Observations on the variability of corticospinal tract excitability during the reaction time period for simple human finger movements

Mehdi Antonie Johan Van Den Bos

PhD Thesis

Sobell Department of Motor Neuroscience and Movement Disorders

Institute of Neurology

University College London

Supervisors:

John C. Rothwell

Richard J. Greenwood

Declaration

I, Mehdi Van Den Bos, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

There is extensive evidence that movements are prepared prior to their release. Transcranial magnetic stimulation, and in particular the motor evoked potential produced when stimulating over the primary motor cortex, has given a great deal of insight into the processes involved in preparation for voluntary movements. The excitability of the primary motor cortex remains in a state of dynamic fluctuation even when in the "resting" state, with the TMS MEP being exquisitely sensitive to this as evidenced by its tremendous trial to trial variability. Interestingly there is growing body of evidence to suggest that modulation of signal noise can provide insight into biological processes including movement preparation – indeed the output of the corticospinal tract would logically need to adapt to resting variability to enable the precise reproduction of movements. While much of the TMS literature has addressed MEP variability as a "noisy" signal, this thesis aims to assess whether elements of this "noise" can be utilized as a marker of biologic process during the reaction time period for simple human finger movements.

Through successive chapters we demonstrate that the variability of corticospinal tract output, as evidenced by the TMS MEP, declines during the process of preparation for simple human finger movements. We demonstrate that the reaction time decline in variability is focal to muscles directly involved in the task. Furthermore, the rate of decline in MEP amplitude variability during the reaction time period appears intimately linked to the speed of movement initiation. Additionally, the changes we see here precede changes in mean excitability in

agonists, and indeed are seen to be associated with a decline in mean excitability when surround muscles are tasked with deliberate inactivity. Finally, observations in stroke patients suggest an alteration in variability control during movement preparation and appear to be associated with concordant changes in task performance.

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List of abbreviations

9HPT	Nine-hole peg test
ADM	Abductor Digiti Minimi
ANOVA	Analysis of variance
ARAT	Action Research Arm Test
BnB	Box and blocks test
CMAP	Compound motor action potential
CST	Corticospinal Tract
df	Degrees of freedom
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyography
EPSP	Excitatory postsynaptic potential
FDI	First Dorsal Interosseous
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-amino butyric acid
Gini	Gini coefficient
ICF	Intra-cortical facilitation
IHI	Inter-hemispheric inhibition
I/O	Input/Output (also known as Stimulus-Response)
LED	Light emitting diode
Les	Lesioned
M1	Primary motor cortex

MCP	Metacarpophalangeal joint
MEP	Motor Evoked Potential
MNCD	Mean normalised consecutive difference
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin scale
ms	millisecond
MSO	Maximum stimulator output
mV	millivolt
MVC	Maximum Voluntary Contraction
N	Newton
PMd	Dorsal Premotor Cortex
PSDep	number of positive pairs divided by total number of matched pairs
PSTH	Post-stimulus time histogram
RMT	Resting Motor Threshold
RT	Reaction time
SEM	Standard error of the mean
SICF	Short interval intra-cortical facilitation
SICI	Short interval intra-cortical inhibition
TMS	Transcranial Magnetic Stimulation
μs	microsecond
ULes	Unlesioned
W	Kendall's Coefficient of Concordance
WSRT	Wilcoxon Signed Ranks Test

Chapter 1 Introduction

Barker at al (1985) developed Transcranial Magnetic Stimulation (TMS) as a painless non-invasive probe of cortical excitability. Stimulation over the motor cortex can activate the corticospinal tract, which may be observed as a muscle contraction or 'twitch', recorded by electromyography (EMG) as the motor evoked potential (MEP). Over the 30 years that TMS has been used to generate MEPs, a distinct and challenging property has been observed, that of TMS MEP variability.

MEPs show tremendous trial-to-trial variability in amplitude, morphology and, to a lesser degree, latency. Work is emerging that attempts to ascertain physiological causes of MEP amplitude variability. In general, however, TMS MEP variability has been considered as a signal to noise problem leading to approaches aimed at mitigation.

At the outset of this work, no study had yet attempted to utilise MEP variability as an informative marker in studying human neurophysiological processes. The TMS MEP elicited through the corticospinal tract reflects the sum of excitability across its cortical and subcortical contributing components, with the moment to moment fluctuations seen reflecting ongoing dynamic changes in inhibitory and excitatory synaptic activity. Preparation for even simple finger movements entails the coordinated shaping of corticospinal tract output excitability to produce the precisely required movement, changes that may be expected to be reflected in the alterations of TMS MEP amplitude variability.

The aim of this PhD thesis was to gain further understanding of how the process of human movement preparation might affect the variability of TMS MEP amplitudes in both health and disease.

The thesis commences with a background literature review (Chapter 2) where I cover the phenomenon of transcranial magnetic stimulation, primary motor cortex contributors to the TMS MEP, treatments on the matter of TMS MEP variability, the process of movement preparation, motor control processes for force and grip type, and finally how the motor control system adapts to injury following stroke.

Following a chapter covering general aspects of methodology (Chapter 3), five experimental chapters are presented which provide novel insights into:

- TMS MEP amplitude variability at rest (Chapter 4)
- TMS MEP amplitude variability during the reaction time period for simple human finger movements (Chapter 5).
- TMS MEP variability during independent and coordinated finger movements (Chapter 6).
- The affect of force and grip type on premovement TMS MEP amplitude variability reductions (Chapter 7).
- Alterations in the premovement control of TMS MEP variability following stroke (Chapter 8).

The final chapter (Chapter 9) summarises findings from these experimental chapters, discusses implications, limitations, highlights unanswered questions and possibilities for future works.

Chapter 2 Literature Review

This chapter presents an overview of literature on the following topics:

- The phenomenon of transcranial magnetic stimulation
- Motor cortex origins of the TMS motor evoked potential
- Observations on variability of the TMS motor evoked potential
- Neurophysiological studies of motor control in health and disease (with a focus on the the use of TMS)

2.1 The Phenomenon of Transcranial Magnetic Stimulation

TMS was first tested on the human motor cortex at the National Hospital of Neurology & Neurosurgery, Queen Square (Barker et al, 1985). In contrast to transcranial electrical stimulation of the cortex (Merton and Morton, 1980), which requires significant voltage to pass through the electrically resistant skin and skull (and thereby producing significant discomfort in conscious subjects), a TMS stimulator generates a magnetic field, passing a large and rapidly changing electric current through over-layed copper coils. The skull's low impedance to magnetism allows the time varying magnetic field projected from the coil to painlessly induce, by Faraday's law, an electrical ("eddy") current in the underlying cortical tissue. The magnetic field generated by modern monophasic TMS stimulators lasts a total of ~1ms, rising rapidly from zero to peak intensity within 100μs of onset and decaying more slowly back to zero within this time (Sommer and Paulus, 2008; The Magstim Company, UK). The choice between biphasic and monophasic stimulators had frequently been a fixed feature of hardware design, though more recently stimulators are emerging that allow for the dynamic adjustment of pulse characteristics (Gattinger et al, 2012; Delvendahl et al, 2014).

The focality and penetrance depth of TMS pulses can be fine tuned by alterations to the shape of the coil through which the magnetic field is projected. Penetrance depth is a function of the size of the coil, eg the diameter of a circular coil, and stimulation intensity (Epstein, 2008). Circular coils are efficient but limited in their focality. The frequently used figure of eight coil employs two overlapping circular coils, with current flowing in the same direction at the point of overlap, to produce an induced field which is maximal at their juncture (Epstein, 2008). This relative difference in positional field strength can be employed, in conjunction with adjustments of stimulation intensity, to achieve 'focal' targeting of cortical structures.

2.2 Motor cortex origins of the TMS motor evoked potential

2.2.1 The D and I-wave hypothesis

When projected across the primary motor cortex the TMS pulse stimulates the axons of pyramidal tract neurons and that of interneurons that summate trans-synaptically over 5-10ms to produce the cascade of repetitive volleys known as D and I-waves (Day et al, 1987). In turn the recorded MEP is the product of multiple recruited motor units that summate within its wave form. Though TMS acts over a relatively wide area of motor cortex, by recording EMG signal from specific muscles of interest we are able to effectively isolate and subsample a cortical population of interest that otherwise overlaps with other output populations within the induced electrical field. TMS is therefore able to act as a relatively specific neuronal population probe in neurophysiological studies of human motor control.

Day et al (1989) provided early evidence for the cortical origins of the TMS MEP, experimental work that centered on the use of post stimulus time histograms (PSTH) to develop the “D and I-wave hypothesis”. Within this hypothesis the MEP is thought to be the sum result of several descending volleys of pyramidal tract neuronal depolarisations following both direct and indirect activation of these same neurons by the cortical magnetic stimulus.

Studying the discharge patterns of single fiber EMG PSTHs in the right hand first dorsal interosseous muscle (FDI) following either electrical or magnetic transcranial stimulation, they noted successive peaks of firing probability (in the PSTH), spaced approximately 1.3-1.6 milliseconds apart. Contrasting electrical and magnetic stimuli they further noted that anodal electrical stimuli always produced an early peak (dubbed P0) and subsequent later peaks, by 1-2ms (P1), 2.5-3.5ms (P2) and 4-5.5ms (P3), could be induced with higher intensities or other forms of stimulation. In particular, using TMS with a circular coil, and the current passing clockwise in the brain (recording from the right hand FDI) always produced a P1 peak and usually a P3 peak at threshold, with no P0 peaks demonstrable within the range of intensities practically possible with the histogram technique. Counter-clockwise stimulation never produced the P1 peak with low intensities first recruiting the P3 peak and subsequently, at higher intensities, the P0 and P2 peaks. By analysing the result of individual peaks in terms of threshold related recruitment and saturation they drew parallels with primate studies from where the direct (D) and indirect (I) wave hypothesis had been derived. Importantly, Day et al (1989) also noted that the

discrete manipulation of D and I-wave recruitment patterns by altering coil orientation were only clearly visible at low stimulation intensities.

More direct evidence for the cortical origins of the TMS MEP came about when Di Lazzaro and colleagues (1998) used TMS with three patients who had recently implanted cervical electrodes to control intractable lumbar spine pain. Whilst simultaneously recording from the implanted cervical electrodes and surface EMG electrodes over the first dorsal interosseous muscle (FDI), monophasic TMS pulses were discharged over the contralateral motor cortex hotspot. The recordings were taken under three conditions: at rest, with 20% maximal voluntary contraction and at 100% maximal voluntary contraction. They detected pyramidal tract volleys at I-wave latencies, with greater successive recruitment of I-waves (in number and size) at higher stimulation intensities. D-waves were only present in two out of the three subjects at high intensities. They also noted that voluntary contraction in the target muscle (particularly at 100% MVC) could decrease the threshold for I-waves and increase volley number and size. Di Lazarro et al (1998) postulated this to be indicative of a post-synaptic cortico-neuronal excitability increase. Importantly when stimulator output was adjusted to achieve the same size MEP under the three conditions: at rest a small D-wave and three I-waves were needed, whereas at 20% MVC the I-waves were smaller and there was no D-wave together with an MEP onset 1.5ms earlier, and lastly at 100% MVC only a single I-wave was evident. This later result clearly shows an alteration of the spinal motor neurones' corticospinal threshold following the onset of voluntary contraction.

Further support and clarification of the "D and I-wave hypothesis" would come through the study of two additional patients with cervical electrodes. Recordings had already shown the persistence of D-waves in animals following the removal of their cortical grey matter, supporting the corticospinal axonal origin of the D-wave (Patton and Amassian, 1954). Di Lazzaro and colleagues first studied (2002) a patient with vascular parkinsonism (including extensive lesions of both the putamen and thalamus). In this case the reason for cervical electrode implantation remains unclear. However, though there were other neurophysiological abnormalities when studied, there remained clear evidence of otherwise normal D and I-waves following single pulse TMS. Di Lazzaro et al later (2004) studied another patient with mild peri-central cerebral atrophy in whom cervical epidural electrodes had been implanted for symptomatic treatment of intractable angina pectoris. In this patient, no evidence of early I-wave volleys could be found though D-waves remained. In addition, a raised resting motor threshold was found and felt to be consistent with the need for recruitment of multiple descending volleys to achieve spinal motor neurone depolarisation. No comment was made on the assessment of active motor threshold. Together this evidence clearly suggested the cortical interneuron origin of the Indirect(I)-waves and the corticospinal tract axonal origin for the Direct(D)-wave.

2.2.2 Paired pulse manipulations of TMS MEP amplitude

As we have seen, a single TMS pulse stimulates multiple neuronal axons repetitive discharges of which together, over the course of some 10ms, summate through the corticospinal tract to produce the MEP. Among these are specific groups of interneurons that have been traditionally studied, with the high temporal specificity

of TMS, by use of paired pulses. Variations of the conditioner and test pulse intensities, together with interstimulus interval, can profoundly affect the morphology, amplitude and latency of the MEP. Using short latency intervals, several different phenomena have been elicited, some of which include: short interval intra-cortical inhibition (SICI), short interval intra-cortical facilitation (SICF) and intra-cortical facilitation (ICF). Changes in the balance of this interneuronal milieu may contribute to the trial-to-trial variability of the single pulse MEP.

SICI and ICF were first described in a now classic paper by Kuijirai et al in 1993. Over a series of experiments, they used a figure of eight coil to manipulate a test pulse MEP in FDI of baseline 1.5mV amplitude with a conditioning pulse at a preceding interval of between 1-15ms. In the first experiment, the conditioner intensity in each of the 10 subjects was below resting motor threshold (RMT) though presumably slightly different between subjects. The key outcome was an inhibition of the MEP at between 1-6ms (what we know as SICI) and facilitation at the 10 and 15ms time points (ie, ICF). They then manipulated the conditioner intensity using ISIs of 3 and 15ms. They noted the presence of SICI occurring between 60 and 90% RMT (maximal effect for this ISI reached at 80% RMT). ICF was present at intensities of 90-140% of RMT, only reaching statistical significance at the higher intensities.

Vucic et al (2009) undertook a more detailed study of the effect of conditioner intensities on SICI by using the threshold tracking technique to study FDI MEPs. With the conditioner set at one of either 40%, 70% or 90% RMT (defined with an MEP amplitude of 0.2mV rather than the more traditional 0.05mV), they used a test pulse

threshold-tracking paradigm to track the change in test pulse intensity needed to achieve a test pulse size of 0.2mV over a range of ISIs including those from 1-7ms. At 40% RMT there was minimal inhibition, with a less than 10% increase in test pulse intensity needed at the 2.5ms peak ISI, the effect disappearing altogether by 4ms. The 70% RMT conditioner intensity demonstrated a more robust SICI effect with test intensity at the ISI 1ms peak needing to increase by about 16% and the ISIs of 2.5-3.5ms needing increases of between 25-35% (peak at 2.5ms) before again dropping away to less than 10% by 4ms. Finally at the 90% conditioner a facilitatory effect was seen at 1.5ms, perhaps reflecting SICF (Peurala et al, 2008), before SICI again became evident between 2-5ms requiring test pulse intensity increases of between 20-35%, with maximal effect at 4ms.

A study by Garry and Thomson (2009) evaluated the effect of variations of test pulse intensity on SICI paired pulses using an ISI of 3ms and a conditioner set at 70% of RMT. Test pulses were varied from 90% to 150% of RMT and MEPs recorded in three conditions: at rest, with contralateral homologous (FDI) muscle contraction and with a sustained 10% of maximal force contraction. They initially they found SICI was by far weakest with active contraction, and still significantly weaker with contralateral muscle contraction than at rest. SICI reached a peak in the rest condition at between 120% to 140% RMT Test pulse intensity where inhibition achieved 50-60% reduction of the test pulse amplitude.

SICF, is a paradigm that essentially probes the periodicity of I-wave contributing interneurons, and was perhaps first formally demonstrated by Tokimura et al (1996).

They studied paired pulses at matched intensities (from 80 to 120% of RMT) over a range of ISIs from 1-7ms in 0.5ms steps and noted synergistic facilitation in particular ISI bands from just below threshold intensity (90% RMT) progressively increasing with stimulus intensity. These ISI bands of 1-1.5, 2.5-3ms and 4-5ms were coincident with previously noted I-wave periodicity and as such were suggested to occur by the authors at the cortical level possibly by inducing multiple I-wave discharges sufficient to produce additional motor unit recruitment for the MEP. Further study of SICF was undertaken by Ziemann et al (1998) who confirmed these facilitatory paired pulse interactions with latency peaks between ISIs of 1.1-1.5ms, 2.3-2.9ms and 4.1-4.4ms, the first peak occurring when the first pulse (test) was as low as 70% RMT and the second pulse (conditioner) being fixed at 90% of RMT; higher test pulses intensities were needed to achieve the second and third facilitatory peaks.

The cortical origins of these facilitatory interactions were demonstrated by Di Lazzaro and colleagues in their 1999 paper detailing the change in cervical electrode recordings following paired pulse stimuli in a SICF protocol. Their key finding of increased descending volleys, beyond that induced by the sum of each pulse alone, demonstrated the synergistic nature of this cortical interaction.

Work by Ilic et al (2002) further defined the overlay between SICI and SICF. There extensive work utilized a combination surface EMG MEP and single unit recordings, diazepam and motor activation, to assess TMS paired pulse interactions in their subject cohort across an array of inter-stimulus intervals and stimulus intensities to demonstrate three features. They confirmed that if the first stimulus was less than

RMT intensity and the second pulse greater than RMT, the resultant MEP was inhibited (SICI), mainly the I3 latency component. However, when either both pulses were equal to or just under RMT or the first pulse was greater than RMT and the second pulse less than RMT, SICF was seen through the recruitment of increased I-waves with I-2 periodicity. They proposed that SICF was produced through a 'jump-on' phenomenon, whereby the second pulse further excited the initial segment of the interneuron axonal membranes which had received EPSPs through the first pulse.

Wassermann has studied paired pulse MEP variability (Wassermann, 2002) and noted significant intra-individual and inter-individual variation in facilitatory/inhibitor responses at the same ISIs. Part of this variation will arise from moment to moment fluctuations of interneuron excitability, and in turn, the changeable nature of susceptibilities to specific combinations of conditioner/test pulse intensity and timing. Furthermore, a growing body of work has recognized that individual variations of interneuron anatomy contribute to inter-subject differences during neurophysiological probing (Hamada et al, 2012; Murase et al, 2015). In this light the direct study of inter-neuronal influences on MEP amplitude variability is technically highly challenging, perhaps even more so in the context of the dynamic changes seen in movement preparation. It may however be feasible to study the sum output of these circuits using supra-threshold single pulse TMS MEPs.

2.3 Observations on variability of the TMS MEP

A striking feature of the motor evoked potential is its trial-to-trial variability in amplitude. We have already described the cortical circuitry, the excitability balances

of which contribute to formation of the MEP. Descending cortical input passes through to the lower motor neuron and thereby the motor units and, certainly, at this end point significant variability in the MEP is seen (Amassian et al, 1989). In general studies of MEP variability have taken three approaches: treating variability as a random noise, developing strategies to estimate this noise and improve the signal to noise ratio, secondly by attempting to look at causes of MEP amplitude variability and attempting to account for them, and finally examining pathological states with increased MEP variability.

2.3.1 MEP variability as a signal-to-noise problem

One of the earliest studies investigating MEP variability was by Brasil-Neto et al (1992) who utilized a figure-of-eight coil to establish that resting MEP amplitude variability declined with increasing proximity to the cortical hotspot. This study utilized the coefficient of variation to correct for differences in mean amplitude of the MEP, which may introduce additional difficulties when interpreting results, e.g. the greater affect of near zero means may accentuate the increase in variability when moving away from the cortical hotspot. Curiously, although substantially reduced, variability persisted on the hotspot with recordings taken at maximum stimulator output. Whilst the study also suggested proximal muscles displayed greater variability than more distal muscles, the result itself may partly be due to the use of a fixed stimulator intensity, which not being threshold adjusted was more susceptible to a floor effect (proximal muscles typically have higher thresholds). This study also demonstrated that the maximum percent error for the mean amplitude plateaus at around 10-20% from between 5-10 samples depending on the exact muscles being

tested. The important question as to whether MEP variability on and off the cortical hotspot, when mean amplitude is closely matched, has not been addressed though clues in others works below hint at an answer.

Kiers et al (1993), demonstrate that a decline in MEP amplitude variability (as measured by both normalised mean consecutive difference and the coefficient of variation) is seen concurrent with an increase in stimulus intensity. Additionally, they note a progressive increase in MEP mean amplitude together with evidence of plateau/saturation in both. Pre-contraction of the target muscle at 5% of maximal voluntary contraction (MVC) also reduced MEP amplitude variability, and though the experiment was also undertaken at 20% MVC, those results are not presented or directly commented upon. Importantly whilst this reduced variability finding comes with substantially increased mean amplitudes, a closer look at their presented data suggests that when amplitudes are matched (across rest/contraction) variability is not consistently different. Later work by Darling et al (2006) looks at this in more detail and again comes to the same conclusion, with a slight pre-contraction of just 5% MVC being sufficient to reduce MEP variability. Interestingly contractions at greater %MVC do not make a substantial further reduction). However as with the earlier paper by Kiers et al (1993), when MEP mean amplitude is accounted for (as shown in the paper's figures), a significant difference in MEP variability does not appear likely.

At the other end of the TMS MEP recording, assessments have been made by Dunnewold et al (1998) to improve the process of sampling raw EMG, reducing MEP

variability caused by technical factors. These authors have demonstrated, for a given individual, while small surface area (pinhead size) electrodes could contribute to MEP variability, once electrodes had increased to 1cm2 surface area and were appropriately placed over the muscle's motor point, these sources of measurement error were no longer significantly contributions to MEP variability.

Kiers et al (1993) suggested that MEP variability might change over time particularly with long, "monotonous" sessions of stimulation due to changes in cortical arousal. They addressed this area in several ways. First they examined whether cortical arousal state (as induced by the concurrent performance of mental arithmetic) would alter MEP variability as compared to the uncontrolled rest state. Surprisingly they found no significant difference in MEP amplitude mean or variability. Based on this same principle they also studied whether MEP's variability changed with time. Firstly, when comparing whether stimuli given more rapidly (5 seconds vs 15 seconds) would alter variability they found no significant difference. Within this same experiment they also analysed variance across a sequence of 300 stimuli first by comparing successive blocks of 50 stimuli and then with a Fast Fourier transform on a mean normalised measure of the MEP area looking for consistent frequency of variation – neither of these analyses were successful in detecting changes with time.

Whilst MEP variability shows no discernable periodicity over these longer stimulation periods there is evidence for an initial transient state which may affect variability. Schmidt et al (2008) that suggested there existed initial transient hyper-excitable state, as demonstrated over the first 15 trials in their recordings of 120 sequential

TMS pulses. Notably their use of the cumulative mean statistic, rather than a block average in the Kiers study, amplified the effect of these initial excitable MEPs. A dedicated assessment on the impact of this initial hyper-excitable state has not yet been made for MEP variability.

A review of the literature on MEP variability reveals an additional challenge. How to measure MEP variability? Each of the papers uses a range of specific statistical tools that are appropriate to their methodology and experimental questions. The diversity of statistics (which include standard deviation, the coefficient of variation, the relative mean consecutive difference and various other measures of estimator error) does pose added complexity when putting into context the work already undertaken. Furthermore, the complex relationship between MEP size (including floor and ceiling effects), stimulus intensity and MEP variability does lead to challenges when utilising measures such as the coefficient of variation which are strongly affected by near-zero mean amplitudes and/or outliers. Ideally, prior to assessing the utility of MEP amplitude variability as a biological signal, a direct comparison of these variability measures should be undertaken to understand the strengths and limitations of each.

2.3.2 Accounting for MEP amplitude variability

Researchers have attempted to reduce MEP variability by systematically targeting its origins. Early studies examined general physiological parameters and practical recording details. Subsequently more complex approaches were undertaken, highlighting both spinal and cortical causes of variability and where possible targeting

them. Some of the more recent work looking at cortical markers of MEP variation may provide insight into the physiological significance of MEP variability.

A few articles have examined more controllable aspects of general physiology as they relate to MEP variability. Amassian et al (1989) had assessed whether both the cardiac and respiratory cycles had any affect on MEP variability, with the idea that changes in cerebral circulation pulse pressure would cause small variations in cortex-to-coil position that would lead to MEP amplitude changes. The study failed to find any such association as did similar work by Ellaway et al (1998) who also use ECG triggered TMS pulses and again found no effect on variability.

Physical displacement (of the coil with respect to the target cortex) has been further studied as a controllable cause of MEP variability. With the arrival of the more focal figure-of-eight coil and the early work by Brasil-Neto et al (1992), frameless stereotaxy was introduced to TMS practice to reduce coil movement as a source MEP variability. Essentially, whilst there is evidence to support improved targeting of cortical anatomy between sessions when using neuro-navigation (Gugino et al, 2001), the motor evoked potential's variability is not significantly reduced by use of this technique, and it thus may be more essential to studies using stimulation at sites other than the primary motor cortex.

Kier's et al (1993) managed to assess spinal excitability changes (by assessment of the H-reflex) in parallel to monitoring variability of the MEP. They noted that

variability of the H-reflex at rest was substantially less than that of the MEP (with similarly matched amplitudes).

At the motor unit level variability of the motor evoked potential can be caused by one of three possibilities (Roesler et al, 2008): changes in excitability of the motor neuron relative to discharge threshold, variability of motor neuron discharge synchronisation leading to possible phasic cancellations, and lastly, varying occurrence of repetitive motor neuron discharges in response to the descending corticospinal tract input. In their paper, Rösler et al (2008) employed the triple pulse technique (using stimulation at Erb's point and resultant phase cancellation) to demonstrate that a significant component of MEP variability was likely to arise at the spinal segmental level. The triple pulse technique however does cause significant subject discomfort. As Kiers et al (1993) have suggested, it seems likely that both the spinal and cortical sites can contribute independently to MEP variation.

Several studies have helped confirm a cortical contribution for variability of the TMS MEP. Work by Ellaway et al (1998) had studied variability in groups of muscles and noted substantial correlations in variability between ipsilateral regional muscles and bilateral homologous muscles suggestive of a cortical site of contribution to MEP variability. Subsequent work by Zarkowski et al (2006), demonstrated that EEG gamma amplitude had a positive correlation with MEP amplitude whilst alpha amplitude had a negative correlation. Sauseng et al (2009) used multi-channel EEG with simultaneous TMS to demonstrate that large amplitude MEPs were evoked

when the cortex underneath the coil had evidenced low alpha power in the time immediately preceding the stimulus.

Mitchell et al (2007) recognized that sweep to sweep variability in the TMS MEP may represent noise within the motor system. They assessed whether EEG oscillations or changes in background EMG activity were linked to the changes seen in MEP amplitude whilst maintaining a low force (~1N) precision grip. Whilst no significant correlation of EEG phase/amplitude to MEP amplitude was seen they did note a mild correlation with FDI MEP amplitudes for beta-oscillatory power in pre-stimulus EMG (r^2=0.2). The strength of this correlation was diminished, but not altogether lost, when pre-stimulus EMG in an uninvolved muscle (ADM) was correlated with FDI MEPs (and vice versa). Mitchell et al (2007) suggested that the lack of EEG correlation reflected the phenomenon of event related desynchronisation (Leocani et al, 2001).

2.3.3 TMS MEP variability observations in stroke patients

Few clinical studies have addressed MEP amplitude variability in pathological states and those that have tended to treat it as hindrance rather than potential source of information. Koski et al (2007) assessed MEP variability in 10 patients who had experienced strokes affecting the motor system (either cortical or subcortical) between eight to 17 months earlier. The patients were assessed twice whilst taking part in a constraint induced movement therapy trial. In their study they estimated that some patients require a significantly higher amount of samples to obtain and accurate estimate of the mean amplitude (up to 36 in some cases), but less for

measures such as the cortical silent period and MEP latency. Closer examination of the data presented in their paper suggests that such high MEP variability at rest is not necessarily consistent between sessions for any given patient. Butler et al (2005) suggested that increased trial-to-trial MEP variability found in their chronic stroke patients complicated extensor muscle mapping in their work.

2.4 Neurophysiological studies of motor control

Primate movements can be reproduced with a significant degree of precision, and it would seem probable that potential output variability of the corticospinal tract observed at rest (such as seen with the TMS MEP) would need to be tightly controlled to facilitate the accurate reproduction of precision movements.

2.4.1. Recordings of movement preparation in non-human primates

The possibility that movements must first be prepared then executed rather than instantaneously executed was first alluded to following the work of Herman von Helmholtz (1853) who demonstrated the long and variable nature of behavioural reaction times. Whilst this work failed to account for sensory causes of delay, since then the idea of the need for movement preparation has been accepted and various models of movement control have been proposed to explain the necessary physiological processes observed. An understanding of some of the work carried out in primate studies of movement preparation may lend insight on what might be observed when studying TMS MEPs (including their variability) to explore human movement preparation. Importantly however, the invasive recordings of individual neuronal firing rates in non-human primates cannot be directly translated to the non-

invasive recording (e.g. TMS or MRI) methods used in experimental observations of human motor control.

Evarts (1965) presented a series of papers in the mid-1960s that described electrophysiological recordings taken from the pyramidal tract of Macaque monkeys during movement preparation. Spontaneous movements of the monkeys were observed and neuronal activity recorded by microelectrodes in pyramidal tract neurons identified by their response to anti-dromic stimulation from medullary pyramid electrodes. His initial paper (1965) determined that large diameter neurons (Betz cells) were relatively quiescent in the absence of movement and displayed phasic bursts of activity during movement of the contralateral arm. In contrast smaller diameter neurons showed tonic bursts in the absence of movement and firing rates could drift upward or downward with movement. Subsequent to this Evarts (1966) trained Macaques on a visually cued wrist extension/flexion task and determined that firing rates in large diameter cells rose prior to EMG onset at 100ms following the cue, with EMG onset at 170ms and task reaction time of 220ms. Small diameter neurons would also show changes in firing rate (rising or falling) with for example, firing rates increasing prior to extension on a background of tonic firing during flexion.

Additional work by Evarts (1968) extended these initial findings, demonstrating corticospinal neuron firing rate correlated with both the direction and amplitude of force exerted in a muscle, whilst later work by others (Georgopoulos, 1988) provided evidence for preparatory cortical coding of limb displacement vectors as a functional

of neuronal populations. Prut and Fetz (1999) suggested downstream spinal interneurons, rather than being merely the late stage output platform for the cortex, would also appear to have a dynamic role throughout the preparatory period. They assessed spinal interneuron activity in two monkeys performing an instructed delayed flexion/extension task at the wrist. First they highlighted the significant effect of the delayed period on about 30% of interneurons, including a significant decline in activity amongst some interneurons. Contrasting flexion/extension responses they teased out further delay period modulations on small subsets of these interneurons, for example some interneurons showed increased but low-level activity early during the delay period instructing an extension movement and escalated with movement initiation, changes that were less pronounced, though importantly appear to be still there during a flexion trial. Some interneurons only activated with movement onset. From the evidence presented (Prut and Fetz, 1999; Fetz et al, 2002) it appears reasonable to conclude that spinal interneuron activity sees early modulation in the preparatory period, however, the primacy of the cortex during movement preparation is in no way diminished by these findings.

Returning to the initial theme of movement preparation stabilizing corticospinal tract output - the optimal subspace model (Churchland et al, 2006) proposes that, at the moment a movement is cued, neurons may occupy a variety of states and only a fraction of these states will be appropriate for the generation of an accurate movement. The movement preparation period thus consists of bringing the population of functionally relevant neurons into the optimum state and once complete the movement is initiated. Churchland et al (2006) utilised an implanted

electrode array in three Macaque monkeys to study variance in neuronal firing rates in the premotor cortex during the preparatory period of a stereotyped reaching movement. Their key finding was that individual neuronal firing rates reduced substantially in variability during the movement preparation period, independent of both the neuron's direction of change in firing rate and to its change in mean firing rate. Additionally they showed that lengthening the delay period between the instruction and go signal allowed for increased preparation, evidence by a further reduction in neuronal firing variability and concomitant reduction in reaction time. They later corroborated this finding by studying changes in neuronal behaviour following microstimulation (as a perturbator) of PMd in Macaques (Churchland et al, 2007) again using a reaching movement – demonstrating that 1) microstimulation early on in movement preparation did not significant delay reaction or compromise movement, and 2) microstimulation late in the preparatory period significantly delayed movement but did not compromise the movement itself. Later work (Afshar et al, 2011) extended the premovement reduction in variability to Macaque M1 neurons, as well as developing new insights on trial-to-trial variability. Together they propose these findings to support the hypothesis that neurons adjust firing rates during the premovement period to achieve the 'optimal' subspace prior to the initiation of movement.

Interestingly Churchland et al (2010) noted a similar phenomenon with respect to sensory stimuli, demonstrating a reduction in neuronal firing rate variability following presentation of visual stimulus. The changes were seen not just in the visual cortex but extended widely over much of the cortex (including motor areas)

presumably through downstream cascading effects. The phenomenon was present even when the cats had been placed under anesthetic.

2.4.2 Movement preparation in humans within a simple reaction time paradigm

Simple reaction time experiments, where a predetermined movement is rapidly executed following the onset of a go cue signal, provide a ready means of studying human movement preparation. Transcranial cranial magnetic stimulation is able to assess the combined excitability of motor cortex and spinal neurons during this movement preparation period. Again, whilst excitability is not the same as firing rate, it nevertheless provides a useful perspective on the cortical preparation of human movement.

Electrical transcranial stimulation had been used by Rossini et al (1988) within a simple reaction time paradigm and demonstrated a facilitation of the motor evoked potential. Using a simple auditory signal as the go cue for a thumb opposition task they stimulated subject's motor cortex by subthreshold anodal electrical stimulation, recording the resultant MEP using surface EMG, and also used the movement EMG onset to determine the subject's reaction time per trial. They noted a rise in probability of evoking MEPs from 100-60ms prior to EMG onset. From 60ms until 20ms premovement, they noticed a more rapid rise in MEP probability together with a rise in amplitude and reduction in MEP latency. The peak of mean amplitude occurred within the 20ms prior to movement onset whilst MEP latency remained about 3-5ms faster than the resting MEPs. In two subjects they also utilised a magnetic stimulator at different intensities and reported a similar pattern of rising

amplitude and shortening latency of MEPs towards movement onset. Utilising TMS with a subthreshold intensity stimulus Pascal-Leone et al (1992) demonstrate a similar rise in excitability 80ms preceding EMG onset in the agonist (APB), also within an auditory cued simple reaction time paradigm.

Hoshiyama et al (1996) more formally studied excitability changes in movement preparation following a visual (LED) go signal comparing changes in radial extensors and ulnar flexors during first a wrist extension response and then later a wrist flexion response. Simultaneous recordings were also made of the thenar mucles. Titrating stimulator intensity to achieve a 0.5-1mV resting amplitude, MEPs were then measured at random time points from 0 to 200ms following the go signal and then recalibrated based on the subjects' reaction time performance (taken from EMG). During wrist flexion ulnar flexors demonstrated a rise in amplitude with radial extensors showing a decline, with the reciprocal agonist-antagonist pattern being reversed for the wrist extension movement. These results are compatible with firing rates changes of primate cortical neurons in the previous mentioned work by Evarts (1965). Thenar muscles (which may be considered a related surround muscle) showed no consistent pattern across subjects – in some amplitude remained static, in others they rose and others still fell. However, interpreting the timing of these changes must be met with some caution as the response locked analysis may be susceptible to the influence of the TMS pulse on reaction time responses (Day et al, 1989b).

A review of the prevailing literature would suggest there is some disagreement as to the exact timing of the rise in premovement motor cortex excitability with MacKinnon and Rothwell (2000) demonstrating excitability onset only within the final 15-30ms prior to EMG activity onset. This difference in results may be a product of differing methodologies, with the MacKinnon and Rothwell (2000) paper accounting for baseline EMG preceding the MEP, sub-threshold TMS and group averaging across time bins 10ms preceding RT (EMG) onset. MacKinnon and Rothwell (2000) also documented a similarly late rise in H-reflex excitability, occurring with the onset of the agonist EMG burst. In essence, there is likely to be an initial slow smaller rise in cortical neuron excitability during movement preparation (between 100-30ms before EMG onset) followed by a very late (30-10ms before movement onset) and rapid, almost exponential, rise in corticospinal tract excitability.

Whilst the TMS work on cortical excitability in human movement preparation can be paralleled with studies of cortical firing rates in primates, TMS paired pulse paradigms provide a unique opportunity to study the role of GABA-ergic inhibitory circuits. Two studies (Reynold's and Ashby, 1999; Nikolova et al, 2006) have highlighted an initial early rise in SICI from 140-160 to 100-120ms before movement, followed by a subsequent decline from 100ms onwards. Gilio et al (2003) also supported a decline in SICI from 100ms (prior to movement onset) onwards. However, they did not find the previously noted earlier rise in SICI, which may be due the use of a higher intensity conditioning pulse (80%RMT) than the other studies. Work by Peurala et al (2008) has shown a significant overlap of SICI/SICF (2003) at higher conditioning intensities and the intensities used by Gilio et al (2003) may have

inadvertently sampled SICF circuits. In essence then there is probably an initial very early slight rise in SICI during movement preparation, and more certainly following this there is substantial decline in SICI as preparation progresses.

At the outset of this work TMS MEP variability had not been utilized to study the process of human movement preparation. Mitchell et al (2007) had suggested the trial to trial variability of TMS MEPs reflected noise inherent in the motor system. From our perspective it seemed reasonable to propose that for simple human finger movements one would expect a downward modulation of TMS MEP amplitude variability during the preparation for movement, reflective of the corticospinal tract's ability to tightly regulate excitability in the reproduction of fine finger movements required for dexterous tasks.

During the preparation of this work colleagues (Klein-Flugge et al, 2013) reanalyzed data from a choice reaction time task they had previously published (Klein-Flugge et al, 2012) with a view to analyzing changes in MEP amplitude variability. Klein-Flugge et al (2013) demonstrated a decline in MEP amplitude variability in the period between the "Go" signal and movement onset, suggesting this represented a decline in corticospinal variability analogous to that observed in Churchland's primate work. They speculated that as neural firing patterns became less variable, corticospinal tract excitability stabilised, causing the observed decline in MEP variability.

The task of Klein-Flugge et al (2013) required interpreting a movement instruction

(cognitive preparation) and execution following the “Go“ signal. This contrast’s with Churchland’s (2006) cued delayed reach task, more akin to a simple reaction time (RT) task with the focus on execution. Though variability changes in Klein-Flugge’s task occurred across the latter half of movement preparation, it remains uncertain how much of the change observed was due to movement selection or execution. An additional technical challenge in this work was the substantial change in MEP mean amplitudes noted across the RT period, and the possible confounding effects this may have, given what is known of the effect of amplitude at rest (Van der Kamp et al, 1996). Whilst this was accounted for through standardization against an input-output curve, collected through a large but separate subject cohort, it remains unclear whether the relationship between amplitude and variability is reliably transferable between individuals and/or across states (ie. in task vs at rest).

2.4.3 Further aspects of motor control

Even the ordinary processes of everyday human movement preparation must encompass a vast repertoire of specific movement paradigms including confinement of activity to discrete muscle groups, complex dexterous tasks, and the scaling of force. TMS has provided some insight into the vast repertoire of physiological paradigms required in even these everyday processes of human movement preparation, such as the scaling of force and the discrete control of activity in individual muscle groups.

Beck et al (2008) demonstrated the presence of inhibition (relative to rest) in APB MEPs during the preparation (premotor) and initiation (phasic motor) components

of a well practiced low force index finger button press task (where FDI was activated). They proposed that the phenomena of "surround inhibition" enabled the discrete activation of individual muscles required for fine finger movements. Given a concomitant rise in excitability of the H-reflex, a cortical origin for the inhibition of the APB MEP was suggested. A similar result at movement initiation had first been demonstrated by Sohn and Hallet in 2004 and by others since (Kassavetis et al, 2014). Interestingly Beck et al (2008) demonstrated patients with dystonia demonstrated an absence of surround inhibition, which they related to a similarly timed absence of SICI in these subjects. Subsequent studies have demonstrated altered surround inhibition in variety of subjects including musicians (Shin et al, 2012). It should be noted that the phenomenon is not found universally in all subjects (Kassavetis et al, 2014). Interestingly Kassavetis et al (2014) highlight the volitional aspect of the surround inhibition task (with subjects tasked to deliberately down regulate activity in surround muscles). The volitional aspect of surround inhibition may allow for the direct assessment of MEP amplitude variability during preparation for a task where mean excitability is expected to reduce.

Two very basic primate grip types are the precision and power grips. In the precision grip individuated movements of the the index finger and thumb allow for the dexterous manipulation of small objects – a task requiring substantial independent control of fine force vectors at the digit tips (Ehrsson et al, 2000). In 1968 Lawrence and Kuypers reported on experimentally performed bilateral lesions of the pyramidal tract in non-human primates, resulting in a remarkable loss of dexterous abilities

(Lemon et al, 2012). In contrast the power grip requires palmar opposition to an object with digits flexed around the object for grip stability (Ehrsson et al, 2000).

Classically the power grip utilizes the intrinsic hands muscles for object fixation and low level force application, with progressively greater levels of force involving scalar activation of the extrinsic hands muscles such as those located in the forearm flexor compartment. However, the ability of a muscle to exert force is frequently dependent on the specifics of muscle mechanics and manipulandum positioning with the first dorsal interosseous being a case in point. FDI exerts the greatest force (70%) across the metacarpophalangeal (MCP) joint of all the flexors (Schreuders et al, 2006), though can be heavily dependent on the angle of the MCP joint with greater force being generated across the mid-range than either extremes.

Datta et al (1989) contrasted various movement tasks and demonstrating increased FDI MEP amplitudes during index finger abduction when compared with a power grip task (both a 20% maximum force as demonstrated by FDI EMG). Their results contrasted with the later work Flament et al (1992) demonstrated grip task complexity influenced the degree of cortical motor neuron recruitment – TMS during the more complex tasks (including power grip and precision grip) resulted in greater MEP mean amplitudes than the simple index finger abduction tasks for each subject, though of interest the ordering of MEP amplitudes across complex tasks varied inconsistently across individual subjects. Hasegawa et al (2001) demonstrated the active motor threshold was significantly lower in precision grip than in a power grip task corroborating work by Huesler et al (1998) who found that greater amplitude

responses from TMS were generated during a precision grip than the power grip. Coming full circle Kouchtir et al (2012) demonstrated that the steepness of the input output (I/O) curve and the level at which the curve plateaued with both greater in the precision grip than in simple index finger abduction. Contrasting methodologies are present in many of these studies, with conflicting results present even when certain variables are seemingly identically controlled for across tasks (eg EMG force feedback at 20 % MVC (Datta et al, 1989) vs 5% MVC (Flament et al, 1993)). Whilst significant work has been conducted assessing changes in mean activity across tasks requiring different levels of dexterity, the idea that dexterous movements require tight control of corticospinal activity leads us to ask whether differences in the control of premovement MEP amplitude variability may be apparent across tasks with differing levels of dexterity.

Work utilizing TMS to study the process of force generation in M1 is confined to the period following movement preparation, ie. during sustained contraction, and is perhaps best summarized by the work of Perez and Cohen (2009). TMS input/output MEP curves were recorded in a forearm flexor muscle at rest and across a range of force levels (10%, 30% and 70% of maximal voluntary contraction) during a sustained power grip contraction. They demonstrated that the slope and peak amplitude plateau appeared greater with high force levels (70%) than at rest or low force levels (10%). This reflects the greater extent of corticospinal tract activation/involvement at higher force levels and may have implications for the requirements of movement preparation. As much it may be possible to see differences in control of TMS MEP

amplitude variability during the preparatory period when comparing tasks of varying force.

2.4.4 Motor control following stroke

Neuronal death following stroke leads to significant debility including motor system dysfunction. Patterns of functional recovery and reorganisation, while obviously highly variable on an individual basis, have been suggested to reflect a hierarchical order. Within this model damage compromising functioning of the corticospinal tract leads to recruitment of the ipsilesional premotor cortex and sequentially the contralateral premotor cortex (Johansen-Berg et al, 2002). Disruption of these secondary motor areas (by use of TMS to create a temporary virtual lesion) compromised reaction time performance in a manner proportional to their recruitment during fMRI sequences recorded performing the same reaction time task (Johansen-Berg et al, 2002). Though the exact pattern of recruitment may well dependent on individual circumstance, in general the degree of secondary motor area recruitment would appear inversely proportional to the integrity of the corticospinal tract (Ward et al, 2006). Importantly the recruitment of secondary motor areas has been thought to compromise the quality and performance of movement following stroke (Ward et al, 2006; Takeuchi et al, 2007).

Two studies report direct recordings of M1 corticospinal tract excitability during movement preparation following stroke. Hummel et al (2009) studied CST excitability in stroke subjects using TMS. Even in their high functioning patient group a deficiency was seen with the persistence of SICI late into movement preparation, though

changes in single pulse MEP responses were not seen between patients and aged matched controls. In contrast, Battaglia et al (2006) had demonstrated a reduced levels of excitation (compared to intact hemisphere and healthy controls) in TMS MEPs generated from the M1 contralateral to the lesioned cerebellar hemisphere of patients with isolated cerebellar stroke. Though no study to date specifically assesses the extent of MEP variability changes during movement preparation, given the impairment of movement performance and quality (Ward et al, 2006; Takeuchi et al, 2007) one might expect this to be manifest in an alteration of the relationship between signal and noise during movement preparation.

2.5 Conclusion

Across the course of this literature review we've noted TMS MEPs elicited over M1 display significant amplitude variability, a likely consequence of the significant array of excitatory and inhibitory inputs seen at the confluence of corticospinal tract output. Work in non-human primates (Churchland et al, 2006; Afshar et al, 2011) has suggested that noise in the motor system at the single neuron level is modulated through the process of movement preparation and may be a useful biological marker.

In human's TMS MEP variability has been postulated to reflect noise within the motor system (Mitchell et al, 2007). Positive preliminary steps have been made to assess changes in MEP amplitude variability during human movement preparation (Klein-Flugge et al, 2013). The work in this thesis proposes to examine MEP amplitude

variability during preparation for simple human finger movements using specifically designed paradigms, to assess the extent of signal within the noise!

However, prior to these experiments we will clarify several aspects relating to the treatment of MEP variability in the resting state. Firstly, we assess the significance of proximity to the cortical hotspot in assessing MEP amplitude variability, when mean amplitudes have been appropriately matched. Secondly whether TMS MEP amplitude variability is significantly influenced by the initial transient state noted by Schmidt et al (2009). Thirdly we will undertake a dedicated study to assess limitation of different statistical markers of central dispersion in the assessment of MEP amplitude variability at rest seen across an input/output curve. Finally, given the findings of Churchland et al (2010) the sensory influence on neuron firing rate variability we will clarify whether significant changes in MEP variability are seen with a visual stimulus.

With respect to movement preparation we assess MEP variability in simple human finger movements including paradigms where mean excitability is specifically tasked not to increase (ie. surround inhibition). Furthermore, we will contrast tasks of varying force and dexterity. Finally, we will assess how the control of MEP amplitude variability during movement preparation is altered in subjects following stroke, where motor preparation is speculated to be less efficient (Ward et al, 2006; Takeuchi et al, 2007).

Chapter 3 General Methods

The application of TMS over the primary motor cortex allows for the neurophysiological assessment of corticospinal tract excitability at rest and during movement preparation through study of the resultant MEP. This chapter describes the methods commonly used throughout the experimental portions of this thesis.

3.1 Subjects

Subjects in each of the experiments were recruited in accordance with the principals of the Declaration of Helsinki. The studies, consent and information sheets had been approved by the Joint Ethics Committee of the Institute of Neurology, UCL, and the National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Foundation Trust, London. Handedness was determined using the Edinburgh Handedness inventory (Oldfield et al, 1971).

3.2 Procedural points

3.2.1 EMG recording

Silver disc electrodes (area ~ 1cm^2) were placed over the muscle(s) of interest in a belly-tendon montage. The ground electrode was placed over the ulnar styloid. Raw EMG signal was recorded (with gain set at x1000) and band-pass filtered (10Hz to 2000Hz) through a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, UK). This amplified signal was then digitally sampled at 5000Hz using a CED Power-1401 device (Cambridge Electronic Design, Cambridge, UK) and Signal Software version 4.08. Data was acquired and electronically stored for later analysis offline.

3.2.2 TMS recordings

TMS study design and practice took into account the safety recommendations of Rossi et al (2009). TMS was delivered using a Magstim 200^2 stimulator (The Magstim Co. Ltd, UK) that passes a monophasic pulse (rise time 0.1ms, decay to zero ~ 0.8ms) through a figure-of-eight coil (Magstim D70 model, 70mm ring diameter with peak field ~1.5 Tesla). Pulse timings were set and delivered using the aforementioned Signal software and CED 1401 device with digital TTL control. Digital TTL signals from the Power 1401 device were also used to turn on (and off) a green visual LED signal that was to be used in the behavioural tasks.

Pulses were delivered with the figure of eight coil placed tangentially to the scalp and the handle angled backwards at 45° to the sagittal plane thereby inducing a posterior-anterior current across the pre-central gyrus. Identification of the cortical hotspot for a muscle utilized the functional method, whereby systematic movements of the coil were made following TMS pulses, using 0.5-1cm increments in the anterior-posterior and medial-lateral directions, ultimately resulting in localization of a muscle "hotspot", the position with the highest and most reliable MEP response.

Once the cortical hotspot for the target muscle was identified the position was recorded for later use. In all experiments this was done directly onto a securely fitted cap (or directly onto the scalp in some cases) using a chinagraph pencil to draw a short crescent line along the anterior bifurcation of the coil, together with a straight-

line at its junction to indicate coil orientation. A second additional semicircle was drawn along the inner lateral aspect of of the medially located loop of the figure of eight coil. This method facilitated easy retargeting between blocks. If there was concern of inadvertent displacement the position of the hotspot was rechecked.

In initial experiments a neuro-navigation system (ANT Visor supplied by ANT Neuro) was additionally utilized for recording of TMS hotspots. For positioning this system utilized an optical tracking system with spherical reflective markers attached to the coil (secured via a detachable clamp), subject (secured to the forehead via tight adjustable circumferential elastic band) and a marking tool each being monitored through an NDI Polaris camera. Following an initial registration protocol whereby the reflective spherical clusters where registered and the scalp surface mapped, TTL signaling through BNC cables could be used to register a TMS target of interest. Subsequently TMS coil re-targetting was guided by a three plane gyroscope interface to maintain the TMS stimulation targeting to within 2mm of the original stimulation site, a task reliably undertaken following initial training and familiarisation.

Once the hotspot had been identified neurophysiological assessment could commence. Determination of the resting motor threshold (RMT) utilised the standard definition, ie. the minimum stimulator intensity able to produce a 50 microvolt MEP on at least five out of ten trials. When a different target intensity was to be utilized (~1mV) a similar procedure was utilized to determine stimulator intensity. The 1mV intensity was used to minimize floor effects closer to resting threshold without the saturation/ceiling effects seen at higher intensities.

3.2.3 EMG processing

CED Signal software was used to review each collected frame offline. Trials were rejected from further analysis if they were contaminated by artefact, there was non-task behaviour, and premature or delayed responses. With respect to EMG artifact due to pre-activation, frames were rejected if EMG spikes greater than 25 microvolts were detected in the 250ms preceding a TMS pulse.

Signal software was then used to determine the MEP peak-to-peak amplitude for each trial (here after referred to as amplitude) using a series of custom scripts made for each experiment. A custom script for Signal software was also used to determine the reaction times (RTs) as follows: first the baseline EMG signal variation was determined by calculating the standard deviation of rectified EMG data in the 100ms preceding the 'Go' signal. The RT was taken as the point following the 'Go' signal at which the rectified EMG signal rose above six times this value.

3.3 Statistical methods

Data collation and statistical analyses were undertaken with Microsoft Excel (version 15; Microsoft, Seattle USA) and IBM SPSS (v21; International Business Machines Corp., USA) respectively. The decision was made to focus on the utilization of non-parametric methods wherever possible, given the non-normal distribution of MEP amplitudes and the relatively small sample sizes traditionally

used in TMS experiments. Typically, the non-parametric Friedman's analysis of variance by ranks was used which allowed repeated measures assessment across only one factor. Significance was set at the 0.05 level with post-hoc testing employing the Wilcoxon Signed Ranks Test (WSRT). If correlation analysis was undertaken Kendall's Tau was utilized.

Although conventionally non-parametric effect sizes have been considered difficult to determine, we provide them here as an internal reference between experiments only. In particular, when reporting Friedman's analysis of variance by ranks we also report Kendall's coefficient of concordance (W) which expresses consistency of effect across cases (0 representing no agreement and 1 being unanimity). Furthermore, when utilizing the Wilcoxon Sign Rank Test we define the effect size as PSdep (in the manner of Grissom and Kim, 2012) which relates the proportion of positive (favourable) difference scores relative to the total number of matched pairs.

Chapter 4 TMS MEP variability in the resting state

4.1 Introduction

The MEP generated by TMS over the motor cortex displays significant trial-to-trial amplitude variability (Amassian et al, 1989). Whilst traditionally treated as a signal to noise problem we wished to assess if MEP amplitude variability could yield valuable physiological information in movement preparation. Prior to undertaking this work, we undertook four preliminary control experiments examining resting MEP variability and the influence of proximity on the cortical hotspot, the initial hyper-excitable state, optimizing the choice of statistical markers of variability and the influence of potential confounders, such as visual stimuli.

4.1.1 Variability and the cortical hotspot

Work by Brasil-Neto et al (1992), using a fixed intensity (100% of stimulator output) with a figure of eight coil, highlighted the existence of TMS cortical hotspots for numerous muscles, sites where variability appeared lowest. Our first experiments assess whether proximity to the cortical hotspot still significantly affects MEP variability at the lower intensities more typically used in TMS experiments. Additionally, work by Van der Kamp et al (1996) has shown that increases in stimulus intensity can decrease the variability of the resting MEP. We wondered whether how significant the affect of positional differences in coil placement on variability would be once amplitudes were matched through changes in stimulator intensity.

4.1.2 The initial hyper-excitable state

Schmidt et al (2009) have demonstrated an initial hyperexcitable state during sequential pulse recordings of the MEP. As described above, amplitude and variability in the resting state are closely linked when manipulated through adjustments in stimulus intensity. In our second experiment wished to establish whether this initial series of MEPs would also show reduced variability, thereby necessitating modifications in planned experimental procedures. Furthermore, most TMS protocols randomise stimulation parameters - would this initial state will still be present under randomised conditions?

4.1.3 Measures of dispersion in the study of MEP amplitude variability

During the literature review of TMS MEP amplitude variability studies we noted the diversity of statistical approaches. Whilst these approaches are often well reasoned we wished to explore practical differences between variability statistics, in particular indices of dispersion that would allow comparison across different means and conditions. To do so our third experiment generates MEP amplitude variability data through the use of input-output curves. Analysis of the data using various markers of variability allows us to identify the advantages and weaknesses for each of these markers, prior to assessing variability in a more dynamic state.

4.1.4 The influence of an LED light on MEP amplitude variability

Finally work by Churchland et al (2010) had elegantly demonstrated that neuronal firing rate variability was influenced by sensory inputs to the visual cortex, across a wide range of cortical areas through downstream cascading effects.

Interestingly this finding was seen even even in anaesthetized animals. We planned to study changes in MEP variability during movement preparation. Prior to utilizing the visual cue as the imperative signal within a simple reaction time paradigm, we wished to assess whether a visual LED signal could potentially influence MEP amplitude variability of itself (i.e. could the visual signal be a potential confounder for our anticipated results).

4.2 Methods

4.2.1 – Variability and the cortical hotspot

In this experiment we wished assess the influence of proximity to the cortical hotspot on MEP variability. Two studies were performed – the first assessed MEP amplitude variability on and off the cortical hotspot with stimulation intensity fixed at 120% RMT – an intensity representative of many TMS studies. The second study examined variability on and off the hotspot under conditions where mean amplitude had been equalized. Here MEP variability is assessed on and off the hotspot, but the stimulator output adjusted to achieve equivalent mean amplitudes at both positions.

4.2.1.a Subject profile

In total of thirteen healthy subjects participated in these studies. For the first study the mean age was 36 years with one left hander and three females amongst them. In the second study subjects had a mean age of 32 years, with four females and one left handed subject.

4.2.1.b Study procedure

We studied TMS motor evoked potentials in FDI of the subjects' dominant hand at rest. Subjects were seated with arms placed comfortable in their lap, forearms midway between pronation and supination. EMG electrodes were applied to FDI in a belly tendon montage with raw EMG recorded for off-line analysis as previously described. TMS was delivered as a monophasic pulse through a figure of eight coil and generated by Magstim (UK Ltd) 200^2 mono-block. A neuro-navigation suite (ANT

Neuro, The Netherlands) was used to provide frameless sterotaxy and enable accurate reacquisition to within 2mm of the designated position.

The FDI hotspot was identified using the functional method and registered with the neuro-navigation software (and additionally marked off on a secured cap). An additional site was targeted 1.5cm medially. Resting motor threshold (RMT) for FDI was determined on the cortical hotspot and the stimulator intensity set to 120% of RMT. For the second study a brief run of MEPs were made on the cortical hotspot at 120% RMT to provide an approximation of the mean MEP amplitude on the hotspot – the secondary site was then re-targeted, stimulator intensity incrementally adjusted to provide a similar mean response and the required intensity recorded. In each study, record blocks of twenty MEPs were then made (each frame separated by between four to six seconds) both on and off the FDI hotspot (the order randomized in each subject with a five minute rest between blocks).

4.2.1.c Analysis

Each frame was analysed off-line with frames contaminated by EMG artefact discarded. The FDI MEP amplitude mean and coefficient of variation were then determined on and off the hotspot. A direct comparison between conditions within each study was undertaken using Wilcoxon Sign Ranks Test for related samples, with alpha set at 0.05 (as for subsequent experiments).

4.2.2 The initial hyper-excitable state

In this experiment we sought to assess impact of the initial hyper-excitable state on MEP amplitude variability across a recording session under two conditions. The first design involved the use of a single pulse (with constant intensity) given sequentially for a total of sixty pulses. The second series interspersed a series of single pulses (again with unchanged pulse intensity) randomly with a second paired pulse condition.

4.2.2.a Subject profile

For these two experiments 18 subjects were recruited. All were healthy volunteers – in the first series nine subjects (mean age 37) were recruited six of whom were female and three left handed. The second study recruited nine subjects with a mean age of 36 (six females and one left handed).

4.2.2.b Experiment procedure

Recordings were made from FDI in the dominant hand. A monophasic TMS pulse was delivered through a D70 figure of eight coil using two Magstim (UK Ltd) monophasic blocks linked via a Magstim "Bistim" module, enabling paired pulse TMS delivery. Frameless stereotaxy was again utilized, through the same neuro-navigation system supplied by ANT Neuro. In all subjects the FDI hotspot in the dominant hemisphere was identified using the functional method and registered with the neuro-navigation suite (registration on a secured cap was also undertaken as an additional precaution). The RMT for FDI was determined for each subject.

Two recording blocks were undertaken with subjects comfortably at rest. One in which 60 single TMS pulses were delivered at 120% of RMT, with between four to six seconds between pulses (the exact timing randomized to prevent anticipation). In the second recording block, a total of 120 pulses were delivered, either as single or paired pulses. Paired pulses utilized a conditioning pulse set at 70% of RMT and preceding the test pulse (still set at 120% RMT) by 3ms. The delivery of single and paired pulses was semi-randomised, with sixty pulses under each state delivered.

4.2.2.c Analysis

For our studies of MEP variability during the initial transient state we took the single pulse MEP amplitude data for each subject and placed them in order of acquisition for processing (paired pulse data from the randomised block were not used in this analysis). For each of these subject data blocks a rolling 5 sample windowed mean and coefficient of variation were obtained. We then normalised these to the overall session results for each statistic. A trial –by-trial grand average across subjects in each condition was determined, which we subsequently regressed by trial number to obtain the across session trend.

4.2.3 Measures of dispersion in the study of MEP amplitude variability

In this control experiment we wished to assess the utility of various measures of central dispersion in assessing resting MEP variability generated through a simple output curve.

4.2.3.a Subject profile

A total of nine subjects were recruited for this study. They had a mean age of 31 years, and among them were two females and one left hander.

4.2.3.b Experiment setup

Subject preparation was undertaken much as for earlier experiments. With the FDI hotspot and RMT and the hotspot recorded, we generated an input/output curve in subjects at rest. TMS pulses delivered at five levels starting at 100% of RMT, with increments of %5 RMT, resulting in coverage from 100-120% of RMT. A block of twenty trials was recorded at each intensity, with the exact order of intensities randomized.

4.2.3.c Analysis

After careful analysis of each frame the TMS intensity blocks for each subject were utilized to generate the mean peak to peak amplitude, as well as a battery of dispersion statistics: the coefficient of variation, the mean normalised consecutive difference, the kurtosis coefficient and the gini coefficient. The coefficient of variation is well known, and consists of the standard deviation/mean. The mean normalised consecutive difference (MNCD) was taken as the absolute difference between consecutive trials, divided by the block mean with the trial results averaged across the session as the final statistic. For the kurtosis coefficient we used Pearson's definition of the fourth moment of the data, where higher kurtosis is the result of infrequent extreme deviations and lower kurtosis the result of frequent moderate sized deviations. Finally the Gini coefficient is derived from the Lorenz curve (Bendel

et al, 1989; Wittebolle et al, 2009), which plots the rank order cumulative sum of all MEPs as percentage of their sum total against the corresponding proportion of the cumulative sample number. From this the Gini coefficient is determined as the area between the Lorenz curve and the line of perfect equality (the diagonal line where each plotted sample has the same incremental value) divided by the total area under the line of equality (as shown in figure 4.1). Subsequent comparisons across intensity blocks used Friedman's analysis, with the p-value set at 0.05 and the Wilcoxon Signed Ranks Test used for post-hoc testing. All statistical analyses and development of regression models were undertaken in SPSS.

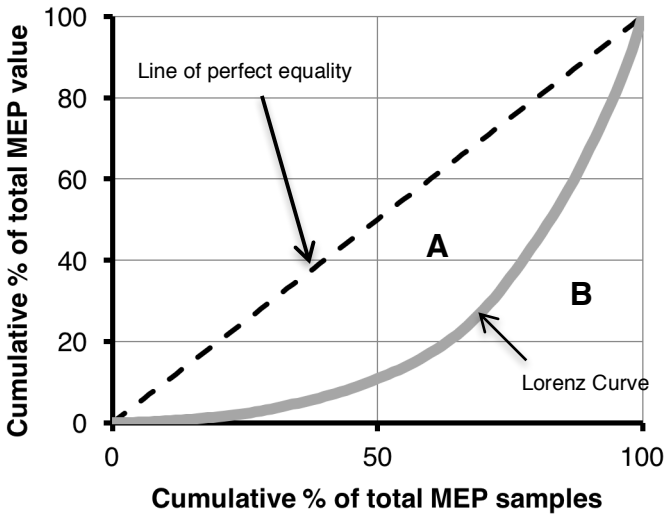


Figure 4.1 The Lorenz curve, the line of perfect equality and the Gini coefficient.

4.2.4 The influence of a visual LED light on MEP amplitude variability

This small control study assesses for the influence of a small LED light on MEP amplitude variability changes. We planned to used the LED light within a simple reaction time paradigm, and wished to be assured MEP variability would not be significantly affected by the stimulus alone.

4.2.4.a Subject profile

Eight healthy volunteer subjects (mean age 25 years, six females, all subjects right handed) participated in this control experiment. All participants were naïve to the other experiments in this thesis.

4.2.4.b Experimental setup

Following careful localisation of the hotspot for FDI in the dominant hemisphere as per earlier experiments, the stimulator intensity was adjusted to achieve an average resting peak to peak intensity of 1mV. A $1cm^2$ LED light was then placed centrally in their field of vision. The LED light was externally triggered to come on for a period of 200ms randomly somewhere between every four to six seconds. Subjects were asked simply to focus on the visual LED. A TMS pulse was delivered at either -500ms or +100ms with respect to the LED's onset, the timing semi-randomized across trials. With forty trials in the recording block, twenty MEPs were recorded under each timing.

4.2.4.c Analysis

Offline screening was performed for each recorded frame. For each subject the mean MEP amplitude and the MEP amplitude Gini coefficient were determined at each TMS pulse timing. Comparison of mean and the Gini coefficient was undertaken utilising the Wilcoxon Signed Ranks Test with a p-value for significance set at 0.05.

4.3 Results

4.3.1 Variability and the cortical hotspot

Here we wished to assess changes to variability on and off the FDI cortical hotspot under two scenarios. In the first series (figure 4.2.a-b) we recorded TMS MEPs on and off the FDI cortical hotspot using a fixed intensity (120% RMT; average stimulator intensity was 51% MSO), on and off the cortical hotspot. In the second series (figure 4.2.c-d) we again recorded TMS MEPs on and off the cortical hotspot, however here care had been taken to adjust stimulator intensity to deliver similar mean amplitudes when recording from the displaced site. The average stimulator output across subjects for the FDI hotspot was also 51% MSO (120% RMT), with stimulator intensity off the hotspot being on average 57% MSO.

The first series, using a fixed stimulator intensity, demonstrates a rise in MEP amplitude variability and drop in mean amplitude when off the cortical hotspot. With respect to the MEP amplitude coefficient of variation (figure 4.2.a), a Wilcoxon Signed Ranks Test was significant with Z=-2.201 p=0.028 and PSDep 1.0. The Wilcoxon Signed Ranks Test confirmed the expected reduction in mean MEP amplitude (figure 4.2.b) off the cortical hotspot with Z=-2.201 and p=0.028 (PSDep 1.0).

For the second series however a significant difference in MEP amplitude variability was not apparent. In analyzing MEP amplitude variability, the coefficient of variation on and off the hotspot displayed no statistically significant difference (Z=-0.169,

p=0.87; figure 4.2.c). Confirmatory checking for a difference in FDI MEP mean across the two sites (with the dynamically adjusted stimulator intensity) demonstrated the absence of a significant difference (figure 4.2.d) with the Wilcoxon Signed Ranks Test delivering a Z=-1.183 and p=0.24.

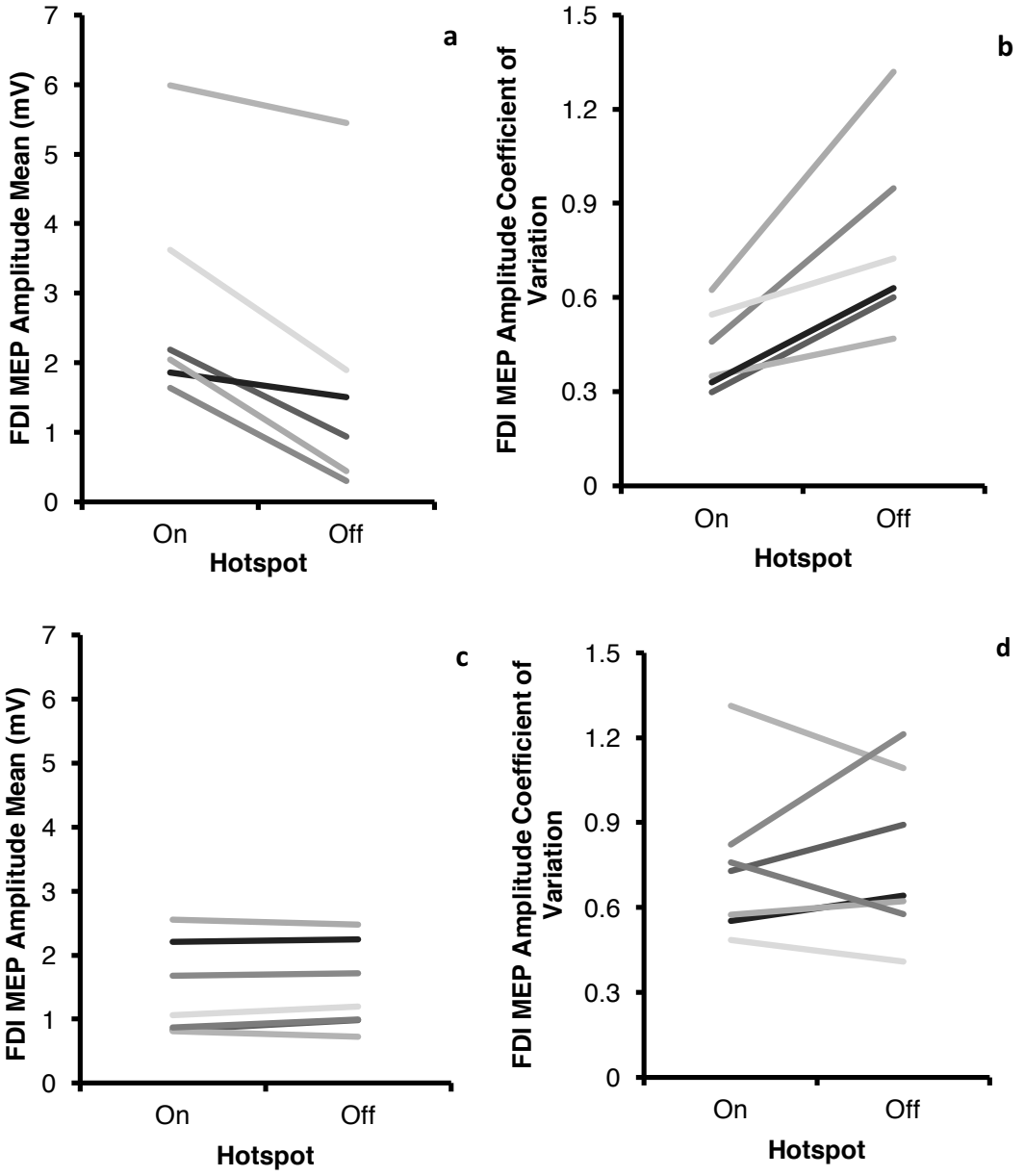


Figure 4.2.a-d MEP amplitude mean and coefficient of variation On and Off the FDI cortical hotspot, plotted for individual subjects. A straight monochromatic line links the result for each subject at both sites. For series one a fixed stimulator intensity was used (mean (a) coefficient of variation (b)). For series two (mean (c) coefficient of variation (d)) stimulator intensity "Off the FDI Hotspot" was adjusted to produce mean amplitudes similar to that seen on the hotspot where stimulator intensity had been set to 120% RMT. One data point is closely overlapped in (c).

4.3.2 Variability and the initial hyper-excitable state

Results for single pulse MEPs are displayed in figure with 4.3.a-b reporting the sequential pulse data analysis and figure 4.3c-d the single pulse data obtained from the randomised sequence. Visual inspection shows that the initial increase in excitability is not accompanied by any change in variability. For the sequential single pulse data mean the initial rise in excitability appears to be delayed.

Using formal linear regression analysis of the rolling 5-sample mean revealed a significant relationship with trial number for both the sequential single pulse and randomised single pulse MEP data. For sequentially obtained single pulse MEPs the data had a slope of -0.747 (t=-3.998) and intercept of +125 (t=18.43) with a regression F ratio of 15.984 and R^2 of 0.23. For randomised single pulse MEPs the data showed a similar slope of -.689 (t=16.026) and intercept of 121.7 (t=78.05), with the F-ratio=256.8 and an R^2 of 0.826. The data for sequentially and randomly obtained single pulse rolling mean can be seen in figures 4.3.a and 4.3.c.

Analysis of the rolling coefficient of variation revealed no linear relationship across trials for both sequential and randomised single pulse MEPs. For sequentially obtained MEP trials the F-ratio for the rolling coefficient of variation average was 0.336 with an R^2 of 0.006, and for single pulse MEPs delivered randomly the F-ratio was 0.978 with an R^2 of 0.018. Figure 3B and 3D display the result for sequential and randomly obtained single pulse MEP data.

In a bid to further assess whether individual moment to moment fluctuations in a subject's mean amplitude influenced the MEP variability we pooled all subject data points (normalized five MEP sample mean and coefficient of variation) and assessed for a correlation between MEP mean and coefficient of variation. For both the sequential and randomized MEP recordings the normal strong relationship between the MEP amplitude mean and coefficient of variation was not seen, suggesting fluctuations in MEP amplitude variability were not linked to changes in mean MEP amplitude (figure 4.4.a-b).

Of additional note most subjects found prolonged use of the headband associated with the neuro-navigation system uncomfortable for prolonged use greater than 25-30 minutes. Given this feedback, our observation that an experienced operator could maintain targeting of the hotspot reliably, and the earlier work by Gugino et al (2001) the neuro-navigation system was not used for further experiments.

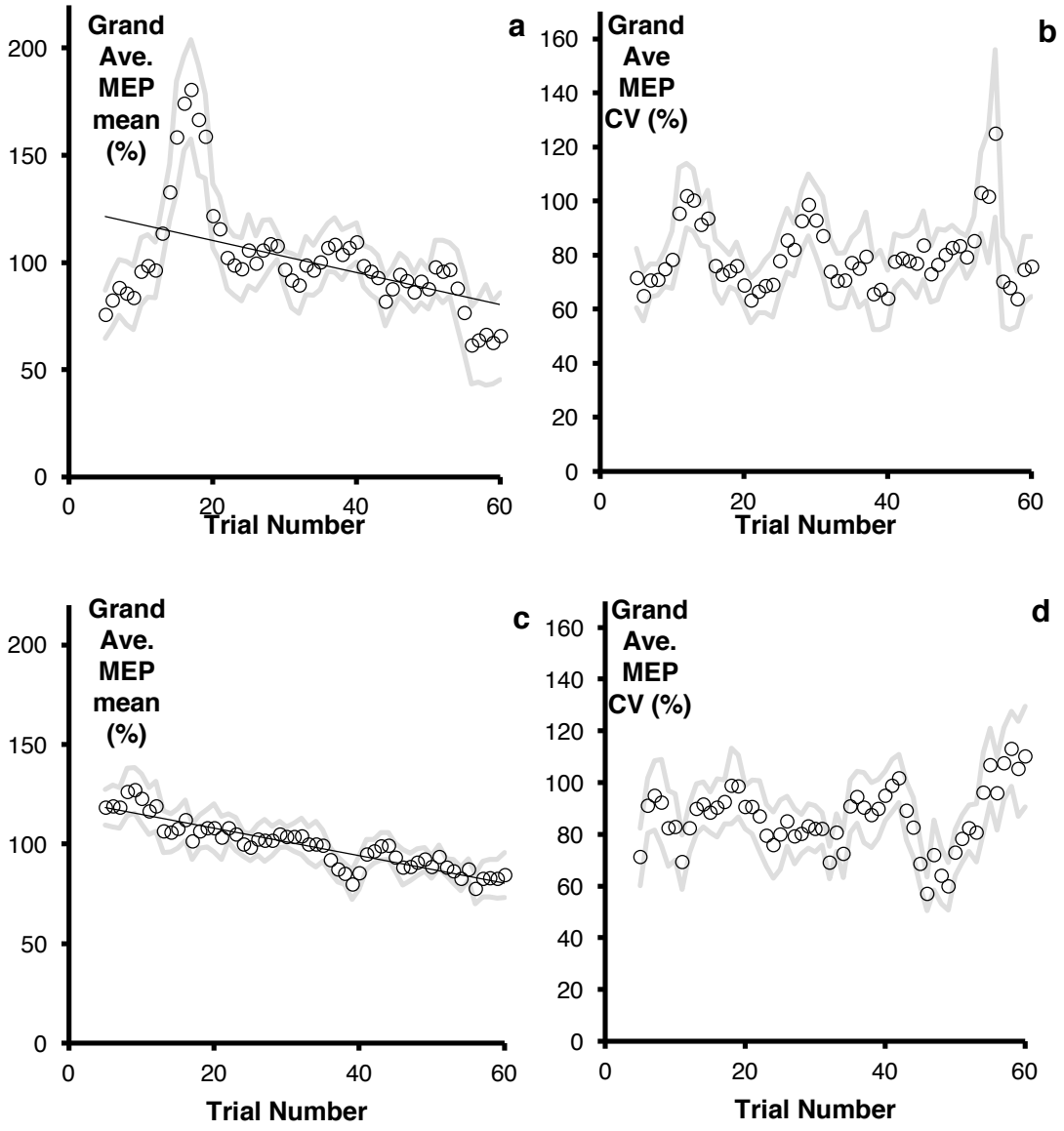


Figure 4.3.a-d Single pulse recordings over the FDI cortical hotspot undertaken in sequence (a and b) or having been randomly interspersed with paired pulses (c and d). Rolling 5-sample average relative statistics (a and c shows the grand average across subjects for MEP amplitude mean, c and d show the across subject grand average for MEP amplitude coefficient of variation) across subjects with each subject's rolling average value transformed into a percentage of their session mean prior to grand averages being generated across all subjects. Data points represent the grand average at each point with the lighter outer lines linking points ±1 SEM.

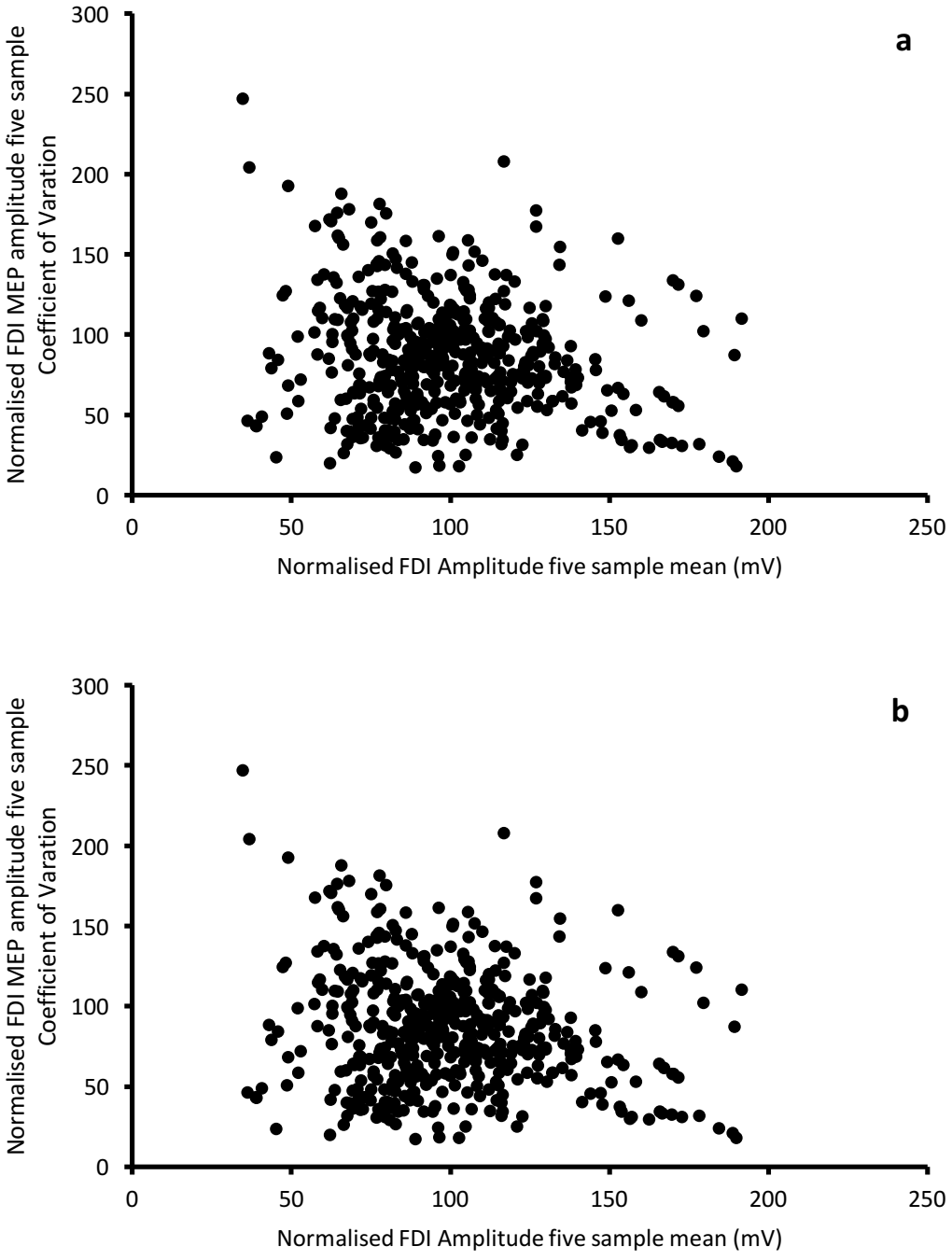


Figure 4.4.a-b Pooled subject data points of normalized MEP amplitude five sample means and coefficients of variation acquired during sequential (a) and randomized (b) TMS pulses.

4.3.3 Comparing measures of dispersion in the study of MEP amplitude variability

As expected increasing TMS intensity increased mean MEP amplitude, however for MEPs up to about 110% RMT (~0.5-1mV) the variability remains relatively constant with most variability markers before tending to decrease at higher intensities (figure 4.5.a-e).

4.3.3.a Dispersion indices' interpretation of MEP variability - overview

A Friedman's analysis revealed significant different across stimulus intensity blocks for MEP amplitude mean (p=0.000), the coefficient of variation (p=0.001), the MNCD (p=0.002), and the Gini coefficient (p=0.003) but not for the kurtosis coefficient (p=0.062). Interestingly post-hoc testing showed that whilst mean amplitude was essentially significantly different between each intensity, variability markers needed at least a 10% change in intensity before a difference became apparent. In all cases, baseline variability markers are relatively high and there was never a difference in variability markers between 100% and 105% RMT. Interestingly the Kurtosis coefficient displayed a tendency to reduce close to threshold, prior to rising and dropping back to equivocal values around 110% RMT. Given the lack of a significant effect for stimulus intensity on the Kurtosis coefficient further exploratory use of this measure was curtailed.

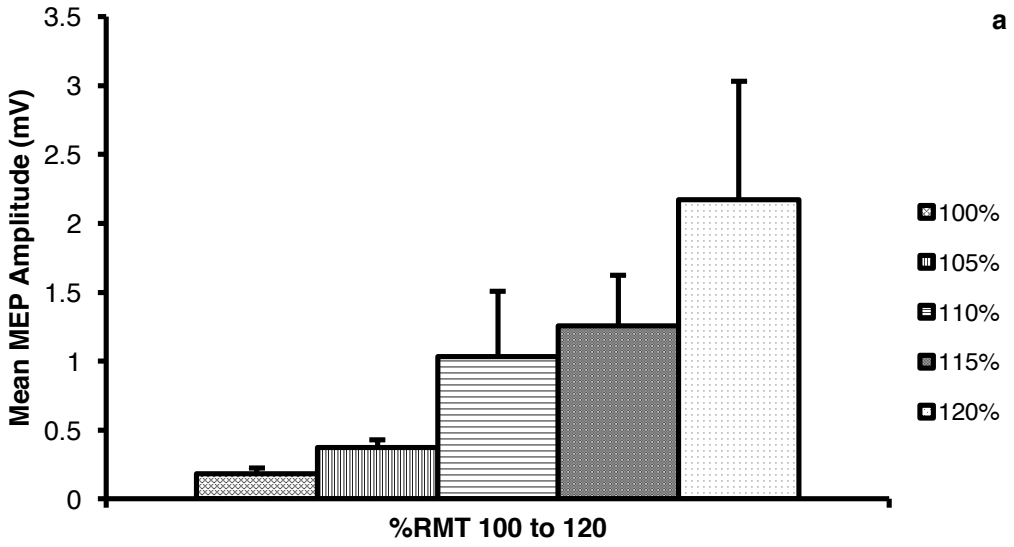
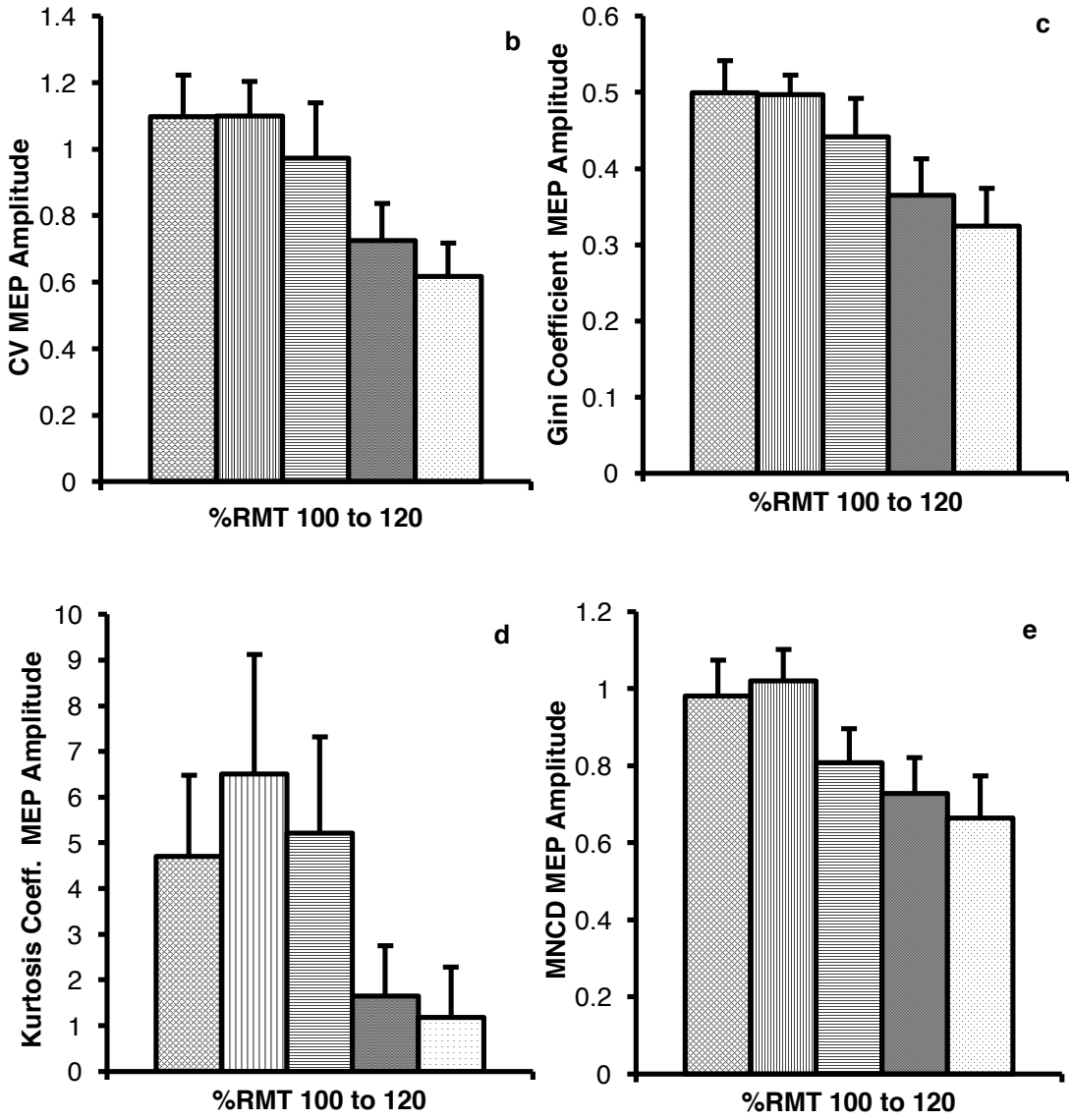


Figure 4.5.a-e *(current and following page).* Results of FDI MEP amplitude statistics (across subject average + SEM) following recordings of an input/output curve from 100%RMT to 120% RMT in 5% RMT increments. (a) MEP amplitude mean (b) Coefficient of Variation (c) Gini Coefficient (d) Kurtosis Coefficient (e) Mean Normalised Consecutive Difference. Legend for each intensity level of the I/O curves is immediately adjacent to figure 4.5.a.


b
CV MEP Amplitude
1.4
1.2
1
0.8
0.6
0.4
0.2
0
%RMT 100 to 120
c
Gini Coefficient MEP Amplitude
0.6
0.5
0.4
0.3
0.2
0.1
0
%RMT 100 to 120
d
Kurtosis Coeff. MEP Amplitude
10
9
8
7
6
5
4
3
2
1
0
%RMT 100 to 120
e
MNCD MEP Amplitude
1.2
1
0.8
0.6
0.4
0.2
0
%RMT 100 to 120

In an attempt to provide a more detailed perspective we pooled the data from each subject recording block to allow exploration of variability through regression models. In our first model (figure 4.6.a-c), the relationship between mean amplitude and variability statistics is particularly strong. Linear regression by MEP amplitude mean is highly significant ($p<0.001$) for each of the Gini coefficient ($R^2=0.41$), the MNCD($R^2=0.32$) and the coefficient of variation ($R^2=0.29$). A better fit for the relationship of these three variability markers to the mean MEP amplitude is held by an exponential decay regression with a corrected R^2 of 0.497 for the Gini coefficient, 0.397 for the coefficient of variation, and 0.39 for the MNCD (in each case the relevant model constants were significant with $p<0.05$). These models are weakened by paucity of high amplitude data points.

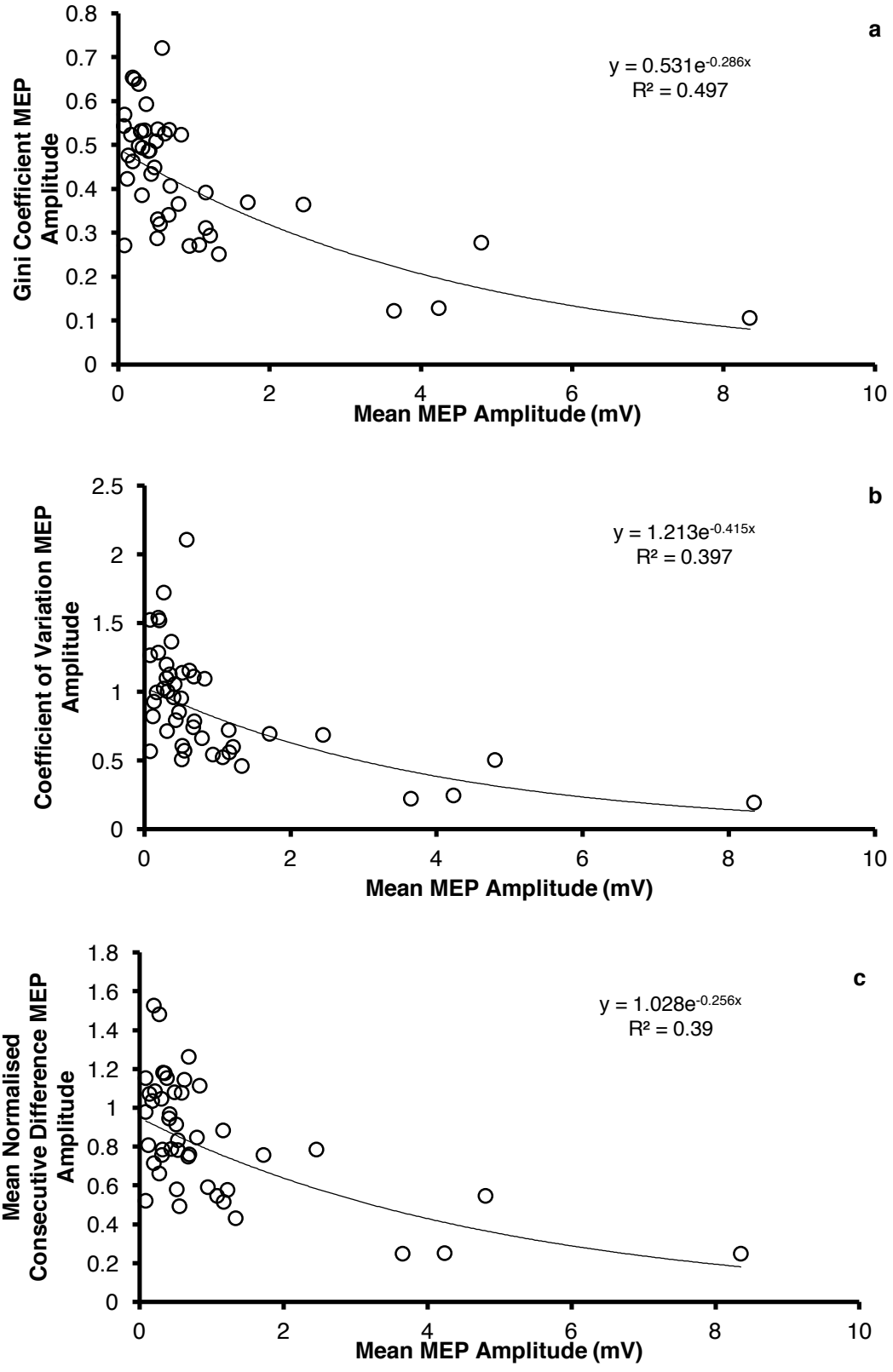


Figure 4.6.a-c Exponential decay models against the mean MEP amplitude for the Gini coefficient (a), coefficient of variation (b) and mean normalised consecutive difference (c).

4.3.3.b Behaviours near threshold

Theoretically the coefficient of variation is sensitive to both outliers and near zero mean amplitudes. Within our dataset, the increased sensitivity of the coefficient of variation to small changes in variability at low MEP amplitudes is perhaps suggested by the high decay constant within the exponential model in figure 4.6.b. Further evidence for this is suggested by logarithmic models regressing the gini coefficient and the mean normalised consecutive difference to the coefficient of variation (figure 4.7.a-b). From these graphs it can be seen that at high levels of variability (typically low amplitude means as per figure 4.6.b) there is more change in the coefficient of variation than either of the other two markers.

4.3.3.c The continuously varying distribution

We examined the coefficient of variation's susceptibility to outliers by plotting the skewness coefficient (as an index of outliers) and with each of the coefficient of variation, the Gini coefficient and the MNCD. The coefficient of variation is most strongly affected by skewness and has the strongest relationship (figure 4.8.a) with the skewness coefficient of the sample (R^2 of 0.75 vs 0.58 for the Gini coefficient and 0.39 for MNCD; all significant with $p<0.001$).

Given the threshold nature of the MEP, the next question to be confirmed is if amplitude has a relationship with skew. A moderate relationship is supported (see figure 4.8.b), R^2 =0.307, with low mean amplitudes tending to be associated with a high skewness coefficient. Additionally, the progressive alteration of skew throughout the amplitude range is suggestive of a continuously varying non-normal

distribution that maybe difficult to reliably transform into a normal distribution via a single manipulation.

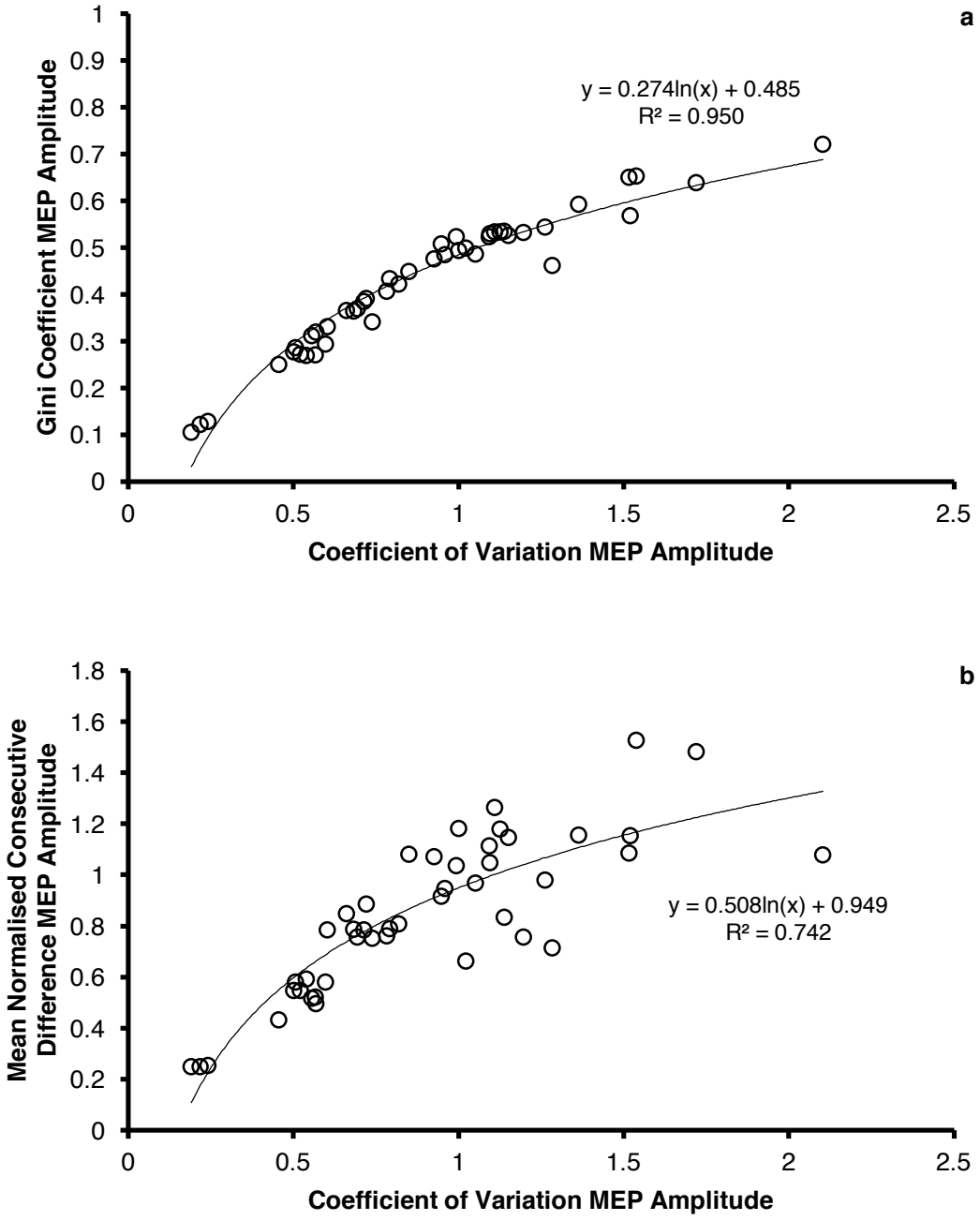


Figure 4.7.a-b Logarithmic regression models comparing the coefficient of variation to the Gini coefficient (A) and the MNCD (B). All model constants significant with $p<0.05$.

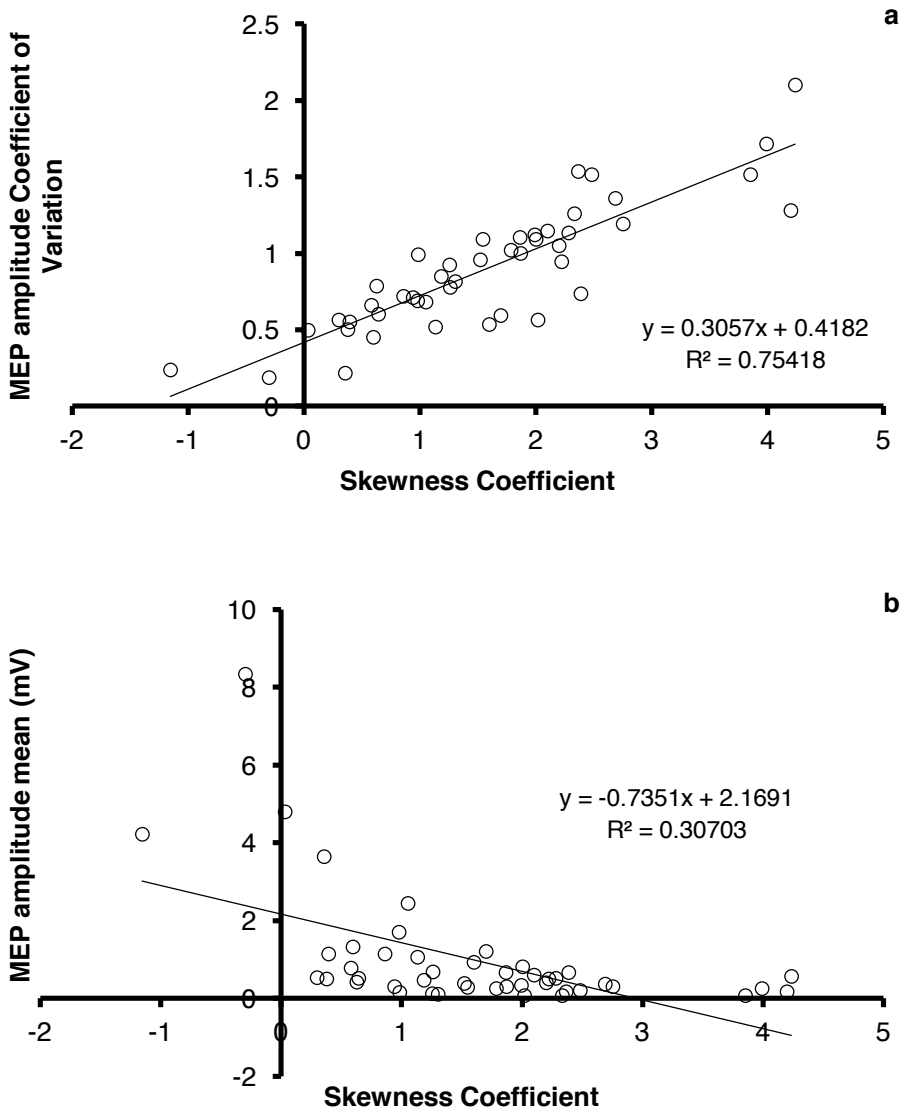


Figure 4.8.a-b The relationship of MEP amplitude skew with the MEP amplitude coefficient of variation (a) and the MEP amplitude mean (b). Both regression models significant with $p<0.001$.

4.3.4 The influence of a visual LED light on MEP amplitude variability

Here we wished to undertake a preliminary screen as to whether a visual LED light alone could influence MEP variability to a practically significant degree. Comparing blocks of twenty TMS pulses recorded before (-500ms; baseline) and after (+100ms) the visual LED turning on, no significant difference was found with respect to either the mean FDI amplitude ($Z=-0.840, p=0.401$), or the mean FDI Gini coefficient ($Z=-0.700$, $p=0.484$).

4.4. Discussion

This series of control experiments were undertaken as necessary background work to facilitate the study of TMS MEP amplitude variability during movement preparation.

4.4.1 The cortical hotspot, amplitude and variability

Previous work has demonstrated that proximity to the cortical hotspot had reduced MEP amplitude variability (Brasil-Neto et al, 1992). However, those experiments had used a fixed 100% of stimulator intensity. We wished to assessed whether a significant effect on amplitude variability for proximity to the cortical hotspot was still present when lower stimulation intensities were used (120% RMT). Our results demonstrate that for a fixed stimulator intensity, a significant effect of proximity can be seen with just 1.5cm displacement.

Worked by van der Kamp et al (1996) noted the effect of stimulator intensity on MEP amplitude variability – with rising intensity decreasing variability together a concomitant rise in mean amplitude. Given our initial findings from the first experiment we wished to assess whether a practically significant difference in variability was still apparent when stimulation intensity had been adjusted off the hotspot so as to match mean amplitudes. Our findings suggest that the influence of proximity on MEP amplitude variability can be mitigated through the adjustment of stimulus intensity. Whilst FDI amplitude means are similar across both TMS sites, no significant difference with respect to MEP amplitude variability was found. Greater stimulation intensities more consistently recruit contributors to the MEP

morphology, with coil displacement able to be compensated for by adjustments to stimulation intensity. However, it should be noted that substantial, seemingly independent fluctuations in variation are still to be found in individual cases.

Overall the results clearly support the importance of TMS stimulus intensity when evaluating resting MEP amplitude variability across conditions. In contrast the precise localization of a cortical hotspot may be less important in assessing MEP variability, if amplitudes are matched via stimulation intensity.

4.4.2 The initial transient state does not influence variability

Our second experiments have demonstrated that the initial transient hyper-excitable state demonstrated by Schmidt et al (2009) is present during both sequential single pulses and when randomly interspersed with paired pulses. Our data was somewhat weaker for sequential single pulse MEPs in terms of regression strength though the slope constant and intercept were of similar values and each statistically significant. This difference may in part be due to the smaller sample numbers in our study (7 vs 20) – however looking at individual response traces from the work of Schmidt et al (2009) reveals considerable heterogeneity despite the strong group effect.

The cause of the initial hyper-excitable state was not examined in this study, though it is clear the phenomenon is readily demonstrable. Schmidt et al (2009) suggested it was unlikely to be due to the subject's TMS naïve state as subjects in their study, though TMS naïve, had spent some time already within the session becoming familiar with the TMS experience. Within the current study, all subjects had previously

experienced motor cortex TMS suggesting this affect can be generalised to all subjects irrespective of previous exposure. Schmidt et al (2009) had used neuro-navigation for a significant proportion of cases, as have we here, and as such coil position changes are unlikely to be a cause of this transient state. Perhaps just as importantly despite the use of neuro-navigation significant fluctuations in variability are still ongoing, consistent with past work suggesting M1 TMS response variability could not be significantly abated through the use of a neuro-navigation alone (Gugino et al, 2001). Given the above, our results assessing MEP variability and hotspot proximity, and significant discomfort experienced by subjects when using the neuro-navigation system, the ongoing use of neuro-navigation equipment was not felt necessary for the purposes of this thesis.

From our perspective, the most significant finding was that variations in mean amplitude seen across a session when using a fixed stimulator intensity at rest do not appear to have a significant influence on MEP amplitude variability. Neither the initial hyper-excitable state nor moment to moment fluctuations in mean excitability, appeared to influence MEP variability. Variability however can be seen to fluctuate significantly across the session and, as such, for our work studying movement preparation comparisons across conditions should ideally be randomized across these fluctuations.

4.4.3 MEP amplitude variability from Input/Output curves

Here our results demonstrate that whilst MEP amplitude mean has a clear graded rise with stimulus intensity blocks, variability markers were generally less sensitive to

fine changes of intensity. This may be because other influences beyond intensity and mean amplitude alone contribute to resting MEP amplitude variability. Both intensity block comparisons and our regression analysis show that MEP amplitude variability is substantially higher at lower intensities, before a rapid decline, with regression hinting at a later plateau. This overall picture is consistent with previous work by Van der Kamp et al (1996).

Our earlier review of the literature had demonstrated multiple contributing generators for the TMS MEP – the progressive decline in variability with increasing intensity is likely to represent a phenomenon of progressive saturation across each of these contributing components. At low intensities variability will be compounded by excitability state fluctuations across each one of these contributors. The progressive increase of stimulator intensity leads to progressive saturation recruitment for each of these contributors, the exponential relationship of MEP variability with mean amplitude providing supportive evidence for the non-sequential nature of this recruitment process.

In choosing a measure of MEP amplitude variability the choice depends very much on the purpose at hand. Our pooled regression data confirms the theoretical susceptibility of the coefficient of variation to outliers, in contrast to the Gini coefficient and MNCD that appeared more robust. Within our data pool these outliers, reflected by shifts in the skewness coefficient, tended to occur at low MEP amplitude means closer to threshold. Early on the assessment Kurtosis, while scale insensitive, appeared to be relatively imprecise. The MNCD, thought robust to

outliers, compares sequential trials, which would not be appropriate for experiments with randomised conditions. The Gini coefficient would appear a practical and robust (consistent with its non-parametric nature) alternative to the coefficient of variation when dealing with MEP amplitude variability recorded under randomised conditions.

4.4.4 The influence of a visual LED light on MEP amplitude variability

Previous work in animals (Churchland et al, 2010) reported a decline in the variability of neural firing rates (in both visual cortex and downstream cortical sites) following presentation of a sensory stimulus across a range of behavioural states, from awake and performing a task, to being anaesthetised. In the present experiment an influence on amplitude variability (or mean excitability) was not apparent when comparing TMS MEPs recorded 500ms and 100ms after an LED light signal.

For our planned assessment of MEP variability within movement preparation we had planned to utilize a visual go signal within a simple reaction time paradigm. Though we cannot rule out the influence of visual inputs to MEP amplitude variability under different conditions, for practical purposes within a visually cued simple reaction time paradigm, the direct influence of the visual signal alone would appear not to be significant. A direct influence of the visual signal on MEP variability would clearly have confounded our results.

Chapter 5 TMS MEP variability during the preparation for simple human finger movements

5.1 Introduction

In a voluntary reaction time task, there is good evidence that while potential movements are prepared in advance (Touge et al, 1998), they continue to evolve after delivery of the imperative signal until release of the descending command to move (Day et al, 1989b; Schluter et al, 1998; Schluter et al, 1999). A substantial proportion of preparation is focused on controlling the precise state of corticospinal output (Churchland et al, 2006; Churchland et al, 2007; Afshar et al, 2011). On the basis that noise in the output of the corticospinal system would compromise movement performance (Mitchell et al, 2007), it may be reasonable to expect that a substantial goal of movement preparation entails control of output variability. The exquisite sensitivity of TMS MEPs to fluctuations in corticospinal tract excitability hints at the potential use of TMS MEP variability as a physiological probe in the study of movement preparation.

Studies in non-human primates have already demonstrated a significant reduction in individual cortical neuronal firing rate variability (Churchland et al, 2006; Afshar et al, 2011) across the movement preparation period. There is further suggestion (Shenoy et al, 2011) that the variability of neuronal firing rates in primates also decline with respect to one another during movement preparation, in a controlled convergence of population activity to a desired end state, however such invasive recordings are clearly not practicable methods for human observations. Population level output changes are known to have dynamic influences on movement control (Georgopoulos, 1988) and are readily observable through non-invasive methods in

humans using techniques such as TMS.

TMS MEPs display exquisite variability (Amassian et al, 1989), with their variation from trial to trial due to the fluctuations of the mean population excitability in cortical and subcortical components of the output pathway (Burke et al, 1995, Ellaway et al, 1998, Funase et al, 1999). It is well known that MEP amplitude variability will reduce during a sustained voluntary contraction (Kiers et al, 1993), though the interpretation of this may be complicated by the reciprocal relationship of mean amplitude with amplitude variability described at rest (Van der Kamp et al, 1996; Brasil-Neto et al, 1992). However, mean excitability changes for task relevant muscles are typically only seen late in the movement preparation process where they rise in a very dramatic fashion (MacKinnon and Rothwell, 2000). With the use of TMS MEP amplitude variability as a surrogate of corticospinal tract output variability we might to expect to see an early modulation of MEP variability during the movement preparation process, prior to a net rise in mean excitability.

On this basis we made several discrete hypotheses. Firstly, that TMS MEP amplitude variability would decline for task relevant muscles during the process of movement preparation within a simple reaction time paradigm. Secondly that the timing of a reduction in MEP variability would precede a mean rise in excitability. And, finally, that the rate of MEP variability decline would bear a direct relationship to speed of task performance.

5.2 Methods

5.2.1 Unilateral index finger abduction in a simple RT paradigm

With this experiment we wished to assess whether a simple movement was able to generate a change in MEP variability in a task relevant muscle. The simple RT paradigm was chosen to ensure the speed of subjects' preparation remained relatively constant whilst also providing a performance discriminator in the subsequent assessment of variability changes.

5.2.1.a Subject profile

A total of 11 subjects participated in this experiment, with a mean age of 36 years. Amongst the participants there were six female subjects, and two of the 11 were left handed. All subjects were free of neurological disease and impairment.

5.2.1.b Experimental setup

We utilized an unwarned simple RT paradigm with a visual imperative signal. The response task was brisk index finger abduction (FDI acting as agonist) in the dominant hand. The subject's dominant hand was placed resting on a table, palmar surface down such that index finger abduction would occur in the plane parallel to the table surface. FDI was chosen as the task relevant muscle and ADM recorded from as an uninvolved muscle – EMG electrodes were applied to both muscles in a belly-tendon montage with ground placed over the ulnar styloid. Capture of raw EMG signal was undertaken as described in chapter three on

general methodology. The 'Go' signal was a green visual LED (1cm^2) that came on for 200ms, and was centered within the subject's field of vision. Subjects were tasked with performing the brisk index finger abduction as swiftly as possible. TMS recording blocks were commenced after ensuring the subjects were able to correctly perform the task and had sufficient practice to stabilize performance.

TMS pulses (delivered as described in the general methods chapter) were utilized to identify the hotspot for FDI which was then marked off for retargeting on a tightly secured cap. Stimulator output was then titrated to achieve ~ 1mV resting MEP in FDI, whilst also ensuring a reliable MEP (consistently above threshold on all trials) was also able to be elicited from ADM. TMS was delivered relative to the Go signal with timings of -500ms, -100ms, +50ms and +100ms. Two widely spaced pre-Go timings were utilized in this first experiment in a bid to assess for TMS pulse induced "false starts". The post-Go timings were chosen to capture the earlier phases of movement preparation (Chen et al, 1998; MacKinnon and Rothwell, 2000, Nikolova et al, 2006) and to minimize the effect of mean excitability changes on MEP variability. Each recording block consisted of 32 trials with between four to six seconds between trials (the exact time randomized to prevent anticipation), the sequence of pulses semi-randomized to eight trials for each TMS time point. Three TMS recording blocks were undertaken with a five minute rest between blocks. In addition, a recording block without TMS was recorded to measure RTs without perturbation by the TMS pulse.

5.2.1.c Analysis

Offline analysis was undertaken for all recorded frames. Each frame was individually reviewed and discarded if contaminated by resting EMG or other artefact. Frames were discarded if subjects took more than 400ms to perform the task or if premature responses were present (< 100ms).

For each subject we determined the MEP amplitude mean and Gini coefficient at each TMS time point (for both FDI and ADM). With respect to corticospinal excitability, knowing that the distribution of MEP amplitudes has a tendency to be skewed to the right, we have chosen to use the mean of peak-to-peak amplitudes in each subject to increase the influence of outliers generated by this skewed distribution and thereby make our measure more sensitive to rises in excitability. Given the non-normal distribution of MEP data seen in the preceding chapter we chose to use the Gini coefficient as a non-parametric measure of central dispersion that while sensitive was less responsive to extreme outliers than the coefficient of variation.

For subject RTs, after processing them in accordance with the general methodology (chapter three), we pooled individual subject trials and determined the median RT for each subject. We then utilized a Friedman's analysis to examine for within subject change across one factor (TMS time point) in each muscle set for the MEP amplitude mean and Gini coefficient, using the Wilcoxon Sign Ranks Test for post-hoc analysis (p-value set at 0.05). To further assess whether

movement preparation changes affected performance we utilised Kendall's Tau in correlation analyses examining the change from the pre-Go baseline in MEP amplitude, for both mean and Gini coefficient, during movement preparation and contrasting this with subject median RT performance along the X-axis.

5.2.2 Bilateral button press task with a simple RT paradigm

In this experiment we wished to assess whether the decline in MEP variability was present when TMS timings were referenced to subject performance on a trial-by-trial basis, thereby gaining a perspective on the process of within subject movement preparation.

5.2.2.a Subject profile

Nine healthy subjects participated in experiment two. Here the mean age was 34 years, and there were four females amongst the participants. All were right handed.

5.2.2.b Experimental procedure

Here we also used a simple RT paradigm, where the response task was a synchronous bilateral button press task requiring flexion at the metacarpophalangeal joint of the index finger in each hand (FDI acting as co-contractor). The button press action was to be brief and brisk. Bilaterally FDI and ADM were again prepared for recording EMG signal, acting as agonist and control

respectively. After first establishing subjects' ability to perform a synchronous bilateral button press task (see statistical analysis and results), this experimental setup allowed us to estimate the RT (and thereby determine the timing of the MEP with respect to movement preparation) with reasonable certainty on a trial-by-trial basis.

Following familiarization and training for the task, subjects performed the button press task in a block of forty trials without TMS, to establish the synchrony of subject movements. TMS setup was then initiated as for experiment one – the hotspot for FDI was then identified, with titration of stimulator output to achieve a reliable MEP in FDI (peak to peak amplitude ~ 1mV) and ADM.

In blocks of TMS the pulse timings and LED parameters remained as for experiment one. Following at least two practice blocks subjects underwent eight recording blocks, each block stimulating one hemisphere, the order semi-randomized to give four blocks either side. Each block consisted of 40 trials, semi-randomized to give 10 trials per TMS time point and with between four to six seconds separating trials, randomized to prevent anticipation. Each block was followed by a five minute rest, and at the switch between sides a ten minute break was instituted to minimize task fatigue.

5.2.2.c Analysis.

To confirm subjects' ability to perform a bilateral button press of reasonable synchrony we pooled all subject RT responses from the initial non-TMS block. We then analyzed the responses between sides using a paired t-test and subsequently undertook regression modelling to analyze the relationship between right and left responses.

Following this assessment, we were able to utilize our experimental setup to estimate the proportion of movement preparation the subject had undertaken for that trial's MEP recording by dividing the TMS stimulation time point by that same trial's RT (as measured in the arm ipsilateral to TMS) and then converting this value into a percentage. We then pooled all the post-go time results for a subject, ranking them in order of percentage RT (%RT) and grouped them in lots of 15 trials. For each group we determined the results for FDI and ADM MEP amplitude mean and Gini coefficient, as well as the mean %RT. We also determined the baseline (-500ms and -100ms) MEP amplitude mean and Gini coefficient for both FDI and ADM, using the average result between these two time points as the 'baseline average'. In turn we then used this baseline to transform the post-go recording blocks into a percentage of baseline for each subject. Finally, we binned these subject results into %RT bins of 5% from 15-20% to 55-60% across all subjects. For each of these bins we determined the across subject grand average for %RT, and MEP amplitude mean and Gini coefficient for both FDI and ADM. These grand averages were then regressed using SPSS.

5.3 Results

5.3.1 Unilateral index finger abduction in a simple reaction time paradigm

The primary purpose of this first experiment was to assess variability in the amplitude of MEPs evoked in the target (agonist) muscle as well as MEPs in a non-involved nearby muscle during a simple reaction time task. The target muscle was the FDI, the non-involved muscle was ADM.

5.3.1.a Changes in FDI (agonist)

Figure 5.1 illustrates how the variability of MEPs declined in the reaction period of two volunteers who had different median reaction times (205ms for subject A, 143ms for subject B). MEPs were evoked in the FDI muscle at different times before (-500ms and -100ms) and after (+50, +100ms) the "go" signal. In participant B, the mean amplitude of the MEPs as well as the amplitude variability was smaller at +50ms than at -500 and -100ms. Reduced amplitude variability persisted at +100ms. In participant A, who had a longer RT, the mean MEP amplitude was approximately constant over all time points but amplitude variability only began to decline at +100ms. Thus the time at which the reduction in MEP variability occurred appeared to be later in the participant with the longer reaction time.

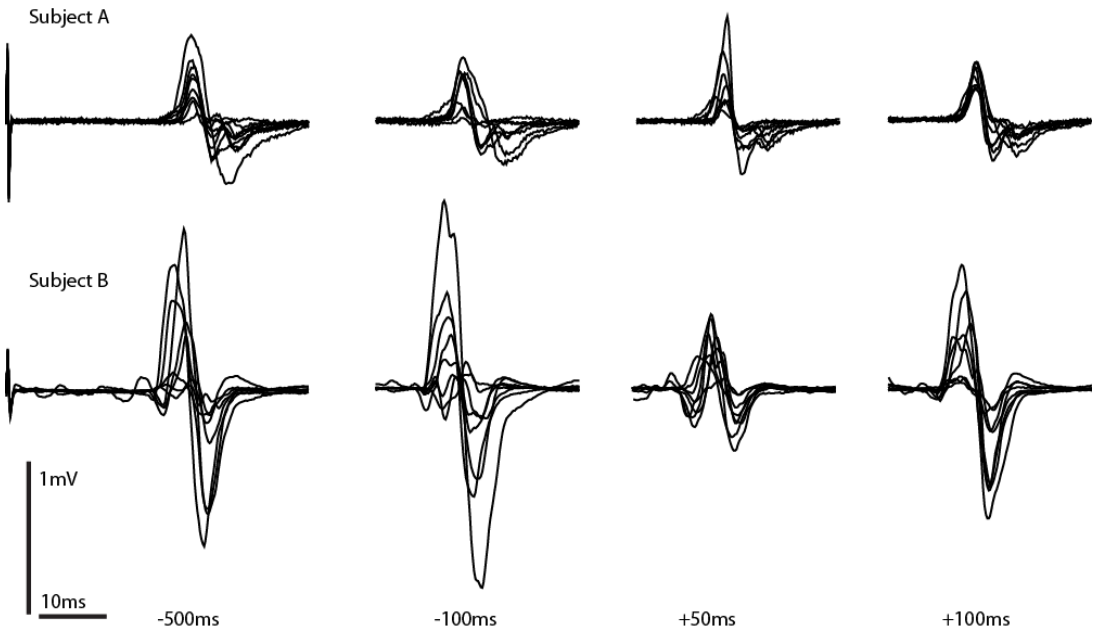


Figure 5.1 TMS MEP changes at baseline and during movement preparation for two subjects, one with a slow reaction time where amplitude variability changes by +100ms (A; 205ms), and another with a faster median reaction time (B; 143ms) where variability has already reduced by +50ms. One complete block of recordings is displayed for each subject with eight traces overlaid for each of the -500ms, -100ms, +50ms and +100ms TMS time points. Statistical calculations for the traces are as follows: Subject A MEP amplitude at -500ms mean 0.480mV Gini 0.292, -100ms mean 0.415mV Gini 0.281, +50ms mean 0.428mV Gini 0.342, +100ms 0.495mV and Gini 0.128; Subject B MEP amplitude at -500ms mean 0.901mV Gini 0.411, -100ms mean 0.885mV Gini 0.426, +50ms mean 0.544mV Gini 0.199, +100ms mean 0.807mV and Gini 0.357.

Premovement changes for FDI MEP data are shown in figures 5.2a-b for all participants. There was no significant change in mean MEP amplitude over time (Friedman's Analysis of Variance by Ranks, χ^2(df=3, N=11)=5.5, p=0.14). In particular, there was no pairwise difference in MEP amplitude at -500 ms vs. +100 ms (Wilcoxon Z= -1.511 and p=0.131). In contrast, there was a significant change in MEP variability over the same time points, due to a decline in Gini coefficient at +100 ms. Statistical analysis showed a significant effect of time (Friedman: χ^2 (df=3, N=11)=15.545, p =0.001, with Kendall's coefficient of concordance = .471 indicating a moderately strong consistency across subjects for the four time points). Follow-up pairwise comparisons demonstrated that subjects at the +100ms time point had a lower Gini coefficient than at -500ms (Z=2.667, p=0.008, PSdep=0.818) and at +50ms (Z=2.934, p=0.003, PSdep=1.0), whilst there was no significant difference between -500ms to -100ms (Z=.445, p=0.657) or to +50ms (Z=.711, p=0.477) as expected. Similar results were found when using the -100ms time point as baseline.

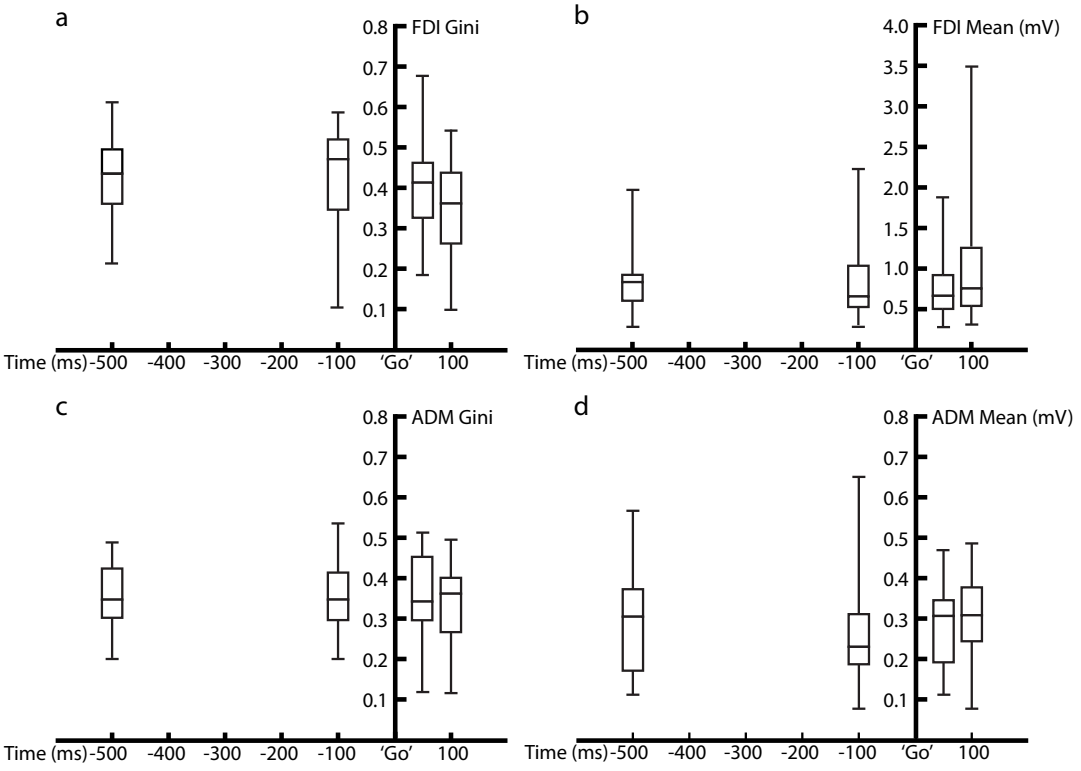


Figure 5.2.a-d MEP amplitude changes in the premovement period relative to the 'Go' signal for the FDI amplitude Gini (a), the FDI amplitude mean (b), for the ADM Gini (c) and the ADM mean (d). Data displayed for each TMS point (spaced appropriately on the X-axis) with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima.

5.3.1.b Changes in ADM (non-involved)

Figure 5.2.c-d shows the mean data from ADM (non-involved muscle) collected simultaneously in the same individuals. There was no effect of time on either MEP amplitude mean (χ^2[df=3, N=11]=2.345, p=0.504) or amplitude Gini ((χ^2[df=3, N=11]=1.582, p=0.664). As further confirmation the pairwise comparison between -500ms and +100ms time points for the ADM amplitude Gini was also not significant (Z=-1.156 and p=0.248).

Finally, we compare the task-related changes in amplitude variability between the FDI (agonist) and ADM (non-involved) by determining the change in Gini coefficient from -500ms to +100ms and expressing the effect as a percentage of baseline variability. There was a significantly larger reduction in variability at +100ms for FDI than ADM (FDI median 15.5% vs ADM median 3.70%, Wilcoxon SRT Z=1.956, p=0.05, PSDep=0.727). We conclude the variability in MEP amplitude of the agonist muscle is smaller 100ms after the "go" signal whereas it is unchanged in a nearby non-involved muscle.

5.3.1.c Changes in FDI MEP amplitude across subjects' reaction times

Examination of the data from each individual (such as that in figure 5.1) suggested that amplitude variability in people with longer reaction times declined later after the "go" signal than it did in people with shorter reaction times. To explore this inter-individual effect we assessed how the reduction in amplitude variability correlated with the reaction time by calculating the difference between the FDI Gini coefficient

at the -500ms time point and the +50ms and +100ms time points, and correlating this with their median task reaction time. There was a significant relationship between the median reaction time and decline in Gini coefficient at both +50ms (Kendall's tau: r=0.6, p=0.01) and +100ms (r = 0.56, p = 0.016) (Figure 5.3.a), confirming that people with shorter median reaction times appear to reduce MEP variability earlier following the "go" signal than those with a longer reaction time.

We also performed the same analysis with mean MEP amplitude by calculating the difference between the mean FDI MEP amplitude at baseline (-500ms relative to 'go') and the mean at +50ms or +100ms. Despite the fact that there was no overall change in MEP amplitude in the mean data of Figure 5.3.b, there was a significant relationship between an individual's median reaction time and the mean MEP amplitude at +50ms (Kendall's tau: r=-0.6 and p = 0.01(Figure 5.3.b) but not for the +100ms time point (r=-0.018 and p=0.938). Specifically the result suggests that subjects who reacted faster had a smaller MEP at the +50ms time point than at baseline (ie pre-"Go").

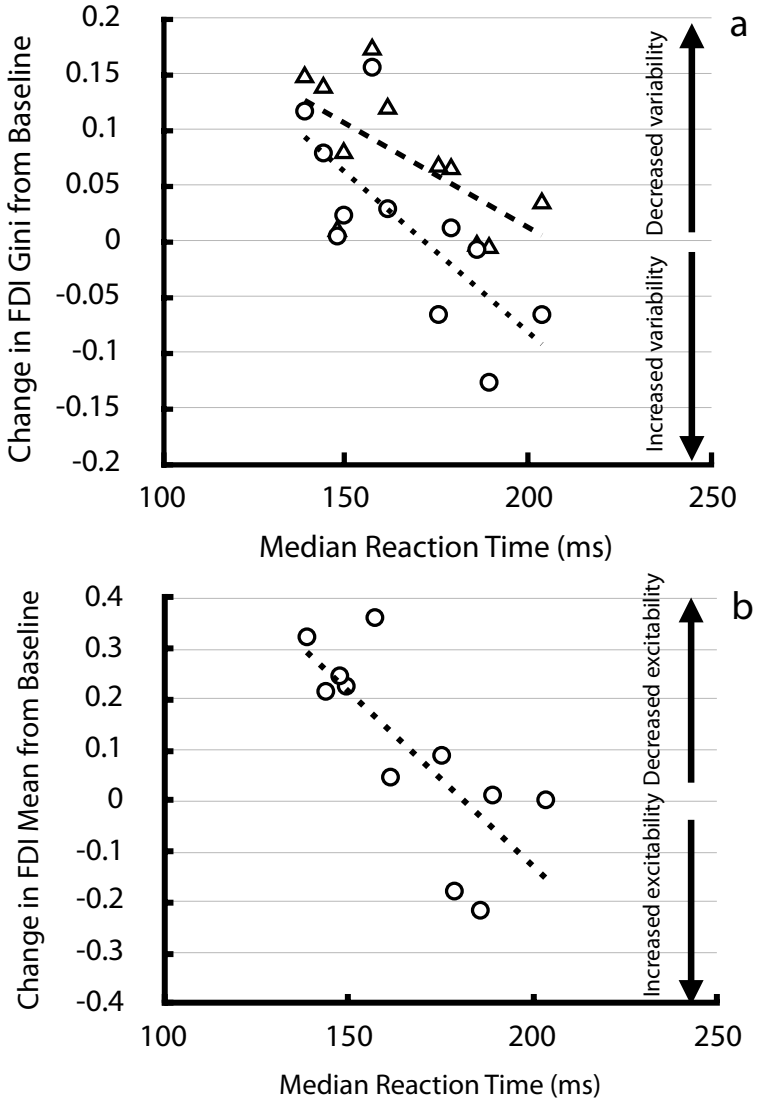


Figure 5.3.a-b Premovement change in FDI MEP amplitude Gini (a) and mean (b) as a function of a subject's median reaction time. Change determined by subtracting either the +50ms or +100ms time value from the -500ms baseline value. In both graphs the open circle and dotted line represent the change to the +50ms time point, the open triangle and dashed line represent the change to the +100ms time point. Trend lines are provided only as a visual aid.

5.3.2 Within subject variation in trial-to-trial RT: influence on MEP amplitude and variability

The results from this chapter's first experiment suggested that the time course of changes in amplitude variability as well as MEP mean amplitude depend on reaction time. However, the result could also be due to differences between individuals rather than a direct link between reaction time and variability; that is, there could be a third factor that makes individuals who have fast reaction times also reduce MEP variability quickly in response to a "go" signal.

To test the hypothesis more directly, this experiment examined whether trial-to-trial variation in reaction times within an individual was related to MEP variability in that trial. This had not been possible with the initial experiment because measurement of the voluntary EMG reaction time in each trial could have been contaminated (and even prolonged (see Day et al, 1989b)) by the MEP. This second experiment overcame this limitation by using a bimanual synchronous reaction time task. Participants had to react to the "go" signal by simultaneously depressing the index finger of both hands. Analysis of control data from trials in which no TMS was employed, showed that reaction times were highly correlated (linear regression: right hand RT= 0.994 * (left hand reaction time) + 1.22; R^2=0.967). We could therefore undertake a response-locked analysis of the trial-by-trial evolution of changes in MEPs in the stimulated (i.e. right) hand while measuring the reaction time in the unstimulated (i.e. left) hand.

Grand average results from all participants for FDI MEP amplitude mean and amplitude variability are shown in figure 5.4.a. The timing of the MEPs has been normalized across trials and across participants by expressing it as a percentage of the total (=100%) reaction time on each trial. Methods for deriving grand averages are described earlier. With this individualized form of analysis it is now clear that FDI MEP amplitude mean shows a small initial decline around 30%RT, followed by a gradual increase and later rapid rise; FDI amplitude variability (Gini) declines linearly throughout the reaction time.

The time course of changes in MEP amplitude mean were better fitted by a second order polynomial/quadratic (R^2=0.953, $F_{2,6}$=82.6, p<0.001) with all constants significant (p<0.001)) than a simple linear regression (R^2=0.648, $F_{1,7}$=12.889 (p=0.009)). Direct comparison of the polynomial and linear regression models suggested the improvement in R^2 of 0.317 (F change of 54.224) was highly significant, with p<0.001. Changes in MEP amplitude variability were best fitted by a linear regression (R^2=0.68; $F_{1,7}$=14.898 p=0.006; slope=-0.475).

This contrasts with the data from ADM (figure 5.4.b) in which there were no significant effects of time on either MEP mean amplitude (R^2=0.090; $F_{1,7}$=1.790, p=0.223) or amplitude variability (R^2=-0.023; $F_{1,7}$=0.821, p=0.395).

Our analysis here confirms the data from the initial experiment: amplitude variability of the MEP in FDI declines over the first half of the reaction time period. Decreases

in variability after this time point are more challenging to interpret since they occur when there are very large changes in MEP amplitude, changes that are known to affect amplitude variability measures, albeit in the resting state.

Furthermore, MEP amplitudes appear to decline by a small amount approximately one third of the way through the reaction time period. This corroborates a similar finding from the unilateral finger abduction task. Although this finding has not been noted directly in previous TMS studies of simple reaction times (Chen et al, 1998; Niklova et al, 2006; Kumru et al, 2008; Levin et al, 2011), a similar phenomenon has been described by Aoki et al (2002). If a phasic contraction is superimposed on a small steady tonic contraction, then a short period of silence in the ongoing EMG can sometimes be demonstrated just prior to the phasic EMG burst. They found that MEPs were reduced in amplitude if elicited during this premovement EMG silence compared to those at baseline and those trials without pre-movement EMG silence.

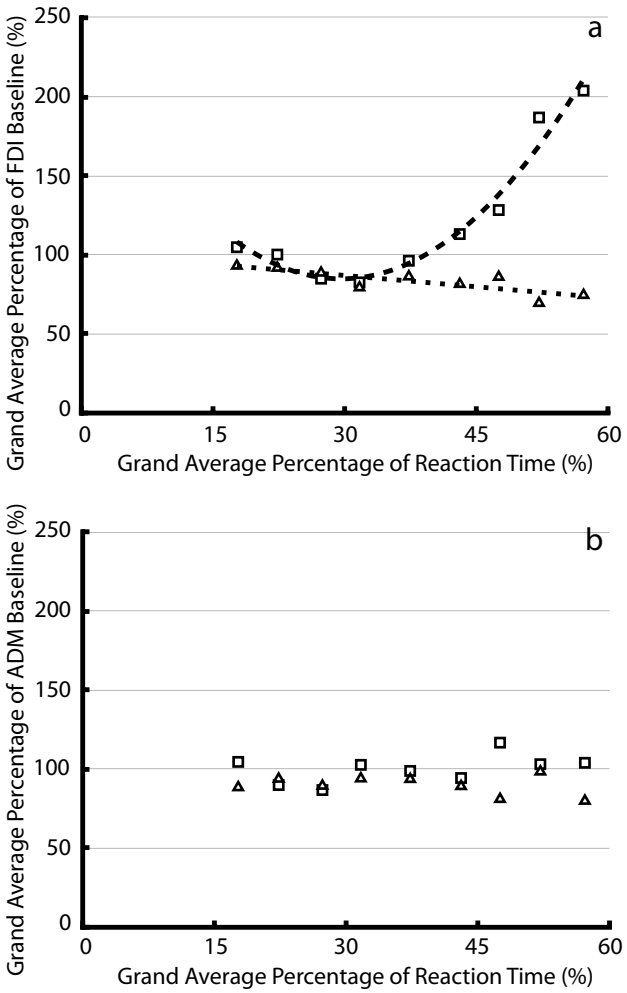


Figure 5.4.a-b Premovement change in MEP amplitude mean and Gini for the FDI (a) and ADM (b) muscles presented as a grand average across all subjects during a simple reaction time bilateral button press task. A strong linear trend is shown for the decline in FDI MEP amplitude Gini (R^2=0.680) and for amplitude mean (not shown) though the best fit for the latter is a 2nd order polynomial (R^2=0.953, as shown in (a)). No significant regression model was found for ADM data (b). Open triangles and dotted regression line represent data points for the amplitude Gini coefficient, open squares and dashed regression line represent the amplitude mean.

Finally, we wished to assess whether the TMS pulse itself introduced a perturbation to the non-TMS hand, that is the hand ipsilateral to the TMS stimulation. First we determined the median reaction time of the ipsilateral hand and compared results for the +50ms and +100ms using a Wilcoxon Signed Ranks Test – these showed no significant difference on the left ($Z=-0.652$, $p=0.515$) or the right ($Z=-0.652$, $p=0.515$). Next we compared left and right hand ipsilateral RTs for each subject at the +50ms and +100ms time points and again found no significant difference using the Wilcoxon Signed Ranks Test with $Z=-0.889$ ($p=0.374$) and $Z=-1.244$ ($p=0.214$) respectively.

5.4 Discussion

The present experiments tested whether variation in corticospinal excitability is controlled prior to movement onset. We argued that controlling variation in corticospinal excitability prior to movement execution allows the motor system to respond more precisely and reliably to a voluntary motor command. The data confirm that variation in the amplitude of MEPs in task relevant muscles declines during the reaction period. The effect is not seen in nearby muscles that are uninvolved in the task. The reduction in variability is seen without a significant rise in mean amplitudes, as might be expected in the resting state. Within individuals, the trial-by-trial variation in the decline in variability is tightly linked to trial-by-trial changes in reaction time, suggesting a close relationship between the timing of a voluntary movement and the variability of corticospinal excitability.

5.4.1 Variability and amplitude of MEPs

At rest, MEP variability declines as MEP amplitude increases (Brasil-Neto et al, 1992; Van der Kamp et al, 1996). MEP amplitude increases dramatically during a reaction time task, complicating the interpretation of changes in variability. One approach to tackling this problem is to use a control experiment to characterise the relationship between MEP variability and amplitude, and use this to factor out amplitude effects on variability in reaction time data (e.g. Klein-Flugge et al, 2013). However, this necessarily introduces some assumptions in the analysis that we wished to avoid here by assessing movement preparation during the earlier portion of the reaction time period, when excitability remains relatively stable (Chen et al, 1998; MacKinnon

and Rothwell, 2000).

5.4.2 Timing of decreases in variability

In the first experiment, the drop in variability at 50 or 100ms after the "go" signal was greater in individuals who had fast reaction times. Conversely, people in whom variability declined slowly, reacted slowly. This would be consistent with the idea that movement starts after MEP variability has declined by a certain amount. However, the result could also be due to trait differences between individuals: rather than a direct link between reaction time and variability, there could be a third factor that makes individuals who have fast reaction times also reduce MEP variability quickly in response to a "go" signal.

The second experiment was designed to eliminate the influence of potential "trait differences" between individuals by examining variation within each individual on a trial-by-trial basis. Using simultaneous bilateral movements we could estimate trial-by-trial reaction times while simultaneously obtaining estimates of MEP variability. These showed that across all individuals, variability declined linearly when reaction times in each trial were normalized to 100%. This strongly supports the idea that variability converges on a specific state prior to release of the motor command to move. If convergence occurs slowly, reaction times are long; if convergence is fast, reaction times are short. Although we cannot confirm a causal connection, it implies that MEP variability is intimately related to the preparation for movement execution. Importantly, the decline in variability also precedes the late net increase in excitability.

5.4.3 Link to previous work in animals and humans

Klein-Flugge et al (2013) suggested that reduced MEP variability in a reaction task is a direct reflection of changes in the variability in relative firing rates of individual cortical neurons as described by Churchland and colleagues (2010) in animal experiments. However, the MEP reflects the sum excitability of all rapidly conducting corticospinal neurons that project to the target muscle. Variations in MEP amplitude are therefore more likely to reflect variations in the mean excitability of the whole population rather than the variation in firing rates for individual neurons. Field potentials are one possible measure of population activity, which in humans can be studied using EEG. Many studies (eg. Romei et al, 2008; Sauseng et al, 2009) of the motor and visual cortex have shown that the response to a TMS pulse (the MEP or a visual phosphene respectively) varies with pre-stimulus EEG power. In addition, the frequency with which TMS evokes a phosphene or the amplitude of an MEP also depends on the phase of the alpha activity, changing by about 15% between opposite phases (McAllister, 2012). Thus it is possible that trial to trial variations in the amplitude or phase of EEG activity during the reaction period might parallel those of the MEP. If so, reduced MEP variability in the present experiments could be linked to the well-characterised alpha/beta EEG desynchronisation prior to movement onset (Leocani et al, 2001).

5.4.4 Reduction in MEP mean amplitude

A number of studies have examined corticospinal excitability during a simple voluntary reaction but none have noted a small initial reduction in excitability that is

maximal about 30% into the reaction period. Reduced excitability in the early reaction period have been observed during experiments involving choice reactions (e.g. Duque et al., 2014) but this has been linked to response selection rather than movement initiation. The effect observed here during simple reactions that involve no response selection may not have been observed in most previous reports because measurements were not normalized on a trial-by-trial basis to individual reaction times. Indeed, the raw data of experiment 1 show that when the time of TMS pulses is linked to onset of the "Go" stimulus rather than reaction onset MEPs show no early suppression.

A likely explanation is that smaller MEPs are a necessary consequence of less variable MEPs. As noted many times (Nielsen, 1996; Kiers et al, 1993) and our own work, low to medium stimulation intensity MEP amplitudes have a large positive skew, meaning that infrequent very large MEPs occur more commonly than very small MEPs. Indeed, the latter are constrained by the fact that they cannot have a negative amplitude. Thus, if variability declines, there will be proportionately fewer large amplitude MEPs, and the mean MEP amplitude will decline. The effect was seen transiently during the first part of the reaction period before it was overwhelmed by increased corticospinal activity caused by the impending motor command to move. The time difference between these effects suggests a surprising conclusion. If motor output was converging onto the optimal state to perform the intended movement, we might expect that excitability of the projection to the agonist muscle would increase at the same time as the variability in output excitability was reduced. The fact that the latter precedes the former implies that variability is first controlled before a final rise in

excitability and eventual movement.

The small reduction in MEP amplitude may also be related to the premovement EMG-silent-period, which can be seen prior to sudden forceful contractions superimposed on a steady background muscle contraction (Mortimer et al, 1987; Aoki et al, 2002). It has been suggested to focus movement preparation, although variability has not been measured (Aoki et al, 2002).

In summary, the experiments show that MEP amplitude variability declines in a task relevant muscle during the reaction period of a simple voluntary movement. The decline is independent of the known resting relationship between MEP variability and mean excitability. Importantly the decline in amplitude variability is intimately and predictably linked with trial-to-trial variations in reaction time, and occurs prior to the increase in excitability of the agonist muscle that signals movement onset. We hypothesise that reduced variation of corticospinal excitability, as reflected in the amplitude of MEPs, is part of a CNS strategy to optimise movement accuracy and reproducibility.

Chapter 6 Independent and coordinated finger movements

6.1 Introduction

The resting state relationship of MEP amplitude variability with mean excitability dominates the past literature as the primary influence known to control MEP variability. Our work in this thesis is starting to demonstrate that such past works need to be place in context, by demonstrating the powerful influence movement preparation can have on MEP variability. Work in the preceding chapter demonstrated a focal specific reduction of MEP amplitude variability in task relevant muscles during the premovement reaction time period, with greater reductions in MEP variability found in subjects with faster RT performance. When trial by trial changes in reaction time were accounted for, the reduction in MEP variability appeared to show a progressive linear decline across the RT period, across both initial declines in mean excitability, as well as later exponential rises in mean excitability.

MEP trial to trial variability across different muscles is known to be correlated both within a limb and between limbs (Ellaway et al, 1998), in keeping with common central influences affecting moment to moment changes in excitability, e.g. cortical oscillations in the resting state (Keil et al, 2014). Interestingly the resting state correlations of MEP amplitude fluctuations across muscles have been shown to be lost when a muscle is taken out of the resting state by deliberate activation (Pearce et al, 2005). One might then naturally ask if a change toward concordant variation would be seen in muscles that are deliberately tasked with co-activation within the same motor task, a question which could be assessed by examining premovement

changes in MEP amplitude variability within intrinsic hand muscles involved in a power grip task.

Movement initiation typically sees a rise in mean corticospinal excitability for muscles actively recruited for performance of a task. As has been mentioned earlier, literature describing MEP variability in the resting state has a traditional relationship with mean MEP amplitude (Van der Kamp et al, 1996), and whilst work in an earlier chapter (Chapter 4) suggests this relationship is not absolute, it does provide a conceptual challenge when studying MEP variability in movement control.

Motor surround inhibition tasks (Sohn and Hallett, 2004) have been described which demonstrate that when muscles ("within the surround") uninvolved in a movement are tasked with "deliberate inactivity", they demonstrate a reduction in mean activity (i.e. a reduced MEP mean amplitude) both during movement preparation (Beck et al, 2008) and at movement initiation (Kassavetis et al, 2014). Surround inhibition has been suggested as one mechanism by which independent dexterous finger movement can be achieved (Sohn and Hallett, 2004). To further highlight the importance of context in understanding the relationship of MEP variability with mean amplitude we will study changes in MEP variability using motor surround inhibition. Using a well established motor surround inhibition paradigm (Kassavetis et al, 2014) we hope to demonstrate that premovement MEP variability declines concomitantly with a decline in mean excitability. Furthermore through use of an inhibitory paradigm, and thereby independence from the inferred relationship with mean

excitability, we hope to demonstrate the continuity of MEP variability reduction in the premovement and movement initiation phases.

6.2 Methods

6.2.1 Power grip experiment

Here we wished to see if two intrinsic hand muscles would demonstrate a concomitant decline in variability. For this experiment this we utilized a power grip task with the grasp manipulandum adjusted to optimize action across the metacarpophalangeal joints and thereby maximize intrinsic hand muscle involvement, allowing us to utilize MEPs recorded from FDI and ADM as task relevant muscles.

6.2.1.a Subject profile

A total of ten healthy subjects participated in this experiment with a mean age of 32.9 years (range 20-51 years). Five subjects were male, five female and all were right handed.

6.2.1.b Study procedure

For this experiment subjects were asked to perform a brief, individually calibrated isometric power grip contraction in response to a visual LED signal within a simple reaction time paradigm. After first positioning the subject and optimizing the power grasp dynamometer (Biometrics Ltd., UK) for each subject hand, we next determined the maximal grip force each subject was able to produce over a series of six trials. Using the maximal force value from the dynamometer we determined the 10-20% proportion (of maximal force) subjects would be trained to target during the task.

Training was undertaken in a series of stages. Subjects were first taught to perform a brief focused contraction to the calibrated 10-20% (of maximal force) window, with peak force to be reached within 200ms from onset of EMG activity. Feedback following trials was given using the onscreen EMG and dynamometer sweeps and verbal instruction, whilst the experimenter also confirmed appropriate pre-contraction relaxation and subsequent activation for both FDI and ADM. Once the power grip contraction could be performed reliably by a subject, they were asked to performed the task within a simple reaction time paradigm using a visual "Go" (Green LED activated on for 100ms) signal placed centrally within the subject's field of vision. The instruction subjects were given for the task was to perform the power grip contraction as quickly and consistently as possible in response to the "Go" signal. Visual and verbal feedback was again provided for subjects between trials, and at least forty training trials were performed for each subject to ensure familiarity with the task.

When subjects were able to perform the simple reaction time task reliably TMS MEP recordings were commenced. Using a figure of eight coil we first localized the MEP hotspot for FDI and ADM for each subject using the functional method. Subsequent to this we adjusted the targeting position of the figure of eight coil and stimulator output intensity over the to achieve resting MEPs in FDI of approximately 0.5-1.0mV, whilst ensuring that a reliably MEP could also be achieved in ADM on every trial (resting amplitude typically ~0.5mV). Targeting position was marked on a firmly secured cap to facilitate retargeting across recording blocks and stimulator intensity recorded.

Recording blocks of trials were then commenced with single TMS pulses delivered each trial (spaced randomly between four to six second intervals) at one timing of either -300ms, +60ms, +90ms and +120ms with respect to the "Go" cue. Pre-testing suggested the median subject RT for the power grip task would be approximately 200ms. We wished to optimise capture of variability changes prior to a rise in mean excitability, and the choice of TMS time points was optimized to do so based on results from the previous chapter. Timings were randomized across trials, with 40 trials per block delivering ten trials per TMS timing. After an initial training trial to familiarize subjects to task performance with concurrent TMS, three blocks of trials were recorded in each subject, with a five minute rest between recording blocks.

6.2.1.c Study analysis

Analysis was undertaken off-line. Each recorded frame was individually inspected and frames with excess EMG pre-activation or task inappropriate responses were excluded from further analysis. For each MEP response in FDI and ADM we recorded the peak to peak amplitude. In each subject we then determined the FDI and ADM MEP amplitude mean and Gini coefficient for teach TMS time point. Non-parametric analyses using Friedman's test for repeated and the Wilcoxon Signed Ranks Test were undertaken using SPSS and the p-value was set at 0.05.

6.2.2 Surround inhibition experiment

Here we wished to assess whether MEP variability would decline in a muscle explicitly tasked to demonstrate "inactivity" throughout movement preparation. To achieve this we utilized a combination of explicit instruction and training for a surround inhibition paradigm similar to that previously tested by Beck et al (2008) in the premovement phase and Sohn and Hallet (2004) at the moment of movement initiation.

6.2.2.a Subject profile

We recruited 15 healthy volunteer subjects for participation in this experiment. The mean subject age was 32.1 (range 20-72) years. Of these subjects nine were female and all were right handed.

6.2.2.b Study procedure

We trained each subject to perform a button press in a specific force range with the index finger of their dominant hand. The task required FDI activity for the button press to utilize no more than 5% of FDI maximum voluntary contraction in a brisk action (onset to maximal force to take no longer than 150ms) whilst ADM EMG activation was to be minimized. Visual feedback of the EMG and button force traces was provided between training trials, together with verbal feedback from the experimenters. Once the task was mastered further training blocks were undertaken within the simple RT paradigm (a visual LED signal, as before, providing the 'Go' signal), with subjects tasked to perform the contraction as

quickly and consistently as possible.

The TMS hotspot for ADM was determined using the functional method, with location then marked on a firmly secured skull cap for retargeting. The stimulator output was titrated to achieve a resting ADM MEP amplitude of at least 1mV. Using these parameters, TMS recordings were made within a simple RT paradigm under two different scenarios, one where TMS pulses were delivered during the premovement phase and another to assess MEP variability in the surround muscle at the moment of movement initiation. The order of TMS recording paradigms was randomized in each subject, with the alternate recording session performed sequentially on the same day.

For the premovement study TMS pulses were delivered at -300ms, +60ms, +90ms and +120ms with respect to the 'Go' signal and were interspersed with "catch trials" undertaken without TMS, as an aid to minimize premature responses. Recordings were undertaken in blocks of forty trials with TMS timing semi-randomised to give eight trials for each time point per block and eight TMS free trials. Trial were separated by an interval randomized at between four to six seconds to prevent anticipation. Following an initial practice block with TMS for familiarization, three recording blocks were undertaken.

In the peri-movement recordings, the same visual "Go" signal was used within a simple reaction time paradigm. TMS pulses were delivered with either movement

onset (active state) or approximately five seconds following movement initiation (rest state). For the active state TMS was triggered using FDI EMG activity, which was actively monitored by Signal software and the EMG trigger adjusted in each individual to produce a TMS pulse typically within 20ms of the onset FDI. Approximately ten seconds was allowed between trials. Following at least twenty TMS trials to allow familiarization, a complete recording block of 40 trials was recorded, with pulse timings semi-randomized to give 20 trials each for the rest and active conditions.

Whilst subjects had to balance the dual requirements of task speed and the need for focal activation in this task, we utilized the training period to guide subjects to perform a swift yet precise response, with rapidity and consistency, whilst striving to maintain minimal overflow into the surround ADM muscle.

6.2.2.c Study analysis

Analysis was undertaken offline, with each recording frame was inspected and if task inappropriate responses or excessive pre-movement EMG detected, the frames were excluded from further analysis. In the peri-movement recording setup, frames were discarded if the TMS pulse was delivered more than 50ms after movement initiation.

For both recording setups we determined the ADM MEP amplitude mean and

Gini coefficient at each TMS time point. For the pre-movement setup this resulted in corticospinal tract excitability measurements at -300ms, +60ms, +90ms and +120ms with respect to the “Go” signal for each subject. For the peri-movement recording procedure, ADM excitability measures were determined in the ‘rest’ and ‘active’ states. Non-parametric tests were then utilized (within SPSS) to undertaken further analysis, using either a Friedman’s analysis or the Wilcoxon Sign Ranks Tests as appropriate with the p-value set to 0.05.

6.3 Results

6.3.1 Power grip experiment

In this experiment we wished to assess the variation in excitability of corticospinal tract output to two task relevant muscles during the premovement phase of an isometric power grip contraction. We hypothesized that both muscles would demonstrate a premovement decline in MEP variability, and hope to see this occur prior to a significant rise in mean excitability.

6.3.1.a Premovement changes in FDI MEPs

Collating the changes in FDI MEP mean amplitude we performed a Friedman's analysis of variance across the factor of TMS time point (box plot in figure 6.1.a) – this was significant with χ^2(df=3, N=10)=8.04, p =0.045, and Kendall's W=0.268. With respect to baseline post hoc testing with the Wilcoxon Signed Ranks Test demonstrated only a trend towards reducing excitability at the +60ms time point when compared to the (+60ms Z=-1.784, p=0.074), whilst the differences from baseline to +90ms and +120ms was not significant time points (Z=-.357, p=0.721 and Z=-1.376, p=0.169 respectively).

We next assessed the FDI MEP amplitude Gini coefficient using a Friedman's Analysis, again with the factor of TMS time point (box plot of changes in FDI MEP amplitude variability seen in figure 6.1.b). This analysis was highly significant with χ^2(df=3, N=10)=19.560, p <0.001, and Kendall's W=0.652. Post-hoc testing with the Wilcoxon Signed Ranks Test demonstrated a significant drop in the FDI Gini coefficient from

baseline by the +60ms time point (Z=-2.497, p=0.013, PSDep=0.9), and again at the +90ms (Z=-2.803, p=0.005, PSDep=1.0) and +120ms time points (Z=-2.803, p=0.005, PSDep=1.0).

6.3.1.b Premovement changes in ADM MEPs

ADM MEP amplitudes were assessed for changes in mean excitability using the Friedman's repeated measures analysis across time (shown in figure 6.2.a). Here again a significant effect was found - χ^2(df=3, N=10)=11.40, p =0.010 and Kendall's W=0.394. Post-hoc testing showed mean ADM excitability had risen from baseline by the +120ms time-point (Z=-2.395, p=0.017, PSDep=0.875), whilst only a trend was seen towards reduction by the +60ms time point (Z=-1.886, p=0.059) and no significant difference from baseline was seen at the +90ms (Z=0.561, p=0.575).

Variability of ADM MEP amplitudes also demonstrated a highly significant effect for TMS time point (shown in figure 6.2.b), and here the Friedman's analysis showed χ^2(df=3, N=10)=14.455, p =0.002, with Kendall's Coefficient of Concordance equal to 0.482. Post-hoc testing with the Wilcoxon Signed Ranks Test showed a significant reduction was noticeable by the +60ms time point (Z=-2.497, p=0.013, PSDep=0.8). A significant reduction was also noted at +90ms (Z=-2.803, p=0.005, PSDep=1.0) and +120ms time points (Z=-2.090, p=0.037, PSDep=0.9).

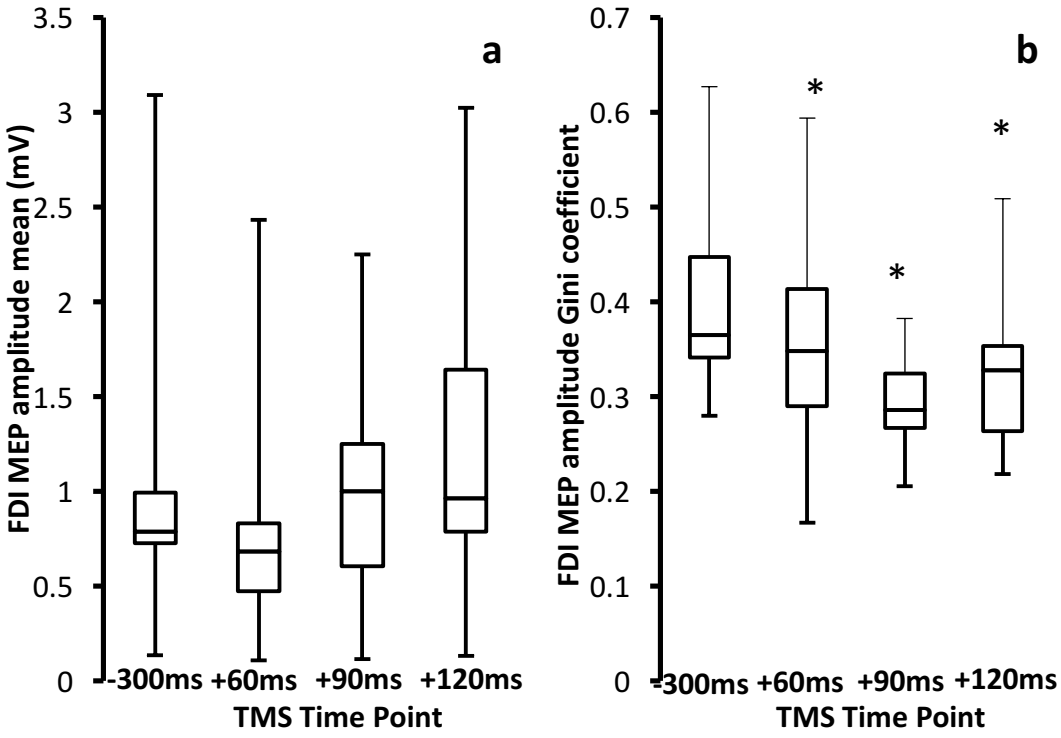


Figure 6.1.a-b Box plots demonstrating premovement changes of the FDI MEP for the power grip task in amplitude mean (a) and amplitude Gini coefficient (b). Data displayed for each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. An asterisk (*) indicates a statistically significant difference from baseline (-300ms).

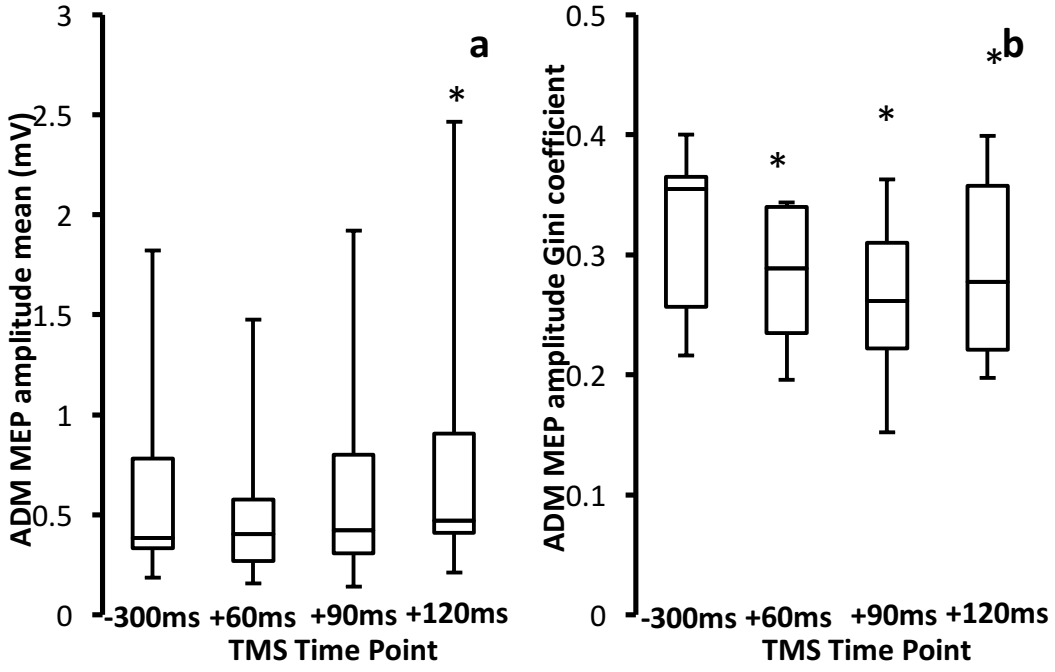


Figure 6.2.a-b Box plots demonstrating premovement changes of the ADM MEP for the power grip task in amplitude mean (a) and amplitude Gini coefficient (b). Data displayed for each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. * indicates a statistically significant difference from baseline.

We next assessed whether the change in the FDI MEP Gini coefficient corresponded to the change seen in ADM. We determined the change in Gini coefficient from baseline (-300ms) for FDI and ADM to the TMS point prior to the rise in mean excitability (ie. +90ms time point). A correlation analysis was then performed relating the change in FDI to that of ADM, where Kendall's Tau demonstrated a correlation coefficient of 0.600 and p=0.016 as shown in figure 6.3.

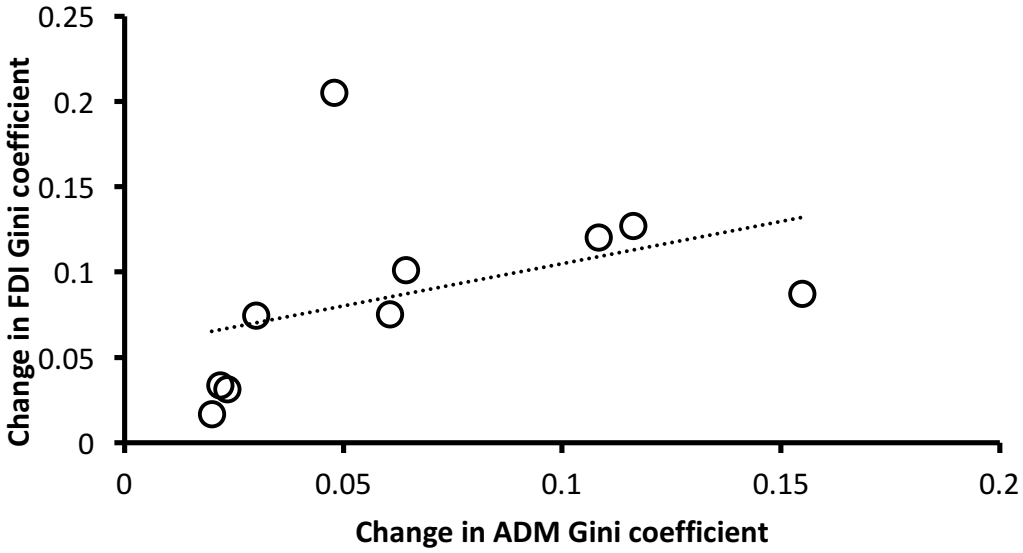


Figure 6.3 Correlation of premovement changes in the Gini coefficient from baseline for FDI (vertical axis) and ADM (horizontal axis). Dotted trendline presented as a visual aid only.

6.3.2 Surround inhibition experiment

For this experiment we wished to assess whether MEP variability would decline within a muscle which had given explicit instruction to be inactive (the 'inhibited' surround muscle), whilst another acted as agonist. In the surround inhibition paradigm (Sohn and Hallet, 2004; Beck et al, 2008; Kassavetis et al, 2014) subjects are required to press a button with the forefinger in a controlled isometric contraction (activating FDI), but in addition subjects must pay attention to activity in the ADM muscle, being instructed to keep EMG activity here as silent as possible. TMS intensity and stimulation site for this experiment were optimized for responses in the ADM muscle (given the expected inhibition we wished to minimize the chance of a floor effect reducing ADM variability), with a stimulator intensity titrated to achieve a resting amplitude of at least 1mV in ADM. Because of this, responses in FDI were of variable amplitude, often much larger than the standard 1mV amplitude. As such we did not analyse them in detail.

6.3.2.a Premovement changes in ADM

In this experiment we expected to see a decline in mean ADM amplitude during premovement preparation, in accordance with the surround inhibition paradigm (Beck et al, 2008). Here the Friedman's analysis for the effect of time point χ^2(df=3, N=15)=10.28, p=0.016, with Kendall's coefficient of concordance=0.228) was significant and can be seen in figure 6.4.b. Post hoc (WSRT) testing revealed that MEPs were significantly smaller at +60ms (Z=2.101, p=0.036, PSDep=0.733) and +120ms (Z=2.045, p=0.041, PSDep=0.867) compared with baseline (-300ms), though

not at +90ms (Z=1.250, p=0.211).

Variability of ADM amplitude (figure 6.4.a) also showed a significant reduction during the task (Friedman's analysis for time point χ^2(df=3, N=15)=13.880, p=0.003, with Kendall's coefficient of concordance=0.308). Post-hoc testing demonstrated ADM amplitude variance had only reduced significantly by the +120ms time point with respect to the -300ms baseline (Z=2.329, p=0.020, PSdep=0.867).

Whilst this result is significant, in practical terms the size and consistency of the effect across subjects is substantially less than that evident in FDI during the chapter five experiments (as partly suggested by the relatively low values for Kendall's coefficient of concordance). This observation perhaps reflects the difficulty in maintaining consistency in the balance between fulfilling the additional task requirement and maximizing the speed of response. Specifically, we found the mean percentage drop in ADM variability was relatively small at 7.7%, whilst the inter-subject coefficient of variation (CV; as an approximate measure of the consistency of effect across individuals) was 3.3 for ADM in this experiment. By way of reference the percentage drop in FDI during the first experiment in chapter 3 was 25.2% whilst the inter-subject coefficient of variance was 1.2. The effect on ADM mean amplitude was also small and variable across individuals (mean reduction of 6.8% with CV=4.5).

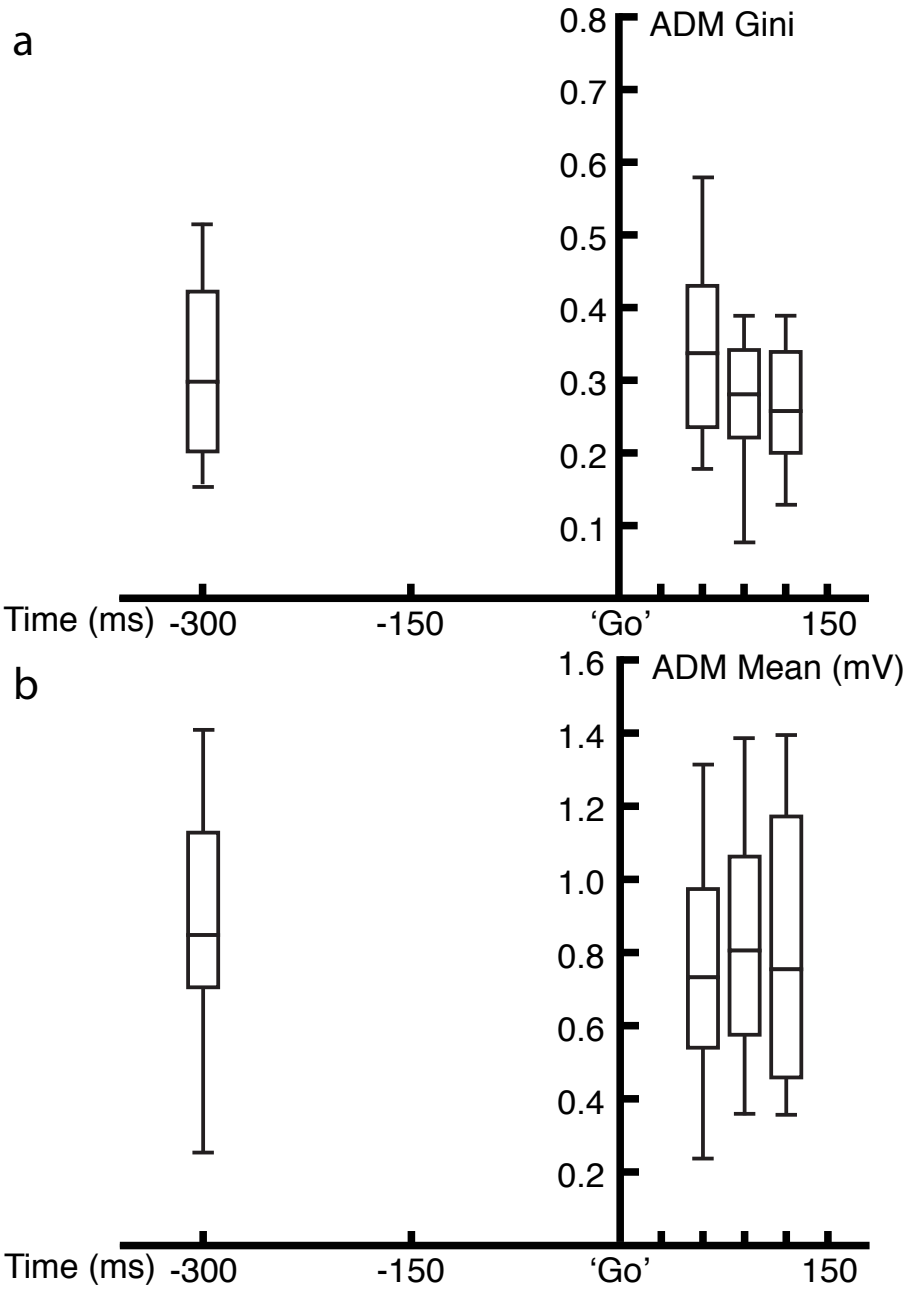


Figure 6.4.a-b Box plots of the time dependent changes in ADM MEP amplitude Gini (A) and mean (B) during the preparatory period of a surround inhibition task which emphasized the importance of keeping uninvolved fingers still (represented by ADM) during a practised button press task. Data displayed for each TMS point (spaced appropriately on the X-axis) with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima.

6.3.2.b Changes in ADM seen at movement initiation

We next assessed for changes in ADM, using the same surround inhibition movement paradigm, at the moment of movement initiation. Using the Wilcoxon Signed Ranks Test we see that ADM amplitude variability had reduced significantly at the moment of initiation with Z=-2.726, p=0.006 and PSDep=0.8. However, a similar comparison of mean ADM amplitude was no longer significant, showing only a trend, with a two-tailed Wilcoxon Signed Ranks Test demonstrating Z=1.647 and p=0.1. Four out of 15 subjects did not demonstrate surround inhibition at the moment of movement initiation. These results are graphed in figure 6.5.a and 6.5.b respectively.

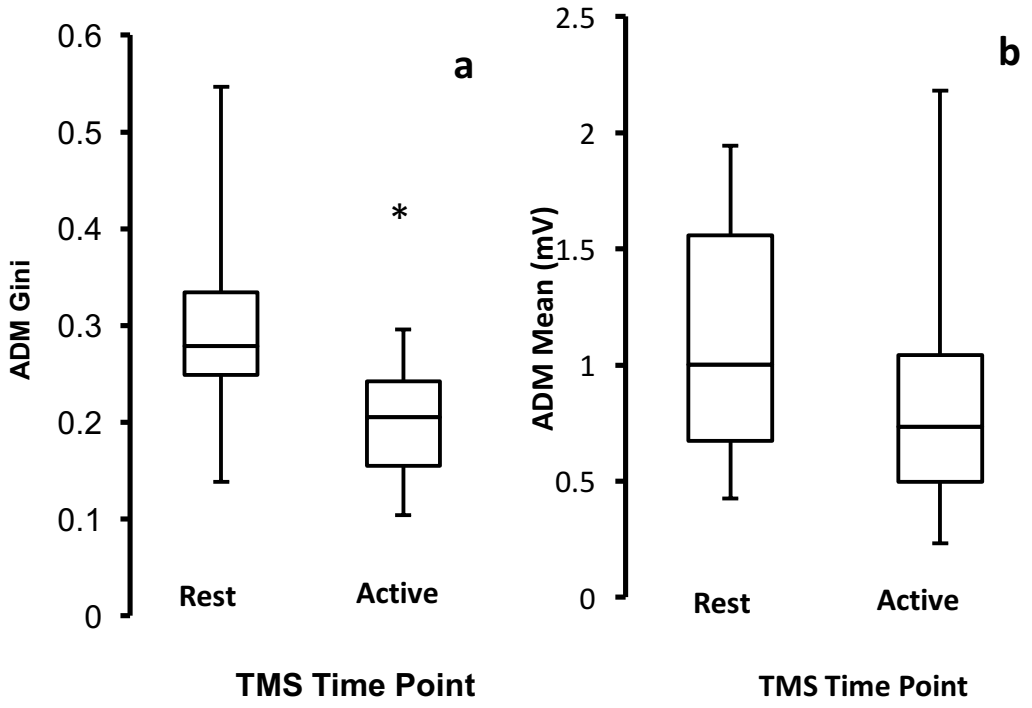


Figure 6.5.a-b Changes in mean excitability (a) and MEP variability (b) in the surround muscle (ADM) at rest and the moment of movement initiation. Data displayed for each TMS point (spaced appropriately on the X-axis) with centre lines representing the median, box limits indicating. Asterisk (*) indicated a statistically significant difference.

6.3.2.c Comparison of premovement to movement initiation phase

We wished to assess whether there was consistency in the changes from rest seen in ADM mean excitability and amplitude variability during the premovement and subsequent initiation of movement. We determined the change in both ADM mean and Gini coefficient from rest/baseline (-300ms) to movement initiation/late in movement preparation (+120ms time point, respectively. Firstly, for the Gini coefficient, we performed a correlation analysis of the change seen premovement to that seen at movement initiation using Kendall's Tau. As demonstrated in figure 6.6.a, the correlation was significant with r=0.478 and p=0.013. Additionally, in almost all subjects a greater reduction in variability is seen at movement initiation than during the premovement phase. We repeated an identical assessment for the change in ADM amplitude mean, again using Kendall's Tau, which was significant with r=0.543 and p=0.005 as shown in figure 6.6.b.

From these results we conclude that when participants are specifically instructed and trained to minimize the amount of activity in a muscle (ADM) not directly activated in a task, there is a tendency for this to be reflected in reduced variability of corticospinal output to the controlled muscle. Though there are variations in individual's consistency between tasks, across the entire subject sample changes in the premovement phase are reasonably concordant with that seen at movement initiation.

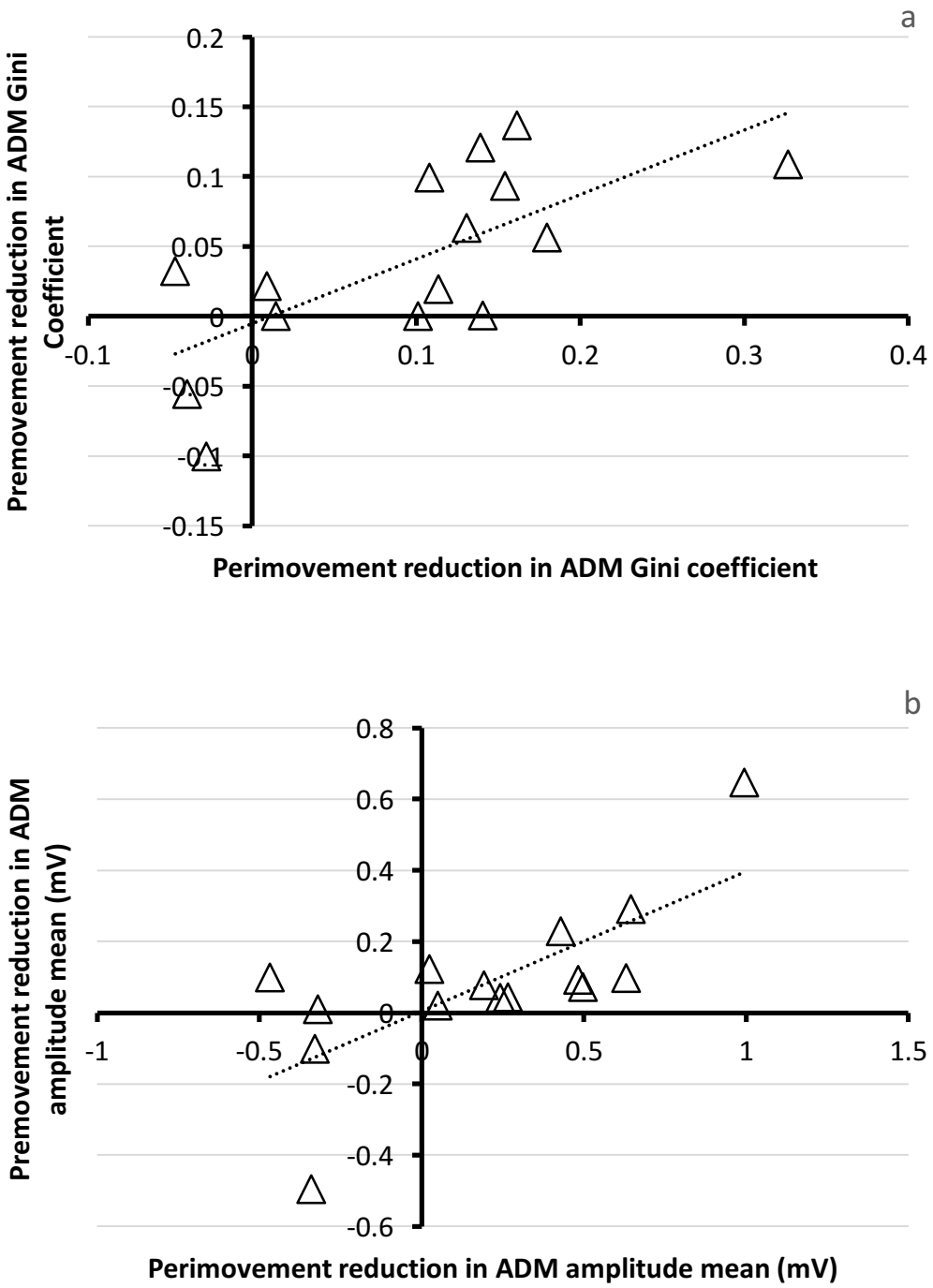


Figure 6.6.a-b Correlation of changes from baseline in ADM MEP amplitude Gini coefficient (a) and mean (b) in premovement variability to that seen at movement initiation with respect to their respective baselines.

6.4 Discussion

The two experiments in this chapter highlight the significant effect of context in manipulating MEP amplitude variability during the preparation for enactment of a motor program. MEP amplitude variability, and its experimental manipulation, needs to be assessed in the appropriate context. Whilst in traditional resting state observations mean amplitude and amplitude variability bear an inverse reciprocal relationship, the two experiments in this chapter study MEP amplitude variability changes in the context of movement preparation and demonstrate that substantial changes can be seen across the reaction time period independent of the traditional relationship with mean amplitude. Our first experiment demonstrated that MEP variability recorded from two separate hand intrinsic hand muscles (FDI and ADM) declined concordantly during the reaction time period of a power grip task, and did so prior to any significant rise in excitability. Our second experiment demonstrated that a surround muscle, specifically tasked to display an inhibitory state (ADM within a surround inhibition paradigm), demonstrate reduced amplitude variability, despite a reduction in mean amplitude. Moreover, across the subject pool, variability and mean excitability changes preceding movement onset were well correlated with the excitability state seen at movement initiation.

6.4.1 Concordant changes during the power grip task

Our study of MEP amplitudes demonstrate in both FDI and ADM, a late rise in mean excitability, together with an earlier decline in MEP amplitude variability. Whilst no significant rise in excitability from baseline is evident at the +60ms and +90ms time points, a significant change in mean excitability starts to become evident by the

+120ms time point, consistent with our findings in the bilateral button press task, and in keeping with the late premovement rise of excitability seen in other works (Anson et al, 2002).

With respect to variability, a significant decline in MEP amplitude variability is seen early, well before the late rise in mean excitability. As early as the +60ms time point a decline in variability is seen for both FDI and ADM MEP amplitudes, a time point at which there is the hint of a decline in MEP mean amplitudes. The concordant time changes again hint at common mechanism driving variability changes, and there appears to be a moderate correlation between FDI and ADM with respect to this reducing variability, as might be expected within the context of an evolving motor program.

6.4.2 MEP variability changes within a surround inhibition paradigm

In these experiments we demonstrate a significant decline in MEP amplitude variability during movement preparation in a surround muscle tasked with inhibition during a trained low force button press task. In the pre-movement phase MEP mean excitability declined for ADM, in most subjects consistently by the +120ms time point, consistent with the premovement decline in surround muscles seen by Beck et al (2008). When the task was reproduced and recordings undertake at the phase of movement initiation, we still see a trend toward reduction in mean amplitude, though the changes are not statistically significant. Whilst Sohn and Hallet (2004) demonstrated the presence of surround inhibition using 12 healthy controls, Kassavetis et al (2014) studied the phenomena with a larger pool of some 31 subjects,

acknowledging that not all subjects will demonstrate the phenomena and highlighting the importance of volition for the paradigm. Furthermore, Kassavetis et al (2014) had studied surround inhibition within a free-movement paradigm, allowing subjects to initiate movement when they were ready. The significant variability we find here, both within and across recording sessions, may be a consequence of the dual task nature of the paradigm, which required subjects to produce both a rapid response (within the simple reaction time paradigm) and to ensure the focality of muscle activation. It is important to note, however, that across the entire subject pool, the magnitude of pre-movement changes in mean excitability were well correlated with the large changes in mean excitability seen at movement initiation.

Changes in amplitude variability were seen more consistently across both experiments, with a significant reduction in ADM MEP variability seen both pre-movement and at movement initiation, despite the reducing amplitudes. It should be noted that the effect we see within the surround inhibition paradigm appears more consistent than that seen with mean excitability. Some subjects demonstrated a rise in mean excitability in one or both recording scenarios, i.e. volitional activation instead of inhibition. A net reduction in MEP variability may still be seen under both conditions as long as the task is performed consistently.

Importantly, again, on the whole changes in MEP amplitude variability seen during the pre-movement phase were correlated with larger reductions at movement initiation, reflective of the phenotypic continuity between the process of preparation for movement and the moment of the movement's initial release.

6.4.3 MEP amplitude mean and variability – the effect of context

The resting state relationship between MEP amplitude mean and variability is well described (Van der Kamp et al, 1996; Brasil-Neto et al, 1992). Researchers have utilized the effect of a tonic contraction on mean excitability to reduce MEP variability (Kiers et al, 1993). However, in most if not all of those paradigms, the resting state MEP output is manipulated through adjustments in stimulator output, adjustments which are reasonably likely to drive a progressive excitatory saturation of corticospinal tract output.

In our work thus far we have demonstrated MEP variability in task relevant muscles is reduced during the process of movement preparation. This chapter demonstrates that movement preparation can generate concordant changes in task relevant muscles. Furthermore, not only are such reductions in task relevant muscles seen prior to any rise in excitability, the reduction in amplitude variability can be seen concomitant with a reduction in amplitude size. Within the reaction time window on corticospinal excitability the key feature driving control of corticospinal excitability is the process preparing for the initiation of volitional movement. When the explicit goal of movement preparation is to reproduce the same movement paradigm consistently, the output of task relevant cortical populations through the corticospinal tract will tend to follow the same pattern. As such MEP variability in task relevant muscle(s) will tend to decline during the period of movement preparation, irrespective of whether output to that muscle is inhibitory or excitatory.

Chapter 7 The influence of force and grip complexity on MEP variability

7.1 Introduction

The reaction time period sees the coordinated and dynamic influence of numerous inputs to the corticospinal tract, much of which is focused on optimizing output for the moment of movement initiation. Previous chapters have demonstrated that for a consistently reproduced movement, the reaction time fore period sees a progressive decline in the corticospinal tract output variance, as manifested by the decline in variance of MEP amplitudes we see in task relevant muscles. The decline in variability can be focal, context specific and independent of the relationship of variability with mean amplitude we traditionally note from resting state dynamics. In this chapter we wish to assess whether the reaction time decline in MEP variability seen thus far will be influenced by the movement parameters of force and task complexity.

The reciprocal relationship between force and extent of primary motor cortex activation is well established – M1 fMRI measured activity has been shown to scale with force and EMG activity (Dai et al, 2001), though there is extensive evidence for a distributed role in force generation across higher order cortical and subcortical structures (Dai et al, 2001; Spraker et al, 2007; Clark et al, 2014). With respect to TMS studies of the contralateral M1 during force generation, through the use of input/output curves at rest and graded force levels Perez and Cohen (2009) demonstrated a progressively increasing responsiveness to TMS over M1 with the scaling of force. Similar dose-response relationships had been found by Taylor et al (1997) in graded increases of biceps brachii contraction force and Semmler et al

(1998) using FDI – greater amplitude MEPs were seen with greater force in the active muscle when tested using single intensity pulses. However, to date, published TMS studies have not assessed changes in corticospinal excitability during the reaction time period. We wished to assess whether, with the greater activation of cortical M1 neurons during higher forces, commensurately tighter control of M1 variability would be seen within the preparatory period.

Regarding our previous findings of an early MEP amplitude decline and the parallels to the premovement silent period documented by Aoki et al (2002) – Mortimer et al (1987) had demonstrated an increase in the frequency of premovement silent periods elicited when a ballistic task was carried out at maximal force, findings also confirmed by Tanii (1996). Noting changes were present only in task relevant muscles, they had suggested that the variable presence of the the premovement silent period was evidence of its learned and volitional nature. With respect to the MEP variability decline during the RT fore period, if a similar mechanism is at play, for higher force tasks we may expect see a greater early decline in mean MEP amplitude, with a concomitant effect on MEP variability.

Numerous studies have already studied the effect of grip type on TMS assessments of mean corticospinal tract excitability. In general, increasing task complexity has seen greater mean MEP amplitudes in task relevant muscles (Flament et al, 1993; Kouchtir-Devanne et al, 2012); Works by some others have found functional differences in intrinsic hand muscles during sustained precision vs power grip contractions (Hasegawa et al, 2002; Geevasinga et al, 2014) though these differences

are less apparent when assessed during the reaction time fore period (Anson et al, 2002). Here we wished to assess the influence of grip type on reaction time MEP variability, positing that the greater task complexity of the precision grip would lead to the need for tighter corticospinal control which would be manifest as a greater reduction in MEP amplitude variability in FDI during the preparatory period.

7.2 Methods

7.2.1 Subject Profile

All subjects were free of neurological and psychiatric illness and had not taken any psychotropic substances in the preceding two weeks. In accordance with the Declaration of Helsinki all subjects gave full and informed written consent prior to the commencement of their involvement in the research and were free to withdraw at any time, though none chose to do so.

7.2.1.a - Power Grip Force Modulation Experiment

For this experiment a total of nine subjects were recruited through a database of healthy volunteers. Three subjects were female and all were right handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). The mean age of subjects was 27.7 years (range 22-45 years).

7.2.1.b - Pinch versus Power Grip Experiment

For this experiment we recruited 12 subjects through a database of healthy volunteers. There was no subject overlap between the two experiments. As in the preceding experimental group, all subjects were right handed, five were male, and the mean subject age was 25.4 years (range 19-38 years).

7.2.2 Study Procedure

TMS was delivered by a Magstim 200^2 monoblock through a D70 figure of eight coil as described in the general methods. At the start of the session subjects were seated

in the experimental chair, with their arms placed in their lap, forearms positioned midway between pronation and supination to maintain a relaxed state. TMS recordings were then commenced to identify the cortical hotspot for FDI. With the optimum position marked off on the scalp, TMS output was then titrated to achieve a target 1mV amplitude MEP at rest, with the simulator intensity recorded for later use.

Training for the behavioral task was then commenced, utilising grasp and pinch dynamometers with digital outputs from Biometrics Ltd (Newport, UK) being used. Both experiments were performed within a simple reaction time paradigm. A visual LED (using a $1cm^2$ green LED) was placed in the center of subjects' field of vision and used as the 'Go' cue.

7.2.2.a Power Grip Force Experiment

We trained each subject to precisely perform an isometric power grip task with the position of the dynamometer within the hand adjusted to achieve maximum force across the MCP joints in each subject, thereby maximizing FDI involvement. We first determined the maximum voluntary contraction based on grip dynamometer output trace during six trials of a maximal effort power grip with their dominant hand. The parameters for the high and low tasks were set at 30-40% MVC for the high force condition and 2-5% MVC for the low force power grip.

Subjects were trained to perform either the high or low force task, with the initial force target chosen randomized in each subject. Task performance was performed

within a simple reaction time paradigm, with subjects instructed to respond as quickly and consistently as possible. The resultant isometric contraction subjects produced was required to rapidly attain the target force window (from onset to peak <200ms).

During training visual (post-trial review of the trial trace with cursors to delineate the power window) and verbal feedback were provided to fine tune performance. Once subjects had learned to perform the task consistently, a reaction time record was made from twenty trials. TMS was then introduced, targeting FDI at the intensity measured earlier. Upon completing TMS recordings for one force intensity, following a short ten-minute break subjects were retrained on the alternate intensity, reaction time recording made again and the TMS recording repeated.

TMS timings for the task were -300ms, +60ms, +90ms and +120ms with respect to the 'Go' signal. Three block of forty trials were undertaken for each intensity, with a short break of at least five-minutes being taken between recording blocks. Each block recording 10 trials at each TMS timing with the order of delivery randomized.

7.2.2.b – Pinch versus Power Grip Experiment

Subjects were trained to perform the isometric pinch and power task within a simple reaction time paradigm. On this occasion subjects were trained to perform the task to one target force window, calibrated individually at 10-20% of a subjects' maximum force for each grip type. Maximum force recorded for each grip type, with using their respective dynamometers, was determined from six maximum effort trials.

Each subject was asked to perform the grip task with a rapid and consistent response to the visual “Go” signal. During training for both grip types we required subjects to perform the isometric contraction swiftly, aiming to achieve peak target force within 200ms. Once the subject was able to perform the task reliably, a block of 20 trials to record the task reaction times was made. Subsequent to this TMS recordings were undertaken with two recording blocks undertaken each containing forty trials, occurring randomly at four to six second intervals to prevent anticipation. A short five-minute break was ensured between these recording blocks. In each block, TMS was randomly delivered at one of two timings (- 300ms and +100ms with respect to the ‘Go’ signal). Our previous experiments had used up to three TMS time points following the Go period to track changes in variability. Here a reduced number of time points was utilized in an effort to shorten the required protocol, and thereby facilitate greater utility in the assessment of clinical subjects (e.g. serial study following acute stroke). Training and recording was completed for one grip type (the order was randomized for each subject) before repeating the procedure for the alternate task after a ten-minute break.

7.2.3 Analysis

All trials were recorded and stored for off-line analysis in line with procedures stated in the general methods chapter. We used CED Signal software to inspect each recorded frame to confirm appropriate task performance and screening for artefacts that would lead to frame exclusion. We then determined the FDI MEP peak to peak amplitude using a custom script. Reaction time measures were made only to

determine equivalence across conditions but were not intended to be part of the main analysis. Non-parametric methods, using SPSS software (version 21), were used to analyse both experiments with the p-value set at 0.05. Box-plots were utilized to visualize results.

7.2.3.a - *Power Grip Force Experiment*

Our primary interest was the effect of force level (high vs low) on the evolution of changes in MEP amplitude variability during the premovement phase. As such we first determined the FDI MEP amplitude mean and Gini coefficient under the high and low force conditions at each TMS time point. For each force level we separately performed a non-parametric Friedman's analysis across the factor of TMS time point for the amplitude mean and Gini coefficient. If significant, further targeted post-hoc analysis was undertaken using the Wilcoxon Signed Ranks Test. Finally, in an effort to reduce the number of comparisons across force levels, we chose to first calculate absolute changes from baseline (-300ms) to the final TMS time point (+120ms) under each condition. The Wilcoxon Signed Ranks Test was then utilized to make a direct comparison across force levels for the generated mean and Gini coefficient premovement change.

7.2.3.b - *Pinch versus Power Grip Experiment*

In this experiment we wished to assess the influence of grip type (pinch versus power grip) on MEP amplitude variability changes during the premovement period. Here we again determined the FDI MEP amplitude mean and Gini coefficient for the baseline (-300ms) and +100ms TMS time points recorded with each grip type. In this case, the

Wilcoxon Signed Ranks Test was initially used to assess premovement change in the MEP amplitude variables within each task. Subsequently in making direct comparison we again determined absolute changes from baseline to the +100ms post “Go” time point in both the mean and Gini coefficient. The Wilcoxon Signed Ranks Test was again used to assess across tasks for task differences in this premovement change for the Gini coefficient and mean.

7.3 Results

7.3.1 – Power grip force experiment

In this experiment we wished to assess the impact of contraction strength on changes in the FDI MEP amplitude mean and variability across the premovement period of a power grip task. Subjects performed the two tasks with similar speed. The average median RT time for the low force task was 181ms (range 139-197ms), whilst for the high force task the average median reaction time was 171ms (range 143-252ms). A Wilcoxon Sign Ranks Test found no significant difference in median RT across the two levels of force (Z=-1.244 and p=0.214).

7.3.1.a - Low force (2-5% MVC) power grip

Results for the analysis of the FDI MEP amplitude Gini coefficient and mean in the low force task are displayed in figure 7.1.a-b. We first assessed whether significant changes were found across time points with respect to the Gini coefficient. A Friedman's repeated measures analysis (across time points) was significant with χ^2(df=3, N=9)=17.133, p =0.001, and Kendall's W=0.635. Post-hoc analysis with the Wilcoxon Signed Ranks Test demonstrated the Gini coefficient had declined significantly from baseline (-300ms) by the +90ms time point (Z=-2.66, p=0.008, PSDep=1.0) and was still significantly reduced by the +120ms time point (Z=-2.66, p=0.008, PSDep=1.0). There was no significant difference at the +60ms time point (Z=-1.244, p=0.214).

We next assessed premovement changes in the low force task for FDI amplitude mean, where a Friedman's analysis was also significant - χ^2(df=3, N=9)=14.467, p =0.002, Kendall's W=0.536. Post-hoc analysis suggested that there was a trend toward amplitude rise at the +120ms point (Z=-1.955, p=0.051, PSDep=0.778). Interestingly whilst there was no significant rise from baseline by +60ms (Z=-1.362, p=0.173) and +90ms (Z=-0.059, p=0.953), there was a significant difference between +120ms and +60ms (Z=-2.66, p=0.008, PSDep=1.0) and +90ms (Z=-2.66, p=0.008, PSDep=1.0).

7.3.1.b - High force (30-40% MVC) power grip

In the high force power grip task the FDI MEP amplitude Gini coefficient again reduced during movement preparation. The Friedman's analysis was significant for TMS time point with χ^2(df=3, N=9) =10.733, p=0.013 and Kendall's W=0.398. Post-hoc testing with the Wilcoxon Signed Ranks Test demonstrated a significant reduction by the +120ms time point (Z=-2.666, p=0.008, PSDep=1.0) as shown in figure 7.2.a. A significant reduction from baseline was not found for either +60ms or +90ms (Z=0.553, p=0.594 and Z=1.007, p=0.314 respectively).

The high force task also demonstrated a significant effect on FDI amplitude mean across time points (Friedman's χ^2(df=3, N=9) =19.0, p<0.001, W=0.704). Wilcoxon Signed Ranks Test again demonstrated a significant rise from baseline (-300ms) by the +120ms time point (Z=-2.666, p=0.008, PSDep=1.0) but not the +60ms (Z=0.889, p=0.374) and +90ms time points (Z=1.362, p=0.173).

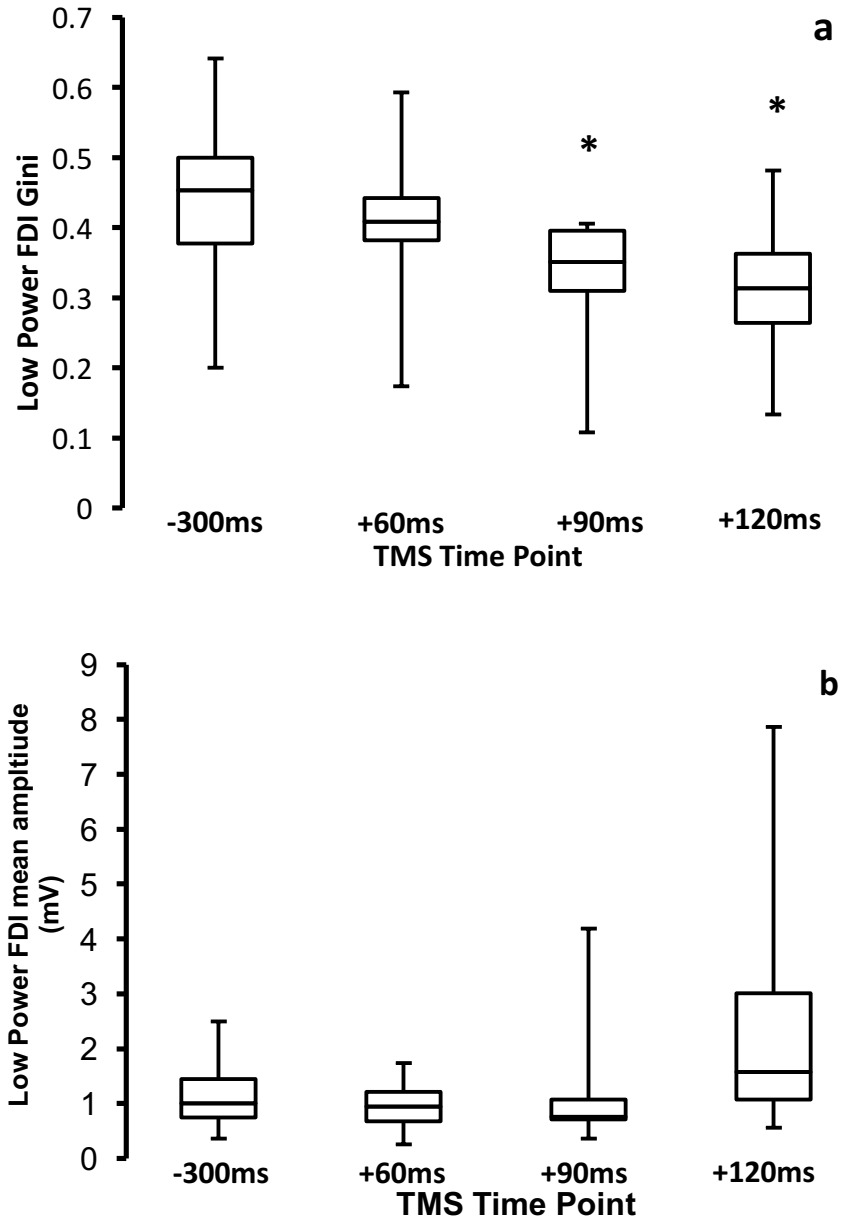


Figure 7.1.a-b Changes in the FDI MEP amplitude Gini coefficient (a) and mean (b) during preparation for the low force power grip task. Data displayed for each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. * denotes a significant difference from baseline (-300ms).

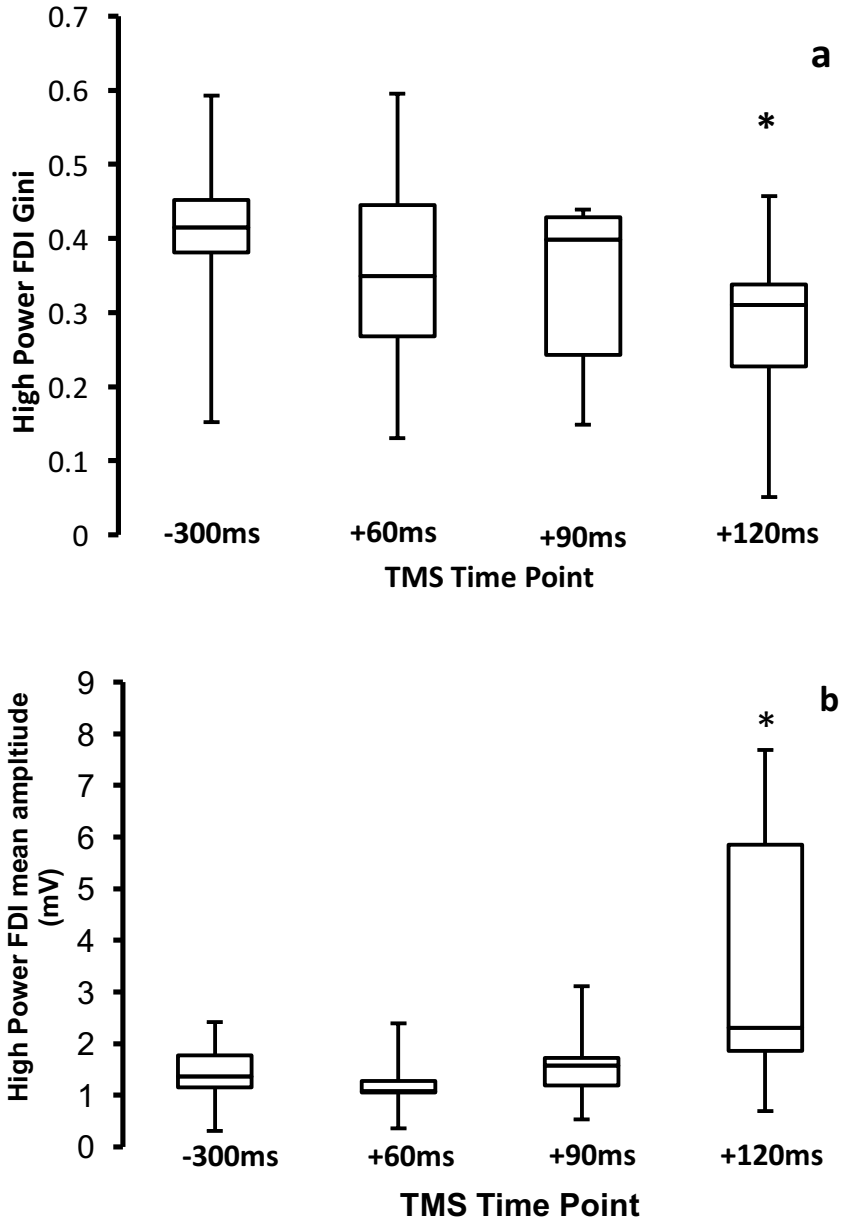


Figure 7.2.a-b Changes in the FDI MEP amplitude Gini coefficient (a) and mean (b) during preparation for the high force power grip task. Data displayed for each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. * denotes a significant difference from baseline (-300ms).

7.3.1.c - Comparison of low and high force conditions

We wished to assess the effect of force in the power grip task on mean excitatory drive at two points. Firstly, we wished to whether the high force condition might be associated with a greater reduction in MEP amplitudes at the early +60ms time point (corresponding to roughly 35% of the movement preparation). To assess this, we compared the change in the mean FDI amplitude from -300ms to +60ms across the high and low force conditions. A Wilcoxon Signed Ranks Test found no significant difference between the two conditions (Z=0.296, p=0.767).

We next compared the premovement difference in mean amplitude from -300ms to +120ms across the two groups using the Wilcoxon Signed Ranks Test. Again we found no significant difference between the two conditions (Z=0.770, p=0.441). Finally, we assessed whether a significant difference was present with respect to the change in the Gini coefficient from baseline (-300ms) to the final TMS (+120ms) time point. Here the WSRT was also not significantly different with Z=0.296, p=0.767.

7.3.2– Pinch versus power grip experiment

One subject recruited towards the end of enrolment was unable to perform the task reliably and was excluded from the study. In the majority of trials the subject responded to the TMS pulse with a triggered movement (across all time points) and was unable to complete the task without these false starts. Whilst the subject was reportedly healthy, the data was considered unreliable and as such excluded from the final analysis. For the remaining 11 subjects the mean age was 25.7 (range 19-38) years, all right handed with five males and six females. Average Median RT across

the grip tasks were not significantly different at 173.3ms (Power grip) and 183.0ms (Pinch grip), Wilcoxon Signed Ranks Test Z=-0.445, p=0.656.

7.3.2.a - Pinch grip task

We first assessed for a difference in FDI mean MEP amplitude (shown in figure 3.3.a), from baseline (-300ms) to +100ms. The Wilcoxon Signed Ranks Test found no difference between the two time points with Z=0.178 and p=0.859. We next assessed for a difference in the FDI amplitude Gini coefficient at rest and +100ms following the 'Go' cue. Here the Wilcoxon Signed Ranks Test demonstrated a significant reduction in variability (graphed in figure 7.3.a) with Z=-2.135, p=0.033 and PSDep=0.909.

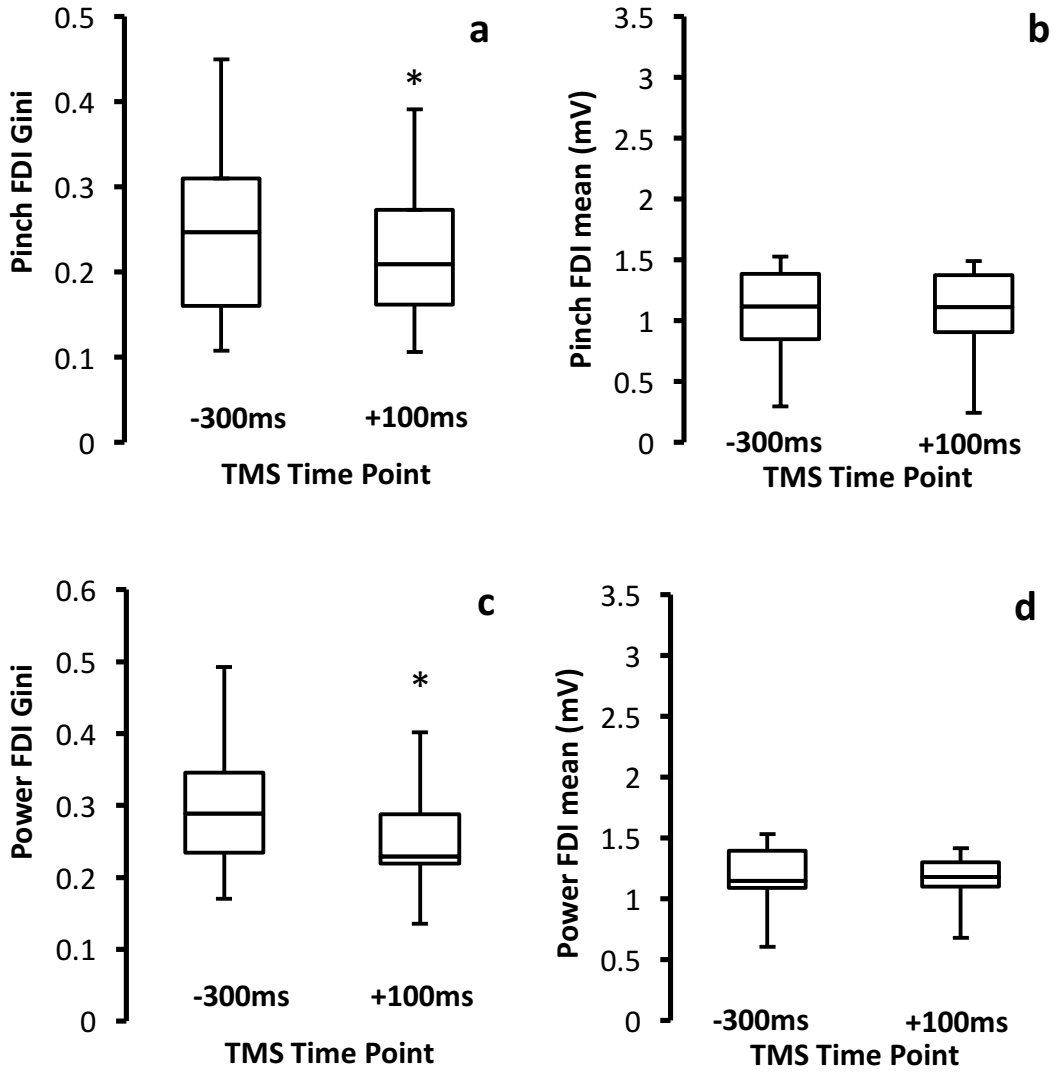
7.3.2.b - Power grip task

The power grip task demonstrated similar results, as graphed in figures 3.3.c and 3.3.d. Whilst the Wilcoxon Signed Ranks Test demonstrated no significant difference in amplitude at +100ms following the Go signal (Z=0.000, p=1.000), a significant reduction in the Gini coefficient had been found following the Go signal with Z=-2.845, p=0.004 and PSDep=0.909.

7.3.2.c - Comparison of pinch vs power grip changes

We had anticipated the possibility of a greater rise (from baseline) in FDI mean excitability during the pinch task when compared to the power task, however a Wilcoxon Signed Ranks Test comparing this change across tasks was not significant (Z=0.267, p=0.790). A comparison of the change from baseline for the FDI MEP

amplitude Gini coefficient was also not significant with Z=1.067, p=0.286. Some individuals displayed significant differences in the rise of mean excitability across tasks and such we performed a comparison of the change in the Gini coefficient which corrected for the change in FDI amplitude mean. Here we divided the change (from -300ms to +100ms) in the Gini coefficient by the change in mean across the same period. Again a Wilcoxon Signed Ranks Test was not significant with Z=-0.356 and p=0.722.



Figures 7.3.a-d Changes in the FDI MEP amplitude Gini coefficient and mean during movement preparation for pinch grip (a and b respectively) and power grip (c and d respectively) tasks. Data displayed at each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. * denotes a significant difference from baseline (-300ms).

7.4 Discussion

The experiments we present in this chapter assess the influence of grip force and grip type in modulating the variability of corticospinal tract activity during the preparation of movement. Consistent with past works (Perez and Cohen, 2008; Taylor et al, 1997; Semmler et al, 1998) our results suggest there is a tendency for force to influence mean excitability at the later stages of movement preparation for a power grip task. However, whilst both low and high force tasks demonstrated a reduction in variability during the reaction time period, the difference in mean excitability did not have a concordant effect on MEP variability. Similarly, in a comparison of pincer vs grip type, whilst a premovement decline in the Gini coefficient was found for both tasks, no differential effect was seen across tasks during the preparatory period. These results appear consistent with the hypothesis that the decline in the Gini coefficient seen across the early to mid reaction time period is primarily a marker of the progress of movement preparation, rather than a secondary consequence of the physiologic modulation of mean corticospinal excitability.

7.4.1.a - Mean excitability changes with grip force

The power grip force experiment documented a significant rise in mean excitability at the +120ms time point for the high force group. A similar late was seen in the low force group though the change was not statistically significant. Though a visual trend appeared to be present, a direct comparison of the mean change in excitability from baseline to the +120ms time point did not demonstrate a statistically significance difference between force levels. With respect to this result it should be noted that in

pretesting, median reaction times across both force levels were reasonably comparable. Our results would appear to fall within the envelope of past results, which show a tendency for the higher force level to result in greater mean excitability (Perez and Cohen, 2008; Dai et al, 2001), with the specific results we see a consequence of deliberate methodological choices.

Perez and Cohen (2008) demonstrated that the I/O curve elicited during 70% maximal contraction generated a steeper curve than when elicited during a 10% contraction; similarly Dai et al (2001) demonstrated the scaling influence of force on M1 bold signal using fMRI, though we also know that other regions such as basal ganglia (subthalamic nucleus and globus pallidus internus), portions of the ventral thalamus (Spraker et al, 2007) and higher-order motor areas (Dai et al, 2001) each demonstrate linked scalar changes during dynamic force generation tasks. Each of these experiment assess contraction during a sustained contraction, beyond the preparatory period. In the RT fore period excitability changes typically occur most dramatically within the last 10-20ms immediately prior to movement initiation (Chen et al, 1998; Anson et al, 2002).

TMS studies undertaken during sustained contraction have demonstrated differences with force with similar sample numbers to our work here (N=10 for Perez and Cohen (2009) vs N=10 in our work). The results we see here at the +120ms time point is likely on the cusp of a more significant rise and it is possible that with greater study numbers a statistically significant difference may become apparent, even in the premovement phase. However, given our interest in variability control prior to more

dynamic mean excitability changes, the timings we have chosen would appear to be optimum.

7.4.1.b - Variability changes with grip force

With both force levels we demonstrated a significant decline in CST output variability (i.e. MEP amplitude variability) during movement preparation. The decline in variability appeared to be seen earlier in the low force group (by +90ms) than in the high force group (+120ms). However, in direct comparisons, no significant difference was found between the two groups in the amount of variability change, even when accounting for individual differences in the mean excitability change. These results do not suggest MEP amplitude variability seen during the premovement period is influenced by target force levels and remains consistent with the declining variability having a primary relationship to the rate of movement preparation, rather than occurring as a consequence of other movement preparation parameters.

The reason for the slightly earlier decline in the low force group remains unclear. It is possible that the narrower band of accepted force in the low force group (3-5% vs 30-40% of maximal contraction respectively) required tighter control of corticospinal tract excitability during the preparatory period. Commensurate with this greater task difficulty one might expect longer reaction time periods (Heitz, 2014) for the low force task. However, reaction times across both force levels were not significantly different, with the difference in median reaction times being less than 10ms in the majority of subjects, and no consistent difference in reaction time due to force across all subjects. Alternatively, the broader force envelope for the high force condition

may have led to less consistent reproduction of isometric contractions, and in turn diminished the protocols ability to detect earlier variability changes. However, subjects had been extensively trained prior to the task, and were given corrective feedback if task performance deteriorated during MEP recordings, making this outcome less likely.

7.4.1.c Early movement preparation MEP inhibition

Aoki et al (2002) and Mortimer et al (1987) had suggested that the premovement EMG silent period was a centrally derived strategy for bringing corticospinal tract output under control and thereby optimizing force generation during ballistic actions. In the power grip task we specifically hoped to see greater evidence of the early movement inhibition of MEP amplitudes of which we gained brief glimpses in earlier chapters. The task had required the generation of a brisk forceful isometric power grip contraction in accordance with our past experiments, conditions which both Mortimer et al (1987) and Aoki et al (2002) and identified as producing the 'premovement EMG silent period'. However even by contrasting across the high and low force conditions a significant difference was not apparent.

Whilst the experimental settings are obviously not the same as those in past works, comments by these past authors may be helpful in identifying why results in the present paradigm are not as expected. Aoki et al (2002) and Mortimer et al (1987) both acknowledge the highly variable nature of the premovement silent period, with Mortimer et al (1987) going on to suggest that it may be an acquired/learned strategy for individual subjects. In this regard participating subjects were not involved in

experiments from previous chapters. Given the relatively small sample sizes used in TMS experiments, the phenomena may, by simple sampling error, be less apparent in the current subject pool. To combat this, a potential training method to facilitate acquisition of the 'skill' could be undertaken, though none is as yet documented. Mortimer et al (1987) also noted that the premovement silent period was more readily seen in self-paced tasks than those performed under reaction time conditions. For practical purposes this was not undertaken in the current paradigm. Finally, it must be recognized that the transient reduction in mean MEP amplitudes we see during our premovement paradigms and that of the premovement silent period noted by Aoki et al (2002) may not be generated by a common neurophysiological mechanism.

7.4.2 Grip type – changes in mean excitability and variability

For this experiment we see no significant rise in mean CST excitability at the +100ms time point, in either pinch or power grip tasks, from baseline (-300ms) suggesting that by the mid-reaction time period mean excitability still remained relatively stable. A direct comparison between the two tasks across subjects also failed to detect a significant difference in mean excitability changes for FDI. Whilst several papers within the TMS literature note increasing mean MEP amplitudes in precision versus power grip tasks (Hasegawa et al, 2001; Kouchtir-Devanne et al, 2012; Geevasinga et al, 2014) each of these experiments have generated these measurements during sustained contractions. In comparing premovement changes of excitability, Anson et al (2002) did not demonstrate a discernible difference between precision and power grip task in FDI MEP amplitudes. Furthermore, in their task excitability only rose in

the very later stages of movement preparation, consistent with past works (Chen et al, 1998; MacKinnon and Rothwell, 2000). Given our focus on detecting differences in MEP variability control, that discernible differences in mean excitability are not yet present in either tasks, is helpful in excluding a substantial effect of mean excitability alone.

Corticospinal tract output variability was modulated by preparation for both precision and grip tasks. FDI MEP variability declined significantly from baseline (-300ms) by the +100ms time point in both tasks. Reaction times in both tasks had been commensurate. A direct comparison of the absolute change between baseline to the +100ms time point found no significant difference between subjects.

For a few individual subjects a change in mean excitability was present. As such a correction to allow for individual differences in mean excitability across the two TMS time points was performed. The hope was that such a correction might amplify the differences across tasks. However again no significant difference across tasks was found with this method also. All-in-all our results would suggest that no significant developing difference in corticospinal tract output variability is present during the early-to-mid reaction time period.

Logically it seems reasonable to suggest that task complexity may influence corticospinal tract output, and in turn that preceding corticospinal tract variability may need to be more tightly controlled during movement preparation. However, our results do not support either statements. In assessing validity we must ask whether

our tasks have significant differences in complexity, and that this differences might be captured in our M1 recordings. As mentioned earlier, numerous experiments have noted a difference in M1 output between pinch and power grips experiments (Anson et al, 2002; Ehrsson et al, 2000; Flament et al 1993; Hasegawa et al, 2001; Kouchtir-Devanne et al, 2012; Geevasinga et al, 2014). However results across these experiments are frequently not concordant – M1 output during precision grip may be lower (Ehrsson et al, 2000), greater (Hasegawa et al, 2001; Kouchtir-Devanne et al, 2012; Geevasinga et al, 2014) or equivocal (Flament et al, 1993; Anson et al, 2002) and discordant results appear to persist even when correcting for differences in task complexity (Ehrsson et al, 2000; Hasegawa et al, 2001).

Our own experiment assessed excitability changes in a muscle (FDI) in which a difference between power and pinch grip tasks had been previously found using TMS (Kouchtir-Devanne et al, 2012). Furthermore, we optimized grip task performance for each subject and explicitly controlled for force through training and ongoing feedback. It may be that during the premovement stages task complexity is not substantially different between precision and power grip tasks across a pool of subjects (Flament et al, 1993), a conclusion that might be reasonably consistent with the equivalence of reaction times across subjects.

Chapter 8 Premovement control of TMS MEP variability in stroke patients

8.1 Introduction

Disturbances in the control of movement in paretic stroke patients have been extensively studied within experimental paradigms. In general, reaction time paradigms (including simple RT paradigms) have shown delayed responses on the paretic side when compared to the non-paretic side for patients who are at least moderately impaired (Battaglia et al, 2006; Bi and Wan, 2013). Of interest to this thesis is the suggestion that stroke patients display an increase in resting TMS MEP amplitude variability, more significantly for the lesional hemisphere (Koski et al, 2007). This chapter examines the possibility that a lesional hemisphere disturbance in control of MEP amplitude variability contributes to an impairment of reaction time performance on the paretic side.

Deficiencies in the control of corticospinal excitability during movement preparation have previously been suggested to contribute to the impairment of RT performance seen in stroke patients (Di Lorenzo, 2013). Only two studies have reported single pulse M1 responses to TMS during movement preparation within a simple reaction time paradigm for stroke patients. In addition to demonstrating alterations in SICI at rest and in movement preparation, Hummel et al (2009) provided data demonstrating the paretic hemisphere displayed a similar rise in mean excitability to healthy controls. Their study, however, examined patients who had recovered exceedingly well from their initial vascular event, with the mean RT performance for stroke subjects being lower than the mean for healthy controls (though not to a statistically significant degree). Battaglia et al (2006) reported on a cohort of

cerebellar stroke patients who did display differences in warned simple RT performance between paretic and non-paretic sides, and also when compared to healthy controls. In keeping with the known facilitatory dentato-thalamo-cortical drive to the contralateral primary motor cortex (Di Lazzaro et al, 1994; Meyer et al, 1994; Grimaldi et al, 2014), subjects with a cerebellar stroke displayed a reduced late premovement rise in mean MEP amplitudes in the contralesional M1 when movements were being made with the ipsilesional hand. Whilst ataxia scores appeared correlated with reaction time, a direct correlation with mean excitability changes had not been examined.

Our earlier findings had noted a reduction in TMS MEP variability prior to a rise in mean M1 excitability during movement preparation, with the decline in MEP variability being predictive of RT performance. Several authors have noted an increase in resting MEP variability seen in stroke patients (Butler et al, 2005; Koski et al, 2007; Wheaton et al, 2009), though a closer look at specific details suggests that this is not a consistent finding across all subjects. However, in light of our own findings in healthy controls, we speculated that the difference in RT performance between stroke patients' paretic and non-paretic sides might be influenced by their respective abilities to modulate MEP amplitude variability during movement preparation. Specifically, we postulated that within preparation for a simple reaction time-paradigm a reduced rate of decline in MEP amplitude variability might be seen in stroke subjects' lesional hemispheres when compared to the contralesional hemisphere. Furthermore, the difference in premovement control of MEP amplitude

variability might explain some of the difference in RT performance between paretic and non-paretic sides.

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8.2 Methods

8.2.1 Patient profile

A total of 12 patients were included in this study. All were recruited through the National Hospital for Neurology and Neurosurgery (London, UK). Each had suffered their first primary ischemic cerebrovascular event with resultant upper limb weakness (defined as grade 4 or less by the Medical Research Council (MRC) Scale for Muscle Strength in at least one muscle group). Exclusion criteria included 1) history of post-stroke epilepsy, prior major neurological or psychiatric comorbidity, 2) the ongoing use of psychotropic medication, 3) dysphasia sufficient to compromise the process of informed consent, 4) significant visual field defect or neglect which would render subjects unable to perform the simple reaction time task using the visual imperative cue and 5) co-concomitant acute medical illness. In accordance with the Declaration of Helsinki each subject gave full and informed written consent, and the experiment was approved by the local ethics committee.

8.2.2 Clinical rating scales

Clinical assessment of patients involved the use of standardized procedures as described below, and were completed for both the paretic and non-paretic sides:

Power grip strength. In each patient we assessed their maximum grip strength using a power grip dynamometer (Biometrics Ltd., UK) – for each side six measures of maximal effort were taken with the best result recorded for further use.

Action Research Arm Test (ARAT). The ARAT was administered according to the protocol described by Hsieh et al (1998) using the same table base height across all subjects (Hsueh et al, 2002).

Nine-Hole Peg Test (9HPT). For each subject the 9HPT was administered according to the methods described by Noskin et al (2008) – briefly the task is a validated measure of finger dexterity and requires subjects to use a single hand to move nine pegs from a shallow container into the nine allotted peg holes. For our data set we used the total time recorded and converted this into the number of pegs per second.

Box and Blocks (BnB) Test. The BnB test, administered as described by Mathiowetz et al (1985), entails subjects using a single hand to move 2x2x2cm wooden blocks from one large open container, over a divider 15 cm high and into a second open container – subjects are asked to move as many blocks as possible within a 60 second timed period.

In determining hemispheric dominance for each subject we utilized the Edinburgh Handedness inventory (Oldfield et al, 1971).

8.2.3 Transcranial Magnetic Stimulation

TMS was performed using a Magstim 200^2 stimulator, with monophasic output delivered through a figure of eight coil with 70mm internal wing diameter (Magstim Model D70). One hemisphere was tested at a time, the order randomised across subjects. In each subject EMG electrodes were applied over the contralateral FDI in a belly-tendon montage with raw EMG signal recorded as described in the general methods chapter. After mapping out the FDI hotspot for the hemisphere the site was marked off on a secured cap for later retargeting. We then titrated stimulator output

to achieve a resting FDI MEP that was reliably clear of threshold, aiming for a peak to peak amplitude of approximately 0.5-1.0mV. This stimulator intensity was recorded for later reuse.

8.2.4 Behavioral task

Subjects were trained to perform an isometric power grip task during which TMS could later be delivered. We had chosen this task as it required little dexterous skill and our clinical cohort were anticipated to have significant impairments in dexterity. The power grip dynamometer was individually adjusted for each subject so that the primary action occurred across the metacarpal-phalangeal joint when in mid-range, conditions under which a significant role for FDI in the generation of force could be assured. The maximal power grip force was recorded as the best of six trials. Subjects were then trained to perform a rapid (from onset to peak power less than 200ms) power grip task at 10-20% of their maximal effort within a simple reaction time paradigm (using a 1cm2 green LED placed in the center of each subject's field of vision for visual "Go" imperative).

When the task could be reliably and consistently reproduced TMS recordings during the task were commenced. Prior to recording, we first reconfirmed the accuracy of the earlier recorded hotspot and stimulator intensity. TMS pulses were delivered in-task at timings of -300ms, +60ms, +90ms and +120ms with respect to the "Go"-signal. Recordings were undertaken in blocks of trials with each "Go" signal delivered at between four to six seconds, randomized to prevent anticipation. Trials were semi-randomized to recorded 10 evoked responses at each of the TMS timings, randomly

interspersed with 10 additional trials without TMS to determine subject reaction time, giving a total of 50 trials per block. Following one training block to familiarize subjects to performing the task with concurrent TMS, three blocks were recorded per hemisphere (giving 30 trials for each condition) and then the entire procedure was repeated on the contralateral side. The choice of initial hemisphere targeting was randomized for each subject.

8.2.5 Statistical analysis

For each subject recorded data was processed offline. Each recorded EMG sweep was individually inspected and trials with obscuring artefact, non-task behaviour and premature EMG activity/responses were excluded from further analysis. In each subject we derived the FDI MEP amplitude mean and Gini coefficient for the four TMS time points, separately for the affected and unaffected hemisphere. Furthermore, using the non-TMS catch trials we determined each patient's median reaction time and reaction time variability (again, using the Gini coefficient), separately for each side.

Our earlier experiments had suggested that the decline of the Gini coefficient occurred in a linear manner. As such, to allow for easier comparison between hemispheres we determined the rate of change (across TMS time points) in the Gini coefficient using a slope function across time (ms), with the baseline (-300ms) time point treated as 0ms. TMS time points were denoted along the horizontal axis (x) and the corresponding Gini coefficient value along the y-axis.

Within this linear model, the least squares method could be utilised to determine slope (b) as follows:

$$b = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sum(x - \bar{x})^2}$$

The Gini coefficient is free of unit notation, and as such the units for the slope measure are given as ms^{-1}.

With respect to the clinical assessment scores, all tasks were completed on both affected and unaffected sides and normalized through expression as a percentage measure of the unaffected hemisphere (i.e. lesioned hemisphere score divided by the un-lesioned hemisphere score and the result multiplied by 100) for each test.

Data across all subjects was collated in Excel (version 15) and SPSS (version 21) used to complete analysis using non-parametric tests (as described in the general methods section) where possible. Repeated measures comparisons across two conditions utilized the Wilcoxon Signed Ranks Test. For repeated measures across more than two conditions (e.g. mean MEP amplitude or MEP amplitude Gini coefficient), a Friedman's analysis was utilized, and post-hoc testing testing conducted using the Wilcoxon Signed Ranks Test. Correlation analyses utilized Kendall's Tau. The p-value was set at 0.05.

8.3 Results

8.3.1 Subject profile

Clinical details for patients are provided in table 8.1. The patient group consisted of nine males and three females, with ages ranging from 30-86 (mean age of 54.2 years) all but one of whom were right handed. On average the time since each patient's stroke was 29.7 months (range from one month to 9 years and 2 months). The site of stroke involved cortical areas in all but two subjects - one a capsular lacune, another a ventral pontine infarct. Haemorrhage complicated the primary ischaemic event in two patients, one following reperfusion post thrombolysis. Our cohort of stroke patients had a mean mRS of 2.9 (range 1 to 4) at the time of assessment.

In two subjects, whilst an MEP could be elicited on the affected side, the elicited potential was of low amplitude and frequently dropped below threshold despite maximizing stimulation intensity. Given the susceptibility to floor effects on variability under these circumstances we elected not to include data from the lesioned hemisphere in these subjects. However, their data are still retained for calculations involving the unaffected hemisphere.

Subject #	Age (years)	Gender	Handedness	Time since stroke	Site of Stroke
1	40	Male	Right	1 yr 6 mo	Right MCA
2	62	Male	Right	7 yr	Right MCA
3	63	Female	Right	1 yr	Right MCA
4	64	Male	Left	6 yr 6 mo	Right MCA
5	55	Male	Right	9 yr 2 mo	Left IC
6	30	Female	Right	2 yr 11 mo	Left MCA
7	45	Female	Right	1 mo	Left MCA with haem. txf
8	46	Male	Right	3 mo	Multifocal right PCA, BG, Thal
9	86	Male	Right	3 mo	Right MCA
10	46	Male	Right	3 mo	Left MCA/PCA with haem. txf BG
11	64	Male	Right	1 mo	Left MCA/ACA + MCA/PCA watershed
12	56	Male	Right	2 mo	Right ventral pons

Table 8.1 Patient clinical profile. Abbreviations as follows – ACA = anterior cerebral artery, MCA = middle cerebral artery, PCA = posterior cerebral artery, IC = internal capsule, BG = basal ganglia, Thal = thalamus, Haem. txf = haemorrhagic transformation; yr = year, mo = months.

8.3.2 Clinical indices

Each patient's functional ability in the upper limbs had been assessed using the ARAT, 9HPT, the BnB test, and grip strength. As shown in the box plots of figure 8.1, a clear reduction in performance in seen on the paretic side for all assessments. A significant difference between the paretic and non-paretic upper limb performance was seen for each of the 9HPT (Z=-2.312, p=0.021, PSDep=0.917), BnB (Z=-3.059, p=0.002, PSDep=1.0), power grip strength (Z=-2.589, p=0.01, PSDep=0.917) and ARAT(Z=-2.023, p=0.043, PSDep=0.417) assessments, though the ARAT was complicated by a ceiling effect.

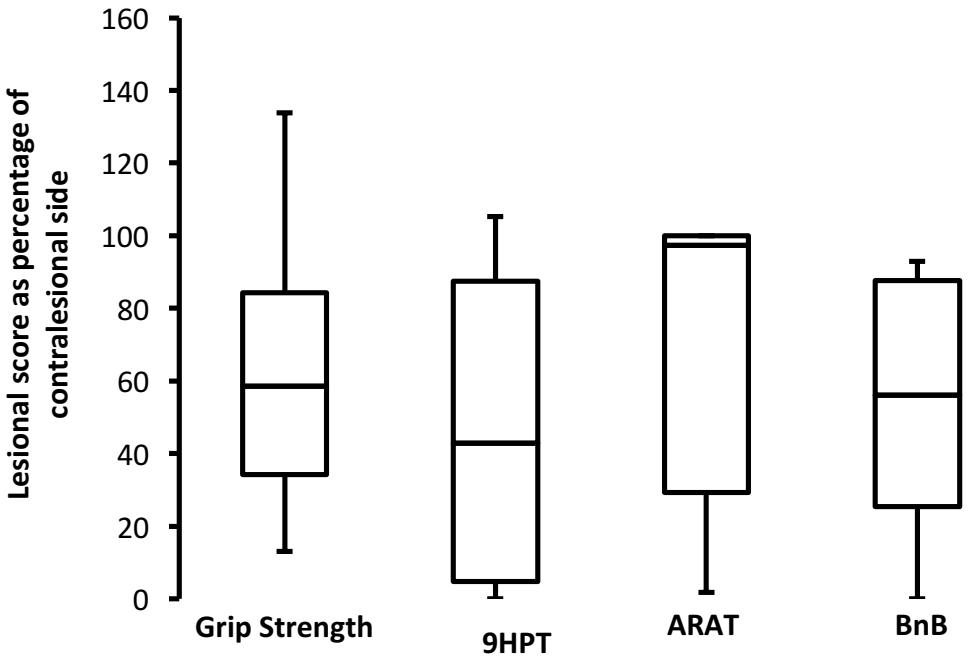

Figure 8.1 Box plot of clinical scores for patients including maximum grip strength, 9HPT, ARAT, and BnB test. Data displayed here show only the affected side (normalized as a percentage of the unaffected side) with centre lines representing the median result, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima.

8.3.3 The lesional hemisphere

In the lesional hemisphere performance for the power grip task demonstrated an average median RT of 269.6ms (range 163.8-539.8ms) across all subjects. Collating TMS MEP results from all subjects in whom an FDI MEP could reliably be obtained, we examined whether FDI MEPs demonstrated a reduction in amplitude variability across time points from baseline toward movement execution. The change in the FDI MEP amplitude Gini coefficient across time points demonstrated a visual trend towards reduction as shown in figure 8.2.a. The Friedman's analysis, though close, was not significant with χ^2 (df=3, N=10)=6.960 and p =0.073. Given this borderline p-value an exploratory analysis, using the Wilcoxon Signed Ranks Test, was performed showing a significant drop from -300ms to +120ms (Z=-2.6 p=0.009, PSDep=0.9). Results were not significant for a reduction in variability from -300ms to +60ms (Z=-1.886 p=0.059, PSDep=0.7) or +90ms (Z=-1.376 p=0.169, PSDep=0.6).

Further analysis of this data revealed a significant effect of TMS time point on the mean MEP mean amplitude (χ^2(df=3, N=10)=16.680, p =0.001, W=0.556), and post-hoc testing confirming a rise in amplitude from baseline (-300ms) by the 120ms time point (Z=-2.701, p=0.007, PSDep=0.90), as shown in figure 8.2.b. An earlier small reduction in MEP mean amplitude from baseline is seen at the +60ms time point (Z=-2.090, p=0.037, PSDep=0.80)

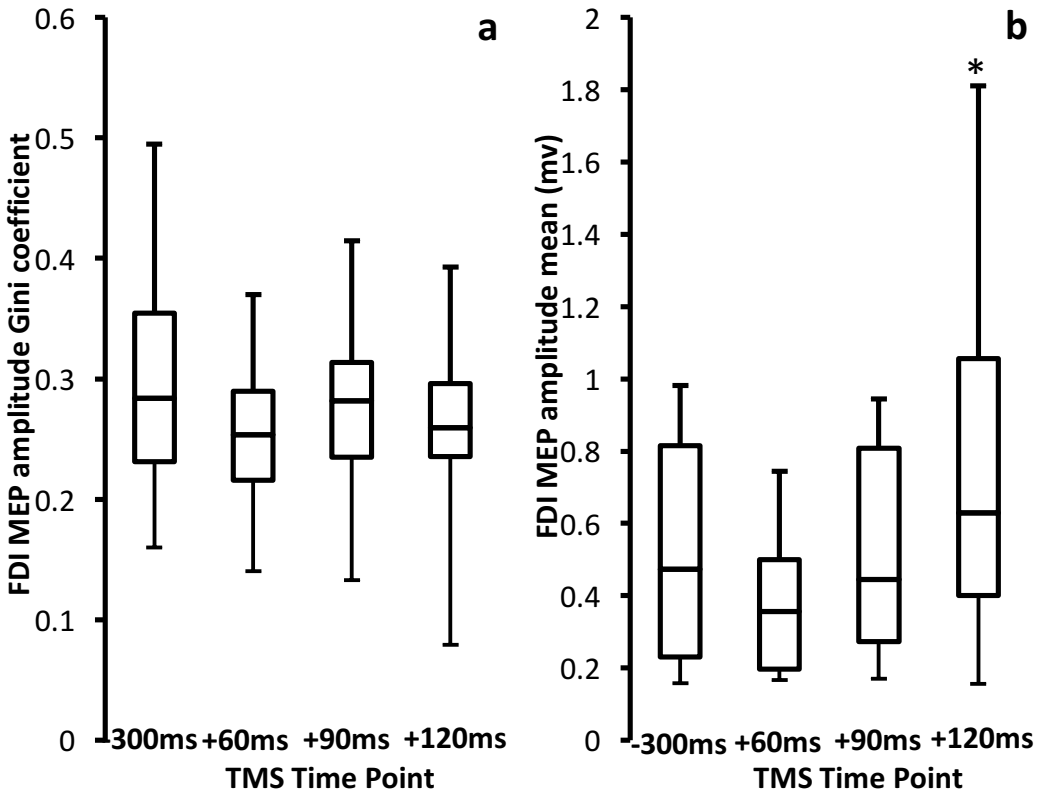

Figure 8.2.a-b Box plot for lesional hemisphere FDI MEP changes for amplitude Gini (a) and mean (b) across TMS time points -300ms, 60ms, 90ms and 120ms. Data displayed for each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. Asterisk (*) denotes a statistically significant difference from baseline (-300ms).

8.3.4 The contralesional hemisphere

The average median RT for the power grip task on the non-paretic side was 224.1 ms (range 154.3ms – 377.4ms) across all subjects. Again, here we wished to assess whether FDI MEPs elicited from the unaffected hemisphere, during movement preparation for the power grip task by the unaffected hand, demonstrated a reduction in amplitude variability. A Friedman's repeated measures analysis across the factor of TMS time point, was significant with χ^2(df=3, N=12)=25.90, p <0.001, and Kendall's coefficient of concordance = 0.719. As shown in figure 8.3.a, post-hoc testing with the Wilcoxon Sign Ranks Test revealed that MEP variability decreased from baseline (-300ms) by the +90ms (Z=-2.903, p=0.004, PSDep=0.909) and +120ms (Z=-3.059, p=0.002, PSDep=1.0) time points, whilst a only a trend toward reduction appeared by the +60ms time point (Z=-1.647, p=0.099).

A similar assessment undertaken for FDI mean MEP amplitude was also significant with a Friedman's analysis across time points showing χ^2(df=3, N=12)=11.9, p =0.008 with the coefficient of concordance, W=0.331. Importantly post-hoc testing demonstrated that whilst a significant rise in mean excitability had taken place by the +120ms time point (Z=-2.589, p=0.01, PSDep=0.833), a rise in excitability was not evident by the +60ms (Z=1.020, p=0.308) or +90ms (Z=-0.157, p=0.875) time points with medians in figure 8.3.b Boxplot hinting at a subtle drop (though not statistically significant) among some subjects.

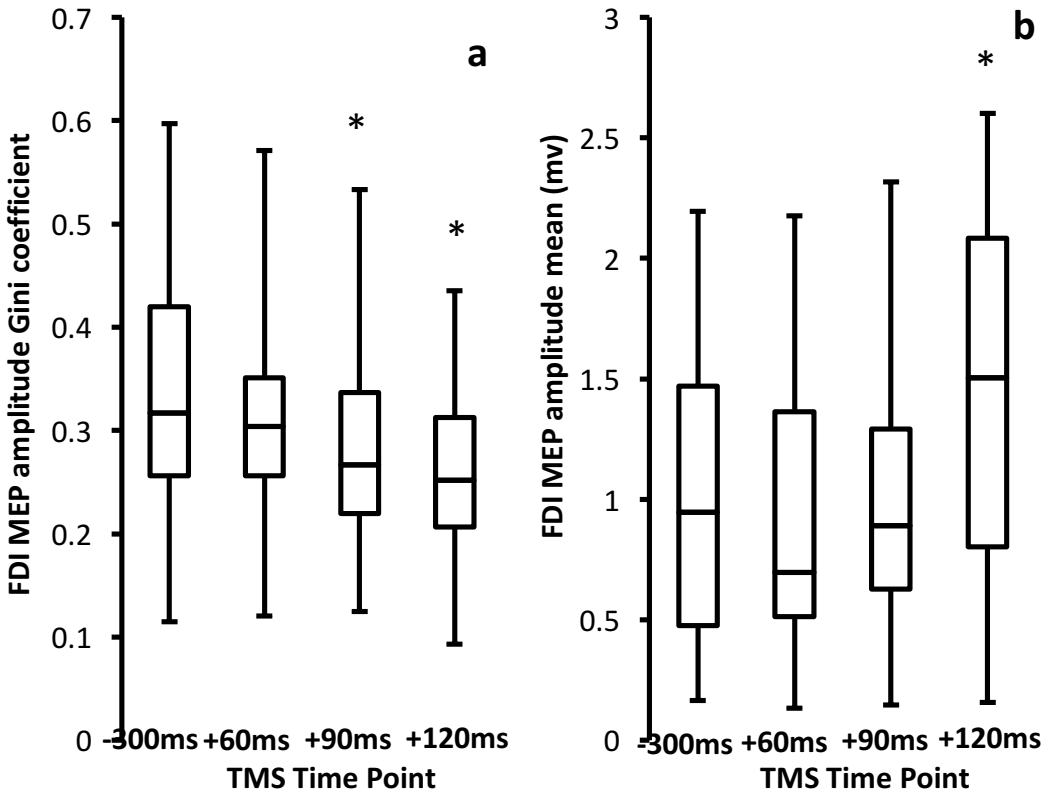

Figure 8.3.a-b Box plot for contralesional hemisphere FDI MEP changes for amplitude Gini (a) and mean (b) across TMS timepoints -300ms, 60ms, 90ms and 120ms. Data displayed for each TMS point with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. Asterisk (*) denotes a significant difference from baseline (-300ms).

8.3.5 Lesional vs contralesional hemisphere

Whilst the differing Gini results for the affected and unaffected side are suggestive of a difference in the change to MEP amplitude variability during preparation for the power grip task, a direct comparison is needed. Friedman's repeated measures analysis is limited to one factor, precluding the addition of a second factor to document hemispheric lesion status. An analysis using multiple comparisons for differences from baseline across each time point would become unwieldy.

Work in an earlier chapter had demonstrated a close to linear decline in MEP amplitude variability during the observed period of movement preparation. Given this, we chose to determine the slope of the Gini coefficient's decline across the recorded time points separately for each hemisphere in all subjects for use in a direct comparison. With the TMS time points placed along the X-axis (and the -300ms time point referenced at 0ms) and Y representing the respective Gini coefficient, we determined the slope for the line of best-fit as describe in methods for this chapter (section 8.2). Preliminary assessment found no statistically significant differences in the mean amplitude and Gini coefficient between the lesional and intact hemispheres at baseline (-300ms).

A direct comparison of the slope of Gini decline in each hemisphere by subject was undertaken (for the 10 subjects in whom MEPs could reliably be elicited bilaterally) using the Wilcoxon Sign Rank Test. As can be seen in figure 8.4.a, a difference was present, with subjects showing a shallower rate of Gini decline in the lesioned

hemisphere when compared to the contralesional hemisphere. The Wilcoxon Sign Ranks Test showed Z=-2.803, p=0.005 and PSDep=1.0.

A similar analysis of the mean MEP amplitude was precluded by work in earlier chapters which demonstrated a 2nd order polynomial relationship with reaction time resulting in a late but dynamic rise in mean excitability. As an alternative we performed a single comparative analysis (lesioned vs intact) of the excitability change from -300ms to +120ms, the later of which is known to show a statistically significant difference in mean excitability for both hemispheres. Using the Wilcoxon Sign Rank Test the result was not significant with Z=-0.357 and p=0.721 (fig. 8.4.b).

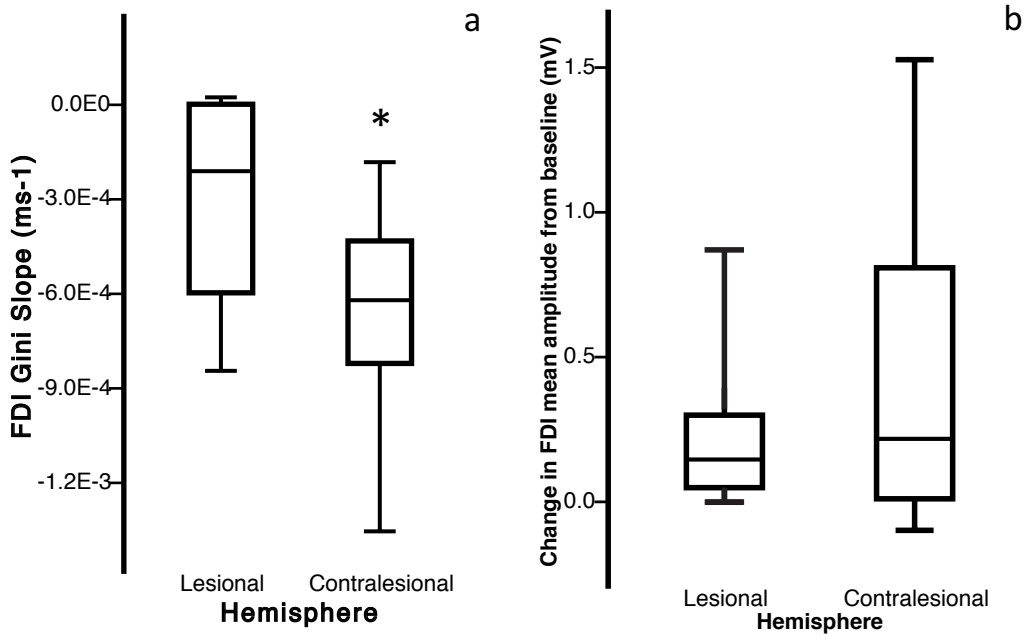

Figure 8.4.a-b Comparison of FDI MEP changes from baseline (-300ms) through movement preparation between lesional and contralesional hemispheres. For FDI MEP amplitude variability (a) the rate of decline in the Gini coefficient across movement preparation is expressed as a slope for the lesioned and unlesioned hemisphere respectively. For the mean (b) the change from baseline to the +120ms time point only is expressed in each subject (expressed as the the FDI MEP amplitude mean at +120ms minus the baseline mean). Data displayed for each side with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. Asterisk (*) denotes a significant difference between sides.

8.3.6 Relationship between task performance and neurophysiological parameters

Having established a significant difference in the slope of Gini decline between the affected and unaffected hemisphere we wished to probe further as to the consequences of this difference. Given the relationship between the decline in the Gini coefficient and reaction times in previous chapters, and a notable difference between hemispheres in patients' pooled median RTs (figure 8.5.a), we anticipated a statistically significant slowing of patient median RTs in the lesional hemisphere. A statistically significant difference within subjects, as assessed by the Wilcoxon Sign Rank Test (Z=-1.961, p=0.05 and PSDep=0.833) was found between the lesional and contralesional hemispheres. In our clinical cohort no difference in task median RT was found on the basis of hemispheric dominance alone (Z=0.652, p=0.515).

To confirm the impact on reaction times we assessed the correlation of the Gini slope with the median reaction time in both the lesional and contra-lesional hemisphere. Figure 8.5.b (lesional) and 8.5.c (contralesional) demonstrate visually that in both hemispheres the faster the median reaction reaction time the greater the slope of declining change in the Gini coefficient. Using Kendall's Tau this relationship is confirmed with a correlation coefficient of 0.600 and p=0.008 for the lesional hemisphere (n=10), whilst in the contralesional hemisphere (n=12) the correlation coefficient was 0.545 and p=0.007. If we were to only utilise subjects in whom MEPs could be elicited in both hemispheres (n=10), then the contralesional correlation coefficient (using Kendall's Tau) rises to 0.644 with p=0.005.

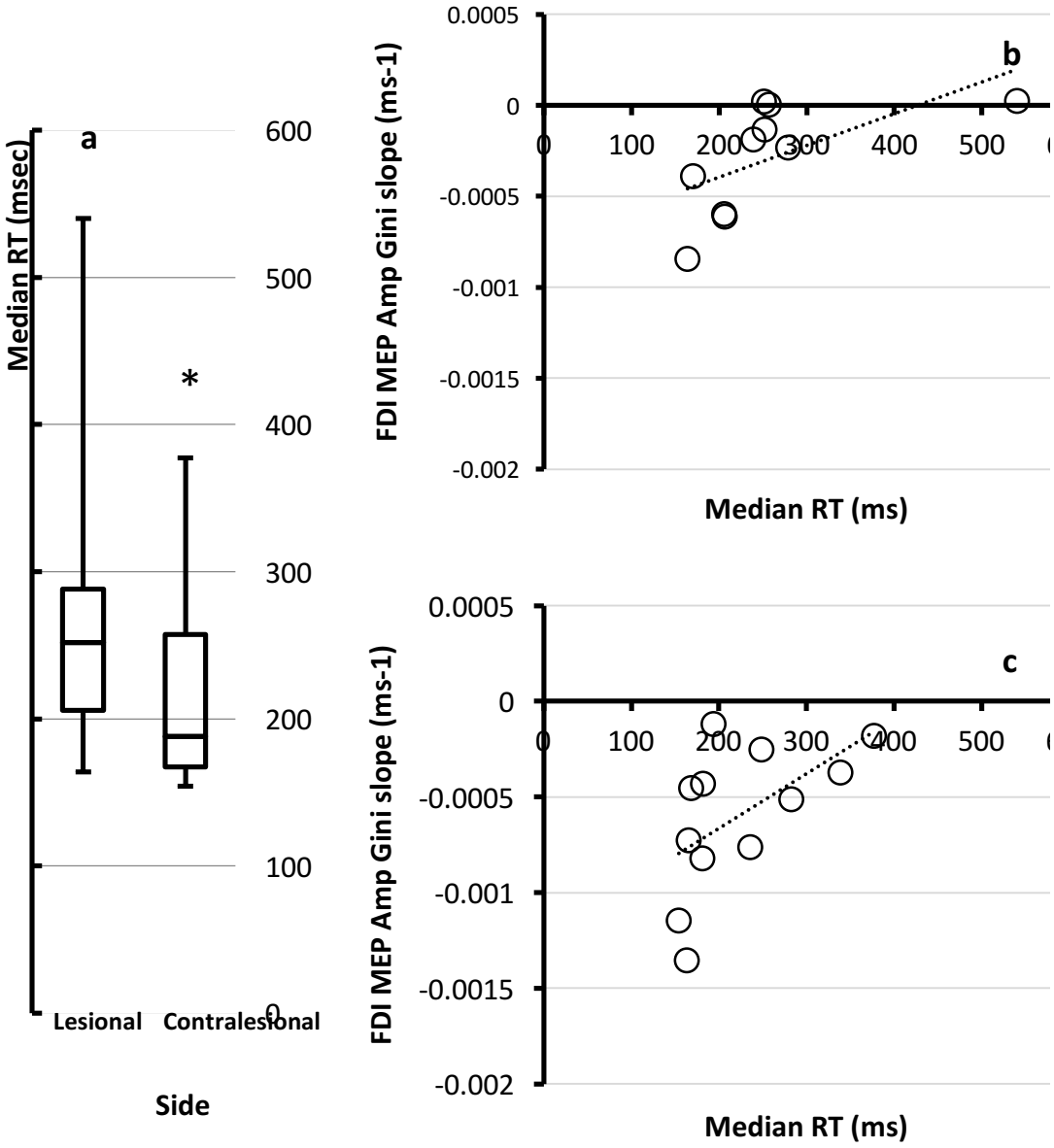

Figure 8.5.a-c Representation of hemispheric median reaction times for subjects and their relationship with slope for the declining Gini coefficient. Median RT are displayed by hemisphere in (a). The relationship of the Gini coefficient slope with the corresponding median RT is shown for the lesional hemisphere in (b) and contralesional hemisphere in (c). For figure 8.5.a data displayed for respective sides with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. Asterisk (*) denotes a significant difference between sides.

Visual inspection of the relationship between the Gini slope and the median RT, suggests a shallower relationship for the lesioned hemisphere when compared to the contralesional hemisphere, i.e. for a given median reaction time the rate of decline in variability will be less in the affected hemisphere than in the unaffected hemisphere.

To further contrast the findings between the affected and unaffected hemisphere we developed a regression model which related the percentage change between the affected and unaffected hemisphere for two variables, the median RT and the slope of premovement change in the Gini coefficient. The graph of resultant values is shown in figure 8.6, and is best fit by a linear model, with an R^2 of 0.504 ($F_{1,9}$=8.134, p=0.021; slope=-0.571 and intercept of 113.6 ($p<0.001$ for both)). A visual inspection of the relationship reaffirms that subjects generally reacted more slowly on the affected side and this corresponded to a proportional reduction in the slope of the Gini coefficient.

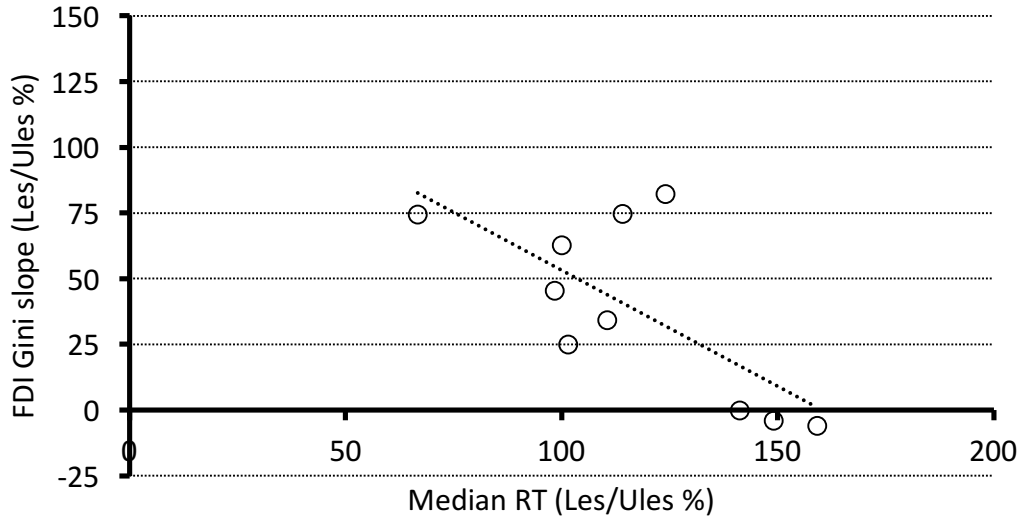

Figure 8.6 Linear regression model relating the percentage change between lesional and contralesional hemisphere for the median RT along the horizontal axis (x = paretic median RT / non-paretic median RT as %) and the premovement Gini slope of decline along the vertical axis (y = lesional FDI MEP Gini slope / contralesional FDI MEP Gini slope as %).

Careful review of the regression model highlights an additional feature – the aforementioned difference in the relationship between Gini slope and median RTs. Consider the point of equivalence (X=100, Y=100; where changes in either slope or the median RT would be evenly matched across hemispheres) and note that the linear model passes beneath this point, confirming that a less substantial decline in the Gini coefficient (lower Gini slope) is potentially seen is in the lesional hemisphere, when compared with a similar median RT for the contralesional hemisphere.

We had earlier shown the lesional hemisphere performed more slowly than the contralesional hemisphere. Whilst this may have contributed to an overall difference in slope it does not account for the difference in the degree of slope required for a given median RT, as highlighted by figures 8.5b-c and 8.6. Essentially a performance trade off should be present.

During training and performance for the power grip tasks subjects were instructed to perform with both speed and consistency. We further assessed the consistency of subject performance by measuring the variability of the reaction time performance. Here again we utilized the Gini coefficient as a non-parametric measure of dispersion for the lesional/paretic and contralesional/unaffected reaction time recordings. Using the Wilcoxon Sign Ranks test to compare the RT Gini coefficient between hemispheres we again find a significant rise in variability on the lesional side with Z=-2.197 and p=0.028 (PSDep=0.917), shown in figure 8.7.

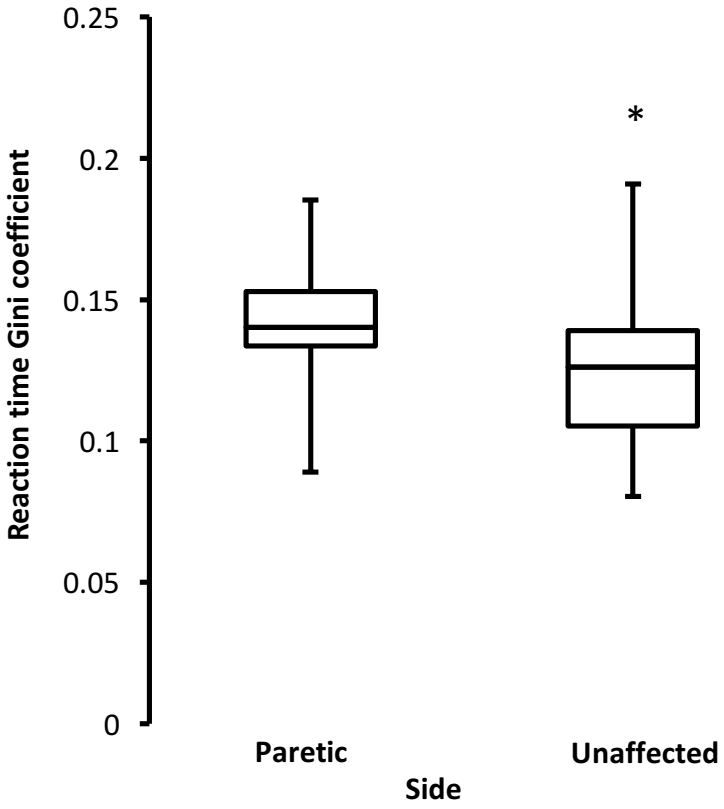

Figure 8.7 Variability of simple reaction time responses (captured using the Gini coefficient) from catch trials without TMS during recording blocks. Data displayed for paretic and unaffected sides with centre lines representing the median, box limits indicating the 25th and 75th percentiles and whiskers extending to the maxima/minima. Asterisk (*) denotes a significant difference between sides.

8.3.7 Correlation between task performance and clinical parameters

We had earlier shown there was a significant difference in the performance in clinical scale parameters between the affected and unaffected hemispheres. Here we wished to assess whether a relationship could be seen between these clinical indicators and cortical function in the reaction time task. As such we again expressed each parameter in the lesional hemisphere as a percentage of the contralesional hemisphere. We then performed multiple bivariate correlation analysis using Kendall's Tau. No statistically significant relationship was present when comparing the Gini Slope to our battery of clinical markers (grip strength, 9HPT, ARAT, Box and Blocks test).

We next used first the time since stroke (in months) and then the subject age in a further batch of bivariate correlation analyses (again using Kendall's Tau) with the clinical scores and the Gini slope. Again no significant relationships with subject age or time since stroke were present.

8.4 Discussion

This study assessed premovement excitability changes for a simple reaction time task, using a controlled brief isometric power grip, in a diverse clinical cohort of ischemic stroke patients who each had experienced upper limb weakness. The key finding is of reduced control of corticospinal tract output variability in the lesional hemisphere. This neurophysiological finding appears to be associated with deficits in speed and consistency of task performance in the paretic hand.

8.4.1 Clinical cohort

All recruited subjects were patients of the National Hospital for Neurology and Neurosurgery, and entered into the study via either outpatient or inpatient settings. It should be noted that in all subjects an MEP was able to be elicited in the paretic limb, a neurophysiological finding associated frequently associated with improved clinical outcomes (Talleli et al, 2006; Stinear et al, 2007). As a consequence, across the cohort functional outcomes were reasonably good, with a mean mRS of just 2.9, and only three subjects with an mRS of 4. The majority of patients in our cohort had lesions involving both cortex and subcortical regions, with only two patients suffering purely subcortical events. Subject mean age for the group was relatively young at 54 years (Hummel et al, 2009; Swayne et al, 2008), though the age range spanned both extremes, from 30-86 years. All subjects were able to perform the behavioral task, with a clearly distinguishable burst of FDI activity associated with power grip dynamometer activation. Functional differences between paretic and non-paretic sides were clearly distinguishable for both the task, and the other clinical indices.

8.4.2 Excitability changes in movement preparation

Corticospinal tract mean excitability in both hemispheres show similar late rises during movement preparation for the power grip task. Of the observable time points only the +120ms time point displays a statistically significant rise in mean MEP amplitude, a finding seen bilaterally. A direct comparison of the change from -300ms to the +120ms time points found no significant difference between the lesional and contra-lesional hemispheres for mean excitability rise.

In contrast the results for MEP amplitude variability during movement preparation appear different for the lesioned and unlesioned hemispheres, with findings suggesting reduced control of variability for the lesional hemisphere during the preparation of movement. MEP amplitudes in the unlesioned hemisphere display a significant drop in variability during movement preparation for the task. In this hemisphere the Gini coefficient shows a trend toward reduction as early as the +60ms time point, with statistical significance emerging by the +90ms time point and continuing through the +120ms time point. The reduction in MEP variability for the intact hemisphere appears well before any rise in mean excitability. Using the slope of Gini decline across time points, a moderate correlation with median RT was shown, consistent with our prior observations in healthy controls.

For the lesional hemisphere, despite the visual trend seen in figure 8.2.a, the Friedman's analysis was close but ultimately did not support a statistically significant decline in MEP variability during movement preparation across time points. An

exploratory analysis did suggest there remains a significant reduction from baseline by the +120m time point in the lesional hemisphere, however this results need to be viewed with caution. When the slope of Gini coefficient change across time points was derived for each subject a relationship with median RT was still observed, again with moderate correlation, preserving the relationship whereby a more pronounced slope of decline was associated with a faster reaction time. It seems likely that a combination of generally slower median RTs, a greater range of median RTs (164-540ms) and a lower number of subjects with reliably elicited MEPs led to the non-significant result for the Friedman's Analysis in the lesional hemisphere. However even if a significant effect were found in both hemispheres, this does not preclude the presence of a difference between hemispheres in the manner in which variability could decline.

8.4.3 Contrasting variability changes between the hemispheres

In both hemispheres the slope of Gini coefficient decline was found to proportionally correspond to the median reaction time for a hemisphere – the more negative the slope the more rapid the median reaction time. During the analysis we noted a visual difference in the relationship between the FDI MEP amplitude Gini coefficient's decline and the median RT between the hemispheres, with the lesioned hemispheres demonstrating a shallower relationship. We subsequently confirmed a reduced rate of decline in the Gini coefficient in the lesioned hemisphere (when compared to the contralesional hemisphere) during movement preparation through a direct comparison.

To some degree this result might be expected – with the loss integrity of integrity for the corticospinal tract and variable loss of higher order and transcallosal inputs, the ability to modulate pyramidal tract output might be expected to be reduced (Murase et al, 2004; Duque et al, 2005; Talleli et al, 2006; Hummel et al, 2009), inline with our hypothesis that supra-spinal elements significantly modulate variability of corticospinal tract output during movement preparation.

Whilst our work did note a difference in median RT between paretic and non-paretic sides, one would not expect this alone to alter the median RT relationship with the slope of decline for the Gini coefficient. Assuming appropriate TMS time point coverage, potentially the same slope could be maintained either side, with median RT points merely shifting along in distribution. Our subsequent confirmation of the reduced slope on the unaffected side, sparked search for a consequence. Instructions for the task had specified for subjects to perform the power grip task as rapidly and consistently as possible. With respect to the latter, one of the observed variables was the consistency of RT performance, quantified by the RT Gini coefficient (derived from the non-TMS RT trials interspersed in recording blocks). A comparison of the paretic and non-paretic sides demonstrated an increased RT Gini coefficient for the paretic side. This result suggests that whilst subjects performed overall more slowly on the affected side, they also performed with greater inconsistency, despite identical amounts of preparation.

Putting together these results suggests that although subjects were able to produce performances on the paretic side with a proportionally lower decline in the Gini

coefficient this was associated with a trade-off, the worsening of their performance consistency. Whilst RT variability is a possible novel marker of performance variability, ideally this result needs to be corroborated through observation of similar variability in other motor performance markers of movement quality for stroke subjects.

8.4.4 Correlation with clinical indices

Past work has suggested a significant correlation of even simple reaction times with clinical indices (Battaglia et al, 2006; Bi and Wan, 2013). Despite the reasonable correlation of reaction time performance with the rate of decline in the Gini coefficient, no correlation could be found when comparing the lesional hemisphere's slope (normalized by the contralesional slope) to our indices of clinical function (paretic function again normalised by non-paretic performance). For our relatively small cohort (10 subjects) no substantial correlation was seen with clinical scales utilized. Whilst the result is disappointing it is not entirely unexpected, given the small number and diversity of clinical subjects involved.

8.4.5 In the context of the literature

The present study provides novel observations regarding movement preparation in stroke subjects through the quantification of MEP amplitude variability. Firstly, TMS revealed an overall decrease in the rate of reduction for MEP variability within the lesional hemisphere across movement preparation. This reduction may be contributory to the reduction in median RT performance and increase RT

performance variability from the paretic side seen in our clinical cohort. The combination of findings is novel and finds no direct parallel in the literature to date.

No significant difference in single pulse premovement mean MEP amplitudes (generated by single pulse TMS) was seen between healthy controls and stroke subjects on their paretic sides in a study by Hummel et al (2009), though their simple reaction time paradigm had applied TMS only in the later half of movement preparation. Furthermore, their study had examined movement preparation in high functioning stroke subjects, whose reaction times were similar to that of healthy controls, whilst our study examined a clinical population with a greater range of debility. Finally, no observations on changes in variability or RT performance between stroke subjects' paretic and non-paretic sides had been made in the study by Hummel et al (2009).

8.4.6 Causative mechanisms

The reduction in control of corticospinal tract variability may be attributed to the utilization of secondary pathways for motor control, a well described phenomenon in stroke subjects (Turton and Lemon, 1999; Ward et al, 2003a; Ward et al, 2003b; Lemon, 2008). Movement preparation utilising secondary pathways may be inherently less efficient (Ward et al, 2006; Werhahn et al, 2003) in achieving the optimum motor control, with resultant performance trade offs.

Several caveats should be raised with respect to the above line of thought. Firstly, it should be recalled that in all clinical subjects for this study a TMS FDI MEP could be

obtained – a key finding placing them among patients likely to have better motor recovery outcomes following paretic stroke (Stinear et al, 2007). Furthermore, while work (Johansen-Berg et al, 2002) has shown that in stroke patients disruption of secondary motor areas (PMd) during movement preparation can compromise performance of even simple human finger movements, only contralesional M1 TMS had been reported on for those subjects, with integrity of the ipsilesional M1 not directly reported in that study. It remains unclear to what substantial degree secondary motor areas contribute to the direct motor control of distal muscles (such as FDI) within simple motor control tasks in patients such as ours, who possess CST continuity, following motor stroke.

Results from our earlier chapters had suggested the possibility that movements were initiated once output variability had been reduced by a pre-specified amount. The current data suggests that among stroke patients the relationship between M1 output variability and movement initiation has been altered. Observations for the lesional hemisphere show that in fact less control of variability is seen in practice for a given RT performance.

Other possibilities exist which may explain the data we show here. A vascular event causing motor weakness might reduce the M1 output projections available to prepare for a movement, which in turn will lead to an increase in noise within the motor system and/or the reduced ability to control noise within the system. As a consequence, reduced control of M1 output variability during the preparation of movement following stroke may necessitate a compromise (learned or otherwise),

that of the reduced control of final output variability (with consequent reduced fidelity to the desired movement) prior to the release of a movement.

8.4.7 Parallels in non-human primate studies

Previous work by Afshar et al (2011) had explored the origins of trial-to-trial performance variability through recordings of M1 and premotor cortex neuronal firing rates in primates during a delayed cued arm reach task. They found a tendency toward slower task performance on individual trials the further individual neuronal firing rates were from their optimal movement-specific subspace. Our work here has no direct corollary – the use of population measures for excitability (the MEP) and the measurements of variability requiring multiple trials (our measure of dispersion, the Gini coefficient) preclude such a comparison. Yet our work might allow for an extension of Afshar et al's (2011) hypothesis, suggesting that task relevant neuronal populations may require regulation into a more cohesive whole prior to their coordinated release towards the final goals of a motor program. When that process is impaired, as in the case of our stroke subjects' lesional hemispheres, movement may be reproduced with less fidelity.

8.4.8 Implications and future work

Our small cohort of stroke patients displayed a diversity of clinical lesions including direct M1, capsular and pontine disruptions of the corticospinal tract. Future cross sectional studies with larger clinical cohorts may be able to focus on delineating contributory roles at each of these levels. In turn a more complete assessment of any relationship with clinical outcomes and function may be better determined. Similarly,

the longitudinal observation of changes in the relationship between RT performance (and other markers of movement quality) and the premovement control of MEP variability may aid our understanding of the evolution of adaptive and maladaptive motor control processes following stroke in both the sub-acute and chronic settings. Contrasting the results of either of these experiments with those of healthy age matched controls will aid in the identification of pathological process.

Chapter 9 General Discussion

9.1 Our main findings

This thesis has sought to assess how the process of movement preparation affects the variability of TMS MEP amplitudes, mean amplitude being an established marker of corticospinal tract excitability. In the setting of precisely repeated movements it seemed reasonable to suggest that variability of corticospinal tract excitability would be progressively reduced across the period of movement preparation. Utilising TMS we demonstrate that MEP amplitude variability stabilizes during the reaction time fore period for simple human finger movements.

In the first experimental chapter we demonstrate that changes in MEP amplitude variability at rest are not predicted by spontaneous changes in mean MEP amplitude, when stimulation intensity is fixed. However, we do demonstrate that changes in stimulation intensity can drive changes in MEP amplitude variability, a factor we successfully utilize to mitigate the affect of distance from the cortical hotspot. Various statistical indices of dispersion are explored as assessments of MEP amplitude variability, allowing us to select the Gini coefficient due to its non-parametric nature, relative robustness to extreme outliers and utility in the setting of randomized data acquisition.

The second experimental chapter assessed TMS MEP amplitude variability during the reaction time period for simple index finger abduction. We noted a decline in MEP amplitude variability in the agonist muscle (FDI) whilst a nearby uninvolved muscle (ADM) was relatively unaffected. This decline in variability appeared earlier and was greater in subjects who reacted faster for the task. We next utilised a different

paradigm, a synchronous bilateral button press task, that allowed for a trial by trial correction by reaction time speed, and demonstrated a progressive linear decline in FDI (synergist) MEP amplitude variability which preceded a later rapid rise in mean MEP amplitude. Again neither of these changes were seen in a nearby but uninvolved muscle (ADM).

Our third experimental chapter highlights that a concordant decline in MEP amplitude variability can be seen across two involved intrinsic hand muscles (FDI and ADM) during movement preparation for a power grip task. In this same chapter we utilize the phenomenon of motor surround inhibition to demonstrate that a surround muscle, tasked with deliberate inactivity, can demonstrate a decline in MEP amplitude variability whilst also seeing a decline in mean excitability. Moreover, changes in both mean amplitude and amplitude variability during the premovement phase were predictive of states seen at the moment of movement initiation.

Chapter seven examined whether changes in force and task complexity individually impacted upon the premovement decline in MEP variability. Contrasting high and low force conditions within the power grip task did not reveal a significant difference in the degree of premovement MEP amplitude variability decline though there appeared to be a trend toward a greater rise of mean excitability in the high force task. A comparison of power grip vs pincer grip tasks also failed to demonstrate a significant difference across tasks for the premovement decline of MEP amplitude variability. Both results would suggest that the primary relevance of reaction time changes in MEP amplitude variability relate to its reflecting the chronometric

progression of movement preparation rather than other aspects of corticospinal tract control, at least within our specific experimental paradigms.

Our final experimental chapter assesses MEP amplitude variance during movement preparation in stroke patients for a power grip task, contrasting the lesional and contralesional hemispheres in a heterogeneous clinical cohort. We observed that a premovement decline in variability could be seen in both hemispheres (though more reliably in the contralesional hemisphere) and both hemispheres retained a relationship with RT performance such that more rapid declines in variability were observed in patients with faster performance. Changes in MEP amplitude variability were seen prior to a mean rise in excitability for the contralesional hemisphere.

Overall, findings in the lesional hemisphere appeared to show a lower rate of decline and this was associated with slower median RT performance. However, there was an asymmetry (between hemispheres) in the relationship of the rate of decline in variability with RT performance – movement preparation by the lesional hemisphere appeared to require a lower rate of decline in MEP variability for a given RT performance. Further analysis suggested an increased variability in RT performance for the paretic side (lesional hemisphere) – this may have limited the sensitivity of the analysis or alternatively represent a novel marker of performance degradation. Though the decline in MEP amplitude variability correlated well with task performance, correlations with other clinical indices were not found, most likely as a consequence of the small and heterogeneous clinical cohort.

9.2 Unanswered questions, limitations and possible future works

The results of our experimental chapters provide reasonable support for our primary hypothesis that TMS MEP amplitude variability in task relevant muscles declines across the reaction time fore period for simple human finger movements within the simple reaction time paradigm. However, pertinent to this matter several questions remain unanswered.

Our studies focused on probing corticospinal tract excitability relatively early during the reaction time period – from 50ms to 120ms following the go command, which amounted to observations between approximately 15-65% of the reaction time period. Shifts towards reduced variability were seen as early as the +50ms time point with later time points demonstrating reductions of approximately 30% in variability. Observations in agonists at time points beyond this window have not been made within this thesis, complicated by intrusion of the MEP waveform into movement and conceptually clouded by the concomitant rise in mean excitability.

One approach to this problem was exemplified by Klein-Flugge et al (2013). Reanalyzing MEP data obtained from a previously published choice reaction time paradigm, they transformed MEP variability observations generated from the original experiment by referencing against data (describing the relationship between mean amplitude and variability in a separate cohort) obtained from input-output curves in the resting state. Whilst this is a reasonable approach, the challenge is that not all change in MEP amplitude variability is driven by changes in MEP mean amplitude (as suggested in chapter 4). Additionally, it is possible that the relationship

between MEP amplitude mean and variability may differ across individuals, just as is the case with respect to SR curves and mean amplitude (van der Kamp et al, 1996). Finally, results in chapter six suggest that within movement preparation the "traditional" relationship between mean amplitude and amplitude variability can be altered, whereby a decrease in variability can be seen with a concomitant decline in mean amplitude.

Despite the above focus on mean amplitude, it would also be reasonable to suggest that both the decline in MEP amplitude variability and subsequent rise in mean excitability may be separate processes, initiated during the preparation to move. Within this thesis our focus has been on the relationship with the speed of task performance, as evidenced by the median RT. We have suggested that the decline in MEP amplitude variability across preparation for a given movement reflects stabilization of CST output. This, in turn, may allow for the more accurate reproduction of a desired movement. Future work could contrast the decline in MEP amplitude variability during movement preparation against other measures of movement quality. One such performance measure could involve subject's ability to precisely reproduce a specific force target (in contrast to the wider windows we use here) during isometric index finger abduction. In general, one might expect the ability to modulate premovement MEP variability to be reflected in a subject's ability to more consistently achieve the desired force target. In a complimentary approach, this work might also be extended to training paradigms, where as a task was learned (and the speed-accuracy relationship improved), a more pronounced decline in variability may be seen.

Whilst we have suggested that the variability of corticospinal tract output (i.e. MEP amplitude variability) is stabilized during movement preparation the mechanisms by which this might occur have not been directly examined by this thesis. One possible influence initially mooted within an early chapter was of a moderating influence by the premovement silent period. Several authors (Mortimer et al, 1987; Aoki et al, 2002) had suggested that the premovement silent period (a cortical inhibitory origin for which had been demonstrated by Aoki et al in 2002), a phenomenon seen when a rapid ballistic movement was initiated from a pre-activated muscle, in effect aided in ballistic movement preparation by bringing cortical neurons into a similar initial state of excitation. Whilst we saw a small dip in mean excitability coincident with the initial decline in variability for our chapter five experiments (finger abduction and bilateral button press experiments) this finding was not replicated across other experiments. The very specific nature of the phenomenon of the premovement silent period (variable and transient premovement EMG inhibition within a pre-activated muscle prior to ballistic activation) may reduce its relevance to the more general process of corticospinal tract output stabilization we see across our experiments.

In assessing excitability, the TMS MEP samples across a wide swathe of the motor system – from the site of stimulation within the primary motor cortex through to the end point, the muscle from which the EMG signal is being recorded (in our case the intrinsic muscles of the hand). Whilst there is potential for influences on MEP variability at every level, the changes in premovement MEP variability we see here are consistent with a primarily cortical origin. Our traditional understanding of

fractionated finger movement control in primates is dominated by the influence of the primary motor cortex and its output through the CST. Though there is an emerging appreciation for the complimentary role of other pathways (e.g. the reticulospinal tract) in coordinating hand movements (Baker, 2011; Honeycutt et al, 2013) it is still reasonable to focus on the control of CST output as the likely exclusive site of modulation during the preparation of individuated finger movements. Whilst spinal and peripheral reflex circuits have a role in modulating and maintaining movements once a movement is initiated a significant role does not appear to be present during preparation for initiation of individuated finger movements (Takei and Seki, 2010; Pierrot-Deseilligny and Burke, 2012). Furthermore, assessing variations of excitability in the motor neuron using F-waves has been shown to be unreliable (McNeil et al, 2013), and the H-reflex is not reliably elicited in the intrinsic hand muscles we examine here, though it should be noted that past works have suggested a lack of correlation between H-reflex and TMS MEP variance (Kiers et al, 1993). Our earlier experiments highlight that by implicit instruction the control of variability (and mean excitability) can be modified, further emphasizing the primacy of the cortex in generating the variability changes we see here.

Along this line, given the known influence of cortical oscillations on the amplitude of MEP response (Romei et al, 2008; Sauseng et al, 2009; McAllister, 2012; Keil et al, 2014), it is possible that the co-utilisation of TMS and EEG recording may help further delineate the cortical origins of the premovement decline in MEP variability. Leocani et al (2001) noted that the characteristic alpha/beta desynchronisation (also known as event related desynchronisation) seen prior to the onset of voluntary movements

could be seen within the simple reaction time paradigm. Such a study could directly examine whether beta desynchronisation progressed at a similar rate as the decline in MEP variability, and in turn if the rate of decline also paralleled the relationship with reaction time performance seen for MEP variability in task relevant muscles. However, it should be noted that recordings of beta desynchronisation represent activity across a wider swathe of cortex than that which ultimately projects to the target muscle – given that not all muscles show a decline in MEP variability, correlated changes may not be seen, or alternatively if they are seen may merely reflect a parallel phenomenon not directly causal to the decline in MEP variability. Interestingly, recording of local field potentials from subthalamic nucleus electrodes in Parkinson's disease patients have demonstrated that the rate of beta frequency desynchronisation paralleled the reaction time performance within a reaction time paradigm (Kühn et al, 2004). Furthermore, MEG recordings in stroke patients (Rossiter et al, 2014) also noted a relative decline in lesional hemisphere premovement beta desynchronisation when compared to patients' intact hemisphere.

The stroke patients studied within this thesis (chapter 8) were a heterogeneous cohort within factors such as age, stroke chronicity, functional state and lesional site. Across this broad group, however, asymmetries between the lesional and contralesional hemisphere were still seen. A direct comparison to aged matched healthy controls has, as yet, not been made representing a limitation of the current work. In addition to such a comparison, future studies that utilize more discrete

clinical profiles when recruiting patients may aid in understanding the relative role of cortical and subcortical structures in the control of variability.

Given our findings relating to performance and MEP variability changes in the reaction time period in healthy subjects/stroke patients and postulations on the significance of event related desynchronisation in generating these changes, the work of Kühn et al (2004) suggests that the assessment of MEP amplitude variability during movement preparation of Parkinson's disease patients may also provide insights into the pathophysiology of this hypokinetic disorder. Performance variability within a reaction time task has been observed at early stages of Parkinson's Disease (Camicioli et al, 2008). Whilst this pathological performance may have its origins in subcortical structures (Kuhn et al, 2004), these structures can still be expected to exert an influence on corticospinal tract output. The use of a similar neurophysiological paradigm in this population may extend our understanding of contributory motor control pathophysiology in early Parkinson's Disease.

In sum this thesis has provided novel evidence that in simple externally cued finger movements the variance of TMS MEP amplitudes (recorded from task relevant intrinsic hand muscles) progressively declines across the early to mid portions of movement preparation. Such a decline in variability occurs independent of and precedes a rise in mean excitability, and can predict motor performance, suggesting that this decline in variability represents an important step during movement preparation. Asymmetries in the control of corticospinal variability during movement preparation are present when comparing the lesional and contralesional

hemispheres of stroke patients and appear to be associated with in-task performance impairments. While several of these findings are novel, they also invite further investigation aimed at understanding the mechanism and imperative for control of this phenomenon. Similarly, potentially useful further work could be undertaken to further explore the implications of a failure to control the variability of corticospinal tract output (during movement preparation) on task performance in both health and disease.

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