Inhibition and Oscillatory Activity in Human Motor Cortex

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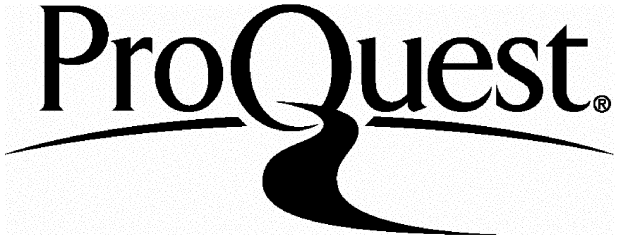

ABSTRACT

Using transcranial magnetic stimulation (TMS) important information can be obtained about the function of motor cortical circuitry during performance of voluntary movements by conscious human subjects. In particular, pairs of TMS pulses can probe inhibitory pathways projecting onto corticospinal neurones, which themselves project to motoneurones innervating hand muscles. This allows investigation of inhibitory circuitry involved in the performance of specific motor tasks, such as the precision grip. Previous studies have shown that pronounced synchronous oscillatory activity within the hand motor system is present at both cortical and muscular level when subjects maintain steady grasp of an object in a precision grip. The origin of this synchronous activity is unknown. However modelling studies have suggested that inhibitory pathways are likely to play an important role in the generation of cortical oscillations, and therefore TMS was used in this Thesis to investigate the origin of synchrony present during the precision grip task.

In the first study, parameters of the paired-pulse test used to measure intracortical inhibition were examined. It was found that by modifying the intensities of the stimuli, and the interval between the paired-pulses, different phases of inhibition could be measured. This enabled specific use of TMS to investigate inhibitory pathways. Both single and paired-pulse TMS were then delivered to the motor cortex of subjects performing a precision grip task. It was found that low intensity TMS could reset the phase of muscle oscillatory activity, consistent with corticospinal neurones being part of the circuitry that generates the oscillatory rhythm. When, in the paired-pulse test, a low intensity stimulus was followed a few milliseconds later with a larger TMS stimulus, in the paired-pulse test, strong intracortical inhibition could be measured. This suggested that inhibitory interneurones activated by low intensity TMS could play an important role in the rhythm-generating network.

An additional study looked at the importance of cutaneous receptor feedback on synchrony, by studying the effects of local anaesthesia of the index finger and thumb. Whereas low intensity TMS was shown to enhance synchronous activity between muscle pairs, suppression of cutaneous feedback from the digits reduced it.

Results in this Thesis suggest that inhibitory interneurones within the motor cortex are important in the generation of synchronous activity within the hand motor system. This synchrony is also under the influence of cutaneous afferent input.

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TABLE OF CONTENTS

CHAPTER 6: LEVELS OF INTRACORTICAL INHIBITION AND EMG-EMG COHERENCE

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Inhibition and oscillatory activity in the human motor system

The motor cortex and corticospinal tract play an essential role in the control of hand function (Lemon 1993). This includes the direct cortico-motoneuronal connection from the cerebral cortex to motoneurones, innervating arm and hand muscles, as well as indirect influences mediated by spinal interneurones. Lesion work performed in monkeys has demonstrated a severe deficit in precise finger movements following damage to the corticospinal tract, highlighting the importance of this pathway in hand function (Lawrence and Kuypers 1968).

The axons of pyramidal tract neurones (PTNs) descend from the motor cortex through the internal capsule and cerebral peduncle. Most of the fibres then project through the pons and medulla before decussating in the lower medulla and descending in the lateral corticospinal tract (Figure 1.1). This crossing of fibres was highlighted by the fact that simulating the motor cortex electrically evoked responses in contralateral hand muscles (Porter and Lemon 1993). Fibres of the corticospinal tract also originate from many other areas of the cerebral cortex. These include the premotor area, supplementary motor area, cingulate areas in the frontal lobe and postcentral gyrus of the parietal lobe. All these brain regions, and additional subcortical areas such as the cerebellum, or basal ganglia contribute to the coordination of skilled hand movement (Lemon 1993). This Thesis focuses on some of the neuronal pathways involved: in particular the cortico-motoneuronal pathway itself, local inhibitory inputs to PTNs within the motor cortex and peripheral afferent pathways which modulate the activity of cortico-motoneuronal connections either at the level of the motor cortex, or at the spinal cord.

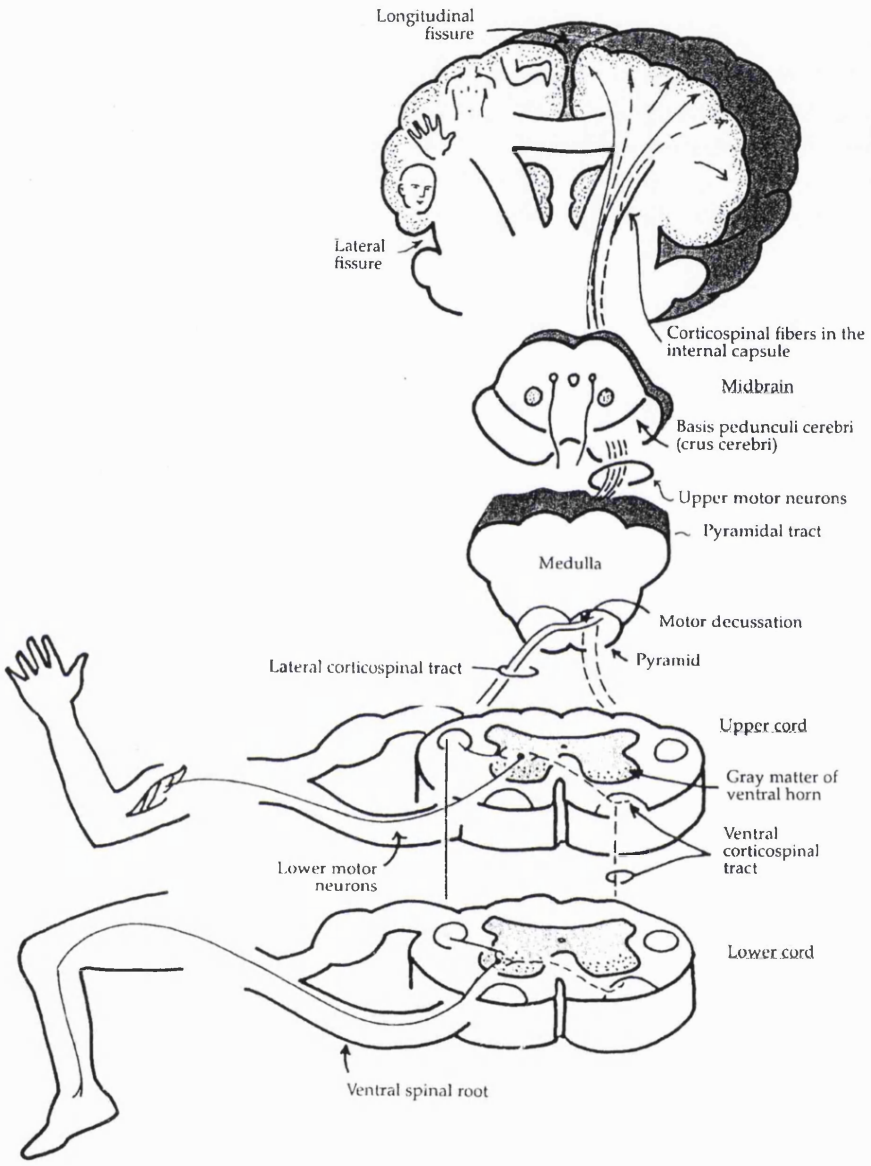


Figure 1.1: Anatomical features of cortico-motoneuronal connections. Axons of pyramidal tract neurones descend from the motor cortex, through the internal capsule and cerebral peduncle, before decussating in the lower medulla. Fibres then descend in the lateral corticospinal tract, and make connections with motoneurones innervating contralateral hand and forearm muscles. Connections to lower limbs are also shown in this diagram. (Adapted from Gertz 1991).

Work in this Thesis involves the use of transcranial magnetic stimulation (TMS) of the motor cortex and corticospinal tract. TMS is a useful tool because it can be used to evoke responses in hand muscles termed motor evoked potentials (MEPs). The amplitude of such responses is related, at least in part, to the level of motor cortex excitability, and can be used to assess the extent to which the motor cortex is activated during a specific hand movement or task. In addition, TMS is used in a clinical setting to measure corticospinal conduction times, or the amplitude and length of activation and suppression of electromyographic (EMG) activity, to gain more information about the pathophysiology of many disease states (Ridding et al. 1995a; Ikoma et al. 1996; Abbruzzese et al. 1997; Ziemann et al. 1997; Greenberg et al. 1998; 2000; Hanajima and Ugawa 2000). It is therefore important to fully understand the nature of the corticospinal pathways activated by TMS. Despite its wide and prevalent use, there is still some doubt as to what TMS evoked responses really tell us about the cortico-motoneuronal system. Part A of this Introduction focuses on ways in which TMS can be used to measure inhibitory pathways within the motor cortex. This is an exciting technique, because information about intracortical circuitry can be gained non-invasively, in conscious human subjects.

Work using TMS often studies the cortical projection to single hand muscle in isolation. However, it is also necessary to consider corticospinal pathways controlling multiple muscles simultaneously, during skilled hand function. Hand movements are achieved by the contraction of many muscles that act on many different joints. This complex process requires peripheral feedback to the cortico-motoneuronal system from muscle, joint and cutaneous receptors and this Thesis focuses on the neuronal pathways involved. Part B of the Introduction describes the use of TMS to assess the importance of peripheral input either at the level of the motor cortex, or at the spinal cord, during simple voluntary activation, or specific motor tasks. This work helps us to understand the importance of peripheral feedback pathways during the manipulation of objects.

It is also informative to compare the EMG activity of hand muscles activated during a specific motor task. Part C of the Introduction explains how detailed analysis of EMG signals has provided evidence of synchronous oscillatory activity present in co-contracting hand and forearm muscles. Comparison of EMG activity with oscillatory activity in the sensorimotor cortex suggests that a functional cortico-muscular coupling exists. Work of this kind is important as it may explain how areas of the motor system communicate with one another. However it remains unclear what neuronal elements or pathways are involved in maintaining synchrony within the cortico-muscular system. Work in this Thesis investigates both the role of cortical pathways using TMS and peripheral feedback using anaesthesia.

PART A: Cortical effects of Transcranial Magnetic Stimulation

1.2 Stimulation of the motor cortex

Stimulating the motor cortex electrically or magnetically can evoke multiple descending volleys in the corticospinal tract. The terms D- (direct) and I- (indirect) waves were introduced to describe the volleys observed when single electrical stimuli were applied to the exposed motor cortex of cats and monkeys (Patton and Amassian 1954). Following on from this, much work has been done in humans to see if a similar mechanism operates during the delivery of transcranial electrical stimulation (TES), and TMS.

This Thesis concerns the effects of TMS of the human motor cortex on contralateral hand and forearm muscles and so this review will focus upon work using similar techniques. However, observations made in animal work and with TES provide useful comparisons.

Particularly relevant to this part of the Introduction are chapters 3 and 6, in which TMS was used to measure inhibition within the motor cortex.

Whereas TES is achieved relatively simply by passing current across two surface electrodes placed on the scalp close to the vertex, TMS requires the use of a special machine called a magnetic stimulator. The magnetic stimulator is a collection of capacitors that can hold charge of up to 4 kV. By an electronic switch mechanism, this energy is rapidly released into the insulated copper coil. This coil is placed on the scalp, over the area of the motor cortex. The current flowing through the coil reaches a peak of 5000 A and generates a magnetic field of approximately 2 tesla. The pulse generated is brief, with a rise-time of 200 μs (Mills 1991). In early studies a circular coil was used to activate the motor cortex. A figure-of-eight, or double-cone coil (types used for work in this Thesis) were then designed to focus the induced current.

1.3 Recordings of descending volleys in monkeys

It is possible to record the descending volleys elicited in the corticospinal tract, when TES or TMS are applied to the motor cortex. It soon became clear that in monkeys and humans, whereas TES evoked similar volleys to those observed by Patton and Amassian (1954), effects evoked by TMS were a little more difficult to interpret.

In experiments performed on anaesthetised monkeys (Edgley et al. 1990; 1997), it was reported that both forms of stimulation could elicit D-waves and I-waves. To concentrate first on effects observed with TES: this stimulation was more effective than TMS at evoking both D-wave and I-wave responses at all levels of intensity. That D-wave responses to both TMS and TES were inversely related to conduction velocity of the axon, supported the earlier observation in cats that D-waves arise from activation of the corticospinal neuron at the axon hillock (initial segment) or nodes of ranvier (Landau et al. 1965). The origin of I-waves remained unexplained. TMS preferentially evoked I-waves, and was not able to evoke a D-wave in slow conducting axons. However a D-

wave response, similar to that evoked at threshold by TES, was evoked in fast conducting axons.

This study also provided evidence that TES even at threshold intensities was able to excite axons deeper below the surface than TMS, as shown by the fact that the volley elicited by TES arrived at the spinal cord earlier (Edgley et al. 1997). When the intensity of TES was increased to suprathreshold levels, there was a step-like decrease in latency of responses. Authors argued that this was due to a shift in activation to a site deeper in the cortex, corresponding in the macaque to the level of the cerebral peduncle. The latency of D-waves evoked by TMS remained unchanged when the intensity was increased (Edgely et al. 1997). It should be noted that this study was carried out in the presence of anaesthesia; this has been shown to affect I-wave responses in both humans (Burke et al. 1993) and monkeys (Baker et al. 1994), and may thus have influenced results.

1.4 Effects observed in humans

The jump in latency of responses evoked with TES (observed with the high intensities in the Edgley study) has also been observed in humans (Nielsen et al. 1995). This study involved recordings from leg muscles. The site of activation of TES was shown to depend on the precise location of the anode. Placing the anode on the vertex, resulted in D-waves with a similar latency to TMS, however, moving the anode more laterally, evoked responses of a faster latency. It was suggested that both stimuli activated the same population of PTNs, but possibly at a different site along the axon. Evidence supporting this came from the observation that a strong anodal stimulus with electrodes lateral to the vertex, could evoke an MEP with similar latency to that evoked by brainstem stimulation.

The presence of multiple descending volleys following TES was highlighted by measuring the force generated in muscles (Day et al. 1987a). The twitch force in 1DI elicited by high intensities of TES greatly exceeded that produced by supramaximal

stimulation of the peripheral nerve and a single antidromic volley from the peripheral nerve was unable to obliterate the cortically evoked response. Results suggested that a single cortical shock could cause repetitive excitation and firing of motoneurones (Day et al. 1987a). A major factor contributing to excitation of motoneurones by cortico-motoneuronal inputs is temporal summation of EPSPs at the motoneurone (Porter and Lemon 1993). In the relaxed state, a single volley in the corticospinal tract results in a single EPSP, which in most cases is not capable of causing motoneurone firing. Normally two or more descending volleys are required. The fact that a single stimulus can elicit a response in relaxed muscle, has been cited as evidence for the existence of multiple descending volleys (Rothwell 1991).

It is also possible to observe differences between TES and TMS effects (complementary to those seen recording descending volleys) in surface EMG recordings. MEP responses to TMS occur at a longer latency than those evoked in the same muscle by TES (Hess et al. 1987; Day et al. 1989). It is important to establish from where the latency difference observed in EMG between TES and TMS arose as this might then provide a clue as to the mechanisms by which electrical and magnetic stimuli excite the motor cortex. Two main factors seem to contribute. First, TES preferentially excites a larger D-wave than TMS, and at least some of this D-wave activity arises from corticospinal axons stimulated deep to the cortex. Therefore motoneurones responding to this input have a short latency (Edgley et al. 1990; 1997). Second, because TMS evokes smaller D-waves (and in some cases no D-wave) temporal summation with I-wave activity is needed before the motoneurone can be brought to firing threshold. This period of summation lengthens the response latency to TMS compared to TES. Studies involving recordings from the epidural space in conscious humans have used a TMS current within the cortex that is posteriorly directed, to evoke only I-waves (Nakamura et al. 1996; Di Lazzaro et al. 1998a,b), whereas a laterally directed current to preferentially evoke D-waves (Nakamura

et al. 1996). This shows that it is possible to use TMS to evoke only I-waves, which would then explain the longer latency of effects observed in muscle.

1.5 Single motor unit recordings in humans

More detailed results regarding the latency of responses evoked by TMS and TES can be obtained by intramuscular needle recordings from a single motor unit, for example in an intrinsic hand muscle. The subject is asked to produce a steady discharge of the single motor unit and both TES and TMS stimulation can then be used to modulate the firing rate of this unit. It has been shown that the motor units in hand muscles activated by increasing intensities of TMS, follow the same pattern of recruitment as those activated by voluntary activity (Gandevia and Rothwell 1987; Bawa and Lemon 1993). This shows that, at least within hand muscles, that recruitment of motor units follows Henneman's size principle (Henneman 1957) even when stimulated artificially. It should be noted that this might not be the case for leg muscles (Awiszus and Feistner 1999).

Information about the effects of stimulation on single motor units can be displayed in a post stimulus time histogram (PSTH). The occurrence of spikes, representing the firing of a single motor unit is represented as time points relative to the stimulus (Mills 1991). Following a stimulus, a rise in firing probability at a latency of ~25 ms (normal conduction time from cortex to muscle) is observed as a peak in the PSTH. This primary peak is followed by a secondary peak appearing around 50–90 ms after the stimulus (Mills et al. 1991). The possible origin of this peak will be discussed in part B of this Introduction. At present attention will be focussed on the primary peak; its short and invariant latency suggest it is due to monosynaptic activation of the single motor unit from the cortex (Day et al. 1989; Boniface et al. 1991).

It is possible to split the primary peak into subpeaks, the presence of which is argued to give further indication of multiple volleys elicited by cortical stimulation. In a study by

Day et al. (1989), anodal TES evoked multiple subpeaks within the primary peak, the first of which was termed P0. Subsequent subpeaks were separated by intervals of roughly 1.5 ms. TMS was delivered with a circular coil, within which current was flowing in an anti-clockwise direction. Note that current induced within the cortex was opposite to this and so in this case was anteriorly directed. This TMS current always elicited a P1 peak and usually a P3 peak, but never a P0 peak. Applying TMS current within the coil in a clockwise direction (directed posteriorly within the cortex) never recruited a P1 peak, but did recruit later subpeaks, In addition at higher intensities with this current direction P0 and P2 peaks were observed (Day et al. 1989).

These results were later supported by a study using a figure-of-eight shaped coil (Werhahn et al. 1994; Sakai et al. 1997). Authors concluded that TMS current within the cortex directed medially or anteriorly preferentially recruited the P1 peak, whereas TMS current within the cortex in the lateral or posterior direction preferentially recruited later peaks. In addition at higher intensities the laterally directed current could evoke an early (P0) response similar to that evoked by TES.

In summary, both electrical and magnetic stimulation elicit multiple descending volleys yet TES appears to have effects of a shorter latency. TES is therefore believed to excite the corticospinal tract directly. Effects of a similar latency can be observed with TMS if the intensity is increased, and the correct current direction is used. That the current direction of TMS within the cortex is important, implies that this determines whether PTNs can be directly stimulated. This is different from electrical stimulation, which evokes both D-waves and I-waves. However correct placement of electrodes in TES is important. In addition increasing the intensity of the TES gains access to deeper levels of the cortex, which are inaccessible with TMS.

1.6 How do TES and TMS stimulate the motor cortex?

The fact that TMS preferentially excites I-waves, but that TES excites D-waves, is best explained by the differences in the neuronal elements excited by the current flow induced in the motor cortex (Rothwell 1991).

To begin first with D-waves: In PTNs giving rise to the corticospinal tract, the action potential is initiated in the axon hillock or initial segment of the axon. This region is close to the soma or cell body of the neurone, and has a high concentration of fast voltage-gated sodium channels. The most effective method of activation of these neurones is for extracellular current to flow along the axis of the neurone towards the cell body and initial segment. This current passes into the PTN via sodium channels, which leads to depolarization and action potential initiation (Stuart et al. 1997).

In monkey experiments, it was suggested that the D-wave elicited by TES or TMS arose from or close to the initial segment (Edgley et al. 1990; 1997). Evidence for this came from comparing the latency of the D-wave volley, with conduction delays defined by other approaches, such as antidromic activation of the PTNs from the pyramidal tract. If the D-wave arises from the initial segment then it might be subject to the ongoing level of excitation and inhibition of the PTN, which would affect the threshold of D-wave activation, but not the latency of effects. This is highlighted by the observation that the D-wave latency is consistent and has little temporal jitter (Kaneko et al. 1996; Di Lazzaro et al. 1998a).

PTNs located in the convexity of the precentral gyrus would be oriented perpendicular to the surface, with their dendrites extending upwards and axons extending downwards. Electric current evoked by TES can flow both vertically and horizontally. The vertical component would thus be ideal to excite PTNs (Rothwell 1991). On the other hand, it has been shown that current induced by TMS is largest at the surface and flows parallel to it (Roth et al. 1991). This would mean that at least at threshold intensities, TMS would be

ineffective at evoking direct responses. Studies have shown that such TMS intensities elicit I-wave responses (Day et al. 1989; Werhahn et al. 1994; Nakamura et al. 1996; Sakai et al. 1997; Di Lazzaro et al. 1998a,b). This model therefore supports the observation that TMS preferentially excites PTNs indirectly. The latency difference observed between D-wave and I-wave responses, or surface EMG responses evoked by TES or TMS could then also be explained in terms of the synaptic delay involved when the corticospinal tract is indirectly excited (Rothwell 1991).

The mechanism by which TMS evokes I-waves will be discussed in some detail in a following section. For now it is important to decide if this simple model can indeed explain the observed effects using TES and TMS. The primary motor cortex lies in the anterior bank of the central sulcus and distal hand muscles are represented in deep convolutions, in which the pyramidal cells are aligned with their long axes horizontal to the cortical surface (Rothwell et al. 1991; Werhahn et al. 1994; Geyer et al. 1996). However, it has been suggested that neurones on the crown of the precentral gyrus may be preferentially activated by TMS, as they are closer to the surface of the cortex. Here PTNs do have a more perpendicular orientation with regard to the surface (Rothwell 1991). This theory does assume however that there are cells in this region that project to the hand muscles. Nothing is really known about the activation of PTNs in the depth of the central sulcus by TMS.

In summary, TES excites PTN axons directly, most likely one or two nodes from the cell body. The latency jump observed when the intensity of TES is increased (Nielsen et al. 1995; Edgley et al. 1990; 1997), represents excitation of TES deeper in the brain, possibly at the cerebral peduncle. TMS preferentially activates the corticospinal tract indirectly, by activating neuronal elements that synapse onto PTNs. At higher intensities, TMS stimuli can directly activate PTN axons to elicit D-waves. Considering the nature of the TMS

current, it is likely that bends in the axon would be preferentially excited (Amassian et al. 1987). It has already been discussed that D-waves could be elicited by TMS if the current direction evoked was changed to a lateral-medial direction. Maybe this current is comparable with that evoked by TES. Another possibility is that the PTNs may themselves be preferentially sensitive to this current direction. This is all speculation, the claims of which have not been tested experimentally. In terms of TMS effects and the generation of I-waves, it is far from clear what effects the current direction of TMS has (Di Lazzaro et al. 2001). This is likely to be due to the complexity of the cortex both in terms of geometry and synaptic organization.

It is clear that TMS can be used to indirectly excite the corticospinal tract and is therefore a useful tool for investigating motor cortex circuitry. Also, if it is accepted that TMS and TES have the same effects at a motoneurone level, then it could be assumed that any effects evoked by TMS that are over and above those following TES, could well be of a cortical nature. This makes a comparison between TES and TMS effects very useful. However it should be noted that above threshold TES also excites I-waves (Edgley et al. 1990; 1997) so when using high intensities of simulation, the TES/TMS comparison becomes less clear.

1.7 Measuring intracortical inhibition with the paired-pulse test

The paired-pulse paradigm was first introduced by Kujirai et al. (1993) as a method of measuring inhibition in the motor cortex. There are two important parameters in the test. These are the intensity of the two stimuli used, and the interstimulus interval (ISI). To measure inhibition, the first stimulus (termed the conditioning stimulus) is set at a subthreshold intensity (no MEP elicited). This is then followed by a larger suprathreshold stimulus (evoking an MEP of approximately 1–2 mV). If the ISI is 1–5 ms, then the conditioning stimulus acts to suppress the response evoked by the test stimulus. Inhibition

is measured by comparing the amplitude of the paired-pulse response, to the MEP elicited by the test stimulus alone.

Kujirai et al. (1993) suggested that inhibition occurred because the conditioning stimulus activated inhibitory pathways within the motor cortex, which make synaptic connections onto the PTNs. The assumption that the effects of the conditioning stimulus were cortical was on the grounds that no EMG response was evoked by this low intensity stimulus. In addition TES as the conditioning stimulus was much less effective at inhibiting the test TMS response, and MEP responses elicited by TES as the test stimulus were not inhibited, by a TMS conditioning stimulus. The cortical nature of the inhibitory mechanism was later proved using epidural recordings of TMS responses in conscious human subjects. A subthreshold conditioning stimulus (which itself had evoked no descending volleys) could inhibit the descending I-wave activity evoked by the test stimulus (Nakamura et al. 1997; Di Lazzaro et al. 1998b). Surface EMG recordings confirmed that this reduction in I-wave activity evoked by the test stimulus, was accompanied by a reduction in the MEP evoked. However, it cannot be directly proved, that all descending volleys recorded in the corticospinal tract were destined for the muscle of interest.

1.8 The generation of I-waves by TMS

It still remains unclear how the conditioning stimulus inhibits I-wave activity generated by the test stimulus. In order to try to understand this mechanism it is necessary to consider current models of I-wave generation. I will begin by stating the model I consider to be the most likely, and a very brief outline of how inhibition operates. This will then be explained in full.

It is possible that the test TMS stimulus activates a population of excitatory and inhibitory interneurones, which synapse onto PTNs. The synchronous activation of these

interneurones by TMS could result in high frequency repetitive firing of PTNs, which results in descending I-wave volleys. The synaptic connections involved in I-wave generation would be subject to inhibitory control, to which the conditioning stimulus gains access.

Perhaps the key to understanding I-waves evoked by TMS is to focus on the remarkable synchronization of volleys. The earliest volley (I_1-wave) occurs 1–2 ms after the D-wave. Following I-waves (I_2-waves and I_3-waves) occur at 1–2 ms intervals. In general I-waves have a more variable latency than the D-wave response between subjects or species (Lemon 2002); but when I-waves are elicited, they always follow this pattern of an average interspike interval of approximately 1.5 ms. However, before any models can be formed it is important to try to establish what neuronal elements do actually synapse onto the PTNs, and which could be stimulated by TMS. It is feasible to suggest that TMS would preferentially activate thick axons; in a study in monkeys it was shown that small neurones could not be directly excited by TMS (Edgley et al. 1997). Candidates would be PTN collateral fibres, or thalamo-cortical projections. However, both these elements were ruled out by a study performed in monkeys and cats (Amassian et al. 1987). I-waves were not evoked by white matter stimulation, thereby proving that antidromically excited collaterals in the white matter did not form part of the I-wave generating mechanism (although it is possible that the very strong recurrent inhibition from these collaterals suppressed any subsequent I-wave discharge). In addition it was still possible to elicit I-waves following lesions causing degeneration of thalamo-cortical afferents (Amassian et al. 1987).

This leaves two other candidates: cortico-cortical afferents projecting to the primary motor cortex from other motor areas, and local interneurones (De Felipe et al. 1986). It has been shown that surface stimulation of premotor and supplementary motor areas

causes large and repetitive I-wave activity in the corticospinal tract (Amassian et al. 1987; Maier et al. 1997). However, lesion studies have not been performed to conclusively test whether these afferents are actually playing the major role in I-wave generation.

Other authors have tended to explain I-wave mechanisms in terms of stimulation of smaller local interneurones (Ziemann and Rothwell 2000). Such neurones are in abundance in the motor cortex (~30 % of the cortical neurones are non-pyramidal interneurones). For simplicities sake, from now on, in order to describe the neuronal elements involved in I-wave generation the term interneurone will be used. It is possible that both inhibitory and excitatory interneurones are involved.

1.8.1 Interneurone chains

Amassian et al. (1987) proposed the theory that following TMS, PTNs are bombarded with activity from a population of interneurones (Figure 1.2A). This population behaves much like a pacemaker, and has fixed temporal characteristics. Such a population of interneurones has been described in thalamo-cortical networks (Blumenfeld and McCormick 2000). However, no evidence exists to show that these networks can operate at frequencies of around 600 Hz (necessary for I-wave generation). An alternative would be that pacemaker cells are involved; a class of pyramidal neurones termed 'chattering cells' has been reported to exist in the cortex; these cells exhibit pronounced oscillations in membrane potential during visual stimulation (Gray and McCormick 1996). However, there have been no reports of such cells in the primary motor cortex.

Other authors have argued that the periodicity of I-waves is caused by activation of independent interneurone chains (Day et al. 1989; Sakai et al. 1997). The time between I-waves is explained by the presence of synapses (Figure 1.2B). Thus an I_1-wave is evoked by interneurones making monosynaptic connections onto PTNs, and I_2-waves and I_3-waves elicited via di- or tri-synaptic connections respectively.

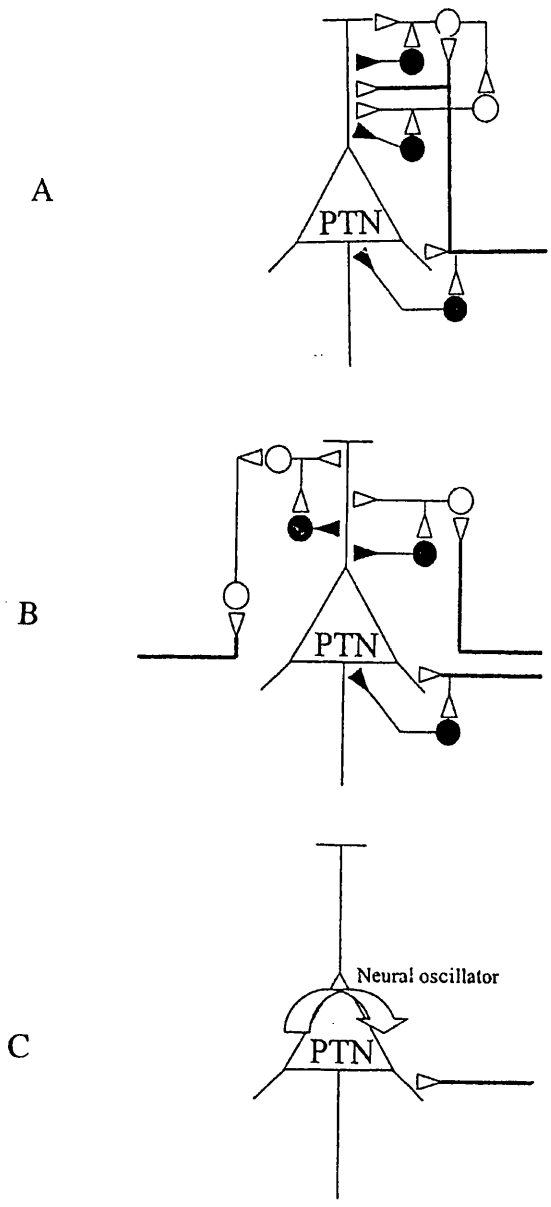


Figure 1.2: Hypothetical models for I-wave generation. (A) TMS may activate a network of inhibitory (black) and excitatory (open) interneurones which synapse onto dendrites of pyramidal tract neurones (PTN). Cell bodies of the interneurones are represented as circles and terminal boutons as triangles. (B) Chains of excitatory interneurones, which synapse onto dendrites of pyramidal tract neurones may be stimulated by TMS. Each chain is under inhibitory control. (C) Following TMS the membrane properties of the PTN may cause it to fire repetitively. (Adapted from Ziemann and Rothwell 2000).

It was shown that cooling of the surface of the motor cortex abolished later I-waves before earlier ones (Amassian et al. 1987). In particular this supports the interneurone chain theory; it is possible that interneurones furthest away (more synapses) from the PTN are closer to the pial surface and are therefore more affected by temperature.

1.8.2 Preferential activation of later I-waves

In addition it has been shown that it is possible to preferentially excite later I-waves depending on TMS current direction within the cortex. That certain orientations are able to avoid evoking the I_1-wave (Day et al. 1989; Sakai et al. 1997; Hanajima et al. 1998) argues that independent chains of interneurones are involved in I-wave generation. However there appears to be a disparity amongst the literature as to which orientation elicits which I-waves. That current direction within the cortex is important has already been illustrated, by the fact that lateral-medial TMS was able to elicit D-waves, unlike posteriorly directed TMS current, which evoked I-waves (Nakamura et al. 1996). This group then went on to use anterior-posterior TMS to elicit I-waves (including I_1-waves), and measure inhibition via recordings of descending volleys in the corticospinal tract (Nakamura et al. 1997). Both Sakai et al. (1997) and Hanajima et al. (1998) in studies using single motor unit recordings, specifically used anteriorly directed TMS to elicit early peaks in the PSTH (I_1-waves). They showed that a posteriorly directed current recruited only later peaks in the PSTH (I_3-waves).

Such studies differ from work done by Di Lazzaro et al. (1998a; 2001), who made epidural recordings of descending volleys. They used posterior-anterior TMS to elicit multiple I-waves including the I_1-wave.

Such disparities are perhaps explained by the observation that the relationship between the size of the descending volleys and subsequent EMG responses were different for anterior-posterior and posterior-anterior TMS currents (Di Lazzaro et al. 2001). It is difficult to know what proportion of the descending volleys are destined for the hand

muscle of interest. In this Thesis, TMS was always used in an anterior-posterior direction. This avoided any preferential activation of later I-waves as shown in single motor unit recordings (Sakai et al. 1997; Hanajima et al. 1998).

1.8.3 Intrinsic properties of the PTN

Other pieces of evidence tend to disagree with the theory that synapses between chains of interneurones explain the timing of I-waves. It has been reported using single motor unit recordings that recruitment of later subpeaks in the PSTH, probably reflecting I_3-waves, had a higher threshold of excitation (Hanajima et al. 1998). This does not agree with the idea that interneurones responsible for these I-waves are closer to the surface of the cortex (as implicated by the cooling study by Amassian et al. 1987).

An alternative theory for the generation of I-waves could be to consider the excitability properties of the PTNs themselves (Figure 1.2C). It is possible that a large and highly synchronous input from a population of interneurones synapsing onto the PTN, may cause it to fire repetitively. This would then explain the fixed interval between I-waves, which arises once the first I-wave has been elicited.

It has been shown in the cat that stimulating corticospinal neurones can cause repetitive discharge at high frequencies (Stafstrom et al. 1984). However, such behaviour following stimulation has not been recorded from PTNs in monkeys. Ghosh and Porter (1988) investigated synaptic effects on monkey PTNs via stimulation of cortico-cortical afferents. EPSPs in PTNs at latencies of approximately 1–6 ms were evoked by stimulation of premotor or somatosensory areas. Following EPSPs was a long inhibitory response roughly 2 ms later and lasting 50–100 ms. The rise-time of EPSPs in cortical PTNs recorded from rat tissue *in vitro* can last several ms (Markram et al. 1997; Thomson 1997). This is not quick enough for repetitive firing approximately every 1–2 ms (as would be necessary to elicit I-waves). Discharge at high frequencies could only be

achieved if EPSPs were interspersed by IPSPs arriving every 1–2 ms (Ziemann and Rothwell 2000). Such inhibitory behaviour has not been shown (Ghosh and Porter 1988). However, the long rise time of excitability of PTNs would also make it unlikely that input from excitatory interneurones at regular intervals (as predicted in the chain or pacemaker theory) could explain I-wave discharge. Inputs arriving after the first had caused the EPSP might prove ineffectual.

Two further points add to the complexity of this problem. It should be noted that a single PTN can give rise to *both* D-waves and I-waves, but D-waves and I_1-waves are rarely seen together (Day et al. 1989; Edgley et al. 1997). Also under 'normal' circumstances PTNs never fire at the high frequencies that would be required for I-wave generation (Maier et al. 1993; Porter and Lemon 1993). The precise neuronal mechanism underlying I-wave generation therefore remains unclear. What is clear is that TMS changes the excitability of PTNs such that they fire repetitively either due to extrinsic interneuronal input, or due to intrinsic 'pacemaker' like properties.

1.9 I-waves and inhibition

Having established some of the possible mechanisms that may be involved in the generation of I-waves by a single TMS stimulus, it is now possible to speculate on how such I-waves could be inhibited. In the paired-pulse test, the subthreshold conditioning stimulus (which does not elicit descending volleys, or at least not ones large enough to produce an MEP response) is able to suppress I-wave generation. Epidural recordings in humans have shown that the I_1-wave is not affected by the conditioning stimulus, whereas I_3-waves nearly always are (Di Lazzaro et al. 1998b). Similar results have been shown in single motor unit recordings by observing the peaks in the PSTH (Hanajima et al. 1998). The preservation of the I_1-wave has led authors to suggest that the conditioning stimulus cannot be directly affecting the PTNs (Di Lazzaro et al. 1998b) but can disrupt the mechanism by which additional waves are formed.

It is important to distinguish between neuronal elements excited by the conditioning stimulus, and those that are activated by the test stimulus. With this in mind, a basic model for inhibition could be as follows. Within the primary motor cortex inhibitory interneurones could have a lower threshold of activation than excitatory interneurones. A similar theory of differences in excitatory and inhibitory thresholds has been suggested in connection with the paired-pulse test (Ziemann et al. 1996c). A low intensity conditioning stimulus could activate inhibitory interneurones. These are then able to inhibit the excitatory effects of the higher intensity test stimulus, which are involved in I-wave generation. It is likely that inhibitory circuits are also activated by the test stimulus. However an imbalance between excitatory and inhibitory effects is created, due to additional inhibitory effects from the conditioning stimulus and this results in suppression of I-waves.

I-wave generation has been explained in terms of synaptic input to PTNs. It is easy to imagine how this system could be affected by inhibition (Figure 1.2B). If the interneurone chain theory for I-wave generation is correct, it is possible that the pathway involved in the generation of the I_3-wave (three synapses) would be more susceptible to inhibition, than that necessary to produce the I_1-wave. Hence the preservation of I_1-waves. It is also possible that the effects of the conditioning stimulus alter input to the PTN in such a way that the PTN is unable to fire repetitively. Clearly more relevant and direct information about the excitability of PTNs is needed before the effects of conditioning and test stimuli can be fully explained.

One interesting aspect to mention again is the effect of different currents of TMS within the cortex. Ignoring any disparity between single motor unit studies and recordings of descending volleys (Di Lazzaro et al. 2001), it has been shown in a single motor unit study that anterior-posterior TMS current within the cortex preferentially elicits I_1-waves,

and posterior-anterior TMS elicits I_3-waves (Hanajima et al. 1998). These authors then investigated whether TMS orientation would also cause conditioning stimulus effects to change. They reported that the conditioning stimulus in both orientations was effective in suppressing I_3-waves evoked by the test stimulus. This means that activation of inhibitory interneurones by the conditioning stimulus is not specific to the direction of the induced current. This differs from the I-wave generating mechanism. It could be speculated then that the direction-specific element in the I-wave generating network is the PTN itself. It may be that under certain circumstances (synchronized excitatory input via interneurones elicited by a preferred TMS current), the PTN fires repetitively. However, this phenomenon can be disrupted, if the right balance of excitatory and inhibitory input is not received.

1.10 I-waves and facilitation

In the paired-pulse test, if the conditioning stimulus is increased to suprathreshold levels, then facilitation between paired-pulses can be observed. Note that a suprathreshold TMS intensity is one that elicits an MEP response (roughly 1–2 mV), and will be a lower intensity for active muscles. Facilitation is believed to involve a different mechanism to inhibition, mainly because the conditioning stimulus now evokes descending volleys. In the facilitation paradigm, two TMS stimuli are delivered using ISIs of 0.5–6 ms. The MEP response evoked by the paired-pulse is sometimes bigger, than the predicted sum of the MEP responses each stimulus would evoke when alone. Facilitation has been shown to occur not only between two threshold TMS stimuli (Tokimura et al. 1996), but also between a suprathreshold conditioning stimuli and a subthreshold test stimulus (Ziemann et al. 1998b). The important finding was that facilitatory effects were only seen when the interval between the two stimuli was 1.1–1.5 ms, 2.3–2.9 ms and 4.1–4.4 ms. This timing was related to the intervals present between I-waves, and the mechanism of facilitation was suggested to be one of I-wave interaction. If the second magnetic stimulus was

substituted for an electrical stimulus, facilitation did not occur (Tokimura et al. 1996). This suggested that facilitatory effects were of a cortical nature. If facilitation were occurring at the motoneurone level, then it would have been observed using TES also. However, by directly stimulating PTNs, TES was bypassing the facilitatory mechanism. That facilitation was a cortical mechanism, was then supported by epidural recordings. It was shown that paired TMS pulses in the facilitation paradigm (ISI 1–1.4 ms) were accompanied by larger and more numerous I-waves than expected by the sum effects of each stimulus alone (Di Lazzaro et al. 1999b).

In a further study concentrating on a single ISI of 1.2 ms, Amassian et al. (1998) applied two anteriorly directed electrical pulses to human motor cortex. No facilitation in MEP responses was observed, and this result was explained as refractoriness of corticospinal fibres (Tokimura et al. 1996; Amassian et al. 1998). However, when two TMS stimuli were delivered (either anterior or posterior, the first suprathreshold, the second subthreshold) facilitation was seen at the same ISI of 1.2 ms. It was argued that this was due to hyperexcitability induced by the conditioning stimulus. It was implied that excitatory interneurones or even PTNs, which did not fire after the first stimulus, were however, sufficiently depolarized to be raised in excitability. Therefore the second stimulus could have additional excitatory effects.

An alternative idea emerges if I-wave generation is linked with abnormal excitability characteristics of the PTN following TMS. The fact that the first TMS stimulus causes the PTN to fire repetitively at abnormally high rates, would suggest that (in this artificial situation) additional inputs (if timed correctly) might be able to boost the system. It may be that the synaptic nature of TMS excitation is necessary for this summing mechanism to operate (hence this is not observed with electrical stimulation).

1.11 Pharmacological experiments

Interneurones have been implicated in both the I-wave generation mechanism, and the inhibitory effects of a low intensity conditioning stimulus. The important role of GABAergic inhibitory interneurones, has been shown by studies employing various pharmacological agents. Volatile anaesthetics with a GABAergic potentiation mechanism, suppressed I-wave responses measured by epidural spinal recordings (Burke et al. 1993). In addition, in facilitation paired-pulse TMS experiments, GABAergic agonists suppressed facilitation of MEPs, whereas other drugs affecting motor cortex excitability by other modes of action had no effect (Ziemann et al. 1998a). This would suggest that GABAergic interneurone activity is either involved in I-wave generation or can influence it.

In inhibition experiments using single motor unit recordings, it was shown that suppression of I-waves by the conditioning stimulus lasted up to 20 ms, a time-course corresponding with the activation of $GABA_A$ receptors (Hanajima et al. 1998). In a study looking at the effects of anti-epileptic drugs on motor cortex excitability, baclofen ($GABA_B$ agonist) was shown to significantly enhance intracortical inhibition (Ziemann et al. 1996a). The benzodiazepine lorazepam, which potentiates $GABA_A$ currents, caused a trend of enhancement of intracortical inhibition (Ziemann et al. 1996b). Possibly contradictory to this, was the finding that a GABA uptake blocker tiagabine, was shown to reduce intracortical inhibition (Werhahn et al. 1999). However, this result was explained by the fact that $GABA_B$ receptors can be found pre-synaptically, on axon terminals of inhibitory interneurones. Such receptors normally act in a feedback mechanism to regulate the activity of the neurone. It is possible then that the uptake blocker, despite increasing concentrations of GABA, reduced the inhibitory activity of the interneurones.

It should be pointed out that when discussing results of this kind, it is impossible to be precise about mechanisms on a receptor level. However, it is quite interesting that there

are so few reports of significant potentiating effects of GABAergic compounds on intracortical inhibition. Perhaps more detailed tests of intracortical inhibition, such as that described in this Thesis, would help clarify this situation. However, the main conclusion here is that GABAergic inhibitory interneurones can be implicated in some of the intracortical mechanisms tested with TMS.

1.12 Biological significance of intracortical inhibition

In summary, it is possible to use TMS to measure intracortical inhibition. This is a very useful and exciting technique, because it allows the testing of the fully functional motor cortex in conscious human subjects. However, it is clear that a complete understanding of the action of TMS on the brain is lacking. It is also unlikely that the effects of TMS represent physiological mechanisms normally operational during voluntary movements.

PART B: Descending corticospinal connections and peripheral input

1.13 Short-latency responses evoked by TMS

Strong evidence was presented in part A that subpeaks in a PSTH (indicating increases in the firing probability of single motor units) were representative of multiple descending volleys evoked by TMS (Day et al. 1989). In addition these peaks could be facilitated by a second TMS stimulus, applied at particular intervals determined by I-wave periodicity (Tokimura et al. 1996; Ziemann et al. 1998b). The implication was that both D-wave and I-wave activity evokes monosynaptic EPSPs in upper limb motoneurones and that these inputs generate the multiple peaks in the PSTH.

It has been argued however, that a population of excitatory propriospinal interneurones, similar to those within the spinal cord of cats (Alstermark and Lundberg 1992), may also constitute part of the corticospinal pathway from motor cortex to motoneurones in humans (Pauvert et al. 1998; Nicolas et al. 2001). Later peaks in the PSTH could then be generated by non-monosynaptic corticospinal connections. This work involved interacting descending volleys evoked by TMS with peripheral muscle afferents. A brief description of these important inputs will be provided.

1.13.1 Muscle afferent fibres

Group I afferent fibres, of which there are two subsets (Ia and Ib) have the largest diameter and therefore the lowest threshold for electrical stimulation. Group Ia fibres arise from primary spindle endings and make monosynaptic connections to motoneurones. Homonymous excitation occurs when Ia fibres from one muscle activate motoneurones innervating the same muscle. This contrasts with heteronymous excitation, when Ia afferents from one muscle activate motoneurones supplying other synergistic muscles (Figure 1.3). These also terminate on group Ia inhibitory interneurones, which mediate reciprocal inhibition between antagonist muscles. Recurrent inhibition of

homonymous and synergistic motoneurones also occurs and involves group Ia inhibitory interneurones and Renshaw cells (Rothwell 1994).

Muscle spindle afferent fibres respond to changes in muscle length and are activated by stretch and changes in velocity. Their sensitivity is controlled by γ- and β-motoneurone innervation (as distinct from α-motoneurones, which innervate extrafusal muscle fibres). Group Ia afferent fibres have a lower threshold than α- motoneurones, and can therefore be stimulated with low intensities of electrical stimulation to evoke what is termed the Hoffman or H-reflex. Stimulation intensity and location are adjusted to minimize the size of the direct response (known as the M response) elicited by activation of the α-motoneurone axons innervating the muscle of interest. This H-reflex test provides a useful test of spinal cord excitability (Rothwell 1994).

1.13.2 Non-monosynaptic corticospinal connections

Activation of group Ia afferents by stimulation of the ulnar nerve and musculo-cutaneous nerves facilitates both TMS evoked MEP responses, and increases firing probabilities of single motor units (Pauvert et al. 1998; Nicolas et al. 2001). Increasing the TMS intensity resulted in the facilitation reversing to inhibition. These data were suggested to provide evidence for the existence of interneurones onto which cortical and musculo-cutaneous inputs converge and whose outputs inhibit transmission through propriospinal neurones (Pierrot-Deseilligny 1996; Pauvert et al. 1998; Nicolas et al. 2001). These studies showed that the initial part of the central volley was spared from modulation, as indicated by the fact that the first 0.5–1 ms of the TMS evoked response was unaffected by Ia inputs (Pauvert et al. 1998; Nicolas et al. 2001). This supports the existence of monosynaptic corticospinal connections in man. However, given these data, later subpeaks in the first peak in the PSTH could feasibly be generated by propriospinal neurones. This interpretation is clearly at odds with work outlined in part A.

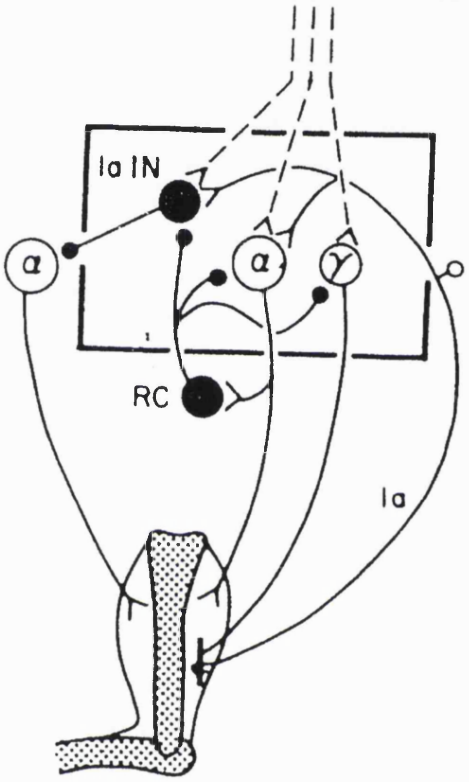


Figure 1.3: Ia afferent connections within the spinal cord. Ia afferent fibres make monosynaptic connections to homonymous α-motoneurones, and disynaptic connections via Ia inhibitiory interneurones (black cells labelled IaIN) to α-motoneurones of antagonist muscles (heteronymous connections). Renshaw cells (black cells labelled RC) are activated by axon collaterals of α-motoneurones, and project back onto the same α-motoneurones and also γ-motoneurones and associated Ia inhibitory interneurones. Dotted lines represent descending inputs from the cortex. This is a schematic diagram; α-motoneurones and γ-motoneurones within the box receive a much denser and more complicated array of inputs than shown here. (Adapted from Rothwell 1994, p131).

Detailed studies in monkeys were unable to find evidence for the existence of C_3-C_4 propriospinal neurones mediating excitation of upper limb motoneurones (Maier et al. 1998; Nakajima et al. 2000; Olivier et al. 2001). It was suggested that the macaque monkey was a better model for the organisation of corticospinal function in man than was the cat, in which such propriospinal transmission has been well established (Alstermark and Lundberg 1992). Work studying threshold TES evoked responses in humans has supported the existence of monosynaptic corticospinal connections (Maertens de Noordhout et al. 1999). Authors compared the PSTH peak of increased single motor unit firing in the flexor carpi radialis forearm muscle evoked by TES, to that evoked by Ia afferent fibres (median nerve stimulated). As the afferent stimulation produced a monosynaptic H-reflex response, authors argued that if the duration of the cortical response was shorter or equal to the H-reflex response, then the cortico-motoneuronal connection must also be monosynaptic. TES was chosen as this was considered the simplest type of cortical stimulation, avoiding the multiple descending volleys elicited with TMS. Single motor units responded to weak anodal TES with a single, narrow peak, the latency of which was consistent with monosynaptic connections. These results are, however, complicated by the fact that profound inhibition of single motor unit discharge after the initial peak may have obscured any non-monosynaptic excitation (Olivier et al. 2001). It was concluded that non-monosynaptic connections might exist in human subjects, though it is difficult to be sure whether such connections play a prominent role in natural cortico-motoneuronal activity (Maertens de Noordhout et al. 1999).

1.14 Long-latency responses evoked by TMS

In addition to the short-latency primary PSTH peak, it is also possible to observe a later second peak at approximately 63–98 ms following the TMS stimulus (Mills et al. 1991). It is important to consider where this secondary peak originates. Chapter 4 in this Thesis deals with the effects of low intensity TMS on ongoing EMG activity in a contracting

hand muscle. This work was primarily done to study the effects of TMS on the frequency of EMG oscillations (discussed in part C). However, the latencies of effects discussed in chapter 4 are equivalent to the timing of the secondary peak in the PSTH mentioned here, and the two findings may be connected.

One observation made by Mills et al. (1991) suggested that the secondary PSTH peak originated, at least in part, from the cortex. Single motor unit recordings from 1DI muscle were made from patients with multiple sclerosis that had normal peripheral nerve conduction, but abnormal central conduction. That the secondary peak in the PSTHs from these patients was delayed suggested that the origin of this peak could not be a purely peripheral one. Authors concluded that a combination of cortical and spinal effects contribute to the secondary peak in the PSTH (Mills et al. 1991). A possible cortical origin was suggested to be a slower-conducting corticospinal pathway, different to that transmitting the earlier response. Alternative hypotheses were a reafferent reflex response in the spinal cord, whereby the motor unit of interest was activated by heteronymous Ia afferents, or that γ-motoneurones supplying muscle spindles were activated by TMS, which then set up the Ia afferent reflex response.

Sammut et al. (1995) suggested that the secondary peak in the PSTH could result from a long-latency reafferent reflex elicited via stimulus-evoked movements of the hand. This suggestion was tested by Garland and Miles (1997) who recorded from the flexor digitorum profundis (FDP) muscle, chosen because a posture could be adopted whereby the muscle was disengaged from the joint about which it acted. This ruled out the effects of an MEP in this muscle affecting inputs from other muscles, and discounted movement of the hand as a contributing factor. In this posture, a secondary peak was still observed in the PSTH of single motor unit firing recorded from the FDP muscle. In addition, secondary peaks similar to those observed in chapter 4 of this Thesis were observed in surface EMG recordings.

Garland and Miles (1997) made raster plots showing that during steady, regular discharge of motor units, motoneurones were unresponsive to synaptic inputs during their usual period of after-hyperpolarization, and return of membrane potential to excitable levels. However when TMS was applied relatively late in this recovery phase, the corticospinal inputs were large enough to cause the motor unit to fire again (Olivier et al. 1995). This was often at an earlier time than would have otherwise occurred at the natural discharge rate. Garland and Miles (1997) argued that this 'moving forward' of spikes in time by TMS, left gaps in the subsequent spike train. This appeared as a trough or silent period in excitability, within single motor unit or surface EMG recordings. Thus the suppression of activity was attributed to a realignment of spikes to a fixed latency after corticospinal input (Garland and Miles 1997). The return of many units to firing threshold was synchronized by the earlier TMS-induced events and this grouped resetting of discharge led to the secondary peak of EMG activity.

To extend this observation further, it is reasonable to suggest that TMS exerts relatively long-term effects on the temporal characteristic of EMG activity through its actions on the motor cortex. When subjects are maintaining a voluntary contraction, silent periods in EMG activity caused by TMS are partly due to disfacilitation of tonic cortical input to motoneurones. It is likely that following this suppression, motoneurones resume responsiveness to cortical input at similar times and therefore again reach their discharge threshold in a synchronous manner. In Chapter 4 I discuss the possibility that the tonic cortical input to motoneurones is of an oscillatory nature, the phase of which can be affected by TMS, and that this mechanism leads to oscillatory changes in the EMG in the 50-250 ms following TMS.

1.15 The effect of voluntary activity

In many of the studies in this Thesis TMS was applied when subjects were voluntarily activating hand muscles. This was during simple abduction of a single intrinsic hand muscle (1DI or AbPB) or during a specific precision grip task involving multiple hand and forearm muscles. That voluntary activation of hand and forearm muscles affects MEP responses evoked by TES and TMS is well accepted, however it is still not clear what the respective contributions of the cortex and spinal cord are to this phenomenon.

Spinal effects have often been used to explain the differences observed in MEP responses between relaxed and active states. It was shown that the latency of MEPs elicited by TES in active 1DI muscle could be 2–3 ms shorter than those elicited in relaxed muscle (Day et al. 1987a,b). A major factor contributing to effects observed can be explained by summation of EPSPs at the motoneurone. At rest, temporal summation of D- and subsequent I-waves is required before threshold of the inactive motoneurones is reached and this prolongs MEP latency. During voluntary activity, motoneurones in the population have increased excitability and are closer to firing threshold. Thus under these conditions a single EPSP from the first descending volley evoked by TMS may well be sufficient to cause motoneurones to discharge, giving rise to a shorter latency MEP (Rothwell 1991). This may also explain the threshold differences for evoking MEP responses in the relaxed and active states, which is also observed using TMS (Hess et al. 1987).

One simple study investigated both spinal and cortical effects of voluntary contraction by using three forms of stimulation: TMS, TES and foramen magnum level stimulation (Ugawa et al. 1995). By exerting low levels of voluntary contraction it was found that the MEP responses to all three stimuli were facilitated in a similar manner. This would suggest that in the transition from relaxed to active states, voluntary activity has

predominantly spinal effects. However, when a higher level of voluntary activity was maintained (e.g. 50 % maximum voluntary contraction), responses to TMS were more significantly facilitated than responses to the other forms of stimulation. Authors concluded that there was also a cortical contribution to the effects of voluntary activity (Ugawa et al. 1995). Indeed Turton and Lemon (1999) showed that for hand muscle, it was likely that much of the additional facilitation of responses to TMS, observed when subject's increased the level of ongoing voluntary contraction, was of cortical origin.

A study by Di Lazzaro et al. (1998a) showed that the descending I-wave volleys recorded epidurally in awake patients, and elicited by TMS, were increased by voluntary activity, thereby confirming a cortical contribution. This conclusion was supported by the fact that voluntary activity had no effect on the amplitude of the corticospinal volley evoked by transcranial electrical stimulation exciting PTNs directly (Di Lazzaro et al. 1999a). The threshold for eliciting descending volleys with TMS was reduced during voluntary activity, but this was a small change in comparison to the increase in descending activity (Di Lazzaro et al. 1998a). Authors concluded that it was likely that spinal mechanisms also played an important role in the facilitatory effects of voluntary activity observed in surface EMG activity. It was possible that descending volleys may have exerted both excitatory and inhibitory post-synaptic effects, and of course the proportion of the descending volleys destined for the muscle of interest is unknown (Di Lazzaro et al. 1998a).

Cortical effects of muscle activation have also been observed by studying the effects of voluntary activity on intracortical inhibition measured by the paired-pulse test (Ridding et al. 1995b). It was shown that low levels of voluntary activity could reduce the amount of inhibition elicited by the conditioning stimulus on test stimulus responses. This suggests

that inhibitory interneurones are influenced by the increase in motor cortex excitability generated by muscle activation.

1.16 Cortical excitability during specific motor tasks

In addition to the modulation of TMS evoked responses by voluntary activation, there are effects due to the nature of the task performed. Work in this Thesis involved applying TMS whilst subjects performed a precision grip task. Subjects squeezed together two levers using index finger and thumb to bring them into a narrow target zone. MEP amplitudes during the hold phase of this precision grip task were measured in different hand and forearm muscles.

Task modulation of MEP sizes evoked by TMS has been confirmed in studies comparing MEPs evoked whilst 1DI is activated to abduct the index finger, to responses evoked whilst subjects perform various grip tasks (Datta et al. 1989; Flament et al. 1993). Datta et al. (1989) found larger MEP amplitudes elicited in 1DI muscle, when a simple index finger abduction task was carried out. This was in comparison to MEPs elicited in the same muscle whilst subjects performed a power grip, suggesting that a higher level of cortical activity was involved during the isolated finger contraction (Datta et al. 1989). In complete contrast Flament et al. (1993) found larger MEPs in 1DI when subjects performed various precision grip tasks, in comparison to simple adduction of 1DI.

1.16.1 Pitfalls in measuring TMS evoked responses

Conflicting data of this kind may have been due to the pitfalls involved in experiments of this kind. As accounted for in the study by Flament et al. (1993), it is important that a constant level of background EMG activity is maintained for each of the tasks used in the experiment. The effect of low levels of voluntary activity (in comparison to the relaxed state) in facilitating TMS responses is dramatic, and it saturates very quickly at levels of around 10 % maximum voluntary contraction (Hess et al. 1987; Turton and Lemon 1999).

In addition using too high levels of voluntary contraction in experiments (e.g. 30 % maximum voluntary contraction) can result in the inability to measure any changes in MEP size between tasks (Kasai and Yahagi 1999).

Similarly, it is important to use the correct intensity of TMS and to understand whether MEP amplitudes reflect spinal and/or cortical excitability. However, this is made difficult by the fact that the input/output relationship between TMS intensity and MEP amplitude is complex. For 1DI muscle, the relationship has been shown to be sigmoidal, and therefore highly nonlinear (Devanne et al. 1997). This is despite the fact that the discharge probability of single motoneurones, as measured by the firing probability of single motor units, increases linearly with TMS intensity. One factor contributing to the sigmoidal nature of the input/output curve is that as the intensity increases, bigger motor units (with larger potentials) are recruited (Henneman 1957). However it is argued that an additional important factor is the number and amplitude of multiple volleys elicited by TMS, and their nature depends on the level of motor cortex excitability (Devanne et al. 1997).

It has been shown that whilst suprathreshold TMS intensities evoke more reproducible and measurable MEP responses in active muscle than lower intensities, there is the risk that subtle changes in motor cortex excitability go unobserved (Lemon et al. 1995; Kasai and Yahagi 1999). It was shown that high responses to suprathreshold TMS were roughly proportional to background EMG levels, implying that cortical excitability had little additional modulatory effect (Lemon et al. 1995).

1.16.2 Corticospinal drive during power and precision grip tasks

A recent study that controlled for all these different factors still found consistent differences in MEP sizes between power and precision grip tasks (Hasegawa et al. 2001b). With the same levels of background activity, MEPs were larger in 1DI muscle in the precision grip task than the power grip (cf Flament et al. 1993). A possible

explanation for this difference is that in the precision grip, intrinsic hand muscles such as 1DI are important (Long et al. 1970) whereas in the power grip, the extrinsic hand muscles provide the major grip force (Schieber 1995).

When MEP size evoked in 1DI was correlated with background EMG activity, it was found that there were different regression coefficients for each task, with a steeper relationship for the precision grip than the power grip (Hasegawa et al. 2001b). This suggested that the nature of the task modulated the influence of background EMG levels on TMS responses. Authors were able to conclude that the differences in MEP size reflected differences in the central motor commands required for precision versus power grip tasks.

In an additional study, focussing on the precision grip, the importance of grip aperture was addressed (Hasegawa et al. 2001a). Subjects gripped levers that were either 20 mm or 80 mm apart. For each grip aperture MEP amplitude evoked in 1DI was correlated with background EMG levels, and different correlation coefficients were obtained depending on grip aperture. For the narrow grip aperture, the relationship between MEP amplitude and background EMG level was dramatically modulated by TMS intensity, whereas this was not the case for the wider aperture. This suggests that when comparing MEP sizes in different grip tasks, a similar grip aperture should be used.

With regards to dynamic phases of muscle contraction (such as the ramp phase of the task used in this Thesis), MEP amplitudes evoked in 1DI were higher during a step or fast contraction, than during a slower smoother contraction (Kasai and Yahagi 1999). Authors argued that whereas the step contraction was a preprogrammed ballistic movement in which corticospinal excitability could not be altered, modulation possibly by afferent input (see below) did occur during the slower ramp. When higher force levels were used, despite the fact that background activity between tasks changed, there were no differences

in MEP sizes. This was explained by correlation coefficients, showing that MEP amplitudes in ramp conditions were dependent on levels of background activity, but MEPs evoked in step contractions were not (Kasai and Yahagi 1999).

1.16.3 Corticospinal drive during different phases of the precision grip task

Other studies have measured cortico-motoneuronal activity during different phases of the same precision grip task. Clear modulation of the firing rate of corticospinal neurones projecting to intrinsic hand muscles was observed (Muir and Lemon 1983; Bennett and Lemon 1996). When monkeys performed the ramp phase of a precision grip task there was a higher rate of cortico-motoneuronal cell firing, than during the hold phases of the task (Bennett and Lemon 1996). Such task modulation was similar to that in active hand muscles, so may have reflected the typical 'muscle-like' properties of most motor cortex neurones; in addition it was argued that since independent movements of the digits were particularly important during the ramp or movement phase of the task, this required greater fractionation of hand muscle activity. This is known to be associated with enhanced activity amongst cortico-motoneuronal cells. Authors suggested that the production of relatively independent digit movements, unlike those involved in co-contraction, required a distributed command signal to different motoneurone pools (Bennett and Lemon 1996).

In a study involving human subjects reaching out, gripping and lifting an object, it was shown that the first contact made with the object was accompanied by important changes in corticospinal activity (Lemon et al. 1995). MEP responses in the intrinsic hand muscles, 1DI and AbPB were strongest during the initial touch of the object. It has been shown that during grip, intrinsic hand muscles come into play and in particular, cutaneous inputs of the digits are essential for muscle coordination during the grasp (Johansson and Westling 1987). Different responses were observed in forearm muscles FDS and EDC, in

which highest MEP amplitudes were observed during the reach phase of the task, reflecting the importance of forearm muscles in palm orientation and finger position during this phase (Lemon et al. 1995). MEP amplitudes were lowest in the static phase of the task when the object was held in the air. Authors argued that whilst important processes involved in parametizing physical properties of the object were performed in the early phases of the task (hence higher cortical excitability), the hold phase was a more stable and automatic task requiring less cortical input (Lemon et al. 1995).

1.17 Peripheral afferent input

During precision grip tasks, such as the one used in the studies in this Thesis, it is likely that input from the periphery plays an important role in the cortical control of hand muscles. Investigators have therefore studied the consequences of activation or block of muscle, cutaneous and joint afferents, on MEP amplitudes and motor performance. These afferent fibres project to the spinal cord and then travel in ascending tracts, projecting to different parts of the sensory-motor network, including the motor cortex. It is likely that afferent inputs could affect MEP responses at several different levels, including those acting at a spinal segmental level, at the level of propriospinal neurones, and at supraspinal levels. Of particular significance in this last group is the effect of fast peripheral input to motor cortex regions which, when stimulated, gives rise to the MEP.
It is first necessary to review the anatomical features of peripheral ascending pathways, details of which have been taken from Rothwell (1994) unless otherwise stated.

Afferents supplying muscle, joint and cutaneous receptors terminate in the upper laminae, particularly lamina IV. Afferent fibres from muscle and joint receptors are classified in terms of diameter (group I – IV). Muscle afferent fibres projecting from muscle spindles have already been reviewed. Other muscle receptors include Golgi tendon organs, which respond to mechanical deformation, Paciniform corpuscles and free nerve endings. Group

Ib afferent fibres project from Golgi tendon organs, whereas Paciniform corpuscles have group II fibres. Group III fibres project from free nerve endings. Sensory fibres terminate in the dorsal horn of the spinal cord, which is divided into various laminae (Rexed).

Sensory receptors within synovial joints of the hands and forearms include free nerve endings, Golgi endings and Ruffini endings. Group I fibres project from Golgi endings, group II fibres from Ruffini endings and group II fibres from nerve endings. Joint receptors respond to deformation of the joint and to extremes in joint angle.

Afferents of cutaneous receptors are termed A, B or C fibres. The fastest conducting fibres are the myelinated A fibres. B fibres are also myelinated, but are slower conducting. Finally the slowest conducting fibres are the unmyelinated C fibres. These fibres project from three types of cutaneous receptor in the skin, of which thermoreceptors and nociceptors are less relevant to studies of motor control. Stretching of skin caused by muscle contraction, or skin deformation following object manipulation, activate mechanoreceptors found in glabrous skin of the hand. Mechanoreceptors are classified in terms of their adaptation rates following activation. There are slow adapting (SA) Merkel cells (called SAI receptors) and Ruffini corpuscles (SAII) and fast adapting (FA) Meissners corpuscles (FAI) and Pacinian corpuscles (FAII). There is a high density of type I units in the fingers. The type II units have larger receptive fields, with FAII receptors located both in the fingers and palm (Johansson 1991). In primates there is significant overlap between the conduction velocity and size of fibres supplying the muscle spindles and cutaneous receptors (Porter and Lemon 1993).

1.17.3 Ascending pathways

A number of ascending tracts exist, which carry sensory information from the periphery towards different parts of the brain. As far as sensory information most likely to directly

influence the motor cortex, the dorsal column-medial lemniscal and spinothalamic pathways are the most important (Porter and Lemon 1993; Rothwell 1994). Low threshold cutaneous afferents, and rapidly adapting cutaneous mechanoreceptor afferents travel via the dorsal column-medial lemniscal pathway (Figure 1.4A). Afferents from more slowly adapting receptors in joints and muscles also travel in this pathway, but by a slightly different route (Figure 1.4B). In addition joint and muscle afferents travel in the spinothalamic pathway (Figure 1.4C).

On entering the spinal cord at lumbar or cervical regions, peripheral afferent fibres ascend by relaying in the dorsal column nuclei and then again in the thalamus. Thalamo-cortical projections are widespread, and it is likely that sensory input to the motor cortex travels both directly to the motor cortex, and indirectly via the somatosensory cortex (Porter and Lemon 1993, Figure 1.5). Figure 1.5 highlights some additional pathways by which activity of the motor cortex can be modulated: afferent fibres project to the cerebellum, from which there are connections to the motor cortex via the thalamus.

1.18 Cutaneomuscular reflex

When cutaneous and/or muscle afferents are activated, a reflex in ongoing EMG activity can be observed (Caccia et al. 1973; Jenner and Stephens 1982). This reflex is composed of three parts. An initial short-latency excitatory phase (E1) occurs approximately 30–40 ms following peripheral stimulation; after that is a longer-latency inhibitory phase (I1) occurring at 40–50 ms; finally there is a secondary excitatory phase (E2) occurring at 50–70 ms. Cutaneous and muscle afferents can be activated naturally by stretch or by electrical stimulation of digital nerves of the hand (activates cutaneous afferents) or nerves at the wrist (activates muscle and cutaneous afferents).

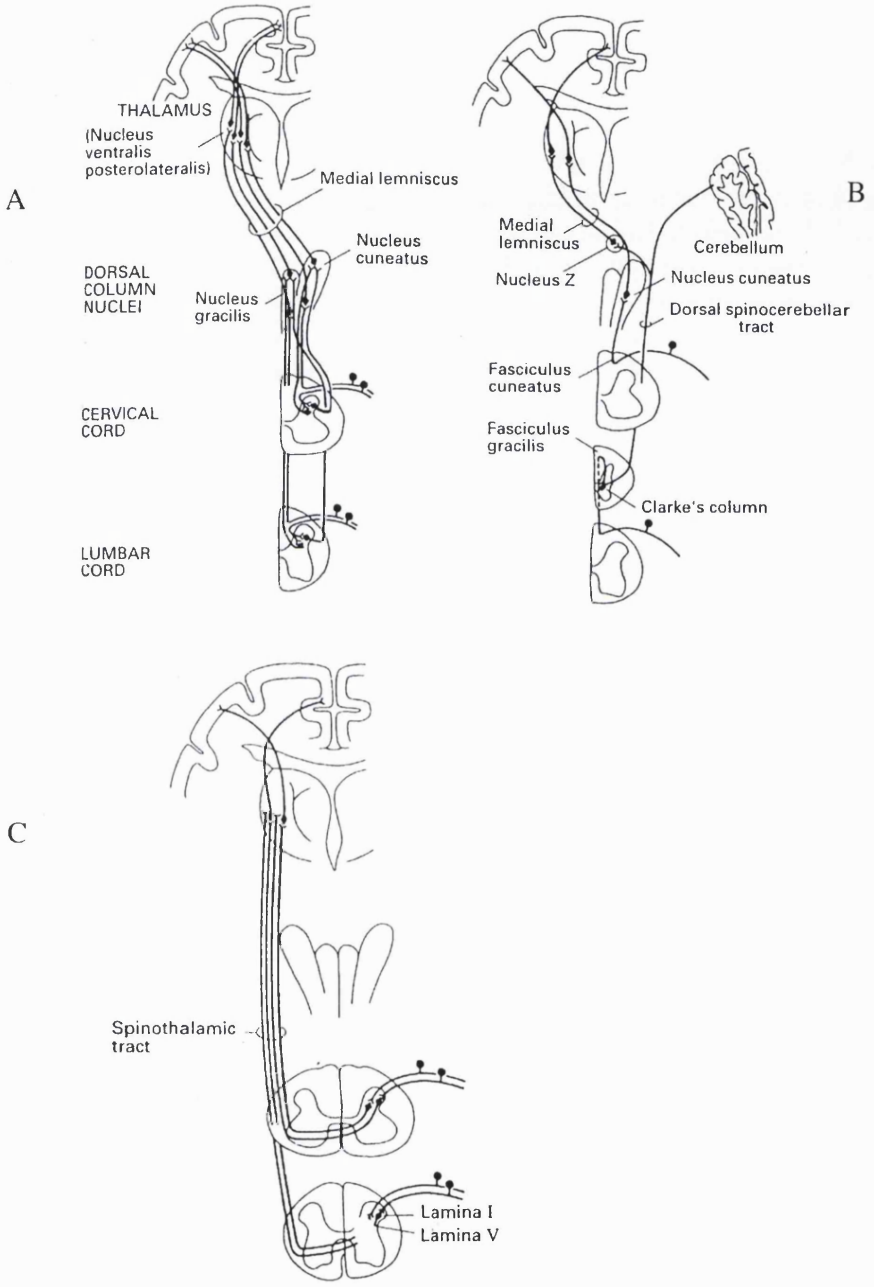


Figure 1.4: Main anatomical features of the dorsal column-medial lemniscal pathway and spinothalamic pathway. (A) The main route in the dorsal column-medial lemniscal pathway taken by fibres projecting from cutaneous receptors. (B) Afferent fibres from receptors in joints and muscles ascend this pathway by a different route and also travel in (C) the spinothalamic pathway. (Adapted from Rothwell 1994, p219, 221).

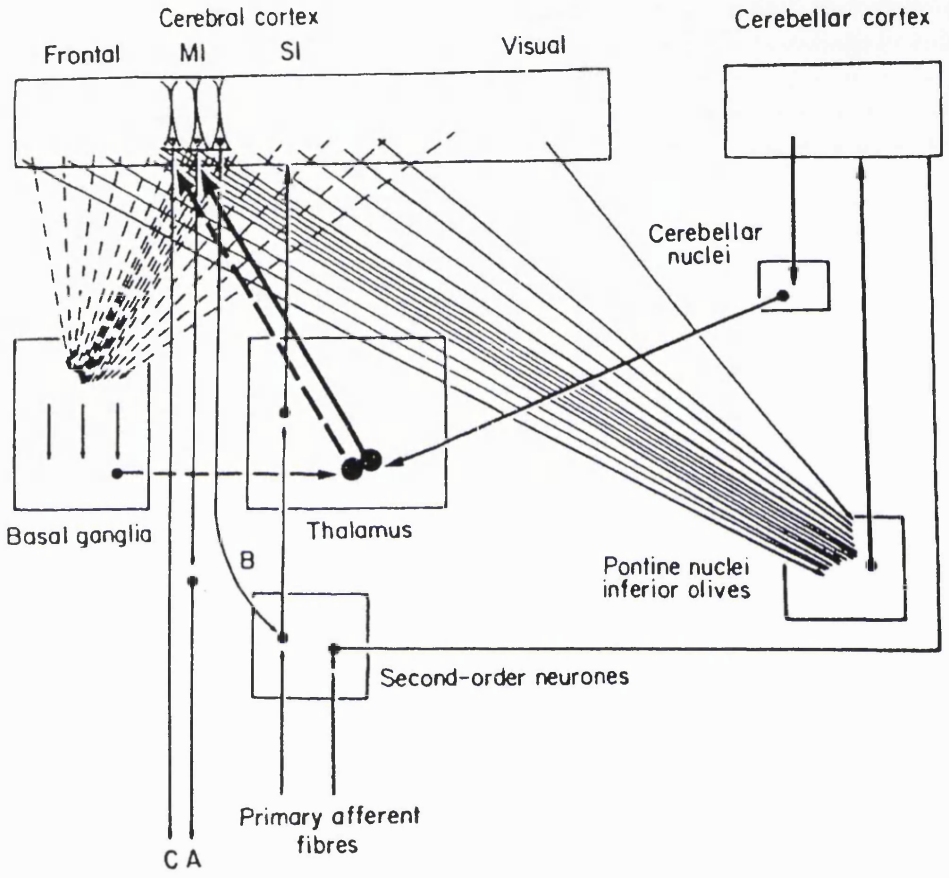


Figure 1.5: Connections between subcortical brain regions and the motor cortex. Afferent fibres that arrive at the thalamus make direct connections with the motor cortex (M1) or indirect connections via the somatosensory cortex (S1). The thalamus also receives input from cerebellar nuclei, via which additional afferent fibres projecting to the cerebellum can eventually modulate motor cortex activity. In addition to the corticospinal tract (C), cortico-reticulo-spinal projections descend from the sensorimotor areas of the cortex (A). There are also descending pathways that modulate sensory input from the periphery (B). Note the dense projections from the basal ganglia and inferior olive to the motor cortex. (Adapted from Porter and Lemon 1993, p26).

Jenner and Stephens (1982) suggested that at least part of the cutaneomuscular reflex involved transcortical pathways and studies of patients with mirror movements has provided strong evidence to support this claim (Farmer et al. 1990; Mayston et al. 1997). When these patients make voluntary movements of the fingers of one hand, there is an involuntary mirroring of the same movements in the fingers of the other hand. Mayston et al. (1997) showed that this was probably due to an abnormal bilateral fast conducting corticospinal pathway. This was indicated by the fact that TMS evoked MEPs of similar latency were observed in contralateral and ipsilateral (to the stimulated motor cortex) hand muscles (Mayston et al. 1997). Digital nerve stimulation on one side, resulted in E2 responses in the 1DI muscles on both sides of the patient. Taken together, TMS evoked responses and cutaneomuscular reflexes in mirror movement patients, provide evidence that the reflex pathway giving rise to the E2 phase involves the corticospinal tract. Sensory evoked potentials recorded over the sensory cortex in mirror movement patients were similar to control subjects suggesting that the afferent pathways in these patients were normal (Mayston et al. 1997).

Despite the fact that the early inhibitory (I1) phase has been considered to be of a spinal origin (Jenner and Stephens 1982; Farmer et al. 1990), bilateral inhibitory responses have also been observed in some patients with mirror movements, suggesting a transcortical pathway may also be involved in this phase of the cutaneomuscular reflex (Mayston et al. 1997).

It therefore seems likely that activation of cutaneous and muscle afferents can have effects on the excitability of the motor cortex. This prompted work investigating the effects of peripheral input on TMS evoked MEP responses. The latency of effects on TMS evoked responses was then compared to the timing of the reflex response in ongoing EMG activity. In this way, any inhibitory or excitatory effects of peripheral input at the cortex, could then support claims about the origins of the transcortical reflex.

1.19 Effects of peripheral input on TMS evoked responses

Tokimura et al. (2000) reported that stimulation of the median nerve at the wrist and digital nerves of index finger and thumb could suppress MEP responses evoked in hand muscles by TMS. Peripheral nerve stimulation was applied approximately 20 ms before TMS was applied at the motor cortex, which is the approximate conduction time from periphery to cortex. Inhibitory effects of the peripheral nerve stimulation on TMS evoked volleys were then indicated by the suppression of the MEP response, evoked in 1DI activity some 25 ms later. The total time-course of effects (approximately 45 ms following peripheral nerve stimulation) corresponded to the latency of the inhibitory period (I1) of the reflex response in the ongoing EMG activity, which would have occurred following application of the median nerve stimulation alone. Recordings of descending volleys in the spinal cord, showed that later I-waves evoked by TMS were suppressed by median nerve stimulation (Tokimura et al. 2000). Authors concluded that cortical inhibition observed following peripheral nerve stimulation is likely to be responsible for the early period of inhibition in the long-latency reflex (Tokimura et al. 2000). A similar conclusion was made by Delwaide and Olivier (1990) who showed that H-reflex responses evoked by forearm stimulation were unaffected by peripheral nerve stimulation of the wrist, suggesting that inhibitory effects observed were of a cortical nature.

Work has been done to see if peripheral inputs can also have excitatory effects on the motor cortex; this would then explain the cortical nature of the E2 phase (that follows the I1 phase) of the long-latency reflex. One study investigated the effects of digital nerve stimulation on MEPs evoked by TMS and TES (Maertens de Noordhout et al. 1992). Activation of cutaneous inputs by digital nerve stimulation caused suppression of TES evoked MEPs, at latencies equivalent to the inhibitory (I1) phase. When longer intervals between digital nerve stimulation and TES were used, excitation was observed, at

latencies equivalent to the E2 phase of the reflex. Modulation of TMS evoked responses by digital nerve stimulation followed a similar pattern of inhibition and excitation although effects were observed over a different time-course. Suppression of responses occurred at a time equivalent to the early inhibitory phase, however excitatory effects were delayed. These results were confirmed in single motor unit recordings. TMS was set at an intensity to increase the firing probability of single motor units, as indicated in PSTH analysis. The digital nerve stimulus also increased the firing probability of the single motor unit at a latency equivalent to the E2 phase. When both stimuli were applied, and TMS was delivered at a latency such that it would have an effect at the onset of the E2 period, the increased firing of single motor units was less than would be expected by the sum of combined effects. This indicates a prolongation of the earlier inhibitory phase, into the expected E2 phase (Maertens de Noordhout et al. 1992).

Nevertheless, it was concluded that, in addition to inhibitory effects, peripheral input could also have excitatory effects on motor cortex activity (Maertens de Noordhout et al. 1992). This was most clear for effects observed on TES evoked MEPs, although authors admit that this is somewhat difficult to explain, given that TES excites PTN axons directly, at a site far enough away from the cell body to be relatively unaffected by motor cortex excitability. However, note that TES can evoke both D-waves and later I-waves (Edgely et al. 1997), the latter of which can be modulated by peripheral nerve stimulation (Tokimura et al. 2000). That TMS preferentially evokes I-waves, might then explain why inhibitory effects of the peripheral inputs on TMS evoked responses were prolonged.

In the same study (Maertens de Noordhout et al. 1992), peripheral afferent activation by stretch was shown to have both inhibitory and excitatory effects on TMS evoked MEPs (corresponding to I1 and E2 phases respectively), although the prolonging of the inhibitory phase (found using digital nerve stimulation) was not observed. This difference

in effects of electrical cutaneous afferent stimulation and natural activation of peripheral inputs, was partly explained by the fact that stretch also activated muscle afferents. In addition, it should also be noted that long-latency reflex responses evoked by electrical stimulation are generally weak and require averaging over trials, before clear effects in EMG are observed. This is in contrast to strong reflex responses evoked by natural activation of cutaneous inputs that can be observed on a single trial basis (Johansson et al. 1994). Indeed marked facilitation of TMS evoked MEP responses by natural cutaneous activation (which may have accounted for the lack of prolonged inhibition observed in the Maertens de Noordhout study), was shown in a study by Johansson et al. (1994). Subjects used a precision grip to restrain an object, which was subjected to pulling loads away from the hand. This loading effect caused an increase in firing of cutaneous afferents recorded within the median nerve. At a latency of 40–140 ms following load application, MEPs evoked in hand muscles were greatly facilitated, in a manner separate to the smaller increase in ongoing EMG activity. This suggested a 'boosting' effect of task-related cutaneous afferent input on TMS evoked responses. Authors argue that, given the latency of effects, this facilitation was likely to have occurred in the motor cortex (Johansson et al. 1994).

1.20 Effects of peripheral input on intracortical inhibition

Tokimura et al. (2000) suggested that the inhibition of MEP responses observed following peripheral nerve stimulation may be similar to the cortico-cortical inhibition measured in the paired-pulse test. The main reason for this was that the same pattern of I-wave effects was shown (sparing of I_1-wave; inhibition of I_2-waves and I_3-waves). This implies that not only can peripheral input access the cortical mechanisms that contribute to MEP generation, but that interneurones involved in intracortical inhibition may also be affected.

In a study looking at the effects of digital nerve stimulation, Ridding and Rothwell (1999) confirmed that cutaneous input produced clear suppression of the TMS evoked test MEP response in an intrinsic hand muscle. This agrees with the work studying the inhibitory phase of the long-latency reflex (Delwaide and Olivier 1990; Maertens de Noordhout et al. 1992; Tokimura et al. 2000). They compensated for this change in the test response, when using the paired-pulse paradigm to measure intracortical inhibition. Digital nerve stimulation was shown to suppress intracortical inhibition induced by the conditioning stimulus on the test stimulus evoked MEP. Authors concluded that the digital nerve stimulus had a different effect on circuits involved in intracortical inhibition (excitation) and those involved in the MEP response (inhibition).

In a similar study, the effects of stimulation of muscle afferents on TMS evoked MEPs in forearm muscles were assessed (Aimonetti and Nielsen 2001). Activation of muscle afferents originating in antagonist muscles had no effect on the size of the MEP responses evoked in the test muscle (e.g. median nerve stimulation had no effect on MEP responses evoked in extensor carpi radialis, ECR). However, similar to the findings of Ridding and Rothwell (1999), there was a reduction in intracortical inhibition. When the homonymous afferent nerve of the test muscle was stimulated, there was again no effect of MEP responses, but also no effect on intracortical inhibition (e.g. radial nerve stimulation had no effects on TMS responses evoked in ECR). These authors found that there were reflex inhibitory and facilitatory responses occurring in ongoing EMG activity of the test muscle following stimulation of the muscle afferents originating from the antagonist muscle. The facilitatory responses occurred with the same latency as the decrease in intracortical inhibition. This study highlighted the reciprocal pattern of excitatory effects of peripheral input on the motor cortex, possibly involved in the co-contraction of wrist extensors and flexors (Aimonetti and Nielsen 2001).

Thus both studies (Ridding and Rothwell 1999; Aimonetti and Nielsen 2000) showed that peripheral afferent input could reduce intracortical inhibition, possibly reflecting an excitatory influence on the motor cortex circuitry involved. However, when similar latencies between peripheral nerve stimulation and TMS was used, inhibition of MEP responses was observed or there was a lack of effect on this parameter. This difference in the modulation of intracortical inhibition and MEP amplitudes is surprising, and suggests that it is important to make a distinction between the neuronal circuitry activated in the two mechanisms when measuring changes in motor cortex excitability.

1.21 The role of cutaneous afferent input during object manipulation

The importance of sensory information during manipulation of objects is highlighted by behavioural changes caused when peripheral feedback is perturbed by nerve block, or ischaemia. In the study described in chapter 7 of this Thesis, digital nerve anaesthesia of the index finger and thumb was used to perturb cutaneous feedback whilst subjects performed a hold-ramp-hold precision grip task. The main aim of this study was to investigate the importance of peripheral feedback on the maintenance of EMG oscillatory activity. Work directly relating to this topic will be covered in part C of the Introduction, which introduces oscillatory activity within the motor system. The following section will focus on behavioural effects of digital nerve anaesthesia, and the effects of sensory feedback perturbation on TMS evoked responses.

The importance of cutaneous afferent input during the manipulation of objects has been highlighted by extensive work performed by Johansson and colleagues (Johansson and Westling 1984; Johansson and Westling 1987; Johansson 1991; Johansson 1996). Microneurography recordings showed task-related activity within cutaneous mechanoreceptors on initial contact with objects during a precision grip task. This was most distinct in the FAI receptor afferents, which have well-defined cutaneous receptive

fields, permitting high spatial acuity (Johansson 1991). This sensory information was shown to be necessary for the subsequent release of motor commands required for further manipulation of the object. These motor responses involve a build up of both grip and load forces as attempts are made to lift or move a gripped object (Johansson 1991). An important objective during the lifting of objects is to avoid slips, and cutaneous mechanoreceptors have been shown to signal the friction between the hand and object surface, enabling the appropriate grip/load force to be achieved (Johansson and Westling 1987). When objects with predictable physical properties (passive objects) were grasped, motor commands were primarily based on the initial experiences of the object, which helped to 'paramatize' the object in terms of sensorimotor control. However, when slips occurred, brief bursts in mechanoreceptor afferents signaled to the motor system that the grip/load ratio needed altering. The new ratio was then stored in the somatosensory memory (Johansson and Westling 1987; Johansson et al. 1992).

Digital nerve anaesthesia applied to the middle phalanx of the fingers or thumb had two main affects. The initial contact force with the object became excessive, indicating the lack of sensory afferent input, and consequently motor grip forces responses were delayed. In addition the afferent input involved in frictional adjustment was disrupted, resulting in high grip forces and an incorrect ratio of grip/load force (Johansson and Westling 1984; Johansson et al. 1992). In terms of behavioural effects, subject's manipulation of passive objects was degraded, and there were more slips. In order to improve performance, subjects had to adapt their largely automatic strategy and voluntary effort was required to update the somatosensory memory with the afferent information available (Johansson and Westling 1984). This approach was similar to that adopted when less predictable (or active) objects were manipulated. These objects exert their own load forces against which subjects grip forces must counteract (Johansson et al. 1992). Using normal afferent feedback to manipulate active objects, subjects relied much more

on sensory information than internal representations of the object. Also under these conditions, digital nerve anaesthesia reduced and delayed grip forces, and induced slips (Johansson et al. 1992).

An interesting observation was that for precision grip tasks involving both finger and thumb, afferent input from both digits was required for normal object manipulation (Johansson et al. 1992). This means that afferent input from a single digit is not enough to predict grip forces required for the other digit, and that an independent digit control strategy operates.

That subjects were able to adapt grip forces during digital nerve anaesthesia suggests that some afferent input was available (Johansson et al. 1992). It is hypothesized that the sensorimotor system may switch to alternative afferent signals if those normally employed are consistently unavailable (Johansson 1991). It is possible that more proximal mechanoreceptors located on the palm of the hand and proprioceptors in the forearm supplied afferent information, however the lack of proximity to the object surface may have resulted in a longer processing time (Johansson et al. 1992). This may explain the delayed responses observed during digital nerve anaesthesia. The lack of fast automatic responses which would have been obtained had the normal afferent inputs been in place, certainly explains the degradation in performance (Johansson et al. 1992).

1.22 Effects of digital nerve anaesthesia on movement detection

During object manipulation, it is important for the subject not only to obtain sensory information about the object, but also about the position of their digits in space. For accurate detection of digit movement it is assumed that a contribution of afferent information from cutaneous, and also joint and muscle receptors at the digits would be required. However, it is not clear what the relative importance of these signals is. Digital nerve anaesthesia (applied distal to the web space of each digit) has been used to assess

the importance of cutaneous and local joint inputs for accurate digit proprioception (Refshauge et al. 1998).

When digital nerve anaesthesia was applied with index finger and thumb, both in the extended position, detection of movement was impaired for both digits. This suggested that digital nerve input from local cutaneous and joint receptors played an important role in digit proprioception. Paralysis of cutaneous afferent input from receptors located in the dorsum of the thumb and the wrist (radial nerve block) had no effect on thumb movement detection (Refshauge et al. 1998).

Thumb anaesthesia has also been shown to change the perception of weights lifted by the thumb (Marsden et al. 1979; Kilbreath et al. 1997). Increased heaviness perceived on thumb flexion was accompanied by increased activity in flexor pollicus longus activity whereas decreased heaviness on thumb extension was accompanied by a decrease in extensor pollicis longus activity (Marsden et al. 1979). This illustrates changes in muscle activation of the prime mover caused by thumb anaesthesia and suggests that afferent information from the thumb is important for the correct balance of flexor and extensor activity.

1.23 Effects of digital nerve anaesthesia on EMG activity

Changes in EMG activity following digital nerve anaesthesia when subjects manipulate objects have also been observed during index finger movements (Collins et al. 1999). In this study two main control conditions were used: an object present condition where subjects reached out to grasp and then lift an object, and an object absent condition in which there was no object present for the subjects to grasp. Subjects were unaware of whether an object would be present or not. It was shown that during trials in which no object was present, due to the lack of contact-evoked sensory input, EMG levels in 1DI and thenar muscles of the hand changed. The direction and amplitude of this difference in

EMG levels (in comparison to EMG levels when the object was present) was variable. However a typical response was contact-driven enhancement of activity in 1DI (prime mover). That the index finger and thumb were co-activated in the precision grip task, was shown by typically less activation of thenar muscles when the object was present. Digital anesthesia of the index finger and thumb affected these differences in EMG activity between the object present/object absent conditions, highlighting the importance of cutaneous afferent feedback during object manipulation (Collins et al. 1999). In agreement with conclusions made by Marsden et al. (1979), this study illustrated the importance of cutaneous feedback for cortico-motoneuronal control of hand muscles. It was also shown that contact with the object generated changes in EMG activity at a latency of 34–54 ms after initial object contact, making it feasible that transcortical pathways were involved.

1.24 Effects of anaesthesia and ischaemia on TMS evoked responses

To investigate further the effects on the cortex of perturbing sensory input from the hand, the effects of median and radial nerve anaesthesia on TMS evoked responses were studied (Rossini et al. 1996; Rossi et al. 1998). Local anaesthesia was applied to these nerves at the wrist, causing a complete envelope of anaesthesia over 1DI muscle whilst sparing the ADM muscle. Authors first tested the latency difference observed when MEPs were evoked in active versus relaxed muscle. The reduction in latency in MEP response evoked in 1DI muscle during active contraction, compared with the relaxed condition, was not observed following local anaesthesia. This was due to an increase in the latency of MEPs evoked in the active muscle. However in ADM (not anaesthetized), latency differences between MEPs evoked in relaxed and active muscles were preserved. Authors interpreted this data by suggesting that 'cutaneous input from stretched skin enveloping the contracting muscle is essential in energizing the corticospinal output toward that specific muscle' (Rossini et al. 1996).

In the relaxed condition, there was also a reduction in the variability of MEP amplitudes evoked in 1DI during anaesthesia. However similar modulation of F-waves (evoked by supramaximal electrical stimulation of the ulnar nerve at the wrist) suggested there were also changes in spinal excitability, although it was difficult to decide if these were 'in parallel' or 'in series' to cortical excitability changes (Rossi et al. 1998). These subtle effects on the variability of MEP responses during anaesthesia implied that during normal conditions a tonic sensory input from skin and joint receptors to the cortico-motoneuronal system exists.

Ischaemia of the forearm can also be used to block afferent feedback. MEP amplitudes evoked by TMS and recorded from the deltoid muscle, proximal to the site of ischaemia were increased (Brasil-Neto et al. 1993). This was interpreted as disinhibition of deltoid muscle by suppression of afferent feedback from active muscles distal to the site of ischaemia. Similar results were obtained in leg muscles during ischaemia applied above the knee. There was a decrease in the amplitudes of MEPs evoked in the toe muscle, extensor digitorum brevis, which was distal to the site of ischaemia. This was in contrast to the larger MEPs evoked in the thigh muscle, vastus medialis, which was proximal to the cuff. That TES evoked MEPs in leg muscles were not affected by ischaemia, was taken as evidence that the effects of sensory feedback perturbation were of a cortical nature. This theory was supported by the fact ischaemia did not affect H-reflex response elicited from the vastus medialis muscle by electrical stimulation of the femoral nerve (Brasil-Neto et al. 1993).

To summarize, in this part of the Introduction evidence has been presented to show that voluntary activity can affect TMS evoked MEPs by acting at both spinal and cortical levels. Voluntary contraction also activates peripheral input, which has additional effects on TMS evoked responses. Although these effects can be mediated at a spinal level, it is

clear that peripheral input can also exert effects on the excitability of the motor cortex. Perturbing afferent feedback from cutaneous receptors or blocking sensory nerves, highlights the importance of afferent ascending pathways. There are deleterious behavioural consequences of digital nerve anaesthesia, which might be linked with excitability changes within cortico-motoneuronal system. It is therefore clear that peripheral afferent input plays an important role in the cortical control of hand function.

PART C: Synchronous oscillations in the motor system

1.25 Cortical oscillations and the motor system

Several chapters in this Thesis include investigation of EMG oscillatory activity. There is strong evidence to suggest that frequencies within the EMG signal arise from a central rhythm generator. In this Thesis, the involvement of the motor cortex in the generation of synchronous oscillatory activity within the motor system is investigated with TMS. In addition digital nerve anaesthesia is used to assess the effect of peripheral feedback on this system. This part of the Introduction provides explanations as to why investigating synchrony in the motor system is important, as well as describing current knowledge about the function and origins of oscillatory activity. A large part of the work in this Thesis, and work reviewed here, includes coherence analysis. Calculating coherence is a means by which to measure the functional connectivity between different cortical areas, between cortex and muscle, or between muscle pairs. Cortico-muscular coherence in the motor system can be viewed as the mechanism by which 'cortex speaks to muscle' (Mima and Hallett 1999).

Brain waves from human cortex are most commonly recorded using electroencephalography (EEG). Rhythms or frequencies of oscillations within cortical activity are generally referred to in bands. The alpha band, recorded over posterior scalp regions, includes frequencies from 8–13 Hz, whereas the beta band refers to frequencies from 14–40 Hz. Higher or gamma frequencies from 40–60 Hz also exist.

Oscillatory activity within the sensorimotor cortex of humans has been recorded using EEG, or the more sensitive technique of magnetoencephalography (MEG). Predominant frequencies of oscillations around 10 Hz and 20 Hz are interpreted as components of the mu rhythm. There is also evidence for higher frequencies around 40 Hz, which are similar to the frequency of the Piper rhythm, found in muscle (Salenius et al. 1996; Brown et al. 1998). Only the lower frequencies will be discussed in this review.

1.26 Movement effects on oscillations within the sensorimotor cortex

Movement of the hand clearly affects oscillatory activity in the sensorimotor cortex. The amplitude of 10 Hz and 20 Hz components of oscillatory activity recorded with EEG is reduced immediately before and during finger movements, and is larger just after movement (Stancak and Pfurtscheller 1996; Pfurtscheller et al. 1996). Mu rhythm recorded with MEG is similarly suppressed during movements of digits of the hand, and increased following movement termination (Salmelin and Hari 1994).

Pfurtscheller et al. (1996) suggested that the suppression, or desynchronization of cortical oscillatory activity during movement, and its rebound synchronization following movement, indicated that cortical synchrony was no more than an idling rhythm, occurring between meaningful activation of the motor system.

However MEG studies by Hari and colleagues (Hari and Salmelin 1997) illustrated a clearer task modulation in the mu rhythm, suggesting a more functional role for cortical oscillations. The source of the 20 Hz oscillations was shown to originate predominantly from the anterior bank of the central sulcus (the motor cortex), whereas the 10 Hz signals arose from the hand somatosensory area in the postcentral cortex (Salmelin et al. 1995; Hari and Salmelin 1997). Despite the fact that both 10 Hz and 20 Hz components of the mu rhythm were affected by movement, there was clearly a different time course of effects for each component. The 20 Hz rhythm was suppressed earlier, and was first to resume synchronization when movement was terminated (Salmelin and Hari 1994); this was taken to reflect a close association with the motor output (hence its closer proximity to the motor cortex). These latency differences were also observed by Pfurthscheller et al. (1996). In addition, the origin of the 20 Hz rhythm changed systematically depending on the body part moved, whereas 10 Hz sources remained stable (Salmelin et al. 1995). Authors suggested that whereas 10 Hz rhythms might be primarily modulated by sensory input, a stronger connection existed between 20 Hz cortical rhythms and motor output.

This link between the mu rhythm, (and in particular the 20 Hz components) and the motor cortex, was of particular interest. To test the involvement of the motor cortex in the rebound of synchronization following movement, TMS was used (Chen et al. 1998). A decrease in the amplitude of MEPs evoked 500 ms–1 s following movement termination, was said to reflect a reduction in corticospinal excitability due to the return of cortical synchronization. Desynchronization of the cortex induced by movement, lasted approximately 200 ms after termination of movement, as indicated by an increase in the size of MEP responses in this period. These results could be interpreted by the Pfurtscheller theory as inactivity of the motor cortex during synchronization, and this may result because functionally relevant inhibitory mechanisms within the motor cortex operate during periods of synchronization.

In a similar manner to voluntary movement, median nerve stimulation caused an immediate suppression of both frequencies around 10 Hz and 20 Hz in sensorimotor cortex (Salmelin and Hari 1994; Salenius et al. 1997b). There then followed a rebound in amplitude or synchronization of these oscillations, with 20 Hz effects appearing earlier (similar to effects occurring when movement was terminated). A reduction in MEP size, 200 ms–1 s after median nerve stimulation, again indicated reduced cortical excitability associated with synchronization (Chen et al. 1999). This provided further evidence to suggest that peripheral input, which is essential in normal hand function, modulates cortical oscillatory activity.

To summarise the results of these investigations within the cortex, it is clear that there is a strong connection between cortical oscillatory activity and the motor system. In addition both 10 Hz and 20 Hz rhythms are affected by sensory input, such as that induced during movement, or by median nerve stimulation.

1.27 Synchronous activity in muscle

Synchrony at the motoneuronal level was first demonstrated by investigating the respiratory system. Short-term synchronization of intercostal motoneurone activity, was suggested to be the result of branched common stem pre-synaptic fibres (Sears and Stagg 1976). Synchronization was caused by the fact that units tended to fire at the same time because they received branched input from a common source (McAuley and Marsden 2000).

Similar short-term synchronization was then observed when recording single motor units from intrinsic hand muscles (Datta and Stephens 1990). A number of lines of evidence suggested that the presynaptic inputs producing synchronization of hand muscle motoneurone firing were of corticospinal origin (Datta et al. 1991; Farmer et al. 1993b).

It is also possible that single units fire at a certain frequency because of a common driving oscillation, which results in tuned synchronization (McAuley and Marsden 2000). Time-domain analysis of motor unit firing data can differentiate short-term synchronization and tuned synchronization. Branching of non-rhythmic inputs to motoneurones results in a narrow central peak in the cross correlogram, whereas an oscillating drive results in a central peak surrounded by a series of peaks or 'side lobes' displaced at regular intervals from the zero (McAuley and Marsden 2000). However, tuned synchronization is best detected by coherence analysis, which is analogous to cross-correlation, but is performed in the frequency-domain.

When the firing rates of single motor unit pairs were compared with coherence analysis, it was shown that there was a correlation at frequencies between of 1–12 Hz, but also in the range of 16–32 Hz (Farmer et al. 1993a; Mills and Schubert 1995). Evidence of firing modulation at frequencies around 20 Hz, despite the fact the mean firing rate of motor units is around 10 Hz, indicated the presence of an oscillating corticospinal drive (Farmer 1998).

1.28 EMG oscillations and tremor

Predominant frequencies present in surface EMG, are similar to those found between pairs of synchronously firing motor units and gross electrical activity in the sensorimotor cortex. Thus, during low levels of voluntary activity, the frequency of oscillations within the EMG signal falls within two main bandwidths, peaking around 10 Hz and 20 Hz. The origin of frequencies within the EMG has been investigated in studies of physiological tremor. In normal subjects, tremor is observed in EMG signals when the hand is outstretched. Lower frequencies of tremor around 10 Hz were first documented by Elble and Randall (1976), however it was later evident that tremor was apparent at frequencies around 20 Hz and 40 Hz (McAuley et al. 1997). In pathological tremor, frequencies in the 5–10 Hz range dominate the EMG oscillatory signal (Deuschl et al. 2001). This section will focus on tremor recorded from normal subjects.

It is possible that surface EMG oscillations are a reflection of oscillatory activity of populations of motor units synchronized at similar frequencies (Farmer et al. 1993a). There is a correlation at frequencies around 10 Hz, between single motor unit firing and surface EMG (Elble and Randall 1976) and coherence between frequencies around 10, 20 and 40 Hz present in surface EMG and tremor recordings (McAuley et al. 1997). As the corticospinal tract has been implicated in the driving of motor unit synchronization, it is thus likely that cortical oscillations also play a role in maintaining EMG oscillatory activity.

However there are other possible origins of physiological tremor observed in the hand to consider first, one of which is a mechanical component. This is caused by the resonant frequency of the hand, which is affected by stiffness of muscle and inertia. Resonant frequencies of the fingers are around 25 Hz, whereas for the hand, which is much heavier they are 6–8 Hz (Deuschl et al. 2001). The contribution of resonance frequency to overall EMG activity can be reduced by adding mass to the hand or fingers, and this is used to

separate these frequencies from those from another source. Modulation of mechanical resonance does not change the predominant frequencies within the EMG activity and this indicates that mechanical influences are not the main origin of EMG oscillations (Elble and Randall 1976; McAuley et al. 1997).

Another peripheral contribution to oscillatory EMG activity could be from reflex mechanisms. For this to occur, the latency of these reflex effects has to be appropriate (Deuschl et al. 2001). However, studies in which afferent feedback from the hand or forearm have been perturbed, either by ischaemia (McAuley et al. 1997) or muscle vibration (Mima et al. 2000) have also failed to demonstrate any changes in predominant frequencies within EMG activity.

Studies of 10 Hz activity present in EMG activity during slow finger movements also dispute the involvement of reflex effects. Kinematic analysis indicated that voluntary movements of fingers were not smooth, but showed pulsatile variations which appeared at frequencies of 8–10 Hz (Vallbo and Wessberg 1993). This modulation was also observed in surface EMG recordings. In order to try to find the origin of such frequencies, recordings from single muscle afferents were made (Wessberg and Vallbo 1995). No evidence was found that the speed variations in finger movement were due to the stretch reflex, as indicated by a lack of consistency between afferent firing and finger kinematics. It was suggested that these 8–10 Hz modulations in movements were caused by a pulsatile descending motor command (Vallbo and Wessberg 1993; Wessberg and Vallbo 1995).

Evidence therefore suggests that physiological tremor originates mainly from a central rhythm generating system. Several investigations have compared EMG oscillatory activity with that observed in the sensorimotor cortex. Some have found little evidence of cortico-muscular coherence at frequencies around 10 Hz when subjects perform stable

voluntary contractions (Conway et al. 1995; Salenius et al. 1997a; Kilner et al. 1999; 2000). This makes it difficult to explain the proposed central origin of physiological tremor in the 8–12 Hz range. However others have reported 10 Hz coherence between sensorimotor cortex and muscle during isometric contraction of forearm muscles (Mardsen et al. 2001) and arm extension (Raethjen et al. 2002) and between muscles during slow movements (Conway et al. 1997). However, these reports are scarce compared to the wealth of literature on cortico-muscular coherence at frequencies centred around 20 Hz.

To summarise, 10 Hz oscillatory activity within the muscle are predominant during movement, and in particular during pathological tremor. Although a central origin for at least some of the EMG oscillatory effects around 10 Hz has been suggested, it has proved difficult in healthy subjects to link cortical 10 Hz oscillations with those in the muscle. This is in contrast to EMG oscillations at frequencies centred around 20 Hz, and ranging from 14–31 Hz.

1.29 Cortico-muscular coherence in the 14–31 Hz range

Evidence exists for a strong connection between frequencies around 20 Hz in the sensorimotor cortex and EMG oscillations of the same frequencies (Conway et al. 1995). Synchronous oscillations in the local field potential recordings from monkey motor cortex in the same 15–30 Hz range were reported by several laboratories (Murthy and Fetz 1992; Sanes and Donoghue 1993; Murthy and Fetz 1996). However no obvious relationship between bursts of oscillatory activity and behaviour was established. Baker et al. (1997) first showed that 20–30 Hz oscillatory activity was particularly pronounced when monkeys performed a precision grip hold task. In addition, they showed that there was strong coherence between this cortical activity and that recorded from the contralateral hand muscles used to perform the task. They also showed that coherence at the same frequencies was found between muscle pairs. These authors suggested that EMG

oscillatory activity within different hand muscles was due, at least in part, to a common cortical drive (Baker et al. 1997).

Similar cortico-muscular coherence was recorded in humans performing a stable hand muscle contraction. Coherence at frequencies in the 15–30 Hz range was observed between MEG signals over the sensorimotor cortex, and EMG activity (Conway et al. 1995; Salenius et al. 1997a; Kilner et al. 1999; 2000). It was also possible to measure coherence at these frequencies using EEG and EMG (Halliday et al. 1998).

These studies provided clear evidence of a cortical origin of oscillatory activity at frequencies around 20 Hz present in hand and forearm muscles. This theory was supported when during weak contraction, the sites within the sensorimotor cortex where this coherence was strongest, correlated with the optimum sites for obtaining motor responses in hand muscles with TMS (monkey: Baker et al. 1997; humans: Mima et al. 2000).

Of particular interest for this Thesis, was the observation that coherence could be measured between hand and forearm muscle pairs (Baker et al. 1997; Kilner et al. 1999; 2000). This provided a means by which to measure indirectly cortical effects on muscle oscillatory activity. This approach allows the use of TMS to probe mechanisms underlying cortico-muscular coherence, without having to directly record from the motor cortex, which would have been subject to artefactual interference.

1.30 Task modulation of 14–31 Hz cortico-muscular coherence

Although synchronous oscillations in the sensorimotor cortex around 20 Hz were strong when monkeys performed a precision grip (to retrieve food from a Kluver board), there was no reliable relationship between components of this movement and oscillatory activity (Murthy and Fetz 1996). The observation that oscillatory episodes were less when monkeys performed repetitive wrist movements, led authors to suggest that synchrony in the sensorimotor cortex was indicative of attentional processes (Murthy and Fetz 1996).

However, work using a precision grip hold-ramp-hold task, has provided evidence to suggest that cortico-muscular coherence has a task-related function (Baker et al. 1997; Baker et al. 1999; Kilner et al. 1999; 2000). This argues against the possibility that cortico-muscular coherence is an epiphenomenon caused by oscillatory activity within the central nervous system 'spilling over' into interneurone and motoneurone pools (Kilner et al. 2000). These studies built on the observation that coherence between cortex and muscle, and between muscle pairs within the frequency range of 15–30 Hz, was strong during the hold period of the precision grip task. A precision grip was used to squeeze two levers between the index finger and thumb, and involved an initial hold phase, followed by a slow increase in grip force (ramp phase), and then a second hold phase at a higher force level. It was shown that cortico-muscular coherence disappeared whenever subjects moved their digits at the beginning of the trial (movement into the first hold phase) and when they removed their digits from the levers at the end of a trial (Kilner et al. 1999; 2000). This observation agrees with work reporting the effects of movement on cortical oscillations by Pfurtscheller et al. (1996), and Salmelin and Hari (1994). Thus coherence was present in the first hold period, disappeared during the ramp phases and then reappeared, approximately 500 ms after subjects enter into a second hold period, in a similar manner to the rebound phenomenon of synchronization within the cortex observed following movement. Coherence between EMG signals recorded from muscle pairs also followed the same task modulation (Kilner et al. 1999; 2000).

Clear evidence of additional task modulation of coherence was provided when the compliance of the levers was changed. The conditions were set so that subjects had to exert the same grip force even though the compliance had changed. Thus, in the more compliant condition, subjects had to move their index finger and thumb closer together to reach the required force level. It was found that hold period coherence between cortex and muscle in the 15–30 Hz range was stronger when subjects had squeezed together compliant (or springy) levers in order to reach this hold phase, than it was for less

compliant levers, or coherence during a steady isometric contraction (Kilner et al. 2000). These authors suggested that there may have been increased peripheral feedback following movement of the more compliant levers, which (given the work with median nerve stimulation) could have contributed to changes in cortical oscillatory activity, and therefore cortico-muscular coherence (Kilner et al. 2000). Following movement of fingers or ankle dorsiflexion, an increase in spindle afferent activation during the subsequent relaxation period can be observed (Edin and Vallbo 1990; Wilson et al. 1995). Given the fact that spindle afferents will be activated differently by the manipulation of levers with different compliance, it is likely that this mechanism may contribute to the difference in levels of coherence observed between tasks. It is known that the peripheral feedback present during the task, is used to parametize grip forces for the hold phases and to define the appropriate motor set for steady grip (Johansson 1996). This motor set may be reflected in the levels of coherence present in the hold periods of the task, especially in the hold period following the movement (Kilner et al. 2000).

1.31 Function of 14–31 Hz cortico-muscular coherence

In order to interpret these task-related changes in cortico-muscular coherence, it is necessary to consider the transmission of oscillatory activity from cortex to muscle. An important model, presented by Baker et al. (1999), suggested that coherence was a coupling mechanism, designed to provide an optimum means by which to recruit motoneurones. Synchronous input has been shown to be more efficient in recruiting cells, than an asynchronous source (Baker et al. 1999). This idea was applied to a model incorporating the corticospinal tract and motoneurone pool, which showed that synchrony in the descending corticospinal input could increase the force output (Baker 1997). Hence under conditions of synchrony, a given force generated in muscle could be achieved with minimum firing rates of PTNs. This model also sought to explain why synchronous oscillations are most prominent during relatively low level or monotonous tasks (Hari

and Salmelin 1997). The spread of oscillatory activity within the cortex will have effects on cortical processing; cells fire at defined rates, leaving little scope for modulation by novel inputs. This effectively reduces the amount of information that can be carried by the system (Baker et al. 1999). So in terms of the precision grip hold-ramp-hold task, used in this Thesis: although the system works well in conditions of low computational load, such as during the hold phases, when the task becomes more complex, as in the movement or ramp phase, the system cannot operate. Hence synchrony disappears, until the system becomes stable again (as in the second hold phase), when synchrony resumes. That coherence is often strongest during this second hold phase (Kilner et al. 1999; 2000) can now also be explained. It is possible that the first hold period acts as a preliminary phase in which subjects practice the hold. A repeat of this task, in the hold 2 phase, demands even less information processing, hence an increase in synchrony within the system.

A popular and more general theory to explain the function of synchrony within the motor system stems from observations made in the visual system (Engel and Singer 2001). The idea that synchrony within the cortex enables activity within different visual areas to be bound together, can also be applied to the motor system (Farmer 1998). It would certainly provide an attractive theory as to how different areas of the brain that contribute to the preparation and coordination of skilled movement, can interact and communicate. However, when this binding theory of synchrony was studied in detail with complex computer modelling, flaws were reported (Shadlen and Movshon 1999). On a more basic level, it is clear that cortico-muscular coherence within individuals is a variable phenomenon, and that some subjects perform the precision grip task with little evidence of synchrony between cortex and muscle activity. This would suggest that although coherence may play an important role in the output of the motor system, it is unlikely that

it is an essential feature, and it would appear that it varies considerably from subject to subject.

1.32 Origin of cortico-muscular coherence

The focus of studies in this Thesis is the origin of synchronous oscillatory activity within hand and forearm muscles. As explained previously, a major source of frequencies (in particular around 20 Hz) within EMG activity is likely to be from the motor cortex. It has been suggested that oscillations could be transmitted via the corticospinal tract from the motor cortex to muscle (Baker et al. 1999; Gross et al. 2000). This implies that the motor cortex is in some way involved in the generation of oscillations.

When evidence of cortico-muscular coherence was first observed, an obvious question was how the mechanism operates (Conway et al. 1995). The phase of oscillations at a particular frequency within cortical and EMG signals were compared, in order to ascertain whether the cortex led the muscle or vice-versa. If the cortex leads the muscle, then the phase lag, as it is called, should equate to the latency of corticospinal conduction (approximately 25 ms). However, Conway et al. (1995) were unable to find a consistent phase lag between signals that corresponded with corticospinal conduction delay (Rothwell 1991). Such inconsistencies were explained further by Halliday et al. (1998) who used EEG and EMG to measure cortico-muscular coherence. Their calculations of phase, implied that the delay between cortical and EMG signals would change with frequency, making it difficult to explain observations of 15–30 Hz cortico-muscular coherence. When EMG triggered averaging of the cortical MEG signal was performed, it was possible to calculate delays between cortical and EMG signals, although the wide latency range of approximately 12–50 ms did not readily fit with a simple corticospinal delay (Salenius et al. 1997a). Short phase lags of around 15 ms, were also reported in a study measuring coherence between EEG and EMG (Mima et al. 2000).

Such inconsistencies in the literature, reflect the complex nature of the analysis of coherent oscillatory activity. This is highlighted by the fact that synchronization is maintained over considerable distances, and that phase reversal may occur as a function of depth (Murthy and Fetz, 1992; 1996). In addition, it is likely that modes of recording oscillatory activity within the cortex, (local field potentials, EEG or MEG) also affect phase calculations, and may contribute to the lack of clear explanations within the literature (Brown 2000). One recent study, however, has matched the phase lag between MEG and EMG signals to a typical corticospinal delay (Gross et al. 2000).

Despite this complexity, two relatively simple theories explaining the source of coherence between cortex and EMG activity were suggested by Conway et al. (1995). One was a closed loop mechanism between cortex and motoneurones (permitting very short phase lags) and incorporating both afferent and descending connections. The other was that synchrony arises from a common oscillatory input to both the motor cortex and muscle, possibly from subcortical brain regions such as the basal ganglia, brainstem or thalamus.

Arguments against the closed loop theory have included the observation that coherent frequencies around 20 Hz are usually similar in arm and leg, despite longer reafference latencies associated with the leg (Salenius et al. 1997a). In addition there has been no clear evidence to support the claim for an essential role of peripheral feedback in the maintenance of EMG oscillatory activity. McAuley et al. (1997) found that ischaemia of the hand and forearm, which effectively disrupts peripheral feedback, did not change the amplitude of predominant frequencies in 1DI during abduction of the index finger. In addition loading of the finger, which lowers peripheral feedback, also had no effect on the frequency of EMG oscillations. These observations argue against mechanical resonance, or tonic stretch reflex loops as origins of oscillatory activity in EMG. Further evidence to dispute the involvement of reflex loops comes from measurements of EEG and EMG

oscillations (Mima et al. 2000). Muscle tendon vibration did not affect the amplitude of oscillations within either signal, or the cortico-muscular coherence. One chapter in this Thesis makes further investigations regarding peripheral effects on synchrony in the motor system, by studying the effects of digital nerve anaesthesia on coherence between muscle pairs.

1.33 Patient Studies

It remains then to decide which central oscillators might affect the motor cortex and corticospinal tract. Given the distributed nature of activity throughout the 'motor network' it is likely that many different structures are involved. Patient studies provide an alternative means by which to investigate this. Should an abnormality within the motor system of patients affect EMG oscillatory activity, then it could be that the neuronal elements involved normally play an important role in the maintenance of synchrony within the motor system.

With regard to 10 Hz oscillations within EMG, the lack of coherence between cortical and EMG signals, at least during stable contractions, has thrown doubt on cortical origins of these frequencies. However, as mentioned previously, work by Wessberg and Vallbo (1995) has implied that cortical activity around 10 Hz may play a role in EMG activity during movement. Evidence of connections between motor cortex and muscle at frequencies around both 10 Hz and 20 Hz, comes from the studies of patients who have mirror movements (Farmer et al. 1990; Mayston et al. 1997; Mayston et al. 2001). These patients receive a shared voluntary command from the motor cortex which is transmitted by activity in corticospinal axons, which project both contra- and ipsilaterally. This pathway gives rise to abnormal synchronization between homologous left and right muscle motor unit firing (Farmer et al. 1990; Mayston et al. 1997). Coherence analysis was performed in patients with X-linked Kallmanns syndrome who showed mirror movements. Like control subjects, patients showed coherence between right index finger

EMG (extensor digitorum muscle) and tremor signals at frequencies between 6–12 Hz and 15–40 Hz. However, unlike control subjects, in which no coherence was observed across the body, patients with mirror movements showed coherence in the same frequency ranges (maxima at 8 Hz and 22–23 Hz) when left EMG signals were compared to right EMG signals and vice versa. Similar observations were obtained from correlations of left and right tremor signals. Finally, left tremor signals were coherent with right EMG signals and vice versa in the patients with mirror movements and not control subjects (Mayston et al. 2001). These data suggest that the corticospinal tract is involved in EMG oscillations and tremor, at frequencies around both 10 Hz and 20 Hz. The fact that in the patients with mirror movement, short latency spinal reflexes were shown to elicit normal ipsilateral responses, suggests that the bilateral effects on oscillatory EMG activity were not due to spinal mechanisms (Mayston et al. 1997). Transcortical reflex responses, shown to elicit abnormal bilateral effects in ongoing EMG activity, may have contributed to oscillatory effects. However authors found it hard to correlate the latencies involved with the transcortical loop, with the wide range of frequencies observed in EMG oscillatory activity (Mayston et al. 2001). In addition similar sensory evoked potentials measured following median nerve stimulation, were recorded in both control subject and patients with mirror movements (Mayston et al. 1997). This shows that afferent inputs involved in the ascending part of the transcortical loop are normal in patients with mirror movements.

These studies with patients with mirror movements, provide further evidence to suggest that the motor cortex and corticospinal tract are involved in the generation of oscillatory activity within the motor system. Other patient studies suggest that other brain areas may contribute to this mechanism. In Parkinson's disease, synaptic connections between the basal ganglia, the cortex and the thalamus are disrupted, and this has deleterious affects on motor function. Should oscillatory activity be affected in Parkinson's disease, this would suggest that structures within the basal ganglia, cortical and thalamic loop might be

involved in the normal generation of cortical oscillations. It was observed that in Parkinson's patients, the normal predominance of EMG activity at frequencies between 15–30 Hz was reduced, leaving frequencies around 3–5 Hz to dominate. When the disease was treated with drugs or deep brain stimulation, frequencies between 15–30 Hz in EMG could be restored (Brown 2000). Further observations have implicated the cerebellum; in patients suffering from tremor due to cerebellar disease, local field potentials recorded from thalamic relay nuclei with cerebellar inputs were coherent with activity within sensorimotor motor cortex and with EMG activity in the 20 Hz range (Marsden et al. 2000). That the cerebellum is involved in pathological tremor would suggest that its activity might be suppressed during normal motor activity.

1.34 Generation of oscillatory activity in the cortex

The presence of oscillations at frequencies around 10 Hz and 20 Hz recorded using EEG or MEG is thought to arise from activity of populations of synchronized neurones (Baker et al. 1999). Synchrony could be the consequence of a common input, either from a remote source, or from within the structure examined (Whittington et al. 2000). The source usually includes neuronal elements with pacemaker qualities, which provide an internal oscillating mechanism. This is then projected to other cells, entraining them to the oscillating rhythm. It is also possible that a network exists, of either inhibitory cells or excitatory cells, or a combination of both. Activation of synaptic connections within this system creates an oscillating wave of activity, as components in the network interact (Whittington et al. 2000). Synchronous oscillations may therefore arise due to the fact that a brain region well known for its oscillatory features, may project onto cells within the motor cortex. Alternatively, PTNs which descend the corticospinal tract, may themselves be part of an oscillatory network.

A likely subcortical source of synchrony is the thalamus. Thalamic neurones have been shown to display oscillatory behaviour in vitro at frequencies around 5–10 Hz (Jahnsen

and Llinas 1984). Interest has been centred on the activity of these cells as the basis for oscillatory bursts at 7–14 Hz, or spindles, observed in EEG during sleep (Lopes da Silva 1991). However, it was shown that in isolation, each thalamic nucleus was incapable of generating spindle oscillations (Steriade et al. 1985). This suggested that a network of neurones generated the oscillatory activity. Indeed both reticular neurones and local interneurones have been shown to exert inhibitory effects on thalamo-cortical cells, and these cells form an inhibitory feedback loop mediated by GABA (Lopes da Silva 1991). So in terms of the internal oscillatory activity of the thalamic system, it appears a population effect prevails rather than the effects of pacemaker cells (though the two mechanisms may operate together). How is this relevant to oscillations observed in the motor cortex? On the one hand it is possible that thalamo-cortical cells projecting to the cortex, provide an oscillating drive. Indeed such a mechanism has been implicated by the fact that many forms of tremor can be treated by thalamotomy or thalamic stimulation (Elble 2000). To assess thalamo-cortical drive to the cortex, cortico-cortical coherence was compared with thalamo-cortical coherence by a method called partialization. That still appreciable cortico-cortical coherence remained following removal of thalamic signal effects suggested that thalamic drive is not the main source of synchronous activity within the cortex (Lopes da Silva 1991).

The alternative theory is that neuronal elements within the motor cortex are part of similar oscillating networks, yet to be fully investigated. Work in this Thesis supports such a proposal. It is possible that PTNs within the motor cortex contribute to oscillatory networks, which operate in a similar manner to those found in other brain regions, such as the thalamus (Lopes da Silva 1991) or hippocampus (Traub et al. 1998). Certainly all the components necessary for an oscillating network are available within the motor cortex. Modelling work has suggested that inhibitory interneurones, in particular those mediated by GABA, play an essential role in rhythm generation (Lytton and Sejnoski 1991; Pauluis

et al. 1999; Whittington et al. 2000). The location of inhibitory input was shown to be important, with inhibitory interneurones projecting on or near the soma of PTNs being particularly effective in producing precise timing (Lytton and Sejnowski 1991). This is in contrast to input on the dendrites, which would elicit broad postsynaptic potentials, with more prolonged latencies. This modelling work has also suggested that $GABA_A$ receptor mediated effects would be likely to be involved, given the rapid conductances shown by these receptors. The conductances of $GABA_B$ receptors, formed on pyramidal cell dendrites, are likely to be too slow (Lytton and Sejnowksi 1991). Pauluis et al. (1999) by a similar modelling method, highlighted the necessity for inhibition strong enough to cancel excitation and reset the network on each cycle of oscillation.

The existence of GABA inhibitory interneurones within the motor cortex has been extensively discussed earlier in part A of this Introduction. That similar inhibitory interneurones may be involved in generating synchronous oscillations within the cortico-muscular system, makes investigations with TMS (chapters 4,5 and 6) all the more exciting.

1.35 The role of the motor cortex in generation of oscillatory activity

Clear evidence has been presented to suggest that neuronal elements within the motor cortex, form part of the rhythm generating system that projects to motoneurones (Pinches et al. 1997; Jackson et al. *in press*). The fact that firing of PTNs are phase-locked to oscillatory activity recorded over the motor cortex via local field potentials, suggests that PTNs are involved in oscillation generation (Pinches et al. 1997). Further work supporting this claim showed that stimulation of PTNs reset the phase of oscillatory activity at frequencies around both 10 Hz and 20 Hz recorded in local field potentials. In addition the phase of frequencies around 20 Hz in EMG oscillatory activity were also reset in a similar manner (Jackson et al. *in press*). This suggests that rather than being driven by a separate oscillating system, residing in a subcortical region of the brain, the

motor cortex is directly involved in oscillation generation. It also suggests that despite lower frequency (around 10 Hz) and higher frequency (20 Hz) rhythm generators being present, only the higher frequency oscillations are normally transmitted to EMG signals. This explains the fact that during stable contractions of hand and forearm muscle, cortico-muscular coherence is strongest at frequencies between 15–30 Hz (Kilner et al. 1999; 2000). Work in this Thesis continues with investigations of this theory, using TMS.

CHAPTER 2: GENERAL METHODS

2.1 Recordings

In this thesis bipolar surface EMG recordings were made from up to four different muscles of the hand and forearm. These were right first dorsal interosseous (1DI) and abductor pollicis brevis (AbPB) of the hand, and flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC) muscles of the forearm. In all studies apart from the one in chapter 3, EMGs were amplified (x 500 or 1000 Hz), high-pass filtered (30 Hz) and sampled at 5000 Hz by a PC-compatible computer attached to a 1401+ interface (Spike 2 CED Ltd. Cambridge, UK). In chapter 3 details specific of that study are given about surface EMG recordings from AbPB and single motor unit recordings made from 1DI.

Surface EMGs were recorded using Ag-AgCl adhesive surface electrodes (Kendall, Germany or Dantec, UK) which picked up electrical activity produced by populations of motor units in the muscle of interest. For each muscle, one electrode was placed on the belly and one placed approximately 10 –15 mm away preferably close to bone, to act as a reference in this differential recording. In order to ensure a good contact of electrodes, prior to placement, the subjects skin was rubbed with abrasive gel (Omni prep, Dantec UK) and cleaned with absolute alcohol. In some experiments conducting electrode gel (Dantec UK) was used if pre-gelled electrodes were not available. All subjects were earthed by means of a metal strap or electrode attached to an earth lead, in order to reduce 50 Hz noise on the EMG recording. Efforts were made to record EMG signals as consistently as possible across subjects (electrode placement, reduction of noise).

2.2 Behavioural tasks

In all studies apart from the one in chapter 3, subjects performed precision grip tasks designed previously to investigate coherent EMG oscillatory activity (Kilner et al. 1999;

2000). In the chapter 3 study, experiments were performed at rest, or when subjects maintained a steady state of voluntary activity in 1DI by abducting their index finger. In this study auditory and visual feedback of subject's activation of 1DI was provided (see chapter 3 for details). In all other studies an alternative form of EMG feedback was used (see below).

2.2.1 Hold-ramp-hold task

In most studies, a hold-ramp-hold task was performed with the aid of a manipulandum that was under robotic control. Subjects gripped two levers between the tips of the thumb and index finger of their right hand; the forearm was supported in the fully pronated position, and the other digits were flexed out of the way (Figure 2.1A). The levers were each 120 mm long, 40 mm wide and 1.5 mm thick and each lever was fixed to the shaft of a DC motor. The motors were controlled by a robotic device (Phantom Haptic Interface, Sensable Technologies Inc, Cambridge MA) which could generate a variety of different force conditions, and was used in these experiments to create a 'virtual spring' so that the levers felt like a compliant object. The spring constant of the levers was under computer control. As subjects squeezed, lever position was encoded optically (resolution of ~33 counts per mm movement of the lever tip). This data was used offline to assess subjects performance during the task (Figure 2.1C). In chapter 7, lever position data was used to measure the velocity of subject's movement (see chapter 7 for more details).

2.2.2 Visual feedback

Visual feedback of the forces exerted on the levers was given by square cursors (position indicators) displayed on a screen mounted at the subjects' eye level (Figure 2.1B). Subjects were instructed to keep these cursors within two target boxes, also shown on the screen. At the onset of each trial, an initial force of 1 N was required to move the levers from their rest position. Subjects then had to produce a rapid contraction to increase the

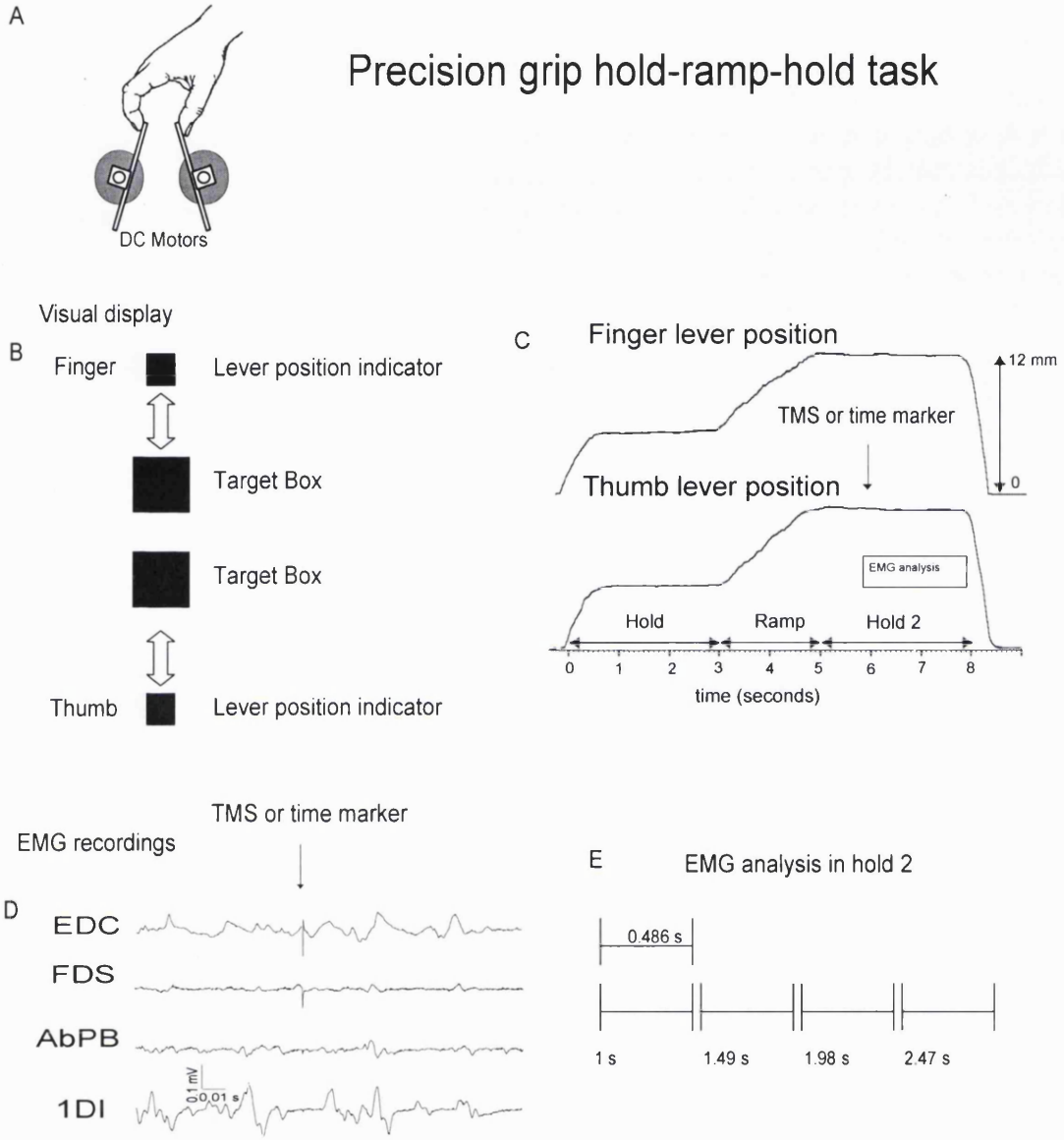


Figure 2.1 : Details of the hold-ramp-hold task. Subjects performed a precision grip task by squeezing two levers (A) and following target boxes displayed on a computer screen (B). Lever position indicators were provided as feedback (B), and their position in time was also recorded online (C). Subjects performed a hold-ramp-hold task and EMG recordings (D) were taken from first dorsal interroseous (1DI), abductor pollicis brevis (AbPB), flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC). In some experiments,TMS was applied during the hold 2 phase of the task. In 'no stimulus' trials a time marker was given at the time when TMS would have been applied. EMG analysis was performed on data from the hold 2 phase of the task (C), following TMS or the time marker. (E) FFT analysis of 128 points permitted a window of analysis of 0.486 s length. In chapter 4 only one window was used. In most other studies 4 windows of analysis were used giving a total analysis period of approximately 2 s.

force on each lever to 1.3 N in order to get the cursors into the target boxes. This force was then maintained for 3 s (hold 1). The subjects then tracked a slow linear increase of the force up to 1.6 N over a 2 s period (ramp), followed by an additional hold at this force level for 3 s (hold 2). The target boxes then disappeared, and subjects released the levers.

2.2.3 Spring constant of the hold-ramp-hold task

A spring constant of 0.05 N/mm was used for the hold-ramp-hold task. From the starting position of each lever a displacement of approximately 6 mm was required to reach the 1.3 N target force in the hold 1 phase. For the hold 2 phase, a displacement (from the starting position) of approximately 12 mm of each lever was required to reach the 1.6 N force target. Starting positions of the levers required a grip aperture of approximately 60 mm and the grip aperture during the hold 2 phase of the task was approximately 40 mm.

2.2.4 EMG feedback

In chapters 5 and 6, EMG feedback of 1DI muscle activation was provided for the subjects. This was done in order to keep the level of 1DI muscle activation consistent over trials (in particular for the hold 2 phase of the hold-ramp-hold task from which EMG data was later analysed). Feedback was achieved with the aid of a custom made display (Electronic Design and Engineering, Cambs) of rectified and smoothed EMG (time constant 500 ms). The display was fixed to a stand beside the computer screen in front of the subject. There were 10 lights on the display, arranged in a vertical column. Calibration of the display was carried out by defining a range of EMG levels, with a lower limit (subject at rest) and a top limit (e.g. maximum voluntary contraction of the target muscle). The target level of EMG activity could then be set at any desired level (e.g. 5 % maximum voluntary contraction) or to match a certain level of EMG typically used by subjects during a particular phase of the task.

2.2.5 Isometric task

In chapters 5 and 6, as well as the hold-ramp-hold task, subjects also performed an isometric task. In this task the index finger and thumb levers were fixed with the aid of screws at the bottom of each shaft. A metal block was placed between the levers to ensure they remained rigid. A grip aperture of approximately 40 mm was required to grip the static levers between index finger and thumb. This matched the final aperture required to reach the hold 2 phase of the hold-ramp-hold task. EMG feedback was provided with the aid of the EMG display, which gave feedback of 1DI activation only. No other feedback was provided in this task. Subjects were instructed to keep activation of 1DI constant by matching a target similar to that given in the hold-ramp-hold task. Visual inspection by the experimenter of the signals from all other muscles recorded online, ensured that a suitable activation level was maintained. The isometric task lasted approximately 3 or 6 minutes.

2.3 Transcranial Magnetic Stimulation

For the study in chapter 3, TMS was used (Magstim 200 stimulator; figure-of-eight coil; outer diameter of each loop 9 cm; Magstim Company Ltd, Dyfed UK). Stimulation was given over the left motor cortex (current flow through the junction region of the coil evoked an anterior-posterior current within the cortex). Minor adjustments of coil position were made in order to find the 'hotspot' on the scalp at which TMS evoked the most consistent MEP response at the lowest TMS intensity in right AbPB. In the surface EMG experiments for this study, subjects lay on a bed and the coil was fixed in place with a clamp. During the single motor unit recordings, subjects sat in a chair, and the coil was held in place by the experimenter.

Studies described in chapters 4, 5 and 6 also involved TMS, although a different coil was used for these experiments (Magstim 200 stimulators; double-cone coil, outer diameter of each loop 13 cm; Magstim Company Ltd, Dyfed UK). The coil was positioned on the

subjects head whilst they were sitting in a chair. Stimulation was given over the left motor cortex (current flow to induce an anterior-posterior current within the cortex). Minor adjustments of coil position were made in order to find the 'hotspot' on the scalp at which TMS evoked an optimal response in right 1DI, at the lowest intensity. The coil was then fixed in place with the aid of velcro strapping around the subject's head and the heavy lead was supported in a harness system above the subject's head. In the hold-ramp-hold task TMS was delivered once during each trial, 1 second into the hold 2 phase. This choice of timing was based on the fact that 15–30 Hz coherence has been reported to be most pronounced in this period (Kilner et al. 1999; 2000). In the isometric task, during which subjects maintained a steady contraction for a period of approximately 6 minutes, TMS was given every 6 s.

2.3.1 TMS intensities

Resting motor threshold (RMT) is usually defined as the minimum stimulus intensity required to elicit at least 5 out of 10 MEPs with amplitude of approximately 0.05 mV at high recording gain. In this thesis this definition of RMT was adapted slightly. A specific tracking technique was used in chapter 3, which measured small MEP responses evoked in relaxed muscle repeatedly throughout an experiment. In order to achieve this, a target RMT response of 0.2 mV peak-to-peak in unrectified EMG data was set, and the intensity required to achieve this response was followed automatically by a computer program. For consistency, in chapters 4,5 and 7, an equivalent RMT was used i.e. the lowest intensity to elicit a small MEP (approximately 0.1-0.2 mV peak-to-peak) *on all trials.*

In chapter 3, 4 and 6 attention was focussed on low TMS intensities which were below RMT. In the case of chapter 3 many experiments were done at rest. In chapters 4 and 6, experiments were done with subjects performing a precision grip task. Note that although responses to TMS during voluntary activity were higher than during rest, RMT was still

used as a reference point in these experiments. In chapter 4, a particular effect was looked for in the oscillatory EMG activity following the stimulus. Intensities were chosen based on whether this effect was elicited, and also on the size of the MEP evoked. In other studies in this thesis (chapter 5 and 6), the RMT intensity of each subject was used to evoke suprathreshold MEPs in active muscle.

However, despite the fact that RMT provided a suitable reference throughout the thesis, TMS intensities are presented as % maximum stimulator output (that is the raw units of the magnetic stimulator). This was to avoid confusion, particularly when dealing with data from both experiments performed at rest and when subjects were active. In should be noted that RMTs for all subjects used were in the range of 18–35 % maximum stimulator output. Chapter 3 includes a comparison of results presented in terms of % maximum stimulator output or as % RMT; these results were found not to differ.

2.4 Time-domain analysis

This was used to analyse EMG responses to TMS. All analysis of EMG was carried out on full-wave rectified, but unsmoothed EMG recordings. Background EMG levels were measured by sampling 400 ms of rectified EMG data immediately before the TMS stimulus. Average background EMG levels were calculated for each condition or task. EMG data were not normalized, in an effort to obtain the most sensitive measurement possible. In the case of background EMG levels, which were very small, measurements such as M-wave amplitude, or maximum muscle activity were considered too large to provide a suitable normalizing factor.

Two measures of the responses evoked by TMS were used. In the first, sections of rectified EMG data 600 ms in length were aligned to the stimulus (400 ms pre-stimulus and 200 ms post-stimulus) and then averaged over trials. This method was used to measure the amplitude of the average MEP response, and also to detect effects of TMS on ongoing oscillatory activity in the time-domain (chapter 4).

A second approach to MEP measurement (chapters 5 and 6) was to measure the MEP size for each response, rather than measure the average of many responses. Cursors were used to define the time window in which the MEP was expected to occur. The highest value in the EMG data (or peak height of the MEP) between these times was then taken for each response. In order to obtain an average MEP value for each condition or task, the mean of all peak values was then calculated together with the standard error.

In general large MEPs (typically 10 times larger than the ongoing, background EMG) were not corrected for the size of background activity (e.g. chapter 5). It was decided that studying effects of background EMG level on unadjusted MEP amplitudes would be more informative than potentially adding more variability to the data. Smaller MEPs (obtained when low intensity TMS was used e.g. chapters 4 and 6) were corrected for background EMG, since this represented a significant fraction of the MEP amplitude.

2.5 Frequency-domain analysis

Analysis of frequencies present in the EMG signals was predominantly carried out on EMG data recorded during trials in which there was no TMS stimulus. However in all cases a time marker was provided in the data where a TMS stimulus would have been elicited. This meant that comparable analysis on both 'no stimulus' and 'stimulus' trials could be performed.

For the hold-ramp-hold task, frequency-domain analysis was performed on EMG data recorded during the hold 2 phase (Figure 2.1C). All windows of analysis started approximately 1 s into the hold 2 period, and used data immediately after the TMS stimulus (or time marker if no stimulus was given). In the isometric task analysis was performed immediately after the time marker, which was given every 6 s.

Within each study, EMG data from an equal number of trials of each task were used for frequency-domain analysis. This permitted linear statistical comparisons to be made between tasks, and across subjects. For the hold-ramp-hold tasks, index finger and thumb

lever position profiles were examined offline. Trials in which the subjects moved overtly during hold periods or were inconsistent in their performance were excluded.

In all studies where frequency analysis was performed EMG data was initially sampled at 5000 Hz. The unrectified signal was high-pass filtered at 30 Hz to remove any slow frequency movement artefacts. Note that this filtering was permitted despite the fact that frequencies of interest in subsequent frequency-domain analysis were around 30 Hz. The important point is that in the raw *unrectified* surface EMG signal, low frequency oscillations were carried by higher frequency signals. The data was then rectified and downsampled offline to 263 Hz (or 263 sampling points per s). A Fast Fourier transform (FFT) algorithm was then applied to the EMG data to produce Fourier coefficients. The FFT essentially fitted sine waves to the EMG data to pick out the predominant frequencies (note this process required EMG data to be rectified). An FFT of 128 points permitted a rectangular window of analysis of 0.486 s or 486 ms (128/263) and a frequency resolution of 2.06 Hz (263/128). This frequency resolution determined the bin width for all frequency-domain graphs.

It is possible to use more than one window of analysis within each trial for frequency-domain calculations. However, in chapter 4 a single section of data 50 ms following the stimulus in each trial was used (Figure 2.1E). This was because it was expected that the effect of TMS would be transient, and analysing larger sections of data might have 'washed out' the effect. For each subject power spectra, stimulus-locked power spectra and coherence spectra, were calculated from EMG data recorded during 58 trials.

In chapters 5, 6 and 7, coherence analysis was performed on four rectangular, non-overlapping windows of analysis for each trial (Figure 2.1E). For each condition 60 trials of EMG data were used. The first section of data began immediately after a standard time marker. For hold-ramp-hold tasks this was 1 s into the hold 2 period. The second section of data then began at 1.49 s, the third at 1.98 s and the last at 2.47 s into the hold 2 period.

The four sections covered EMG data of approximately 2 s in length (4 x 486 ms) and conveniently sampled data to the end of the hold 2 phase (which was 3 s long). In the isometric task, the same length of analysis was performed after the time marker (1 every 6 s) throughout the performance period.

2.5.1 Power spectrum calculation

Power spectra are particularly referred to in chapters 4 and 7. A simple overview of the calculations will be presented here, before the formulae are given. The power calculation was used to measure which the predominant frequencies in a single EMG signal were. The FFT analysis effectively split up each EMG signal into its component frequencies. For each frequency within each trial of data, a Fourier coefficient was produced. This can be represented as a vector, with length representing the amplitude of the oscillation, and angle representing the phase (Figure 2.2A). The power of a given frequency within a single trial is its amplitude squared (in this example the amplitude of 5 vectors representing 5 trials were denoted a_1, a_2 etc; Figure 2.2B). The phase component or angle of the Fourier coefficient vector was ignored. The squared amplitudes were then averaged over trials.

For each frequency:

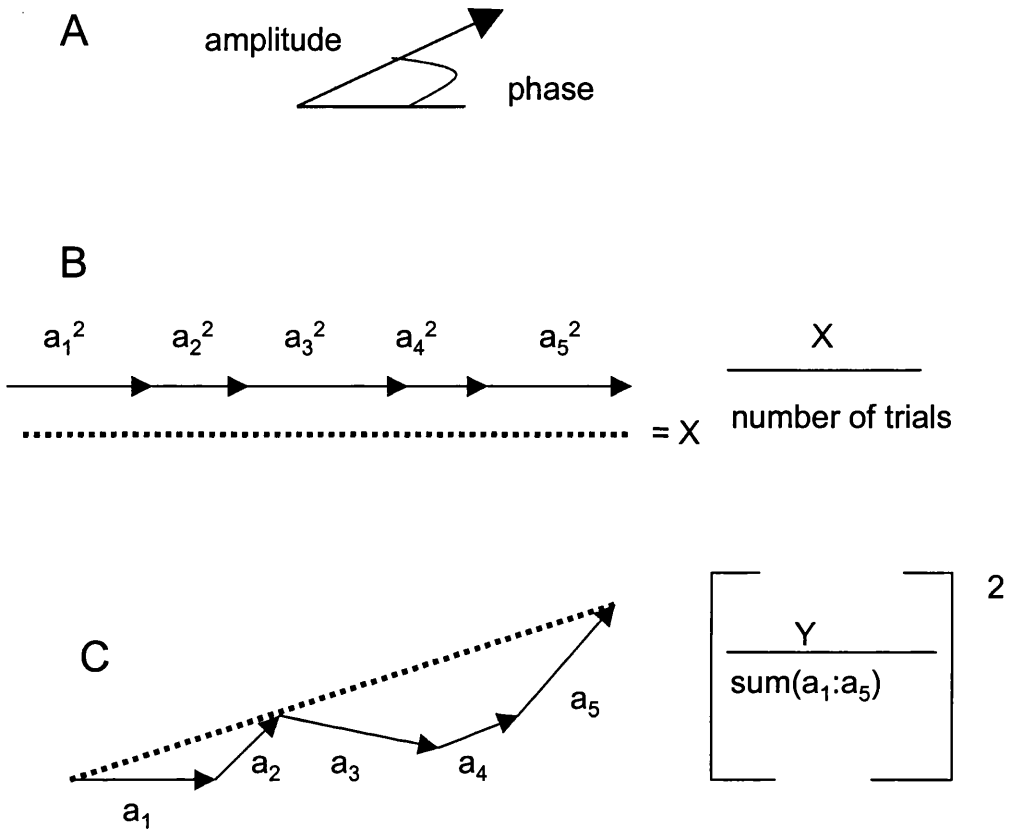


Figure 2.2 : Power and coherence calculations explained. (A) The FFT algorithm was applied to each trial to produce a Fourier coefficient for each frequency. This can be represented as a vector, the length of which represents the amplitude and the angle of which represents the phase. (B) In power calculations, for a given frequency, the amplitude component only was squared before averaging over trials (in this case 5 trials; a_1 is amplitude for trial 1). (C) In coherence calculations, for a given frequency, vectors were summed over trials. The resultant value (Y) was then divided by the sum of vector amplitudes in order to calculate a ratio, which was then squared.

2.5.2 Coherence calculation

Coherence calculations were required for all chapters except chapter 3. Coherence is measured between muscle pairs. The calculation compares the phase of a given frequency within one EMG signal, with the same frequency present in the other EMG signal. FFT analysis similar to that used for constructing power spectra was initially used on both EMG signals to extract the frequency components of each (represented as Fourier coefficient vectors). However for coherence calculations both the amplitude and phase components of the Fourier coefficient vector were important. In addition, as two EMG signals were compared, the phase component of each vector was phase *difference* between a given frequency within the two EMG signals. Vectors were then summed over trials and rather than obtaining an average value of the sum of vectors (i.e. retaining the same units as vector amplitude), coherence is calculated as a ratio (Figure 2.2C). It is a measure of how consistent the phase difference between the two EMG signals was over trials. The vector sum was divided by the sum of vector amplitudes to calculate this ratio which was then squared. For example, if a given frequency within the two EMG signals had exactly the same phase difference over all trials, then vectors would all line up in a straight line. The vector sum would equal the amplitude sum, and coherence would be 1. At the other end of the scale if there was no consistent phase difference over trials, coherence would be zero.

2.5.3 Formulae for power and coherence analysis

The calculations for coherence are described in Baker et al. (1997). Essentially the power spectrum of the signal from each muscle ($P_i(f)$) was calculated as:

$$P_i(f) = \frac{1}{N}\sum_{n=1}^{N}\left|\frac{2}{L}F_{i,n(f)}\right|^2 \qquad (1)$$

where $F_{i,n}(f)$ is the Fourier coefficient at frequency F, for the nth section of data, for signal i.

The cross spectrum between two signals (or between muscle pairs) *i=1,2*, was:

$$X_{12}(f) = \frac{1}{N}\sum_{n=1}^{N}\left(\frac{2}{L}F_{1,n}(f)\right)\left(\frac{2}{L}F_{2,n}(f)\right) \qquad (2)$$

The estimate of the coherence was calculated:

$$C_{12} = \frac{\left|X_{12}(f)\right|^2}{P_1(f)P_2(f)} \qquad (3)$$

In chapter 7, coherence between the single muscle pair 1DI/AbPB was compared across subjects. Coherence values between 14–31 Hz were summed for each subject and then multiplied by the bin width (2 Hz) to calculate the area of coherent activity ('frequency area').

In chapter 4, 5, 6 and 7 in order to calculate an overall coherence score for each subject, coherence values for the 6 muscle pairs first had to be normalized.

This was achieved by using a transformation as described previously (Kilner et al. 1999; 2000). The calculation used was:

$$Z = \arctan h(\sqrt{C_{12}})x\sqrt{2N} \qquad (4)$$

where C is the coherence value (the dependence of C and Z on frequency is suppressed for simplicity of notation) and N is the number of sections of data used in the calculation (e.g. 60 x 4 = 240).

For a given subject, transformed muscle pair coherence values at each frequency *(f)* were then combined as follows (see appendix):

$$\xi = \frac{1}{\sqrt{M}} \sum_{p=1}^{P} Z_p \qquad (5)$$

where M is the number of muscles used and P is the number of muscle pairs combined.

Combined values for each subject were then summed within a particular frequency range (4–12 Hz or 14–31 Hz). This value was then multiplied by the frequency bin width (2 Hz) to obtain the area of 'all muscle pair' coherence for that subject.

Note that for one part of the study in chapter 6 and the study in chapter 7 only 5 transformed muscle pair coherence values were combined (see appendix).

2.5.4 Statistics

Coherence larger than S may be considered significantly different from zero with $P<\alpha$ where

$$S = 1 - \alpha^{1/(N-1)} \qquad (6)$$

$\alpha = 0.05$ was used, and 95% confidence limits were added to coherence spectra. However, no assumptions were made about the variance of coherence. This applied to both single muscle pair coherence (chapter 7) and the measure ξ (see formula 5; all muscle pair coherence) when testing for significant differences between tasks or conditions. Instead, all coherence values or combined ξ values within a particular frequency range were summed and used for analysis. Significant differences in values of coherence were instead tested with a one tailed paired t-test run across subjects. All mean coherence values are quoted with the standard error value.

2.6 Appendix – 'All muscle pair' coherence calculations

The calculation used to combine muscle pair coherence values after they were normalized was

$$\xi = \frac{1}{\sqrt{M}} \sum_{p=1}^{P} Z_p \qquad (7)$$

where M is the number of muscles used in the recording (i.e. 4) and P is the number of muscle pairs combined (on the majority of occasions 6).

This combination calculation is different from that used by Kilner et al. (1999; 2000).

$$\xi = \frac{1}{\sqrt{P}} \sum_{p=1}^{P} Z_p \qquad (8)$$

where P is the number of muscle pairs combined.

A comparison of both methods is shown in table 1. With regards to calculations using 6 muscle pairs, the combination calculation used in this thesis resulted in slightly larger final coherence values (e.g. for this subject a frequency area of 204 Hz) than would have been calculated by the previously reported method (frequency area of 166 Hz). Note that no comparisons on absolute coherence values have been made to previous work by Kilner et al. (1999; 2000).

In two cases in this thesis (chapter 6 and chapter 7), only 5 muscle pairs were combined to given an overall muscle pair coherence score. In some subjects, the FDS/EDC muscle pair coherence spectra was contaminated with white noise from an unknown source, and so this muscle pair had to be excluded from each subject's data. In the combination calculation used in this thesis, M remained at 4 (still 4 muscles involved in the analysis), but P was now 5. This resulted in a lower final score for coherence (frequency area of 184

Hz) compared to when 6 muscle pairs were combined (frequency area of 204 Hz), indicating that one muscle pair less was used for analysis.

The combination calculation used by Kilner et al. (1999; 2000), however served to equal out coherence scores regardless of the number of muscle pairs used in the analysis. Note that using this method, both final coherence values were very similar (frequency area of 166 Hz for 6 muscle pairs; 165 Hz for 5 muscle pairs).

In this thesis all results presented concern *changes* rather than absolute values of coherence. All conclusions were made after statistical comparisons between tasks or conditions, using a paired t-test. With regard to all studies in which all muscle pair coherence was calculated, comparisons were only made on coherence calculated using the same number of muscle pairs.

Both methods of combination are valid, and gave the same overall result, as shown by confirmation in this thesis of observations made by Kilner and colleagues.

1	B	C	D	E	F	G	H	I	J	K
2		COHERENCE VALUES - SEE EQUATION 6								
3	Frequency	14.39	16.45	18.50	20.56	22.62	24.67	26.73	28.78	30.84
4	Muscle pairs									
5	1DI/AbPB	0.12	0.03	0.06	0.28	0.16	0.14	0.11	0.08	0.09
6	1DI/FDS	0.02	0.01	0.01	0.04	0.05	0.06	0.06	0.03	0.05
7	1DI/EDC	0.00	0.00	0.02	0.04	0.01	0.01	0.01	0.02	0.02
8	AbPB/FDS	0.00	0.01	0.01	0.02	0.02	0.02	0.05	0.04	0.03
9	AbPB/EDC	0.01	0.00	0.01	0.03	0.01	0.03	0.00	0.02	0.02
10	FDS/EDC	0.02	0.00	0.01	0.00	0.00	0.02	0.00	0.04	0.01
11										
12		TRANSFORMATION - SEE EQUATION 4 e.g. for 1DI/ABPB and 14.39 Hz SQRT(2*240)*ATANH(sqrt(C5))								
13		Note 240 is number of sections of data (4 windows * 60 trials)								
14										
15	1DI/AbPB	7.80	3.73	5.52	13.00	9.18	8.53	7.69	6.25	6.82
16	1DI/FDS	2.83	2.22	1.66	4.36	4.98	5.68	5.44	3.74	4.84
17	1DI/EDC	1.49	0.99	2.72	4.26	2.53	2.45	2.58	3.43	3.30
18	AbPB/FDS	1.07	2.49	1.76	3.37	3.20	3.27	4.95	4.31	4.03
19	AbPB/EDC	1.76	0.28	1.69	4.13	2.64	4.10	1.53	3.43	3.13
20	FDS/EDC	3.30	0.39	2.17	1.47	1.23	3.19	0.55	4.42	2.28
21										
22		COMBINATION CALCULATION USED IN THIS THESIS- SEE EQUATION 7								
23		e.g. for 14.39 Hz and 6 muscle pairs SUM(C15:C20)/SQRT(4); for 5 muscle pairs SUM(C15:C19)/SQRT(4)								
24	6 muscle pairs	9.12	5.05	7.76	15.30	11.88	13.61	11.37	12.79	12.20
25	5 muscle pairs	7.47	4.86	6.67	14.56	11.27	12.01	11.10	10.58	11.05
26										
27		SUM BETWEEN 14 -31 Hz			MULTIPLY BY 2.0559 Hz FOR COHERENCE AREA					
28	6 muscle pairs	99.08	SUM(C24:K24)		**204**	**Hz**				
29	5 muscle pairs	89.58	SUM(C25:K25)		**184**	**Hz**				
30										
31		COMBINATION CALCULATION USED BY KILNER et al 1999,2000- SEE EQUATION 8								
32		e.g. for 14.39 Hz and 6 muscle pairs SUM(C15:C20)/SQRT(6); for 5 muscle pairs SUM(C15:C19)/SQRT(5)								
33	6 muscle pairs	7.45	4.13	6.34	12.49	9.70	11.11	9.29	10.44	9.96
34	5 muscle pairs	6.68	4.34	5.97	13.02	10.08	10.75	9.93	9.46	9.89
35										
36		SUM BETWEEN 14 -31 Hz			MULTIPLY BY 2.0559 Hz FOR COHERENCE AREA					
37	6 muscle pairs	80.90	SUM(C33:K33)		**166**	**Hz**				
38	5 muscle pairs	80.12	SUM(C34:K34)		**165**	**Hz**				

Table 2.1: Summary of coherence calculations used in this thesis. Data from a single subject is shown. Coherence was calculated for all frequencies between 2 Hz and 40 Hz for each muscle pair. Coherence calculations for frequencies between 14.39 Hz and 30.84 Hz only are shown here. In order to calculate an all muscle pair coherence score for each subject, coherence values for each muscle pair were first transformed. Transformed values were then combined over muscle pairs for each frequency. Note that in some cases only 5 muscle pair values were used. The combination calculation used in Kilner et al. (1999; 2000) is provided as a comparison. Final coherence values calculated using each method are highlighted in bold.

CHAPTER 3: TWO PHASES OF INTRACORTICAL INHIBITION EXPLORED BY TRANSCRANIAL MAGNETIC THRESHOLD TRACKING

3.1 Introduction

The paired-pulse test, in which a subthreshold TMS pulse is used to condition the EMG response to a larger test stimulus, was described by Kujirai et al. (1993) as a method to study inhibitory mechanisms in the human motor cortex. Further information about the intracortical mechanisms involved has come from more invasive studies. Indirect activation of the corticospinal tract with a single test stimulus evokes multiple descending volleys in the spinal cord, termed I-waves (Nakamura et al. 1997; Di Lazzaro et al. 1998a,b). When a conditioning stimulus preceded the test stimulus by 1–5 ms, the later I-waves evoked by the test stimulus were inhibited. Suppression of the I_3-wave by a conditioning stimulus has also been inferred, without exposure of the spinal cord, from recordings of single motor units (Hanajima et al. 1998).

The mechanisms of I-wave generation and inhibition by TMS are still far from clear (Ziemann and Rothwell 2000). Kujirai et al. (1993) proposed that the intracortical inhibition is mediated by GABAergic interneurones. Hanajima et al. (1998) showed that I-wave suppression lasts up to 20 ms, a period that corresponds with the activation of $GABA_A$ receptors. Further support for this theory has come from studies in which several GABA-potentiating compounds were found to enhance intracortical inhibition (Ziemann et al. 1996a,b). However, not all GABAergic compounds had this effect (Ziemann et al. 1996a), and tiagabine, a GABA uptake blocker, has been found to reduce intracortical inhibition (Werhahn et al. 1999). This apparently contradictory result might be explained by enhanced activation of $GABA_B$ receptors, acting pre-synaptically to inhibit the $GABA_A$ mediated inhibition (Werhahn et al. 1999; Sanger et al. 2001).

The paired-pulse test has been extensively used in patient studies to test for alterations in intracortical inhibition in neurological disease. The results were initially promising, but since a similar reduction in intracortical inhibition has now been found in a wide range of neurological conditions (Ridding et al. 1995a; Ikoma et al. 1996; Abbruzzese et al. 1997; Ziemann et al. 1997; Greenberg et al. 1998; 2000), the current test has little diagnostic value and its implications for disease pathophysiology are as yet unclear. Factors that may have helped obscure disease-specific alterations in intracortical inhibition include: (i) the use of a measure of inhibition (response reduction) that is restricted in range (since reduction is limited to 100 %), (ii) the averaging together of inhibition levels over a range of interstimulus intervals (ISIs), and (iii) the dependence of paired-pulse inhibition on other factors than the integrity of particular synaptic pathways in the cortex (e.g. the resting level of synaptic drive on PTNs).

Recently, Awiszus et al. (1999) addressed the first of these issues by applying a threshold tracking procedure (Bostock et al. 1998) to the paired-pulse test. Inhibition was measured by the increase in test stimulus required to maintain a constant response, rather than by the reduction in response to a constant stimulus. The present study has additionally addressed the other two issues to further develop the paired-pulse test, in the hope of improving its clinical usefulness. In normal subjects, the dependence of cortical excitability on the timing and amplitude of a subthreshold conditioning stimulus, and on voluntary muscle activity, has revealed two distinct components of intracortical inhibition, which may differ in their susceptibility to disease.

3.2 Methods (1)

3.2.1 Subjects

16 healthy volunteers (8 males and 8 females, 23-55 years old) were the subjects. Informed written consent was obtained from all subjects. A local ethics committee approved the experimental procedures, which were performed according to the Declaration of Helsinki.

3.2.2 EMG recordings

Surface EMGs were amplified (1mV/V), filtered (2 kHz with a time constant of 100 ms) and sampled at 10 kHz. Recordings were taken from the right abductor pollicis brevis (AbPB) using 5 mm Ag-AgCl surface electrodes (Dantec, UK). Sweeps of data 300 ms in length (100 ms pre-stimulus) were saved via a custom made interface and computer program (QTRAC, H Bostock) onto a computer. Subjects were provided with audio feedback of the EMG signal, to ensure total EMG silence in the experiments performed at rest. For active muscle conditions, subjects were instructed to perform a low level of voluntary activity by simple thumb abduction. The activity was quantified by full-wave rectification and leaky integration of the EMG signal (time constant 1 s). A target level was provided, by a simple dial scale (calibrated to maximum voluntary contraction), and the subject was requested to maintain a ~10 % maximum contraction.

In some experiments, single motor units were recorded from the right first dorsal interroseous (1DI) muscle with a concentric needle electrode (Medelec). Signals were amplified (100 μV/V), filtered at 1 or 3 kHz with a time constant of 3 ms and sampled at 5000 Hz. Data was sampled in 100 ms sweeps via a 1401 interface system and computer running Sig-Av software (CED, Cambridge UK). Subjects were instructed to activate a single motor unit at a steady, low rate with the aid of audio-visual feedback. Action

potential waveform was monitored with an oscilloscope to ensure that the same motor unit was recorded during each experimental session.

3.2.3 Stimulation

TMS was applied over the hand area of the left motor cortex through a figure-of-eight-shaped coil (outer diameter of each loop, 9cm) using two high-power Magstim 200 magnetic stimulators (Magstim Company Ltd, Dyfed UK). The coil was oriented to evoke a current within the cortex flowing in an anterior-posterior direction. Both stimulators were connected to the same coil through a Y connector or bistim module (Magstim Company Ltd, Dyfed UK). For the surface EMG experiments, subjects were laid on a bed and the coil was held in a fixed position with a clamp. In the single motor unit recordings the coil was held on the head by an experimenter and care was taken to retain a constant position.

Experiments were conducted in a conditioning-test design and the ISI was varied. For the purposes of threshold tracking (see below), resting motor threshold (RMT) was defined as the stimulus intensity needed to produce a 0.2 mV (peak-to-peak) MEP response. Active motor threshold (AMT) was defined as the intensity needed to produce the same response during low level voluntary activity (~ 10 % maximum). The 0.2 mV 'threshold' response level was about twice as high as that commonly used (0.05 mV rectified EMG), and was preferred for two reasons: it enabled more efficient threshold tracking (see below), and it enabled AMT to be tracked in the presence of low level spontaneous activity without signal averaging.

3.2.4 Surface EMG threshold tracking

In order to measure intracortical inhibition the paired-pulse paradigm was used. This involved three conditions: a single conditioning stimulus intensity, a single test stimulus intensity and a paired-pulse consisting of the conditioning stimulus preceding the test stimulus (Figure 3.1A). Conventionally in the paired-pulse test, the conditioning stimulus is set at an intensity below threshold and the test stimulus is suprathreshold and set to evoke an MEP of approximately 1–2 mV. The ISI between the stimuli is usually between 1–5 ms and 3 conditions are usually mixed randomly during each experimental block. When the test stimulus is preceded by a conditioning stimulus, the MEP evoked is smaller than the response elicited by the test stimulus alone (Figure 3.1B). In the conventional paired-pulse test, quantification of how much the MEP elicited by the paired-pulse has been reduced, in comparison to the test stimulus response alone, indicates the inhibitory effect of the conditioning stimulus (Figure 3.1D).

For this study the paired-pulse test was modified. A fixed target MEP response (MEP amplitude) was set at a threshold level of 0.2 mV (peak-to-peak in unrectified EMG). Inhibition produced by the conditioning stimulus was measured as the increase in test stimulus required to achieve the target response (Figure 3.1C,E). This technique is analogous to the measurement of impulse-dependent excitability changes in peripheral nerve by threshold tracking (Bostock et al. 1998) and in order for this test to be optimal, conditions could not be randomised. Motor thresholds were measured by automatic adjustment of the test stimulus intensity by computer to maintain the MEP at the target level of 0.2 mV. MEPs were measured online, peak-to-peak and from raw unrectified EMG data, on a trial to trial basis. To achieve this the computer program set up a sampling window in the EMG data of approximately 15 ms in length, starting 20–30 ms after the stimulus. This was an appropriate latency to record MEP responses in each subject. Each paired-pulse combination comprised a fixed conditioning stimulus delivered by one magnetic stimulator followed by a varying test stimulus from the other magnetic

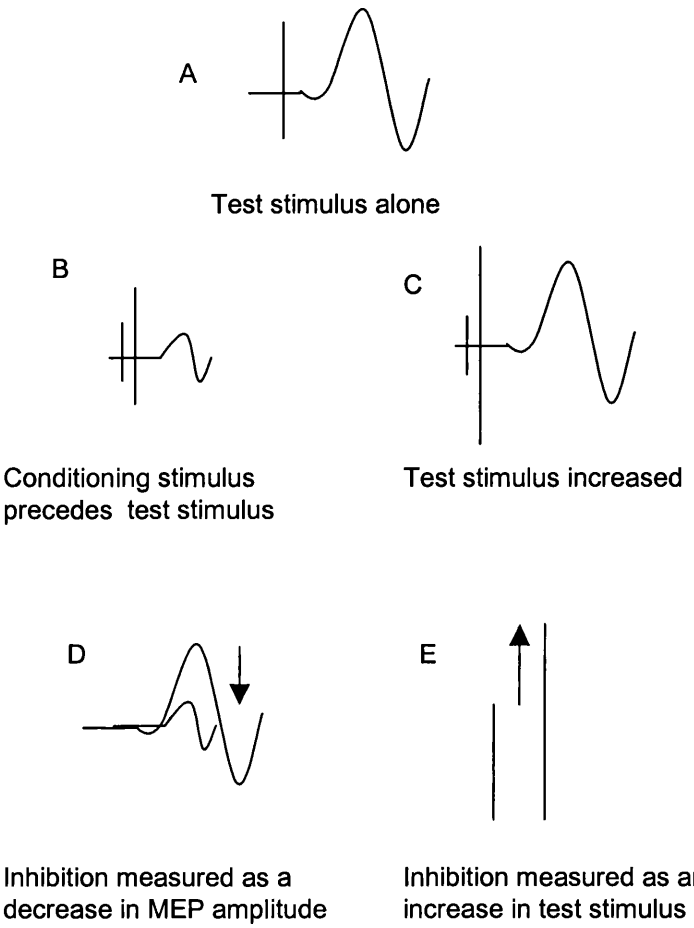


Figure 3.1 : Measuring inhibition with the paired-pulse test. (A) Test stimulus alone evokes an MEP response. (B) When a conditioning stimulus precedes a test stimulus, the MEP evoked is smaller than the response evoked by the test stimulus alone. (C) In this study, the MEP amplitude was kept constant, by increasing the test stimulus intensity in the paired-pulse condition. (D) In the conventional paired-pulse test, inhibition is measured as the reduction in MEP size when the paired-pulse response is compared to the response evoked by the test stimulus alone. (E) In this study inhibition was measured by the amount the test stimulus had to be increased in order to evoke the target MEP response in the paired-pulse condition.

stimulator. If a paired-pulse evoked an MEP response lower than the target (0.2 mV), then the test stimulus was automatically stepped up until the response reached its target. Thereafter the test stimulus was automatically stepped up or down, depending on whether the previous response was less or greater than the target response.

A tracking strategy was developed on the basis of preliminary observations of the stimulus-response characteristics of the MEPs. Figure 3.2 shows data from a single subject. Stimuli were given in 1 % maximum stimulator output steps, and presented in a upward ramp from 10 % to 50 %, and then back down again. The corresponding MEP amplitudes were recorded (Figure 3.2A). For small response levels, the stimulus-response relationship was approximately exponential, so that the relationship between the logarithm of the MEP amplitude and the stimulus was close to linear over a hundred-fold range of responses, from about 0.02 to 2 mV (Figure 3.2B). A small target response of 0.2 mV was therefore selected, in the middle of this 'linear' range. The tracking program used a modified form of proportional tracking (Bostock et al. 1998), in which the stimulus was changed in proportion to the error: *log (0.2 mV)–log (MEP response).* However, the precision of this proportional tracking was limited by the fact that the magnetic stimulus was limited to integral values from 1 to 100 % of maximum stimulator output. Since the RMT was typically close to 30 % maximum stimulator output, the changes in test stimulus were restricted to multiples of about 3.3 % of RMT.

Figure 3.3 illustrates this threshold tracking by showing part of a typical experiment, in which the effect of changing ISI on inhibition was being investigated. The black circles in Figure 3.3A,B indicate the control test stimuli (i.e. without conditioning stimulus), and their responses plotted on a logarithmic scale. The responses were variable, as expected, but fluctuate uniformly about the target level, with the larger deviations resulting in an increase or decrease in the next stimulus level. The control test stimuli were alternated with paired pulses, in which the test stimulus followed a conditioning stimulus at different ISIs. Alternation of control test stimuli with paired pulses provided a continuous

monitoring of RMT and enabled any slow changes in cortical excitability to be allowed for.

The paired pulses are represented by open circles in Figure 3.3: the conditioned test stimuli in A, their responses in C, and the ISIs in D. After each change in ISI (or in other experiments in the conditioning stimulus), paired-pulses were given with the same conditioning parameters until 5 responses were considered valid. A response was considered valid if it differed from the target response by less than 30 %, or if the last 2 responses bracketed the target response. Thus for ISI=2 ms, the first 4 responses were close to target level, whilst the last one bracketed the response, so all 5 stimuli were considered to provide valid threshold estimates and no more were required. In contrast, when the ISI was increased to 2.4 ms, many responses were too small to provide a valid threshold estimate until the test stimulus had been increased considerably, so a total of 12 stimuli were required.

For simplicity, in Figure 3.2 only the control and one set of conditioned test stimuli (with conditioning stimuli 28 % maximum stimulator output) are illustrated. In fact, to save time, three different stimulus conditions were alternated (test alone, 28 % conditioning + test, and 18 % conditioning + test), with a 4 sec interval between each single or paired pulse, so that 5 stimuli of each type were delivered per min. In most tracking experiments, the control threshold (RMT or AMT) was compared with two types of paired-pulse, but in some cases two ISIs were fixed, and the conditioning stimuli stepped over a range of intensities. Experiments investigating the effect of ISI were performed on a separate subject group (n=10) and a separate day, to those investigating conditioning stimulus intensities (n=7).

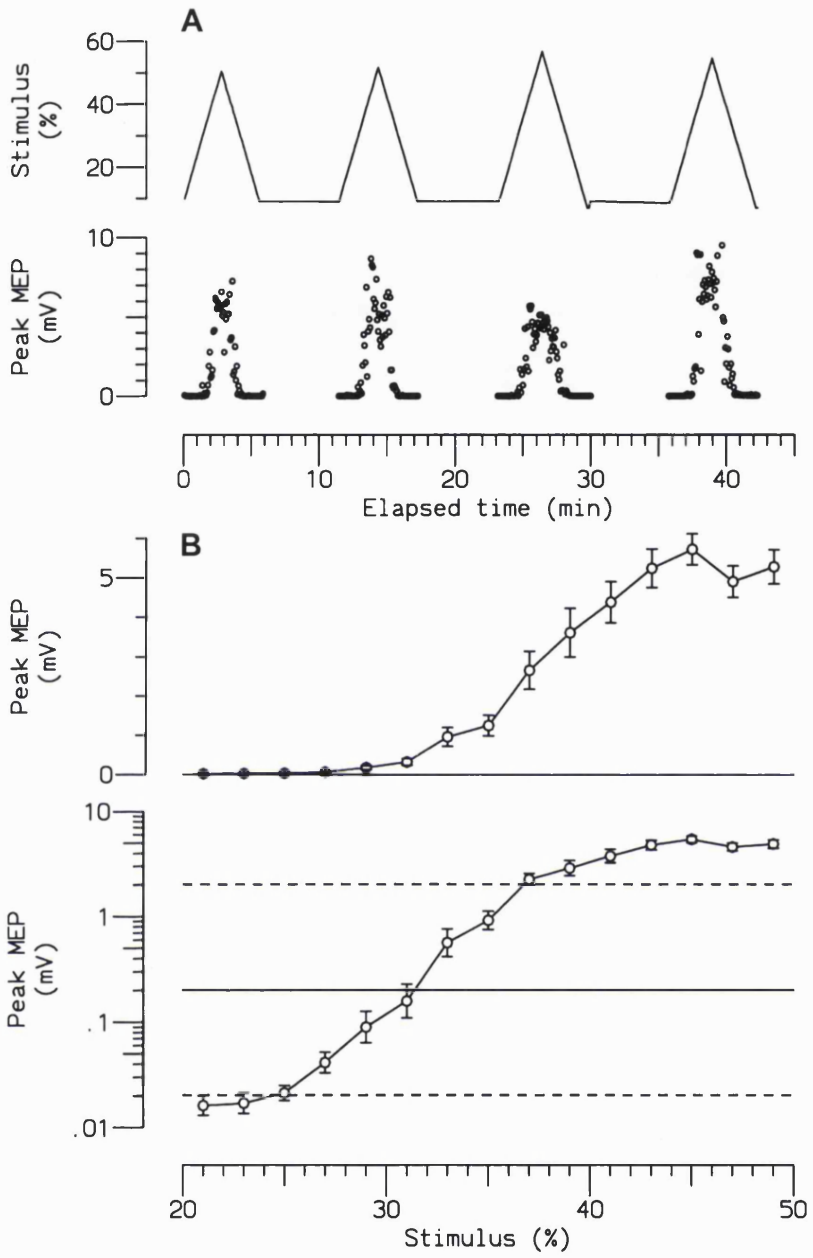


Figure 3.2: (A) Stimulus/response relationship for MEPs recorded from AbPB of a relaxed subject by stepping the TMS stimulus up and down in 1% steps. (B) Stimulus/response curves plotted for data in (A), showing mean amplitudes plotted on a linear scale and geometric means plotted on a logarithmic scale. Each point is a mean of approximately 16 values, error bars are ± SE. Note that when plotted on a logarithmic scale, the stimulus/response relationship was approximately linear for responses in the range 0.02–2 mV (indicated by dashed lines). The target response for threshold tracking is indicated by the line at 0.2 mV.

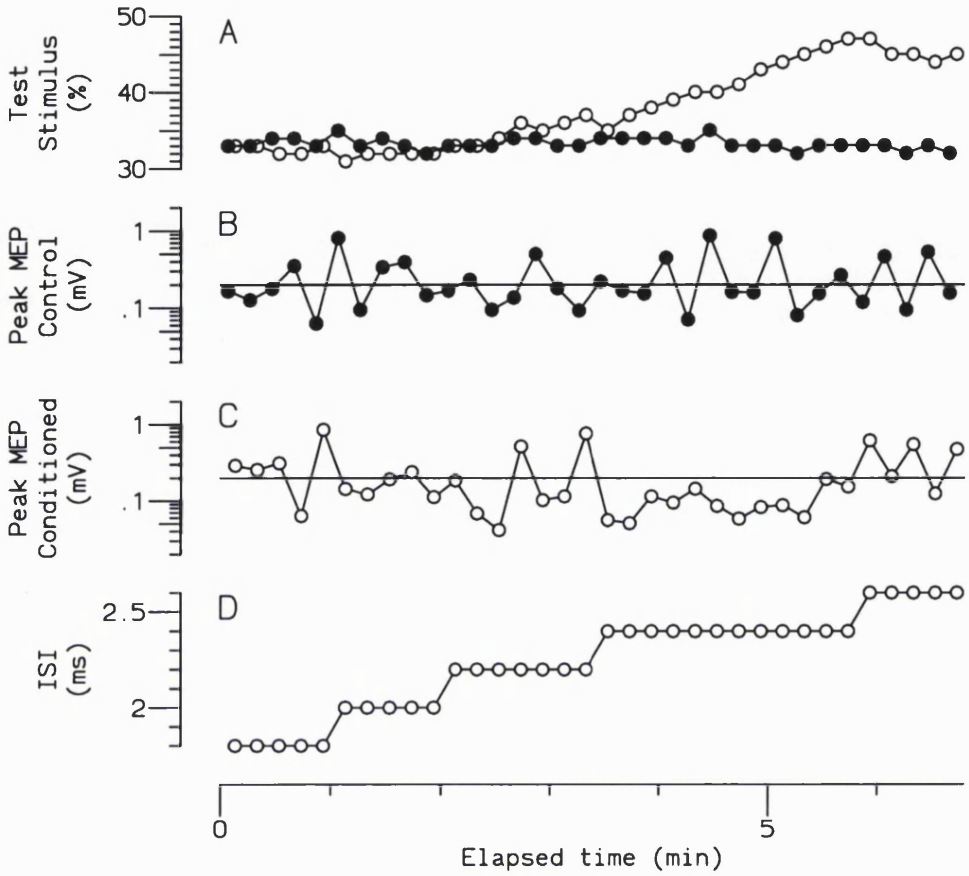


Figure 3.3: An example of threshold tracking. (A) Black circles indicate control test stimuli and open circles the conditioned test stimuli, during tracking of a 0.2 mV (in unrectified EMG) target response in a relaxed subject. The black circles provide a running estimate of the RMT. (B) Peak MEP responses to control test stimuli in (A), are plotted on a logarithmic scale, showing even fluctuation about the target response. (C) Responses to conditioned test stimuli in (A; i.e. paired-pulses). (D) Interstimulus interval (ISI) was increased in 0.2 ms steps when 5 stimuli were accepted as estimates of conditioned threshold. Note excess of low responses in C when ISI was increased to 2.4 ms, while test stimuli increased to high level, showing prominent inhibition. Conditioning stimuli were fixed at 28 % maximum stimulator output. The test stimulus was altered by 1 % for each factor of 2 by which the last response differed from the target response of 0.2 mV.

3.3 Data Analysis for surface EMG threshold tracking

3.3.1 Estimation of thresholds and calculation of inhibition

To calculate an overall estimate of the test stimulus intensity required to evoke the target response for a particular condition, *log (response)* was plotted against test stimulus intensity (as in Figure 3.2) for each paired-pulse, and the test stimulus corresponding to the 0.2 mV target was estimated by regression analysis. To limit the contribution of data points to those in the linear part of the stimulus/*log (response)* relationship, each point was given a weight (1–abs[*log (response/target)/log(10)*]. Those with negative weighting, i.e. those more than 2 mV or less than 0.02 mV, were excluded from the analysis.

Inhibition due to a conditioning stimulus was measured as the increase in test stimulus intensity required to achieve the target response. When preceded by a conditioning stimulus, the new test stimulus intensity required to evoke the target response was referred to as the conditioned threshold. Calculating inhibition was then as follows:

For relaxed muscle, Inhibition = (conditioned threshold–RMT)/RMT×100.

For active muscle, Inhibition = (conditioned threshold–AMT)/AMT×100.

To allow for slow changes in cortical excitability, the values of RMT or AMT in the above expressions were estimated from the unconditioned data obtained over the same time period as the conditioned threshold. Thus in Figure 3.3, the RMT used to calculate inhibition at an ISI=2.4 ms was obtained from the unconditioned stimuli and responses (black circles) between 3.5 and 5.9 minutes.

3.3.2 Statistics

Changes in the level of inhibition, depending on the parameters used in the paired-pulse test, were assessed with a two tailed paired t-test (unless otherwise stated).

3.4 Methods (2) Single motor unit recordings

3.4.1 Paired-pulse paradigm

3 subjects were used to make single motor unit recordings. Each subject was tested on a number of separate days, and a different motor unit was recorded from each time. On each occasion, having first obtained an estimate of RMT using a surface EMG recording, the intensity of stimulation was then reduced by approximately 5 % maximum stimulator output for the test stimulus to be used in single motor unit recordings. Subjects were instructed to recruit a single motor unit in 1DI by abduction of their index finger. A minimum level of voluntary activity was used, and subjects were comfortably able to maintain a constant firing rate. The test stimulus was then finely adjusted to a value that produced an increase of approximately 50 % in the firing probability of the motor unit, and consequently a peak in the post stimulus time histogram (PSTH). A conditioning stimulus of 30–40 % RMT (14–18 % maximum stimulator output) was then added 1 ms before the test stimulus and finely adjusted until inhibition at ISI=1 ms was observed. Both stimuli then remained fixed. 3 conditions were then delivered in a random order: test stimulus alone, the conditioning and test stimulus with ISI=1 ms and conditioning and test stimulus with ISI=2.5 ms.

3.4.2 Data Analysis: Post stimulus time histograms

PSTHs were constructed for 3 conditions: test stimulus alone (control), and for the paired-pulses, using ISI=1 ms and ISI=2.5 ms. During the experiment sweeps of data (or trials) were recorded, beginning with the time TMS was delivered, and lasting for a period of 100 ms. For each single motor unit, each condition had the same number of trials. Care was taken during each experiment to record the firing of only one single motor unit by aid of audio-visual feedback and offline, only spikes indicating firing from this motor unit were used for analysis. Sometimes after the higher intensity of the test stimulus, compound motor unit discharges were elicited. Trials in which this occurred were

rejected. For each condition, the times at which spikes occurred within each trial were recorded and used to construct a PSTH with bin width 0.2 ms. A peak in the PSTH was defined as a period of increased firing of the single motor unit, induced by the stimulus. For each single motor unit, the peak in the test stimulus PSTH was determined by eye. The start and end times used to define this peak, were then used to define the peaks for the paired-pulse conditions. In order to quantify the responses, counts within the peak for each condition were summed and the result divided by the total number of trials. This calculated the firing probability of the single motor unit for each of the 3 conditions.

Note that during the recordings there was no online PSTH accumulation. This made choosing optimum test stimulus intensities sometimes difficult (too high risked compound motor unit discharges, too low resulted in the lack of a clear test stimulus peak in the PSTH). This meant that the data from many single motor unit recordings had to be disguarded. 8 single motor units were considered suitable for the pooled analysis, and the mean firing probability was calculated for each condition.

3.4.3 Statistics

Firing rate probability was compared across conditions (test and ISI=1 ms, test and ISI=2.5 ms) with a two tailed paired t-test.

3.5 Results

3.5.1 Surface EMG threshold tracking

Three threshold-tracking protocols were used. The dependence of inhibition on ISI was first tested for different levels of conditioning stimulus in relaxed subjects. Then the dependence of inhibition on conditioning stimulus intensity was tested for two ISIs, first with the subject relaxed, and immediately afterwards with the subject making a minimal voluntary contraction.

3.5.2 Dependence of inhibition on ISI

Figure 3.4A illustrates an experiment in which a range of ISIs from 1–4.4 ms was investigated in 0.2 ms steps. The conditioning stimulus was fixed at either 18 % maximum stimulator output (squares) or 28 % maximum stimulator output (circles). The target test response was 0.2 mV and subjects remained relaxed. With the conditioning stimulus of 18 %, inhibition was maximal at ISI=1 ms (the minimum interval allowed with the Magstims). In contrast, the 28 % conditioning stimulus produced a strong peak of inhibition at ISI=2.4 ms, with a second peak at ISI>4 ms, but no inhibition at 1 ms. Similar results were obtained from other subjects with conditioning stimuli in the range of 18–28 % maximum stimulator output. However, the results were variable between subjects, and in some cases inhibition was evident at both ISI=1 ms and ISI=2.2–2.8 ms. To determine the optimum ISIs for measuring inhibition, the 2 sets of data in Figure 3.4A were averaged with a further 24, obtained from a total of 10 subjects, and plotted in Figure 3.4B. The conditioning stimuli ranged from 18–28 % maximum stimulator output (or 50–87 % of RMT), with more than 2 levels of conditioning stimuli recorded for some subjects. The results show 2 clear peaks of inhibition, at an ISI less than or equal to 1 ms and at an ISI between 2.4 and 2.6 ms, with a slight suggestion of a third peak at 4.2 ms. ISIs of 1 and 2.5 ms were therefore chosen to investigate the effect of the conditioning stimulus on inhibition in more detail.

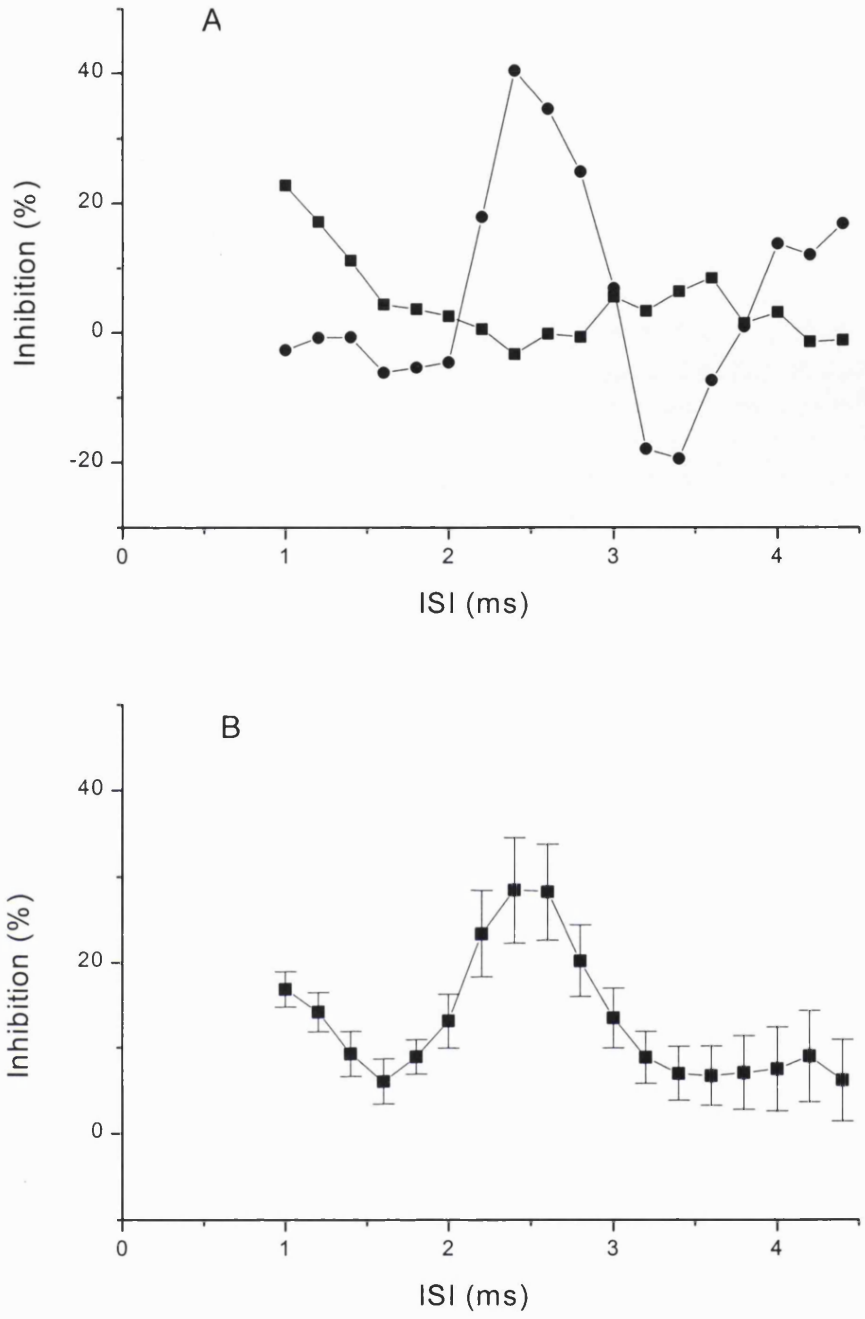


Figure 3.4: Dependence of inhibition on interstimulus interval (ISI). (A) Results of a single subject, similar to those in Figure 3.2, showing inhibition recorded for conditioning stimuli of 18 % (squares) and 28 % (circles) maximum stimulator output. Inhibition, expressed as percentage increase in threshold above RMT peaked at ISI=1 ms and ISI=2.4 ms respectively. (B) Pooled data for conditioning stimuli in the range 18–28 % maximum stimulator output from 10 subjects, means ± SE (n=26). Inhibition was consistently recorded peaking at ISI=1 ms, or ISI=2.2–2.8 ms, or both.

3.5.3 Dependence of inhibition on conditioning stimulus and voluntary activation

Figure 3.5 illustrates the mean inhibition data for 7 subjects, for conditioning stimuli from 0 to 32 % maximum stimulator output in 2 % steps, recorded for ISI=1 ms (circles) and ISI=2.5 ms (squares). Experiments were performed both in the relaxed state (open symbols) and with a low level of voluntary activity (black symbols). The relaxed ISI=1 ms and ISI=2.5 ms data in Figure 3.5A are replotted in Figures 3.5B and 3.5C respectively with standard error bars.

Inhibition at ISI=1 ms started at a lower level of conditioning stimulus than inhibition at 2.5 ms (Figure 3.5A) and the maximal level of inhibition was greater at ISI=2.5 ms. Inhibition at ISI=1 ms first became significantly different from zero ($P<0.05$, one tailed paired t-test) when the conditioning stimulus reached 16 % maximum stimulator output, whereas inhibition at ISI=2.5 ms only became significant at 22 % maximum stimulator output. The difference in inhibition first became significant ($P<0.001$) when the conditioning stimulus reached 18 % maximum stimulator output (~46 % RMT).

It might have been expected that the onset of inhibition would be more consistent across subjects when expressed as a percentage of RMT, rather than as a percentage of maximum stimulator output. To test this, the level of conditioning stimulus at which inhibition started ('inhibition threshold') was estimated for each subject. The (interpolated) conditioning stimulus at which inhibition first rose above 5 % (provided it continued to increase beyond 10 %) was expressed either as % maximum stimulator output or as % RMT of that subject before averaging across subjects. Inhibition thresholds were no more consistent, as measured by their coefficient of variation (CV), when expressed as % RMT than when expressed as % maximum stimulator output. For ISI=1 ms, inhibition thresholds averaged 16.1 ± 1.7 % (mean±SD) maximum stimulator output or 42.0 ± 4.5 % RMT, with CVs 11 % in each case, whereas for ISI=2.5 ms, inhibition thresholds averaged 21.4 ± 1.6 % maximum stimulator output or 56.2 ± 7.6 %

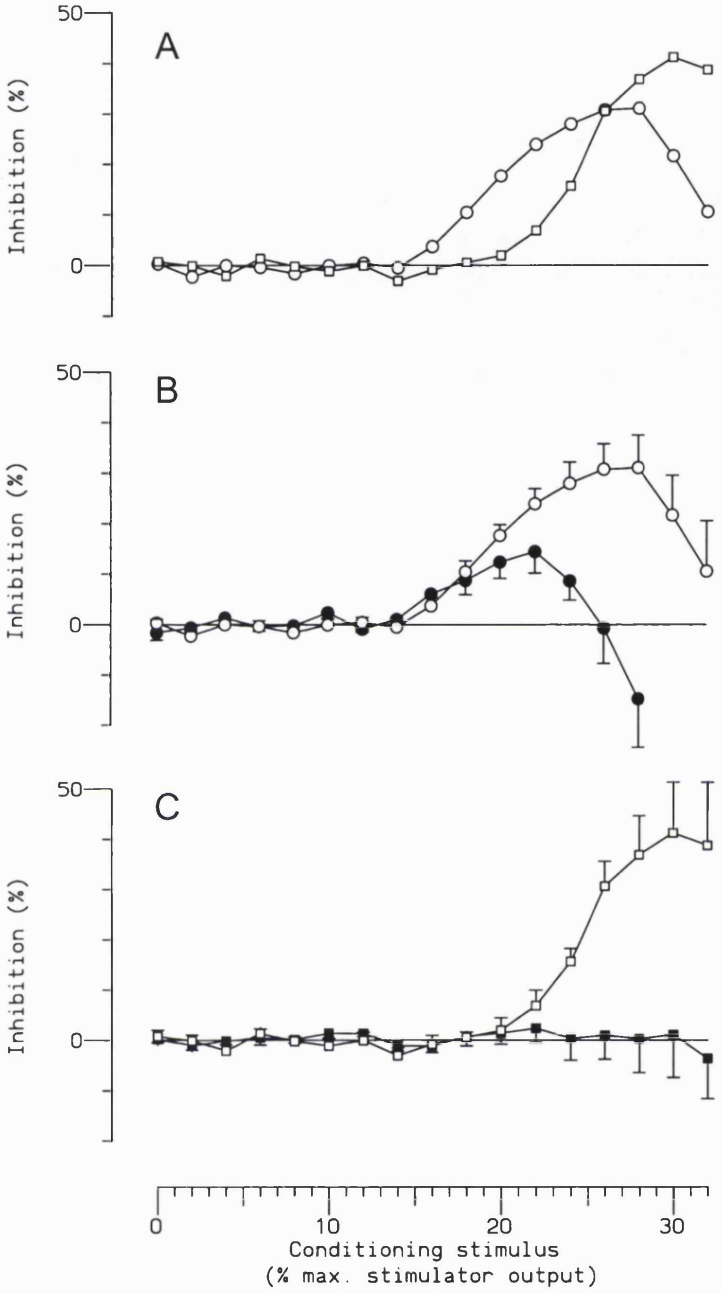


Figure 3.5: Inhibition plotted as a function of conditioning stimulus. (A) Comparison between ISI=1 ms and ISI=2.5 ms for relaxed subjects (n=7). (B) Effect of voluntary activity on inhibition at ISI=1 ms. (C) Effect of voluntary activity on inhibition at ISI=2.5 ms. Note total suppression of inhibition. Circles, ISI=1 ms; squares, ISI=2.5 ms. Open symbols, muscles relaxed; black symbols, muscles active. Error bars in (B,C) are ± SE.

RMT, with CVs 8 % and 14 % respectively. Inhibition thresholds were lower at ISI=1 ms than at ISI=2.5 ms by an average of 5.3 ± 1.7 % maximum stimulator output (P=0.0003), or 14 ± 6 % RMT (P=0.0008).

A low level of voluntary activity (10 % maximal rectified and integrated EMG) reduced inhibition at both ISI=1 ms (Figure 3.5B) and ISI=2.5 ms (Figure 3.5C). At ISI=1 ms the inhibition threshold was not significantly affected by voluntary activity (17.3 ± 2.3 % maximum stimulator output, P=0.2), but the peak inhibition was much reduced. In contrast, inhibition at ISI=2.5 ms was virtually abolished at all levels of conditioning stimulus. The effects of voluntary activity first affected inhibition significantly (P<0.05) when conditioning stimuli reached 24 % maximum stimulator output at both ISIs.

3.5.4 Relationship between paired-pulse inhibition and facilitation

It is not clear from Figure 3.5 what happens at high values of the conditioning stimulus, especially at ISI=2.5 ms. When the first stimulus reaches RMT it must excite a threshold response, and from other studies it is expected that a second stimulus at the interval of 2.5 ms should produce I-wave facilitation (Tokimura et al. 1996; Ziemann et al. 1998b). Figure 3.6 shows results from an additional experiment performed in a single subject to investigate the transition from inhibition to facilitation at ISI=2.5 ms. This has already been done for paired-pulses at an ISI close to 1 ms (Awizsus et al. 1999). The experiment involved paired-pulse stimulation only, the combination of which was set to elicit the target output response of 0.2 mV. In the first half of the experiment, the first stimulus acted as a conditioning stimulus, in a manner consistent with inhibition experiments previously described. Conditioning stimulus intensities were increased in 2 % steps. The second stimulus acted as a test stimulus, and tracked the MEP response of 0.2 mV. When the first stimulus equalled the second stimulus, then the roles of the stimuli were reversed.

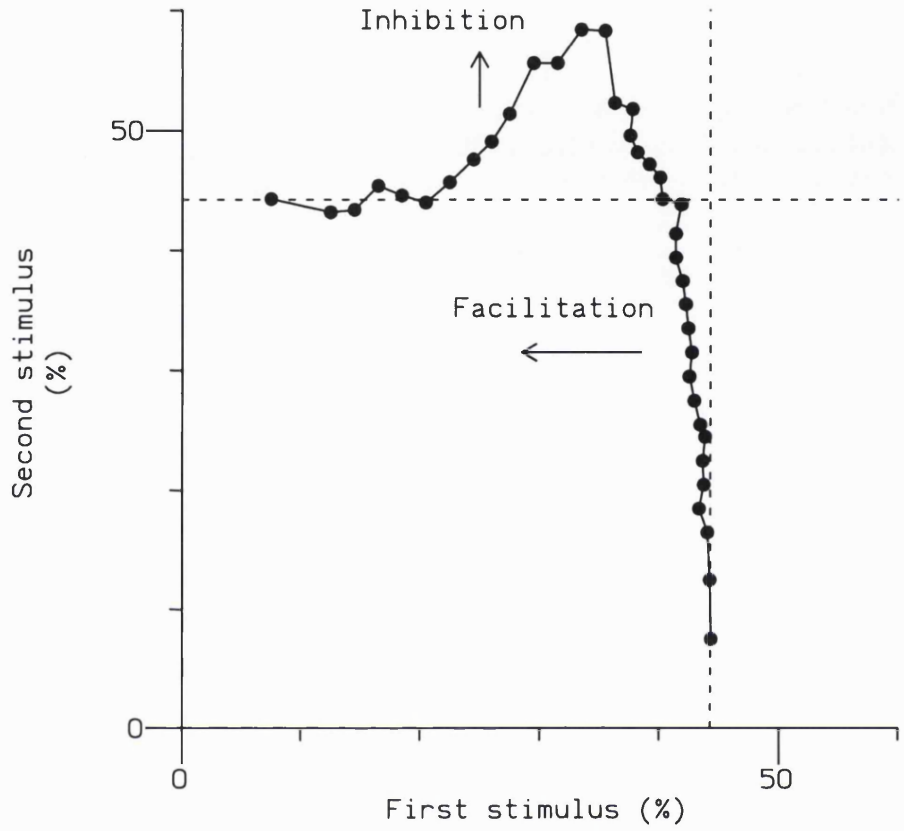


Figure 3.6: Plot of stimulus pairs causing threshold responses at ISI=2.5 ms in a relaxed subject. Dashed lines indicate RMT. The inhibitory part of the plot was generated as in Figure 3.5 B. The first stimulus acting as the conditioning stimulus, was increased in 2 % steps of maximum stimulator output. The second stimulus tracked the target response of 0.2 mV. When the first stimulus equalled the second stimulus, their roles were reversed. The second stimulus was stepped downwards and the first stimulus had to track the target response. This formed the facilitatory part of the experiment.

The first stimulus now tracked the target MEP response of 0.2 mV and the second stimulus was stepped down in 2 % steps. In this way facilitation evoked by the second stimulus could be measured, by the reduction in the first stimulus required to elicit a threshold response. Figure 3.6 demonstrates that both inhibition and facilitation occur at ISI=2.5 ms (as was shown with ISI=1.2 ms; Awizsus et al. 1999).

3.5.5 Single motor unit study

Figure 3.7 illustrates the control and conditioned PSTHs obtained from a single motor unit in 1DI for 3 combinations of conditioning and test stimuli. The test stimulus alone (Figure 3.7 A) produced a peak of increased firing (firing probability 0.67). In the conditioned PSTHs the conditioning stimulus was given 1 ms or 2.5 ms prior to the test stimulus (Figures 3.7B,C). With ISI=1 ms the peak was reduced in size (firing probability 0.37), but with ISI=2.5 ms the peak remained unaffected (firing probability 0.59).

Similar results were obtained from 7 other single units, and details from all the 8 single motor units tested are given in Table 3.1. For ISI=1 ms the total mean firing probability was significantly reduced from 0.60 ± 0.05 (mean ± SE) in the test PSTH to 0.39 ± 0.06 in the conditioned PSTH (P=0.0004). With ISI=2.5 ms, however, the firing probability remained unaffected at 0.61 ± 0.06.

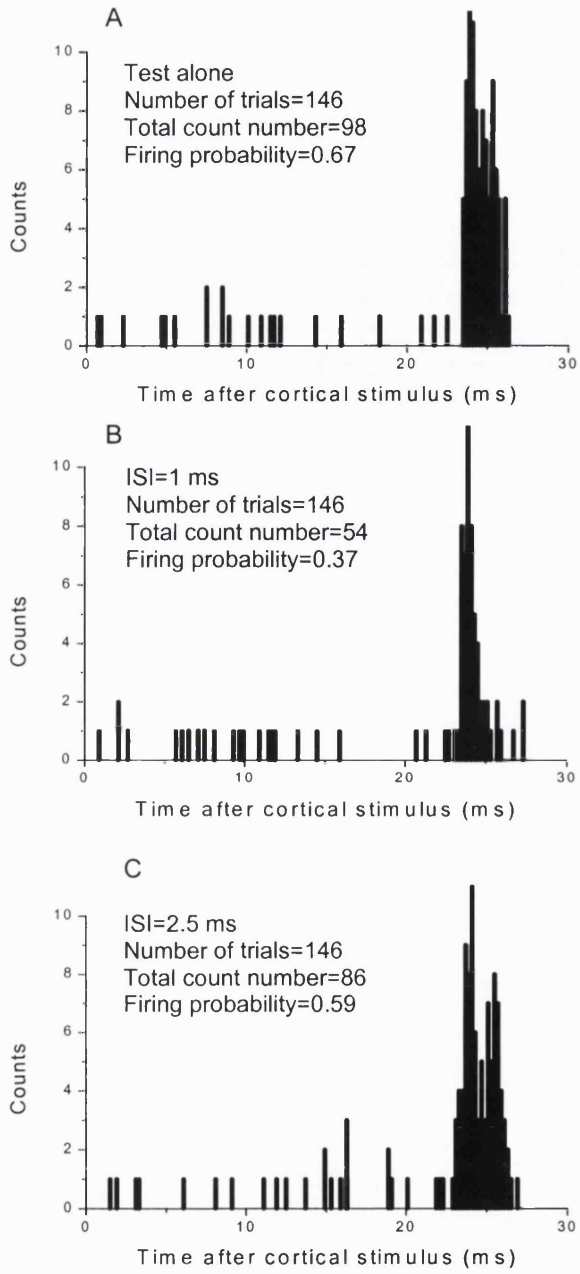


Figure 3.7: Post stimulus time histograms for firing of a single motor unit in right 1DI. Stimuli were a single test stimulus, manually adjusted to give a firing probability of about 0.5 (A), or a similar stimulus, preceded by a conditioning stimulus at ISI=1 ms (B) or ISI=2.5 ms (C). At ISI =1 ms, the firing probability was considerably reduced, due to selective inhibition of longer latency responses.

Unit	No. of trials	Test alone total counts	Test alone firing probability	ISI=1 ms total counts	ISI =1 ms firing probability	ISI=2.5 ms total counts	ISI=2.5 ms firing probability
1	146	98	0.67	54	0.37	86	0.59
2	78	57	0.73	39	0.50	62	0.79
3	71	43	0.61	28	0.39	48	0.68
4	117	96	0.82	76	0.65	92	0.79
5	115	76	0.66	61	0.53	81	0.70
6	75	37	0.49	13	0.17	28	0.37
7	67	32	0.48	10	0.15	29	0.43
8	113	42	0.37	36	0.32	55	0.49
		mean ± SE	0.60 ± 0.05		0.39 ± 0.06*		0.61 ± 0.06

Table 3.1: Inhibition of single motor unit discharge evoked from a test stimulus by a conditioning stimulus given 1 or 2.5 ms earlier.
* P=0.0004.

3.6 Discussion

In this study a threshold tracking procedure was applied to the paired-pulse test in order to investigate inhibitory intracortical mechanisms in more detail. Results have shown that using surface EMG recordings it is possible to distinguish between two types of inhibition, occurring at ISIs of 1 and 2.5 ms. These two phases of inhibition differ in terms of the conditioning stimulus levels required to elicit the effect, and in their susceptibility to voluntary activity.

3.6.1 Benefits of using the threshold tracking procedure

The threshold tracking technique provides a flexible method of testing motor cortex excitability. Throughout the experiment, the subject's RMT was measured repeatedly, as the test stimulus alone tracked the target MEP response. The thresholds to the paired-pulse stimuli were always compared with the unconditioned thresholds measured at the same time. This provided a more efficient method of allowing for drift or fluctuations in cortical excitability than the normal method of randomising different stimulus conditions, and enabled the conditioning stimulus parameters to be altered in a sequential fashion, to minimise the threshold changes (and therefore the tracking time) between consecutive stimulus conditions.

The method of assessing inhibition by keeping a constant MEP output and systematically varying the stimulus enables a wide range of effects of the conditioning stimulus to be assessed. Conventional measurement of inhibition becomes insensitive to small changes when inhibition approaches 100 %, and the failure of several studies to demonstrate an action of the $GABA_A$ agonist lorazepam on intracortical inhibition has been attributed to this 'floor effect' (Boroojerdi et al. 2001). The tracking method avoids this limitation.

A further advantage of threshold tracking, as applied to the measurement of intracortical inhibition, is that it helps to limit the contribution of spinal and peripheral elements to the measurements. When comparing patients with controls, for example, it is normally

assumed that a quantitative reduction in inhibition reflects changes in the cortical circuitry. This assumes that the input-output relationships of the spinal and peripheral mechanisms converting the output of the cortex to the EMG response are unchanged. Measurement of inhibition at a constant level of EMG response avoids this assumption.

Awiszus et al. (1999) used a threshold tracking procedure to investigate both intracortical inhibition and facilitation. In the inhibition experiments, the conditioning stimulus was fixed and the test stimulus 'hunted' or tracked the target response, in a similar set-up to the one used in this study. They reported that when using a fixed ISI of 1.2 ms, the threshold for elicitation of inhibition was at a conditioning stimulus intensity of 40–50 % RMT. This is in agreement with results in the present study when investigating the effect of conditioning stimulus intensity with ISI=1 ms.

3.6.2 Two phases of inhibition

The threshold tracking method revealed that the paired-pulse inhibition curve (Kujirai et al. 1993) consists of two distinct phases, with maximum inhibition at ISIs of around 1 ms and 2.5 ms. The optimal ISI for the first phase of inhibition was probably less than 1 ms (Figure 3.4 B), but could not be determined precisely because of the limitations of the equipment. It could not have been very much shorter than 1 ms, however, since as the ISI approaches 0 ms, a conditioning stimulus of 60 % RMT must cause a *facilitation* of 60 %. Inhibition at ISI=1 ms had a lower threshold than inhibition at an ISI=2.5 ms, but tended to decline more rapidly with increasing stimulus intensities than inhibition at ISI=2.5 ms. This suggests that averaging across an ISI range runs the risk of obscuring some results. Similarly, in studies using individual ISIs (Hanajima et al. 1998, ISI=4 ms; Nakamura et al. 1997, ISI=2 ms, 5 ms), maximum inhibition may not have been measured, simply because an optimal ISI was not used.

Previous studies of low intensity TMS indicate that both phases of inhibition are likely to depend primarily on cortical mechanisms. Subthreshold conditioning stimuli reduce the I-waves that descend down the corticospinal tract, following a test magnetic stimulus 1–5 ms later (Nakamura et al. 1997; Di Lazzaro et al. 1998b), but no inhibition is seen if the test stimulus is electrical, and excites a descending volley directly (Kujirai et al. 1993). There are two possible explanations for the higher threshold for inhibition at ISI=2.5 ms than at ISI=1 ms: (a) that a different class of neural elements has to be excited by the stimuli, or (b) that the extra delay is due to an extra interneurone interposed between the neural element excited by the first stimulus and the PTN. The first stimulus therefore has to be suprathreshold for excitation of that interneurone before any effect can be transmitted to the PTN.

The inhibition at ISI=2.5 ms is thought to be synaptic in origin since it is affected by drugs that act on GABAergic systems in the cortex (Ziemann et al. 1996a). This would support explanation (b). However, inhibition at ISI=1 ms has not been studied in great detail. The fact that it is less affected by voluntary activity, suggests that an axonal element is involved. This would support explanation (a). If this were the case, then we might speculate that inhibition occurs because the first stimulus activates axons normally involved in the response to the second stimulus. At an ISI=1 ms, these axons would be relatively refractory and therefore the response to the second stimulus would be reduced. Although this explanation is attractive there is one potential problem: post-synaptic potentials evoked by the conditioning stimulus might be expected to summate after a 1 ms interval with the synaptic potentials evoked by the test stimulus, so that the net synaptic effect of the two stimuli would be almost equal to that of the second stimulus alone. In this case refractoriness could not account for the inhibition observed.

However, this model of synaptic summation may not be appropriate for cortical PTNs. A single TMS shock produces highly synchronised activation of PTNs that then discharge repetitively at about 600 Hz to produce a series of I-waves (Ziemann and Rothwell 2000).

This behaviour could be due to high frequency discharge of a population of interneurones synapsing onto the PTN, or the intrinsic membrane properties of the neurone itself, or a combination of both. The mechanism most likely involves both synaptic excitatory and inhibitory effects. If some neuronal elements are refractory when the test stimulus activates the motor cortex, then there will be asynchronous activation of the PTN. This should result in an initial discharge that is much less effective in generating subsequent I-waves than that evoked by a more synchronous input. Indeed, direct recordings of descending volleys evoked by paired pulse stimulation at ISI=1 ms show that the initial I_1-wave is unaffected by a prior conditioning stimulus, but that the I_2-wave and later waves are virtually abolished (Di Lazzaro et al. 1998b).

It is in fact possible that a combination of (a) and (b) is probably true. Inhibition at ISI=1 ms could be due to refractoriness of cortical axons activated by the first stimulus, coupled with a peculiar sensitivity of PTNs to synchronised synaptic inputs. The resulting inhibition would, at least in part, be unaffected by voluntary activity or GABAergic treatment. Inhibition at ISI=2.5 ms requires a higher conditioning stimulus intensity i.e. one that is suprathreshold for excitation of inhibitory interneurones synapsing onto the PTNs. The resulting inhibition *would* be affected by voluntary activity or GABAergic treatment. There is no time for this synaptic inhibitory mechanism to occur with ISI=1 ms due to the synaptic delay. Changes in axonal excitability may play a part in inhibition measured using ISI=2.5 ms, although these are unlikely to be the main inhibitory mechanism operating at this ISI, as indicated by the greater susceptibility of inhibition to voluntary activity.

3.6.3 Relationship of inhibition to I-wave facilitation

There is a surprising correspondence between the ISIs at which intracortical inhibition is observed in Figure 3.4, and the ISIs at which short-lasting, I-wave facilitation has been

observed (e.g. Figure 1 in Tokimura et al. 1996, Figure 1 in Ziemann et al. 1998b). The timing of the peaks of inhibition at 1.0 and 2.5 ms is close to that of the peaks of facilitation at 1.2–1.3 ms and 2.5–2.8 ms (Ziemann et al. 1998b). However, this correspondence is probably coincidental. Direct recordings of paired-pulse inhibition at ISI=2–3 ms (Di Lazzaro et al. 1998b) show that inhibition affects only the I_2-waves and I_3-waves and later waves, and not the I_1-wave. Thus for ISI=2.5 ms the actual timing of inhibition is around 5 ms (ISI=2.5 ms + I_2-wave of test stimulus at 2.5 ms) rather than at 2.5 ms.

Nevertheless, there is no doubt that facilitation can co-exist with inhibition at ISIs of 1 and 2.5 ms. This can be seen in the data for ISI=1.2 ms from Awiszus et al. (1999). They found that when the first stimulus exceeded approximately 65 % of RMT, it became responsible for MEP initiation and was facilitated by the second stimulus, whereas weaker first stimuli (<65 % RMT) only inhibited the response to the second stimulus. Their threshold data was conveniently summarised in a plot of all stimulus pairs that evoked a threshold response (Figure 4 in Awiszus et al. 1999). Figure 3.6, from the present study, shows that this plot has a similar form for ISI=2.5 ms. At either ISI it appears as if the paired stimuli can excite threshold responses by two independent mechanisms: the first stimulus may be facilitated by the second, or the response to the second stimulus may be inhibited by the first. Threshold is determined by whichever of the two mechanisms provides the lowest threshold. At the point of cross-over (first stimulus < RMT, second stimulus > RMT), the interaction of the paired-pulse could be regarded either as facilitation (of the first stimulus by the second) or as inhibition (of the second stimulus by the first).

The mechanism of facilitation is probably quite different from that producing inhibition. Since all the axons excited by a larger first stimulus will be refractory to a smaller stimulus 1 ms later, facilitation must occur through action at a different site. A possible candidate is the axon hillock (or first node) of either the PTN or the interneurones

providing excitatory input to PTNs, as proposed by Amassian et al. (1998). At the PTN, the second stimulus could sum with the EPSP corresponding to the first I-wave at ~1 ms (after 1 synaptic delay), or with the EPSP corresponding to the second I-wave at ~2.5 ms (after two synaptic delays) to facilitate PTN excitation. The latency of the facilitated response would correspond to the first (ISI=1 ms) or second (ISI=2.5 ms) I-wave to the first stimulus, but to a D-wave from the second stimulus. If the interaction occurred at interneurones, the latency of the facilitated response would correspond to the I_1-wave (or later waves) from the second stimulus.

3.6.4 Conclusions

There are advantages in measuring intracortical inhibition at constant response, using a threshold tracking procedure, as compared to conventional measurements with constant stimuli. This method has provided evidence that different mechanisms of inhibition can be tested at ISIs of 1 and 2.5 ms, and this may prove useful when measuring intracortical inhibition levels in various disease states. Inhibition threshold might also be an important parameter to consider in future tests of intracortical inhibition. It is likely that inhibition measured using ISI=1 ms involves refractory mechanisms, and thus it is possible that inhibition may arise primarily through loss of the synchrony of PTN inputs that is required for the generation of multiple I-waves. This suggestion awaits further and more invasive investigation into the origin of I-waves generated by TMS.

CHAPTER 4: LOW INTENSITY TMS RESETS EMG OSCILLATIONS AND ENHANCES EMG-EMG COHERENCE

4.1 Introduction

It has been shown in the previous chapter, that low intensity TMS can be used to demonstrate intracortical inhibitory phenomena. The present study investigated the effects of low intensity TMS on the cortico-motoneuronal system and on oscillatory EMG activity. In addition low intensity TMS was used to investigate the origin of synchronous oscillations and their coherence between cortex and muscle and between muscle pairs. Experimental and computer modelling studies have highlighted the importance of inhibitory interneurones in the maintenance of synchronous oscillations within the cortex (Baker 1997; Pauluis et al. 1999; Whittington et al. 2000). Effects of TMS in this study may provide support for this claim.

Predominant frequencies present in surface EMG signals recorded during low levels of voluntary activity fall within the two main bandwidths of 6–12 Hz and 14–31 Hz. Origins of such EMG oscillatory activity have been investigated by studying physiological tremor. Although mechanical or reflex mechanisms could be involved in generating tremor frequencies, there is strong evidence to suggest that a central mechanism plays a dominant role (Elble and Randall 1976; McAuley et al. 1997; Mayston et al. 2001). Indeed synchrony between motor unit firing has been observed at both 1–12 Hz and 16–32 Hz (Farmer et al. 1993a) and patient studies have suggested that such synchrony has a corticospinal origin (Farmer et al. 1993a, b). It is possible that surface EMG oscillations are a reflection of synchrony within populations of motor units.

Frequencies around 10 Hz in the EMG are dominant in various pathological forms of tremor (Deuschl et al. 2001). In normal subjects, during slow unloaded voluntary

movements of fingers, small recurring variations in speed occur, which appear at a frequency of 8–10 Hz (Vallbo and Wessberg 1993; Wessberg and Vallbo 1995). Recordings from single muscle afferent fibres have shown that the origin of these speed variations was not a closed reflex loop. It was suggested these 8–10 Hz modulations in movements were caused by a pulsatile descending motor command (Wessberg and Vallbo 1995), inferring a central 10 Hz rhythm generating system exists that can influence motor output. Indeed there has been a report of coherence between muscles at frequencies around 10 Hz, during slow movement (Conway et al. 1997). In addition a few reports have been made of cortico-muscular coherence at these frequencies during arm extension (Raethjen et al. 2002) or isometric contraction of forearm muscles (Marsden et al 2001). These results provide further support for a central origin of the 10 Hz component of physiological tremor (McAuley et al. 1997; Mayston et al. 2001). However, there has been no evidence of cortico-muscular coherence or coherence between muscle pairs at frequencies around 10 Hz during a precision grip hold task (Kilner et al. 1999; 2000). The present study provides further insight into the origins of EMG oscillations in the 10 Hz frequency range during this task.

With regard to frequencies around 20 Hz in EMG activity, there is much clearer evidence to suggest that these originate from a common cortical drive. During stable contractions cortico-muscular coherence in the 14–31 Hz range has been observed between EMG signals from hand and forearm muscles and oscillatory activity in the sensorimotor cortex (Conway et al. 1995; Baker et al. 1997; Halliday et al. 1998; Salenius et al. 1997a, Kilner et al. 1999; 2000). Coherence is due to the presence of common frequencies in the 14–31 Hz bandwidth, which display a fixed phase relationship. It has been shown by phase analysis that for a given frequency, the phase of cortical oscillations precedes that within EMG signals (Conway et al. 1995; Halliday et al. 1998; Gross et al. 2000; Mima et al. 2000), suggesting that the cortex drives EMG oscillatory activity at frequencies between

14–31 Hz. However, the mechanism underlying this phenomenon remains unknown. The corticospinal tract has been suggested as a suitable candidate for the transmission of oscillatory activity from cortex to muscle (Baker et al. 1999). Indeed one report has shown that the phase lag between cortical and EMG oscillatory signals agrees with corticospinal conduction time measured with TMS (Gross et al. 2000).

TMS is a useful tool to activate indirectly the corticospinal tract. The aim of this study was to see if PTNs and local inhibitory interneurones are involved in the generation of synchronous oscillations within the cortico-muscular system. In order to provide evidence for this it would be necessary to show that TMS can affect the phase of oscillations present in EMG activity. Changes in the amplitude of frequencies within the EMG signal could be caused by asynchronous firing of motor units (Hepp-Reymond et al. 1996), and would not be sufficient proof that neuronal elements excited by TMS were directly involved in oscillation generation. One aim of this study was to investigate TMS effects on activity within a single muscle (Figure 4.1A,B). If neuronal elements within the motor cortex are not involved in oscillation generation, then low intensity TMS will have no effect on the phase of ongoing EMG oscillations (Figure 4.1A). However, if TMS causes a disruption in phase or resetting of EMG oscillations, then it could be argued that the neuronal elements excited by TMS form part of the rhythm generating circuit (Figure 4.1B). A similar study has already been done in monkeys (Jackson et al. *in press*). These authors activated PTNs by antidromic stimulation of the medullary pyramidal tract. They showed that this could reset the phase of ongoing local field potential oscillations of the primary motor cortex and have similar effects on oscillatory EMG in contralateral muscles. The authors argued that this resetting effect must be due to the fact that PTNs form part of the rhythm generating network. It would therefore be interesting to compare the effects of indirect stimulation of the corticospinal tract with TMS, to those seen by direct pyramidal tract stimulation in monkeys.

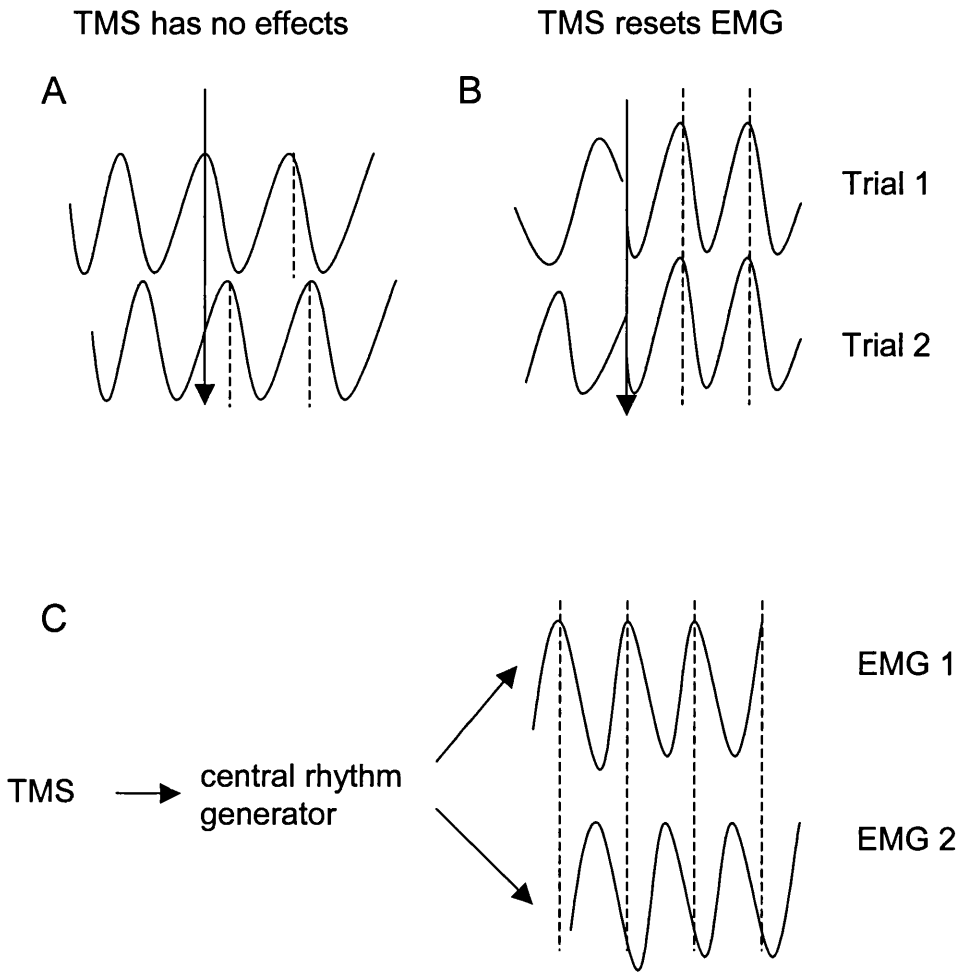


Figure 4.1: Possible effects of TMS on oscillatory EMG activity and EMG-EMG coherence. (A) If TMS has no effect on ongoing oscillations at a given frequency, then subsequent oscillations over trials will not have a consistent phase relationship with the stimulus (dashed lines indicate phase of oscillations). (B) However if TMS resets ongoing EMG oscillations, then oscillations of a particular frequency will be phase-locked to the stimulus over trials. (C) Coherent EMG oscillations at a particular frequency between two muscles have a consistent phase relationship (dashed lines). For 14–31 Hz coherence, this could be due in part to drive from a central rhythm generator. In order for TMS to effect EMG-EMG coherence it will have to gain access to the cortical oscillator driving the coherent EMG signals.

In the present study the effects of TMS on coherence between muscle pairs was also investigated. EMG activity between muscle pairs is coherent in the same frequency range as cortico-muscular coherence (Baker et al. 1997; Kilner et al. 1999; 2000), suggesting that EMG-EMG coherence at least in part originates from a common 15–30 Hz drive from the cortex. Coherence between muscle pairs also shows the same task modulation as cortico-muscular coherence and is increased during the hold phases of a precision grip task, and suppressed during the movement phases (Baker et al. 1997; Kilner et al. 1999; 2000). As TMS cannot be delivered during MEG and its artefact would seriously disturb the EEG signal for a short period, the most suitable measurement of coherence for this study was considered to be that between the EMG activity of muscle pairs.

Coherence calculates the phase difference between oscillations of a particular frequency in two EMG signals. If TMS can affect the phase of oscillations within single EMG activity, it is likely that coherence between muscle pair activity would also be affected. Note that coherence calculated in this study is a global measure that takes the phase relationship between *all* muscle pairs recorded from into account. Should TMS gain access to the central oscillator driving the EMG of all these muscles, effects on EMG-EMG coherence should be observed. This would provide clear evidence that intracortical interneurones and the corticospinal tract activated by TMS, are involved in the generation of synchrony within the cortico-muscular system (Figure 4.1C).

4.2 Methods

Experiments were performed on 14 healthy human volunteers (aged 20-35 years old; 7 females). The recordings had ethical committee approval, and all subjects gave informed consent.

4.2.1 Behavioral task

Subjects performed a precision grip task designed previously to investigate coherent EMG oscillatory activity (Kilner et al. 1999; 2000). Details of the hold-ramp-hold task used in this study are given in chapter 2. A spring constant of 0.05 N/mm was used.

4.2.2 EMG recordings

Bipolar surface EMGs were recorded from right first dorsal interosseous (1DI), abductor pollicis brevis (AbPB), flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC) muscles. EMGs were amplified (x 1000), filtered (30 Hz) and sampled at 5000 Hz by a PC-compatible computer attached to a 1401+ interface (Spike 2 CED Ltd. Cambridge, UK).

4.2.3 Stimulation

The TMS coil (Magstim 200 stimulator; double-cone coil, 13 cm loop diameter; Magstim Company Ltd, Dyfed UK) was positioned on the subject's head. The coil was placed over the motor cortex (current flow within the cortex in an anterior-posterior direction) and the hotspot for 1DI activation found. The coil was then fixed in place with the aid of velcro strapping around the subject's head and the heavy lead was supported in a harness system. A single TMS shock was delivered once during each trial, 1 second into the hold 2 phase of the task in 'stimulus' trials. This choice of timing was based on the fact that 14–31 Hz coherence has been reported to be most pronounced in this period (Kilner et al. 1999; 2000). In some subjects TMS stimuli were interspersed at random with zero intensity stimuli ('zero' trials) to give a mixed block of trials.

TMS intensities used during the experiment were chosen on the basis of online averaging of approximately 10 MEP responses in 1DI. For most subjects an intensity was chosen that elicited a discernable, but close to threshold response, with no silent period. In

addition in some subjects, subthreshold intensities (no obvious MEP) and suprathreshold intensities (MEP>0.2 mV in rectified EMG data with clear silent period) were chosen.

4.2.4 Protocol

Subjects started the experiment by performing a block of 60 hold-ramp-hold trials ('no stimulus' trial condition). A further 2–3 blocks of 60 hold-ramp-hold trials were then performed, with TMS delivered each trial ('stimulus' trials). A different intensity of TMS was used for each 'stimulus' trial block. In some subjects an extra block of 120 hold-ramp-hold trials were performed with 'zero' trials mixed in with the TMS stimuli. The TMS coil was then removed, and after a 10 minute rest period, a final set of 60 'no stimulus' hold-ramp-hold trials ('washout no stimulus') was performed.

4.3 Data Analysis

For offline analysis (in both the time and frequency-domain), results for 'stimulus' trials were pooled based on mean 1DI MEP responses. MEP measurements were made by averaging rectified EMG data aligned to the stimulus, and taking the mean peak value. Sections of data 400 ms before the stimulus and 200 ms post-stimulus were used. Average background EMG activity (average of 400ms EMG data pre-stimulus) was subtracted from the mean MEP values. Results were then assessed as effects of subthreshold intensities (0–0.015 mV MEP), threshold intensities (0.015–0.2 mV MEP), and suprathreshold intensities (MEP> 0.2 mV).

During the experiment approximately three TMS intensities were used for the same subject. Sometimes these intensities elicited similar average MEPs. For example, offline analysis showed that for a single subject a TMS intensity of 19% maximum stimulator output elicited an average MEP of 0.02 mV and 20 % elicited an average MEP of 0.18 mV; both were termed threshold intensities. For pooling frequency-domain data, only results obtained from one intensity per range (e.g. one subthreshold, one threshold or one

suprathrehsold intensity) were used for each subject. In examples of this kind, the intensity chosen for pooled analysis was the one that elicited an MEP response most similar to other MEP responses within that range elicited from other subjects.

4.3.1 Stimulus-locking effects

Index finger and thumb lever position profiles were examined offline. Trials in which subjects made large movements during hold periods or were inconsistent in their performance were excluded. Note that TMS was delivered in the hold 2 phase of the task during each trial, and so EMG analysis was only applied to this section of the data. Analysis was performed in the time-domain by averaging the rectified EMG data aligned to the stimulus. Sections of EMG data 50 ms before the stimulus and 200 ms after the stimulus were averaged. This method clearly showed any oscillations that were phase-locked to the stimulus as peaks and troughs in the aligned average recording.

For all frequency-domain analysis (stimulus-locked power and coherence), the first 58 correct trials of an approximate 60 trial block were used in order to keep the trial number equal for all subjects. Analysis was performed using FFTs of EMG data taken from the hold 2 period of the task. An FFT of 128 points permitted a 2 Hz resolution, and a 486 ms rectangular window was used, starting 0.05 s after the stimulus (so as not to include the MEP) or equivalent time marker in 'no stimulus' trials or 'zero' trials (see chapter 2).

4.3.2 Stimulus-locked power calculation

Power and stimulus-locked power analyses were performed on single EMG data. An overview of the calculations will be presented here, before the formulae are given. The FFT analysis effectively split up each EMG signal into its component frequencies. For each frequency within each trial of data, a Fourier coefficient was produced. This can be represented as a vector, with length representing the amplitude of the oscillation, and angle representing the phase (Figure 4.2A). A power spectrum was constructed to

determine the predominant frequencies in each EMG signal. The power of a given frequency within a single trial is its amplitude squared. This value was then averaged over trials (in this example five trials; Figure 4.2B). Stimulus-locked power was also calculated on single EMG data. This time both the phase and the amplitude of the oscillation were taken into account. The Fourier coefficient vector was therefore summed over trials (Figure 4.2C,D). The phase component of each vector was the phase of that oscillation in relation to the timing of the TMS stimulus. The vector sum therefore depended on whether the phase of each oscillation of that frequency was consistent with the stimulus. If TMS caused resetting, the phase of oscillations of a given frequency would be locked to the stimulus in each trial, and the vectors over trials would sum to give a large value (Figure 4.2C). However if no stimulus-locking occurred, phases would all be different over trials, and the vectors would sum to give a small value (Figure 4.2D). Finally in order to calculate stimulus-locked power, the vector sum was averaged, before squaring.

4.3.3 Formulae for the stimulus-locked power calculation

The calculation utilised the FFT algorithm on sections of data aligned to each stimulus (or time maker) of length L sample points (in this case 128). The Fourier coefficient $F_n(f)$ for the nth section of data (n = 1,2,..,N) is a complex number representing the amplitude $a_n(f)$ and phase $\phi_n(f)$ of the component at frequency f:

$$F_n(f) = \frac{L}{2} a_n(f) e^{i\phi_n(f)} \qquad (1)$$

To compute the power spectrum $P(f)$, the squared magnitude of each coefficient was averaged across stimuli:

$$P(f) = \frac{1}{N}\sum_{n=1}^{N}\left|\frac{2}{L}F_n(f)\right|^2 = \frac{1}{N}\sum_{n=1}^{N} a_n(f)^2 \qquad (2)$$

For each frequency:

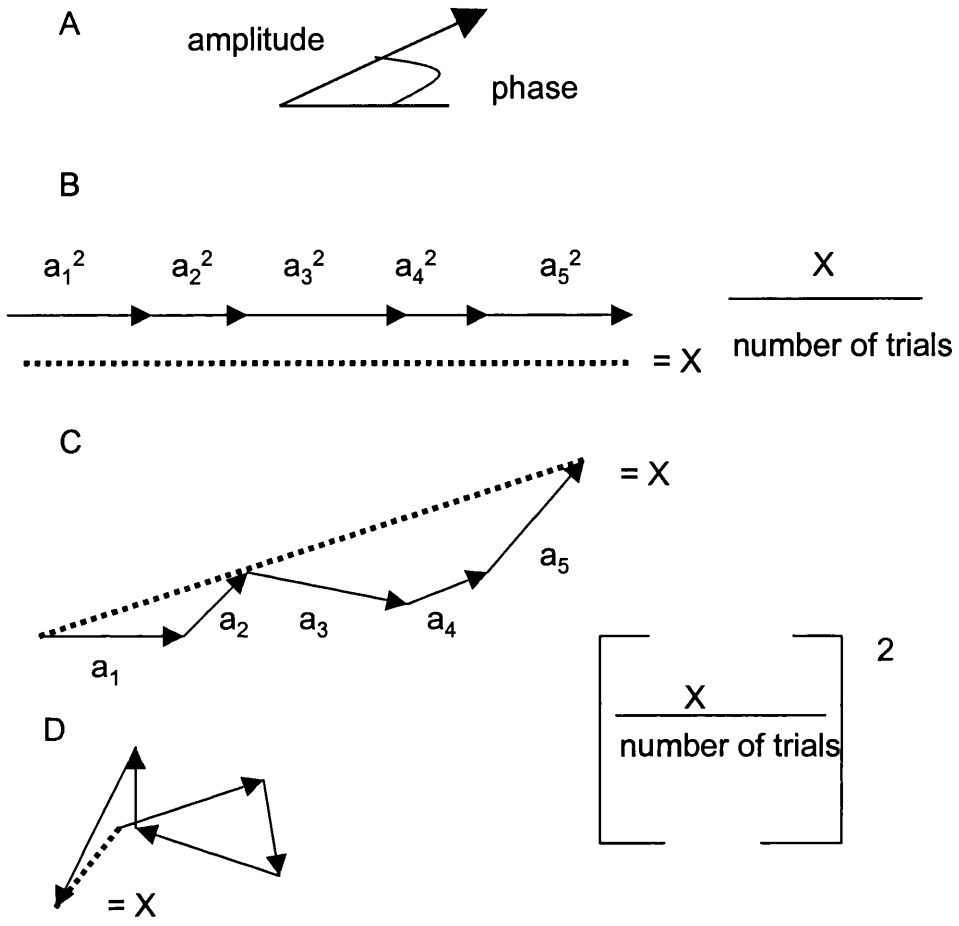


Figure 4.2 : Power and stimulus-locked power calculation explained. (A) The FFT algorithm was applied to each trial to produce a Fourier coefficient for each frequency. This can be represented as a vector. (B) In power calculations, for a given frequency, the amplitude component 'a' only was squared before averaging over trials. In this example there were 5 trials, the amplitude of the frequency in trial 1 represented as 'a_1'. (C,D) For stimulus-locked power calculations the phase component was also taken into account and so the Fourier coefficient vectors for a given frequency were summed over trials. The resultant value was then averaged before squaring. Note that when the phase of oscillations was consistent over trials (i.e. stimulus-locking occurred) vectors summed to create a large overall average (C). However when there was no consistent phase relationship of that frequency with the stimulus, the vectors summed to create a small overall average (no significant stimulus-locking; D).

Stimulus-locked power $P_{s-l}(f)$ was calculated by instead averaging the coefficients *before* taking the squared magnitude:

$$P_{s-l}(f) = \left| \frac{1}{N} \sum_{n=1}^{N} \frac{2}{L} F_n(f) \right|^2 \qquad (3)$$

In this way, the phase of each data section was incorporated within the average. Components without a constant phase relationship to the stimulus averaged out, leaving the stimulus-locked part of the signal only.

4.3.4 Statistics for stimulus-locked power

It was possible to calculate standard error for power spectra from the averaging procedure described in (2). 95 % confidence limits were then calculated by multiplying by 1.96.

For stimulus-locked power spectra, 95 % confidence limits were also given. However the calculation for this was not as straight forward. Note that the stimulus-locked power calculation is equivalent to existing methods for analysing phase-locked power in the time-domain (Kalcher and Pfurtscheller 1995). In order to test whether phase or stimulus-locked power is significant, previous methods have used a period of data prior to the stimulus as a baseline with which to compare the evoked response (Pfurtscheller and Lopes da Silva 1999). It should be noted however, that if the total power in the signal increases following the stimulus, then there will also be an increase in the noise component which will affect the calculation. This problem was avoided, by repeatedly phase-shifting the post-stimulus data randomly 500 times. For each frequency the power values obtained for all 500 shifts were ordered in terms of amplitude and the 475th value taken. This was used as the ± 95 % confidence limit (0.95 x 500) for the expected stimulus-locked power given the same amplitude distribution but with no stimulus-locking. Values greater than this were then assigned statistical significance.

4.3.5 Coherence calculation

Details of the coherence calculation used in this study have been given in chapter 2. Coherence analysis was performed on EMG data from the hold 2 phase of the task only and between muscle pairs. The FFT analysis used for coherence calculations was the same as that used for stimulus-locked power calculations (same window length and MEP avoided). It should be noted however, that in the case of coherence, two different EMG signals were compared and the phase component of the vector calculated was phase *difference* between oscillations at a given frequency within *two* EMG signals.

Coherence was calculated for each frequency between 4–12 Hz, and 14–31 Hz for all six muscle pairs. For each subject, coherence values were normalized and combined across muscle pairs, to give an 'all muscle pair' coherence value. This coherence value was then summed within bandwidths of 4–12 Hz, and 14–31 Hz and multiplied by the frequency resolution of 2 Hz, to express coherence in terms of 'normalized coherence area'.

4.3.6 Statistics for coherence

Significant differences in values of coherence were tested with a one tailed paired t-test run across subjects.

4.4 Results

4.4.1 Stimulus-locking effects on 1DI muscle – TMS intensity

Effects of TMS on ongoing EMG activity were analysed in both the time and frequency-domain. Figure 4.3 shows data from a single subject and from 1DI muscle only. Three different intensities were used. In the time-domain analysis (Figure 4.3A,B) rectified EMG data was aligned to the stimulus. Subthreshold TMS (in this subject an intensity of 12 % maximum stimulator output was used) evoked no discernible MEP (Figure 4.3A), whereas threshold TMS (14 % maximum stimulator output) evoked a small MEP (mean peak amplitude 0.06 mV; Figure 4.3B). In stimulus-triggered averaging of this kind,

oscillations with no consistent phase relative to the stimulus have cancelled out. However, clear peaks and troughs in the EMG, as shown most clearly after threshold TMS (14 %), indicate that this TMS intensity reset ongoing EMG activity. That is subsequent oscillations were phase-locked to the stimulus. This effect was not observed when a suprathreshold stimulus (18 % maximum stimulator output) was used (Figure 4.3A). The MEP (mean peak amplitude 0.71 mV) was followed by a silent period, which resulted in a clear inhibition of EMG activity during the period of interest. Suprathreshold TMS results were therefore not used in further analysis.

To quantify the stimulus-locking effects shown in the time-domain, frequency-domain analysis was performed on EMG data 50 ms following the stimulus (so as to avoid the MEP). The 486 ms window used in the FFT analysis on every sweep of EMG data conveniently sampled the peaks and troughs evident in the time-domain. Plots in Figure 4.3C-F show power spectra and stimulus-locked power in trials when subthreshold (12 %; C, E) and threshold (14 %; D, F) TMS were given. In this subject, there were peaks in both power spectra, which centred around 10 Hz and 20 Hz. However, a significant peak in stimulus-locked power occurred at a frequency of 24 Hz only (Figure 4.3F). In addition, this effect was only observed using threshold (14 %) TMS. The criterion for selecting peaks was that they were at least 1.5 times greater than confidence limits. In addition only the highest of two peaks closer together than two bins was taken. In summary for this subject, although ongoing EMG activity had frequency components within both 4–12 Hz and 14–31 Hz ranges, it was in the higher range that TMS caused resetting of EMG oscillatory activity.

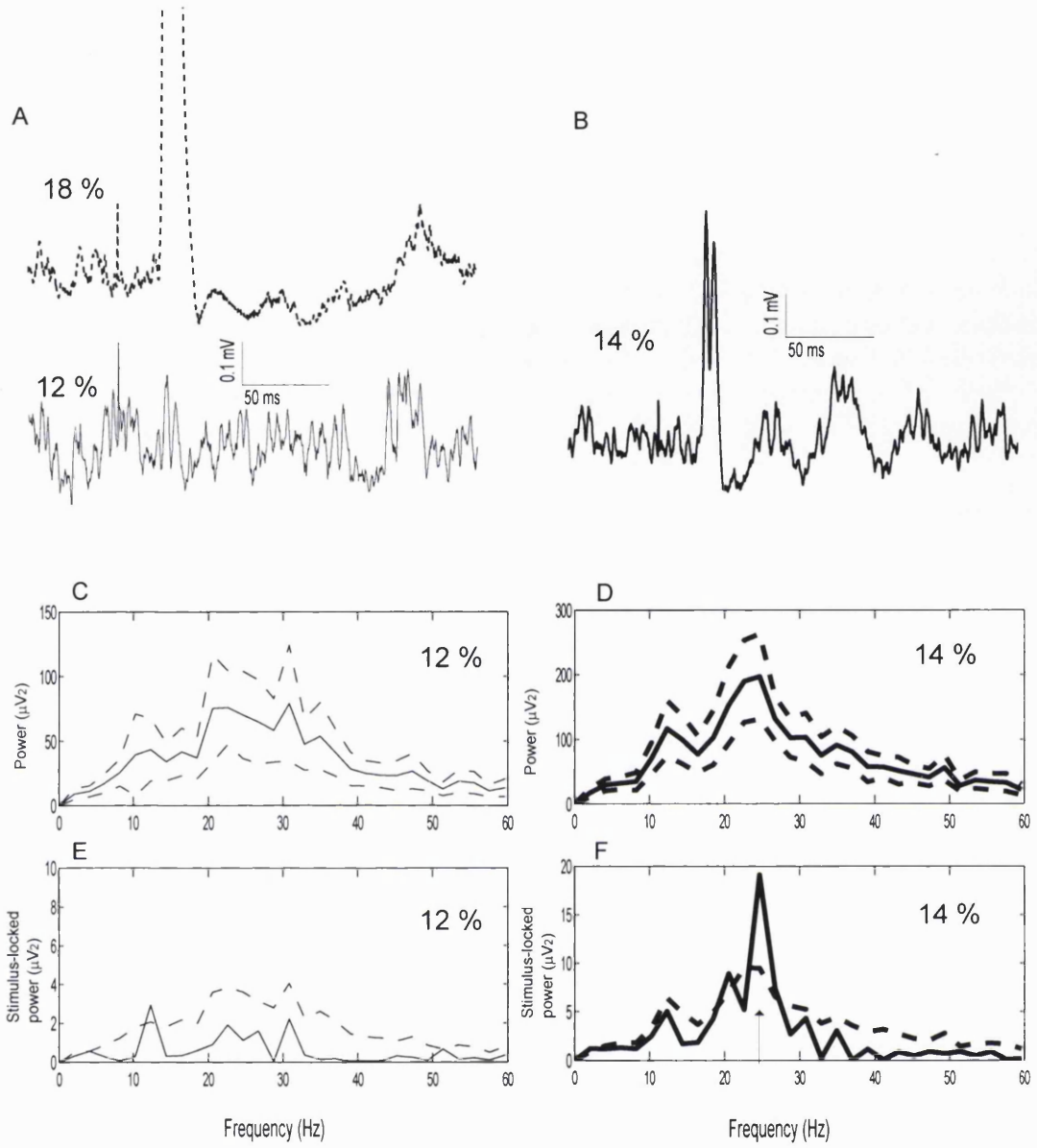


Figure 4.3: (A,B)Time-domain analysis of stimulus-locking effects on 1DI EMG activity of a single subject. Rectified EMG data (58 trials) were aligned to the TMS stimulus and averaged. The effect of intensity was investigated by using subthreshold (12 % maximum stimulator output; A), threshhold (14 % maximum stimulator output; B) or suprathreshold (18% maximum stimulator output; A) stimuli. Oscillations with no consistent phase relationship relative to the stimulus have been cancelled out. Clear peaks and troughs following the MEP indicate EMG activity phase-locked to the stimulus. (C-F) Frequency-domain analysis of stimulus-locking effects of subthreshold (12 %; C,E) and threshold (14 %; D,F) TMS stimuli in the same subject. Graphs show power spectra (C,D) and stimulus-locked power spectra (E,F) for 1DI. Dashed lines are 95% confidence limits. The arrow indicates the significant stimulus-locked power peak (1.5 times greater than confidence limits) evoked by threshold TMS (note scale change on y axis).

4.4.2 Stimulus-locking effects on all muscles – threshold TMS

The pilot experiments showed that significant stimulus-locking effects were elicited by threshold TMS stimuli, and therefore subsequent analysis on pooled data used threshold TMS data only.

Figure 4.4A-D shows histograms of pooled data (n=9) from all four muscles. Frequencies at which significant peaks in the stimulus-locked power spectra occurred were recorded for all nine subjects. Peak frequencies occurred across the whole 4–31 Hz range for all muscles. For the two intrinsic hand muscles there was a grouping of effects in the 4–12 Hz and 14–31 Hz bandwidths, however no clear distribution was observed for the extrinsic muscles. This shows that there were differences in stimulus-locking effects across muscles. An interesting observation is that stimulus-locking effects occurred at frequencies in the 4–12 Hz bandwidth. Note that it has previously proved difficult to show evidence of a cortical origin of frequencies in this range, during the precision grip task. In addition across subjects and muscles, there were 13 instances of peak frequencies in the gamma (30–60 Hz) range (4 AbPB; 6 FDS; 3 EDC; data not shown in histograms). Although there was no clear distribution of peak frequencies when the data were plotted at the highest resolution of approximately 2 Hz, data was still pooled according to the commonly used frequency ranges e.g. 4–12 Hz and 14–31 Hz (Figure 4.4E). This was to see if stimulus-locking effects were any stronger at frequencies around 20 Hz (which have been associated with a stronger cortical drive). There was in fact some indication that greater stimulus-locking effects occurred in the 14–31 Hz range (60 % total number of peaks) than effects observed in the 4–12 Hz range (40 % total number of peaks).

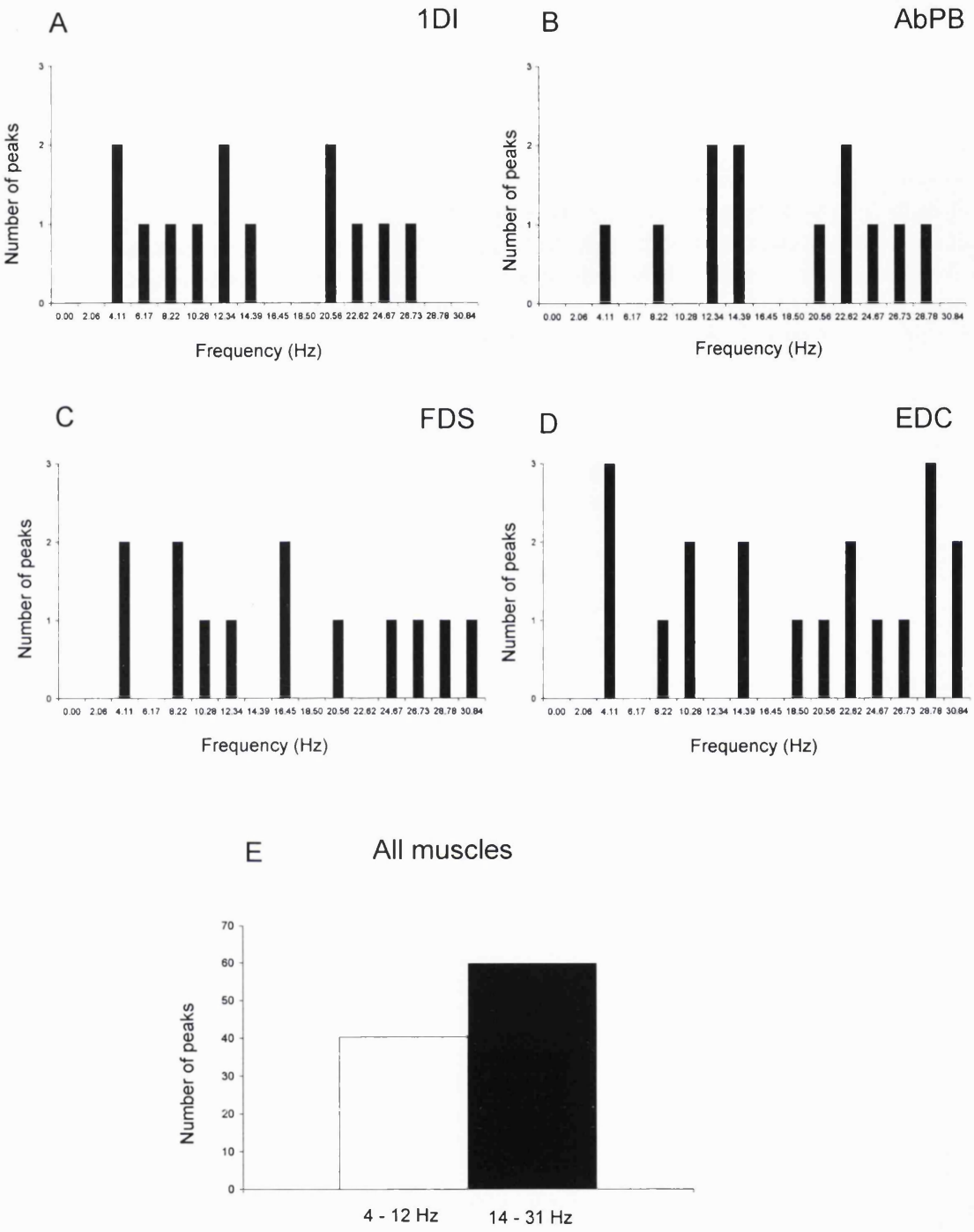


Figure 4.4: Pooled data from frequency-domain analysis of stimulus-locked power elicited by threshold TMS (n=9). Histograms A-D show the number of peaks in the stimulus-locked power spectra that occurred at each frequency for muscles 1DI (A), AbPB (B), FDS (C) and EDC (D).(E) Bar chart showing pooled data from all muscles, indicating the distribution of peaks across 4-12 Hz and 14-31 Hz frequency bandwidths.

4.4.3 Effects of TMS on power

It is worth noting that no measure of stimulus-locked power will ever be exactly zero due to residual noise after averaging. If the total power in the EMG signal increased following the stimulus, there may have been a corresponding increase in the noise component, which could have affected stimulus-locked power. In order to rule out this effect, it was checked whether TMS changed the power of ongoing EMG oscillations. Power spectra were constructed using the same FFT analysis on the same sections of EMG data as those used in the stimulus-locked power calculations. Figure 4.5A shows 1DI muscle power spectra from a single subject during 'no stimulus' and threshold intensity 'stimulus' trials. Arrows indicate that in the 'no stimulus' trials there were two peaks in the power spectra, at 14 Hz and 28 Hz. TMS did not change the frequencies at which these peaks occurred to any great extent (peaks following TMS were 12 Hz and 24 Hz). In addition, the amplitude of these peaks following TMS fell within the standard error limits of the 'no stimulus' trial peaks, indicating that TMS did not change the power of frequencies within the EMG signal. Grouping all subjects together (n=9), the frequencies at which peaks occurred in power spectra of 'no stimulus' trials and 'stimulus' trials are shown in a histogram (Figure 4.5B). Clear peaks in the power spectra were determined by eye. The frequency distribution of peaks in the power spectra was not changed by TMS. Finally the amplitudes of all peaks in the power spectra were taken, and a grand average (all frequencies included) calculated for 'no stimulus' and 'stimulus' trials. There was no significant increase in mean power during 'stimulus' trials (Figure 4.5C).

4.4.4 Effects on EMG-EMG coherence - threshold TMS

As the clearest stimulus-locking effects were elicited with threshold TMS, coherence analysis initially concentrated on comparing threshold 'stimulus' trials with 'no stimulus' trials. Coherence spectra from 6 muscle pairs of a single subject are shown in Figure 4.6.

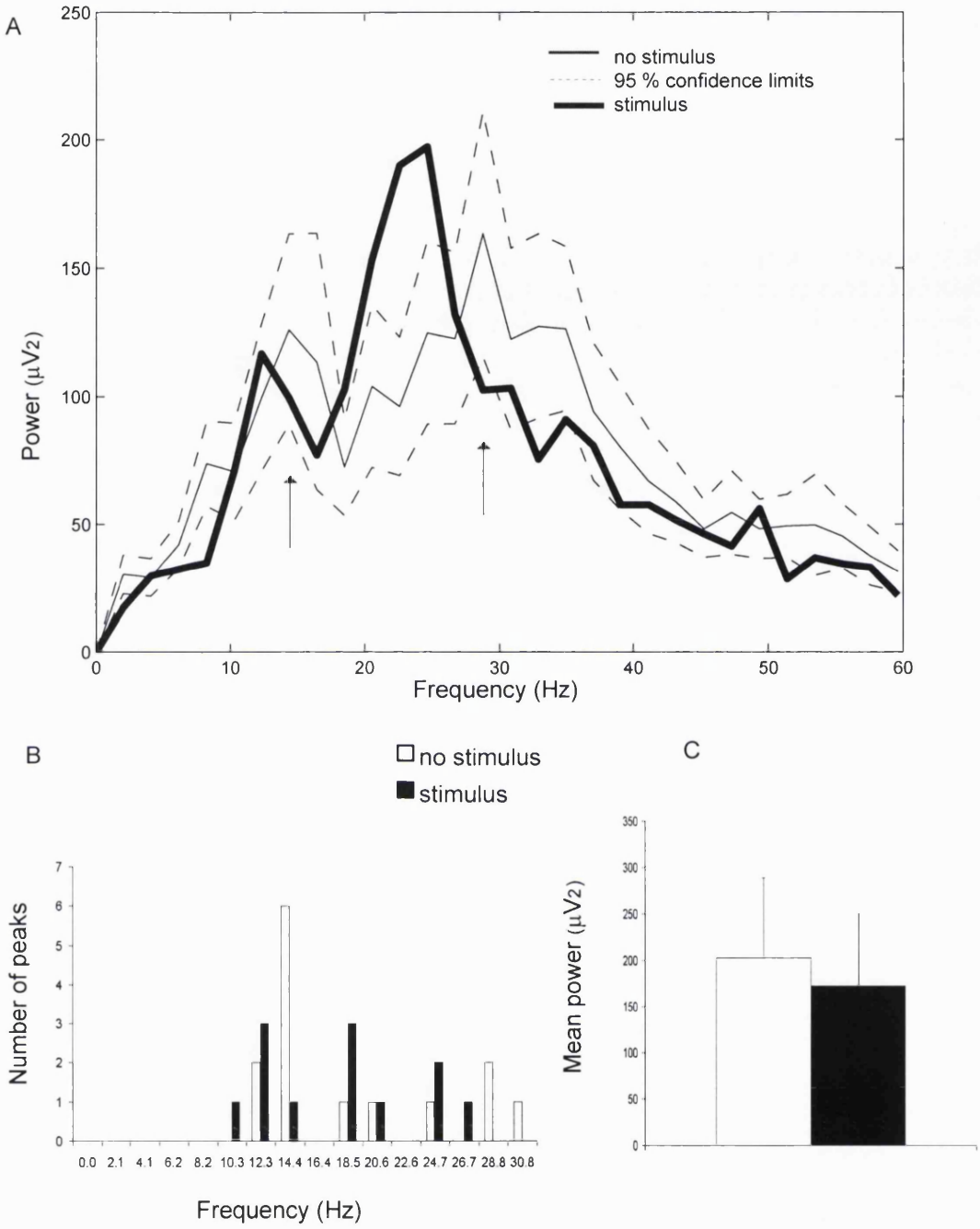


Figure 4.5: Threshold TMS effects on 1DI power spectra. (A) Power spectra of 1DI EMG of a single subject during 'no stimulus' (thin line) and 'stimulus' (thick line) trials. For simplicity, 95 % confidence limits (dashed lines) are shown for the 'no stimulus' trials only. The arrows indicate peaks in the power spectrum during 'no stimulus' trials. Note that neither the frequency at which these peaks occurred, nor the amplitude of the peaks were changed to any great extent by TMS. (B,C) Pooled data from 1DI (n=9). The frequency at which all peaks present in 'no stimulus' trial power spectra (open bars) and 'stimulus' trial power spectra (black bars) were recorded (B). There was no difference in frequency distribution of power peaks following TMS. Similarly, there was no change in the amplitude of power peaks, as shown by plotting the mean peak amplitude for both conditions (C).

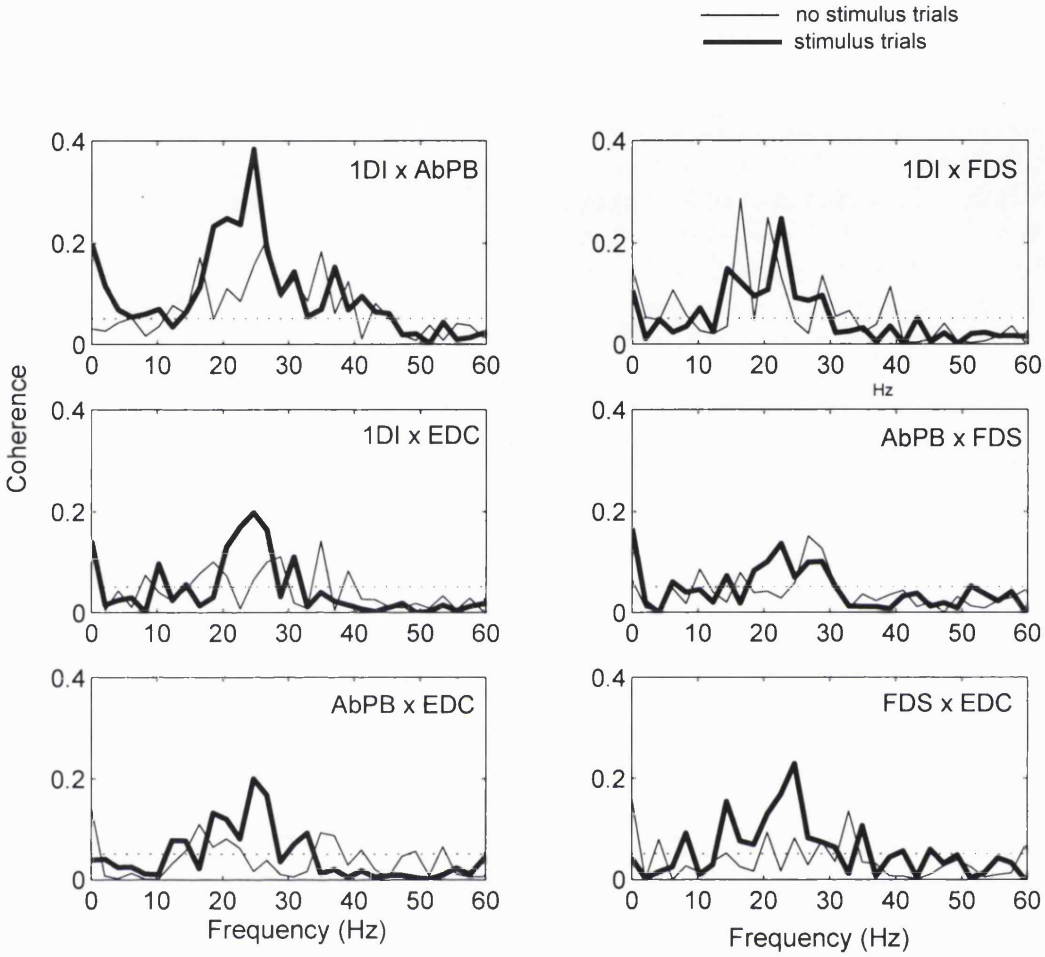


Figure 4.6: Single subject coherence spectra for six muscle pairs. Thin lines show coherence during 'no stimulus' trials, and thick lines indicate coherence levels in trials when threshold TMS was applied. TMS resulted in an increase in coherence, in all muscle pairs. Horizontal dotted lines are 95% confidence limits.

In all muscle pairs of this subject, coherence was higher following threshold TMS than during 'no stimulus' trials. As coherence peaks in Figure 4.6 predominantly spanned the 14–31 Hz range, pooled analysis (n=9) was performed on coherence between these frequencies (Figure 4.7A). Muscle pair coherence values were normalised and combined to obtain an all muscle pair coherence value for each subject. Threshold TMS caused a small but significant increase in 14–31 Hz coherence comparing 'stimulus' trials with 'no stimulus' trials (P=0.03), and when 'stimulus' trials were compared with an additional set of 'no stimulus' trials performed at the end of the experiment ('washout no stimulus' trials; P=0.001). 14-31 Hz coherence was however, significantly lower during the 'washout no stimulus' trials than coherence measured from 'no stimulus trials' (P=0.02). As there was some indication of significant coherence peaks also encompassing frequencies lower than 14 Hz, and stimulus-locking also occurred in the lower frequency band, 4–12 Hz coherence was also calculated and data (n=9) pooled (Figure 4.7B). It should be noted that coherence in this frequency range under normal physiological circumstances is not usually observed, hence the low coherence levels in the 'no stimulus' trials. Threshold TMS significantly increased 4–12 Hz coherence when 'stimulus' trials were compared with 'no stimulus' (P=0.0007) and 'washout no stimulus' trials (P=0.003).

4.4.5 Effects on EMG-EMG coherence -subthreshold TMS

Coherence changes following subthreshold TMS were also assessed (n=6). Note that coherence values are presented in terms of the area of coherent activity with the unit Hz. There was a significant increase in 14–31 Hz coherence when 'stimulus' trials (coherence area of 206 ± 15 Hz) were compared to both 'no stimulus' trials (coherence area of 186 ± 18 Hz; P=0.04) and 'washout no stimulus' trials (coherence area of 147 ± 12 Hz; P=0.001). Note that coherence was significantly lower in the 'washout no stimulus' trials

than in the 'no stimulus' trials (P=0.02). There were no significant changes in 4–12 Hz coherence following subthreshold TMS.

4.4.6 Effects on EMG-EMG coherence – 'zero' trials

In addition to the two control trials used ('no stimulus' and 'washout no stimulus'), in some subjects an extra control condition was added (n=6). This was to randomly intermix TMS stimuli with trials in which TMS intensity was set at zero, in the same experimental block. Effects on coherence during the 'stimulus' trials, and during the 'zero' trials were then compared. Threshold TMS induced a significant increase in 4–12 Hz coherence when 'stimulus' trials (coherence area of 115 ± 14 Hz) were compared to 'zero' trials (coherence area of 81 ± 15 Hz) from the same block (P=0.0008). This increase in coherence was also significant when 'stimulus' trials were compared to the block of 'no stimulus' trials (coherence area of 75 ± 12 Hz) performed previously during a separate block of trials (P=0.0005).

However, in the same subjects there was no difference in 14–31 Hz coherence between threshold 'stimulus' trials (coherence area of 199 ± 23 Hz) and 'zero' trials (coherence area of 181 ± 18 Hz) from the same block. There was also no difference in 14–31 Hz coherence between 'stimulus' trials and the 'no stimulus' trials (coherence area of 192 ± 17 Hz) performed previously during a separate block of trials.

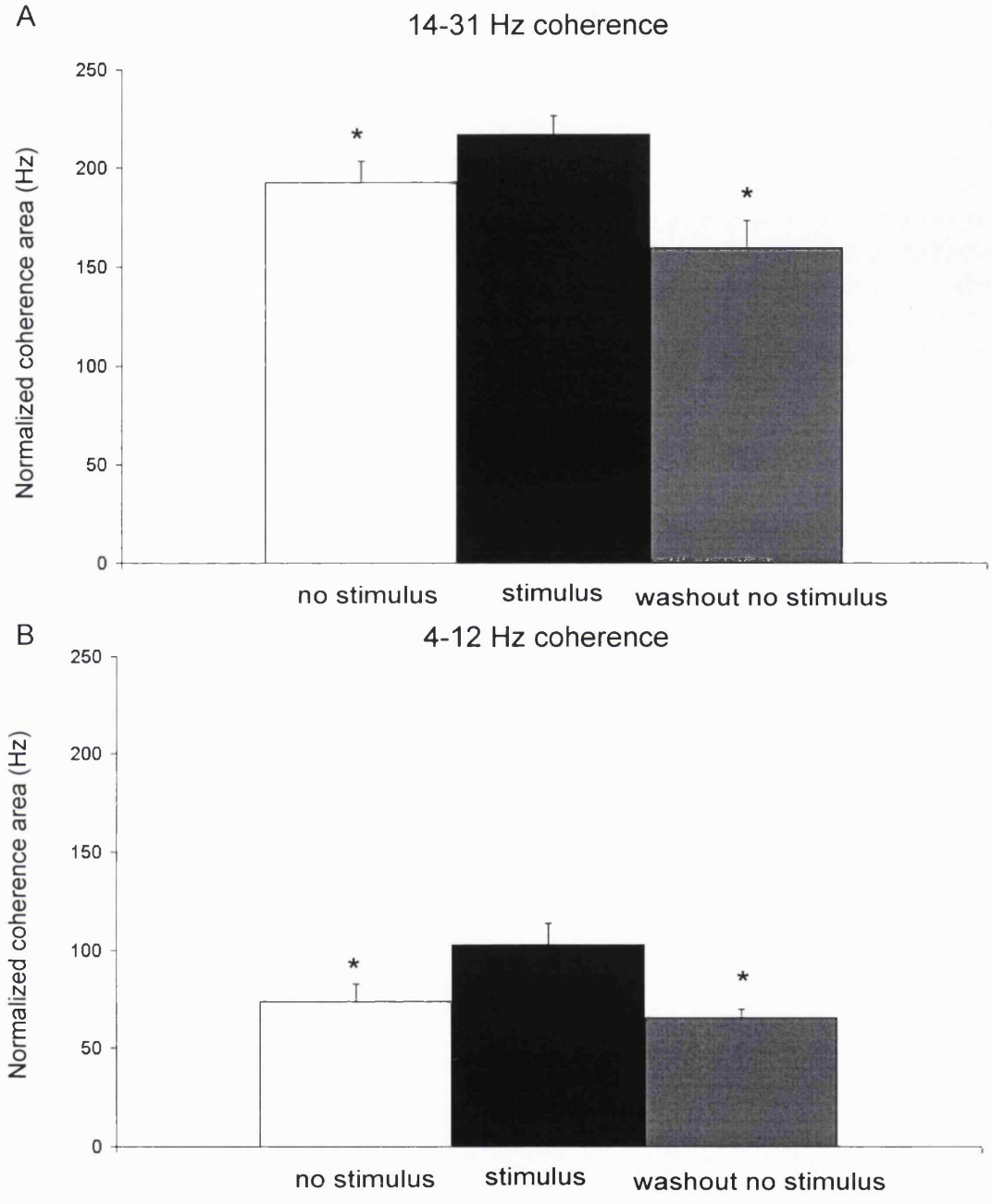


Figure 4.7: Pooled data of normalized all muscle pair coherence (n=9). Threshold TMS evoked a significant increase in both (A) 14-31 Hz ($P<0.05$) and (B) 4-12 Hz coherence ($P<0.005$). This effect was seen both when comparing 'stimulus' trials with 'no stimulus' trials performed at the start of the experiment and also with a set of 'washout no stimulus' trials performed at the end of the experiment. 14-31 Hz coherence was also significantly lower in the 'washout no stimulus' trials, than in 'no stimulus' trials (A; $P<0.05$).

4.5 Discussion

This study has shown that TMS at threshold intensity can reset EMG oscillatory activity. Oscillations following TMS were phase-locked to the stimulus. Stimulus-locking effects occurred in both the 14–31 Hz and 4–12 Hz ranges (with a suggestion of a stronger effect in the higher frequency bandwidth). In addition threshold TMS significantly increased EMG-EMG coherence in both 4–12 Hz and 14–31 Hz ranges. The latter observation is in agreement with work by Mills and Schubert (1995), who reported that TMS increased coherence in the 16–32 Hz range between pairs of single motor unit spike trains. These authors suggested that these effects might be caused by TMS resetting the firing of corticospinal cells, which would result in a synchronous re-firing of motor units. The present study extends these observations. The fact that for some 250 ms following TMS, surface EMG signals were phase-locked to the stimulus, suggests that following TMS motoneurones not only resume responsiveness in a synchronous manner, but remain phase-locked to the oscillatory drive from motor cortex to muscle. It is therefore the phase of the oscillatory corticospinal drive that is reset by TMS.

The optimum intensity of TMS showing stimulus-locking effects was such as to evoke a small but discernible MEP (0.015 – 0.2 mV) in 1DI. This intensity was termed 'threshold' TMS. Intensities evoking no MEP in 1DI did not elicit significant stimulus-locking, in any of the recorded muscles. In addition higher intensities of TMS that evoked MEPs above 0.2 mV could not be tested as a silent period of motoneurone firing resulted in suppression of the EMG signal during the period of interest. The mechanisms underlying the changes elicited by threshold TMS are of considerable interest. Although it is clear that some changes at the spinal level did result from low intensity TMS, the results are also consistent with TMS gaining access to the cortical origin of rhythms around 20 Hz and possibly 10 Hz. This resulted in changes in the phase of ongoing EMG oscillatory activity.

4.5.1 Effects in the 14–31 Hz bandwidth

A common hypothesis is that oscillations in the 14–31 Hz bandwidth of hand and forearm EMG signals are driven, at least in part, by the motor cortex (Conway et al. 1995; Baker et al. 1997; Salenius et al. 1997a; Kilner et al. 1999; 2000). For simplicity, I will refer to the neuronal network involved as the cortical oscillator. Stimulus-locking effects in the 14–31 Hz frequency range support the idea that a cortical oscillator operating in the 20 Hz range drives EMG activity and that TMS was able to reset this mechanism. Within each EMG signal, 14–31 Hz oscillations were thus phase-locked to the stimulus. In addition, TMS also caused an increase in coherence between muscle pairs in this frequency bandwidth. This suggests that a 20 Hz cortical oscillator is involved in establishing coherence between muscle pairs.

What is difficult to decide is whether these stimulus-locking and coherence effects were mutually exclusive. If stimulus-locking effects within each EMG signal were similar, then this mechanism could have resulted in a more consistent phase difference between muscles over trials. This may have caused an increase in coherence. However, the situation was more complicated, as differences in the frequency at which peak stimulus-locking occurred between muscles were observed. This could have resulted in a reduction in coherence, implying that stimulus-locking and coherence effects were mediated by different mechanisms.

It is possible that TMS gained access to the network of cortical neurones generating oscillations and in some way boosted activity, possibly by activating inhibitory interneurones which play an important role in oscillation generation (Lytton and Sejnowski 1991; Pauluis et al. 1999; Whittington et al. 2000). Results in this study support this theory, if it is accepted that effects of subthreshold TMS (i.e. an intensity which did not produce an MEP) *are* of a purely cortical nature. There was an increase in 14–31 Hz coherence when subthreshold 'stimulus' trials were compared to 'no stimulus' and 'washout no stimulus' trials. It is indeed possible that subthreshold TMS could boost

the central oscillator and cause an increase in 14–31 Hz EMG-EMG coherence. What is interesting is that stimulus-locking effects were minimal at this intensity, again suggesting that indeed the two effects may operate by different mechanisms.

It should be noted that there was a significant difference between coherence in the 14-31 Hz bandwidth recorded during 'no stimulus' trials and 'washout no stimulus' trials. This was surprising, given that both sets of trials served as controls, but may have been due to lasting effects of TMS applied in the 'stimulus' trials performed before the 'washout no stimulus' trials. It is however, difficult to explain why such longer lasting effects of TMS would cause a significant reduction in coherence, although subjects may have been adopting a different strategy to perform the task. Note that a reduction of coherence in the 4-12 Hz bandwidth did not occur, and 4-12 Hz coherence was similar during both control trials.

4.5.2 Effects in the 4–12 Hz bandwidth

The presence of predominant frequencies around 10 Hz in EMG activity, during a precision grip hold, raises the question as to the origin of this frequency. To date, apart from two recent studies (Marsden et al. 2001; Raethjen et al. 2002), there has been a lack of evidence to suggest EMG oscillations in the 4–12 Hz range are driven by the cortex during stable contractions. Coherence between muscle pairs in this frequency range during the precision grip task is usually below significance levels (Kilner et al 1999; 2000). It is interesting therefore that threshold TMS caused stimulus-locking in this bandwidth. It is possible that TMS was acting at a spinal level as an MEP was evident in the EMG (at least for the intrinsic hand muscles). As motoneuronal firing rate is naturally around 10 Hz (Farmer et al. 1993a), resetting of motoneuronal firing could explain the stimulus-locking around this frequency. However there were effects as low as 4–6 Hz which are more difficult to explain. Also note that resetting effects in both the 14–31 Hz and 4–12 Hz bandwidths were observed during the same block of trials in a single

subject. It seems unlikely that these results were obtained by TMS operating via two such different mechanisms (one cortical, one spinal) given the wide frequency distribution of stimulus-locking effects (Figure 4.4).

The interesting finding was that 4–12 Hz coherence, negligible in 'no stimulus' trials, was increased to significant levels following threshold TMS. The simplest explanation is that this was a result of stimulus-locking in this bandwidth, which as explained could involve spinal mechanisms. However, a more exciting alternative is that TMS gained access to 10 Hz oscillators, (whether cortical or spinal) which are not under normal physiological conditions driving EMG signals.

4.5.3 Effects observed during 'zero' trials mixed in with 'stimulus' trials

Before addressing the issue of cortical oscillators it is important to consider observations when 'stimulus' trials were intermixed with 'zero' trials within the same block. The assumption would be that effects similar to those observed between separate blocks of 'no stimulus' trials and 'stimulus' trials would be seen. This was not the case. Threshold TMS when mixed (and subsequently compared) with 'zero' trials had no significant effect on 14–31 Hz coherence, but did increase 4–12 Hz coherence. 'Zero' trial coherence was no different from coherence during a 'no stimulus' trial block. Indeed the effect of TMS in both bandwidths held true when comparing 'stimulus' trials to a 'no stimulus' block performed at the start of the experiment.

It would seem that the presence of 'zero' trials nullified the enhancing effect of threshold TMS on 14–31 Hz coherence, but not on 4–12 Hz coherence. Task modulation of EMG-EMG coherence in the 14–31 Hz bandwidth may explain this result (Kilner et al. 2000). It is possible that for the unpredictable hold-ramp-hold task during which there was a random mixture of 'stimulus' and 'zero' trials, that subjects used a different motor set (Johansson et al. 1992), than the one used for a more predictable set of only 'stimulus'

trials. It is possible that this change in motor set was reflected in the lower level of 14–31 Hz muscle pair coherence (Kilner et al. 2000).

4.5.4 Generation of oscillatory activity within the cortex

If we accept that EMG frequencies within the 14–31 Hz bandwidth are driven by a cortical oscillatory drive, which may also under certain conditions operate at frequencies around 10 Hz, it remains to be explained which neuronal components constitute the cortical oscillators previously referred to. One possibility is that a brain region well known for its rhythm generating capabilities projects an oscillatory drive onto the cells within the motor cortex. Such a theory has been raised in patient studies in which structures such as the basal ganglia or cerebellum have been shown to play a role in controlling synchronization of cortical activity (Brown 2000). Whether similar mechanisms operate in healthy subjects is unknown. In addition how did TMS over the motor cortex affect oscillation generation at this remote site? It is feasible to suggest that the TMS stimulus used in this study may have activated afferents projecting from such brain regions onto the motor cortex. Indeed a suitable candidate would be thalamo-cortical afferent fibres. Extensive work has been done on brain slices to show that networks of cells in the thalamus generate oscillatory activity (Steriade et al. 1985; Lopes da Silva 1991) which could then have provided an oscillatory drive to entrain activity within the motor cortex. One argument against this hypothesis is that signal analysis in similar experiments showed that significant cortico-cortical coherence remained after the removal of the thalamic signal (Lopes da Silva 1991).

That both TMS, and direct pyramidal tract stimulation (Jackson et al. *in press*) caused *resetting* of the phase of oscillatory activity in EMG signals, suggests that cells *within* the motor cortex form part of the rhythm generating loop. Stimulating afferents carrying an oscillatory signal generated elsewhere, could have temporarily paused oscillatory activity, but would not have reset the rhythm. An alternative could be that the network of neurones

generating the rhythm extends from one brain region to the other. However, some modelling work has suggested that oscillatory networks are confined to narrow regions of the brain e.g. single columns of the cortex, due to the limited axonal aborization of inhibitory interneurones within these networks, that play a key role in rhythm generation (Lytton and Sejnowski 1991). It is tempting to speculate that cells within the motor cortex form their own oscillatory network, which includes inhibitory interneurones activated by low TMS intensities, which project onto PTNs.

What is interesting is that direct pyramidal tract stimulation caused resetting at frequencies around 10 Hz and 20 Hz in the cortex (local field potential recordings), but only 20 Hz within EMG activity (Jackson et al. *in press*). An explanation for this could be that neurones in the motor cortex are involved in oscillatory circuits operating at both 10 Hz and 20 Hz, but it is only the 20 Hz oscillator that drives EMG activity. Indeed oscillatory activity in both ranges has been recorded from the human sensorimotor cortex using MEG (Salmelin and Hari 1994) and EEG (Pfurtscheller et al. 1996) and the 20 Hz signal has been shown to have stronger connections with the motor cortex (Salmelin et al. 1995). This may explain the clear task modulation of 15–30 Hz cortico-muscular coherence observed in both monkeys and humans (Baker et al. 1997; Kilner et al. 1999; 2000).

It is highly likely that TMS at the low intensities used in this study resulted in trans-synaptic excitation of PTNs via activation of both excitatory and inhibitory interneurones (chapter 3). It could be argued that as a technique it is more likely to have more widespread cortical effects than direct pyramidal tract stimulation. Indeed the fact that 10 Hz stimulus-locking effects in EMG were not observed after direct pyramidal tract stimulation in the monkey but were observed in the present study, suggests 10 Hz stimulus-locking effects of TMS in humans are more likely to be of cortical origin.

4.5.5 Conclusions

This study has provided evidence to suggest that local inhibitory interneurones and PTNs within the motor cortex are involved in the generation of cortical oscillations. These results support the suggestion that the corticospinal tract transmits oscillatory drive from the cortex to muscle EMG activity (Baker et al. 1999) at frequencies in the 14–31 Hz bandwidth (and possibly in the 4–12 Hz range also). A few observations were made in this study of stimulus-locking at frequencies around 40 Hz. EMG oscillations in this frequency range (termed the Piper rhythm) normally occur during higher muscle activation than used in this study. However such frequencies have also been shown to be coherent with cortical activity (Salenius et al. 1996; Brown et al. 1998). TMS may therefore be able to access other oscillatory networks operating at higher frequencies. All of these cortical oscillators may contribute to the wide range of frequencies observed in physiological tremor (McAuley et al. 1997; Mayston et al. 2001).

CHAPTER 5: CORTICOSPINAL DRIVE AND EMG-EMG COHERENCE

5.1 Introduction

In chapter 4 data were presented to show clear evidence that the motor cortex is involved in the maintenance of EMG-EMG coherence between muscle pairs. Low intensity TMS was used to reset oscillatory EMG activity. Using higher intensities of TMS, the excitability of corticospinal pathways projecting to hand muscles can be determined. The amplitude of MEPs evoked by TMS is a measure of corticospinal drive to hand muscles during various tasks (Flament et al. 1993; Kasai and Yahagi 1999; Hasegawa et al. 2001a,b). In this study similar techniques were used to measure the motor cortex excitability during a precision grip task carried out under two different conditions. In one condition, subjects gripped a compliant object and performed a hold-ramp-hold task, whereas in the other a non-compliant and static object was gripped. The aim of the study was to see if cortical drive to muscles covaried with levels of coherence between pairs of muscles. It was shown previously that during the hold phases of a compliant hold-ramp-hold task a higher level of 14–31 Hz EMG-EMG coherence is observed than when a less compliant, or non-compliant object is gripped (Kilner et al. 2000). If the motor cortex is involved in the maintenance of coherent activity between pairs of muscles, there could be a weaker oscillatory corticospinal drive when there is less EMG-EMG coherence. It is possible then that smaller MEPs would be evoked during grip of the less or non-compliant object. Alternatively, it could be that in some way synchrony is maintained by inhibitory mechanisms, or that corticospinal tract neurones are less excitable when discharging in an oscillatory fashion. In this case it is possible that MEPs evoked whilst gripping the non-compliant object would be larger.

5.2 Methods

Experiments were performed on 8 healthy right-handed human volunteers (aged 20-35 years old; 4 females). The experiments had ethical committee approval, and all subjects gave informed consent.

5.2.1 Behavioral task

Subjects performed a precision grip task designed previously to investigate coherent oscillatory activity (Kilner et al. 1999; 2000). Details of the precision grip task have been described in chapter 2. A hold-ramp-hold task was performed under compliant conditions (spring constant 0.05 N/mm) and an isometric task involved precision grip of a non-compliant object. Grip apertures required for the hold 2 period of the hold-ramp-hold task, and for the isometric task were similar (approximately 40 mm).

5.2.2 EMG recordings

Bipolar surface EMGs were recorded from right first dorsal interosseous (1DI), abductor pollicis brevis (AbPB), flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC) muscles. EMGs were amplified (x 500 Hz or 1000 Hz), filtered (30 Hz) and sampled at 5000 Hz by a PC-compatible computer attached to a 1401+ interface (Spike 2 CED Ltd. Cambridge, UK). Subjects were given feedback of the EMG level in 1DI muscle, details of which are described in chapter 2.

5.2.3 Stimulation

The TMS coil (Magstim 200 stimulator; double cone coil, 13 mm diameter of each loop; Magstim Company Ltd, Dyfed UK) was positioned on the subject's head. The coil was placed over the motor cortex (current flow in an anterior-posterior direction) and the hotspot for 1DI activation found. The coil was then fixed in place with the aid of velcro strapping around the subject's head and the lead was supported in a harness system. For

the hold-ramp-hold task, TMS was delivered once during each trial, 1 s into the hold 2 phase. For the isometric task, TMS was delivered once every 6 s.

5.2.4 TMS intensities

RMT was defined as the lowest stimulus intensity to evoke a small but reproducible threshold MEP in relaxed 1DI muscle (approximately 0.1–0.2 mV peak-to-peak in unrectified EMG). 20–30 stimuli at this intensity (20–30 % maximum stimulator output) were then delivered during each condition. Note that this intensity was checked before trials began to ensure that when subjects were active (i.e. during the isometric task), an MEP of at least 1–2 mV was elicited. If the MEP was too small, this intensity was adjusted slightly (by 1–2 % maximum stimulator output only).

5.2.5 Protocol

Subjects began by (a) performing 60 trials of the hold-ramp-hold task (spring constant 0.05 N/mm) with no feedback of 1DI muscle activation and no stimuli. They then performed (b) further trials of the hold-ramp-hold task again (no stimuli), but this time with the aid of 1DI feedback. In order to establish the target level for 1DI activation, subjects were instructed to perform the hold-ramp-hold task as naturally as possible. A comfortable level of 1DI EMG activation was defined for the hold 2 phase of the task. (This was later checked and found to be approximately 5–10 % maximum voluntary contraction). Subjects were then instructed to try to keep this level of 1DI activation as consistent as possible during each trial, with the aid of feedback. Subjects next performed (c) an isometric task of approximately 6 minutes, with no stimulus, again with the aid of 1DI feedback. Efforts were made to ensure that the target level of 1DI EMG activation was the same for both the hold-ramp-hold and isometric tasks. These trials were later used to make coherence measurements for each precision grip task.

Subjects then performed (d) 20-30 trials of the hold-ramp-hold task (spring constant 0.05 N/mm) with no EMG feedback. TMS was delivered once every trial. Subjects then performed (e) a further 20-30 trials of the hold-ramp-hold task but were this time given feedback of 1DI muscle activation. Finally subjects performed (f) approximately 3 minutes of the isometric task again with the aid of 1DI muscle feedback, with TMS delivered every 6 s.

5.3 Data Analysis

5.3.1 EMG-EMG coherence calculations

Details of the coherence analysis employed are presented in chapter 2. An FFT of 128 was used in an analysis involving four rectangular, non-overlapping windows of approximately 486 ms length each. For the hold-ramp-hold task, coherence analysis was performed on the last 2 s of EMG data in the hold 2 phase. For the isometric task, an equivalent section of EMG data was analysed approximately every 6 s in the data. Muscle pair coherence values were normalized and for each subject combined, to give an 'all muscle pair' value. Normalized coherence was then summed between 14–31 Hz. Finally this value was multiplied by 2 Hz, which was the bin width, or resolution of the coherence spectra. This resulted in units of 'normalized coherence area'.

5.3.2 Statistics for coherence analysis

Significant differences in values of coherence were tested with a one tailed paired t-test run across subjects.

5.3.3 MEP measurements

MEPs were measured as described in chapter 2. Briefly, for each task condition all 20-30 trials of rectified EMG data were superimposed. The area of interest (the width of the MEP response) was defined and a single peak amplitude value was taken for each trial.

For each task condition, the mean of the peak values was then calculated with the standard error. Background EMG levels were measured by sampling 400 ms of rectified EMG data immediately before the stimulus. Average background EMG levels were calculated for each task condition. Efforts were made to record EMG signals as consistently as possible across subjects. Raw rectified EMG levels were considered to provide a sensitive measure of background activity and no attempt was made to normalize these data. Similarly MEP values were also left as raw data. It was decided that studying effects of background EMG level on unadjusted MEP amplitudes would be more informative than potentially adding more variability to the data by normalization.

5.3.4 Statistics for MEP measurements

Significant differences in MEP amplitudes and average background EMG levels between task conditions were tested with a one tailed paired t-test run across subjects. Regression analysis between these two parameters was performed with a 95 % confidence limit.

5.4 Results

5.4.1 14–31 Hz coherence during precision grip tasks

Coherence spectra from pairs of EMGs recorded from a typical subject during two conditions of the precision grip task are shown in Figure 5.1. In both cases feedback of 1DI activity was provided. In this subject significant coherence in the 14–31 Hz bandwidth was present during the hold 2 phase of the hold-ramp-hold task. There was clearly less coherence in this bandwidth in all muscle pairs during the isometric task.

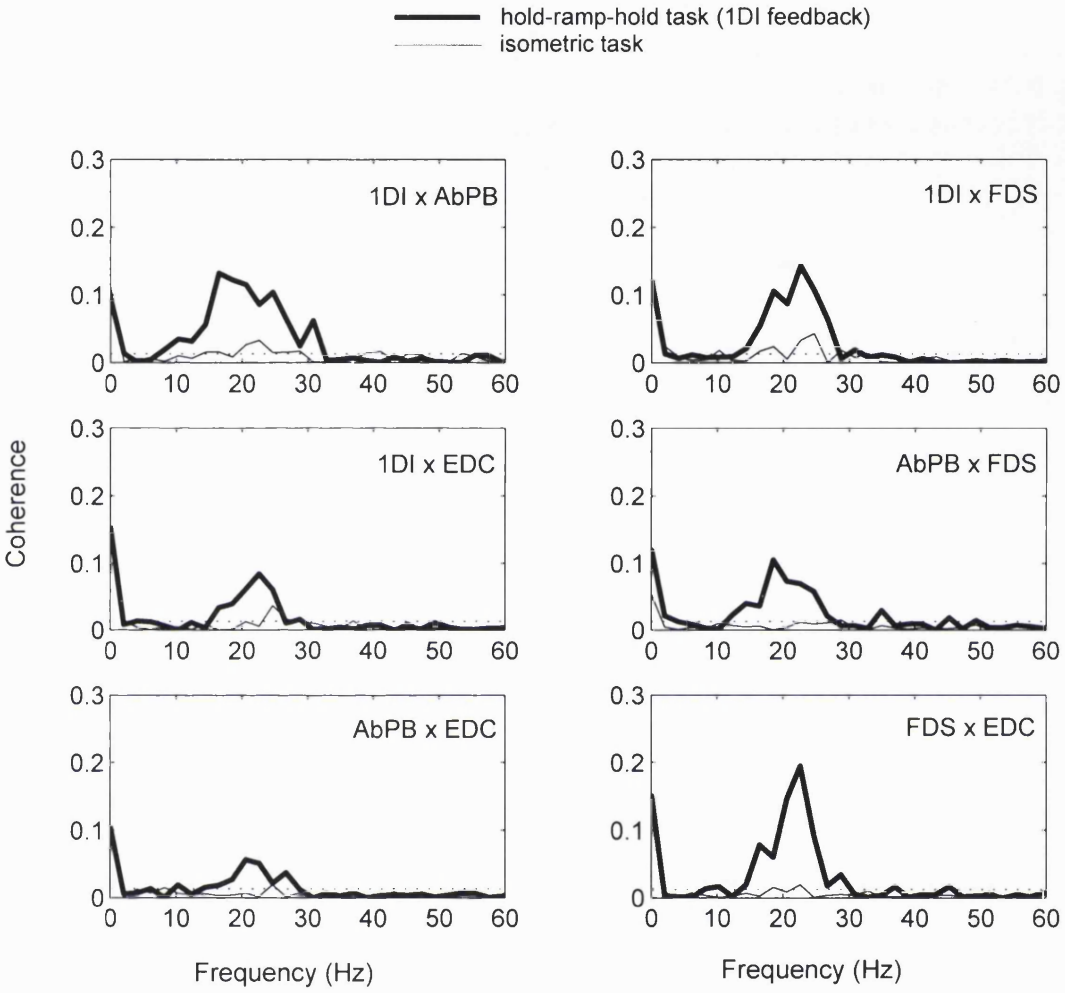


Figure 5.1: Coherence spectra for six muscle pairs of a single subject. Coherence present during the isometric task (thin lines) is less than that during the hold 2 phase of the hold-ramp-hold task (thick lines). Both tasks were performed with the aid of 1DI EMG feedback. Note similar levels of coherence present in the different muscle pairs during each task condition. Dotted horizontal lines indicate 95 % confidence limits.

Muscle pair coherence values between 14 –31 Hz were then combined for each subject to obtain an 'all muscle pair' coherence value. Data was then pooled for all 8 subjects and presented as mean coherence values. Figure 5.2 shows 14–31 Hz coherence present during each of the three task conditions: hold-ramp-hold task with and without 1DI feedback, and the isometric task with 1DI feedback. Note that feedback of 1DI muscle activation did not effect the level of coherence measured during the hold-ramp-hold task. The level of 14–31 Hz coherence was significantly lower during the isometric task when compared to both the hold-ramp-hold task with 1DI feedback ($P=0.01$) and the hold-ramp-hold task without feedback ($P=0.007$).

5.4.2 MEP size differences during precision grip tasks

Mean MEP values from 1DI and AbPB muscles were measured. In 1DI there was no significant difference between mean MEP size elicited in any of the three task conditions (Figure 5.3A). For AbPB, MEPs evoked during the isometric task were significantly smaller than those evoked during either hold-ramp-hold task (Figure 5.3B). Note that there was again no difference between mean MEP size evoked in the hold-ramp-hold tasks (with or without 1DI feedback). Despite the use of feedback, there were still small, but significant changes in background EMG levels for IDI between tasks. Figure 5.4A shows background EMG levels present in 1DI muscle for all three task conditions. When compared to background EMG levels present in the hold-ramp-hold task performed with the aid of 1DI feedback, background EMG levels for the isometric task were significantly lower ($P<0.05$). Background levels during the hold-ramp-hold task performed without 1DI feedback were also lower ($P<0.05$) when compared to those performed with feedback. These were small but significant differences. Note that there was no difference in background EMG level between the hold-ramp-hold task without 1DI feedback and the isometric task. It should be noted that feedback of the level of muscle activity was only provided for the 1DI muscle, and so changes in background EMG levels between tasks,

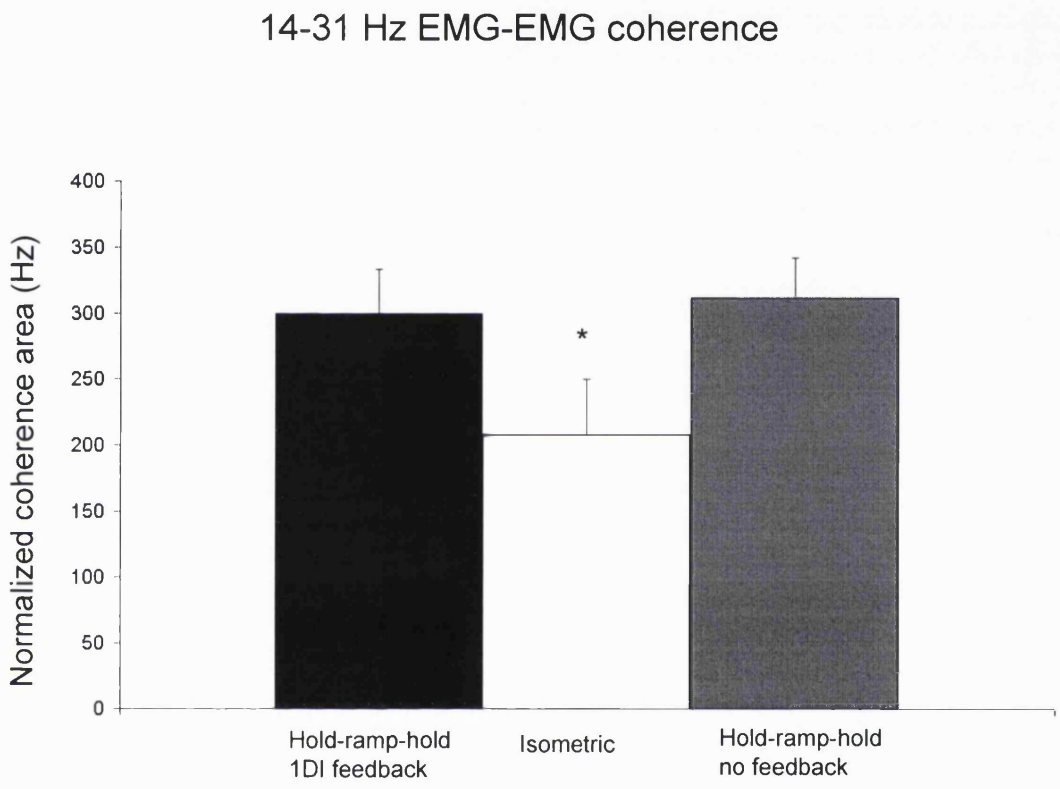


Figure 5.2: Pooled data showing 14-31 Hz EMG-EMG coherence present during precision grip tasks (n=8). In the isometric task (open bars), significantly less coherence was present than that measured during both the hold-ramp-hold task with 1DI feedback (black bars; $P<0.05$) and without feedback (grey bars; $P<0.01$). Note that there was no effect of 1DI feedback on coherence in the hold-ramp-hold task.

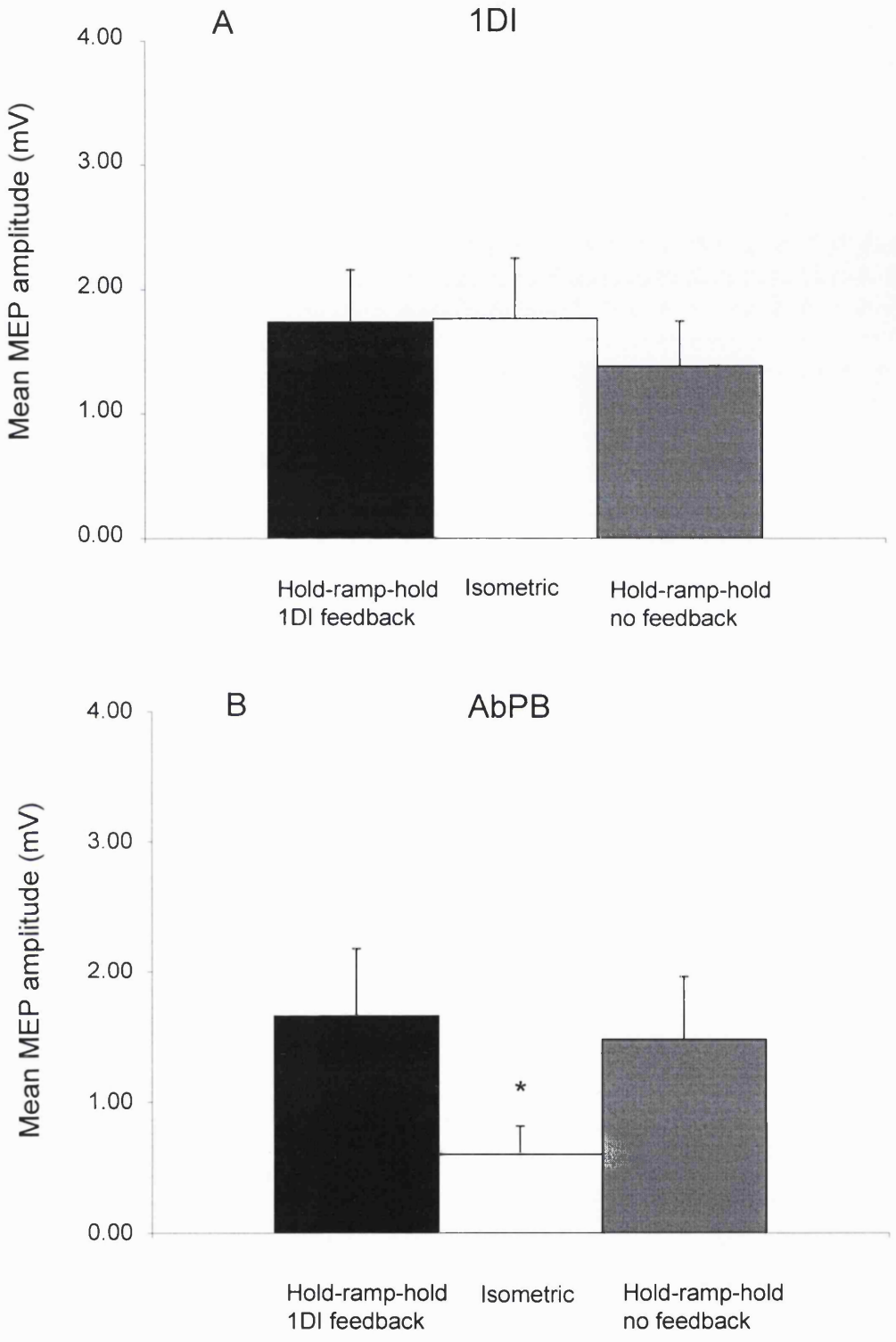


Figure 5.3: Pooled data showing mean MEP amplitudes evoked during precision grip tasks (n=8). (A) In 1DI there were no differences in mean MEP amplitude between any of the tasks. (B) In AbPB, mean MEP amplitude during the isometric task (open bars) was significantly lower compared to the hold-ramp-hold task with 1DI feedback (black bars; $P<0.05$) and without feedback (grey bars; $P<0.05$).

for AbPB might have been expected. That this was indeed the case is shown in Figure 5.4B. Levels during the isometric task were significantly lower when compared with both hold-ramp-hold tasks (Figure 5.4B; $P<0.005$). There was also significantly less background EMG in AbPB during the hold-ramp-hold task with no feedback, compared to the hold-ramp-hold task when 1DI feedback was provided ($P<0.05$).

5.4.3 Correlation between background EMG level and MEP amplitude

Note that because feedback of 1DI EMG level was provided, background EMG levels for 1DI within each task condition over trials were very consistent. It was therefore difficult to make any correlation between background EMG levels and MEP size on a single subject basis. Between subjects, there was a difference in background EMG level. Correlations between background EMG levels and MEP amplitude were therefore made using pooled data for both 1DI and AbPB muscles. Figure 5.5A shows results from 1DI during the hold-ramp-hold task and the isometric task (both performed with 1DI feedback). Mean MEP values from all subjects are plotted against average background EMG level. When all subjects were considered, there was no significant correlation between background EMG level and MEP size, for either task condition. This was also true of the hold-ramp-hold task without 1DI feedback (data not shown). Correlations were also made for AbPB (Figure 5.5B). For the hold-ramp-hold task (with 1DI feedback), when all subject data was used, there was a significant correlation between background EMG level and MEP size (r^2 0.7; $P<0.05$). Note that there was also a significant correlation during the hold-ramp-hold task with no EMG feedback (r^2 0.8; $P<0.005$; data not shown). There was also a significant correlation between background EMG level and MEP size for the isometric task, despite the range of background EMG levels recorded being very small (r^2 0.7; $P<0.01$). Note that for AbPB there *were* changes in MEP sizes between the hold-ramp-hold task (with or without 1DI feedback) and the isometric task, and changes in background EMG level.

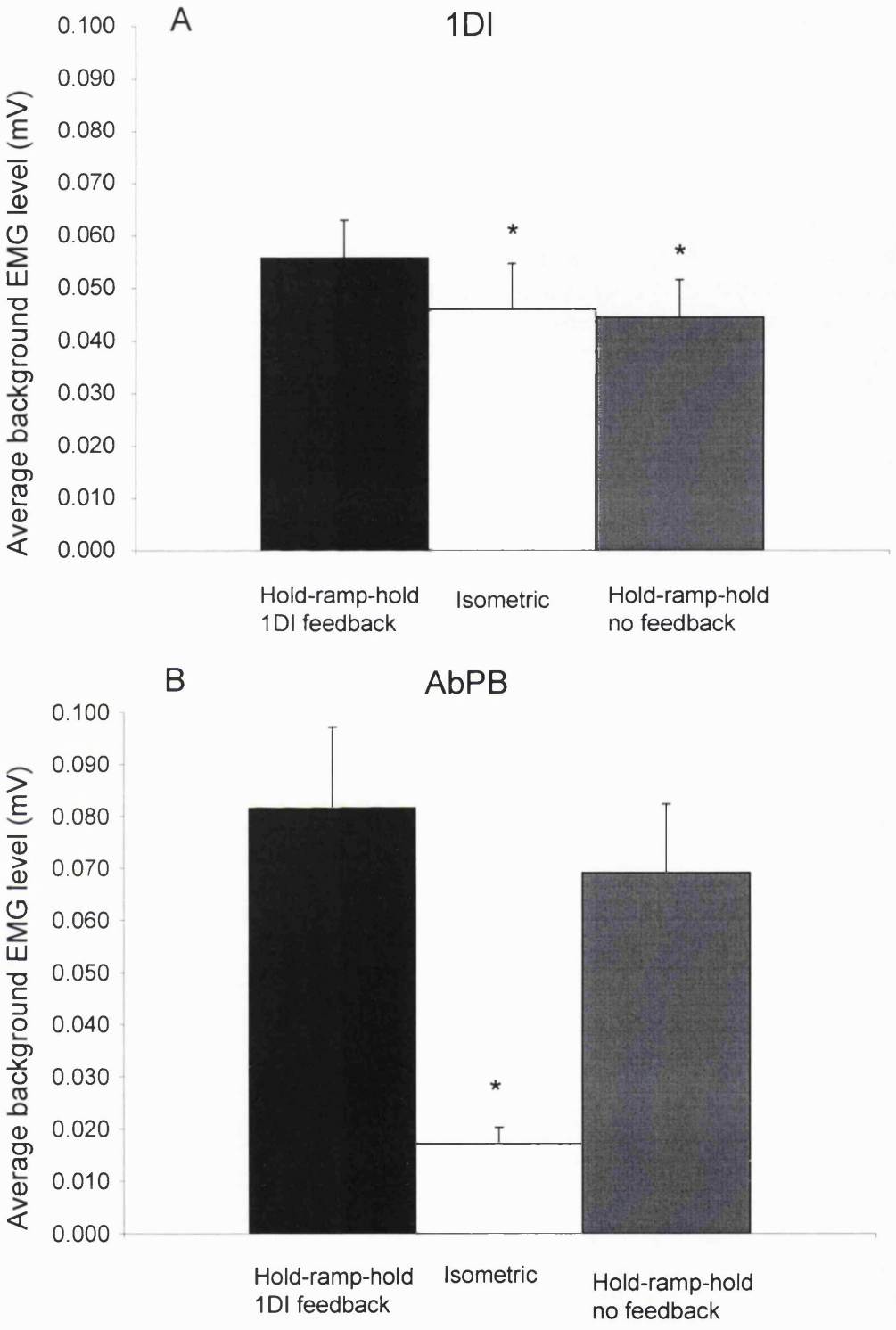


Figure 5.4: Average background EMG levels during precision grip tasks from pooled data (n=8). (A) In 1DI background EMG levels were significantly smaller in both the isometric task (open bars; $P<0.05$) and the hold-ramp-hold task with no EMG feedback (grey bars; $P<0.05$) compared to the hold-ramp-hold task with 1DI feedback. Note there was no difference in background EMG levels between the hold-ramp-hold task without EMG feedback (grey bars), and the isometric task (open bars). (B) In AbPB, background EMG levels in the isometric task were significantly smaller than both the hold-ramp-hold tasks ($P=0.0005$ compared to 1DI feedback; $P<0.005$ compared to no EMG feedback).

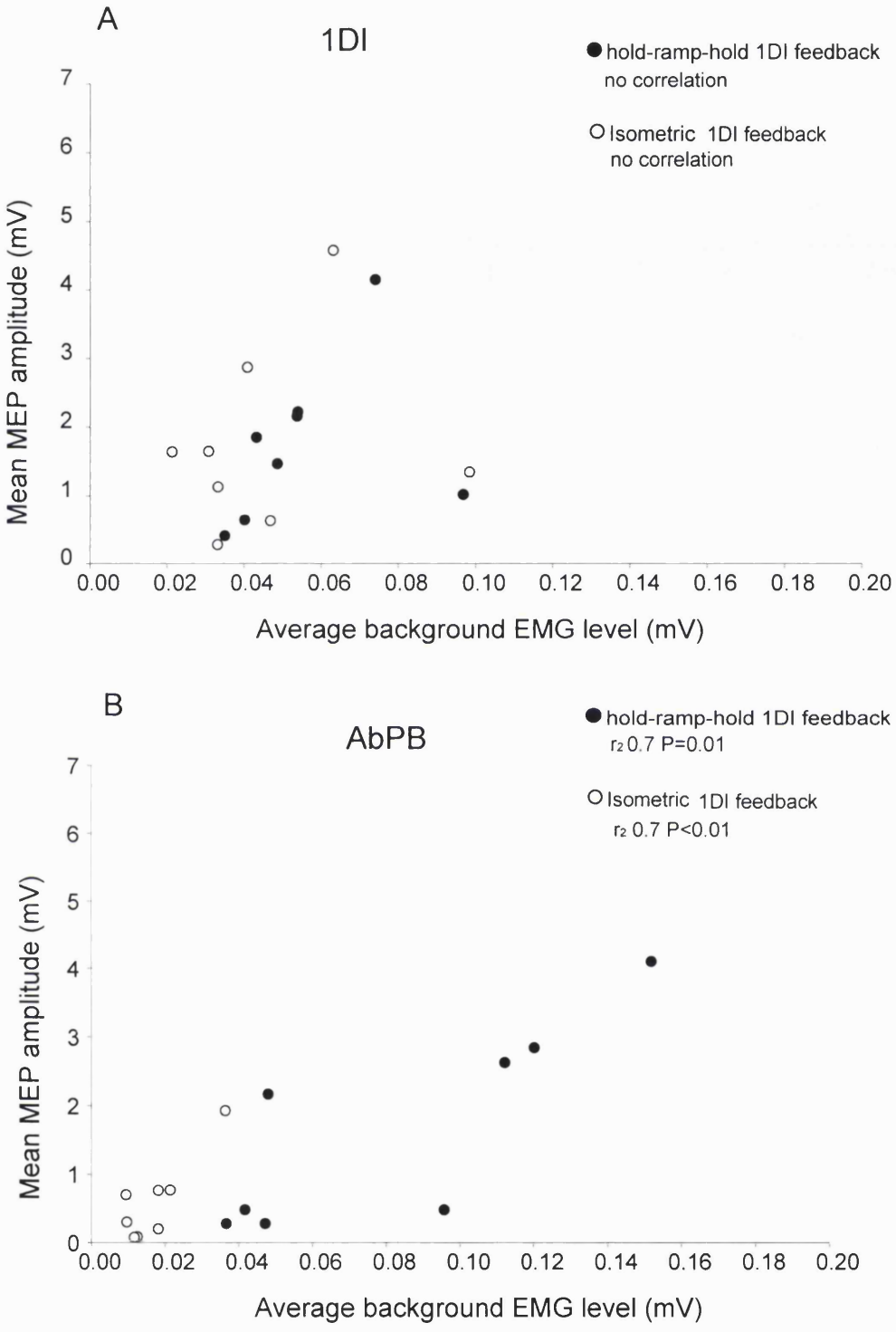


Figure 5.5: Pooled data showing the correlation between average background EMG level and mean MEP amplitude during precision grip tasks. 20-30 trials of data were used from each of the 8 subjects presented. (A) In 1DI there were no correlations in either task. (B) In AbPB, there was a significant correlation in the hold-ramp-hold task with 1DI feedback (black circles) and in the isometric task (open circles).

5.5 Discussion

It has been shown that levels of EMG-EMG coherence in the hold phase of a precision grip task, vary systematically with the degree of compliance of the gripped object (i.e. lever compliance; Kilner et al. 2000). The aim of this study was to examine corticospinal activity in the hold phases of different precision grip task conditions, by measuring MEP responses evoked by TMS. The hypothesis was that if EMG-EMG coherence present between muscle pairs was due to the level of oscillatory activity in the corticospinal projections to these muscles, then this should influence the amplitude of the evoked MEP. The direction change is not predictable. As outlined in a model (Matthews 1999), the excitability of the motor cortex, as measured by the amplitude of a response evoked by an external stimulus, depends critically on the firing rate of the PTN. Chapter 4 provided evidence to suggest that cortical inhibitory interneurones projecting onto PTNs could be involved in the generation of EMG oscillatory activity. Oscillatory activity involving cycles of excitability and inhibition, could thus involve firing patterns that either enhance of depress responses to TMS (Matthews 1999). To summarize the results: despite differences in coherence between hold-ramp-hold (spring constant 0.05 N/mm) and isometric tasks, there were no differences in MEP size in 1DI muscle. In contrast, significantly lower MEPs were evoked in AbPB during the isometric task, than in the compliant hold-ramp-hold task. However there was also a significantly lower level of background EMG during the isometric task and this could explain the difference in MEP amplitude observed.

5.5.1 EMG-EMG coherence measurements

It was not possible to measure coherence during the stimulus trials. This was because the large MEPs evoked were followed by a silent period, which makes analysis difficult. The aim was simply to get a 'signature' coherence level for each subject, for the two task conditions. There was clearly a lower level of 14–31 Hz EMG-EMG coherence in the

isometric task compared to the more compliant hold-ramp-hold task (and 1DI feedback had no effect on this measurement).

5.5.2 Background EMG levels

Despite efforts to keep background EMG levels in 1DI constant throughout some of the task conditions, small but significant differences in 1DI activity were observed between the isometric task, and the hold-ramp-hold task with EMG feedback. This may have resulted because of the difficulty subjects had in maintaining constant EMG levels in 1DI muscle, whilst performing a precision grip of a compliant object. The EMG feedback system was fixed at a coarse setting, which explains why it was possible that during the experiment differences in levels between task conditions went unnoticed. It was decided that making the feedback system more sensitive could have resulted in subjects moving too much, to correct themselves, which may have affected coherence (Pfurtscheller et al. 1996). Indeed the fact that for 1DI, background EMG levels were the same for the hold-ramp-hold task *without* 1DI feedback and the isometric task, indicates that EMG feedback may have been more of a hindrance than a help.

Results for AbPB suggest that smaller MEPs were elicited in the isometric task. However, there were also lower background EMG levels present, which had dramatic effects on MEP size (Figure 5.5B). The drastic reduction in background EMG level in AbPB in the isometric task, highlights a further aspect of trying to keep the EMG level of another muscle constant. Although the nature of the hold-ramp-hold task required subjects to used AbPB, subjects tended to de-activate AbPB in the isometric task.

5.5.3 Background EMG and MEP amplitudes

Correlations between MEP amplitudes and background EMG levels were made on pooled data to assess the overall influence of EMG activity on evoked responses. Overall there

was no correlation between background EMG levels and MEP amplitude in 1DI for either task condition. There was a correlation in AbPB, during both the hold-ramp-hold task and isometric task; as expected the range of background EMG levels was higher for AbPB than 1DI. Therefore it is highly likely that differences in background EMG levels between task conditions may have contributed to the changes observed in MEP amplitude. Indeed it is well known that levels of muscle activation can dramatically modulate MEP size during background EMG levels lower than 10% maximum voluntary activity (Hess et al. 1987; Kasai and Yahagi 1999). For hand muscles, the facilitatory effects of voluntary activity have been shown to saturate at around 10 % maximum voluntary activity (Hess et al. 1987; Turton and Lemon 1999). However higher levels of voluntary activity were avoided in this study, as this has been shown to reduce the sensitivity of TMS responses to motor cortex excitability (Kasai and Yahagi 1999).

In contrast to the difference in MEP size observed in AbPB there were no differences in MEP amplitudes evoked in 1DI, between any of the task conditions. This result was most clear for the comparison between the hold-ramp-hold task without EMG feedback, and the isometric task, as background EMG levels were not different between these conditions. However, even the lower background EMG level in the isometric task compared to the hold-ramp-hold task with EMG feedback, did not result in a reduction in MEP amplitudes.

5.5.4 TMS evoked responses and coherence measurements

These data suggest that despite the fact there was a clear difference in EMG-EMG coherence between the hold-ramp-hold and isometric task, there was no difference in corticospinal drive to muscles. This is surprising, given that an oscillatory drive from the motor cortex has been argued to maintain coherence between muscle pairs (Conway et al. 1995; Baker et al. 1997; Salenius et al. 1997a; Kilner et al. 1999; 2000). Indeed chapter 4

of this thesis showed that low intensities of TMS could enhance EMG-EMG coherence during the hold phase of a precision grip task. Similarly, motor unit synchrony present in the precision grip task was increased following TMS (Huesler et al. 1998). It is however possible that different, possibly slower conducting corticospinal fibres are involved in maintaining synchrony between the cortex and muscle, than large, fast-conducting neurones excited by high intensities of TMS. This difference could explain why it has proved difficult to match phase lags between the cortex and muscle with corticospinal conduction time (Conway et al. 1995; Halldiay et al. 1998; Salenius et al. 1997a; Mima et al. 2000). However a more recent report has found similar latencies (Gross et al. 2000).

An alternative explanation for the lack of correlation between TMS evoked MEP responses and EMG-EMG coherence may be the different nature of each measurement. Coherence measures the phase difference of oscillatory activity between two EMG signals, regardless of amplitude. In addition coherence values used in this study were calculated following a combination of coherence measured between six muscle pairs. This differs from TMS evoked responses, which were sensitive to the amplitude of EMG activity, and were measured in individual muscles separately. That TMS delivered using one coil position, could evoke MEPs of varying amplitudes in intrinsic (and extrinsic) hand muscles during a hold-ramp-hold precision grip task, despite the fact that overall there are similar levels of coherence between muscle pairs (Figure 5.1), illustrates this point. When synergy within multiunit EMG activity of hand muscles during a precision grip, was measured in the time-domain, or as a function of amplitude, there was a lack of agreement in measurements (Hepp-Reymond et al. 1996). This was partly explained by the fact that covariation in the amplitude domain can be based on asynchronous increase in firing rates. In addition, when analysing both single motor unit and multiunit synchronisation in the time domain, there was no clear indication of fixed synergy between muscle pairs either between or within subjects. Instead it was concluded that the

cortico-motoneuronal system operates in a task-dependent and flexible manner allowing the oscillatory corticospinal drive to motoneurones to adapt to a particular task (Hepp-Reymond et al. 1996). Indeed short-term synchronization between single motor units can be modified by both visual and auditory feedback (Schmied et al. 1993). This may explain why it is difficult to obtain any clear differences in MEP sizes evoked in single muscles, in tasks that differ in levels of muscle pair coherence.

It is also important to consider how the oscillatory process in the motor cortex operates and is transmitted to hand muscles. In chapter 4 it was shown that low intensity TMS, likely to activate inhibitory interneurones within the motor cortex, could enhance coherence between muscle pairs, and also reset EMG oscillatory activity. If oscillations are generated by a network of inhibitory and excitatory cells, it is possible that a large TMS stimulus would disrupt the system by overloading it, indicated by the fact that following the MEP there is a silent period of inhibition, before normal oscillatory activity resumes. Therefore the MEP evoked by large intensities of TMS may not be a suitable measurement of the more subtle cortical mechanisms in operation to maintain coherence between muscle pairs.

It is also possible then that the intensity of TMS used in these studies may have been too high. However, in a study in which MEP amplitudes were correlated to background EMG levels, effects observed using intensities of 0.8 x RMT, were similar to those observed using 1.0 x RMT (Hasegawa et al. 2001a). In contrast, in both the study of Lemon et al. (1995), and Kasai and Yahagi (1999), no clear task modulation of MEP amplitudes were reported between tasks, when using TMS intensities of 1.0 x RMT.

5.5.5 Conclusions

The clear difference in EMG-EMG coherence between precision grip tasks differing in the level of object compliance, was not reflected in the amplitude of large MEP responses evoked by suprathreshold TMS. This was despite the fact that coherence between muscle pairs, arising at least in part from an oscillatory cortical drive, was significantly higher in the more compliant task. These findings suggest that despite the fact that suprathreshold TMS can be used to test the activation of the motor cortex during specific motor tasks, this intensity of TMS maybe unsuitable for testing the excitability of neuronal elements involved in synchronous cortico-muscular activity. These results also reflect the amplitude independent nature of synchrony between cortical and muscle activity.

CHAPTER 6: LEVELS OF INTRACORTICAL INHIBITION AND EMG-EMG COHERENCE

6.1 Introduction

The level of synchrony between the cortex and muscle, and between muscle pairs, is modulated during performance of the precision grip task. It has been shown that synchrony in the 14–31 Hz range is stronger during the hold phases of a compliant hold-ramp-hold task, than during an isometric grip task (see chapter 5; Kilner et al 1999; 2000). Chapter 4 showed that low intensity TMS was able to evoke EMG oscillatory activity. Results implied that the neuronal elements excited by TMS are involved in the rhythm generating network responsible for EMG-EMG coherence.

It has been proposed that low intensity TMS excites interneurones within the motor cortex, which are inhibitory to PTNs. Modelling studies have suggested that synchrony within the cortex, is maintained by inhibitory mechanisms (Lytton and Sejnowski 1991; Pauluis et al 1999; Whittington et al 2000) and therefore activation of inhibitory interneurones by TMS could explain its action on synchrony generating mechanisms. Chapter 3 described details of a paired-pulse test, to measure levels of intracortical inhibition. It seems sensible to suggest that if TMS can elicit effects on EMG oscillatory activity and have intracortical inhibitory effects, that the two mechanisms might be related. If they are, it could be predicted that levels of intracortical inhibition could also be modulated by the precision grip task. The prediction would be that higher levels of EMG-EMG coherence in the 14–31 Hz range observed in the hold-ramp-hold task, would be accompanied by higher levels of intracortical inhibition. This is in comparison to the isometric task, during which both parameters would be lower.

It should be noted that the paired-pulse paradigm used to measure intracortical inhibition has parameters that can be altered (see chapter 3 for details). It is possible that different types or phases of intracortical inhibition can be measured depending on the ISI and conditioning stimulus used. A main conclusion of chapter 3 was that optimal ISIs to use to measure intracortical inhibition were ISI=2.5 ms and ISI=1 ms. Those ISIs were therefore used in this study. Should any differences in inhibition between the two precision grip tasks be evident, it would be interesting to see if these changes were consistent no matter what the ISI used.

A difference in conditioning stimulus intensities required for the two phases of inhibition was also reported in chapter 3, permitting another interesting aspect of the present study. Chapter 4 showed that low intensity TMS could increase EMG-EMG coherence, and have stimulus-locking effects on the EMG signal (that is the phase of oscillations in the EMG was reset by the stimulus). The threshold intensities used in chapter 4 (14–23 % maximum stimulator output) were conveniently in the same range as those used to elicit inhibition in the study in chapter 3 (14–28 % maximum stimulator output). (Note that RMTs of subjects were also comparable; 18–30 % maximum stimulator output). As a threshold conditioning stimulus was shown to be optimal for inhibition experiments using ISI=2.5 ms, it was decided that a conditioning stimulus intensity similar to those used in chapter 4 could be used for this paradigm in the present study. It should be noted that a small, but discernable MEP was elicited by such intensities in chapter 4, which is not normally seen with a conditioning stimulus in the paired-pulse test. However, the present aim was to see if a conditioning stimulus that elicited effects on EMG oscillatory activity, could also have inhibitory effects.

By way of contrast, as inhibition using ISI=1 ms was shown to require a lower (subthreshold) conditioning stimulus intensity in chapter 3, it was decided to use this

paradigm to compare the effects of different conditioning stimulus intensities. It was also necessary to use an optimum conditioning stimulus to measure inhibition using ISI=1 ms, and compare this to levels measured using ISI=2.5 ms.

To summarise, questions addressed in this study were:

1. Are there differences in the levels of intracortical inhibition between the hold-ramp-hold and isometric precision grip tasks?
2. Are the results the same for different ISIs and different conditioning stimuli?
3. Can levels of EMG-EMG coherence be correlated with levels of intracortical inhibition?
4. Can the effects of low intensity TMS on EMG oscillatory activity be related to its inhibitory effects?

6.2 Methods

Experiments were performed on 11 healthy right-handed human volunteers (aged 20-35 years old; 7 females). The recordings had ethical committee approval, and all subjects gave informed consent.

6.2.1 Behavioural task

Details of the precision grip tasks have been described in chapter 2. Both the hold-ramp-hold task (spring constant 0.05 N/mm) and the isometric task were used in this study.

6.2.2 EMG recordings

Bipolar surface EMGs were recorded from right first dorsal interosseous (1DI), abductor pollicis brevis (AbPB), flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC) muscles. EMGs were amplified (x 500 Hz or 1000 Hz), filtered (30 Hz)

and sampled at 5000 Hz by a PC-compatible computer attached to a 1401+ interface (Spike 2 CED Ltd. Cambridge, UK).

Subjects were given feedback of the EMG level in 1DI muscle. Details are described in chapter 2. Subjects were instructed to perform the hold-ramp-hold task as naturally as possible. A comfortable level of 1DI EMG activation was defined for the hold 2 phase of the task. Subjects were then instructed to try to keep this level of 1DI activation as consistent as possible during each trial with the aid of feedback. Efforts were made to ensure that this level of 1DI EMG was then reproduced and maintained in the isometric task.

6.2.3 Stimulation

Two Magstim 200 magnetic stimulators were connected to the same coil (double-cone coil, 13 cm diameter) through a Y connector (Magstim Company Ltd, Dyfed, UK). The TMS coil was positioned on the subject's head over the left motor cortex (current flow in an anterior-posterior direction) and the hotspot for 1DI activation found. The coil was then fixed in place with the aid of velcro strapping around the subject's head and the lead was supported in a harness system. For the hold-ramp-hold task, TMS was delivered once during each trial, 1 s into the hold 2 phase. For the isometric task, TMS was delivered once every 6 s.

6.2.4 Paired-pulse TMS paradigm

In order to measure intracortical inhibition, the paired-pulse paradigm was used. Three conditions were mixed randomly during each experimental block: a single conditioning stimulus, a single test stimulus and a paired-pulse consisting of the conditioning stimulus preceding the test stimulus. Approximately 10 trials of each condition were recorded (30 trials in total for each block). In one set of experiments an ISI=2.5 ms was used. In another set of experiments, performed on a separate day, an ISI=1 ms was used.

6.2.5 TMS intensities

RMT was defined as the lowest stimulus intensity to evoke a small but reproducible threshold MEP in relaxed 1DI muscle. The subject was then instructed to maintain voluntary activity by performing the isometric task (with the use of 1DI EMG feedback). On most occasions the RMT intensity evoked an MEP in 1DI of approximately 1 mV during active conditions. This intensity was then used as the test stimulus. If when active, the RMT intensity was too low to evoke an MEP in 1DI of around 1 mV, the intensity was increased by 1 or 2 %, and this new intensity was used as the test stimulus. Note that the RMT for each subject was redefined for each experimental session.

Conditioning stimulus intensities were chosen based on online stimulus-triggered averaging of rectified 1DI EMG data (and on results from chapter 4). Approximately 10 MEP responses in 1DI muscle were averaged whilst subjects maintained voluntary activity by performing the isometric task. An intensity was chosen that elicited a discernable, but close to threshold MEP response, with no silent period. Evidence of peaks and troughs following the MEP were also looked for. In the experiments using ISI =2.5 ms, this was the only conditioning stimulus required. For experiments using ISI=1 ms (which took place on a separate day), a similar threshold conditioning stimulus intensity was used. For some subjects it was exactly the same intensity as used in the ISI=2.5 ms experiments; however, on occasions when the RMT in that subject had changed, it was found that the conditioning stimulus intensity had also to be adjusted. In addition a lower subthreshold conditioning stimulus was used (usually 3 % lower than the stimulus-locking intensity). It was checked online that the lower conditioning stimulus evoked no MEP and no peaks and troughs in the averaged EMG.

6.2.6 Protocol

Experiments were performed on two separate days on the same set of 11 subjects.

Day 1

1. 60 trials of the hold-ramp-hold task with no stimulus.
2. Isometric task of approximately 6 minutes, with no stimulus.

   Paired-pulse test employed using ISI=2.5 ms and a threshold conditioning stimulus in steps 3 and 4 during:
3. 30 trials of the hold-ramp-hold task, with TMS applied during the hold 2 phase of the task.
4. Isometric task lasting approximately 3 minutes. TMS applied every 6 s (30 stimuli in total)
5. 5 subjects performed an extra isometric task (lasting 6 minutes) during which the conditioning stimulus alone was delivered once every 6 s (60 stimuli in total).

Day 2

1. 60 trials of the hold-ramp-hold task with no stimulus.
2. Isometric task of approximately 6 minutes, with no stimulus.

   Paired-pulse test employed using ISI=1 ms and a threshold conditioning stimulus in steps 3 and 4 during:
3. 30 trials of the hold-ramp-hold task, with TMS applied during the hold 2 phase of the task.
4. Isometric task lasting approximately 3 minutes. TMS applied every 6 s (30 stimuli in total)
5. Steps 3 and 4 were then repeated using a subthreshold conditioning stimulus.

Note that the order of presentation of the different blocks of trials was sometimes changed, but the trials in which there was no TMS applied always preceded stimulus trials.

6.3 Data analysis

6.3.1 Inhibition measurements

All MEP measurements were made on 1DI muscle data only. This was because TMS intensities were chosen based on responses evoked in this muscle and EMG feedback was given from this muscle only.

MEPs were measured as described in chapter 2. Briefly, for each condition all 10-12 trials of rectified EMG data were superimposed. The area of interest (the duration of the MEP response) was defined and a single peak value was taken for each trial. Small MEPs were sometimes evoked (e.g. conditioning stimulus responses). In these cases, it was sometimes difficult to distinguish the MEP from the background EMG signal. Efforts were made to define the width of the MEP and thus the area in which to take the peak height from, as accurately as possible. Start and end times (or widths of small MEPs) were later checked by comparison to times used when a large MEP was evoked (in the test stimulus condition), and were found to be comparable.

For each condition, the mean of the peak values was then calculated with the standard error. Background EMG levels were measured by sampling 400 ms of rectified EMG data immediately before the stimulus. Average background EMG levels were calculated for each condition and were subtracted from all mean peak MEP values. This was to ensure that only a TMS evoked MEP was used in comparisons between conditions and tasks, and because it was evident that despite giving subjects feedback of 1DI muscle activation, there were sometimes still significant changes in background EMG level between hold-ramp-hold and isometric tasks.

To obtain an inhibition score for each subject (see correlation results) inhibition was measured by calculating the paired-pulse response as a % of the test stimulus response. In order to assess overall inhibition, mean MEP responses from each subject for each condition were pooled. For each task, mean test stimulus responses were compared to

paired-pulse responses. Inhibition was measured by calculating the mean paired-pulse response as a % of the mean test stimulus response.

6.3.2 Statistics for inhibition measurements

Significant differences between tasks, in mean MEP responses evoked by the test stimulus, and paired-pulse responses were measured using one tailed paired t-tests.

6.3.3 EMG-EMG coherence calculations

Details of the coherence analysis employed are presented in chapters 2 and 4. For the trials performed at the start of the experiments (note no TMS applied), FFTs of 128 points were used in an analysis involving 4 rectangular, non-overlapping windows of approximately 486 ms length each. For the hold-ramp-hold task, coherence analysis was performed on the last 2 s of EMG data in the hold 2 phase. For the isometric task, an equivalent section of EMG data was analysed approximately every 6 s in the data.

Muscle pair coherence values were normalized (see chapter 2). For each subject, normalized values were then combined, to give an 'all muscle pair' value. Normalized coherence was then summed between 14–31 Hz. Finally this value was multiplied by 2 Hz, which was the bin width, or resolution of the coherence spectra. This resulted in units of 'normalized coherence area'. Note that in the experiments using ISI=1 ms, due to technical reasons, it was not possible to include data from the FDS/EDC muscle pair (see appendix in section 2.6 of chapter 2).

6.3.4 Effects of the conditioning stimulus on oscillatory EMG activity

Details of the analysis used to measure effects of TMS in the frequency-domain have been explained in detail in chapters 2 and 4. Note that in order to assess stimulus-locking or coherence effects efficiently, approximately 60 trials of EMG data are required (this is in order to achieve an adequate signal to noise ratio). Only 10–15 trials of the

conditioning stimulus effects alone were recorded during trials in which inhibition was assessed. As effects on low intensity TMS on EMG oscillatory activity have been investigated extensively in chapter 4, it was not considered necessary to fully re-establish these effects in this study. However just to be sure the intensity chosen for the conditioning stimulus was correct, in 5 subjects, a block of 60 trials of the conditioning stimulus only was completed whilst subjects performed the isometric task. This was on the day when the ISI=2.5 ms experiments were performed. Stimulus-locking effects and effects on EMG-EMG coherence of the conditioning stimulus were then assessed in this task. An FFT of 128 was used on a single window of analysis 0.05 s following the stimulus. Chapter 4 includes details of the stimulus-locked power calculation and chapter 2 a summary of the coherence calculation. Note that EMG-EMG coherence present following the stimulus had to be compared to that present in trials in which no TMS had been applied. For this comparison, data from trials performed at the start of the experiment had to be reanalysed, this time using a single window of analysis. This test was carried out to show that TMS effects on EMG oscillatory activity do indeed also occur in the isometric task, as effects were only investigated in the hold-ramp-hold task in chapter 4.

6.3.5 Statistics for coherence analysis

Significant differences in values of coherence were tested with a one tailed paired t-test run across subjects. Regression analysis was performed with inhibition and coherence levels and significance tested with a 95 % confidence limit.

6.4 Results

6.4.1 14–31 Hz EMG-EMG coherence

All muscle pair coherence was measured between 14–31 Hz for each subject. Data was pooled for all 11 subjects and presented as mean coherence values. Figure 6.1A shows

14–31 Hz coherence present during each of the precision grip tasks in which ISI=2.5 ms paired-pulse experiments were subsequently performed. In addition coherence measurements were also made before ISI=1 ms paired-pulse experiments (Figure 6.1B). In both cases the level of 14–31 Hz coherence was significantly lower during the isometric task ($P<0.005$).

6.4.2 Stimulus-locking effects of the conditioning stimulus

It was possible to choose with confidence a stimulus-locking conditioning stimulus intensity based on studies described in chapter 4. However, just to be sure that the appropriate intensities were chosen, in 5 subjects, an extra block of trials were performed with subjects performing the isometric task (on the day when the ISI=2.5 ms experiments took place). In 4 out of the 5 subjects tested, stimulus-locking occurred in both the 4–12 Hz and 14–31 Hz ranges. In the final subject, stimulus-locking occurred in the 4–12 Hz range only.

6.4.3 Effects of the conditioning stimulus on EMG-EMG coherence

In the same 5 subjects, the effect of the conditioning stimulus on EMG-EMG coherence during the isometric task was also measured. EMG-EMG coherence present during the isometric task performed at the start of the experiment (i.e. trials in which no TMS had been applied) was used as a comparison. When data from all subjects was pooled (n=5), 14–31 Hz coherence present during conditioning stimulus trials of the isometric task (176 ± 26 Hz) was significantly higher ($P=0.01$) than that during the trials with no TMS, performed at the start of the experiment (119 ± 14 Hz).

To summarise, it was shown that conditioning stimulus intensities used in the inhibition experiments using ISI=2.5 ms (and half of the experiments using ISI=1 ms) had both stimulus-locking effects, and increased EMG-EMG coherence.

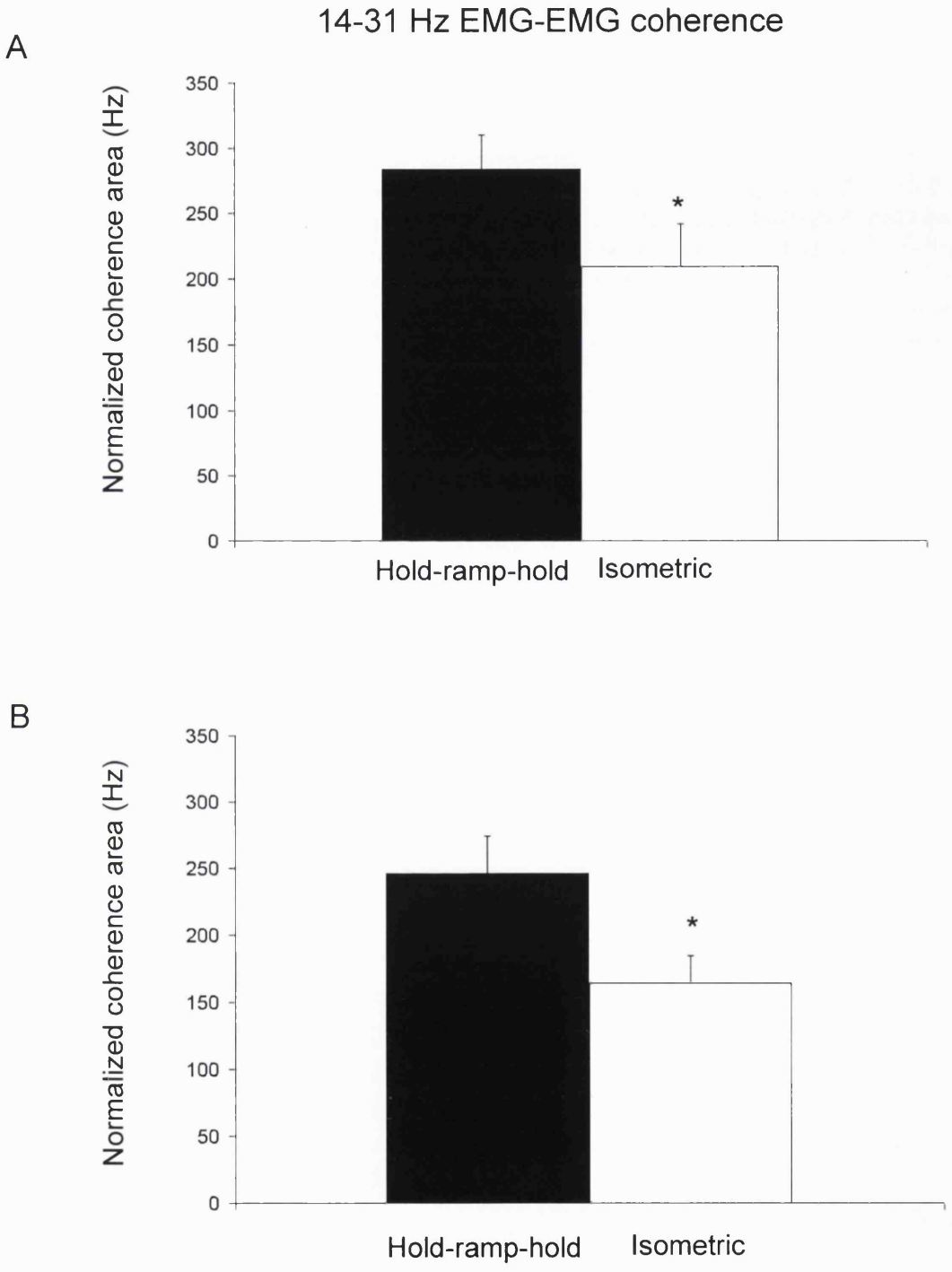


Figure 6.1: 14-31 Hz EMG-EMG coherence present during precision grip tasks. EMG-EMG coherence was measured during blocks of 'no stimulus' trials performed before (A) ISI=2.5 ms (B) or ISI=1 ms was used in the paired-pulse test. In both cases, there was significantly less 14-31 Hz coherence present in the isometric task (open bars) when compared to coherence present in the hold-ramp-hold task (black bars; $P<0.005$).

6.4.4 Intracortical inhibition measured using ISI=2.5 ms

Figure 6.2 shows data from paired-pulse experiments using ISI=2.5 ms. The conditioning stimulus was set at an intensity sufficient to elicit effects on EMG oscillatory activity. Data are pooled from 11 subjects. In both the hold-ramp-hold task and the isometric task the MEP response elicited by the paired-pulse was significantly lower than the test stimulus response ($P<0.001$). For the hold-ramp-hold task, the paired-pulse response was 30 % of the test stimulus response. In the isometric task, the paired-pulse response was 22 % of the test stimulus response.

The test stimulus responses evoked in the two tasks were not significantly different. The paired-pulse responses were however, different. The paired-pulse response during the isometric task (0.43 ± 0.06 mV) was significantly lower than that during the hold-ramp-hold task (0.55 ± 0.09 mV; $P<0.05$). This indicates that the intracortical inhibition was stronger during the isometric task in this part of the study.

Background EMG levels for the 2 tasks were significantly different (isometric 0.058 ± 0.006 mV; hold-ramp-hold 0.044 ± 0.006 mV; $P<0.0001$), however note that there were no differences in the size of test responses. In addition the paired-pulse response for the isometric task was smaller than that evoked during the hold-ramp-hold task, rather than larger as would be predicted by the higher background EMG level.

6.4.5 Inhibition measured using ISI=2.5 ms and 14–31 Hz EMG-EMG coherence

Significant differences were evident in both EMG-EMG coherence and intracortical inhibition (measured using ISI=2.5 ms) between the two tasks. Therefore, for each subject, coherence levels measured during trials performed at the start of the experiment (no TMS applied) were correlated with inhibition (paired-pulse response given as a % of the test stimulus response; Figure 6.3). Note that the lower the percentage of test stimulus response, the higher the inhibition.

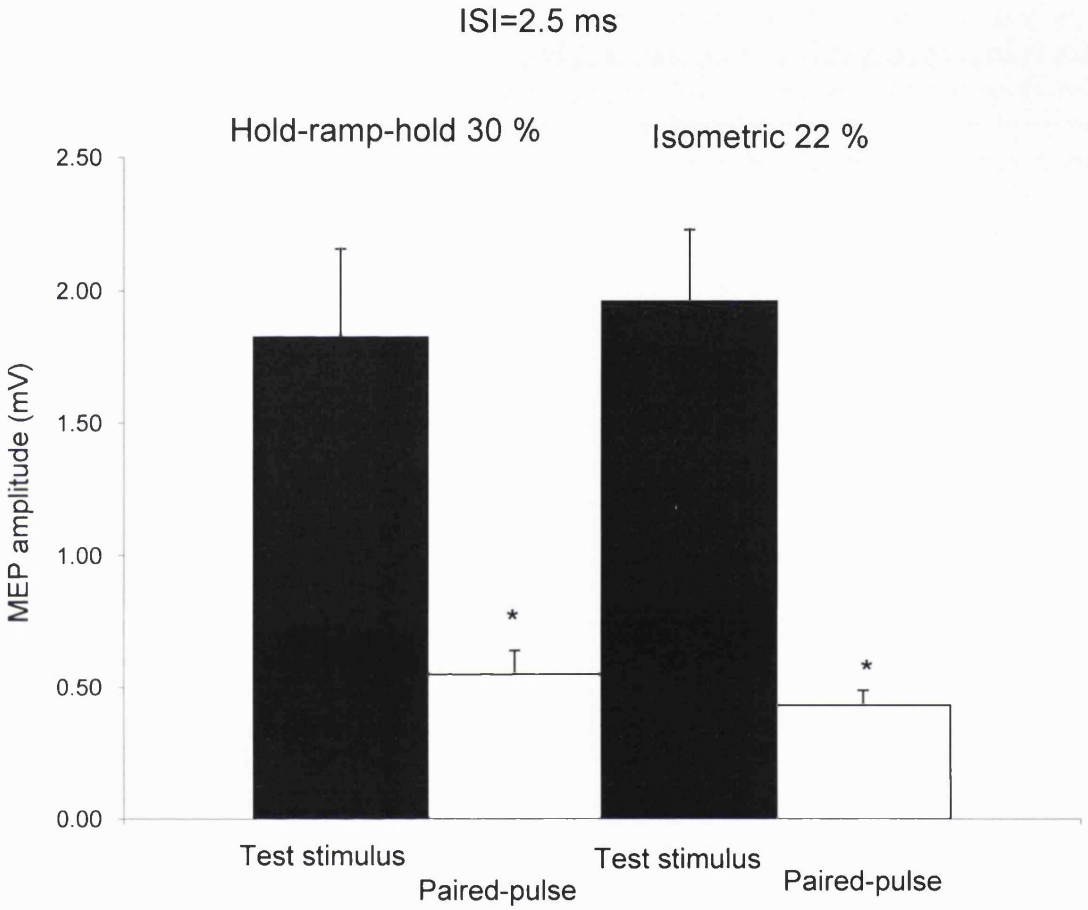


Figure 6.2: Intracortical inhibition measured with ISI=2.5 ms during precision grip tasks. Data from all 11 subjects has been pooled. In both the hold-ramp-hold and isometric task, the paired-pulse response (open bars) was significantly smaller than the response evoked by the test stimulus alone (black bars; $P<0.001$). Inhibition was measured by calculating the average MEP evoked by the paired-pulse as a percentage of the average test stimulus response (figures above graph). Note that a lower percentage indicates a higher level of inhibition. The average paired-pulse response in the isometric task was significantly lower than that evoked in the hold-ramp-hold task ($P<0.05$). This means that the inhibition measured during the isometric task (22 % test response) was significantly higher than that in the hold-ramp-hold task (30 % test response).

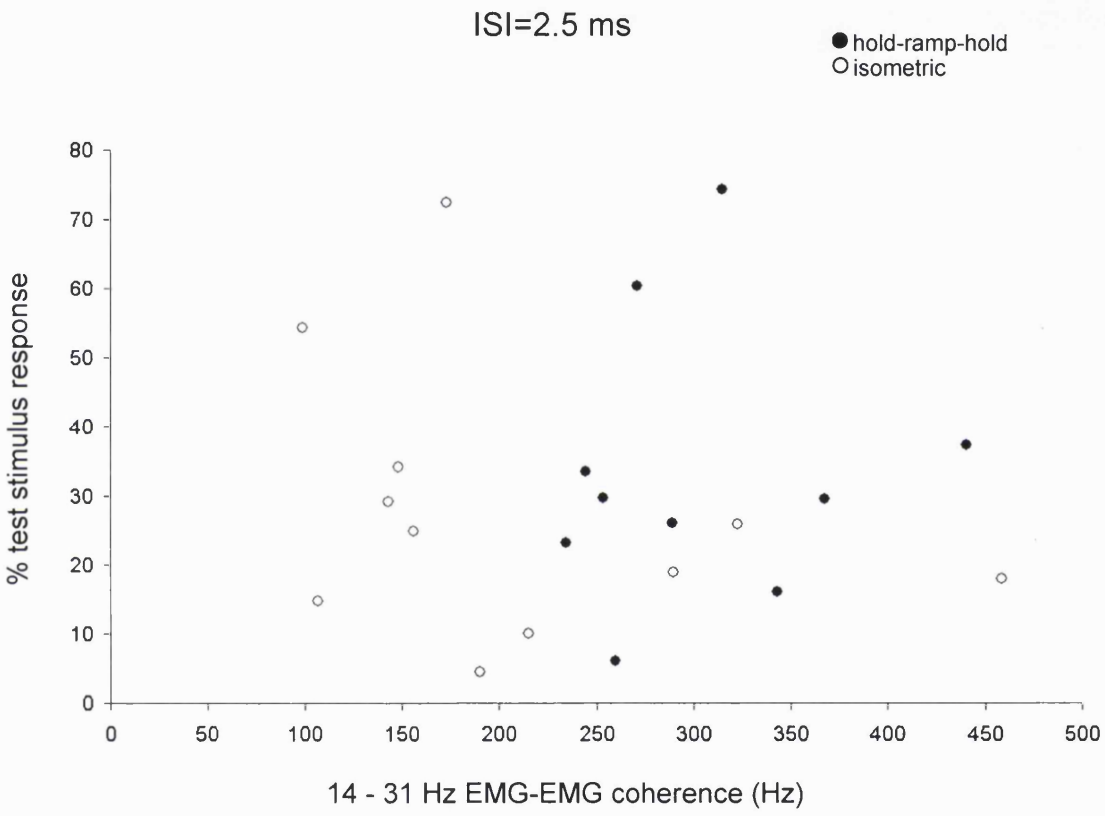


Figure 6.3: Correlation between 14-31 Hz EMG-EMG coherence and inhibition measured during precision grip tasks. Data from all 11 subjects is shown. Inhibition was measured using ISI=2.5 ms, and is presented as % test stimulus response. Note that the lower the percentage, the higher the inhibition. For both the hold-ramp-hold task (black circles) and isometric task (open circles), there was no correlation between the level of coherence measured at the start of the experiment, and the amount of inhibition measured later on.

For neither task was there a correlation between the coherence level measured at the start of the experiment, and the level of intracortical inhibition measured using ISI=2.5 ms later on.

6.4.6 Inhibition measured using ISI=1 ms

The objective of these experiments was to see if a similar result to that described above could be obtained with ISI=1 ms. In addition it was interesting to see if a lower (subthreshold) stimulus would be more effective (chapter 3). Note that tests on stimulus-locking effects were only done on the higher (threshold) conditioning stimulus (and on days when ISI=2.5 ms experiments took place). When choosing the lower conditioning stimulus intensity it was ensured that no effects on ongoing EMG oscillatory activity were evident in the time-domain.

Figure 6.4 shows results when a threshold conditioning stimulus was used with ISI=1 ms (data pooled from all 11 subjects). Again there was a significant reduction in MEP size when comparing the paired-pulse responses, to the test responses (P=0.01). This reduction was observed during both precision grip tasks. For the hold-ramp-hold task, the paired-pulse response was 73 % of the test stimulus response. In the isometric task, the paired-pulse response was 65 % of the test stimulus response. Thus inhibition occurred in both tasks. However, despite using a similar conditioning stimulus intensity, much less inhibition was measured when using ISI =1 ms, than was measured when ISI=2.5 ms was used (ISI=2.5 ms: hold-ramp-hold 30 %, isometric 22 %).

Note that the test stimulus response for the isometric task (1.96 ± 0.23 mV) was significantly higher than that evoked in the hold-ramp-hold task (1.55 ± 0.24 mV; $P<0.05$) for this set of experiments. The background EMG levels were not different (hold-ramp-hold task 0.036 ± 0.03 mV; isometric task 0.037 ± 0.03 mV).

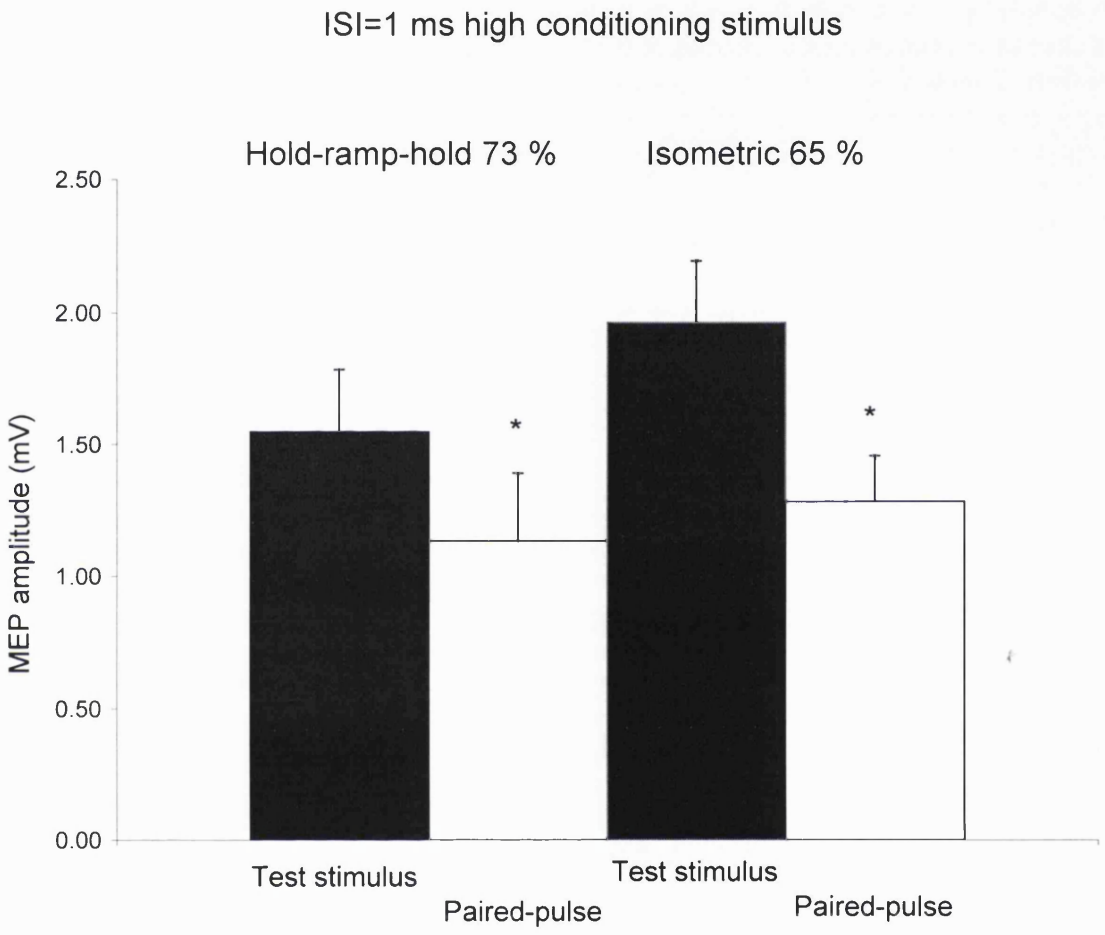


Figure 6.4: Intracortical inhibition measured with ISI=1 ms during precision grip tasks. Data from all 11 subjects has been pooled. A conditioning stimulus similar to that used in ISI=2.5 ms experiments was used. In both the hold-ramp-hold and isometric task, the paired-pulse response (open bars) was significantly smaller than the response evoked by the test stimulus alone (black bars; P=0.01). Inhibition was measured by calculating the average MEP evoked by the paired-pulse as a percentage of the average test stimulus response (figures above graph). Note that a lower percentage indicates a higher level of inhibition. There was no difference between the average paired-pulse response evoked in each task and thus no difference in inhibition. Note that the test stimulus response was significantly higher in the isometric task (P<0.05).

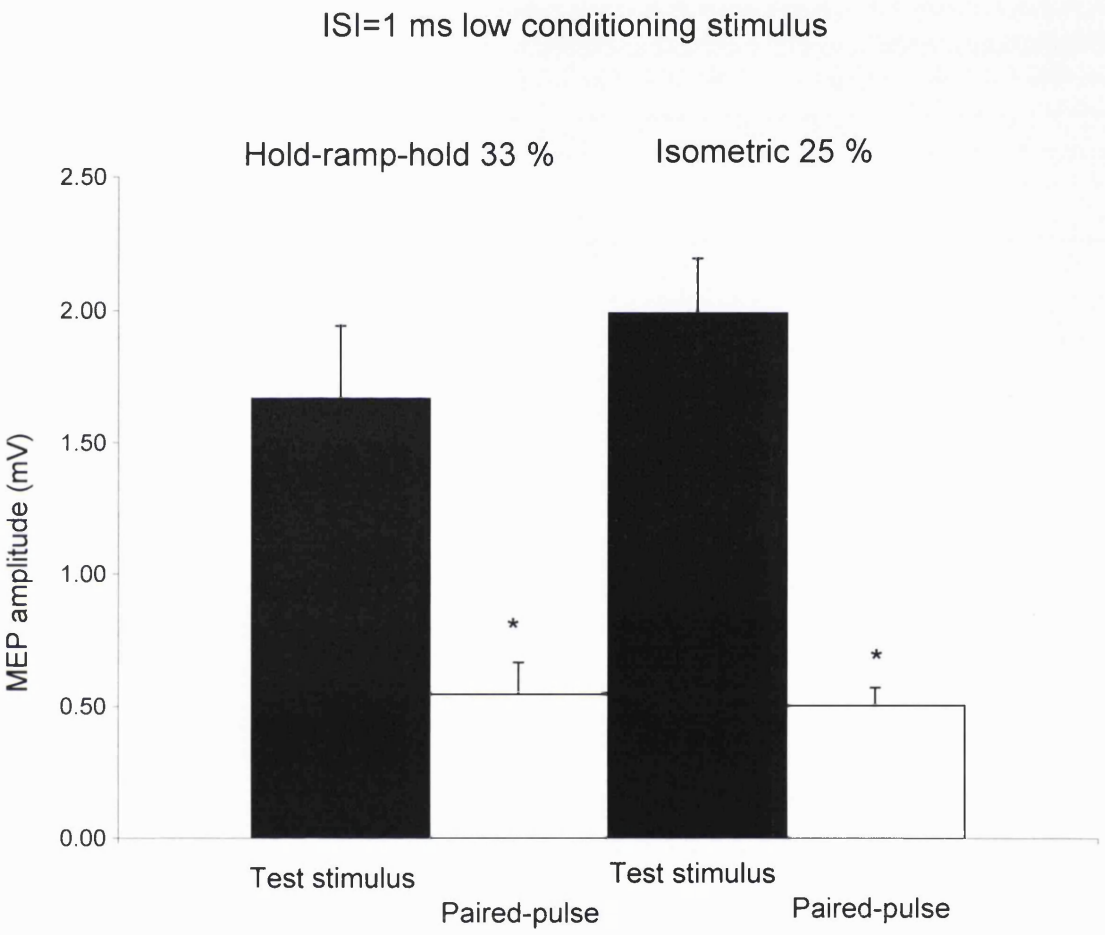


Figure 6.5: Intracortical inhibition measured with ISI=1 ms during precision grip tasks. Data from all 11 subjects has been pooled. A subthreshold intensity of conditioning stimulus was used. In both the hold-ramp-hold and isometric task, the paired-pulse response (open bars) was significanlty smaller than the response evoked by the test stimulus alone (black bars; $P<0.001$). Inhibition was measured by calculating the average MEP evoked by the paired-pulse as a percentage of the average test stimulus response (figures above graph). Note that a lower percentage indicates a higher inhibition. There was no difference between the average paired-pulse responses evoked in each task and thus no difference in inhibition. There was also no difference in test stimulus responses between tasks.

This could have resulted in a significantly higher level of inhibition being measured for the isometric task (larger test stimulus with which to compare the paired-pulse response). However, the paired-pulse response for the hold-ramp-hold task (1.13 ± 0.26 mV) was not different from the paired-pulse response in the isometric task (1.28 ± 0.17 mV). There was therefore no significant difference between levels of inhibition for the two precision grip tasks, when ISI=1 ms was used with the higher conditioning stimulus.

Experiments were then repeated (ISI=1 ms) using a subthreshold conditioning stimulus intensity. Figure 6.5 shows MEP responses evoked in both the hold-ramp-hold and the isometric tasks. There was significant inhibition of the test response in both cases ($P<0.001$). For the hold-ramp-hold task, the paired-pulse response was 33 % of the test stimulus response. In the isometric task, the paired-pulse response was 25 % of the test stimulus response. Note that inhibition measured using ISI=1 ms and a lower conditioning stimulus was much stronger than when the higher conditioning stimulus was used. Also inhibition levels were comparable to those measured using ISI=2.5 ms.

Despite there being a significant difference in background EMG levels (hold-ramp-hold 0.040 ± 0.004 mV; isometric 0.037 ± 0.003 mV; $P<0.05$), there was no significant difference between test stimulus responses. There was also no significant difference between paired-pulse responses between the two tasks, and thus no difference in the level of inhibition measured.

6.5 Discussion

The main results of the study were as follows: there was significantly more 14–31 Hz coherence present in the hold 2 phase of the hold-ramp-hold task, than during the isometric task. When a conditioning stimulus that had both stimulus-locking and coherence enhancing effects was used with ISI=2.5 ms in the paired-pulse test, significant inhibition could be measured in both precision grip tasks. This was also true when using

ISI=1 ms, however a lower conditioning stimulus was shown to be more optimal. Did inhibition differ between tasks? Using ISI=2.5 ms significantly more inhibition was measured during the isometric task. Using an ISI=1 ms there were no significant differences in inhibition between tasks (regardless of the conditioning stimulus intensity used). It should be pointed out that the difference in inhibition levels between tasks (when optimal conditioning stimulus intensities were used) in percentage terms was the same for both ISIs (i.e. 8). (Paired-pulse response given as % test response: ISI=2.5 ms hold-ramp-hold 30 %, isometric 22 %; ISI=1 ms hold-ramp-hold 33 %, isometric 25 %). However, in the trials when ISI=2.5 ms was used, the difference in paired-pulse responses between tasks just reached significance (P=0.0429) unlike trials in which ISI=1 ms was used (P=0.3204). (For both ISIs the test stimulus responses were not different between tasks).

It should be noted that coherence was not measured simultaneously with inhibition. Similar to the argument in chapter 5, it was not possible to obtain accurate measurements of each parameter during the same set of trials. In designing this study it was assumed that the coherence changes observed between tasks in the trials performed at the start of the experiment, were present during similar trials performed later on, in which TMS was applied. However, this may not have been the case, as will be discussed shortly.

6.5.1 Threshold differences in inhibition

When comparing results from this study and those from chapter 3, it is important to note that again two phases of inhibition could be measured. In this study, inhibition could be measured with ISI=2.5 ms when a conditioning stimulus of threshold intensity was used. However, when the same intensity of stimulus was used with ISI=1 ms, inhibition measured was much less. The optimal conditioning stimulus intensity to use to measure inhibition with ISI=1 ms was one that was lower (subthreshold intensity). This supports

the suggestion that different inhibitory mechanisms are measured, when using these two ISIs in the paired-pulse test (chapter 3).

6.5.2 Effects of voluntary activity

In chapter 3, voluntary activity suppressed almost all inhibition measured using ISI=2.5 ms. It is perhaps surprising then that it was possible to measure inhibition using ISI=2.5 ms in this study. As inhibition at rest was not measured also it is difficult to be sure if, or by how much, inhibition was reduced. However, the fact that it was not abolished (as was the case in chapter 3), is most likely to be due to the different methods of measuring inhibition used in these studies. In chapter 3 MEP responses were kept constant at a threshold level of 0.2 mV and inhibition was measured as the increase in test stimulus required to elicit the target MEP response in the paired-pulse condition. Whereas inhibition had a large window in which to be measured when at rest, during voluntary activity the test stimulus required to elicit an MEP of 0.2 mV was lower and scope for measuring inhibition reduced. In this study all inhibition measurements were made with subjects performing a precision grip task, and so the conventional paired-pulse test (inhibition measured as a reduction in MEP amplitude), was considered a more suitable method of measuring inhibition.

6.5.3 Inhibitory effects using ISI=2.5 ms

Chapter 3 concluded that intracortical inhibition mediated by GABAergic pathways could be measured using the paired-pulse test with ISI=2.5 ms. Low intensity TMS (similar to intensities used for conditioning stimuli in the paired-pulse test using ISI=2.5 ms) was then shown to enhance EMG-EMG coherence in chapter 4. This suggested that GABAergic inhibitory pathways activated by low intensity TMS were involved in maintaining coherence between muscle pair EMG activity. That strong intracortical inhibition was measured in the present study during the hold-ramp-hold task, in which

there was strong EMG-EMG coherence, supports this claim. A prediction made for this study was that higher levels of intracortical inhibition would be measured during the task in which there was more EMG-EMG coherence. Why then, was inhibition measured using ISI=2.5 ms not lower during the isometric task? (during which there were lower levels of EMG-EMG coherence). The simplest explanation is that lower levels of cortical inhibition are present during tasks involving *more* synchronous cortico-muscular oscillatory activity. This agrees with the observation of reduced short-latency intracortical inhibition (measured using ISIs 1–5 ms) in patients with epilepsy (Caramia et al. 1996; Brown et al. 1996). However, epilepsy is a pathological condition of pronounced synchronous cortical activity, thought to be due to an imbalance between inhibitory and excitatory mechanisms, with *increased excitability* favouring epileptic activity (Werhahn et al. 2000). In addition, associating lower levels of cortical inhibition with cortical synchrony contradicts numerous theories of the important role of inhibition in the maintenance and control of cortical oscillatory activity (Lytton and Sejnowski 1991; Pauluis et al. 1999; Whittington et al. 2001). In fact synchrony within the cortex of normal subjects has been associated with a reduction in cortical excitability (Chen et al. 1998; Chen et al. 1999).

It is interesting to note that reports of significant increases in short-latency intracortical inhibition, reflecting an increase in ongoing inhibitory activity within the cortex (as predicted for the hold-ramp-hold task) are rare. Using GABA potentiating compounds to investigate intracortical pathways, it has often been the case that a significant reduction in short-latency facilitation (measured using pairs of suprathreshold stimuli separated by intervals of 1–5 ms) can be observed, but only a trend in enhancement of intracortical inhibition (Ziemann et al. 1996b; 1998a; Di Lazzarro et al. 2000). Instead suppression of short-latency intracortical inhibition is a common finding when testing cortical excitability of patients with movement disorders (Hanjima and Ugawa 2000).

Does this mean that the paired-pulse test used in this study was not the most suitable measurement of cortical inhibition? It may have been informative to include measurements of cortical facilitation. In addition there is a wide diversity of GABAergic interneurones in the cortex (Thomson and Deuchars 1994; Gupta et al. 2000) and it is certainly possible that other GABA mediated inhibitory mechanisms may be also in operation during synchronous cortico-muscular activity. Interestingly, in patients with Parkinson's disease and dystonia, whereas short-latency inhibition was found to be similar to levels measured in normal subjects, measuring long-latency inhibition indicated that there was increased cortical inhibition in these patients (Berardelli et al. 1996; Rona et al. 1998). Long-latency inhibition, measured with suprathreshold stimulus pairs separated by intervals of around 100 ms, is likely to be mediated by a different class of GABA interneurone ($GABA_B$) than short-latency inhibition ($GABA_A$) (Sanger et al. 2001). Supporting the suggestion that in fact other GABAergic pathways may be involved in the generation of coherent EMG activity, is the fact that there was no significant correlation between EMG-EMG coherence, and intracortical inhibition measured with ISI=2.5 ms in the short-latency paired-pulse test in this study.

6.5.4 Inhibition measured with ISI=1 ms

What is interesting with regard to inhibition experiments using ISI=1 ms, is that *lower* conditioning stimulus intensities (than those used for ISI=2.5 ms experiments) elicited *stronger* inhibition. It was considered beyond the realm of this study to check the effects of subthreshold stimulus intensities on EMG oscillatory activity, however it was assumed there would be no stimulus-locking effects (chapter 4). Effects on coherence are a little more difficult to interpret as there was evidence of an enhancement in coherence following subthreshold TMS. Nevertheless, if it is accepted that subthreshold TMS has weaker effects on EMG oscillatory activity, but stronger inhibitory effects, it could be argued that, for inhibition measured using ISI=1 ms, the two phenomena have different

mechanisms. Chapter 3 suggested that the lower threshold of inhibition was due to the fact that the paired-pulse test using ISI=1 ms measures predominantly axonal effects of the conditioning stimulus. Inhibition is caused by asynchronous activation of PTNs by the test stimulus, due to refractory mechanisms (rather than GABAergic synaptic pathways). This may explain the lack of significant modulation of inhibition measured using ISI=1 ms between tasks in this study despite clear differences in levels of muscle pair coherence.

6.5.5 Effects of the conditioning stimulus

There is however, a further consideration to make. What were the effects of the conditioning stimulus itself? It is possible that the conditioning stimulus activated the same proportion of inhibitory interneurones (same intensity, same position of coil) during each task. If we accept the suggestion that these same inhibitory interneurones are involved in the generation of EMG-EMG coherence (as suggested in chapter 4) then the conditioning stimulus may have temporarily altered coherence differences between tasks. Indeed in this study low intensity TMS enhanced the area of coherent activity between 14–31 Hz in the isometric task. This effect could well have been reflected in the paired-pulse responses and may therefore have affected measurements of inhibition.

6.5.6 Conclusions

In this study, short-latency intracortical inhibition measured by the paired-pulse test with either ISI=1 ms or ISI=2.5 ms, could not be directly correlated with levels of EMG-EMG coherence. This was despite strong intracortical inhibition being present in both precision grip tasks, and some evidence that more inhibition was present in the isometric task during which less muscle pair coherence was observed. It is concluded that additional tests of cortical inhibition might be necessary to measure subtle changes in the excitability of inhibitory pathways involved in the maintenance of coherent EMG activity.

It is also important to consider the effects of the conditioning stimulus when assessing changes in cortico-muscular synchrony, and this will be addressed in chapter 8.

CHAPTER 7: DIGITAL NERVE ANAESTHESIA DECREASES EMG-EMG COHERENCE

7.1 Introduction

It is now widely accepted that EMG oscillations in the 15–30 Hz range recorded during a precision grip task, are at least in part, driven from central structures, including the primary motor cortex. This conclusion was based on recordings of motor cortex oscillations in the 15–30 Hz range in monkeys (Murthy and Fetz 1992; Sanes and Donoghue 1993; Baker et al. 1997) and measurements in humans of coherence between cortical signals and EMG activity (Conway et al. 1995; Mima et al. 2000). A cortical origin was therefore proposed for MEG-EMG and EMG-EMG coherence observed during hold phases of a hold-ramp-hold precision grip task (Baker et al. 1997; Kilner et al. 1999). Chapter 4 presented data that supports the suggestion that the corticospinal tract is a strong candidate for transmitting oscillatory activity from the cortex to muscle (Baker et al. 1999; Gross et al. 2000).

It is known that 20 Hz EEG beta synchronization is abolished during movements and rebounds after movement (Pfurtscheller et al. 1996). That EMG-EMG coherence is abolished during the ramp phase of the hold-ramp-hold task is therefore not surprising (Kilner et al. 1999; 2000). However, the interesting point is that both MEG-EMG and EMG-EMG coherence during the hold 2 phase of the hold-ramp-hold task *increased* with increasing *compliance* of the levers used to perform the task (Kilner et al. 2000). It was concluded that levels of coherence in the hold phases of this task might reflect important changes in motor state encompassing alterations in grip force and digit position, both of which are critical for grasp of a compliant or springy object (Kilner et al. 2000).

Cutaneous input from the digits is an important source of sensory events controlling precision grip, and provides essential information about object compliance (Johansson

1996). Thus it was of interest to investigate whether interrupting these inputs would affect the coherence between hand muscles during precision grip of a compliant object. Although such coherence has been attributed for the most part to central mechanisms (Salenius et al. 1997a; Hari and Salenius, 1999; Mima et al. 2000), it is known to be influenced by somatosensory input. For example, it has been shown that median nerve stimulation provoked an immediate reduction, then an increase or rebound in the amplitude of sensorimotor 10 Hz and 20 Hz mu rhythms (Salmelin and Hari 1994; Salenius et al. 1997b). This suggests that a surge of afferent input could increase oscillatory activity within the cortex. The present study focussed on changes in the ongoing level of EMG-EMG coherence that occur after a movement into a new steady hold period, since this transition is accompanied by a particularly marked increase in coherence that might involve sensory feedback from the hand.

Digital nerve anaesthesia has been previously used to perturb peripheral feedback and caused a loss of proprioceptive acuity (Refshauge et al. 1998). It was therefore chosen for this study as a suitable tool to investigate the effects of blocking cutaneous afferent input on behaviour and EMG-EMG coherence during a precision grip task. To date the only other study to my knowledge that has used anaesthesia to investigate changes in oscillatory EMG activity used ischaemic anaesthesia (McAuley et al. 1997). During ischaemia it was reported that there was no change in EMG power spectra or EMG-tremor coherence when subjects performed abduction of the 1DI muscle.

7.2 Methods

7.2.1 Subjects

Ten healthy human subjects (6 male and 4 female), all right-handed and aged between 20 and 55 years, were recruited for these experiments. All gave informed consent. The project was approved by the Joint Medical Ethics Committee of the National Hospital for

Neurology and Neurosurgery and the Institute of Neurology and was carried out in accordance with the Declaration of Helsinki.

7.2.2 Behavioural task

Details of the hold-ramp-hold task used in this study are given in chapter 2. A single spring constant of 0.05 N/mm was used. In addition to the original set-up, a screen was positioned to block the subject's view of their hand. This was to ensure that subjects did not try to compensate for lack of proprioception by using visual feedback of their hand. Index finger and thumb lever positions were recorded using optical encoders (resolution of ~ 33 counts per mm movement of the lever tip).

7.2.3 EMG recordings

Bipolar surface EMG recordings were taken for the first dorsal interosseous (1DI), abductor pollicis brevis (AbPB), flexor digitorum superficialis (FDS) and extensor digitorum communis (EDC) muscles. EMGs were amplified (x 1000), high-pass filtered (30 Hz), and sampled at 5000 Hz by a PC-compatible computer attached to a 1401+ interface (Spike 2 CED Ltd. Cambridge, UK).

7.2.4 Digital anaesthesia

Local ring block anaesthesia of the thumb and index finger was performed by the injection of 1 ml of Lignocaine hydrochloride (1% w/v; Antigen Pharmaceuticals) into each side of the base of the proximal phalanx of each digit (2 ml per digit). Prior to the induction of digital anaesthesia the threshold of sensation to electrical stimuli of the tips of the index finger and thumb was determined. For this small surface electrodes were firmly attached to the lateral borders of the two digits along the path of the digital nerves. Electrical stimulation (Digitimer stimulator DS7) was delivered at a randomised rate

(0.2–0.7 mA, 100 μs pulse width), and subjects were asked to report the occurrence of all detectable stimuli. The level of local anaesthesia induced by lignocaine was tested clinically using light touch stimuli, as well as by the threshold to electrical stimulation applied to the digit tips.

7.2.5 Protocol

Subjects performed at least 65 trials prior to ('pre-anaesthesia' trials) and after the induction of digital anaesthesia ('anaesthesia' trials). Anaesthesia trials were started as soon as there was a complete loss of light touch sensation from the digit tips and/or a doubling of the threshold to electrical stimulation. Subjects performed a further 65 trials approximately 2 hours later once the anaesthesia had worn off ('washout' trials), as determined by the return of light touch sensation and a return of the threshold to electrical stimulation to baseline levels.

7.3 Data Analysis

Thumb and index finger position signals during each of the testing phases were examined off-line. Trials in which subjects had failed to follow the target boxes sufficiently for at least part of the trial or had made large movements during the hold 2 phase of the task, were excluded. This applied mainly to the anaesthesia experimental block. In order to have equal trial numbers for each condition, the first 60 trials from each experimental block were then used for analysis.

7.3.1 Behavioural analysis

Index finger and thumb velocities were calculated by differentiating the recorded lever position signals. The velocity profile for each trial was then smoothed (using a 50 point Hanning window), before rectification. The average rectified velocity during a 2 s period in each of the 3 phases of the task (hold 1, ramp and hold 2) for each trial was calculated

(last 2 s of the 3 s hold phases used). These mean velocity values were then averaged over all 60 trials for each phase of the task.

7.3.2 Statistics

Significant differences in velocity of index finger or thumb between pre-anaesthesia and anaesthesia trials, and between pre-anaesthesia and washout trials, were tested with a one tailed paired t-test run across subjects.

7.3.3 Frequency-domain analysis

Details of the coherence calculation used in this study are given in chapter 2. Briefly, EMG signals were rectified and down-sampled to an effective sampling rate of 263 Hz. Power spectra for 1DI and AbPB muscles, and coherence between muscle pairs, were calculated. Fast Fourier Transforms (FFTs) of the EMG data were performed using 4 x 128 point non-overlapping rectangular windows (2 Hz resolution). For the coherence analysis the first window began 1s into the hold 2 period of the hold-ramp-hold task (same as the analysis performed in chapters 5 and 7). For the power analysis, the same 4 windows were used.

For the 1DI/AbPB muscle pair, coherence values between 14–31 Hz were summed for each subject and then multiplied by the bin width (2 Hz) to calculate the 'frequency area' of coherent activity. In order to calculate an overall coherence value from all muscle pairs of each subject, coherence values for muscle pairs had to be normalized using a transformation calculation (chapter 2). For a given subject, transformed muscle pair coherence values at each frequency were then combined and then values for each subject were summed between 14–31 Hz. This value was then multiplied by the frequency bin

width (2 Hz) to obtain the 'normalized coherence area' for 'all muscle pairs' in that subject.

For technical reasons, the muscle pair FDS/EDC was excluded from the analysis of 'all muscle pair' coherence.

7.3.4 Statistics

Significant differences in coherence values between pre-anaesthesia and anaesthesia trials and anaesthesia and washout trials were tested with a one tailed paired t-test run across subjects. Regression analysis was performed with velocity and coherence values and significance was tested with 95 % confidence limits.

7.4 Results

7.4.1 Behaviour

In most subjects, lignocaine injections resulted in the abolition of light touch for approximately 90 minutes. During this period of local digital anaesthesia subjects could still perform the hold-ramp-hold task adequately, but the performance was severely degraded. A selection of 15 lever position traces (from a total of 60 per trial) for two typical subjects (DW and RL) were superimposed for index finger and thumb (Figure 7.1). Performance during anaesthesia (Figure 7.1B and 7.1D) was less accurate and more variable than that achieved pre-anaesthesia (Figure 7.1A and 7.1C). Note that some subjects found the hold 1 phase of the task particularly difficult following anaesthesia (e.g. RL).

Figure 7.2 shows an example of the hand position used by a typical subject to grasp the levers. In anaesthesia trials many subjects changed the position of their thumb, and rotated it so that more of the palmar surface was in contact with the levers. There was also a tendency to curve the index finger round the lever in efforts to squeeze it.

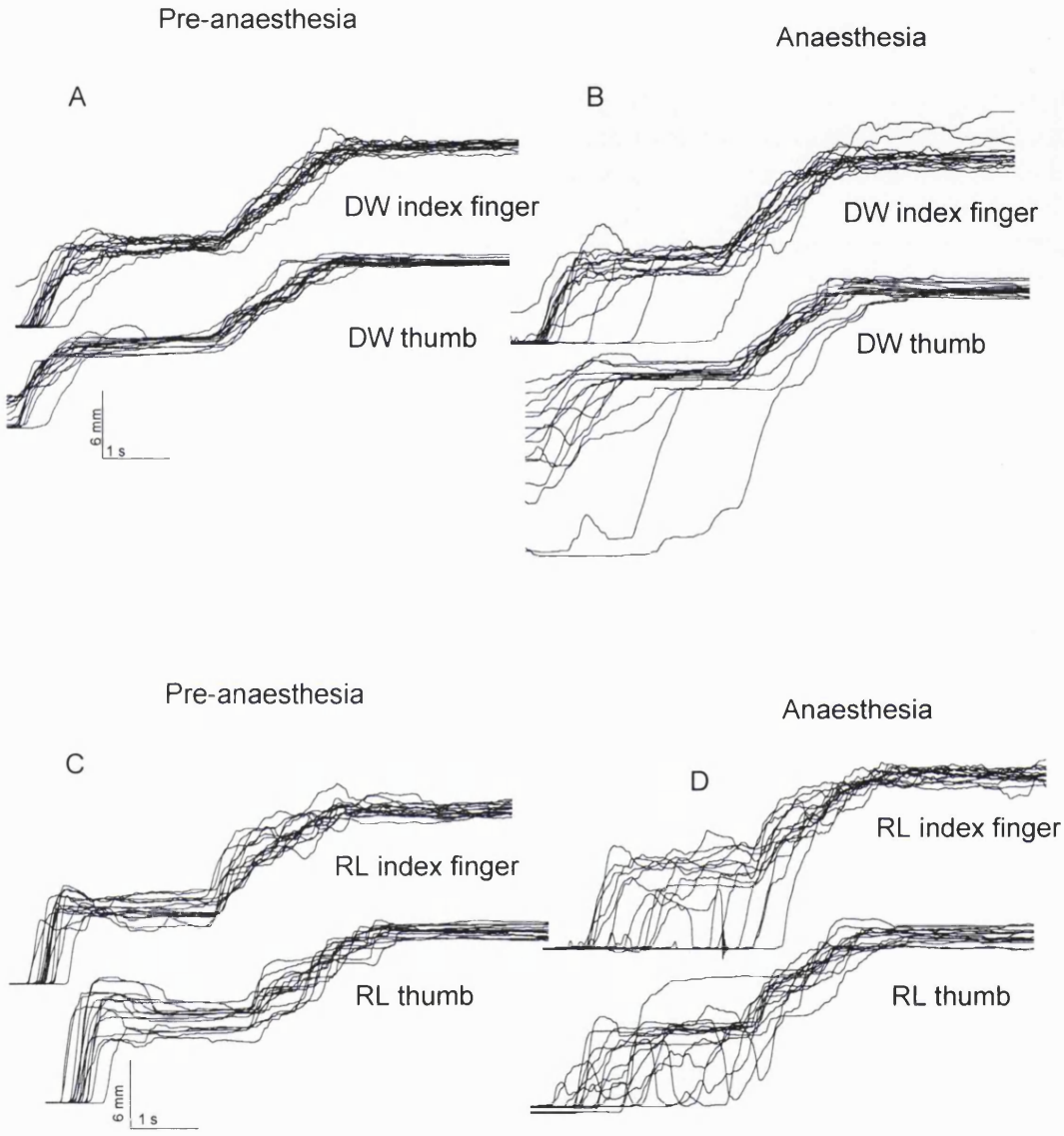
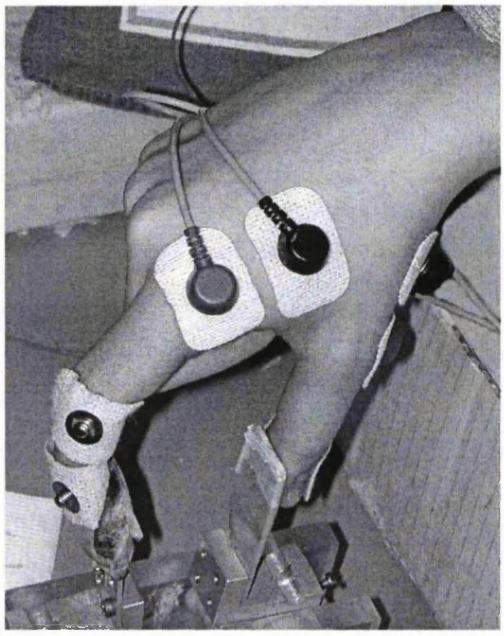


Figure 7.1: Lever position profiles for index finger and thumb before (A,C) and after (B,D) digital anaesthesia. Data from 2 typical subjects DW (A,B) and RL (C,D) are presented. Both subjects show a marked reduction in accuracy in performing the hold-ramp-hold task during anaesthesia. Subject RL was typical of some subjects who found it particularly difficult to achieve steady grip of the levers in the hold 1 phase during anaesthesia. There were however other subjects like DW, who were more able to keep on target during this part of the task.

Pre-anaesthesia



Anaesthesia

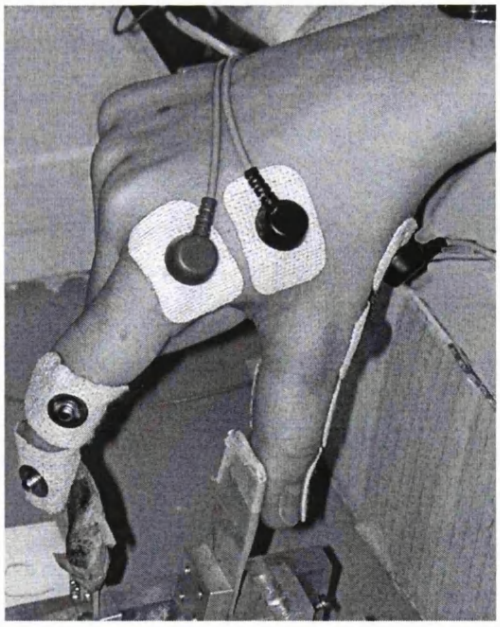


Figure 7.2: Hand position during the precision grip hold-ramp-hold task. Notice that during digital nerve anaesthesia, this subject rotated the thumb so that more of the palmar surface would be in contact with the lever. In addition the index finger was more curved around the lever and both digits tended to drift to the very edge of the levers.

Many subject's digits tended to drift to the very edge of the levers, and in some cases subjects lost the grip of the levers. Subjects were unaware this was the case until they noticed that the lever position indicators on the computer screen failed to respond to their grip command. In such instances digits were replaced in contact with the levers by the experimenter, and that trial was later excluded from analysis.

7.4.2 Digit movement velocity

In order to quantify the behavioural changes induced by digital nerve anaesthesia, absolute mean velocity of both digits during hold 1, ramp and hold 2 phases of the task were calculated. There was a significant increase in index finger velocity when comparing anaesthesia to pre-anaesthesia trials, during all phases of the task (hold 1 and ramp phases $P<0.01$; hold 2 phase $P<0.05$; Figure 7.3A). Thumb velocity was significantly increased during the hold phases, but not the ramp phase of the task (hold 1 phase $P<0.01$; hold 2 phase $P<0.05$; Figure 7.3B). Velocity increases following anaesthesia during hold 1 were larger than in other phases, again showing that some subjects found it difficult to perform this part of the task.

7.4.3 Power spectra

Before studying the effects of digital nerve anaesthesia on coherence between muscle pairs, it was important to see first whether anaesthesia affected ongoing EMG oscillatory activity in single muscles. This analysis was performed on the two intrinsic hand muscles. Power spectra for 1DI (Figure 7.4A) and AbPB (Figure 7.4B) muscles pre-anaesthesia and during anaesthesia of two representative subjects are presented. Power was measured for EMG data in the last 2 s of the hold 2 phase of the task. Peaks in the power spectra were determined by eye and are indicated with arrows. Digital nerve anaesthesia did not affect the frequency at which peaks in the power spectra occurred for these two subjects.

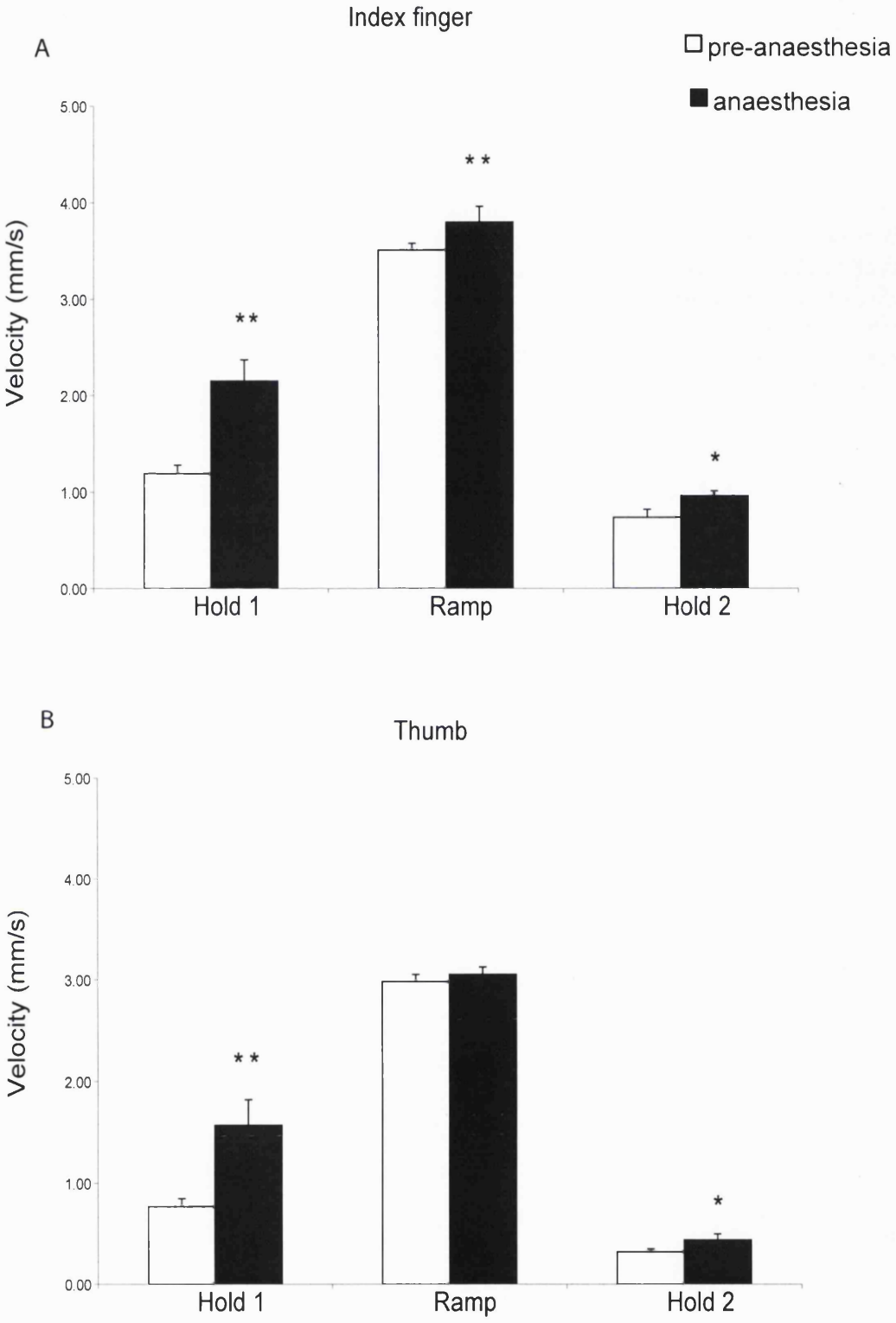


Figure 7.3: Digit velocities during each phase of the hold-ramp-hold task. Velocities were calculated from lever position data for index finger and thumb. Absolute mean values (n=10) for index finger (A) and thumb (B) velocity during pre-anaesthesia (open bars) and anaesthesia (black bars) trials are presented. Note that index finger velocity was significanlty increased in all phases of the task during anaesthesia. Thumb velocity was significantly increased in the hold phases of the task during anaesthesia (* $P<0.05$, **$P<0.01$).

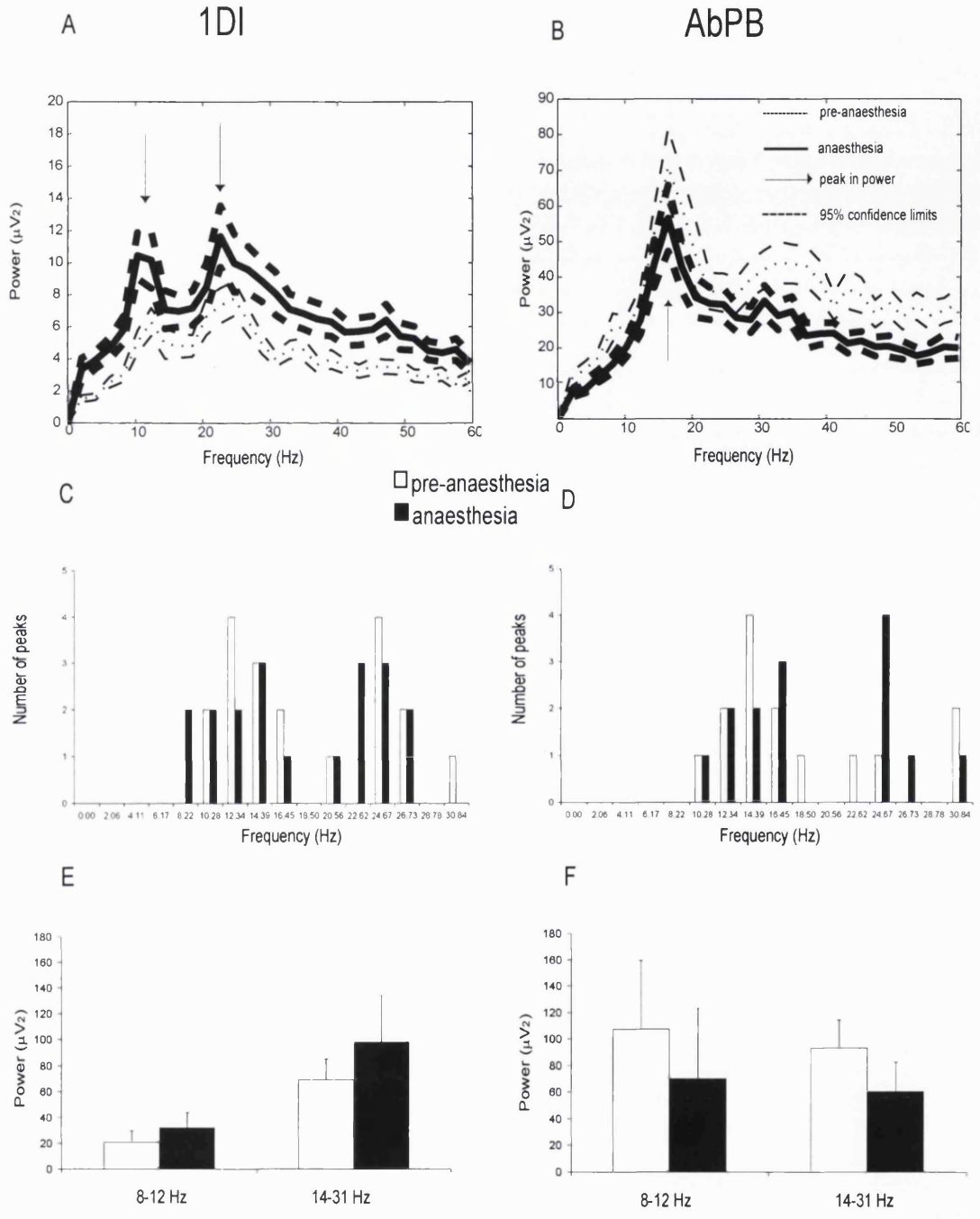


Figure 7.4: Effects of digital anaesthesia on power spectra from 1DI (A,C,E) and AbPB (B,D,F). (A,B) Power spectra from 2 subjects pre-anaesthesia (dotted lines) and during anaesthesia (thick lines). Dashed tram-lines indicate 95% confidence limits. Arrows show peaks in the power spectra. (C,D) Pooled data from all subjects (n=10) showing the frequency distribution of peaks in the power spectra. (E,F) Pooled data showing mean power of peaks in the power spectra of both muscles, within different frequency ranges. Note that in terms of frequency and amplitude, there were no significant differences between peaks in power spectra pre-anaesthesia (open bars) and during anaesthesia (black bars).

The frequency at which peaks in the power spectra occurred were recorded for all subjects. Histograms of pooled data (n=10) from 1DI (Figure 7.4C) and AbPB (Figure 7.4D) show that the frequency distribution of peaks was not changed by digital nerve anaesthesia. Grouping all subjects together, there were peaks in both the 8–12 Hz and 14–31 Hz ranges pre-anaesthesia (8–12 Hz: 1DI n=6, AbPB n=3; 14–31 Hz 1DI n=13, AbPB n=11). This distribution was similar to that occurring during anaesthesia (8–12 Hz: 1DI n=6, AbPB n=3; 14 –31 Hz 1DI n=13, AbPB n=11).

In terms of the amplitude of peaks in 1DI power spectra, there was a increase in power in the subject shown in Figure 7.4A. However, when data from all subjects was pooled, mean power pre-anaesthesia was not significantly changed during anaesthesia (two tailed unpaired t-test; Figure 7.4E). This was for peaks occurring in the 8–12 Hz range (pre: 21 ± 9 μV^2 ; post: 32 ± 12 μV^2) and in the 14–31 Hz range (pre: 69 ± 16 μV^2; post: 98 ± 36 μV^2). Similarly there was no change in mean power in AbPB muscle (Figure 7.4F) in either frequency range (8–12 Hz, pre: 108 ± 52 μV^2 ; post: 70 ± 53 μV^2 ; 14–31 Hz, pre: 93 ± 21 μV^2 ; post: 61± 22 μV^2). Therefore digital nerve anaesthesia had no effect on predominant frequencies present in 1DI and AbPB EMG signals.

7.4.4 EMG-EMG coherence at 14–31 Hz

Coherence spectra from pairs of EMGs recorded from a single subject during the hold 2 period of the task are shown in Figure 7.5. In this subject significant coherence in the 14–31 Hz bandwidth was present in the pre-anaesthesia trials. Digital nerve anaesthesia caused a clear reduction in this bandwidth in most muscle pairs. The change was particularly obvious in 1DI/AbPB, 1DI/FDS and AbPB/FDS, but not in the others (1DI/EDC and AbPB/EDC). Note that this reduction in 14–31 Hz coherence was reversed in washout trials, when the effect of the anaesthesia had worn off.

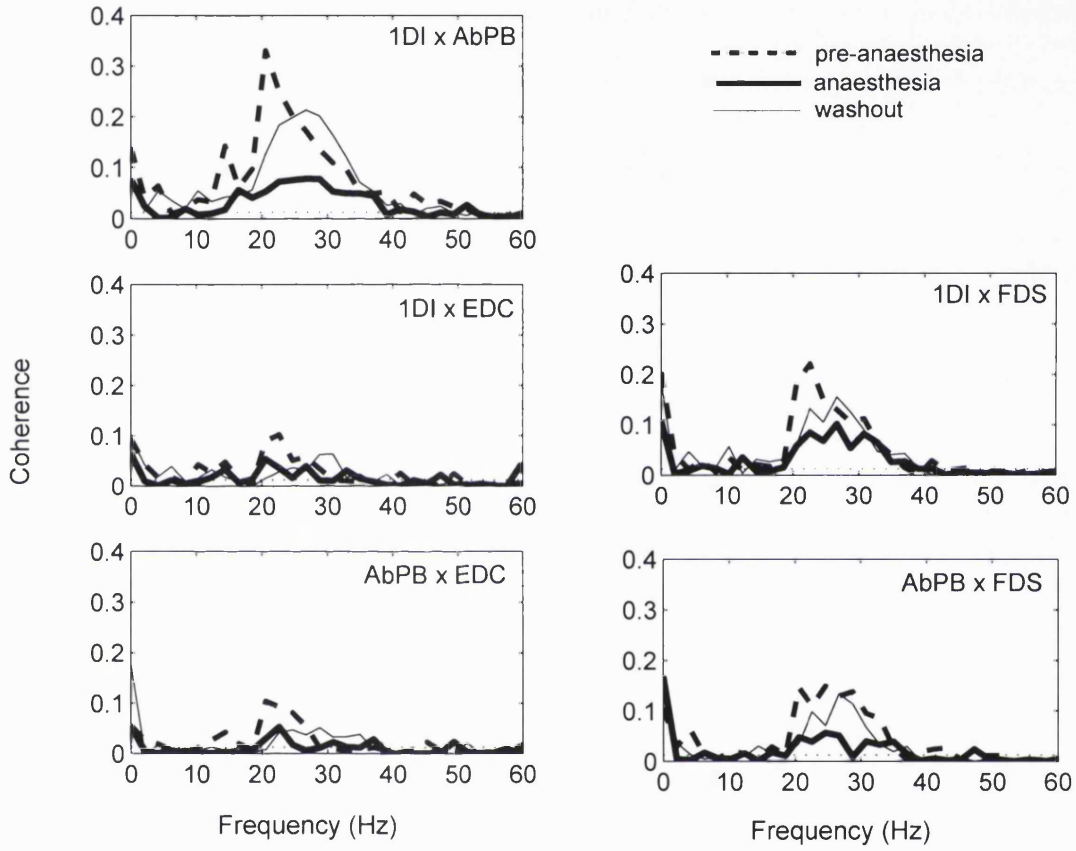


Figure 7.5: Coherence spectra from five muscle pairs of a single subject. Coherence present during pre-anaesthesia trials (dashed lines) is reduced during anaesthesia (thick lines), and returns to pre-anaesthesia values in the washout trials (thin lines). Dotted horizontal lines indicate 95 % confidence limits.

Data from all subjects (n=10) were pooled for the 1DI/AbPB muscle pair. This particular muscle pair was chosen because most subjects showed marked coherence in this muscle pair in pre-anaesthesia trials, and 1DI and AbPB are intrinsic hand muscles selectively acting on the digits which had been anaesthetized. Digital nerve anaesthesia significantly reduced 14–31 Hz coherence when anaesthesia trials were compared to pre-anaesthesia trials (Figure 7.6A). This was significant (P=0.04, n=8) only if data from the two subjects with very low coherence in the 1DI/AbPB muscle pair (coherence area < 0.7 Hz) in the pre-anaesthesia trials were excluded from the pooled data. Removal of data from these subjects was considered valid on the grounds that the low coherence values pre-anaesthesia offered little scope for the effects of anaesthesia to be measured sufficiently. The *a priori* hypothesis was that coherence would be reduced. There was also a significant reduction for anaesthesia versus washout trials (P=0.02, n=8). When the 14–31 Hz normalized coherence for all muscle pairs was pooled for all 10 subjects, a significant reduction during local anaesthesia was observed for all comparisons (Figure 7.6B; versus pre-anaesthesia P=0.02, n=10; versus washout P=0.006, n=10).

7.4.5 Velocity and Coherence

It has been shown that there were small but significant increases in digit velocity during anaesthesia trials. It is well known that during movement cortico-muscular coherence is often reduced or absent (Baker et al. 1997; Kilner et al. 1999; 2000). It should be noted, however, that movement velocities recorded during the hold 2 phase were very small, and not comparable with those at the beginning and end of each trial or even during the ramp phase (Figure 7.3). The movement velocities observed in this study were therefore not considered to confound the application of coherence analysis to this phase of the task. However, to ensure that this was not the case, both parameters were examined on a subject-by-subject basis.

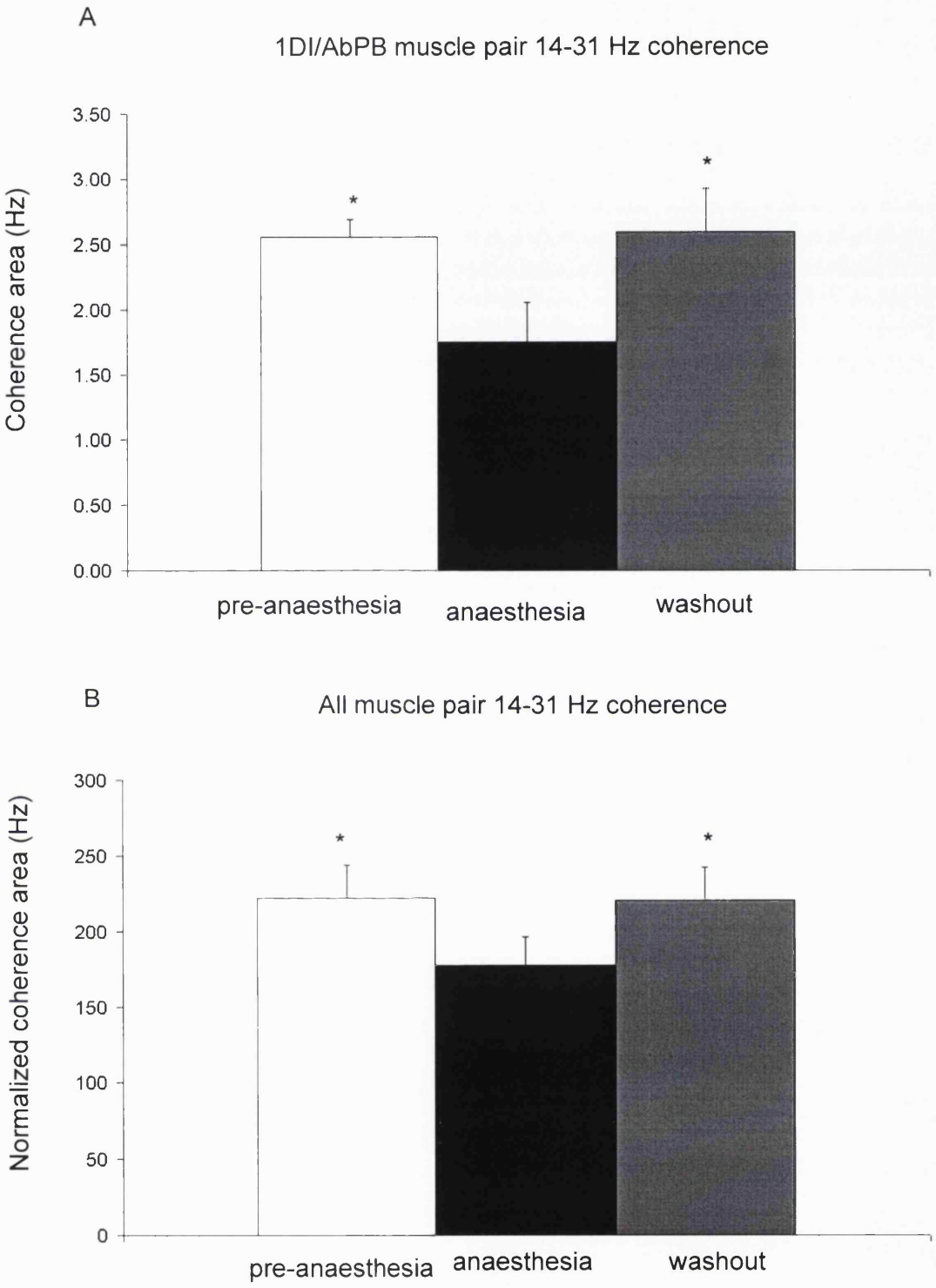


Figure 7.6: Effects of digital anaesthesia on 14-31 Hz coherence during the hold 2 phase of the precision grip task. (A) Pooled data from 8 subjects is presented for the 1DI/AbPB muscle pair. There was a significant reduction in coherence when comparing anaesthesia trials to both pre-anaesthesia and washout trials ($P<0.05$). (B) Pooled data from 10 subjects for all muscle pair coherence. Again there was a significant reduction in coherence during anaesthesia trials when compared to both pre-anaesthesia ($P<0.05$) and washout trials ($P<0.01$).

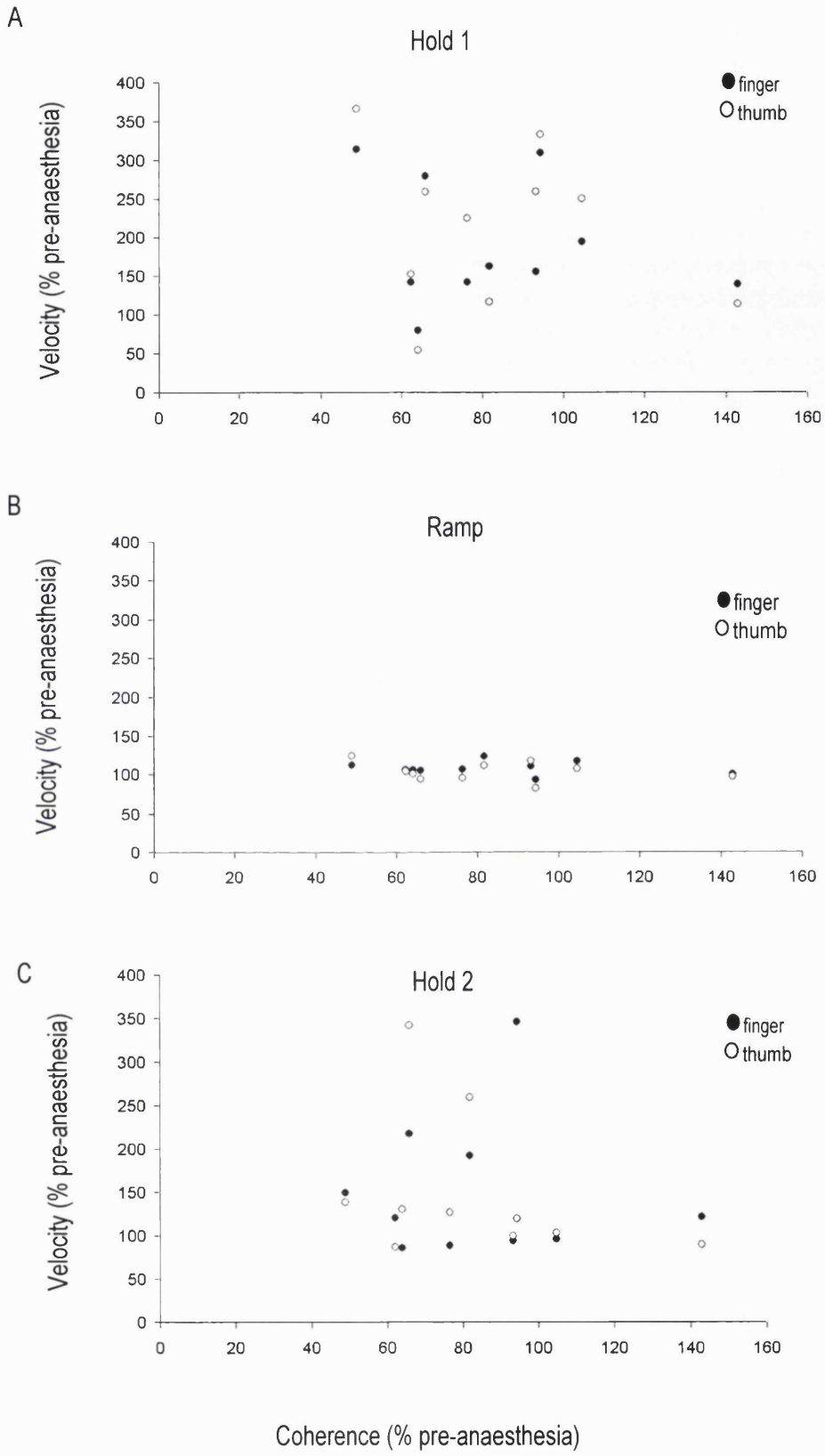


Figure 7.7: Comparison of the velocity of index finger (black circles) and thumb (open circles) with all muscle pair 14-31 Hz coherence. Velocity and coherence data obtained from digital nerve anaesthesia trials are expressed as precentages of pre-anaesthesia data. Velocity data from all 10 subjects are presented for hold 1 (A), ramp (B) and hold 2 (C) phases of the precision grip task. Velocity was calculated from lever position profiles over a 2 second period in each phase. Coherence data was taken from the hold 2 phase in all cases.

The mean velocity of digit movement in the anaesthesia trials, expressed as a percentage of pre-anaesthesia velocity, was plotted against coherence in the 14–31 Hz bandwidth (combined value from all muscle pairs) observed during the hold 2 period (expressed as a percentage of pre-anaesthesia coherence). Results were plotted separately for velocity changes in the hold 1 (Figure 7.7A), ramp (Figure 7.7B) and hold 2 (Figure 7.7C) phases. For the hold 2 phase, there was no correlation between either index finger or thumb velocity and coherence, indicating that coherence changes calculated were not influenced by any slight digit movements. There was also no correlation between the velocity of the digits during the ramp phase, and coherence in the hold 2 phase, nor was there a correlation between velocity of the digits during the hold 1 phase and coherence in the hold 2 phase.

7.5 Discussion

The main results of this study were that local anaesthesia of the thumb and index finger produced marked deficits in the performance of a precision grip of a compliant object. It also reduced, but did not abolish, the 14–31 Hz coherence between EMG activity recorded from different hand and forearm muscles. After local anaesthesia subjects were still capable of performing the hold-ramp-hold task, but their accuracy was impaired. In particular, they had considerable difficulty in performing a steady grip during the hold 1 period of the task.

The reduction of 14–31 Hz EMG-EMG coherence during the hold 2 phase of the task, during anaesthesia, was particularly marked for the coherence between the two intrinsic hand muscles, 1DI and AbPB. These muscles act on the two digits that were subjected to the ring block anaesthesia. Also observed was a reduction in the coherence between extrinsic and intrinsic digit muscles (FDS, EDC with 1DI, AbPB). Since there was no

correlation between the decrease in coherence and the very small increase in digit velocity present in the hold 2 phase of the task (Figure 7.7C) it is concluded that the reduction in coherence was unlikely to have been due to any small movements of the digits during the hold phase. It is more likely to have resulted from the lack of peripheral feedback induced by the digital nerve anaesthesia.

7.5.1 Cortical origin of 10 Hz and 20 Hz EMG activity

It is assumed that at least a proportion of the power of frequencies around 20 Hz present in EMG signals when subjects perform a precision grip task, results from a common cortical input to motoneurones (Baker et al. 1999). Lack of coherence between cortex and muscle at frequencies around 10 Hz during the precision grip task has thrown doubt on the cortical origin of these frequencies (Baker et al. 1997; Kilner et al. 1999; 2000). However, during slow finger movement, Wessberg and Vallbo (1995) showed that the 8–10 Hz discontinuities or motion tremor were inconsistent with afferent firing, and suggested a central descending pulsatile origin. Chapter 4 of this thesis provides further evidence to suggest that the cortex could indeed provide a 10 Hz oscillatory drive to EMG. In the present study, digital anaesthesia did not affect the predominant frequencies around either 10 Hz or 20 Hz in the EMG power spectra compiled from hold 2 recordings. This is in agreement with a previous study in which ischaemia was used to perturb peripheral feedback and no change in 1DI EMG oscillatory activity was observed (McAuley et al. 1997). This would suggest that, in terms of frequencies considered to be driven by the cortex (14–31 Hz), the magnitude of the central drive was not affected by lack of peripheral feedback, but that this did disrupt the mechanism by which synchrony was established. Results also argue that, during a stable contraction, peripheral feedback from cutaneous receptors is not involved in the establishment of frequencies around 10 Hz in EMG.

7.5.2 EMG-EMG coherence as a reflection of motor set

In this study analysis was performed on the EMG activity during the second hold period, because previous work had shown coherence to be greatest in this period (Kilner et al. 1999). In addition, hold 2 coherence was related to the degree of compliance of the gripped object (Kilner et al. 2000). These authors suggested that when object compliance changes, a different motor set is engaged to maintain steady grip of the object; peripheral feedback could be important in signalling the new level of object compliance. The new motor set is reflected in the degree of cortico-muscular coherence, which in turn contributes to the coherence between oscillatory activity in different hand and forearm muscles. For this study we used the same task, but only a single compliant setting for the levers. It is possible however, that lack of proprioception during anaesthesia produced similar effects as the reduction in lever compliance (levers perceived as feeling less springy) and this resulted in a reduction in EMG-EMG coherence. This theory implies a cognitive aspect to the task; and indeed it has been shown that motor imagery can affect cortical oscillations (Schnitzler et al. 1997).

7.5.3 Lack of cutaneous input affects grip force prediction and motor set recruitment

It has been argued that synchronous motor cortex output to motoneurones may provide an optimum means by which to maintain grip force (Baker et al. 1999). However, oscillatory and highly synchronous firing of the cortex could have effects on cortical processing (Baker et al. 1999). Due to the fact that there will be fixed times at which cells can fire, there is a reduced limit to the amount of information carried. The system can operate during periods of low computational load such as sustained contractions or 'hold' periods. However, during movement, much more information processing is required and so it is not possible for the motor cortex to operate in a synchronous state. This would explain why synchrony within the cortex (Pfurtsheller et al. 1996), and between cortex and

muscle, or muscle pairs (Baker et al. 1997, 1999; Kilner et al. 1999) disappears during movement.

In the pre-anaesthesia trials in this study, subjects were able to engage a suitable motor set to achieve the hold-ramp-hold task. Using feedback from cutaneous receptors it was possible to predict the grip forces required for the hold phases of the task (Johansson and Westling 1984), on a trial to trial basis. Synchrony between cortex and muscle, and thus muscle pairs was strong in these hold periods. During anaesthesia subjects clearly found the task difficult. This was shown by lever position profiles showing much greater variability over trials. In addition velocity of digit movements increased. The explanation for this was lack of proprioception. It has been shown previously that digital nerve anaesthesia leaves subjects unable to accurately detect applied movements to index finger or thumb (Refshauge et al. 1998). It was suggested that this was due to the lack of feedback from cutaneous receptors and possibly also joint receptors. The importance of cutaneous receptors in muscle responses to objects has also been shown using local anaesthesia (Collins et al. 1999). Johansson et al. (1992) showed that there was less grip force modulation following digital nerve anaesthesia and the latencies between onset of load changes and grip force responses were prolonged. It is possible then that lack of proprioception made predicting grip forces required in the hold phases of the task in this study difficult. This increase in information processing may have contributed to the reduction in EMG-EMG coherence shown in anaesthesia trials.

7.5.4 Difficulties in the hold 1 period: lack of preparation for grip parameters

During anaesthesia, there were higher increases in both thumb and finger velocity during the hold 1 phase, compared to other parts of the task. This highlights the particular difficulty subjects had in paramatizing grip forces for the hold 1 phase. Note that before hold 1 subjects were required to make a ballistic movement, unlike the smooth ramp phase preceding the hold 2 phase, and this may well have contributed to the difficulties

involved. During anaesthesia, subjects were obviously much more dependent on visual feedback to calibrate their performance. The behavioural data suggests that visual feedback of digit movement acquired in the hold 1 and the ramp periods was then used to guide accurate performance during hold 2 (Figures 7.1 and 7.3). Continued errors during hold 1 and ramp phases throughout the session indicate that subjects may have needed to recalibrate their grip on each trial. If the hold 1 phase acts as a preparatory phase for hold 2 in terms of grip force parameters, then disruption of the earlier hold 1 may have affected how well synchrony could be established in the hold 2 phase.

7.5.5 Role of peripheral feedback in maintaining synchrony between muscle pairs

As some subjects clearly showed EMG-EMG coherence following digital anaesthesia, it cannot be argued that peripheral input is essential to establish cortico-muscular synchrony. Indeed it has been found that cortico-muscular coherence was still observed in a patient with contralateral hemianaesthesia (Marsden et al. 1999) and coherence in the 15 –30 Hz range has been observed between single motor units in a hand of a deafferented subject (Farmer et al. 1993a). It seems more plausible to suggest that peripheral input enhances ongoing synchrony by signalling the fact that the grip force parameters in the hold phases of the task are predictable. The enhancement of coherence that occurs after the transition from movement to steady hold is thus blocked by local anaesthesia. This concept is in keeping with the finding that cutaneous inputs from digits 'boosts' sensorimotor cortical activity during grip responses (Johansson et al. 1994).

7.5.6 Conclusions

In conclusion, this study has highlighted an important complementary role of peripheral feedback, in the establishment of synchronous oscillatory activity between muscle pairs. Although the main generator of 15–30 Hz rhythms lies within the CNS, and possibly the

motor cortex, the activity of this generator can be significantly influenced by cutaneous inputs from hand digits. This is especially in circumstances where these inputs become important for task performance.

CHAPTER 8: DISCUSSION

Particular frequencies of cortical oscillatory activity have been shown to be coherent with EMG activity when subjects perform a stable precision grip with index finger and thumb (Baker et al. 1997; Kilner et al. 1999; 2000). This observation suggests a strong coupling between activity in the motor cortex and hand muscles during this task. That coherence is modulated by the compliance of the object that is grasped suggests synchrony within the cortico-motoneuronal system has a functional role (Kilner et al. 2000).

Short-latency inhibition measured with TMS in the paired-pulse test has been implicated in the control of voluntary movement (Floeter and Rothwell 1999), and modelling studies have suggested that inhibitory interneurones play an important role in oscillation generation within areas of the brain (Lytton and Sejnowski 1991; Pauluis et al. 1999; Whittington et al. 2000). The aim of this Thesis was to establish whether intracortical inhibitory interneurones activated by TMS in the paired-pulse test, could be involved in the maintenance of cortico-muscular synchrony. Evidence suggests that GABA inhibitory interneurones are involved in both mechanisms (Lytton and Sejnowski 1991; Ziemann et al. 1996a,b; Whittington et al. 2000).

8.1 Threshold tracking in the paired-pulse test

In chapter 3, low intensity TMS was used in a paired-pulse test to activate inhibitory interneurones within the motor cortex. A novel threshold tracking technique was described which showed clear differences in the levels of inhibition measured, depending on specific parameters used in the paired-pulse test. That inhibition measured with a very short interval of 1 ms between the conditioning and test stimuli had a clear threshold and could still be measured during voluntary activity suggested that axons of interneurones were activated by low intensity TMS. In addition when the interval between paired stimuli was increased to 2.5 ms, the intensity (or threshold) required to elicit inhibition increased, and inhibition was more susceptible to voluntary activity. This suggested that

another phase of intracortical inhibition could be measured; one which involved activation of GABAergic synaptic pathways (Kujirai et al. 1993; Ziemann et al. 1996c).

8.2 TMS effects on oscillatory EMG activity

Further information regarding the effects of low intensity TMS on the cortico-motoneuronal system was provided in chapter 4. Low intensity TMS reset the phase of oscillations within EMG activity. Intensities required to elicit these resetting effects were slightly higher than the subthreshold intensities normally used in the standard short-latency inhibition test (Kujirai et al. 1993; Schäfer et al. 1997). However, as observed in chapter 6, low intensity TMS that evokes a small MEP (threshold intensities) could still have strong inhibitory effects within the motor cortex. The resetting effects of threshold TMS suggested that cortical neurones within the network that generates cortical oscillations might be similar to those proposed to be part of the I-wave circuit implicated in intracortical inhibition (Ziemann and Rothwell 2000). Further evidence was provided by the fact that threshold TMS and also in some cases subthreshold TMS enhanced coherence between the oscillatory EMG activity of muscle pairs (chapter 4). These data suggest that inhibitory interneurones may indeed be part of the rhythm generating mechanism that operates within the cortico-muscular system.

Although the resetting effects of TMS were measured within EMG signals, and enhancement of coherence measured between muscle pairs, I will first concentrate on possible inhibitory processes *within the cortex* that may be involved in these effects. With regard to frequencies between 14–31 Hz, cortico-muscular coherence and phase-lag analysis has provided strong evidence that the motor cortex drives or leads EMG oscillatory activity during stable muscle contractions (Conway et al. 1995; Baker et al. 1997; Salenius et al. 1997a; Halliday et al. 1998; Kilner et al. 1999; 2000; Gross et al. 2000; Mima et al. 2000). The evidence is less clear for a similar mechanism operating for frequencies around 10 Hz although there are some reports of cortico-muscular coherence

in this range (Marsden et al. 2001; Raethjen et al. 2002). I will consider alternative possibilities for the effects at 10 Hz shortly. At present, results from chapter 4 support the claim that the corticospinal tract transmits oscillatory activity *in both* frequency ranges from the motor cortex to muscle. It is therefore necessary to understand how inhibition is involved in the generation of synchronous oscillations within the cortex.

8.3 The time course of inhibitory mechanisms

Do the stimulus-locked peaks and troughs observed in EMG activity evoked by low intensity TMS represent phases of intracortical inhibition? Note that this oscillatory EMG activity was the result of TMS resetting effects occurring at frequencies around both 10 Hz and 20 Hz. With regard to effects at 20 Hz, time-domain analysis showed that the first *oscillatory* peak in the EMG (note this is after the MEP) started at approximately 50 ms after the stimulus with a subsequent peak roughly 50 ms later (Figure 4.3B). Subjects on whom low intensity TMS had predominantly 10 Hz resetting effects, showed longer latencies for the first oscillatory peak. This is a rough approximation of results: frequency-domain analysis attempts to fit sine waves to the recorded EMG activity and therefore takes the shape of the whole peak into account rather than peak latencies. In addition time-domain effects varied among subjects, a finding reflected in the wide distribution of peak frequencies of stimulus-locking effects observed (Figure 4.4). If intracortical inhibition were responsible for 20 Hz resetting effects and the stimulus-locked troughs in the EMG data, it would need to operate with a time course in which the first IPSP peak occurred at approximately 30 ms after TMS, corresponding to the first trough after the MEP, with subsequent peaks of inhibition approximately every 50 ms.

This highlights the fact that short-latency TMS-induced intracortical inhibition and oscillatory activity within the cortico-muscular system operate on very different time scales. TMS-induced, short-latency inhibition involves interaction with the I-wave

generating circuit, which operates at roughly 600 Hz (Ziemann and Rothwell 2000). In contrast, oscillations within the sensorimotor cortex associated with natural movements and which feature in cortico-muscular synchronization occur at frequencies around 10 Hz and/or 20 Hz (Salmelin and Hari 1994; Salemlin et al. 1995; Conway et al. 1995; McAuley et al. 1997; Kilner et al. 1999; 2000; Mima et al. 2000). Nevertheless if we consider that a network of neurones generates oscillations (Whittington et al. 2000) then the timing of oscillatory cycles of activity may well be controlled by fast IPSPs of $GABA_A$ interneurones (Lytton and Sejnowski 1991). Effects of low intensity TMS in chapter 4 supports the theory that PTNs and local inhibitory interneurones within the motor cortex are involved in such networks and indeed $GABA_A$ receptor mediated effects have also been implicated in short-latency inhibition (Hanajima et al. 1998). It is difficult to be sure why inhibitory phases are more frequent in the I-wave generating circuit; however it may be due to the fact that this is a more localised effect involving a smaller population of neurones (Amassian et al 1987), than the more widespread oscillatory activity (Murthy and Fetz, 1992; 1996). There are some parallels of this discussion with those concerning the very high frequency D-waves and I-waves that are evoked by TMS, since frequencies of this kind are not seen in the natural activity of motor cortex neurones.

8.4 Measurements of motor cortex excitability

There were two main aspects to experiments discussed in chapters 5 and 6. On the one hand, it was interesting to find evidence of strong ongoing inhibition present during a precision grip task during which there are strong levels of muscle pair coherence (e.g. hold-ramp-hold task; chapter 6). However a further aim was to correlate this inhibition with different levels of muscle pair coherence. The hypothesis was that there would be more intracortical inhibition present during tasks in which there was more muscle pair coherence.

In chapter 5 single TMS stimuli were used to evoke MEP responses of 1–2 mV in hand muscles. One hypothesis was that smaller MEPs would be elicited in the hold-ramp-hold task, due to higher levels of cortical inhibition present when more coherence between muscle pairs was observed. This was compared with the isometric task during which muscle pair coherence was less. There were in fact no consistent differences in MEP size between precision grip task conditions despite a clear change in muscle pair coherence. In order to understand why this should be, it is necessary to consider more carefully the effects of TMS. During strong muscle pair coherence observed in the precision grip task there is more synchronous activity within the cortex (Baker et al. 1997; Kilner et al. 1999). PTNs have been shown to be phase-locked with local field potential recordings in monkeys (Pinches et al. 1997). Therefore we could think of peaks and troughs of oscillatory activity in the cortical signal (Baker et al. 1999), reflecting excitatory and inhibitory phases of the motor cortex. In this Thesis TMS stimuli used to measure MEP amplitudes were applied at fixed time intervals with regards to the periods of the precision grip task, yet randomly in terms of ongoing oscillatory activity. From trial to trial it was equally likely that the TMS was applied in the excitatory versus the inhibitory phases of oscillatory activity, rather than being timed to occur in one particular phase. Averaging of responses to TMS might have cancelled out any clear effect and explain why the MEP amplitudes evoked during the two tested tasks were similar, even though the tasks differed in terms of muscle pair coherence. There is however, no immediate solution to this problem. Timing the TMS to be delivered during inhibitory oscillatory phases of cortical activity, would not necessarily ensure a consistent effect over trials: the measurement is also dependent on the percentage of PTNs activated by the stimulus, that are phase-locked to the local field potential.

There are further complications when considering effects at the level of individual PTNs. In a study modelling neuronal excitability, which can probably be applied to cortical

cells, Matthews (1999) suggested that once a neurone 'begins to fire, the measurement of its excitability [by an external stimulus such as TMS] ceases to provide a valid indication of its background synaptic drive'. Indeed at high firing rates excitability changed very little in accordance with synaptic drive, whereas at low firing rates excitability fell rather than rose (Matthews 1999). Applying these ideas to PTNs would suggest that measuring the cortical output in terms of MEP amplitude does not necessarily provide a reliable measure of the level of ongoing excitation/inhibition of motor cortex PTNs.

8.5 Measurements of short-latency intracortical inhibition

What about results obtained using the paired-pulse test, which measured short-latency inhibition more directly? Inhibition was measured as the reduction in MEP response elicited by the test stimulus delivered *after* the conditioning stimulus. Chapter 4 showed that the conditioning stimulus *reset* the phase of oscillatory activity. Indeed it would seem that using a paired-pulse test would at least in part solve the timing problem of the test stimulus in relation to ongoing oscillatory activity. As long as the interval between the conditioning and test stimuli was constant over trials, the test stimulus would always be delivered during oscillatory activity at the same phase over trials (i.e. phase-locked to the conditioning stimulus). If fast $GABA_A$ interneurones mediate the resetting effects of TMS then it should be possible to measure this inhibition by applying the test stimulus a few milliseconds after the conditioning stimulus. If we consider the hold-ramp-hold task only, then the fact that low intensity TMS does reset EMG oscillatory activity (chapter 4) and have inhibitory effects on a subsequent MEP response (chapter 6), is sufficient enough evidence that the two mechanisms are related. However, is this a measurement of ongoing inhibition involved in oscillation generation or are we measuring the inhibition elicited by the conditioning stimulus? It is possible that inhibitory interneurones recruited in the ongoing inhibition are more excitable, and in addition there is a 'subliminal fringe' of excited interneurones yet to fire (Aranyi et al. 1998), that would be activated by the

conditioning stimulus. Using this explanation we would expect to measure more intracortical inhibition in the hold-ramp-hold task, in comparison to the isometric task, which was not the case (chapter 6). One possible reason for this could be that occlusion occurs between ongoing inhibition within the cortex and inhibition generated by the conditioning stimulus, similar to that observed within the spinal cord (Iles et al. 1992; Kudina et al. 1993). That ongoing inhibition was stronger during the hold-ramp-hold task, may then have resulted in reduced intracortical inhibition measured by the conditioning stimulus. However that measurements of intracortical inhibition usually agree with predicted changes in cortical excitability (e.g. reduced intracortical inhibition before movement onset and during movement; Ridding et al. 1995b; Floeter and Rothwell 1999; Reynolds and Ashby 1999) suggests that occlusion is a rare occurrence.

An alternative explanation is that the effects of the conditioning stimulus serve to temporarily reduce the difference in coherence levels between tasks, resulting in similar levels of inhibition measured by the paired-pulse (e.g. results using ISI=1 ms). To use this argument to explain why (using the ISI=2.5 ms) *more* inhibition was measured during the isometric task, it would be necessary to compare the magnitude of stimulus-locking and increases in coherence evoked by the conditioning stimulus during the isometric task, to those observed during the hold-ramp-hold task. It is not possible to adequately test this with data from the same set of subjects in this Thesis, as only the hold-ramp-hold task was used in chapter 4. However, note that TMS did cause significant resetting and enhancement of coherence in the isometric task (as verified with a smaller set of subjects in chapter 6) despite lower levels of muscle pair coherence to those in the hold-ramp-hold task. (In addition note that in chapter 4 threshold TMS caused an equal if not greater increase in 4–12 Hz coherence, compared to 14–31 Hz coherence, despite the fact that 4–12 Hz coherence was much lower in 'no stimulus' trials).

8.6 Long-latency intracortical inhibition

A further possibility arises when we again consider the latency of the inhibitory phases present in EMG oscillatory activity (every 50 ms or 100 ms) following low intensity TMS. It is possible that these effects were caused by inhibition operating over a longer time course. Indeed both $GABA_A$ and $GABA_B$ conductances have been implicated in oscillation generation (Lytton and Sejnowski 1991) and in vitro work has shown that stimulation of the neocortex can produce disynaptic fast and slow IPSPs (McCormick 1989; Diesz 1999). If $GABA_B$ mechanisms also play a role in oscillation generation, these will not have been measured accurately in tests of short-latency intracortical inhibition. Indeed $GABA_B$ receptors have been implicated in long latency inhibition measured by a different TMS paired-pulse test (Valls-Sole et al. 1992; Werhahn et al. 1999). Rather than the conditioning stimulus being of a subthreshold intensity, in this test often a pair of suprathreshold stimuli are used and longer intervals of approximately 60–200 ms separate the two stimuli. Note that in this case the response evoked by the conditioning stimulus is a large MEP, with silent period. This differs from the smaller MEP and subsequent oscillatory peaks presented in chapter 4.

Although short-latency and long-latency intracortical inhibition may operate via different mechanisms (suggested by the fact that increasing the intensity of the test stimulus had different effects on the inhibition measured) the two inhibitory pathways are not independent (Sanger et al. 2001). Authors suggested that a suprathreshold conditioning stimulus activates long-latency inhibition via $GABA_B$ receptor mediated slow IPSPs with time courses of 150–200 ms. This inhibition was shown to suppress short-latency inhibition involving fast IPSPs with a time course of 20 ms, mediated by $GABA_A$ receptors.

8.7 $GABA_A$ versus $GABA_B$ receptor mediated inhibition

Could distinguishing between $GABA_A$ and $GABA_B$ receptor mediated effects help explain the different inhibitory effects implicated in this Thesis? As mentioned in chapter 1, the precise origin of the high frequency I-waves is still unknown, and is somewhat difficult to reconcile with the rather slow rise time of both EPSP and IPSPs observed in PTNs (Ghosh and Porter 1988). However, it is more likely that fast IPSPs operate ($GABA_A$), than slower ones. In order to provide evidence for $GABA_B$ mechanisms in oscillation generation within the context of this Thesis, it would be necessary to use longer intervals between *threshold* conditioning stimuli (low intensity TMS used in chapter 4) and suprathreshold test stimuli. Increasing the interval between a subthreshold conditioning stimulus and a suprathreshold test stimulus beyond 6 ms up to 30 ms, has been shown to result in facilitation of the MEP response (Kujirai et al. 1993; Ziemann et al. 1996c). However, when two TMS stimuli slightly above threshold were delivered, inhibition of the test response was observed at intervals between 5–40 ms, and facilitation at intervals between 50–90 ms (Valls-Sole et al. 1992). When both stimuli were increased to higher suprathreshold intensites, facilitation was observed at intervals between 25–50 ms, and inhibition from 60–150 ms (Valls-Sole et al. 1992). It is interesting to speculate that these periods of inhibition and excitation represent the peaks and troughs of oscillatory activity generated by the resetting effects of the conditioning stimulus. The discrepancy in latencies could simply result from the different strengths and frequencies of stimulus-locking effects of the conditioning stimulus, and whether or not a prolonged silent period was evoked.

A study supporting the suggestion that long-latency inhibition may be involved in cortical oscillatory mechanisms, measured intracortical inhibition in patients with progressive myoclonus epilepsy (Valzania et al. 1999). Intracortical inhibition, measured by pairs of suprathreshold stimuli separated by intervals of 100–150 ms, was reduced. There was also strong facilitation measured using pairs of threshold stimuli separated by 50 ms. Epilepsy

was argued to result from suppression in the activity of $GABA_B$ inhibitory interneurones, resulting in reduced control of cortical excitability necessary for skilled movement (Matsumura et al. 1992).

In summary it may be possible that both $GABA_A$ and $GABA_B$ intracortical inhibitory mechanisms operate in the generation of oscillatory activity within the cortico-muscular system. Though $GABA_A$ mechanisms are favoured by those modelling oscillatory activity (Lytton and Sejnowski 1991), results from paired-pulse TMS experiments imply that $GABA_B$ mediated inhibitory effects may also be important. If the effects of low intensity TMS on oscillatory EMG activity were predominantly mediated by $GABA_B$ intracortical inhibitory mechanisms, then this may explain the reduction in short-latency inhibition (Sanger et al. 2001) observed in a task during which there was strong EMG-EMG coherence (chapter 6).

8.8 Why TMS resetting effects are unlikely to be spinally mediated

Now it is necessary to turn to the level of the peripheral motor apparatus, and explain why it is unlikely that TMS reset motoneurone activity. Certainly this is a tempting explanation for the 10 Hz resetting effects, given that the natural firing rate of motoneurones is at this frequency (Farmer et al. 1993a). But how does this explain effects at frequencies around 20 Hz? Clear evidence suggests frequencies in the 14–31 Hz range have, at least in part, a corticospinal origin (Farmer et al. 1993a; Conway et al. 1995; Baker et al. 1997; Kilner et al. 1999; 2000). Moreover, phase-lag analysis between cortical and EMG signals suggests that the cortex drives muscle activity (Conway et al. 1995; Salenius et al. 1997a; Halliday et al. 1998; Mima et al. 2000). It is also possible to equate such lags to fast corticospinal conduction times (Gross et al. 2000). This provides clear evidence against a peripheral origin for rhythm generation. In order for the phase of EMG oscillatory activity to be locked to the stimulus (chapter 4), TMS must have activated the oscillation generation site. If TMS were simply causing a pause in the firing

of motoneurones, then subsequent oscillations would not be phase-locked to the stimulus over trials. There would simply be a continuation of the ongoing oscillatory drive from the cortex. In addition, it is highly unlikely that low intensity TMS acting at the level of the motoneurone could enhance EMG-EMG coherence in the 14–31 Hz range (chapter 4). Admittedly, given the lack of evidence of EMG-EMG coherence at frequencies between 4–12 Hz during precision grip hold tasks, the situation regarding resetting effects around 10 Hz is more complicated (Baker et al. 1997; Kilner et al. 1999). However why would TMS elicit resetting effects at frequencies around 10 Hz and 20 Hz in the same subject, by such different mechanisms? Surely a more distinct peak frequency distribution for stimulus-locking effects would have been obtained had this been the case. On the contrary two further arguments point towards a cortical origin of the 10 Hz resetting effects observed in chapter 4. Stimulus-locking at frequencies around 10 Hz in EMG was not observed following direct pyramidal tract stimulation in monkeys (Jackson et al. *in press*), suggesting that effects of TMS presented in chapter 4 were not mediated by spinal mechanisms. In addition increasing evidence suggests that the 10 Hz component of physiological tremor is of a cortical origin (McAuley et al. 1997; Mayston et al. 2001; Raethjen et al. 2002).

8.9 Excitatory and inhibitory phases in EMG activity

The results described in this Thesis need to be considered in the light of existing knowledge about effects of TMS on EMG activity. The secondary peak observed in PSTH analysis (Mills et al. 1991) following TMS has a similar latency to the first oscillatory peak observed in chapter 4 of this Thesis. Garland and Miles (1997) suggested that this secondary peak might arise from a realignment or resetting of single motor unit firing in phase with the corticospinal input generated by TMS. This results in a synchronised return of many units to firing threshold and this grouped resetting of discharge leads to the secondary peak of EMG activity.

Work in this Thesis supports and extends this observation. By resetting the oscillatory activity within the motor cortex, TMS may have caused disfacilitation of the tonic input to motoneurones, the timing of which is phase-locked to the stimulus. Following this suppression, responsiveness of motoneurones would therefore resume synchronously, to fire again in response to the oscillatory input from the cortex. That for some 250 ms following the stimulus, this cortical signal is still phase-locked to the TMS stimulus, means that EMG activity remains stimulus-locked. This results in a continuation of peaks and troughs in the EMG activity.

A further relevant observation is that natural activation or electrical stimulation of muscle or cutaneous afferents, can elicit a pattern of peaks and troughs in the EMG activity known as the cutaneomuscular reflex (Jenner and Stephens 1982). There is good evidence to suggest that the E2 phase (50-70 ms post-stimulus) and possibly the I1 phase (40-50 ms post-stimulus) are of cortical origin (Farmer et al. 1990; Mayston et al. 1997; Mayston et al. 2001). Is it possible that these effects are in someway related to the resetting effects of TMS, and that both are affected by diseases affecting the organisation of the corticospinal tract? It is unlikely that stimulus-locking effects in EMG activity, and TMS enhancement of coherence between muscle pairs would occur via peripheral mechanisms. Various pieces of evidence argue against peripheral feedback loops in the generation of cortico-muscular coherence, some of which have already been mentioned. In addition there are similar frequencies of coherent activity within lower and upper limbs despite reafferent times being longer in lower limbs (Salenius et al. 1997a). Muscle tendon vibration did not affect cortico-muscular coherence (Mima et al. 2000) and ischaemia did not change predominant frequencies within the EMG signal (McAuley et al. 1997). Finally, coherence in the 15–30 Hz range between single motor units has been recorded from a hand muscle of a deafferented patient (Farmer et al. 1993a).

8.10 Peripheral inputs and intracortical inhibition

A number of investigations have interacted TMS with peripheral inputs and related this to the phases of EMG activity in the long latency stretch reflex (Delwaide and Olivier 1990; Maertens de Noordhout et al. 1992; Tokimura et al. 2000). In essence this work is similar to the aims of chapters 5 and 6 and it is interesting to observe that problems in measuring inhibition have also arisen. Tokimura et al. (2000) showed that a peripheral stimulus could inhibit descending volleys within the corticospinal tract evoked by TMS, in a manner similar to a TMS conditioning stimulus in the paired-pulse test (Di Lazzaro et al. 1998b). This suggested that peripheral nerve stimulation might also activate intracortical GABAergic inhibitory interneurones. However although digital nerve stimulation given at the correct latency also had inhibitory effects on the cortical elements involved in the MEP response, such inhibition was not reflected when short-latency intracortical inhibition was measured (Ridding and Rothwell 1999). That this intracortical inhibition was suppressed suggested that the digital nerve stimulus also had excitatory effects. Similarly, stimulation of muscle afferent nerves also suppressed short-latency intracortical inhibition despite there being no change in MEP size (Aimonetti and Nielsen 2001). Some of these results are explained by the fact that peripheral nerve stimulation can have both inhibitory and excitatory effects (Maertens de Noordhout et al. 1992), and natural stimulation of cutaneous inputs in a functional context exerts mainly excitatory effects (Johansson et al. 1994).

8.11 Peripheral inputs, movement and oscillatory activity

Part of this Thesis was devoted to demonstrating that peripheral inputs also have effects on oscillatory activity within the cortico-muscular system. Median nerve stimulation has been shown to immediately decrease, and then increase the amplitude of synchronous oscillatory activity at frequencies around both 10 Hz and 20 Hz within the sensorimotor cortex (Salmelin and Hari 1994; Salenius et al. 1997b). Authors concluded that afferent

input immediately desynchronizes the cortex, after which there follows a rebound of synchronization. To test the involvement of the motor cortex in this mechanism in a manner similar to studies investigating the origin of the long latency reflex (and chapters 5 and 6), TMS was used. It was reported that from 200 ms–1 s following median nerve stimulation, smaller MEPs were evoked; this suggested that the motor cortex was less excitable during synchronization (Chen et al. 1999). This also provides evidence that the increased cortical synchrony observed following median nerve stimulation could involve inhibition within the motor cortex. Note that, compared with previous studies referred to in section 8.10, this inhibition was measured over longer latencies which might explain the clearer result.

Moreover, movement of the fingers has similar effects as median nerve stimulation on cortical oscillatory activity, providing a more functional meaning to the changes observed. During movement there is desynchronization of both 10 Hz and 20 Hz oscillations in the sensorimotor cortex, followed by a rebound in synchronous activity within 1 s after termination of movement (Pfurtscheller et al. 1996). These authors reported that this is *time-locked* not phase-locked, event-related synchronization (Pfurtscheller and Andrew 1999). This provides further evidence against a peripheral origin for the generation of oscillatory activity but is consistent with resetting effects of low intensity TMS. Following movement, it is also possible to obtain similar effects on MEP amplitudes to those observed with median nerve stimulation. A decrease in MEP amplitudes 700 ms–1 s following movement termination again reflects a reduction in motor cortex excitability (or increased inhibition) during the rebound of synchronization (Chen et al. 1998).

8.12 The function of synchronous oscillations

That synchronous oscillatory activity within the sensorimotor cortex is affected by movement (Pfurtscheller et al. 1996) and coherence between muscle pairs is modulated

by the compliance of objects grasped (Kilner et al. 2000), suggests that synchrony has a functional role within the cortico-muscular system. Indeed a simple model was presented in chapters 1 and 7 to highlight this point, and the involvement of cortical inhibitory mechanisms was also implied (Baker et al. 1999). Synchrony between the cortex and muscle was said to provide an optimum means by which to activate motoneurones, and would therefore be useful in hold phases of the precision grip task when muscles are co-contracting. In order for synchronized activity to dominate within the cortex, only specific frequencies of oscillatory activity can be permitted, resulting in a limited processing capacity. Other frequencies of activity within the motor cortex are likely to be inhibited, in order to maintain synchrony (Baker et al. 1999).

When fractionation of finger movement is required, (such as in the ramp phase) the motor cortex has to operate in a more sophisticated manner (Bennett and Lemon 1996). This requires more intensive, higher level processing in separate assemblies than could operate in the synchronous state, hence desynchronization is necessary (Baker et al. 1999). Interestingly, movement also reduces intracortical inhibition, indirectly supporting the argument that inhibition is stronger during periods of synchronization (Ridding et al. 1995b).

8.13 The importance of cutaneous inputs

Further support for the function of synchrony within the cortico-muscular system was provided in chapter 7. Observing the effects of digital nerve anaesthesia highlighted the importance of peripheral feedback during the manipulation of objects. Blocking cutaneous feedback resulted in a reduction of coherence between muscle pairs, in parallel with a severe degradation in task performance. At first the reduction in coherence may seem contradictory; it has been argued that movement and at least the initial effects of peripheral input causes desynchronization, so blocking peripheral input should in theory increase synchrony. However oscillatory activity during movement was not assessed;

instead coherence between muscle pairs was measured during steady hold phases of the precision grip task, performed *following* movement. Also note that following desynchronization induced by median nerve stimulation, a rebound of synchronization occurs (Salemlin and Hari 1994; Salenius et al 1997b).

An important observation was that digital nerve anaesthesia did not affect the power or amplitude of frequencies within EMG oscillatory activity. This is similar to the finding of McAuley et al. (1997) who studied the effects of ischaemia on oscillatory EMG activity. Therefore although chapter 7 did show effects of cutaneous input on coherence between muscle pairs, as already mentioned above, there is strong evidence against a peripheral origin of rhythm generation.

So how did blocking cutaneous input affect muscle pair coherence? Many experiments have shown that cutaneous input can have direct effects on motor cortex excitability (Maertens de Noordhout et al. 1992; Johansson et al. 1994; Lemon et al. 1995; Ridding and Rothwell 1999; Tokimura et al. 2000; Aimonetti and Nielsen 2001). These may act to enhance synchronous activity within the sensorimotor cortex (Salmelin and Hari 1994; Salenius et al. 1997b). It is possible then, that lack of cutaneous input may have reduced synchronous cortical activity, thus affecting EMG-EMG coherence.

Another important consideration is that peripheral feedback is essential for parametizing grip forces (Johansson 1996). This is highlighted by the use of inappropriate contact forces and delays in the selection of suitable grip forces during digital nerve anaesthesia (Johansson and Westling 1984; Johansson et al. 1992). A different motor set is required to perform the precision grip on objects with specific physical properties, one of which is object compliance. It is suggested that the motor set is reflected in the level of coherence between the cortex and muscle and between muscle pairs, and this concept favours a possible functional role for synchrony in the 14–31 Hz range (Kilner et al. 2000; chapters 5 and 6). Lack of peripheral feedback during digital nerve anaesthesia, may well have

disrupted subject's ability to select and maintain a suitable motor set, and this was reflected by lower levels of muscle pair coherence (chapter 7).

8.14 Final conclusion

This Thesis provides evidence for the involvement of inhibitory pathways within the motor cortex in the generation of oscillatory activity within the cortico-muscular system. Subthreshold TMS increased coherence between EMG activity of muscle pairs and threshold TMS had clear effects on the phase of EMG oscillatory activity. The hypothesis that these effects were mediated via the activation by TMS of intracortical inhibitory interneurones was tested with specific use of the paired-pulse test. It is possible that inhibition mediated by GABA receptors other than the type mediating the short-latency inhibition measured in this Thesis, may have contributed to the effects of TMS on oscillatory EMG activity. Alternatively, at threshold intensities, TMS may have activated both inhibitory and facilitatory pathways, which may also explain some of the findings observed. Digital nerve anaesthesia caused severe degradation in task performance, and a parallel reduction in coherent muscle pair activity. This supports the argument that synchronous activity has an important functional role within the cortico-muscular system. That work of this kind can be achieved in conscious human subjects provides exciting opportunities for further investigation into the role of inhibition and synchrony within the human motor system.

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