to the motor task, affects on homeostatic plasticity or affects on PNT. Anodal tDCS has been reported to increase motor learning and motor adaptation (Nitsche et al. (2003c); Hunter et al. (2009)) and motor learning has been associated with 10 Hz cortical oscillations (Antal et al. (2008)). Stagg et al. (2009) demonstrated that both polarities of tDCS opposed the linear decreasing habituation effect of the motor task. The intervention induced effects observed in this study did not appear until the second repetition of the task (five minutes post tDCS), a time course that may be suggestive of an interaction with the habituation response (Endroczi et al. (1970); Stagg et al. (2009)). Stagg et al. (2009) did report that anodal tDCS had the largest effect on the habituation effect, but the IMC data here suggest that cathodal tDCS had the largest effect on the (8 - 10) Hz rhythm.

It is interesting to hypothesize about what decreasing motor habituation might mean for motor rehabilitation. It might be suggestive of a different learning state as habituation tends to lead to optimised motor control. Inhibition of habituation may keep the brain in a 'learning state' for longer or alternatively it may diminish the optimisation that is desirable in motor learning. As noted above 10 Hz oscillations have been associated with motor learning and habituation (Antal

et al. (2008)) and Hunter et al. (2009) reported that anodal tDCS enhanced motor adaptation in a robot resistive force field. Unfortunately they did not study the effects of cathodal tDCS.

The other hypothesis suggested above was that of an interaction with the homeostatic response. Vallbo and Wessberg (1993) observed pulsatile (8 - 10) Hz output during slow movement that was central in origin, but appeared to be separate from mechanisms of PNT. Pogosyan et al. (2009) demonstrated that tACS enhanced 20 Hz IMC and slowed the initiation of new movement. In the present study both anodal and cathodal tDCS interventions opposed the action-reaction affect of the motor task. Here we observed that 20 Hz IMC was enhanced and (8 - 10) Hz reduced with both polarities of stimulation. This may suggest that the (8 - 10) Hz IMC seen here is representative of the movement rhythm discussed by Vallbo and Wessberg (1993). Marsden et al. (2001) and Raethjen et al. (2002) have suggested that (8 - 12) Hz CMC is also reflective of PNT but so far the data here does not suggest any link between these phenomena apart from the fact that they share the same frequency range. The incorporation of accelerometer recordings meant that the relationship between (8 - 10) Hz IMC and (8 - 12) Hz PNT could be explored and the findings are discussed below.

The final point worth noting about these data is that cortical stimulation, in the form of tDCS, appears to have interacted with common drive to the muscles, represented by IMC. This may be suggestive of a cortical generator for these oscillations, or, at least, that components of these oscillations are driven by and can be altered at the cortex. This has long been suspected for output in the β frequency range, but there has been less evidence for cortical involvement in the α rhythm.

6.3.2 Alternative Analysis: Average Significant Coherence

In order to determine whether significant changes had occurred after tDCS interventions Power et al. (2006) summed the significant coherence at each time interval for each participant (Figure 4.10), averaged this over all participants and tested for significant differences between baseline and each time interval. The problems with this technique are discussed in the Literature Review (section 3.4.3) but for comparison purposes it was reproduced here. This was particularly suitable since the contraction task used by Power et al. (2006) and the present study were the same. Power et al. (2006) only looked for changes to IMC in the first 10 minutes post intervention and for the purposes of comparison this analysis was restricted to this time interval too.

The data from the present study showed that there were no significant differences from baseline in the averaged area for α or β IMC after anodal, cathodal or sham tDCS. Power et al. (2006) reported no significant changes in α for either polarity of tDCS; however, they did report significant, polarity dependent changes in the β band: an 18% increase following anodal tDCS and a 17% decrease following cathodal tDCS.

There are some possible reasons for the differences in these results. Power et al. (2006) did not test baseline stability; whereas participants in the present study who did not exhibit stable baselines were not included in this analysis. Evidently Power et al. (2006) could not exclude non-stable participants since they did not test for them.

Another issue, that became apparent whilst repeating their analysis technique, was that of zero significant coherence exhibited by some individuals in this study.

Power et al. (2006) did not discuss removing this data and so it was decided to include it in the average analysis here. It is clear that the inclusion of a binary zero coherence in an otherwise analogue scale will have an effect on the average and will skew the ability to quantify the change in coherence. This effect can be seen clearly in both Figures 5.21 and 5.22.

Finally, Power et al. (2006) immediately preceded the contraction task with a TMS investigation; this protocol was not carried out in the present study. As discussed previously in the Literature Review (section 3.9) each of these investigatory tools may be able to alter cortical function within its own right and the combinatory effects of so many interventions is unknown. The separation of TMS and the contraction was one of the main motivations for the current study and may have caused the differences in effects observed here.

In conclusion this analysis regime did not find any significant differences in α and β IMC following either anodal, cathodal or sham stimulation in the current study. Given the concerns with the protocol, that have already been discussed, it was concluded that any changes to IMC by an intervention would include baseline stability tests and would be analysed using the more robust pooled analysis technique.

6.3.3 Physiological Neurogenic Tremor

The addition of the accelerometer in the contraction task was initially undertaken to determine whether coherence between accelerometer and EMG recordings in the (8 - 12) Hz frequency range of physiological neurogenic tremor would reflect tDCS induced changes to cortical activity that could be detected in the periphery. Before this could be assessed stability in individual's accelerometer spectra and muscle-accelerometer coherence without an intervention were evaluated.

Baseline Stability

The development of the contraction task, above, demonstrated that there were significant differences in the participants' input baseline muscle spectra and this lack of stability was also observed in the accelerometer spectral data (Figure 5.25). This was somewhat surprising since many studies assess changes in accelerometer frequency power spectra when assessing tremor (Raethjen et al. (2002) for an example). This demonstrates that caution should be used when employing this spectral data since, for this trial at least, it changed significantly without an intervention.

The χ^2 extended difference of coherence tests highlighted that there were significant differences in individuals' baseline ED-A data, but not in the 1DI-A data which demonstrated stability (Figure 5.27). It is unclear why ED-A did not exhibit stability. The accelerometer was positioned on the middle finger and ED was active whilst the middle finger was extended and yet the (8 - 12) Hz coherence between muscle activity and the limb tremor were not stable. This may relate to ED's joint action as a wrist and finger extensor muscle and may reflect it's role in stabilising the wrist. The instability exhibited between ED-A meant that it was unsuitable as a measure for evaluating any tDCS induced changes to PNT.

1DI-A baseline stability was restricted to the (8 - 12) Hz frequency range; this is probably because higher frequencies are dominated by the mechanical properties of the limb, loading and fatigue and these factors are unlikely to remain stable over different tests. The tremor frequency profile of the neurogenic component of physiological tremor is supposed to be uniform throughout all muscles of the body and the result that 1DI-A demonstrates stable coherence with an accelerometer mounted on the middle finger supports this. The action of 1DI is not related

to the plane the accelerometer was aligned to on the middle finger and so the 1DI-A coherence is likely to be a measure of common drive that links the EMG of 1DI to the tremor signal of synergistic muscles action on the middle finger. Coherence between 1DI EMG and the accelerometer trace was therefore deemed to be an acceptable measure for assessing intervention induced PNT in the (8 - 12) Hz frequency range.

Comparison of Coherence

In order to assess any intervention dependent changes to (8 - 12) Hz PNT the data was treated in the same way as the IMC above: The 1DI-A data from each participant were pooled for each intervention and comparison of coherence tests between baseline and the post intervention tests (1, 5, 10, 15, 20, 25 and 30 minutes) were conducted.

Comparison to Baseline

There was a significant increase in 1DI-A coupling around 9 Hz after anodal tDCS, when compared to baseline, that persisted for the duration of the testing time (Figure 5.28a). In comparison there was very little change following cathodal tDCS (Figure 5.28b). When post intervention IMC data were compared to baseline there were very few changes at this frequency and the results for anodal tDCS on IMC and PNT, in particular, appear to be quite different. This finding does not support the idea of there being a link between (8 - 10) Hz muscle output and PNT.

A test of the effects of the contraction task on PNT showed that there were some small changes to 1DI-A coupling (Figure 5.29). The motor task induced changes to PNT are not as wide spread as those observed for IMC, but they are still present. To further investigate these effects and to distinguish them from those

caused by the tDCS interventions a comparison to sham tDCS was also undertaken.

Comparison to Sham tDCS

The comparison to sham produced interesting results. Anodal stimulation, which seemed to interact significantly with 1DI-A when compared to baseline did not cause any significant changes when the effects of the motor task were controlled for (Figure 5.30a). While the motor task only induced significant changes from baseline at the end of the test period this result suggests that there was a non-significant trend towards increased (8 - 10) Hz across the entire 30 minutes. There is nothing in the literature to suggest that repetitive contraction should enhance (8 - 10) Hz PNT, but it did significantly enhance (8 - 10) Hz IMC (Figure 5.19). Raethjen et al. (2002) reported that in an epilipetic population (6 - 15) Hz CMC was present at the same time as (6 - 15) Hz ECoG-accelerometer coherence and so postulated that there was cortical drive to both the EMG and PNT in this frequency range. At best the non-significant trend towards enhanced (8 - 10) Hz PNT and the significant increase in (8 - 10) Hz IMC found in the present study may suggest a weak link between (8 - 10) Hz IMC and PNT, but ultimately there is very little evidence to link the two phenomena.

The first piece of evidence found in this study to link (8 - 10) Hz IMC and (8 - 10) Hz PNT was seen when the effects of cathodal tDCS were compared to sham tDCS. The IMC data had shown that both anodal and cathodal stimulation suppressed (8 - 10) Hz IMC at five minutes and for cathodal tDCS also at ten minutes post stimulation (Figures 5.20). Anodal tDCS produced the smaller reduction in (8 - 10) Hz IMC and as already noted these data show that it was not associated with a suppression in PNT (Figure 5.30a). Cathodal tDCS caused a larger effect in (8 - 10) Hz IMC and this was mirrored, to a smaller degree, in

the PNT data (Figure 5.30b).

There were three proposals put forward to explain the changes seen in (8 - 10) Hz IMC after the motor task and tDCS: that it represents the cortical input to PNT, habituation to the task, or pulsatile (8 - 10) Hz output associated with slow movement working to oppose the promotion of the constant contraction. Habituation was discussed in the context of the IMC data, but it was hypothesised that the accelerometer data would provide insight on any associations between (8 - 10) Hz IMC and PNT. The data showed that there were very few similarities between changes to (8 - 10) Hz IMC and PNT and they do not appear to represent the same generator mechanisms within the motor system. The decrease seen in both the IMC and PNT data post cathodal tDCS however means that the idea that they are linked in some other way cannot be ruled out.

Compared to the motor task cathodal stimulation also caused increases in (8 - 12) Hz 1DI-A coupling across many of the test times. The significantly enhanced frequency shifted upwards throughout the testing period (Figure 5.30b). These increases in PNT were not mirrored by changes to IMC and the first of them was concomitant with the decrease at (8 - 10) Hz.

Taken together these results for anodal, cathodal and sham tDCS suggest, like McAuley and Marsden (2000) proposed, that there are multiple components expressed in PNT and that it is an output of different, integrated inputs, of which IMC is one. The suppression of both (8 - 10) Hz IMC and PNT following cathodal tDCS suggests that when the IMC input is large enough it will affect PNT. The expression of multiple inputs from various parts of the nervous system probably goes some way to explaining the contradictory results found in the literature (Conway et al. (1995); Marsden et al. (2001); Raethjen et al. (2002)).

There is a strong association between β oscillations and the motor cortex and, as shown above, both polarities of cortical stimulation interacted with β IMC. As discussed in the Literature Review there is controversy about the role of the cortex in α oscillations observed in both (8 - 10) Hz IMC and (8 - 12) Hz PNT. These results seem to suggest that since cortical stimulation altered both (8 - 10) Hz IMC and PNT a component of it is driven by the cortex. There is much debate concerning the origin of the generator for PNT (McAuley and Marsden (2000) for a review) but these results, in parallel with others in the literature, suggest that the generator, or rather, inputs points in the loop, for this rhythm are poorly associated with the motor cortex.

The results for cathodal tDCS are also interesting because inhibition in the cortex appears to have weakly enhanced oscillations farther down the motor pathway. As discussed above imaging studies found that cathodal stimulation affected more areas of the brain than anodal did. This same suggestion has been hinted at in the TMS and IMC studies and the interaction between cathodal tDCS and PNT may be another example of this.

6.4 Inter-Muscular Coherence and Physiological Neurogenic Tremor as Investigatory Tools

The primary aim of this part of the thesis was to develop IMC and PNT as investigatory tools and to use them to evaluate any effects of tDCS.

This study demonstrated the importance of designing a contraction task that allows for the collection of high quality data and good subject compliance. Robust analysis was also demonstrated to be of importance in developing IMC and

PNT as useful measures. The introduction of baseline stability analysis was an integral component as it was vital to demonstrate that IMC and PNT remained stable without an intervention. Interestingly baseline stability analysis showed that both muscle and accelerometer spectra were not appropriate measures for investigating post intervention changes in these trials; however IMC and coherence between 1DI and the accelerometer were. Comparison of coherence tests proved to be a better analysis technique for assessing the post intervention data than the analysis protocol put forward by Power et al. (2006). Finally the employment of these techniques showed that the contraction task used to collect IMC and PNT was itself having an effect on IMC and PNT. This result meant that comparison of coherence between sham and each post-intervention time test would be required to gain a better understanding of the effects of the interventions.

The results from the IMC and PNT measures, as well as the cortical excitability study suggest that tDCS penetrates the skull and interacts with cortical excitability, synchronization and propagation of oscillations at the cortical level. Both anodal and cathodal tDCS promoted the β oscillations that are strongly associated with the motor task. Interestingly they also interacted with α rhythms which are not generally associated with constant contraction or the cortex. The IMC results suggest that tDCS was opposing the effects of the motor task as either an opposition to habituation or homeostatic plasticity.

While the changes to MEPs, IMC and PNT are interesting they were small and it seems unlikely that they would be clinically relevant. The next part of the thesis used these tools to investigate if alterations to the tDCS protocol could promote changes in IMC and PNT.

6.5 Paired Associative Stimulation: Transcranial Direct Current Stimulation and Peripheral Nerve Stimulation

Stimulation of the peripheral nerves results in afferent volleys in the ascending sensory pathways that will influence activity in the brainstem, cerebellar, sub cortical and cortical regions. Studies have reported that PNS enhances cortical excitability and BOLD fMRI (Riding et al. (2000); Charlton et al. (2003); Wu et al. (2005)) and that the effects persist after the stimulation has ended. These reports suggest that PNS is capable of interacting with mechanisms involved in neuroplasticity at the cortical level. Although variability in MEP amplitudes have made the evaluation of PNS induced changes to cortical excitability difficult, PNS appears to require a much longer stimulation duration (two hours) in order to produce the same change in MEP amplitudes caused by 10 minutes of anodal tDCS. There are no prior publications of the effects of PNS on IMC or PNT.

Peripheral nerve stimulation is combined with other NIBS techniques to create paired associative stimulation paradigms which are reported to enhance the effects of either stimulus delivered alone. PNS paired with TMS is well documented in the literature and conforms to the accepted mechanisms of associative plasticity: that the inputs to the postsynaptic cell are synchronous and occur at the same time. PNS paired with tDCS is less well researched; here the tDCS is held constant and the PNS pulsed creating a pair of stimuli that are, arguably, synchronised and concommitant on the post synaptic cell. This kind of stimulation has been reported to, again, enhance the effects of either stimuli delivered alone (Celnik et al. (2009); Rizzo et al. (2014)). There are no reports in the literature of these affects being tested with IMC or PNT.

As noted above the motor task itself has an effect on IMC and PNT, particularly for IMC. These effects were tested again in a new population of participants. The results showed a similar but stronger effect on α IMC compared to those seen in the first group, but there were fewer significant changes to the β band (Figure 5.31). For PNT, again, the results were similar: there was only one very weak change in PNT which occurred at the same frequency as the previous group's (Figure 5.35).

6.5.1 Peripheral Nerve Stimulation

Effects on Inter-muscular Coherence

As previously discussed it is important to understand the effects of each intervention before combining with others. This is because there is the possibility that combinations of stimuli could be causing priming effects on the brain. As such an evaluation of the effects of peripheral stimulation alone was carried out first.

The data showed that compared to baseline PNS significantly increased β range coupling between the muscles at a number of time points throughout the test period (Figure 5.32a). When the effects of the motor task were controlled for the addition of peripheral stimulation was shown to cause increases in β IMC that were restricted to measurements made in the first 10 minutes post intervention (Figure 5.32b). In the α band, however, there were few changes in the first 10 mintues.

The β range IMC enhancements were similar, but weaker, to those observed following both anodal and cathodal tDCS (Figure 5.20). Like tDCS PNS also increases cortical activity as shown by an imaging study (Wu et al. (2005)). The similarity in the IMC results may suggest that PNS and tDCS affect the cortex in similar ways; here PNS causes increased input to the cortex which enhances

the activity of the inhibitory interneurones thus enhancing the propagation of β rhythms in the CST.

The effects of PNS in the α band differed from both anodal and cathodal tDCS. In the first 10 minutes PNS did not interact strongly with the α band, however, both polarities of tDCS reduced (8 - 10) Hz IMC. It was been suggested above that tDCS opposed the increase in (8 - 10) Hz IMC that may be representative of task habituation or the homeostatic response to the constant contraction. PNS, however, did not appear to interact with this. There are a number of possible reasons for this result: In the cortex tDCS will affect all the neurones under the electrode and so may be interacting with a number of neural networks that are responsible for different outputs; whereas PNS is, arguably, a more focussed technique when it arrives in the cortex and is input on a particular subset of neurones within M1 that are strongly linked to sensory feedback and may be involved in sustaining the β rhythm associated with a constant contraction. The result may also be caused by the interaction of PNS with other regions of the brain.

The secondary effects (those that occured in the final 10 minutes) of PNS were mostly restricted to the α band. As noted above the motor task enhanced (8 - 10) Hz IMC and peripheral nerve stimulation seems to have increased the frequency range of this effect to extend over the entire α range. This result was not seen with either polarity of tDCS.

Effects on Physiological Neurogenic Tremor

The effects of PNS on α IMC were also reflected in the coupling between 1DI-A in the (8 - 10) Hz PNT frequency range. Compared to baseline (Figure 5.36a) there were the same increases around 9 Hz that were seen following anodal tDCS (Figure 5.28a), and like anodal tDCS they were no longer significant when the motor

task was controlled for (Figure 5.36b). It was discussed above (section 6.3.3) that this result suggests that the contraction task causes a non-significant increase in 1DI-A coupling at this frequency, and since it also causes a significant increase in (8 - 10) Hz IMC it was postulated that this represents a weak link between the two measures.

A stronger piece of evidence to support the link between (8 - 10) Hz IMC and PNT was seen in the later, secondary effects: PNS significantly increased both IMC and 1DI-A coupling at 15 and 20 minutes post stimulation. This is the second link between (8 - 10) Hz IMC and (8 - 10) Hz PNT seen in this study; the first being the concomitant decrease in (8 - 10) Hz IMC and PNT at five minutes post cathodal tDCS. PNS appears to have increased PNT 15 and 20 minutes post stimulation. The ascending afferent volleys caused by PNS interact with other brain structures and may impact on the role of each of these areas on tremor generating mechanisms. The delayed time course of this result may be suggestive of a rebound affect caused by earlier interactions with other systems, or may be associated with the removal of the stimulation.

This is the first study to investigate the effects of peripheral nerve stimulation on IMC and PNT and they were, generally, weak. This was not unexpected. McKay et al. (2002) and Chipchase et al. (2011) reported that durations of at least 30 - 45 minutes were required to induce persistent changes to cortical excitability. Rizzo et al. (2014) delivered 600 PNS pulses for five minutes and Uy and Ridding (2003) used the same stimulation parameters as the present study; neither observed any changes to cortical excitability. Cortical excitability was not measured here but the effects on β IMC were similar in magnitude to those caused by both polarities of tDCS. As noted above there is controversy about the magnitude of the effects of tDCS on cortical excitability and the similarity between these results suggests

that 10 minutes of tDCS, like PNS, probably has a weak effect.

6.5.2 Paired Associative Stimulation

Reports from the literature suggest that pairing tDCS with PNS may enhance the weak effects of both stimuli further (Celnik et al. (2009); Rizzo et al. (2014)); this was explored here.

Effects on Inter-Muscular Coherence

Compared to baseline both anodal and cathodal tDCS/PNS caused large increases in α IMC and decreases in β, although cathodal tDCS/PNS also caused increases in the β range (Figure 5.33a and Figure 5.34a). These changes were very similar, but stronger, to those caused by the contraction task alone (Figure 5.19 and 5.31). They were much larger than those caused by tDCS alone. This is an interesting result, it suggests that the combination of anodal or cathodal tDCS/PNS enhances the effects of the contraction task; a result not seen for either kind of stimulation alone.

When the effects of the motor task were controlled for it was seen that anodal tDCS/PNS caused few changes in the first five minutes (Figure 5.33b). This fits with the above suggestion that this stimulation was affecting the cortex in the same way as the contraction task. The later effects were dominated by decreases in β IMC and only one, later, increase in α IMC. These results are in direct opposition to the effects of anodal tDCS alone and are reminiscent of the results of the priming rather than the paired stimulation studies. It is possible that the constant contraction task used to establish baseline IMC had a priming effect on the cortex. When the effects of repeating the motor task were investigated the first group's results showed a decrease in β IMC. It was suggested that this is a homeostatic response in accordance with the mechanisms of homeostatic plastic-

ity and priming that were discussed in the literature review (section 3.9); that is an increase in activity induces an opposing response to a subsequent stimulus. It is possible that the combination of anodal tDCS and PNS also enhanced β rhythmicity during the stimulation which induced a heightened homeostatic response to later repetitions of the motor task.

Ziemann and Siebner (2008) also suggested that priming through gating, in which reduced excitability of inhibitory circuits increases Ca^{2+} intake to neurones, can occur when the two stimuli occur simultaneously. When delivered alone anodal tDCS and PNS are reported to have an enhancing effect on cortical excitability and by this mechanism conditioning by one or other of these stimuli would result in a reduction in later excitability levels. It is suggested then that the results of anodal tDCS/PNS seen here are not caused by a paired, associative stimulation to the cortical neurones, but instead are a priming effect caused by suppression or excitation of the background activity that promotes future inhibition/excitation.

Cathodal tDCS/PNS caused effects in the first 10 minutes (enhanced β IMC) that were similar to those induced by cathodal tDCS and PNS alone (Figure 5.34b). It did not include the reduction in the (8 - 10) Hz range that cathodal tDCS induced in both the IMC and PNT; indeed PNS alone had the largest affect on the α range. While the results in the β band were similar in frequency to those of each stimulus alone the combination of the two did not enhance their affects. This result suggests that cathodal tDCS/PNS had a similar, but not stronger, effect on cortical activity as cathodal tDCS and PNS alone. The gating arguments above may also describe these results: the excitability enhancing effects of PNS when paired with the inhibitory effects of cathodal tDCS may result in an overall enhancement in activity.

It is interesting to note that in this study interactions in the α band have been seen to be strongest with PNS and cathodal tDCS; stimuli that have been associated with inducing activity in other regions of the brain (Baudewig et al. (2001); Lang et al. (2005)). Alterations to α may therefore be representative of these non-cortical interactions or the lack of change in α IMC after anodal tDCS may be attributed to a higher selectivity of anodal tDCS to interact only with the cortex.

It is difficult to compare these results to the tDCS/PNS literature since, as noted above, the effects of tDCS on IMC do not seem to translate easily into measures of cortical excitability. Celnik et al. (2009) and Rizzo et al. (2014) both reported that anodal tDCS paired with PNS enhanced MEPs in stroke and normal populations respectively. Rizzo et al. (2014) also tested the effects of cathodal tDCS/PNS and found that the excitability diminishing effects of cathodal tDCS were enhanced. The results from the present study do not appear to be in accordance with these reports: anodal tDCS/PNS shared no similarities with the effects of anodal tDCS alone, and while cathodal tDCS/PNS did the effects were not enhanced. Celnik et al. (2009) delivered 36000 PNS pulses over 2 hours with 20 minutes of tDCS a much stronger set of stimuli than were delivered here. Rizzo et al. (2014), however, paired only 5 minutes of tDCS with 1500 pulses, a more similar pair or stimuli to those delivered in the current study. A study by Chipchase et al. (2011) showed that different frequencies and intensities of PNS affect cortical excitability in different ways. This is not surprising, particularly in the light of the frequency dependency of rTMS. The PNS paradigms amongst this and the other tDCS/PNS studies were very different which may account for the differences in results. As discussed in the literature review there were also concerns about the multiple investigations used by Rizzo et al. (2014) in particular active motor threshold was measured before cortical excitability tests. Active

motor threshold tests require a contraction in the muscle of interest that is concurrent with the magnetic stimulation and this may affect later tests of cortical excitability. Indeed the study conducted by Rizzo et al. (2014) only delived tDCS for five minutes and yet reported significant changes to cortical excitability, a feat, to date, only achieved by Nitsche and Paulus (2000).

Effects on Physiological Neurogenic Tremor

The effects of paired tDCS and peripheral nerve stimulation were also tested on physiological neurogenic tremor. It was unclear what the effects would be since paired associative stimulation and also priming are generally considered to occur in the cortex. Whether changes would interact with inputs to the PNT system was explored.

The combination of anodal tDCS and PNS increased PNT in the (8 - 9) Hz frequency range compared to baseline, but when the effects of the contraction task were controlled for there were no changes. This is the same result that was seen for each stimulus alone, and it has been discussed above (section 6.3.3). The result from this anodal tDCS/PNS PAS protocol suggests that, as for IMC, the paired stimuli did not cause a stronger effect on PNT than the motor task already had.

Both cathodal tDCS alone and paired with PNS enhanced (10 - 12) Hz PNT at 15 minutes and while the results were similar cathodal tDCS/PNS was not stronger. The delivery of both cathodal tDCS and PNS stimuli alone caused changes in IMC that were also reflected in PNS. The combination of the two stimuli, however, did not result in a simultaneous change in the two measures.

Neither polarity of tDCS/PNS had many affects on α IMC and they also had very

few affects on PNT. Where there was an increase in tremor it did not correspond to a change in IMC. The incorporation of ascending stimuli therefore did not influence interactions with the inputs to PNT, supporting the idea that IMC and PNT are, for the most part, distinct measures.

6.6 Transcranial Sinusoidal Current Stimulation

As discussed in the Literature Review (section 3.7.1) there are reports that low current and low durations of tSCS and tACS interact with motor oscillations, motor learning and motor function when delivered at physiologically relevant frequencies (Antal et al. (2008); Pogosyan et al. (2009)). Longer term plastic changes to oscillations have yet to be investigated. This study aimed to use IMC and PNT to explore whether higher intensities and durations of tSCS (see Figure 4.12) can interact with the neuronal environment to induce, or interact with the present oscillations to cause changes in the peripheries that persist after the period of stimulation has ended.

The anodal tDCS signal was modulated by imposing small 5 Hz, 10 Hz and 20 Hz oscillations onto it. Safety concerns meant that maximum current did not exceed 1 mA and the sinusoidal modulations oscillated between (0.95 - 1) mA. The 10 Hz and 20 Hz frequencies were selected to test if they would interact with α or β frequency range coupling; it was postulated that 5 Hz tSCS would have very little effect on IMC and PNT.

6.6.1 Effects on Inter-Muscular Coherence

Compared to baseline all three tSCS frequencies increased β IMC and only 10 Hz interacted with IMC in the α band. When the effects of the repetitive contraction task were controlled for 10 Hz tSCS had the largest primary effects on IMC and

5 Hz had the largest secondary.

Cortical oscillations and inter-muscular coherence at 5 Hz are not generally associated with the performance of constant contractions. As expected there were no changes to 5 Hz IMC compared to both baseline and sham (Figures 5.39), this is in accordance with Pogosyan et al. (2009) who also reported no change to 5 Hz CMC with 5 Hz tACS. In fact there were very few changes to IMC in the first 10 minutes after 5 Hz tSCS suggesting that this kind of cortical stimulation had very little direct effect on common drive in either the α or β bands. Compared to sham there were later, secondary, effects in the β band that, with the exception of a reduction at 20 minutes, were similar to those caused by anodal tDCS. It is not surprising that the effects of these two stimuli are so similar given that this was primarily an anodal stimulation with a relatively slow and small 5 Hz oscillation imposed on it.

Antal et al. (2008) showed that 10 Hz tACS increased motor learning, and motor learning has been associated with 10 Hz oscillations in M1 (Zhuang et al. (1998, 1997)). Similarly Pogosyan et al. (2009) delivered 20 Hz tACS and observed increases in 20 Hz CMC and the slowing of voluntary movement. The results of both these studies suggest that an oscillation must be present in order for sinusoidal stimulation to interact with it. The strong increase in (8 - 10) Hz IMC that was seen during the motor task shows that it is present in the nervous system. Although it is not completely clear that its presence in the IMC reflects its presence in the cortex the current study did show that cortical stimulation in the form of tDCS interacted with the (8 - 10) Hz oscillations suggesting that there may be an input to that rhythm in the cortex. Delivery of 10 Hz tSCS caused no alterations to 10 Hz IMC when compared to baseline; however there was an increase at 15 minutes compared to the motor task (Figure 5.40). As

already noted repetition of the motor task in these trials increased (8 - 10) Hz IMC at most of the time test intervals; however in both sham studies there was a conspicuous gap at the 15 minute test point (Figure 5.19 and Figure 5.31). tSCS at 10 Hz appears to have abolished this 15 minute gap seen for the motor task and this may suggest that it was subtly interacting with 10 Hz IMC.

Enhanced 10 Hz IMC in the motor task was suggested to be comparable to the task habituation that was observed in the imaging studies, or representative of a homeostatic response that suppresses unstable positive β feedback and promotes (8 - 10) Hz rhythms that are linked to new movement. Anodal and cathodal tDCS seemed to abolish this response by enhancing β and decreasing α IMC at the five minute test point (Figure 5.20). The affects of 10 Hz tSCS in the β band were similar to anodal and cathodal tDCS with an increase observed at 20 Hz, however, it did not reduce 10 Hz IMC as those stimuli had. Antal et al. (2008) demonstrated that implicit motor learning was enhanced during 10 Hz tACS and although tDCS, tACS or tSCS induced alterations to 10 Hz oscillations have not been explicitly demonstrated another study has linked 10 Hz oscillations with motor learning (Zhuang et al. (1998)). Taken together these studies and the results from the present study suggest that an oscillatory stimulus of 10 Hz may interact with cortical 10 Hz oscillations that are involved in motor habituation or motor learning.

Maintained contraction is associated with β oscillations and coherence particularly around 20 Hz and Pogosyan et al. (2009) showed that 20 Hz tACS enhanced β CMC. In line with this it was hypothesized that 20 Hz tSCS would also enhance 20 Hz IMC. Compared to baseline there were widespread increases in β IMC, including an increase at 20 Hz which was initially observed at 10 minutes and persisted throughout the test period (Figure 5.41a). When the effects of the

contraction task were controlled for the changes at 20 Hz were no longer present (Figure 5.41b). This suggests that for the motor task there were non-significant increases in 20 Hz IMC and that 20 Hz tSCS did not significantly enhance them past what the contraction task had already achieved. It is possible that further increased durations or intensities of stimulation would enhance 20 Hz IMC more than the contraction task did.

Like 10 Hz tSCS, 20 Hz stimulation also increased 10 Hz IMC at 15 minutes compared to the motor task. The result that both 10 Hz and 20 Hz tSCS changed 10 Hz IMC at 15 minutes is interesting and may hint that interaction with one frequency impacts on another. Why 20 Hz tSCS would interact with 10 Hz in particular is not clear but it was suggested above in the context of homeoplasticity. It may be that enhanced 20 Hz oscillations in a constant contraction task are interacting with motor habituation or a homeostatic response.

These results, and implications from the literature, suggest that the oscillations must already be present in the brain in order for an external intervention to interact with them and that increasing the stimulation intensity and duration alone does not induce oscillations that do not already exist. From a rehabilitation perspective this may be problematic and could mean that patients must have a degree of recovery in order to benefit from this kind of stimulation if these rhythms have practical and functional roles in motor control.

Despite the increased stimulation intensity and duration the affects of tSCS on IMC were small, particularly compared to those reported by Pogosyan et al. (2009) and Antal et al. (2008). There were a number of differences between these studies. Both groups delivered the stimulation during the contraction task and the oscillatory modulations to the waveform were much larger than the ones em-

ployed in the present study. It is possible that larger persistent effects may be induced if the stimulation is delivered during the task and the oscillatory modulations to the signal are larger. As it stands 20 Hz tSCS did not significantly change 20 Hz IMC in the first 10 minutes of the trial compared to changes already induced by the contraction task.

The electrode montage employed by Pogosyan et al. (2009) was also different from the traditional: one electrode was placed over the motor cortex and the other on the ipsilateral side of the neck. The path of the current flow was therefore different from usual and yet there is an effect on the cortical neurones. This result, again, highlights the question of electrode configuration discussed in the Literature Review (section 3.3.1): are the effects caused by the activation of the motor cortex, driven in this case by the resonating stimulus, or alternatively are they driven by the current path? This is a particularly interesting point for tSCS and tACS which being oscillatory may not rely on current flow like tDCS. Current flow is reported to be important for inducing after effects and anodal tDCS enhanced β IMC and the present study employed an anodal DC signal to drive current flow. The report of Pogosyan et al. (2009) suggested that the incorporation of a 20 Hz oscillation would interact with the enhanced β IMC to increase it even further. The effect seen in the current study, however, was smaller than both tDCS and tACS. This may suggest that current flow is less important than presumed, particularly for the purposes of interacting with oscillations.

Another suggestion for the weak effects of 20 Hz tSCS on β IMC may be found in the pharmacological studies of Baker and Baker (2003) and Riddle et al. (2004): Diazepam increased cortical β oscillations but did not enhance β CMC and carbamazepine did not change β oscillations but did enhance cortical β CMC. These effects may have been caused by the drugs interacting with other mechanisms in

the cortex, but they do demonstrate that the propagation and sychronization of oscillations in the cortico-spinal tract may not simply be achieved by enhancing cortical oscillations.

It is of interest to note that even though the effects of these interventions on IMC share characteristics the effects of anodal and cathodal tDCS on MEPs are reportedly literal polar opposites. Like cathodal tDCS each frequency of tSCS caused different results in the IMC. It would be of interest to observe if the different tSCS frequencies would result in opposing effects on other investigatory tools, particularly on motor function; however for IMC it would appear that tDCS produces the largest changes and that these are not enhanced by altering the frequency of the signal.

6.6.2 Effects of Transcranial Sinusoidal Current Stimulation on Physiological Neurogenic Tremor

Both polarities of tDCS interacted with PNT in a small, but significant way. This study was conducted to investigate if it was possible for tSCS to interact with PNT in a frequency dependent way and, potentially, enhance the effects of tDCS. Of particular interest was to ascertain if 10 Hz tSCS would interact with the (8 - 10) Hz component of PNT as both cathodal tDCS and PNS had done.

Compared to baseline 10 Hz tSCS caused significant reductions and increases in (8 - 10) Hz IMC across a range of time tests and these were also reflected in the PNT (Figure 5.40a and 5.43). When compared to the motor task the reduction in IMC at 1 minute post intervention was no longer present but it persisted in the PNT and occurred again, but weaker, at the 10 minute interval measurement. As already noted the contraction task appears to cause a trend towards increasing PNT in the lower frequency range ((8 - 9) Hz); in the first 10 minutes after stim-

ulation 10 Hz tSCS opposed this effect. While this did not occur at exactly 10 Hz it was in a range associated with this frequency. The result suggests that the stimulation can entrain oscillations in PNT that are present in the task and that the effect outlasts the stimulation which was not observed for Antal et al. (2008) and Pogosyan et al. (2009). Those studies differed from the present one in that they delivered the stimulation concurrently with the task, the result here suggests that the induced oscillations are still present in the system after the stimulation has ended and interact with oscillations that are induced by the initiation of a motor task. Whether or not the effect would be larger if delivery of the stimulation and execution of the task had occurred at the same time is worthy of investigation.

This effect of 10 Hz tSCS on PNT seems similar to the effects of cathodal tDCS (Figure 5.30b), however, that was associated with a simultaneous change in IMC (Figure 5.20b) which was not observed with 10 Hz tSCS. It was proposed above that (8 - 10) Hz IMC is representative of an input to PNT and that when large enough is capable of altering the output; however, the lack of correlation between the IMC and PNT measures may suggest that 10 Hz tSCS is selectively altering a different input to PNT.

It is interesting to note that 10 Hz tSCS caused a small increase in 20 Hz IMC. The suppression of (8 - 10) Hz rhythm, in a different region, may indirectly have led to the promotion of β oscillations and the suppression of the homeostatic response. Above it was suggested that IMC is only one input to PNT, but it is also likely that PNT is also an input to IMC. Further evidence for this hypothesis may be found in the results for 20 Hz tSCS: When the effects of the motor task were controlled for both 10 Hz and 20 Hz tSCS increased both IMC and PNT at approximately 10 Hz at 15 minutes post stimulation (Figure 5.43b and 5.44b). The magnitude of the change in IMC was similar for both stimuli, but in PNT

it was larger following 20 Hz tSCS. As noted above the IMC is proposed to be representative of an input to PNT and these changes support this proposal.

Each of the stimuli (5 Hz, 10 Hz and 20 Hz) caused a large decrease at (11 - 12) Hz at the 20 minutes test interval (Figure 5.42 for the 5 Hz tSCS result) the lateness of the onset of this effect may then alternatively suggest that it was a secondary effect, that is, a rebound to the system caused by stimulation effects elsewhere. As previously noted these secondary effects have not been reported in the literature.

6.7 Transcranial Sinusoidal Current Stimulation and Peripheral Nerve Stimulation

Here 5 Hz, 10 Hz and 20 Hz tSCS were combined with peripheral nerve stimulation to investigate if PNS could enhance the effects of each stimulation. Again, the effects on IMC and PNT were tested.

6.7.1 Effects on Inter-Muscular Coherence

Like the tDCS/PNS results there were few similarities between tSCS and tSCS/PNS. Under both paradigms and compared to baseline 5 Hz stimulation strongly enhanced (15 - 20) Hz IMC (Figure 5.45a), 10 Hz tSCS strongly enhanced (10 - 12) Hz and (20 - 25) Hz IMC (Figure 5.46a), and 20 Hz tSCS enhanced 20 Hz IMC (Figure 5.47a). These effects were stronger when tSCS was combined with PNS suggesting that the incorporation of PNS enhanced some of the effects of the cortical stimulation alone; however, they did not persist when the effects of the motor task were controlled for (Figures 5.45b, 5.46b, 5.47b).

The incorporation of PNS to the sinusoidal intervention did not promote the

stimulation frequency for either 5 Hz tSCS/PNS or 20 Hz tSCS/PNS. Compared to sham 10 Hz tSCS/PNS increased 10 Hz IMC at 15 minutes. The relevance of this is perhaps diminished since all tSCS/PNS protocols had the same effect on this point. The importance of this point is unclear, but of all the sinusoidal stimuli 10 Hz tSCS produced the largest affect on IMC and 10 Hz. These data strengthen the propositions above that the oscillation must already be present in order to be enhanced and that the stimulation interacts most strongly with those oscillations when they are of the same frequency.

As noted above this study delivered only 300 pulses in combination with 10 minutes tSCS, much fewer than Celnik et al. (2009) or Rizzo et al. (2014). It is possible that this was not enough PNS pulses to induce an effect, although the TMS-PNS studies used only 90 pulses and achieved positive results (Stefan et al. (2000); Wolters et al. (2003)). If low current cortical stimulation combined with peripheral nerve stimulation does enhance the effects of each stimulus delivered alone then it probably requires more pulses than were delivered here. It is also possible that this is not a paired associative stimulation at all and instead a priming one, this possibility was discussed above (section 6.6).

Associative LTP occurs when the inputs to a postsynaptic cell are synchronous and occur at the same time, or when the input is synchronised and concommitant with depolarisation of the postsynaptic neurone (Buonomano and Merenich (1998)). It is entirely likely that at many points during the intervention there was a phase difference between the cortical and peripheral stumuli resulting in non-synchronised input. An appealing idea that has yet to be explored is to deliver the cortical and peripheral stimuli at the same frequency so that the peak, or trough, of the wave occur in the cortex at the same time as the peripheral stimulation arrives.

6.7.2 Effects on Physiological Neurogenic Tremor

Compared to baseline both 5 Hz and 10 Hz tSCS/PNS produced the low range ((8 - 9) Hz) increases to 1DI-A that have been observed repeatedly in this study (Figures 5.48a, 5.49a). When the effects of the contraction task were controlled for, again, this effect was no longer present (Figures 5.48b, 5.49b).

Compared to sham the tSCS/PNS protocols caused similar results to each other and also to tSCS alone, with large increase in PNT 15 minutes post stimulation followed by reductions at 20 minutes. The enhancements, but not the reductions, were also seen in the IMC but were larger in the PNT. This may suggest that while IMC can influence PNT so too can PNT affect IMC, or that common descending activity is integrated differently in these two systems causing different results.

6.8 Conclusions

The initial aims of this thesis were to assess and extend reports from the literature of the effects of tDCS on cortical excitability and inter-muscular coherence, and to develop IMC and PNT as tools that would provide new insight into how tDCS affects cortical activity.

The secondary aims of this thesis were to investigate whether the effects of the tDCS protocol on IMC and PNT could be enhanced by altering the traditional stimulation. The effects of both paired associative stimuli and sinusoidal stimulation protocols were tested.

6.8.1 Transcranial Direct Current Stimulation and Cortical Excitability

The polarity dependent effects of tDCS on cortical excitability (as measured by TMS induced MEPs) were reproduced, to a certain extent, here and showed that cathodal tDCS is capable of penetrating the skull and significantly altering cortical output. There is controversy in the literature about the magnitude of this effect and the conclusion drawn from the data here is that there appears to be only a small change in excitability after cathodal tDCS. There were no significant changes to MEP amplitudes after anodal tDCS

Variability in MEP amplitudes, both with and without an intervention, however, continue to obscure the ability to evaluate the data effectively. A number of reasons for this were suggested above and each deserves to be addressed as this is an important issue for an investigatory tool.

A defined protocol for TMS investigations of tDCS should also be addressed. Developing an understanding of the effects on the induced electric fields by delivering magnetic stimulation through inactive and active conductive electrodes will be important in developing robust testing regimes. It is even possible that stimulating through an electrode will result in an enhanced effect since the studies that removed the electrodes, including this one, have reported lower after effects.

6.8.2 Inter-Muscular Coherence and Physiological Neurogenic Tremor as Investigatory Measures

The incorporation of baseline stability analysis was instrumental in the development of IMC and PNT as measures of cortical activity and demonstrated that under the correct conditions these measures remain stable when no intervention

has been delivered. The high test-retest reproducibility of IMC and PNT meant that they were therefore deemed as suitable tools for investigating the effects of tDCS. This analysis showed that the implementation of a contraction task that produces good quality EMG and promotes subject compliance is integral in producing stable IMC and PNT measures. Utilizing the pooling regime and the comparison of coherence protocol in Neurospec proved to be effective in analysing changes to IMC and PNT.

It should be noted that comparison of coherence tests showed that IMC and PNT are altered from their baseline values with repetition of the contraction task after sham tDCS. This was observed in two separate, but admittedly quite small, normal populations. This suggests that future studies should control for these effects. The task may be limiting in other ways too. According to the literature complex and challenging tasks tend to improve motor outcomes; however, these are difficult to design for healthy individuals and the preliminary studies showed they result in poor baseline stability outcomes. This may be overcome by carefully selecting the muscles of interest, using auxotonic contractions or using geographic or force targets, but it is clear that good task design will be important in the use of these tool.

6.8.3 Transcranial Direct Current Stimulation, Inter-Muscular Coherence and Physiological Neurogenic Tremor

The polarity dependent effects of tDCS on IMC that are reported in the literature were not entirely reproducible here. With the incorporation of the more robust analysis technique it seems likely from the data collected here that both anodal and cathodal tDCS interact with cortical β oscillations, but perhaps in different ways. It is suggested that anodal tDCS either promotes the neural networks involved in sustaining this rhythm, promotes the propagation of these rhythms in

the CST or enhances cortical activity thus promoting inhibitory interneurones which impose β rhythmicity. Cathodal tDCS may cause inhibition in the cortex which, again, promotes activity amongst the inhibitory interneurones resulting in increased β IMC. The affects on α oscillations are less clear. Changes to α IMC were poorly represented in PNT suggesting that while the may be input on one another (8 - 10) Hz IMC is not purely a reflection of PNT and vice versa. It was suggested that the (8 - 10) Hz IMC observed here was implicated in habituation to the motor task or a central or peripheral homeostatic response. Both IMC and PNT investigations also revealed, later, secondary effects that may be representative of rebound response or feedback from other structures that were affected by the initial stimulation. This has not been observed before in other studies.

PNT was also altered following cortical stimulation. Since there was only a weak link between (8 - 10) Hz IMC and PNT it was suggested that the investigation of neurogenic tremor is distinct from IMC. At some post intervention time points PNT was both reduced and facilitated at different frequencies. This and the observation that some changes to (8 - 10) Hz IMC occurred at the same time as weak changes to PNT supports the hypothesis that PNT is an output that represents multiple input components. This hypotheses may be important in discussions on the origins of oscillations and hint that instead of there being an oscillatory generator there are instead points in the loop that are receptive to interaction from other sources.

6.8.4 Paired Associative Stimulation: Transcranial Direct Current Stimulation and Peripheral Nerve Stimulation

The increase in β IMC seen after PNS alone was postulated to have occurred in two ways: The stimulus increases input and activity in the cortex which promotes inhibitory interneurones to impose a β rhythm. The weak interaction with α IMC however led to the suggestion that the effects of PNS are focussed on the subset of neurons that are strongly linked to sensory feedback and responsible for promoting the β rhythm. PNS did enhance both IMC and PNT suggesting again that under certain conditions these measures are linked and that enhanced sensory feedback can increase PNT.

The pairing of tDCS with PNS did not enhance the effects of tDCS alone. In fact the data suggested that this procedure is not a paired associative one at all, but a priming one instead. This is contrary to some of the literature and warrants further exploration with other investigatory tools as the number of stimuli and the duration of PNS stimulation may be important. As noted above neuroplasticity models suggest that intervention induced synaptic strengthening or depression are dependent on previous activity in the same subset of neurones. Previous activity does not necessarily mean an external intervention and there is evidence to suggest that differences amongst participants' activity and attention may affect their response to the stimulation (Gomez-Pinilla et al. (2002); Lamy and Boakye (2013)). This may actually account for some of the variance and non-normal distributions seen in these data. Priming effects are reminiscent of Heisenberg's classic paradox in which the observation of an event changes its outcome. An understanding of these effects is clearly required to ensure correct experimental design and analysis procedures, and to explore any beneficially enhancing effects it

may impose on neural activity. Tests where the TMS inter pulse intervals, number of pulses and intensities are varied while the tDCS intensity remains constant would be useful in investigating priming. It may also be more appropriate to establish stable baselines and retrieve hotspot data days before the intervention is delivered.

6.8.5 Transcranial Sinusoidal Current Stimulation and Paired Associative Stimulation

For tSCS and tSCS/PNS it was of particular interest to discover if the sinusoidal stimulation could induce or interact with oscillations involved in motor control. The data suggest that it was not possible to induce oscillations that are not already present simply by imposing a sinusoidal stimulus and the introduction of an additional peripheral stimulus did not change this. As noted above this may be problematic for motor rehabilitation, but it is possible that stronger stimuli or a carefully designed task may overcome this obstacle.

The data did suggest that there was a weak interaction between the stimulus frequency and an oscillation that was already present. The effects were, however, weaker than tDCS alone and were not made stronger with PNS. This may suggest that interactions with oscillatory frequencies have more complex and far reaching effects than first thought. Because IMC only provides an insight into some of the descending signals and not the rhythms in the cortex itself then it is unclear whether these cortical oscillations were enhanced, what is clear though is that propagation was not. This proposition is in accordance with the pharmacology studies of Baker and Baker (2003) and Riddle et al. (2004) who have both demonstrated that the link between enhancing cortical oscillations, enhancing propagation of that oscillation down the CST and enhancing synchronisation in other structures is not straight forward.

Overall the largest effects on IMC or PNT were caused by either polarity of tDCS or PNS alone. There is the possibility that tSCS may have stronger effects if the modulation of the signal is larger. Even the strongest effects in these trials, however, do not appear to be large enough to be clinically relevant and it was concluded that tDCS has a limited ability to interact with neuronal activity. Interestingly the magnitude and duration of tDCS after effects were very similar to PNS. It has been demonstrated that PNS requires a much longer stimulation time, of two hours, to induce meaningful changes to cortical excitability. Based on the work of Nitsche and Paulus (2000, 2001); Nitsche et al. (2003b) most studies restrict stimulation duration to 10 minutes but, as noted above, there is considerable controversy about the effects. It is possible that, like PNS, longer durations of tDCS would remove much of this variability and induce meaningful after effects.

There is still ambiguity about the importance of electrode configuration for tDCS. The questions of whether changes are induced by the hyper/depolarisation trends occurring under the electrode or by the path of the current flow have still to be addressed and are important if tDCS will ever be developed for motor rehabilitation of areas other than the hand or arms. There is evidence for both roles as seen by the path dependency of TMS (DiLazzaro et al. (2004)) and the positive results achieved by Pogosyan et al. (2009). Indeed the traditional montage is purely based on the fact that changes to TMS induced MEPs are observed, hence the possibilities for the intervention have been limited by the investigatory technique. Other cortical areas are also important in motor control, for example the pre-motor cortex or supplementary motor area, but since tDCS at those sites did not significantly change TMS induced MEPs they have been, generally, excluded as stimulation sites. This raises the question of why M1 has become the main stimulation site and it is possible that this is not the best, or only target. There

is evidence that motor learning occurs in the motor cortex (Nitsche et al. (2003c); Antal et al. (2008); Doyon et al. (2009)) but improving planning and interacting with pattern generators may also have beneficial effects on motor outcomes in different stages of rehabilitation.

Future studies would benefit from a better understanding of how cortical excitability, cortical oscillations, IMC and PNT translate into functionally relevant motor outputs particularly in terms of the homestatic response to movement, motor adaptation and motor learning. These might take the form of impeditive force-field robotic studies where the effects of motor adaptation/motor learning on EEG, CMC, IMC and PNT can be evaluated with and without interventions, or studies into the effects of long term PNS and TMS/PAS on IMC and PNT.

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