The functional relevance of cortico-cortical interactions in the human motor system

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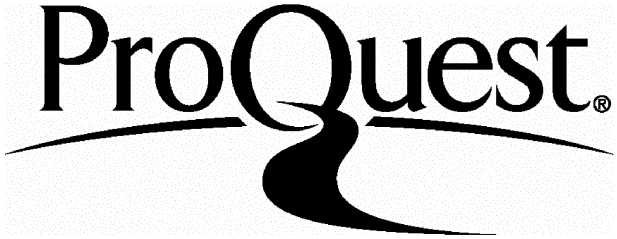

Abstract

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By recording surface electroencephalographic (EEG) data from human subjects, it is possible to calculate measures of functional connectivity and to correlate these with behavioural changes induced with repetitive transcranial magnetic stimulation (rTMS). RTMS is a tool that can be used to cause temporary disruption to an area of cortex so that the effects of such "transient brain lesions" can be studied.

This thesis investigates cortical plasticity in humans and, in particular, the functional relevance of cortico-cortical coherence in the motor system. Specifically, this work explores the effects of high (5Hz) and low (1Hz) frequency rTMS to the motor cortex and supplementary motor area (SMA). It investigates the relevance of such changes in connectivity to motor function using simple behavioural tasks such as finger tapping, reaction times and bimanual coordination. In addition, the cortico-cortical coherence recorded from stroke patients is compared with that from healthy subjects in an attempt to correlate coherence with recovery from stroke.

This work demonstrates that rTMS to motor areas of the healthy human brain causes effects that outlast the duration of the train of stimulation. More specifically, rTMS to motor areas causes changes in cortico-cortical coherence, associated changes in motor behaviour and differential effects dependent on stimulation parameters. Disruption to one motor cortex with rTMS may be compensated for functionally by the opposite motor cortex in healthy humans. Lastly, changes in cortico-cortical connectivity after stroke were shown to relate to functional recovery. These results led to several conclusions. Firstly, rTMS can acutely disrupt cortical function at a behavioural level. Secondly, the effects of rTMS are partly mediated by changes in cortico-cortical coupling. Thirdly, the effects of rTMS are limited by acute plasticity at a cortical level. Fourthly, chronic plasticity in stroke is partly mediated by changes in cortico-cortical coupling.

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Publications of work incorporated into this thesis

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Oliviero A, Strens LHA, Di Lazzaro V, Tonali PA, Brown P (2003). Persistent effects of high frequency repetitive TMS on the coupling between motor areas in the human. Exp Brain Res 149(1): 107-113.

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List of abbreviations used

ANOVA	Analysis of variance
APB	Abductor pollicis brevis
CMCT	Central motor conduction time
CNS	Central nervous system
CT	Computerised tomography
EEG	Electroencephalography
EMG	Electromyography
EPSP	Excitatory postsynaptic potential
ERD	Event-related desynchronisation
ERS	Event-related synchronisation
FDI	First dorsal interosseous
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
IPSP	Inhibitory postsynaptic potential
LFP	Local field potential
MEG	Magnetoencephalography
MEP	Motor evoked potential
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contraction
NHNN	National hospital for Neurology and Neurosurgery
PET	Positron emission tomography
rTMS	Repetitive transcranial magnetic stimulation
SD	Standard deviation
SEM	Standard error of the mean
SMA	Supplementary motor area
TMS	Transcranial magnetic stimulation
TRCoh	Task-related coherence
TRPow	Task-related power
VEOG	vertical electro-oculogram
WE	Wrist extensors
WF	Wrist flexors

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1 Introduction

The human motor system transforms neural information into physical energy by issuing commands that are transmitted via the brain stem and spinal cord to skeletal muscles. The muscles translate this neural information into a contractile force that produces movement. Motor neurons are thus the final common pathway for all such behavioural acts.

There are several different non-invasive techniques that can be employed to investigate the function of the human motor system: electroencephalography (EEG), magnetoencephalography (MEG), functional brain imaging, transcranial magnetic stimulation (TMS) or any combination of the above. These different techniques vary in spatial and temporal resolution. Modern brain imaging, such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), allows a good spatial resolution (in samples per millimetre) and can reveal the brain networks involved in a specific function. However, images take time to acquire and so the temporal resolution is poor. EEG is a measure of mixed frequency electrical signals produced by masses of neurons depolarising in synchronisation. It only reflects neuronal activity in the shallow layers of the cerebral cortex but provides extremely high temporal resolution (in samples per millisecond). MEG exploits the fact that minute electrical currents generated at a cellular level give rise to small magnetic fields. When compared with EEG, MEG has a relatively high spatial resolution and provides a high temporal resolution (in samples per millisecond). TMS can be used to explore the excitability of cortical circuits and to disrupt activity transiently in any focal cortical area, allowing function to be assessed on a millisecond scale.

This thesis describes studies that combine two of these techniques: EEG and TMS. The aim of the experiments described is to investigate whether coherent oscillatory activity in the human motor system has a functional relevance.

1.1 Oscillatory activity in the human central nervous system

Oscillations within cortical and subcortical structures may result from two processes. Firstly, an individual neuron may be able to oscillate as a result of intrinsic membrane characteristics. It can thus act as a pacemaker cell, subsequently entraining a network of cells. Secondly, oscillations may arise as a result of interactions between groups of neurons (network properties). This section briefly discusses the mechanisms of

generation of oscillatory EEG signals and the possible functions of synchronous oscillatory activity in the motor system.

1.1.1 The origin of EEG

The human brain contains approximately one hundred thousand million (10^{11}) excitable nerve cells or neurons (Kandel, 1991). Each neuron consists of a cell body (soma) with radiating branches (dendrites) and an axon. The soma is the metabolic centre of the neuron and the dendrites serve as the main structures for receiving the input to the neuron from other nerve cells. The axon is the main conducting unit of the neuron and is capable of propagating a transient electrical signal called an action potential. The axon arises from a specialised region of the soma known as the axon hillock, where the action potential is initiated once a critical threshold is reached. Near its distal end, the axon divides into fine branches that end in presynaptic terminals. These synapse with dendrites of other neurons or with target structures and are the transmitting elements of the neuron. A single neuron can have several dendritic branches and the more dendrites a neuron has, the more connections it can make with other neurons. The critical signalling functions of the brain (for example, sensory processing, motor programming, learning, memory) are carried out by interconnected sets of neurons.

Neurons, like other cells of the body, maintain a potential difference across their external membrane, known as the resting membrane potential. This results from the unequal distribution of Na^+, K^+, Cl^- and organic anions across the membranes of the cells. An increase in the membrane potential is referred to as hyperpolarisation and a reduction as depolarisation. Neurons and muscle cells are excitable, in that their resting membrane potential can be significantly altered and thus serve as a signalling mechanism.

Stimulation of dendrites from adjoining axons causes small voltage changes, creating a potential difference across the cell's membrane. Depolarisation of the postsynaptic transmembrane potential is represented by an excitatory postsynaptic potential (EPSP). Hyperpolarisation is represented by an inhibitory postsynaptic potential (IPSP) (Martin, 1991). Communication occurs because an electrical disturbance produced in one part of the cell membrane spreads to other parts. Such a disturbance becomes weaker with increasing distance from its source (attenuation) unless energy is expended to amplify it as it travels. Over short distances this attenuation is unimportant, and many small neurons conduct their signals passively, without amplification. However, for long-distance communication such passive spread is inadequate. Thus, larger neurons employ an active signalling mechanism: the

generation of action potentials. If the sum of incoming signals from other neurons (excitatory and inhibitory postsynaptic potentials) reaching the axon hillock exceeds that of the cell's particular threshold potential, an 'explosion' of electrical activity is triggered that is propagated rapidly along the axon's plasma membrane. This transmembrane polarity oscillation is known as an action potential and is sustained by automatic amplification as it travels along the axon membrane towards the terminal buttons. An action potential is triggered when a brief depolarising stimulus causes voltage-gated Na^+ channels to open, making the membrane more permeable to Na^+ and further depolarising the membrane. This positive feedback causes still more Na^+ channels to open, resulting in an all-or-none action potential. In each region of membrane, the action potential is rapidly terminated by the inactivation of the Na^+ channels and, in many neurons, by the opening of voltage-gated K^+ channels (Alberts et al., 1989). Action potentials can convey information without attenuation along axons at speeds of up to 100 m/s (Alberts et al., 1989). The magnitude of the summed postsynaptic potentials directly affects the frequency at which the action potential is regenerated along the axon.

At the terminal buttons, the neuron's information is conveyed to other excitable cells. Information transfer between neurons, encoded as the firing pattern of nerve impulses (see below), is accomplished through chemical secretion at the synapses (Alberts et al., 1989). The electrical signal in the presynaptic axon terminal triggers the secretion of neurotransmitters that, in turn, provoke an electrical discharge in the postsynaptic cell. Glutamate is the most common excitatory neurotransmitter in the human brain whereas glycine and gamma-aminobutyric acid (GABA) are the most frequently occurring inhibitory neurotransmitters. Thus, brief variations in the transmembrane voltages provide the fundamental basis for neuronal signalling.

As well as generating action potentials that propagate along the axon, the postsynaptic potentials also generate local extracellular field potentials due to ion fluxes in the extracellular space around the neurons. Although it might seem that the most obvious source for the potentials recorded in the EEG is the action potential, scalp EEG is known to be the result of the summation of extracellular field potentials generated by populations of neurons, mainly from pyramidal cells (Martin, 1991). This neural activity is transmitted to the recording electrodes by volume conduction and the signal generated decreases in amplitude the greater the distance between the source and point of recording. In order to generate signals large enough to be detected in the surface EEG, the activity of tens of thousands of neurons (around 1 cm^2 of cortical

surface) needs to be summated. In addition, for the activity to be summated effectively, the neurons must be active synchronously (Pfurtscheller and Lopes da Silva, 1999). In the cortex, the summated postsynaptic potentials are felt to be generated from pyramidal cells whose regular, parallel arrangement with their apical dendrites oriented perpendicular to the cortical surface facilitates summation (Martin, 1991). Electrical activity in the deep nuclei produces surface potentials of very low amplitude that are overwhelmed by cortical activity. EEG signals thus reflect the dynamics of electrical activity in populations of cortical neurons underlying the electrode.

But how can the membrane properties of neurons facilitate neuronal transmission? Following intracellular recordings, four cellular types of neuron have been described: regular-spiking (RS), intrinsically bursting (IB), fast rhythmic bursting (FRB; also known as chattering) and fast-spiking (FS) neurons (Steriade, 2001). RS neurons constitute the majority of cortical neurons and display trains of single spikes that adapt to maintained stimulation. IB neurons generate clusters of action potentials with clear spike inactivation, followed by hyperpolarisation and neuronal silence. FRB neurons give rise to high frequency (300-600 Hz) spike bursts recurring at fast rates. FS neurons fire thin (< 0.5 ms) action potentials and tonically sustain very high firing rates without frequency adaptation. In general, RS and IB neurons are pyramidal-type neurons whereas FS firing patterns are conventionally regarded as local GABAergic cells. Neurons displaying FRB firing patterns are either pyramidal-shaped neurons or local circuit, sparsely spiny or aspiny interneurons. However, this is a simplistic classification and each of the above firing patterns does not necessarily apply to a single class of neurons (Steriade, 2001).

The oscillations in membrane potentials of neurons and muscle cells might, therefore, be a mechanism for communication. This could work in two ways. Firstly, an individual neuron could oscillate and act as a pacemaker cell, entraining networks of cells (Traub and Miles, 1991). Secondly, network rhythmicity could arise as a result of interactions between neuron subunits, each of which possesses an intrinsically determined frequency preference (Llinas, 1988). The different firing properties of neurons in the neocortex contribute to its network behaviour. There is good evidence that individual neurons have frequency preferences that enable them either to generate spontaneous membrane voltage oscillations, or to respond best to inputs within a narrow frequency window (Hutcheon and Yarom, 2000). Such intrinsically defined properties of individual neurons will have a role in determining the dynamics of coherent brain activity.

1.1.2 EEG oscillatory rhythms

Five fundamental EEG rhythms have been identified in humans and are defined by the frequency range: delta (<4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (>30 Hz) rhythms (Misulis, 1997). Alpha rhythms are generally associated with a state of relaxed wakefulness and they are recorded best over the temporal and occipital lobes in awake subjects who have their eyes closed. When the brain is aroused and actively engaged in mental activities, it generates beta rhythms, typically low in amplitude and distributed maximally over frontal and central regions. Theta and delta rhythms imply drowsiness, sleep or a deep meditative state of mind (Misulis, 1997). Oscillations greater than 30 Hz are known as gamma rhythms and are implicated in establishing rapid coupling or synchronising spatially separated cell assemblies (discussed below). This EEG rhythm classification only partially reflects the functional variation of rhythmic activities. For example, EEG activities within the alpha range may be distinguished by their dynamics, place of generation and relation to certain behavioural acts (Niedermeyer 1997; Lutzenberger 1997; Pfurtscheller et al., 1997).

To generate an EEG signal distinct from background noise, there has to be sufficient synchronised activity in a large number of neurons proximal to the electrode. There are at least two mechanisms by which such local synchrony can occur. Synchrony can be a consequence of a common input, either remote from the area of cortex being recorded or within it. Synchrony can also be a result of an emergent population oscillation within the network of cells under the electrode. In the latter case, it is the properties of the network as a whole that generate the oscillations observed. Examples of emergent network oscillations (particularly at gamma and beta frequencies) in hippocampus and neocortex show a strong dependence on the activity of inhibitory interneurons within the oscillating area (Whittington et al., 2000).

The generation of beta oscillations in cortical structures seems inextricably linked with the generation of gamma oscillations. Auditory stimuli can 'evoke' gamma rhythms followed by beta (Haenschel et al., 2000). The beta component is strongest in response to novel stimuli, whereas the gamma habituates (as is seen in vitro: Doheny et al., 2000). Gamma, mixed with beta, appears after exposure to a visual stimulus that needs to be remembered briefly (Tallon-Baudry et al., 1999). In response to pictures or words, there is increased temporal/parietal coherence of EEG activity in the 13-18 Hz band (von Stein et al., 1999). In vitro models may provide clues to the mechanism and function of in vivo gamma and beta oscillations. In the CA1 region of rat hippocampus

slices, gamma and beta oscillations, lasting seconds, are most readily induced by two-site stimulation (Whittington et al., 1997). Beta oscillation generation requires that the stimulus be sufficiently strong, so that pyramidal cells and interneurons remain depolarised enough to fire (Faulkner et al., 1999). Interneurons then continue to fire a network gamma rhythm, whereas pyramidal cells skip beats, switching to beta frequency because of increased afterhyperpolarisations (Whittington et al., 1997).

Gamma and beta oscillations are synchronised by different cellular mechanisms in vitro (Traub et al., 1999). IPSPs are critical for shaping gamma oscillations and EPSPs onto interneurons are necessary for long-range synchrony. However, EPSPs between pyramidal cells within rat hippocampal CA1 do not play an essential role in gamma oscillations. In contrast, EPSPs between rat CA1 pyramidal cells do appear to be critical for generating a synchronised beta rhythm, even within a local population of neurons, although such EPSPs may not be necessary for individual pyramidal cells to fire at beta frequency (Traub et al., 1999). The contrasting cellular mechanisms of gamma and beta oscillations allow synaptic plasticity to regulate long-range synchrony of the two frequency ranges by different means.

Gamma oscillations have been found in vivo in various limbic, olfactory and neocortical structures, occurring spontaneously, in response to sensory stimulation, in association with motor performance, or after electrical stimulation of certain brainstem and diencephalic nuclei (Traub et al., 1999). The gamma oscillation is not proposed to represent information itself, but rather to provide a temporal structure for correlations in the neurons that do encode specific information (Jeffreys et al., 1996).

Oscillatory activity is thus a widespread feature of normal brain behaviour. The brain can be considered as a collection of dynamic neural networks, with the activity in any particular area being modulated by the actions of, and interactions with, other functionally related cortical and subcortical structures. Cortical oscillatory activity has been proposed as a mechanism that may bind together distributed networks into coherent ensembles (Singer and Gray, 1995). This concept of 'binding' has been applied to perception, object recognition, attention, memory formation and recall, motor control, sensorimotor integration, language processing and logical inference (Engel and Singer 2001). It is possible that the cortical oscillatory activity acts to select responses and to associate them for further processing by coordinating the temporal pattern of distributed discharges. The planning and execution of movements involves parallel processing in areas of the cerebral cortex that are separated in their activity both

temporally and spatially. However, the mechanism by which these areas combine to produce a coordinated movement is unknown.

1.2 Task-specificity of oscillations in the motor system

A variety of oscillations with different amplitudes and frequencies can be observed within cortical and subcortical sensorimotor regions. Task-related changes in regional brain activation are a consequence of altered firing rates of single neurons involved in the processing of the task. At the level of neuronal assemblies, this can be measured as local field potentials that frequently have oscillatory properties (Gray and Singer, 1989; Murthy and Fetz, 1992). Synchronised groups of action potentials in afferent fibres generate EPSPs in the dendritic areas to produce the corresponding local field potentials and surface potentials in the EEG (Martin, 1991).

Alterations in the power of the EEG signal measured at a given frequency indicate the degree of synchrony between neurons generating the signal. Increases or decreases in EEG power that occur with a task have been termed event-related synchronisation (ERS) or desynchronisation (ERD), respectively. These types of time-locked change are detected by frequency analysis of the EEG signal data and are considered to represent frequency-specific changes of the ongoing EEG activity (Pfurtscheller and Lopes da Silva, 1999). A similar method of analysing EEG in relation to movement is to calculate task-related power (TRPow) and coherence (TRCoh) changes (Andres et al., 1999; Hummel et al., 2002). This method computes power and coherence in the frequency domain during steady-state behavioural tasks so is not time-locked to single events. The latter method is used in the work presented in this thesis.

Considered simply, at rest, relatively widespread synchronised alpha and beta oscillations reflect an idling state of the brain (Pfurtscheller et al., 1996a,b). Idling rhythms are either established in cortical networks or driven by a common thalamic pacemaker. A task-related oscillatory burst in a circumscribed subset of neurons inside a particular cortical region could then occur out of synchrony with the idling rhythm but in the same frequency band. This burst would cause a local decrease in power (ERD or TRPow decrease) through phase cancellation. If bursts were then synchronised with concomitant bursts in other activated areas, there would be increased inter-regional coherence. There is much evidence that such oscillatory changes are task-related.

1.2.1 Event-related desynchronisation

Lower alpha (7-10 Hz) desynchronisation is obtained in response to almost any type of task. It is topographically widespread and probably reflects general task demands and attentional processes. Upper alpha (10-12 Hz) desynchronisation is often topographically restricted and develops during the processing of sensory-semantic information over parieto-occipital areas (Klimesch, 1999). Voluntary movement results in a circumscribed desynchronisation in the upper alpha and lower beta bands, localised close to the sensorimotor areas (Toro et al., 1994a; Stancak and Pfurtscheller, 1996c; Leocani et al., 1997). This ERD starts about 2 s prior to movement-onset over the contralateral sensorimotor cortex and becomes bilaterally symmetrical immediately before execution of movement. Both the alpha and beta ERD are somatotopically organised (Crone et al., 1998) such that with hand movement, ERD can be seen over the hand area of the sensorimotor cortex while surrounding areas demonstrate ERS (Pfurtscheller et al., 1997).

Voluntary finger movement not only results in an alpha band ERD over primary sensorimotor areas, but also in an ERD over the supplementary motor area (SMA; Pfurtscheller and Berghold, 1989; Derambure et al., 1993). The event-related coherence between the contralateral sensorimotor area and the SMA shows high alpha band coherence during rest and a decrease in coherence during planning and execution of movement. Thus, this SMA rhythm is linearly coupled with the central alpha (mu) rhythm during rest, perhaps caused by cortico-cortical connections or mutual influence from an underlying thalamic generator. During planning and preparation of movement, these rhythms are desynchronised when each of the underlying cortical areas becomes active and the degree of synchrony between them decreases.

1.2.2 Event-related synchronisation

Beta band synchronisation has been reported in the first second after termination of movement (Pfurtscheller et al., 1996b; Salmelin et al., 1995). The beta ERS is seen over both hemispheres but is more marked over the contralateral sensorimotor area and has a somatotopic organisation (Salmelin et al., 1995; Neuper and Pfurtscheller, 1996). In addition, the beta ERS coincides with a reduction in motor cortical excitability (Chen et al., 1998) and is also found after an imagined movement (Neuper and Pfurtscheller, 2001).

In the gamma band (around 40 Hz), Pfurtscheller and Andrew (1999) found a short-lasting ERS over the contralateral sensorimotor area and SMA, while no ipsilateral ERS was present. No interhemispheric gamma coherence was found between the left and right sensorimotor areas during movement. However, there was an increase in gamma coherence between the contralateral sensorimotor area and the SMA directly preceding movement onset. This might suggest that functional interaction in the gamma band takes place between SMA and motor areas during the final stages of movement preparation (Pfurtscheller et al., 1993). Oscillations in the gamma band appear appropriate to establish rapid coupling or synchronisation between spatially separated cell assemblies (Singer, 1993).

Therefore, ERD can be interpreted as an electrophysiological correlate of activated cortical areas involved in the processing of sensory or cognitive information or in the production of motor behaviour (Pfurtscheller, 1992; Pfurtscheller and Lopes da Siva, 1999). An increased or more widespread ERD could be the result of the involvement of a larger neural network or more cell assemblies in information processing. In contrast, ERS can be interpreted as the cooperative or synchronised behaviour of a large number of neurons (Pfurtscheller et al., 1996b).

1.2.3 Task-related coherence changes

A significant increase in TRCoh has been demonstrated between the visual and motor cortex during a visuomotor tracking task (Classen et al., 1998) most clearly defined in the low beta frequency range (13-21 Hz). This increase was not seen during purely visual and purely motor control tasks. Increases in EEG-EEG coherence during motor execution occur over bilateral fronto-central regions linking SMA and bilateral sensorimotor areas (Andrew and Pfurtscheller, 1995, 1996; Leocani et al., 1997; Manganotti et al., 1998; Gerloff et al., 1998; Andres et al., 1999; Ohara et al., 2001). Enhanced functional coupling among different cortical regions has generally been considered to reflect excitatory interactions. From these studies, it has become clear that higher task demands are reflected by changes in the functional coupling between cortical areas and not only by changes in regional activation.

There is thus considerable evidence that EEG modulations in the alpha and beta bands may represent activities closely related to the preparation and execution of movement, as witnessed by movement-related modulations in power (Pfurtscheller et al. 1994, 1997; Salmelin and Hari 1994; Toro et al. 1994a, b; Stancak and Pfurtscheller 1996a,b,c; Leocani et al. 1997) and coherence (Gerloff et al. 1998; Manganotti et al. 1998). In particular, EEG activities in the alpha range (Pfurtscheller et al. 2000; Worden

et al. 2000; Hummel et al., 2002) may be related to predominantly inhibitory processes. Inhibition in neural networks is important, not only to optimise energy demands, but also to limit and control excitatory processes. Synchronised alpha band rhythms during mental inactivity (idling) may be important to introduce powerful inhibitory effects (Klimesch, 1996). During an inhibitory motor paradigm, it has been demonstrated that the reduction in cortical excitability (as measured by MEP amplitude) was accompanied by a significant increase of alpha oscillations (11-13 Hz) over the sensorimotor areas (Hummel et al., 2002).

1.3 TMS as a tool for disrupting cortical function

1.3.1 Basic principles of TMS

TMS is being increasingly used as a non-invasive tool for studying human brain physiology (reviews: Chen, 2000; Hallett, 2000; Siebner and Rothwell, 2003; Kobayashi and Pascual-Leone, 2003). It is based on the principle of electromagnetic induction, as discovered by Faraday in 1838. A brief pulse of electrical current is passed through a coil of wire, generating a magnetic field with lines of flux passing perpendicularly to the plane of the coil. This magnetic field in turn induces a perpendicular electrical field. For 'magnetic stimulation' of the human cerebral cortex, the stimulating coil is placed over a subject's head, resting on the scalp overlying a specific cortical area. If the pulse of electrical current passing through the coil has sufficient strength and short enough duration, rapidly changing magnetic pulses are generated that can penetrate scalp and skull to reach the brain with negligible attenuation. If the induced electrical field falls within a conductor, such as nervous tissue, then current will flow. The site of stimulation of a nerve fibre is the point along its length at which sufficient current to cause depolarisation passes through its membrane. The intensity of stimulation can be controlled by changing the intensity of current flowing in the coil, thus changing the magnitude of the magnetic field and of the secondary electrical field. Using a figure-of-eight shaped coil also provides a more focal stimulation than using a circular coil (Rossini and Rossi, 1998).

The current induced by TMS can spread beyond the intended cortical target area, although the precise extent of neuronal activation is not known. Measurements from the surface of the spinal cord have shown that TMS can evoke an early direct wave and up to four further indirect waves (Edgley et al., 1997). At low intensities, TMS over the hand area of the motor cortex activates corticospinal neurons transynaptically, producing indirect responses (Day et al., 1989; Burke et al., 1993; Kaneko et al., 1996; Nakamura et al., 1996; Di Lazzaro et al., 1998). Direct axonal activation only occurs at

high stimulation intensities, or if the magnetic stimulus induces current to flow in a latero-medial direction along the central sulcus (Werhahn et al., 1994; Kaneko et al., 1996; Nakamura et al., 1996; Di Lazzaro et al., 1998). In contrast, transcranial electrical stimulation (TES) appears to stimulate pyramidal tract cell axons in the subcortical white matter (direct responses) and only activates pyramidal neurons transynaptically at higher intensities (Di Lazzaro et al., 2001).

1.3.2 Methods of giving TMS

TMS can be applied in three ways: single-pulse, paired-pulse and repetitive. Single-pulse TMS can be used to measure central conduction time, cortical excitability and to map muscle representations in the motor cortex. Paired-pulse techniques are useful in assessing cortical excitability (discussed further in section 2.5.5). Repetitive TMS (rTMS) can be used to modulate cortical excitability and to disrupt cortical areas transiently in order to investigate their functions.

1.3.3 Clinical applications of TMS

TMS delivered to different levels of the motor system can provide information about the excitability of the motor cortex (i.e. the responsiveness to stimulation), the functional integrity of intracortical neuronal structures, the conduction along corticospinal, corticonuclear and callosal fibres, as well as the function of nerve roots and peripheral motor pathways to the muscles.

When TMS is applied to the motor cortex at appropriate stimulation intensities, motor evoked potentials (MEPs) can be recorded from contralateral limb muscles. The amplitude of the MEP reflects the integrity of the corticospinal tract, the excitability of motor cortex and nerve roots, and conduction along the peripheral motor pathway to the muscles. Patients with dysfunction at any level along the corticospinal pathway can have abnormal MEPs. After a stroke, for example, the absence of contralateral MEPs suggest a poor outcome (Escudero et al., 1998). Single-pulse TMS can be used to measure the motor threshold (see section 2.5.3) of muscles (the lowest stimulation intensity necessary to evoke MEPs in that muscle). Motor threshold is often increased in diseases that affect the corticospinal tract, such as multiple sclerosis, stroke and brain or spinal cord injury (Davey et al., 1998; Chistyakov et al., 2001; Boniface et al., 1991, 1994). Central motor conduction time (CMCT) is defined as the latency difference between the MEPs induced by stimulation of the motor cortex and those evoked by spinal (motor root) stimulation. Marked lengthening of the CMCT suggests

demyelination of pathways and can be seen in multiple sclerosis and cervical myelopathies (Hess et al., 1987; Boniface et al., 1991; de Noordhout et al., 1998).

Therefore, in a clinical setting, TMS measurements can be useful in making an early diagnosis, and in some cases for predicting prognosis, in a variety of neurological disorders (review: Kobayashi and Pascual-Leone, 2003).

1.3.4 Disruptive rTMS and virtual lesions

A train of TMS pulses of the same intensity applied to a single brain area at a given frequency (ranging from 1-20 Hz or more) is known as repetitive TMS (rTMS). The higher the stimulation frequency and intensity, the greater is the disruption of cortical function during the train of stimulation. This disruptive property of rTMS can be used to create a 'virtual brain lesion', thus providing a method of investigating the effects of cortical dysfunction (Walsh and Rushworth, 1999). For example, rTMS of the speech area can cause speech arrest (Pascual-Leone et al., 1991) and if applied over the frontal cortex, can lead to recall deficits (Grafman et al., 1994). The assumption is that if activity in a particular cortical area is essential for a task, then rTMS to that area will disrupt task performance. However, non-specific effects on attention and arousal (such as the noise of the coil discharging or the scalp sensation produced) should be taken into account. Comparing the effects of stimulation at different sites or using sham stimulation can be used as control methods.

1.3.5 Modulating cortical excitability with rTMS

The frequency, intensity and duration of stimulation all influence the effects of rTMS. In addition to the immediate effects during the train of stimulation, rTMS can also induce modulation of cortical excitability that outlasts the duration of the train for several minutes. Low frequency (1 Hz and below) rTMS usually causes suppression of corticospinal excitability (Chen et al., 1997b; Muellbacher et al., 2000). High frequency stimulation (5 Hz and above) leads to facilitation of excitability (Pascual-Leone et al., 1994, 1998; Berardelli et al., 1998; Peinemann et al., 2000; Wu et al., 2000). Stimulation at intensities below resting motor threshold usually requires longer trains before any lasting effect is seen (Maeda et al., 2000a, b).

The mechanisms underlying the lasting modulations in cortical excitability are still unclear. Long-term potentiation (Gustafsson and Wigstrom, 1988) and depression (Christie et al., 1994) of cortical synapses or closely related neuronal mechanisms have been suggested as possible mechanisms to explain the effect of high and low frequency rTMS, respectively. Animal studies suggest that modulation of

neurotransmitters (Ben-Shachar et al., 1997; Keck et al., 2000) and gene induction (Hausmann et al., 2000; Ji et al., 1998) may contribute to these long-lasting modulatory effects of rTMS. Human studies combining rTMS with functional neuroimaging techniques (for example, fMRI and PET) have detected suppressed cerebral blood flow and metabolism in the stimulated area after 1 Hz rTMS to the motor cortex and increased blood flow after 10-20 Hz stimulation (Pascual-Leone et al., 1998; Siebner et al., 1998; Fox et al., 1997).

Several TMS (Wassermann et al., 1998; Gerschlager et al., 2001; Munchau et al., 2002) and functional imaging (Siebner et al., 2000; Paus et al., 2001; Strafella et al., 2001) studies have revealed lasting effects of rTMS at sites distant from the stimulation site. Motor cortex studies demonstrate that the threshold for producing effects at a distance depends on the intensity of stimulation. Local inhibitory circuits are activated first, and projecting neurons at higher intensities. In the motor cortex hand area, corticospinal neurons have a slightly lower threshold than transcallosal projections (Ferbert et al., 1992; Hanajima et al., 2001). Therefore, rTMS effects may be limited to the area of stimulation at low intensities but spread to interconnected areas at higher intensities.

In summary, TMS has become a useful tool for investigating and modulating human brain function. Single and paired pulse TMS techniques can describe changes in the excitability of cortico-cortical and cortico-subcortical connections. TMS can be used to disrupt activity in any accessible cortical area to explore the functional relevance of cortical reorganisation. Repetitive TMS can produce changes in the excitability of cortical circuits that outlast the period of stimulation, creating the possibility of intervening directly with the mechanisms of cortical plasticity in the intact human cortex.

1.4 Functional relevance of cortico-cortical interactions in the human motor system

The functional relevance of cortical oscillations in the motor system is suggested by the modulations seen in EEG power and coherence with motor tasks (section 1.2). However, this is only circumstantial evidence of functional relevance. If cortico-cortical coherence is an activity functionally related to movement and not just an epiphenomenon, then it should be possible to demonstrate that manipulating cortico-cortical coupling could thereby change motor function or vice versa. Manipulation of coupling could be achieved, for example, by using pharmacological interventions or by using rTMS.

1.4.1 Pharmacological interventions for manipulating coupling

Using cortico-cortical coherence as a marker of functional coupling between different cortical areas, Cassidy and Brown (2001) investigated whether functional coupling is impaired in Parkinson's disease, a condition characterised by the relatively selective degeneration of dopaminergic neurons in the ventral mesencephalon. Differences in EEG-EEG coherence were determined in Parkinsonian patients while they performed various motor tasks both on and off the dopamine precursor, levodopa. The subjects tracked a visual target using their wrist or copied the same movement from memory (i.e. a tracking task versus a self-generated task). Imaging studies have compared these two tasks and shown a difference in cortical activation patterns (Jueptner et al., 1996). It was hypothesised that if synchronisation were to have a mechanistic role in binding distributed but related cortical activities, then its pattern should also differ between tasks. Extensive cortico-cortical coherence was seen only in the presence of exogenous levodopa stimulation. Furthermore, this coupling was task-specific with different patterns of inter-regional coherence seen for the different tasks. Failure of the appropriate modulation of cortico-cortical coherence and hence coupling by the basal ganglia may be important in explaining the extensive nature of psychomotor dysfunction in Parkinson's disease.

Recently, the effects of the benzodiazepine diazepam, a GABA(A) agonist, and its antagonist, flumazenil, have been studied on motor cortical oscillations and corticomuscular coherence during different hand motor tasks (Baker and Baker, 2003). Sensorimotor cortex EEG recordings showed oscillations around 10 and 20 Hz that were modulated with task performance, and strongest during periods of tonic contraction. The 20 Hz oscillations were coherent with contralateral EMG. EEG power around 20 Hz increased with diazepam, the effect being reversed with administration of flumazenil. Diazepam injection also reduced 20 Hz corticomuscular coherence. The results suggest that 20 Hz oscillations in the sensorimotor cortex are at least partially produced by local cortical circuits reliant on GABA(A)-mediated intracortical inhibition.

1.4.2 Manipulating coupling using rTMS

RTMS can be used to disrupt cortical activity for periods of time after the end of the stimulation. Several authors have described lasting effects of rTMS on cognitive functions in healthy subjects (Kosslyn et al., 1999; Hilgetag et al., 2001). Effects on mood have been described after rTMS to the frontal cortex (Triggs et al., 1999) and improved analogical reasoning was seen after rTMS over the left prefrontal cortex (Boroojerdi et al., 2001). This feature provides an opportunity to study mechanisms of

acute cortical reorganisation in the healthy human brain. Plasticity of the mature human brain refers to the processes involved in learning, memory and neural repair. Effects on cortical excitability or on functional cortico-cortical connectivity have been demonstrated but it is much more difficult to show effects of rTMS on motor behaviour. For example, low frequency rTMS over the primary sensorimotor area reduces corticospinal excitability as measured using TMS paired-pulse techniques. However, several studies looking at motor behaviour following such stimulation have failed to show significant changes, for example, in finger-tapping speed (Chen et al., 1997b), in peak acceleration or maximum pinch force of brisk finger movements (Muellbacher et al., 2000) and in handwriting movements (Siebner et al., 1999c). Reasons for this lack of measurable effect on motor behaviour include the intrinsic variability of volitional movements and the fact that the motor tasks studied were highly over-learned. Thus, the motor system might be able to compensate for disruption of cortical function by rTMS. Muellbacher et al. (2002) were able to show that suprathreshold 1 Hz rTMS to the primary motor cortex interfered with motor performance during learning but not when the motor skill had been consolidated. RTMS has been shown to interfere with motor behaviour if the tasks studied are more complex (Pascual-Leone et al., 1998).

Only a couple of studies to date have attempted to manipulate EEG-EEG coherence using repetitive TMS. Jing and Takigawa (2000) showed that 10 Hz rTMS given to the left frontal region (100% of motor threshold; given in two trains of 3 sec each) caused an increase in directed, but not standard, coherence in the alpha band between the stimulated cortex and other connected brain areas. However, this was not correlated with any behavioural change in brain function. A recent study by Chen et al. (2003) looking at voluntary thumb movements, demonstrated that low frequency (0.9 Hz) rTMS over the premotor cortex for 15 minutes gave rise to a reduction of the task-related power decrease in the alpha and beta bands, selectively increased the task-related coherence changes between cortical motor areas in the upper alpha band, and caused a decrease in cortico-muscular coherence. These effects lasted about 15 minutes after the end of the rTMS train. Again, behavioural changes were not assessed.

1.5 Aims of this research

Task-related changes in regional brain activation are a consequence of altered firing rates of single neurons involved in the processing of the task. At the level of neuronal assemblies, this can be measured as local field potentials (LFPs), which frequently have oscillatory properties (Gray and Singer, 1989; Murthy and Fetz, 1992).

Synchronised groups of action potentials in afferent fibres generate wave-like excitatory post-synaptic potentials (EPSPs) in the dendritic areas to produce the corresponding field and surface potentials in the EEG (Martin, 1991). Previous studies have emphasised a close relationship between EEG oscillatory activity, oscillatory changes in LFPs and variations of single neuron firing rates, and the generation of EPSPs and inhibitory post-synaptic potentials (IPSPs) (Singer, 1993; Contreras and Steriade, 1995; Donoghue et al., 1998; Fries et al., 2001). Animal studies have shown that changes in inter-regional correlated oscillations can reflect behavioural measures (Murthy and Fetz, 1992; Sanes and Donoghue, 1993; Singer, 1993, Bressler, 1995). Inter-regional functional cortical interactions can be assessed non-invasively in humans using task-related power and coherence analysis of oscillatory activities occurring in different brain regions.

RTMS is a non-invasive method of temporarily interfering with local cortical function (Pascual-Leone et al., 1994; Grafman et al., 1994; Cohen et al., 1997). If changes in regional oscillatory activity reflect cortical function and if cortical function can be disrupted using rTMS, then combining the two techniques allows a method of investigating the relationship between cortical oscillatory activity and motor function. There are no studies in the literature to date, other than those described here, that attempt to manipulate both EEG-EEG coherence and motor function using repetitive TMS.

The aims of this research are to demonstrate that rTMS can both manipulate cortico-cortical coherence and disrupt motor behaviour, and to demonstrate that the changes in coupling and motor behaviour are associated. Chapter 2 describes the methodology common to the experiments. Chapters 3 and 4 investigate the effects of 1 Hz and 5 Hz motor cortex rTMS, respectively, on cortico-cortical coherence and reaction times. Effects of 5 Hz rTMS to the supplementary motor area on coherence and bimanual coordination are studied in chapter 5. The notion that the human motor cortex is capable of acute, temporary changes following disruption of function by rTMS is explored in chapter 6. In chapter 7, the functional relevance of EEG-EEG connectivity is further investigated in stroke patients, correlating changes in coupling with motor recovery from stroke. The experimental results are then discussed together in chapter 8 and conclusions drawn.

2 Common methodology

This chapter describes the methodological and analytical techniques that are common to the experiments performed in chapters 3 to 7. Where necessary, specific methodology is given at the start of each experimental chapter.

This thesis details experiments in which the effects of repetitive TMS on EEG signals and behavioural tasks are studied, including how cortical connectivity is altered after stroke. In chapters 3-6, rTMS was applied to cortical motor areas of healthy subjects, at either high (5 Hz) or low (1 Hz) frequency. EEG was recorded and studied in the frequency domain. As well as comparing recordings before and after stimulation, the connectivity between EEG signals was investigated with the subjects at rest and while performing a behavioural task. The nature of the task varied between experiments. Bilateral tonic wrist extension and finger abduction were used in chapters 3 and 4, as opposed to bimanual hand coordination tasks (chapter 5) and right hand index finger tapping (chapter 6). The effect of rTMS on a simple choice reaction time task was also studied (chapter 4). In chapter 7, EEG signals were recorded while subjects performed a unimanual handgrip task. The sections below describe the techniques involved in making such recordings.

2.1 Experimental subjects

All experiments were performed at the Sobell Department of Motor Neuroscience and Movement Disorders. All subjects were studied with their informed consent and with the approval of the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery, in accordance with the Declaration of Helsinki.

The healthy volunteers who took part in the experiments were recruited from among the staff at the Institute of Neurology. In addition, several friends and relatives of the stroke patient group volunteered as controls for the experiment in chapter 7. This control group was age-matched with the stroke group. Stroke patients (chapter 7) were recruited from the outpatient and inpatient services at the National Hospital for Neurology and Neurosurgery. All subjects, both healthy controls and stroke patients, were right-handed according to the Edinburgh handedness scale (Oldfield, 1971).

2.2 Electromyography recording

For surface EMG recordings (chapters 3, 4, 6 and 7), the skin was prepared with isopropyl alcohol and gentle abrasion using a blunt needle. Bipolar Ag-AgCl electrodes (9 mm diameter) were secured with tape (Blenderm, 3M, St. Paul U.S.A) to the relevant muscle and an earth electrode attached on a body part distant to the recording site. Electrodes were positioned over the muscle belly 2-3 cm apart. In the case of FDI muscles, one electrode was attached over the muscle belly and the second over the first metacarpophalangeal joint. Conducting gel (Parker Laboratories Inc., U.S.A) was inserted between the skin and the electrode to reduce impedance. Once placed, electrodes remained fixed for the duration of an experiment.

Surface EMG recordings were made in the experiments for three main reasons. Firstly, for monitoring subjects performing unimanual tasks to ensure that the non-performing hand was at rest and that there were no mirror movements. Secondly, to provide a way of controlling the force level achieved during a tonic muscle contraction. Thirdly, to ensure that epochs of EEG data used for analysis were taken specifically from periods of EMG activity ('active' data) or from periods of absent EMG activity ('rest' data).

To determine the maximal voluntary contraction (MVC) of a muscle, manual resistance was given to the relevant body part and the mean full-wave rectified EMG level over the most active five-second period calculated. The relative force of contraction was estimated using the ratio between the mean rectified value during contraction and the rectified MVC.

The experiments in chapters 3, 4 and 7 required the subjects to maintain a steady force level during tonic muscle contraction. The importance of controlling force level during cortical activity studies has been suggested by several authors. Positron emission tomography (PET) studies have shown that brain activation in the primary and secondary motor areas was linearly correlated with force up to 40% of maximal (Dettmers et al., 1995, 1996). Mima et al. (1999) showed that during weak to moderate isometric contractions between thumb and little finger (up to 60% of maximal), alpha band power in the contralateral sensorimotor areas was inversely linearly correlated with the force, whereas beta band EEG power did not change significantly. Cortico-muscular coherence has been observed in the gamma band (30-60 Hz) during maximal isometric contraction and in the beta band (15-30 Hz) during weak contractions (Brown et al., 1998; Mima et al., 1999, 2000b, 2001a).

2.3 Electroencephalography recording

Surface EEG recordings (chapters 3-6) were performed using 9 mm Ag-AgCl electrodes attached to the scalp with collodion (SLE diagnostics, Surrey, U.K.). As with EMG electrode placement, the skin was prepared beforehand with isopropyl alcohol and gentle abrasion. Once placed, electrodes remained fixed for the duration of an experiment.

For the experiments in chapters 3-6, surface electrodes were positioned with reference to the International 10-20 System (Misulis, 1997). Electrode montages varied slightly between experiments in terms of EEG recording sites. However, the method of positioning an electrode at each site remained constant. C3 and C4 were positioned using single pulse TMS to locate the left and right motor hand areas, respectively, and electrodes placed at these sites. Further electrodes were placed 2.5cm apart with reference to C3 and C4. These sites were termed, from anterior to posterior, F3/F4, FC3/FC4, and CP3/CP4, in correspondence (+/- 1 cm) with the 10-20 system. EEG electrodes were also sited directly at FCZ, CZ, P3, P4, PO3 and O1 (figure 2.1). F3/4, FC3/4, C3/4, and FZ are likely to overlie the dorsolateral prefrontal and lateral premotor areas, primary sensorimotor cortices and medial prefrontal cortex, respectively, whereas FCZ and CZ were considered to overlie mesial motor areas, particularly the cingulate and supplementary motor areas (Homan et al., 1987; Steinmetz et al., 1989; Andres et al., 1999; Asada et al., 1999).

In chapter 7, a different technique of electrode placement was used. Scalp EEG was recorded from 64 (10 mm Ag-AgCl) surface electrodes mounted on a cap (Easy Cap, Falk Minow Services, Germany) with linked mastoid references (figure 2.2). Conducting gel (Abralyt 2000, Falk Minow Services, Germany) was applied to the skin after gentle abrasion with isopropyl alcohol. Electrode impedance was kept below 5 kΩ, monitored using Neuroscan software (SCAN 4.2, Neurosoft Inc. U.S.A.). In addition, eye movements were recorded with vertical electro-oculogram (VEOG) electrodes.

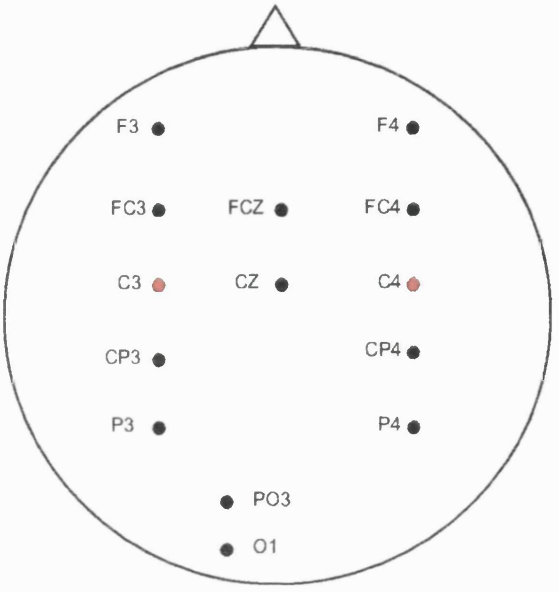


Figure 2.1. The position of EEG electrodes used for experiments in chapters 3-6. Electrode locations are based on the International 10-20 system.

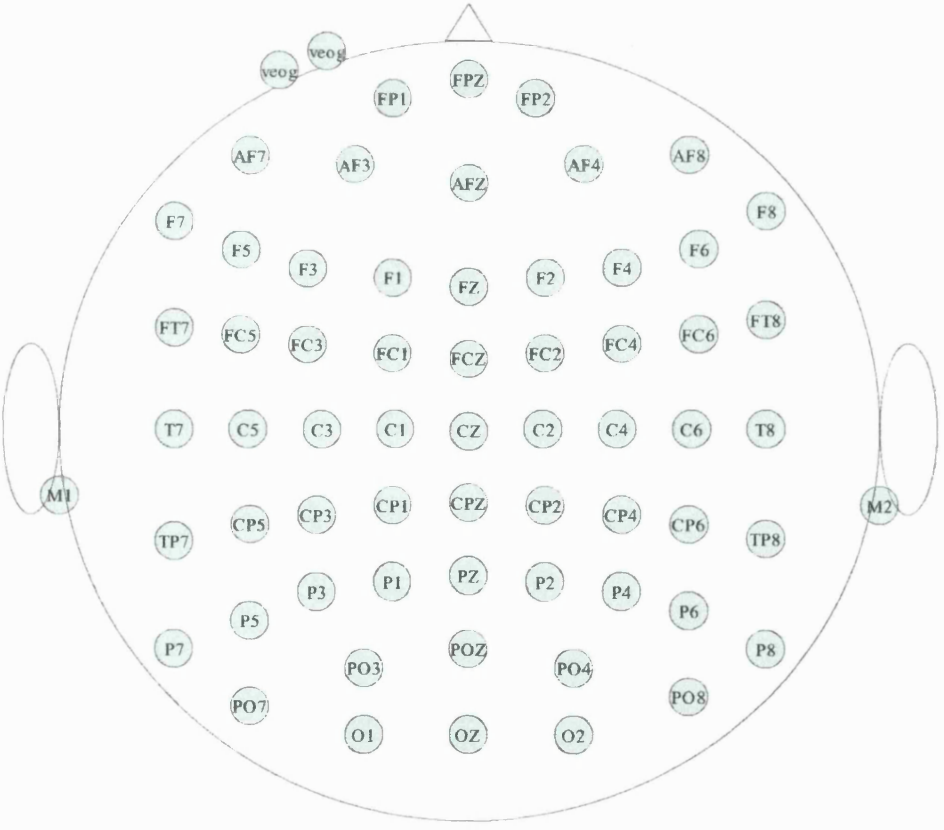


Figure 2.2. The EEG electrode array used in chapter 7. The 64 surface electrodes were mounted on a cap worn by the subject. VEOG = vertical electro-oculogram electrodes.

Electrical currents can spread throughout the conducting tissues of the brain. Scalp EEG therefore reflects intracranial electrical potentials and thereby the activity of neural generators within the brain, although high frequencies may be filtered out by the dura, skull and scalp. Scalp EEG is measured against specific reference electrodes. The underlying assumption is that the reference should be electrically quiet, which is not always the case (Nunez et al., 1997). It has become apparent that the effects of volume conduction (Nunez et al., 1997) and the use of common reference electrodes (Fein et al., 1988; Rappelsberger and Petsche, 1988; Classen et al., 1998) may distort coherence estimates and thus the interpretation of such analysis.

Volume conduction and common reference inflate coherence estimates (Nunez et al., 1997). In addition, they may lead to misleading changes in coherence during dynamic conditions. For example, alpha and beta frequency EEG oscillatory activity over the premotor and primary sensorimotor areas in humans typically decreases in power during motor tasks (event-related desynchronisation; Pfurtscheller et al., 1994,1997; Salmelin and Hari, 1994; Toro et al., 1994a,b; Stancak and Pfurtscheller, 1996a,b,c; Leocani et al., 1997). This might cause a relative increase of the common reference signal and/or occipital alpha (volume-conducted) activity contamination over the areas of interest. This increase in the ratio of common signals, either volume-conducted or reference, might result in an apparent increase of coherence without a change in functional coupling (Andrew and Pfurtscheller, 1996).

There are several different methods that can be used to derive EEG: standard common reference, averaged reference, linked earlobe reference, balanced non-cephalic electrode reference, bipolar recording and current source density estimation (Laplacian derivatives). However, there is considerable debate over which is the most appropriate way to avoid inflated EEG-EEG coherence estimates (Nunez et al., 1997; Nunez et al., 1999). Laplacian derivatives, which remove the common reference effect, may, on the contrary, lead to an underestimate of inter-regional EEG coherence especially for low frequencies including alpha (Mima et al., 2000a).

In chapters 3 and 4, a bipolar derivation was adopted. Electrodes were linked to form a chain of three bipolar leads on each side (F3-FC3, FC3-C3, C3-CP3, F4-FC4, FC4-C4, C4-CP4) and a single ipsilateral occipital bipolar lead (PO3-O1). By using bipolar leads without common electrodes for coherence analysis, the use of a common reference was avoided, but this technique also avoided excessive spatial filtering. The effects of volume conduction were further limited by looking for a change in EEG-EEG coherence following rTMS. In chapters 5 and 6, linked earlobe electrodes were used as a

reference, and in chapter 7, linked mastoid electrodes. This procedure might have introduced a common signal to all other channels, leading to inflated coherence estimates. However, this was limited by using a subtractive approach, with the assumption that movement-related activity is not picked up by the reference electrodes. Therefore, in order to separate the task-related coherence from the background coherence, the values of the resting state were subtracted from those of the active state. This subtraction method also reduces between-subject differences.

2.4 Signal amplification, filtering and pre-processing

EEG and EMG signals recorded from the body surface have very small voltages and this requires considerable amplification to produce a signal large enough for digitising. The output from most amplifiers is analogue (a continuous-valued function). Analogue-to-digital (A/D) conversion essentially converts this continuous-valued function to a series of discrete-valued samples that approximate the original function. Signals can also be broken down into fundamental frequencies, with each frequency having its own intensity. The display of the intensities of all frequency components of a signal is called a power spectrum. However, clinical interest usually lies in signals of a particular frequency range (bandwidth). Filtering the signal is thus required to limit the bandwidth to the appropriate range, in effect filtering out unwanted frequencies. The power density function of surface EMG signals has negligible contributions outside the range 5-10 Hz to 400-450 Hz. The clinically relevant frequency range for EEG signals is usually from 0.1-100 Hz (with the notable exception of the high frequency content of somatosensory evoked potentials). The bandwidth of the filters should therefore be within these ranges.

Thus, EEG and EMG signals are amplified, A/D converted and filtered. One further consideration is the sampling rate of the A/D converter. Digital conversion consists of measuring the voltage at regular intervals and storing the information in digital format (as binary code). The sampling rate is the number of times the signal is measured per second in the A/D conversion process. For a signal whose components vary rapidly with time, if the sampling rate is too low, then these rapid changes may be missed and under-sampled. This leads to the problem of aliasing when higher frequencies can be incorrectly interpreted as lower ones. This is illustrated in figure 2.3. To avoid this ambiguity, the sampling rate should be at least twice that of the highest frequency component of the sample. Put another way, the highest frequency discriminated for a given sampling rate (known as the Nyquist frequency) is equal to half the sampling rate.

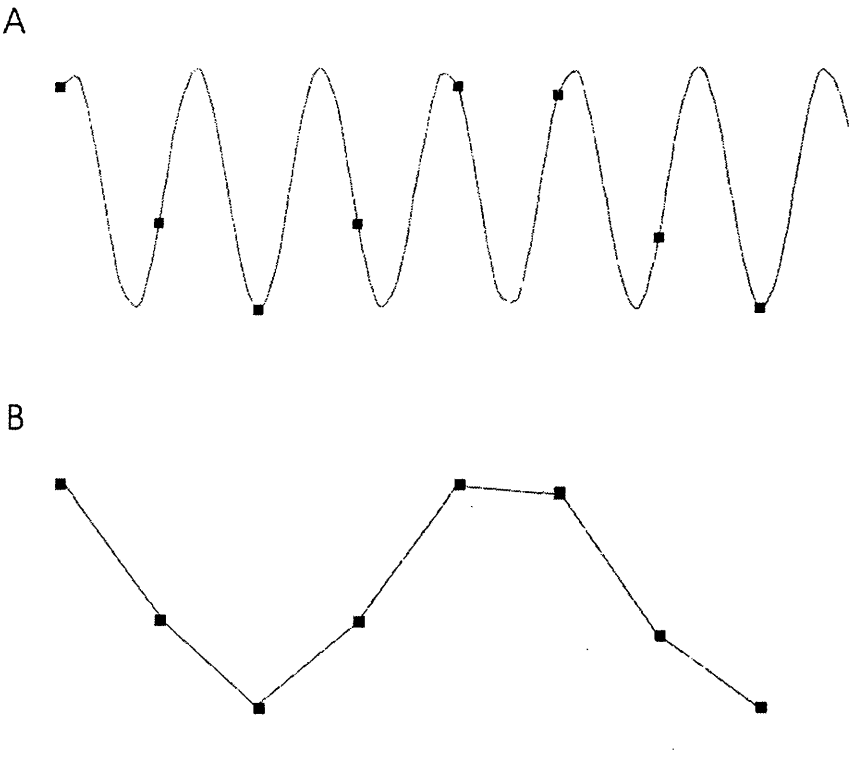


Figure 2.3. The concept of aliasing. A. Sine wave signal. The black dots are the voltages measured at each sampling time as determined by the A/D converter. B. Using the voltages measured in A, a waveform is reconstructed. The frequency of this waveform is much less than that of the original because the sampling time was too slow.

For the acquisition of data in chapters 3-6, EEG and EMG signals were amplified and bandpass filtered with an analogue filter (D150 amplifier, Digitimer, Welwyn Garden City, U.K.). EEG (amplified x 50,000-100,000) was filtered from 0.53 to 300 Hz and EMG (amplified x 1000-5000) from 53 to 300 Hz. After A/D conversion using a **1401** laboratory interface (Cambridge Electronic Design, Cambridge, UK; 12-bit resolution i.e. 4096 [2^{12}] digital voltage levels for each analogue data point sampled) sampling at 1000 Hz, the data were stored on a personal computer as Spike2 data files (Cambridge Electronic Design, Cambridge, UK). All data were sampled at 1000 Hz, more than double the filter high-cut setting, to avoid aliasing. Prior to analysis, all recorded EEG data was visually assessed off-line to remove any artefact due to eye movements, scalp EMG or mains spikes. For analysis of EEG during muscle activation, only data sections when the muscle was active (as determined by the EMG signal) were used. Similarly, data containing any active EMG were rejected for use as 'rest' data. Artefact-free and relevant rest/movement data were exported to new data files using Spike2 software.

In chapter 7, signals were amplified (EEG x 150,000; EMG x 7,500), A/D converted and then digitally filtered (Syn Amps, Neurosoft Inc., U.S.A.). EEG signals were bandpass filtered from 0.3 to 70 Hz and EMG signals from 5 to 100 Hz. The data were sampled at 500 Hz, again high enough to avoid aliasing. After processing, data were stored directly on a personal computer as continuous files using Neuroscan software (Neurosoft Inc., U.S.A.). Eye movement artefact was removed off-line using the ocular artefact reduction script in the Neuroscan software. Ocular artefacts are particularly troublesome for multi-electrode arrays as electrodes placed in the frontal and temporal regions are susceptible to contamination. The Neuroscan software enables the EEG to be 'corrected' for eye movements. The ocular artefact reduction script employs a regression analysis in combination with artefact averaging to produce a reliable method for artefact removal (Semlitsch et al., 1986). Firstly, a search is made of the data for maximum eye movement potentials by scanning for the maximum absolute voltage from the VEOG channel. Secondly, an average artefact response is constructed. Averaging is initiated when the voltage exceeds a percentage (in this case 20%) of the maximum eye movement potential. From this average, transmission coefficients are calculated separately for all EEG channels. The electro-oculogram is then subtracted from the EEG on a sweep-by-sweep, point-by-point basis. An example of EEG data with ocular artefact removed using this method is given in figure 2.4.

Data were then converted into .eeg files (software developed by A. Pogosyan, Sobell) for further off-line analysis, including the removal of further artefact (scalp EMG, mains spikes). Prior to all off-line analysis, a 50 Hz digital notch filter was applied to the data to reduce any mains interference.

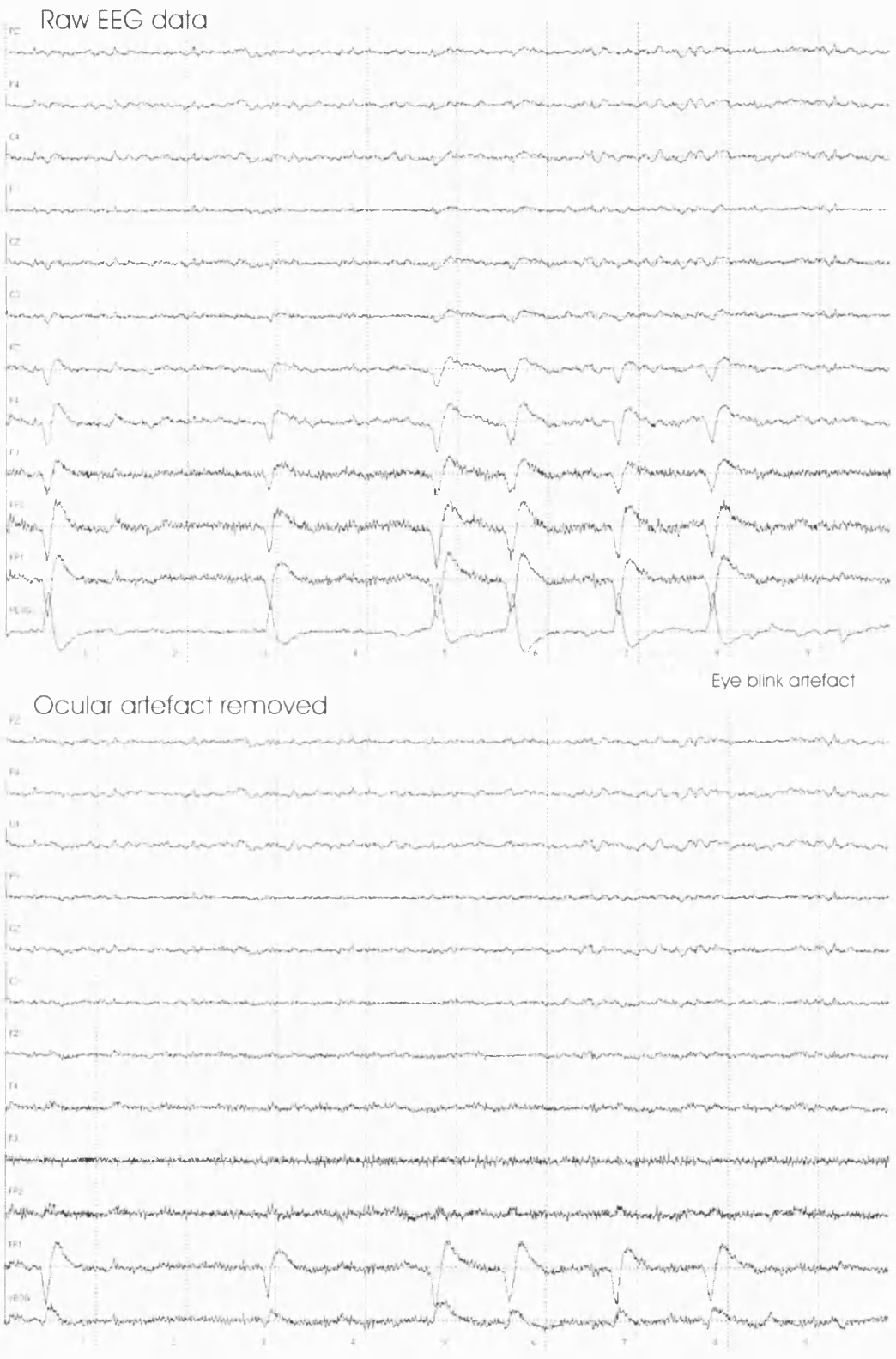


Figure 2.4. An example of raw EEG data recorded from a stroke patient (chapter 7) before and after removal of ocular artefact. Linked mastoid electrodes served as the reference.

2.5 Transcranial magnetic stimulation

Transcranial magnetic stimulation can be applied in several ways, as single pulses of stimulation, pairs of stimuli separated by variable time intervals to the same or different areas of the brain, or as trains of repetitive stimuli at various frequencies. In this thesis, single pulse TMS was used to locate motor hand areas and to determine cortical motor thresholds (chapters 3-6). Paired-pulse TMS was used in chapter 6 to investigate cortical excitability after a train of 5 Hz rTMS. Repetitive TMS was given to the primary motor cortex (M1) at 1 Hz (chapter 3) and 5 Hz (chapters 4 and 6) and to the supplementary motor area (SMA) at 5 Hz (chapter 5). The principle mechanisms of magnetic stimulation have been discussed in chapter 1. The practicalities of using the TMS equipment are discussed below.

2.5.1 Equipment

Repetitive TMS was performed with a Magstim Rapid Rate Stimulator and single-pulse TMS with a Magstim 200 stimulator (Magstim company, Whitland, Dyfed, UK). Figure-of-eight shaped coils were used to apply the stimulation (Magstim company, Whitland, Dyfed, UK). The coils had an outer winding diameter of 70 mm in all experiments except when bilateral motor cortical stimulation was required (chapter 6). Here, to enable two coils to be placed simultaneously on a subject's scalp, coils with smaller diameters (50 mm) were used. Using figure of eight-shaped coils improves the accuracy of stimulation since the induced electrical field is at a maximum directly under the coil centre (where the windings join). However, this region may still be up to 4 cm long, potentially activating a similar area within the brain. The Magstim 200 generates a monophasic coil current. This reduces heat dissipation in the coil, discharge click noise, and stimulus artefact and increases stimulus accuracy. The Rapid Rate Stimulator generates a biphasic coil current. During the first phase of the stimulus, the current in the centre of the coil flows towards the handle.

2.5.2 Localisation of cortical motor areas

The primary motor cortex (M1, Brodmann's area 4) lies in the frontal lobe, anterior and adjacent to the central sulcus. For the localisation of M1 using TMS, the coil was placed tangentially to the scalp with the handle pointing backwards and laterally. It was held at a 45° angle away from the midline, approximately perpendicular to the line of the central sulcus, inducing a posterior-anterior current in the brain. This orientation was chosen based on the finding that the lowest motor threshold is achieved when the induced electrical current in the brain flows approximately perpendicular to the line of

the central sulcus (Brasil-Neto et al., 1992; Mills et al., 1992) and is, therefore, optimal to activate the motor cortex trans-synaptically (Werhahn et al., 1994). To determine the optimal position for activation of the right FDI, the coil was moved in 0.5cm steps around the presumed motor hand area of the left motor cortex. The site where stimuli of slightly suprathreshold intensity consistently produced the largest MEPs in the target muscle was marked on the scalp as the "hot spot". This was repeated to locate the right motor cortex hand area.

The SMA (Brodmann's area 6) is located directly anterior to the leg representation of the primary motor cortex and is at the same depth on the interhemispheric surface (Fox et al., 1985; Geyer et al., 2000; He et al., 1995). The point of stimulation used for the SMA (chapter 6) was located 2 cm in front of CZ. This position represents the hand area of the SMA as established using fMRI (Lee et al., 1999). This site was also anterior to the leg area, as determined by a motor response from the tibialis anterior muscle in each individual subject using TMS. A recent study by Steyvers et al. (2003) showed that the optimum site for disturbing bimanual coordination using rTMS was approximately 2.5 to 4.5 cm anterior to CZ.

2.5.3 Determination of cortical motor thresholds

Motor threshold refers to the lowest TMS intensity necessary to evoke motor-evoked potential (MEPs) in the target muscle when single-pulse stimuli are applied to the motor cortex. Motor threshold is believed to reflect membrane excitability of corticospinal neurons, and interneurons projecting onto them, in the motor cortex, as well as the excitability of motor neurons in the spinal cord, neuromuscular junctions and muscle (Ziemann et al., 1996a).

The resting motor threshold (RMT) intensity, expressed as a percentage of maximum stimulator output, was approached from suprathreshold levels by reducing the stimulus intensity in 1% steps. RMT was defined as the first stimulus intensity that failed to produce an MEP of more than 50 μV in at least 5 out of 10 subsequent trials (Rossini et al., 1994). Similarly, active threshold (AMT) was defined as the lowest stimulus intensity at which 5 out of 10 consecutive stimuli elicited MEPs of 200μV amplitude in the tonically contracting FDI. Both resting and active motor thresholds were determined at baseline immediately before rTMS to any given subject.

2.5.4 *rTMS stimulation parameters*

Adjustable parameters of repetitive stimulation include frequency, intensity and duration of stimulus train. The parameters used for the experiments in this thesis are given in table 2.1 below.

	Frequency (Hz)	Site	Intensity	No. Stimuli
Chapter 3	1	Left M1	90% AMT	1500
Chapter 4	5	Left M1	100% AMT	50
Chapter 5	5	SMA	90% AMT	50
Chapter 6	5	Left M1	90% AMT	150

Table 2.1. rTMS parameters used in the experiments described in this thesis.

Note that all intensities used here are below resting motor threshold and relate to the AMT for each individual subject tested. Hence, with subthreshold stimulation, no direct EMG responses are elicited and so any effects are likely to be central in origin. Motor thresholds were determined prior to affixing scalp electrodes. For the 1 Hz rTMS train, the C3 electrode was removed prior to stimulation and replaced immediately it had finished. This avoided the electrode over-heating during the stimulation train. For the shorter 5 Hz trains, motor thresholds were re-measured, and stimulation given, with the scalp electrodes in situ. RTMS was applied with the subjects at rest except in chapter 6, where subjects were finger tapping during stimulation. For M1 stimulation, the coil was held in an identical way as described for the threshold measurements. Coil orientation over the SMA was with a backward-pointing handle and coil junction in the midline. In the 1 Hz experiment (chapter 3), a total of 1500 stimuli were given. This necessitated switching between two figure-of-eight shaped coils in each run, each time overheating of the coil occurred (usually once per run). Switching between coils took only 3-5 seconds to perform.

2.5.5 *Paired-pulse TMS and measurement of cortical excitability*

Inhibitory and facilitatory interactions in the cortex can be studied by combining a subthreshold conditioning stimulus with a suprathreshold test stimulus. These are given through the same coil at different interstimulus intervals. The effects of the conditioning stimulus on the size of a test MEP vary depending on the stimulus intensity and interstimulus interval. Maximal inhibitory effects are found at short interstimulus intervals of 1-4 ms using conditioning stimuli of 60-80% of the resting motor threshold (Kujirai et al., 1993; Schafer et al., 1997). The amount of inhibition is usually 20-40% of

the test MEP. If the interstimulus interval is 7-20 ms, the conditioning stimulus can facilitate the test MEP (Kujirai et al., 1993; Ziemann et al., 1996c). An example of inhibited and facilitated MEPs taken from data used in chapter 6 is given in figure 2.5.

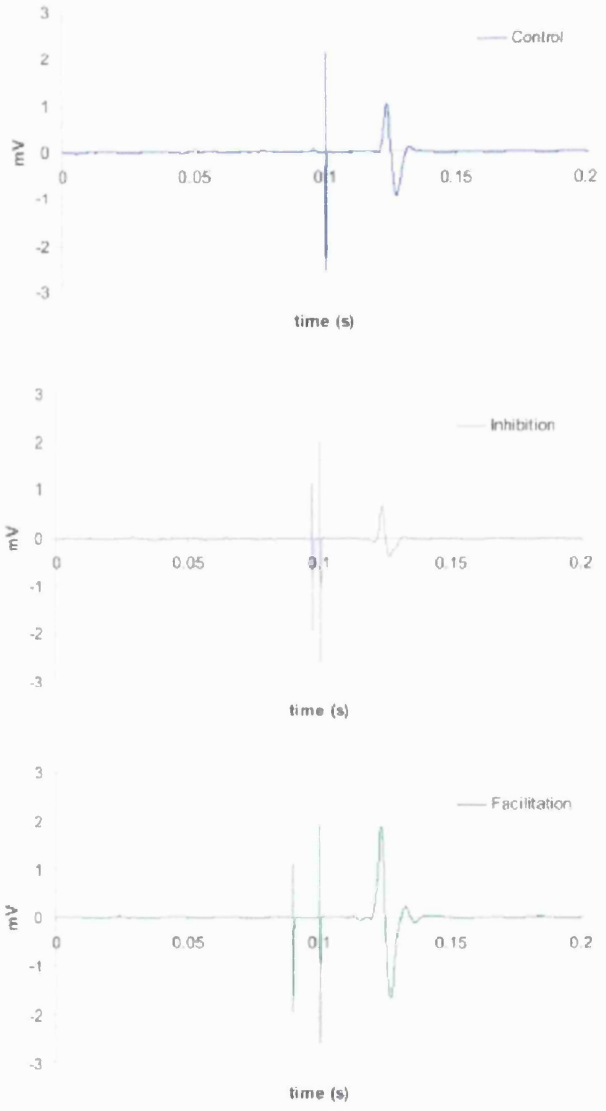


Figure 2.5. Raw data taken from a control subject during paired-pulse TMS (chapter 6). A single control stimulus over the motor cortex generates a MEP in the contralateral FDI muscle. If a second TMS pulse is given just before the control pulse, the resulting MEP can be modulated. If the inter-stimulus interval is short (in this case 3 ms) the MEP is inhibited. If the inter-stimulus interval is longer (10 ms) the MEP is facilitated.

For the paired-pulse paradigm used in chapter 6, the interstimulus intervals were set at 3 ms to achieve inhibition and at 10 ms for facilitation. The intensities were set at 80% of AMT for the conditioning stimulus and 120% of RMT for the test stimulus. Pairs of

stimuli were 3.3 s apart and administered pseudorandomly with single-pulse (control) stimuli. To investigate the effects of repetitive TMS to the left M1 on the excitability of the right M1, paired-pulse TMS was applied over the right M1, and MEP responses recorded from left FDI, before and after the rTMS was given.

2.5.6 TMS safety criteria

When used correctly, TMS is a non-invasive, safe and painless method of activating the human motor cortex. However, attention must be given to the setting of stimulation parameters. The most serious adverse effect of TMS is the induction of seizures. This is usually associated with repetitive TMS of high frequency, high intensity and long duration. Single pulse TMS, with stimuli delivered no more than once every few seconds, does not carry a significant risk. Safety guidelines for the selection of stimulation parameters have been published (Wassermann, 1998). The parameters used for stimulation in chapters 3-6 do not fall outside these guidelines. No seizures were induced in any subject and no ill effects of stimulation were reported. For the purposes of the experiments detailed here, subjects were not considered for TMS if there was a history of previous seizures, they had a family history of seizures, they were pregnant, they were taking medications that lowered seizure threshold, they had a pacemaker fitted or they had a metal implant anywhere in the head. All TMS was performed in the presence of a qualified physician and in a hospital setting.

2.6 Data analysis

The EEG data in chapters 3-6 were analysed in the frequency domain using a suite of programs written by D. Halliday and J. Ogden (Division of Neuroscience and Biomedical Systems, Glasgow). The data in chapter 7 were analysed using a suite of programs written by A. Pogosyan (Sobell Department of Motor Neuroscience and Movement Disorders, London). Both analysis programs were based on methods outlined in Halliday et al. (1995). Subsequent comparisons of spectral power and coherence estimates were performed in Microsoft Excel.

The continuously varying waveforms that constitute EEG signals lead to analysis based on continuous time series. To characterise linear interactions between time series, frequency domain measures were derived using spectral estimation based on the fast Fourier transform (Halliday et al., 1995). Spectral methods are particularly suited to the study of systems displaying rhythmic behaviour (Conway et al., 1995). Several assumptions were made about the data for this technique to be used. Firstly, the surface EEG signals were assumed to be realisations of time series, in other words the

data comprised an ordered sequence of values of a variable at equally spaced time intervals. Secondly, the time series data were assumed to be stationary. Stationarity is defined as a quality of a process in which the statistical parameters (mean, standard deviation) of that process do not change with time (Challis and Kitney, 1990). Thirdly, the data were assumed to meet the requirements for a mixing condition. The mixing condition assumes that sampled values, widely spaced in time, are statistically independent (Halliday et al., 1995). This allows confidence limits to be constructed for parameter estimates and thus hypotheses can be tested.

Local stationarity of the data was achieved by dividing the complete data record into non-overlapping disjoint segments prior to spectral analysis. The segment length used must be short enough to avoid non-stationary sections of data but long enough to obtain the desired level of frequency resolution. The frequency resolution of spectra calculated with the Fourier transform increases as the segment length used increases (see section 2.6.4).

2.6.1 Fourier transform

Mathematically, any signal in a given time interval can be decomposed into a sum of mutually orthogonal sinusoidal waves of different frequencies, amplitudes and phases. The Fourier Transform is a method of uncovering the periodic structure of EEG signals. It is a complex function of frequency used to obtain the frequency spectrum of a given signal. It is thus used to calculate the frequency, amplitude and phase of each sine wave making up a signal.

Spectral estimates were calculated by dividing each time series (total record length R) into L segments (each of length T), where R = LT. A finite Fourier transform was then performed on each segment. For a time series *x(t)*, the Fourier transform of the l^{th} segment at frequency λ is defined as:

$$d_x^{\mathrm{T}}(\lambda,l)=\int_{(l-1)\mathrm{T}}^{l\mathrm{T}} x(t)e^{-i\lambda t}\,\mathrm{d}t \approx \sum_{t=(l-1)\mathrm{T}}^{l\mathrm{T}-1} e^{-i\lambda t}x(t)$$

where $i=\sqrt{-1}$. This transform can be thought of as a decomposition of the sampled waveform into constituent frequency components, which should highlight any distinct periodic components in the data.

The individual data processes are characterised by estimates of the power spectrum, or auto-spectrum, of each process. The pairwise relationships between the processes are characterised in the frequency domain by estimates of coherence.

2.6.2 *Power spectra*

The power spectrum, $f_{xx}(\lambda)$, for the time series $x(t)$ is given by the equation:

$$\hat{f}_{xx}(\lambda) = \frac{1}{2\pi LT} \sum_{l=1}^{L} d_x^T(\lambda, l) \overline{d_x^T(\lambda, l)}$$

where the overbar — represents a complex conjugate. Power spectra then undergo a logarithmic transformation (log10) to stabilise the variance that is then independent of the original value. Since the original power spectral values can be < 1, logarithmic transformation can result in negative values.

2.6.3 *Coherence*

The calculation of coherence is a method used to find out whether the same frequency components of two simultaneously recorded signals have similar phase shifts from trial to trial. The stability of the phase shift observed at certain frequencies may indicate that the corresponding rhythms in two signals are of mutual origin or are interacting with each other.

Coherence was used to assess the linear dependency between two time series, $x(t)$ and $y(t)$, at a given frequency (λ). Coherence is written as the function $|R_{xy}(\lambda)|^2$ and defined by the equation:

$$|R_{xy}(\lambda)|^2 = \frac{|f_{xy}(\lambda)|^2}{f_{xx(\lambda)} f_{yy(\lambda)}}$$

Coherence functions provide a bounded (values lie between 0 and 1), unitless and normative measure of association, where 0 indicates independence of the two signals and 1 indicates a perfect linear relationship, whereby phase differences and amplitude ratios remain constant between the signals (Rosenberg et al., 1989). Coherence functions thus provide estimates of the strength of coupling between two EEG signals. As for the power spectra, a Fisher transform was applied to normalise the underlying distribution of correlation coefficients and stabilise the variances of the distributions

(Rosenberg et al., 1989; Farmer et al., 1993). The Fisher transform takes the hyperbolic inverse tangent ($\tanh^{-1}$) of the modulus of the coherency function, where coherency is given by the square root of the coherence.

2.6.4 Frequency resolution and segment length used

The segment length defines the minimum frequency that can be resolved and thus the spectral resolution, according to the equation:

Frequency resolution = sampling rate / segment length

The segment length in the Fourier transform in chapters 3-6 was 1024 data points and the sampling rate was 1 kHz. Thus the frequency resolution was 0.98 Hz. In chapter 7, data were sampled at 500 Hz with a segment length of 512 data points, again giving a frequency resolution of 0.98 Hz.

2.6.5 Statistical analysis and interpretation

Spectral power and coherence estimates in any given experiment were obtained under identical conditions for each subject. Prior to analysis, all spectral power estimated underwent logarithmic transformation and all coherence estimates underwent a Fisher transformation. Analysis of variance (ANOVA) was then used to compare data for two or more conditions across multiple subjects (chapters 3-6) and between multiple groups (chapter 7).

To reduce the data, log power and transformed coherence estimates for each subject and for each condition were averaged across set frequency bands (the bands used were similar in each experiment). In order to decrease the effect of inter-subject and inter-electrode pair variation in coherence data, task-related log power and task-related transformed coherence were calculated by subtracting the raw values at rest from those during the active state. Coherence was always interpreted in conjunction with power primarily to ensure that changes in coherence were not due to modulations in non-linearly related frequency components (Florian et al., 1998).

Analysis was performed using SPSS (version 10). For parametric data, a repeated measures general linear model analysis was performed. For non-parametric data, a Friedman ANOVA was used. In all parametric ANOVAs used, a Greenhouse-Geisser correction for sphericity was incorporated where necessary. ANOVA results were considered significant for $p < 0.05$. Post-hoc analysis was performed on significant parametric data sets using the Student's paired t-test and on significant non-parametric

data sets using the Wilcoxon 2-sample test. Bonferroni corrections for multiple comparisons were applied where necessary.

3 The effects of 1 Hz repetitive TMS on cortico-cortical intrahemispheric and interhemispheric coherence.

Repetitive TMS can modulate cortical function by enhancing or decreasing cortical excitability depending on the parameters of stimulation (Pascual-Leone et al., 1998). As the effects outlast the duration of stimulation, rTMS is attracting increasing interest as a possible novel non-invasive therapy for movement and psychiatric disorders, particularly depression (Wassermann and Lisanby, 2001).

Nevertheless, the mechanisms underlying changes in cortical function following rTMS remain unclear. RTMS could have its effects through the modulation of local intracortical circuits, or through the modulation of coupling with distant sites. Some evidence in support of the latter comes from studies in which the activity of cortical areas distant to the site of low frequency rTMS has been assessed using single shock TMS or positron emission tomography (Wassermann et al., 1998; Siebner et al., 2000 and 2001).

A possible modulation of cortico-cortical coupling may be more directly investigated through the assessment of changes in cortico-cortical coherence following rTMS. This has the additional benefit that changes in coupling in the alpha band may tend to involve inhibitory mechanisms, although perhaps not exclusively so (Worden et al., 2000; Pfurtscheller, 1992; Pfurtscheller et al., 2000; Hummel et al., 2002). Analysis of EEG-EEG coherence following rTMS has been applied to short trains of high frequency (10 Hz) rTMS delivered at motor threshold (Jing and Takigawa, 2000). This study found changes in directed, but not standard, EEG-EEG coherence in the alpha band (8-13 Hz). Low frequency rTMS (0.9 Hz) over the premotor cortex has recently been shown to reduce the task-related power decrease in the alpha and beta bands, increase task-related coherence changes between cortical motor areas in the upper alpha band, and decrease cortico-muscular coherence (Chen et al., 2003).

In this chapter, the effects of rTMS given at low frequency (1Hz) over the motor cortex are investigated. Such trains cause a short-term decrease in cortical excitability as measured using single pulse TMS (Chen et al., 1997b; Siebner et al., 1999a; Muellbacher et al., 2000; Maeda et al., 2000a; Touge et al., 2001). Low intensity, subthreshold shocks were used to ensure purely local effects. Firstly, this ensured there would be no evoked muscle twitches that could modify central processing through changed afferent input. Secondly, this ensured that changes in cortico-cortical

coherence were due to stimulation of motor cortex rather than of adjacent areas. For example, the threshold for inhibitory effects on MEPs is lower with rTMS over the premotor cortex than motor cortex (Gerschlager et al., 2001). This study hypothesised that coupling between the stimulated motor cortex and functionally related cortical areas would be increased at frequencies predominantly associated with inhibitory effects. Specifically, it was predicted that coherence in the alpha band between the stimulated primary motor cortex and more anterior ipsilateral motor areas (including lateral premotor and prefrontal areas) and that between stimulated and contralateral unstimulated motor cortices would be increased after rTMS. Conversely, it was predicted that there would be no effect on the coherence in the alpha band between the contralateral unstimulated motor and anterior motor cortices. The data confirm these predictions and suggest that the effects on coupling are both prolonged and mechanistically relevant to the inhibitory effects of low frequency rTMS.

An additional experiment investigated the effects of 1 Hz rTMS on a simple choice reaction time task. The results from this are given in the next chapter.

3.1 Methods

Fifteen healthy volunteers were studied, eleven men and four women (mean age 34 +/- 7.6 (SD) years, range 25-50 years). All subjects were right-handed according to the Edinburgh inventory (Oldfield, 1971) and none reported any ill effects after the study.

3.1.1 Experimental design

Subjects were seated in a comfortable reclining chair with their forearms resting horizontally by their sides and the whole body at rest. EEG and EMG electrodes were attached as described below. They were instructed to relax but to keep their eyes open and fixate a visual target directly in front. Recordings were taken prior to, immediately after, 25 minutes after and 50 minutes after rTMS (times pre, post 0, post 25 and post 50, respectively). Subjects sat at rest and were required to contract to verbal command the first dorsal interosseous (FDI) and wrist extensor (WE) muscles bilaterally by fanning the fingers while extending the wrist. Contractions were about 20-30% of maximal strength. Three cycles of 60 seconds of rest and 60 seconds of continuous tonic muscle activation were recorded for each time point.

3.1.2 Magnetic stimulation

The optimal position ('hot spot') over the left motor cortex for activation of the right FDI muscle was determined using previously described methods (section 2.5). This was

repeated to locate the right motor cortex hand area. Both resting and active motor thresholds (RMT and AMT, respectively) were determined at baseline immediately before rTMS (section 2.5). Focal rTMS was applied to the left motor hand area using a frequency of 1Hz, with the subject at rest. The coil was held in an identical way as described for the threshold measurements. The intensity of rTMS was set at 90% of AMT of the left motor cortex hand area for each individual subject. A total of 1500 stimuli were given. Stimulation variables were in accordance with published safety recommendations (Wassermann, 1998).

3.1.3 EEG and EMG recording

The left motor hand area was located using single pulse TMS as described above and a surface electrode placed at this site. Further electrodes were placed 2.5 cm posterior, 2.5 cm anterior and 5 cm anterior to this first electrode. These sites are henceforth termed, from anterior to posterior, F3, FC3, C3 and CP3, as these are the corresponding sites (+/-0.5 cm) in the international 10-20 system. This was repeated on the right side. In addition, EEG electrodes were fixed to PO3 and O1 (figure 3.1).

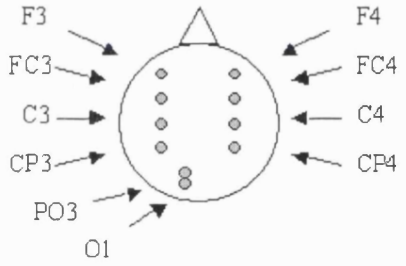


Figure 3.1 Scalp EEG electrode arrangement. The C3 and C4 electrodes were positioned first using TMS to locate the motor hand areas for the left and right hemispheres, respectively. F3, FC3 and CP3 were positioned with respect to C3, with all electrodes 2.5 cm apart. F4, FC4 and CP4 were positioned with respect to C4. PO3 and O1 were positioned according to the international 10-20 system.

F3/4, FC3/4 and O1 are likely to overlie the prefrontal, lateral premotor and occipital areas, respectively (Homan et al., 1987; Steinmetz et al., 1989), whereas C3/4 were considered to overlie primary motor cortex based on the TMS results. The electrodes were linked such that they formed a chain of three bipolar leads on each side (F3-FC3, FC3-C3, C3-CP3, F4-FC4, FC4-C4, C4-CP4) and a single ipsilateral occipital bipolar lead (PO3-O1). EMG data were recorded bilaterally from FDI and WE using pairs of surface Ag-AgCl electrodes.

The signals were amplified and band-pass filtered (EEG 0.53 to 300 Hz; EMG 53 to 300 Hz). After A/D conversion using a 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) the data were stored on a personal computer as SMR files (Spike 2, Cambridge Electronic Design, Cambridge, UK). All data were sampled at 1000Hz.

3.1.4 Data analysis

The data were inspected visually for scalp EMG, eye movement artefact and mains spikes. 110 seconds of clean data were extracted from each recording and examined using the spectral analysis methods outlined in Halliday et al. (1995). Such long total data lengths were necessary to achieve reliable spectral estimates with low 95% confidence limits. The five seconds prior to and after each activation or relaxation of the muscles were excluded to avoid using data that involved transients due to movement preparation, onset or termination. Thus, only steady-state tonic contraction and rest periods were analysed. The Fourier transforms of non-overlapping sections of 1024 data points were calculated, the results averaged across sections, and the power spectra and coherence estimated. The frequency resolution was 0.98Hz. Recordings were Hanning-windowed to control spectral leakage.

The EEG between 7.8-13.7 Hz (alpha) and 14.6-30.3 Hz (beta) was chosen for analysis. There is extensive evidence that EEG in the alpha and beta bands may represent activities closely related to the preparation and execution of movement, as witnessed by movement-related modulations in power (Pfurtscheller et al., 1994,1997; Salmelin and Hari, 1994; Toro et al., 1994a,b; Stancak and Pfurtscheller, 1996a,b,c; Leocani et al., 1997) and coherence (Gerloff et al., 1998; Manganotti et al., 1998). The measurement of power and coherence was performed across the whole alpha and beta bands. An alternative, perhaps more sensitive, method has been described by Doppelmayr et al. (1998) in which power is measured around the mean peak frequency evident in the alpha band in each subject. Nevertheless, measurements across predefined alpha bands have been used by many authors (Manganotti et al., 1998; Dieber et al., 2001; Hummel et al., 2002; Pfurtscheller et al., 2000; Mima et al., 2000b; Andrew and Pfurtscheller, 1997) and, as demonstrated in the results, were sensitive enough to reveal rTMS induced differences in coherence.

The variance of spectral power estimates was stabilised by logarithmic transformation (Halliday et al., 1995). To compare the coherence between EEG signals, the variance of the modulus of the coherency (given by the square root of the coherence) was

normalised using a Fisher transform (Rosenberg et al., 1989). These values are henceforth referred to as log power and ($\tanh^{-1}$)coherence, respectively.

3.1.5 Statistical analysis

The average log power and ($\tanh^{-1}$)coherence values in the alpha (7.8-13.7 Hz) and beta (14.6-30.3 Hz) frequency bands were computed across subjects for both rest and active conditions and for the four time points. The results for each band were entered into separate factorial analyses of variance (ANOVA) incorporating, where necessary, a Greenhouse-Geisser correction for non-sphericity.

For intrahemispheric alpha and beta EEG-EEG ($\tanh^{-1}$)coherence, within-subject factors were condition (rest and active), region (left and right hemisphere), and time (pre and post 0). Interhemispheric coherence was also analysed using the same within-subject factors for condition and time. For EEG log power, the within-subject factors were the same for time and condition but four regions were entered (C3-CP3, F3-FC3, C4-CP4, F4-FC4). EEG log power for the control region, PO3-O1, and mean rectified EMG for FDI and WE during the active condition were also analysed separately by ANOVA. If main effects or interactions were significant, data were further compared post-hoc using the Student's t-test for paired samples.

3.2 Results

There was no significant difference between the mean rectified EMG level in the pre-rTMS period and that in any of the post-stimulation periods.

3.2.1 EEG power

EEG log power was assessed by ANOVA for both alpha and beta frequencies. In both frequency bands, power decreased with muscle activity compared to rest. However, no effect of stimulation was seen in either band.

Alpha power: The graphs of mean spectral alpha log power for F3-FC3 and C3-CP3 are shown in figure 3.2. There was no apparent difference before and after the application of rTMS. For the EEG electrodes F3-FC3, C3-CP3, F4-FC4 and C4-CP4, there was a main effect only for condition ($F[1,14] = 6.669$, $p = 0.022$) and no interactions. This allowed data to be combined across all four regions and two time points. Post-hoc t-tests then confirmed that power decreased by 5% during the active compared to the rest state ($p = 0.011$). Finally, the control region PO3-O1 showed no main effect for time or condition and no interactions.

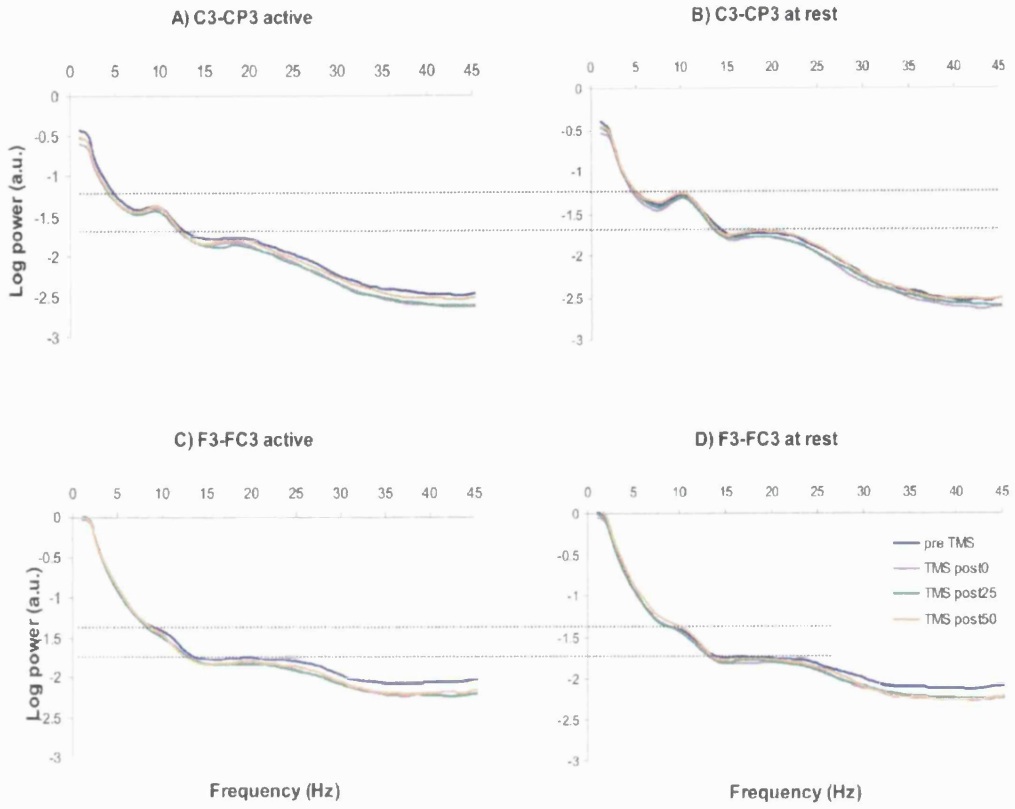


Figure 3.2. Mean spectral power results. Average data (n = 15) for log power spectra during rest and active states at each of the four time points (pre TMS and 0, 25 and 50 minutes post-stimulation). A) C3-CP3 during tonic muscle activation. B) C3-CP3 at rest. C) F3-FC3 during tonic muscle activation. D) F3-FC3 at rest. Dotted lines are included to highlight the change in alpha frequency peak between rest and active states.

Beta power: For the electrodes F3-FC3, C3-CP3, F4-FC4 and C4-CP4, there was a main effect for time ($F[1,14] = 8.212$, $p = 0.012$) and a significant interaction of region and condition ($F[3,42] = 4.809$, $p = 0.006$). The lack of interactions with time allowed data to be combined across the two time points. With muscle activation, C3-CP3 power decreased by 3.1%, C4-CP4 power decreased by 2.8%, F3-FC3 power increased by 0.4% and F4-FC4 power increased by 0.7%. The only change to reach significance was the C3-CP3 power decrease ($p = 0.007$). Again, the control region PO3-O1 showed no main effect for time or condition and no interactions.

3.2.2 Cortico-cortical intrahemispheric coherence

Transformed coherence was assessed for both alpha and beta frequencies with ANOVA. Alpha coherence in the stimulated hemisphere increased after rTMS. However, there were no changes in coherence in the beta band.

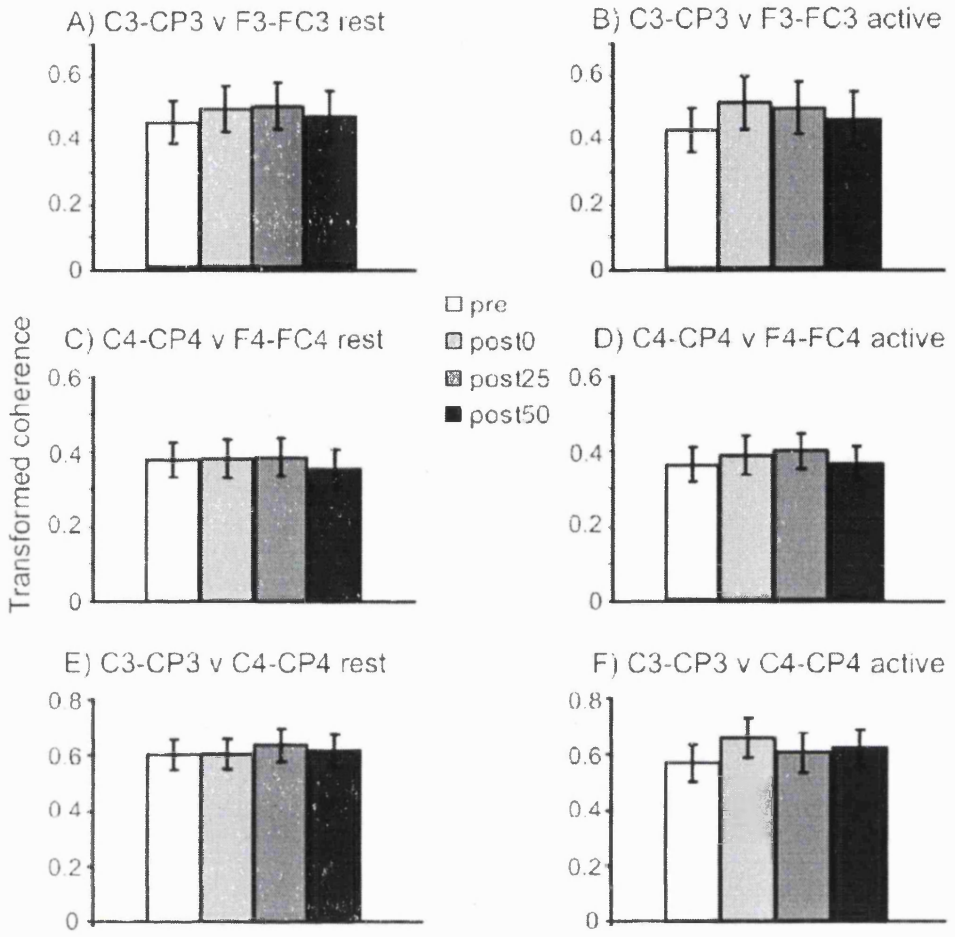


Figure 3.3. Mean transformed alpha coherence results. Average transformed alpha coherence (n = 15) for left and right hemispheres according to timing with respect to rTMS and condition (active and rest). Bars are standard error of mean. Graphs show coherence between A) C3-CP3 and F3-FC3 at rest, B) C3-CP3 and F3-FC3 during tonic muscle activation, C) C4-CP4 and F4-FC4 at rest, D) C4-CP4 and F4-FC4 during tonic muscle activation, E) C3-CP3 and C4-CP4 during tonic muscle activation, F) C3-CP3 and C4-CP4 at rest.

Alpha intrahemispheric coherence: Figure 3.3 (A to D) shows the average transformed coherences in the alpha band for the right and left hemispheres both at rest and during muscle activation. Changes are marked in the left hemisphere. Figure 3.4 shows the average change in coherence between C3-CP3 and F3-FC3 during muscle activation for pre and immediately post-stimulation. Transformed coherences in the alpha band were assessed by ANOVA. There was no main effect for time (rTMS), region or condition, and no interactions with the exception of a significant interaction of time with region ($F[1,14] = 11.003$, $p = 0.005$). This allowed data to be combined for the rest and active conditions. Post-hoc Student's paired t-test confirmed that the increase (of 15%) in coherence in the alpha band immediately following rTMS occurred ipsilateral (p =

0.032) but not contralateral (p = 0.258) to the stimulation. This ipsilateral effect of rTMS was still significant after 25 mins (p = 0.037) but not 50 mins (p = 0.179). Mean ipsilateral transformed coherence pre-stimulation was 0.419 +/- 0.055 (SEM) compared with mean contralateral transformed coherence pre-stimulation of 0.371 +/- 0.035. Mean ipsilateral transformed coherence post-stimulation was 0.480 +/- 0.064 (SEM) compared with mean contralateral transformed coherence post-stimulation of 0.388 +/- 0.041.

Beta intrahemispheric coherence: Beta coherence was also assessed by ANOVA. There were no main effects of time, region or condition and there were no interactions.

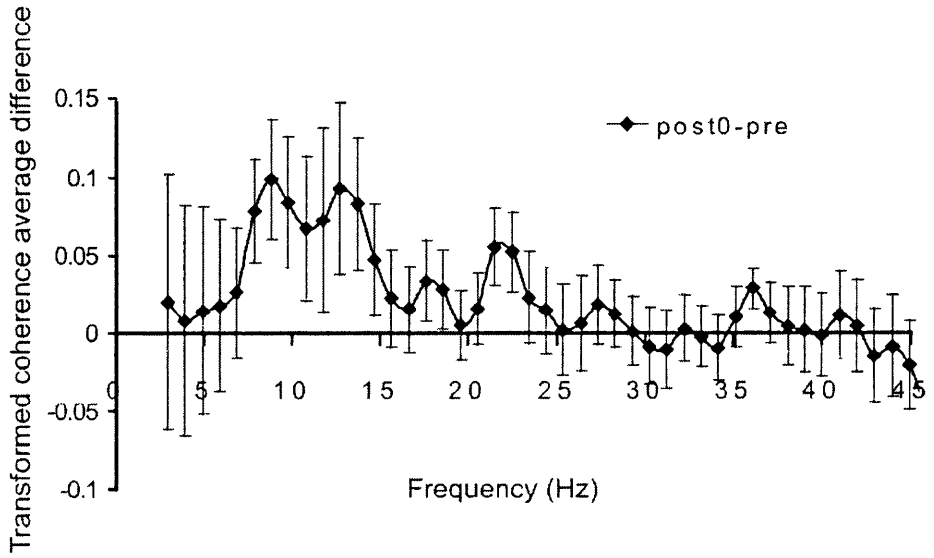


Figure 3.4. Change in alpha coherence with rTMS. Average transformed alpha coherence difference (n = 15) between C3-CP3 and F3-FC3 during tonic muscle activation before and immediately after rTMS to the left motor cortex. Bars are standard error of mean.

3.2.3 Interhemispheric coherence

Interhemispheric transformed coherence between C3-CP3 and C4-CP4 (see figures 3.3E and 3.3F) was also analysed by ANOVA for both the alpha and beta bands.

Alpha interhemispheric coherence: For the alpha frequency band, there was no main effect for time (rTMS) or condition, but there was a significant interaction of time (rTMS) with condition ($F[1,14] = 7.487$, $p = 0.016$). Post-hoc Students paired t-test demonstrated a significant increase (of 16%) in interhemispheric alpha coherence in the active state following stimulation ($p = 0.034$). This effect was not significant at 25 minutes following stimulation. Mean interhemispheric transformed coherence during tonic muscle contraction was 0.569 +/- 0.061 (SEM) pre-stimulation and 0.659 +/-

0.065 post-stimulation. At rest, mean transformed coherences were 0.604 +/- 0.047 pre-stimulation and 0.606 +/- 0.047 post-stimulation.

Beta interhemispheric coherence: For the beta frequency band, there were no significant main effects or interactions of time or condition.

3.3 Discussion

These results show that rTMS given at low frequency (1Hz) causes a persistent increase in the alpha coherence between the ipsilateral stimulated primary motor and more anterior motor areas (intrahemispheric) and in the coherence between the ipsilateral stimulated and contralateral unstimulated motor areas (interhemispheric). The effect of rTMS was relatively focal as there was no change in the coherence between primary motor cortex and more anterior motor areas over the cerebral hemisphere contralateral to stimulation.

The increase in alpha coherence is unlikely to be due to a general alteration in arousal of subjects for several reasons. The intrahemispheric changes only occurred ipsilateral to the rTMS and the interhemispheric changes were confined to periods of tonic contraction. An effect of general arousal would have been expected to involve both left and right hemispheres and both rest and active conditions. Additionally, in a similar experimental paradigm, high frequency (5Hz) rTMS over the motor cortex (100% active motor threshold; data analysed in an identical way) gives the opposite effect, causing ipsilateral cortico-cortical coherence to decrease post-stimulation (chapter 4). The lack of rTMS effects on regional power is also against any non-specific arousal changes during the paradigm. The effect must have been purely central in origin because the intensity of the individual rTMS pulses was less than active threshold. Therefore, no muscle twitches were evoked that could modify central processing through changed afferent input. Neither did differences in non-linear components of the signals account for differences in coherence. There was no change in EEG power with rTMS so that the increases in EEG-EEG coherence in the alpha band reflected a change in the absolute degree of coupling between areas.

Interestingly, although motor and premotor area alpha power changed according to whether the subjects were at rest or being recorded during voluntary muscle contraction, there was no variation in alpha coherence with rest versus contraction. This may be because sustained tonic contraction was examined, avoiding transients occurring during the first few seconds of contraction (Crone et al, 1998). Changes in coherence are, however, reported in phasic tasks (Gerloff et al., 1998; Manganotti et

al., 1998). As expected (Gerloff et al., 1998; Manganotti et al., 1998; Pfurtscheller et al., 2000), there was a significant reduction in power over the left and right motor and premotor areas in the alpha range during muscle contraction, as well as a reduction in power over the motor areas in the beta range. This reduction in power has been interpreted as a sign of regional activation (Pfurtscheller, 1988; Toro et al., 1994a, b; Stancak and Pfurtscheller, 1995; Gerloff et al., 1998).

One can only speculate on the possible mechanisms responsible for the change in the alpha band coherence after rTMS. The stimulus intensity used was below AMT, suggesting that it was insufficient to activate excitatory inputs to corticospinal neurones. On the other hand, such low intensities are used as conditioning pulses in paired-pulse testing of cortical inhibition and facilitation (Kujirai et al., 1993). Thus, the rTMS was probably capable of exciting cortical interneurones. Subthreshold 1 Hz rTMS has been shown to decrease local cerebral blood flow, consistent with TMS-induced activation of local inhibitory mechanisms, whereas subthreshold 20 Hz rTMS increased local cerebral blood flow (Speer et al., 2000).

Since inhibition is a critical factor in the development of cortical oscillations and coherence (Rubin and Terman, 2000; Contreras et al., 1997; Pauluis et al., 1999), it seems possible that long-lasting changes in the excitability of inhibitory mechanisms after rTMS could contribute to the present results. This suggestion is supported by the observation that 1Hz rTMS at 90% resting threshold could increase intracortical inhibition (ICI) in patients with writer's cramp (Siebner et al., 1999b). However, it is more difficult to reconcile with the lack of effect of 1Hz rTMS to motor cortex on ICI in healthy subjects at both 90% resting (Siebner et al., 1999b) and 80% active threshold (Munchau et al., 2002). One possibility is that the paired-pulse method of testing ICI in healthy subjects is prone to 'floor' effects because the baseline levels of inhibition are so strong (Fisher et al., 2002). Perhaps more sensitive measures of inhibition such as the threshold tracking design of Fisher et al. (2002) and Awiszus et al. (1999) would detect subtle effects that might mirror the changes in cortico-cortical coherence.

The pharmacological basis of the short-term plasticity in cortico-cortical coupling induced by rTMS is unclear. Gamma-aminobutyric acid (GABA) transmission is implicated in the effects of TMS on the cortex (Kujirai et al., 1993; Ziemann et al., 1996a,b; Werhahn et al., 1999) and modulation of GABA is felt to be the key mechanism of short-term plasticity in the adult mammalian central nervous system (Jones, 1993; Donoghue et al., 1996).

In summary, rTMS over the primary motor cortex seems to modulate the coupling of this area with distant sites. This modulation preferentially involves the alpha frequency band, which is believed to have mainly inhibitory activities. These results therefore suggest one means whereby low frequency rTMS may, in part, result in decreased excitability of the motor cortex, through an increase in the cortico-cortical and interhemispheric coupling in the alpha frequency band. Changes in cortico-cortical and interhemispheric coupling following rTMS over motor cortex occurred at intensities that were likely to be too low to change cortical excitability as measured by MEP size (Siebner et al., 1999b; Gerschlager et al., 2001), suggesting that cortico-cortical and interhemispheric coherence may provide a more sensitive measure of cortical function following rTMS. More speculatively, rTMS appears to be a non-invasive tool whereby certain connections within the brain may be strategically modulated.

3.4 Summary of key points

- Fifteen healthy subjects received one train (1Hz, 90% of AMT, 1500 stimuli) of rTMS to the left motor hand area.

- Spectral power and coherence estimates were calculated for alpha and beta frequencies between different EEG signals at rest and while muscles of the distal upper limb were tonically contracted.

- RTMS over the left motor hand area caused a significant increase in ipsilateral EEG-EEG alpha coherence and in the interhemispheric alpha coherence between motor areas in the alpha band.

- The effects of rTMS lasted up to 25 minutes post-stimulation. There was no significant change in EEG-EEG alpha coherence over the hemisphere contralateral to stimulation.

- Low frequency, subthreshold rTMS of the motor cortex increases ipsilateral cortico-cortical and interhemispheric coherence in the alpha band that may, in part, mediate the inhibitory effects of low frequency rTMS.

4 The effects of 5 Hz repetitive TMS on cortico-cortical coherence and reaction times.

The mechanisms of action of rTMS are unclear. Studies to date have mainly tried to define rTMS effects by evaluating changes in motor cortex excitability using single and paired pulse magnetic stimulation (Pascual-Leone et al., 1994, 1998; Chen et al., 1997b; Berardelli et al., 1998; Maeda et al., 2000a; Peinemann et al., 2000; Gerschlager et al., 2001) or local glucose metabolism using positron emission tomography (Siebner et al., 2000, 2001).

As discussed in the previous chapter, one possible mechanism of action of rTMS is the modulation of cortico-cortical coupling. This may be investigated through the assessment of changes in the coherence between EEG signals simultaneously recorded over different cortical sites. Coherence between distant electrodes is mainly due to long axon connections coupling different cortical areas (Thatcher et al., 1986), although the effects of volume conduction should not be underestimated. The previous experiment demonstrated increased coupling between premotor and motor areas in the alpha band after a train of 1500 low frequency (1 Hz) stimuli delivered over the primary motor cortex at subthreshold intensity. Changes in regional EEG power in the alpha band tend to involve inhibitory mechanisms (Pfurtscheller, 1992; Chen et al., 1998; Worden et al., 2000; Pfurtscheller et al., 2000; Hummel et al., 2002). If coherence within the same band were to have similar significance, then the increased coupling between the two areas seen previously could contribute to the reduction in excitability of the stimulated primary motor cortex that occurs with low frequency (stimulus rates of 1 Hz or less) rTMS (Chen et al., 1997b). In contrast, higher frequencies (stimulus rates of more than 1 Hz) may promote a short-term increase in cortical excitability (Pascual-Leone et al., 1998; Maeda et al., 2000a; Peinemann et al., 2000; Di Lazzaro et al., 2002a), although a recent study in which the modulatory effects of rTMS were systematically investigated at various frequencies and various train lengths, demonstrated a substantial inter-individual variability of the effects of rTMS (Maeda et al., 2000b). To date, other than work presented in this thesis, there is only one report of EEG-EEG coherence changes following high frequency rTMS. Jing and Takigawa (2000) paradoxically found an increase in directed, but not standard, EEG-EEG coherence in the alpha band (8–13 Hz). As high frequency rTMS seems to promote a short-term increase in cortical excitability a reduction in cortico-cortical coupling in the alpha band might have been expected (Pascual-Leone et al., 1998; Peinemann et al., 2000; Wu et al., 2000). Indeed, a second study in which 5 Hz rTMS was delivered over

the SMA found a reduction in the coupling of the two primary motor cortices in the alpha band, with related effects on inter-limb coordination (chapter 5).

In this chapter, the effects of 5 Hz rTMS on functional coupling are investigated, specifically looking at direct effects on the coherence between motor cortex and more anterior ipsilateral motor areas and between right and left motor cortices. It was hypothesised that coupling between the motor cortex and functionally related cortical areas would be decreased at frequencies at which oscillatory activity is predominantly associated with decreased inhibition. Specifically, it was predicted that coherence in the alpha band between the primary motor cortex and more anterior ipsilateral motor areas (including lateral premotor and prefrontal areas) would be decreased after high frequency rTMS, in line with the reported temporary increases in motor cortical excitability.

Interactions between the premotor and motor cortex are involved in action selection and movement execution (Deiber et al., 1991; Rao et al., 1997). It might be expected, therefore, that any change in cortical coupling should have a functional correlate on a motor task. For this reason, the effects of both 1 Hz and 5 Hz rTMS to the left motor cortex on a simple choice reaction time task with a visual go signal were studied.

4.1 Methods

Sixteen healthy volunteers were studied (ten men and six women, mean age [± SD] 30 [± 6] years, range 21–46 years). All subjects were right-handed according to the Edinburgh inventory (Oldfield, 1971).

4.1.1 Experimental design

As in the previous experiment, subjects were seated in a comfortable reclining chair with their arms resting horizontally by their sides. EEG and EMG electrodes were attached as described below. They were instructed to keep their eyes open, fixate a visual target directly in front and relax. Recordings were taken prior to, immediately after, 25 min after and 50 min after rTMS (times pre, post 0, post 25 and post 50, respectively). Subjects were required to contract to verbal command the first dorsal interosseous (FDI) and wrist extensor (WE) muscles bilaterally by fanning the fingers while extending the wrist for 60 s. Contractions were about 20–30% maximal strength. Three cycles of 60 s of rest and 60 s of continuous tonic muscle activation were recorded for each time point.

4.1.2 Magnetic stimulation

The optimal position ('hot spot') over the left motor cortex for activation of the right FDI muscle was determined using previously described methods (chapter 2). This was repeated to locate the right motor cortex hand area. Both resting and active motor thresholds (RMT and AMT, respectively) were determined at baseline immediately before rTMS. Repetitive TMS was applied to the left motor hand area using a frequency of 5 Hz, with the subject at rest. The coil was held in an identical way as described for the threshold measurements (chapter 2). The intensity of the rTMS was set at 100% AMT of the left motor cortex hand area for each individual subject. Fifty stimuli were given. Stimulation variables were in accordance with published safety recommendations (Wassermann, 1998).

4.1.3 EEG and EMG recording

By using bipolar leads without common electrodes for coherence analysis (for example, comparing F3-FC3 with C3-CP3 and F4-FC4 with C4-CP4) not only can the use of a common reference be avoided but also excessive spatial filtering. The effects of volume conduction were further limited by looking for a change in EEG-EEG coherence following rTMS. Bipolar leads over the sensorimotor cortex may also satisfactorily resolve activities in the lower and upper alpha bands (Pfurtscheller et al., 1981). The left motor hand area was located using single-pulse TMS and a surface electrode placed at this site. Further electrodes were placed 2.5 cm posterior, 2.5 cm anterior and 5 cm anterior to this first electrode (henceforth termed, from anterior to posterior, F3, FC3, C3 and CP3 as before). This was repeated on the right side (refer to figure 3.1). In addition, EEG electrodes were fixed to PO3 and O1. The electrodes were linked so as to form a chain of three bipolar leads on each side (F3-FC3, FC3-C3, C3-CP3, F4-FC4, FC4-C4, C4-CP4) and a single ipsilateral occipital bipolar lead (PO3-O1). EMG data were recorded bilaterally from FDI and WE using pairs of surface Ag-AgCl electrodes.

4.1.4 Data analysis

The data were inspected visually for eye movement artefact, scalp EMG and mains spikes. As in chapter 3, 110 s of clean data were extracted from each recording and examined using the spectral analysis methods outlined in Halliday et al. (1995). The 5 s prior to and after each activation or relaxation of the muscles were excluded to avoid using data that involved transients due to movement preparation, onset or termination. Thus only steady-state tonic contraction and rest periods were analysed. The Fourier transforms of non-overlapping sections of 1,024 data points were calculated, the

results averaged across sections, and the power spectra, coherence and phase relationships estimated. The frequency resolution was 0.98 Hz. Recordings were Hanning-windowed to control spectral leakage.

The EEG bands 7.8-9.7 Hz (low alpha), 10.7-13.6 Hz (high alpha) and 14.6-30.3 Hz (beta) were chosen for analysis. EEG activities in the alpha range (Pfurtscheller et al., 2000; Worden et al., 2000; Hummel et al., 2002) may be related to predominantly inhibitory processes, and it was expected, therefore for coherence to decrease following high frequency rTMS. The alpha band was divided into two as it may consist of two functionally distinct activities, with frequencies above and below 10 Hz. The lower frequency component is involved in a widespread movement-type non-specific event-related desynchronisation pattern, whereas the higher alpha band shows a more focused and task-specific pattern, suggesting that it is this that is more closely related to movement preparation and execution (Pfurtscheller et al., 1981, 2000; Andrew and Pfurtscheller, 1997). In support of this distinction, Manganotti et al., (1998) and Deiber et al., (2001) found that motor tasks exerted different effects on the topography of the lower and upper alpha activities, and Mima et al., (2000b) found that only sensorimotor activity in the upper alpha band was coupled with contralateral muscle activity.

The signals were amplified and band-pass filtered (EEG 0.53–300 Hz; EMG 53–300 Hz). After A/D conversion using a 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) the data were stored on a personal computer as SMR files (Spike 2; Cambridge Electronic Design). All data were sampled at 1,000 Hz.

The variance of spectral power estimates was stabilised by logarithmic transformation (Halliday et al., 1995). To compare the coherence between EEG signals, the variance of the modulus of the coherency (given by the square root of the coherence) was normalised using a Fisher transform (Rosenberg et al., 1989; figure 4.1). These values are henceforth referred to as log power and ($\tanh^{-1}$) coherence, respectively. The average log power and ($\tanh^{-1}$) coherence values were computed across subjects for both rest and active conditions and for the four time points. Based on *a priori* evidence (discussed above) that activities in the lower and upper alpha bands differ functionally, the results from these bands and from the beta band were then entered into separate factorial analyses of variance incorporating, where necessary, a Greenhouse-Geisser correction for non-sphericity. To avoid a disproportionate number of levels relative to the number of subjects studied separate ANOVAs for intrahemispheric and interhemispheric EEG-EEG ($\tanh^{-1}$) coherence were performed in each band.

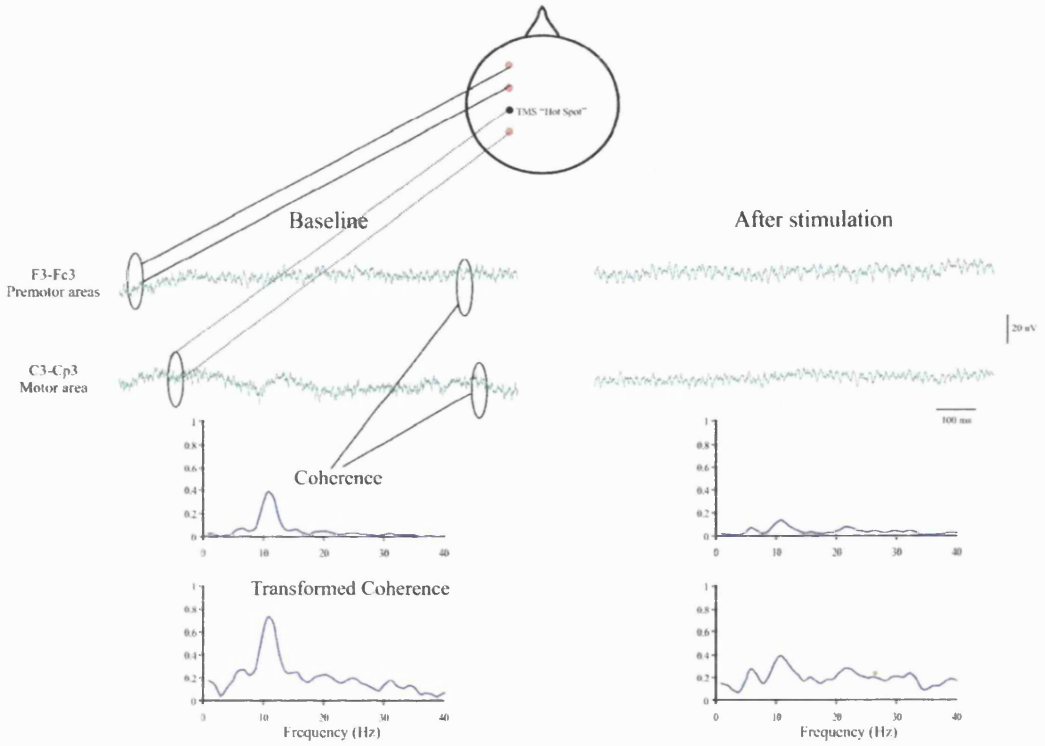


Figure 4.1. Raw EEG recorded during voluntary contraction of first dorsal interosseous and forearm extensor before and after repetitive transcranial magnetic stimulation (rTMS). The EEG recordings were visually inspected off-line and the coherence and the transformed coherence were estimated

4.1.5 Reaction Time Study

Subjects underwent further testing to investigate whether the persistent effects of 1 Hz and 5 Hz rTMS could interfere with a simple choice reaction time task (RT). The task involved reacting manually to a visual stimulus by pressing a switch with the appropriate thumb. 'Go' stimuli were leftward or rightward pointing double arrows ("<<", ">>") that appeared on a screen. Three RT sessions were recorded at one sitting, each consisting of 2 blocks of 40 trials and each lasting 6 minutes. In each block, trials were presented randomly every 4 to 6 seconds.

For each subject, reaction time testing for the different conditions was performed on separate days. For the rTMS conditions, the first RT session was followed by 5 Hz rTMS (50 Stimuli, 100% AMT) or 1 Hz rTMS (1500 stimuli, 90% AMT) to the left motor cortex and the second and third sessions recorded immediately afterwards. For the control condition, the three sessions were acquired at the same times but without any rTMS. Stimulated and non-stimulated sessions were intermixed in a pseudorandom order.

Fourteen (8 males, mean age ± SD = 30.0 ± 6.7 years) of the original 16 subjects took part in the 5 Hz rTMS and control testing. Of these, six subjects (5 males, 34.8 ± 6.8 years) also had 1 Hz stimulation and were thus included in all three testing conditions (1 Hz, 5 Hz, control). Non-parametric statistics were used to compare the percentage change in RT between the control and rTMS conditions.

4.2 Results

There was no significant difference between the mean rectified EMG level in the pre-rTMS period and that in any of the post-stimulation periods.

4.2.1 Cortical power spectra data

For each of the frequency bands of interest (7.8–9.7 Hz, lower alpha; 10.7–13.6 Hz, upper alpha; 14.6-30.3 Hz, beta) an ANOVA was performed using within-subject factors of time (pre and post 0), condition (rest and active) and region (C3-CP3, C4-CP4, F3-FC3, F4-FC4). Spectral power was decreased in active periods compared to rest in the alpha and beta bands, consistent with other reports (Gerloff et al., 1998; Manganotti et al., 1998; Pfurtscheller et al., 2000).

Lower alpha power: There was a main effect for condition ($F[1,15] = 4.736$, $p = 0.046$) and an interaction between region and condition ($F[3,45] = 5.105$, $p = 0.023$). Post hoc t-tests revealed a decrease in power during the active state of 6.5% at C3-CP3 ($p = 0.021$) and a decrease of 8.6% at C4-CP4 ($p = 0.013$).

Higher alpha power: There was a main effect for condition ($F[1,15] = 36.423$, $p < 0.001$), an interaction between region and condition ($F[3,45] = 8.100$, $p = 0.003$) and an interaction between condition and time ($F[1,15] = 7.978$, $p = 0.013$). There was no three-way interaction between region, condition and time. Post hoc t-tests showed that power decreased significantly during the active state at C3-CP3 (12.7% decrease, $p < 0.001$), C4-CP4 (17.9%, $p < 0.001$) and F3-FC3 (5.8%, $p = 0.018$). Despite a significant interaction between time and condition, post hoc t-tests did not reveal any significant change of regional power with time for the rest or active states.

Beta power: There was a main effect for region ($F[3,45] = 3.217$, $p = 0.032$) and an interaction between region and condition ($F[3,45] = 7.164$, $p = 0.010$). Post hoc t-tests revealed a decrease in power during the active state of 4.0% at C3-CP3 ($p = 0.001$) and a decrease of 3.2% at C4-CP4 ($p = 0.028$).

Finally, as a control, the bipolar EEG pair PO3-O1 was also analysed across the three candidate bands. There was no significant power change with time or condition, and no significant interaction between the two factors for any frequency band.

4.2.2 Cortico-cortical intrahemispheric coherence

Figure 4.2 shows the average transformed coherences for the left and right hemispheres in the three frequency bands both at rest and during muscle activation. The greatest change with rTMS occurs in the upper alpha band during muscle activation, and is more marked in the left hemisphere (see arrow A in figure 4.2). Figure 4.3 shows the average change in coherence between C3-CP3 and F3-FC3 (post 0-pre). There is a drop in coherence in the upper alpha band following rTMS, which was most evident over the stimulated (left) hemisphere (see arrow B in figure 4.2).

For each of the three frequency bands of interest (7.8–9.7 Hz, lower alpha; 10.7–13.6 Hz, upper alpha; 7.8-13.6 Hz, beta) an ANOVA for repeated measures was performed using within-subject factors of time (pre and post 0), condition (rest and active) and region (F3-FC3 to C3-CP3 and F4-FC4 to C4-CP4).

There were no significant main effects or interactions for the lower alpha and the beta bands. However, for the upper alpha band, there was a main effect for condition ($F[1,15] = 19.892$, $p < 0.001$) and a significant interaction between time and condition ($F[1,15] = 9.220$, $p = 0.008$). There was no effect of region, allowing data for the two regions to be combined. Post hoc t-tests confirmed that a significant decrease in coherence (of 13.2%) in the upper alpha band occurred immediately following rTMS, only during voluntary muscle activation ($p = 0.010$). This effect of rTMS was not significant after 25 or 50 min.

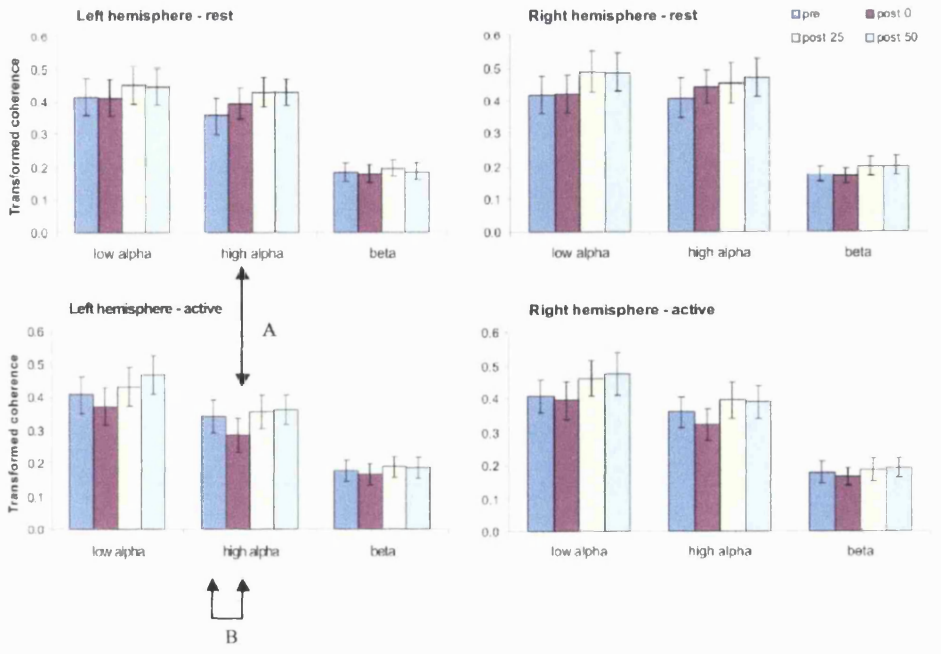


Figure 4.2. Average transformed coherence between F3-FC3 and C3-CP3 and between F4-FC4 and C4-CP4 (n=16) in the different frequency bands of interest according to timing with respect to rTMS, condition (active and rest) and hemisphere. Bars are standard error of the mean (see text for explanation of arrows).

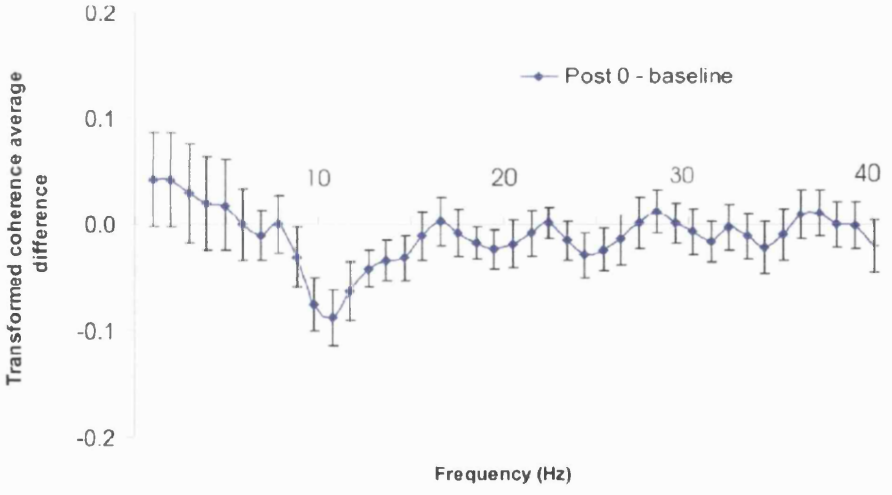


Figure 4.3. Change in average transformed coherence (n=16) between C3-CP3 and F3-FC3 during tonic muscle activation following rTMS to the left motor cortex. Bars are standard error of mean.

4.2.3 *Interhemispheric coherence*

Interhemispheric coherence (between C3-CP3 and C4-CP4) for the three frequency bands was also analysed by ANOVA (figure 4.4). There were no main effects for time or condition or interactions in the upper and lower alpha bands. However, there was a main effect for condition in the beta band ($F[1,15] = 9.111$, $p = 0.009$). Post hoc t-tests showed that there was a decrease in beta interhemispheric coherence of 8.1% with muscle activity ($p = 0.009$).

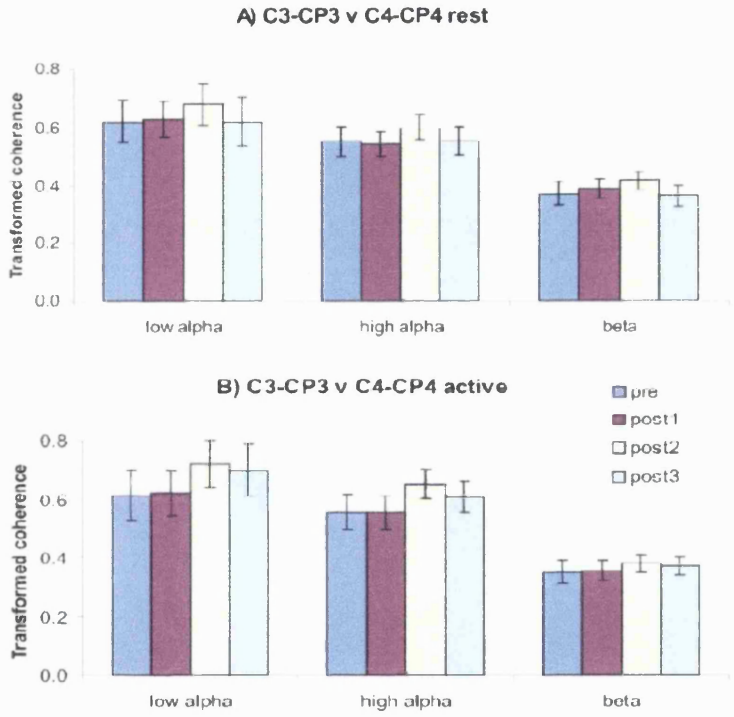


Figure 4.4. Average transformed interhemispheric coherence between C3-CP3 and C4-CP4 (n=16) for the different frequency bands of interest according to condition (rest and active). Bars are standard error of the mean.

4.2.4 *Reaction Time Study*

Could the lasting effects of rTMS interfere with a simple choice reaction time (RT) task? The RT task was thus evaluated under control and stimulated conditions. Figure 4.5 shows the results for the 5 Hz versus control conditions (n=14). Reaction times usually improve slightly with repeated attempts (the practice effect) as can be seen from the reaction times in the control condition. However, in the 5 Hz stimulated condition, the reaction times appear to be slower in the time period immediately after the stimulation (0 to 6 minutes). Reaction time data for both hands at each time point were entered

into a Friedman ANOVA. The difference in reaction times between the control and 5Hz stimulated conditions did not quite reach significance (p = 0.079).

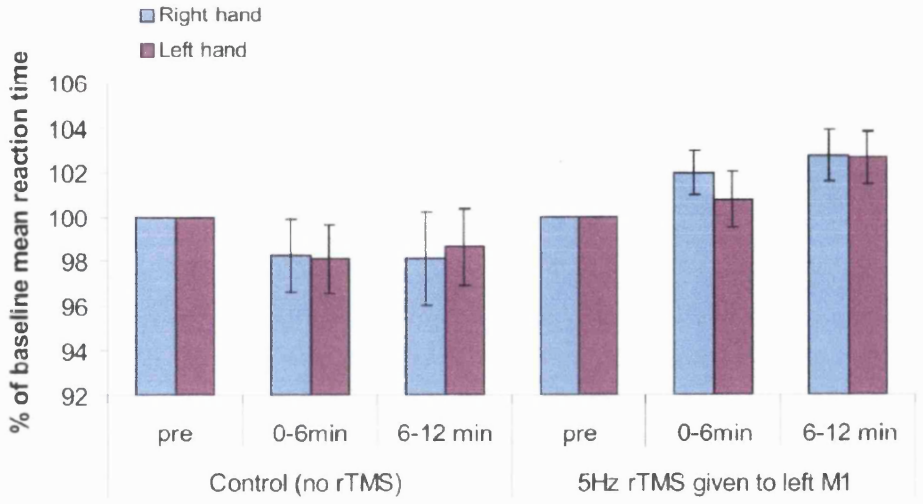


Figure 4.5. Effects of rTMS on reaction times. Graphs show the reaction times for each hand before and after 5 Hz rTMS (n=14) was given to the left motor cortex. For each stimulation condition, subjects also performed a control condition when no rTMS was given. Bars represent the standard error of the mean.

Six subjects were included in all three experimental paradigms and their data (figure 4.6) were thus used to compare directly the differential effects on reaction times of 1 and 5 Hz rTMS compared with no stimulation. Data (n=6) for each hand were entered into separate non-parametric (Friedman) ANOVAs and, if significant, analysed post-hoc using the Wilcoxon 2-sample test. Reaction times for the left hand were not significantly different between conditions. However, for the right hand, there were significant changes in reaction times (Friedman ANOVA, p = 0.003). In the control (no stimulation) condition, reaction times were an average of 10.2 ms slower with practice (p = 0.043). This apparent reversal of the practice effect is likely to represent a type II statistical error due to the small sample size. After subjects had received 5 Hz rTMS, the reaction times were also significantly slower than control (by an average of 14.5 ms, p = 0.028). In contrast, after 1 Hz rTMS the reaction times decreased by 8.0 ms, although this difference was not significant. Reaction times post-stimulation after 1 Hz rTMS were significantly different from the control condition (p = 0.028). However, post-stimulation reaction times after 5 Hz rTMS did not differ from those in the control condition (p > 0.05). It could be assumed that all these differences arise from a possible type II error in the control condition (see above). However, reaction times were also significantly

different post-stimulation after 1 Hz compared with 5 Hz rTMS ($p = 0.028$). Thus, although the sample size was small, there did appear to be a differential effect of 1 and 5 Hz rTMS on reaction times. This was in keeping with the differential effects of low and high frequency rTMS on cortico-cortical coherence.

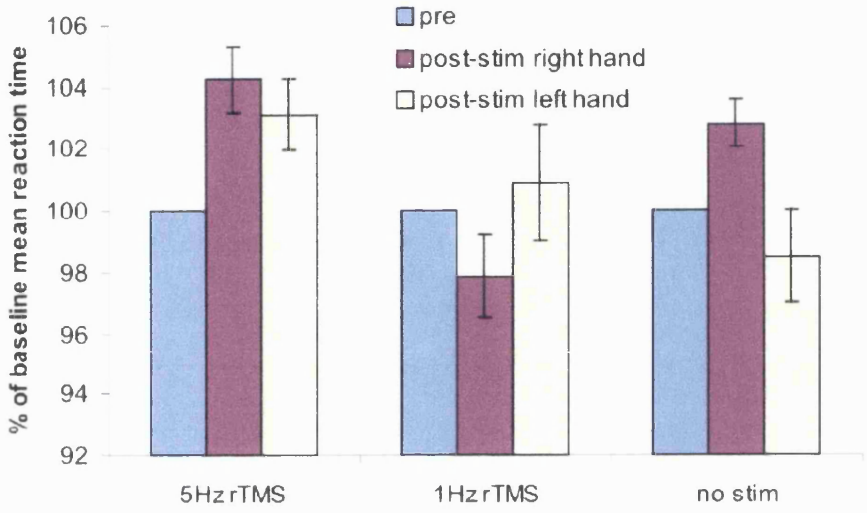


Figure 4.6. The graph shows the differential effects (n=6) of 1 and 5 Hz rTMS on reaction times for each hand. Bars represent the standard error of the mean.

4.3 Discussion

High frequency rTMS caused a significant decrease in the upper alpha coherence between the primary motor and more anterior motor areas. Interhemispheric coherence was not affected by rTMS although there was a decrease in beta coherence with muscle contraction compared with rest. The reaction time data hints at a differential effect of 1 and 5 Hz rTMS.

4.3.1 Effects of 5 Hz rTMS on cortico-cortical interactions

The changes in coherence are unlikely to be due to a general alteration in arousal of subjects for several reasons. In the previous chapter, albeit with many more stimuli, low frequency (1 Hz) rTMS over the motor cortex (90% active motor threshold) gave the opposite effect, causing ipsilateral cortico-cortical coherence to increase post-stimulation. The intrahemispheric changes in this experiment were confined to periods of tonic contraction. An effect of general arousal would have been expected to involve both rest and active conditions. The lack of rTMS effects on regional power is also against any non-specific arousal changes during the paradigm. The effect must have

been purely central in origin because the intensity of the individual rTMS pulses was below resting motor threshold and rTMS was performed at rest. Therefore, no muscle twitches were evoked that could modify central processing through changed afferent input. Neither did differences in non-linear components of the signals account for differences in coherence. There was no change in EEG power with rTMS so that the changes in EEG-EEG coherence in the alpha band reflected a change in the absolute degree of coupling between areas.

There was, however, a significant reduction in the power over left and right motor and premotor areas in the alpha and beta ranges during muscle contraction, as expected (Gerloff et al., 1998; Manganotti et al., 1998; Pfurtscheller et al., 2000). This reduction in power has been interpreted as a sign of regional activation (Pfurtscheller, 1988; Toro et al., 1994a,b; Stancak and Pfurtscheller, 1995; Gerloff et al., 1998). Although power in the upper alpha band decreased during contraction, EEG-EEG coherence in this band remained unchanged. Conversely, coherence in the same band decreased following rTMS at 5 Hz, while regional EEG power remained unchanged. Independent changes in regional EEG power and EEG-EEG coherence have been noted before and it has been suggested that the two measures may relate to different aspects of cortical function that might, to some extent, operate independently of each other (Gerloff et al., 1998).

Previous studies have shown changes in beta interhemispheric coherence with phasic bimanual hand movements. As movement complexity increases, beta interhemispheric coherence increases whereas if the speed of movement increases, coherence decreases since bimanual coordination is impaired (Serrien and Brown, 2002). In this chapter, it has been found that beta interhemispheric coherence decreases with tonic bimanual muscle contraction. This could be a result of the difference in task performed; tonic muscle contraction compared with phasic bimanual hand movements.

5 Hz rTMS changed cortical coupling but the effect was only significant during the active contraction of muscles and in the upper alpha band. Other reports also support the notion that activities in the upper and lower alpha bands are likely to be functionally distinct (Manganotti et al., 1998; Mima et al., 2000b; Pfurtscheller et al., 2000). It is noteworthy that the effects of 1 Hz rTMS on alpha band coupling are not only opposite but also less specific. Thus they do not depend on functional state (rest compared to voluntary tonic activity) and are more generalised, affecting interhemispheric coupling as well. These differences may be a function of the different frequency of stimulation and/or the greater number of stimuli delivered.

The decrease in cortico-cortical coupling brought about by rTMS involved the alpha band. Increases in EEG power in this band have been associated with predominantly inhibitory activities (Pfurtscheller, 1992; Chen et al., 1998; Pfurtscheller et al., 2000; Worden et al., 2000) and it is tempting to assume a similar, overall inhibitory function for the coupling between premotor and motor cortex in the alpha band. Consistent with this, inhibitory connections between the primary motor cortex and more anterior areas have recently been reported in humans by Civardi et al. (2001). RTMS at 5 Hz might modify inhibitory coupling through effects on inhibitory projections from anterior motor areas to primary motor cortex or through effects on local GABAergic interneurones that receive input from excitatory axons coming from the premotor cortex. The GABAergic interneurones might then be modulated by local TMS over the primary motor cortex (Kujirai et al., 1993; Ziemann et al., 1996a,b; Werhahn et al., 1999; Di Lazzaro et al., 2000). Either way, 5 Hz rTMS might reduce the efficacy of inhibitory cortico-cortical inputs to the motor cortex, thereby contributing to the increase in excitability observed following this intervention (Pascual-Leone et al., 1998; Maeda et al., 2000a; Peinemann et al., 2000; Di Lazzaro et al., 2002b). Alternatively, the effects of rTMS on the motor cortex might be more indirect, involving remote after-effects on subcortical structures that then cause secondary changes in cortico-cortical coupling. Such indirect subcortical or transcallosal effects might have also accounted for the change in coupling between the motor cortex and premotor areas contralateral to the rTMS (although fig. 4.2 suggests there was a trend for this change to be less than that occurring over the stimulated hemisphere).

4.3.2 Behavioural effects of rTMS

The previous two experiments have shown that rTMS, given to the left motor cortex at different frequencies, has effects on cortico-cortical coupling that are different. Although the sample size was small, the effects of left motor cortex rTMS on right hand reaction times also differed according to whether high or low frequency stimulation was given. Left hand reaction times were not affected. Reaction times were longer after 5 Hz stimulation than after 1 Hz rTMS. Premotor cortical areas are involved in responses to perceptual cues (Halsband and Freund, 1990; Jeannerod et al., 1995) and are also implicated in the initiation and selection of movement (Deiber et al., 1991; Rao et al., 1997). It could be speculated that 5 Hz rTMS causes an imbalance in the net inhibitory connections between premotor cortex and motor cortex and that this modification in the flow of information between the two cortical structures is responsible for a delayed reaction. In this context, diminished inhibition seems functionally more important than increased inhibition.

In summary, high frequency rTMS over primary motor cortex seems to modulate the coupling of this area with more anterior cortical regions. This modulation involves the upper alpha frequency band and may contribute to the changes in excitability observed after rTMS over the primary motor cortex. Of note, although stimulation was given to the left motor cortex, the changes in coupling were observed in both hemispheres.

4.4 Summary of key points

- Sixteen healthy subjects received a single train (5 Hz, 100% AMT, 50 stimuli) of rTMS to the left motor hand area.
- Spectral power and coherence estimates were calculated between different EEG signals at rest and while muscles of the distal upper limb were tonically contracted.
- There were no significant spectral power changes with rTMS.
- Repetitive TMS over the left motor hand area caused a significant decrease in the intrahemispheric EEG-EEG coherence between motor and premotor cortex in the 10.7–13.6 Hz (upper alpha band) lasting a few minutes after stimulation. There was no significant change with rTMS in interhemispheric EEG-EEG coherence between motor areas.
- Thus, high frequency rTMS of the motor cortex decreases cortico-cortical intrahemispheric coherence in the upper alpha band.
- 1 and 5 Hz rTMS to the left motor hand area may have different effects on right hand reaction times.

5 Repetitive TMS of the supplementary motor area (SMA) degrades bimanual movement control

The previous chapter demonstrated that 5 Hz rTMS to the motor cortex was capable of inducing changes in cortico-cortical coherence between the motor areas that coincided with disruption of a related motor task. It should therefore be possible to disrupt the function of a second cortical area, producing measurable physiological changes and cause alterations in an associated motor task. This chapter explores such a possibility and studies the effects of 5 Hz rTMS to another accessible cortical motor area, the supplementary motor area (SMA).

Bimanual movements of the upper limbs form an essential part of our behaviour and are commonly required during activities of daily living. The planning and execution of such bimanual tasks involves a coordinated organisation of the motor commands to both hands. One mechanism in which the sensorimotor cortices could achieve such an association might be through dynamic networks that rely on synchronised neural activity. This type of coupled processing not only allows for the appropriate selection of networks relevant for task production but also enhances the significance of neural responses since concurrent discharges have a stronger impact than momentarily disorganised inputs (Singer, 1993). Human studies have shown EEG coherence to be a valuable measure of such interregional correlation of oscillatory activities (Andres et al., 1999; Gerloff et al., 1998).

Coordinated behaviour often comprises a high degree of complexity, yet an intrinsic tendency towards simultaneity of movement subsists. It is well recognised that in-phase (symmetrical or mirror) movements are more accurate and stable than anti-phase (asymmetrical or parallel) movements, and require less attention (Kelso, 1984; Monno et al., 2000; Swinnen et al., 1997). These patterns represent preferred coordination dynamics and the additional supremacy of the in-phase mode over the anti-phase mode might be accounted for by differences in processing requirements. This hypothesis has been confirmed in functional imaging work that has demonstrated an increased neural activation in the prefrontal cortex and midbrain during anti-phase as compared to in-phase patterns (Fink et al., 1999; Sadato et al., 1997). Furthermore, data from patient groups have shown that these rhythmical actions, and especially anti-phase movements, are disturbed by prefrontal and mesio-frontal lesions (Luria, 1969; Leonard et al., 1988; Halsband et al., 1993; Stephan et al., 1999).

The imaging data and observations in patients indicate the prominent role of the medial wall areas, including the SMA, in bimanual function (Goerres et al., 1998; Immisch et al., 2001; Jancke et al., 2000a, 2000b; Meyer-Lindenberg et al., 2002; Sadato et al., 1997; Toyokura et al., 1999). To establish further the functional contribution of this area to the control of coordinated behaviour, rTMS of the SMA was used to interfere with the regulation of in-phase and anti-phase movements. Previous studies have shown that fronto-central TMS can successfully interfere with concurrent behavioural tasks, most likely due to disturbance of neuronal processing in the SMA region (Muri et al., 1994, 1995; Cunnington et al., 1996; Gerloff et al., 1997; Meyer-Lindenberg et al., 2002). In this study, the question of whether post-stimulation effects of rTMS could interact with the execution of these coordination modes was addressed. This would determine the impact of a transient disturbance of the SMA on the accuracy of behavioural output in the absence of the confounding influence of stimulation during performance. At the same time, this would establish whether any effects of rTMS could be associated with a change in the coupled activity of the primary motor cortices.

5.1 Methods

For the stimulation plus behavioural paradigm, six healthy volunteers, 2 female and 4 male, (mean age [± SD] 31 [± 6] years) were studied. For the coherence measurement paradigm, nine healthy subjects were studied, 2 females and 7 males (mean age 34 ± 7 years). All were right-handed as measured using the Edinburgh inventory (Oldfield, 1971).

5.1.1 Experimental design

Subjects were seated in front of a desk upon which a device with two response keys was installed. They were instructed to make cyclical tapping movements with the index fingers at a comfortable, self-paced rate onto the response keys. Movements were either in-phase (both index fingers moved simultaneously upward and downward) or anti-phase (one index finger moved upward whilst the other moved downward). Subjects were blindfolded and executed the tasks before (pre) and after (post) rTMS of the SMA. Two post-stimulation sessions were included: immediately after (post 0) and 25 min after (post 25) the stimulation. More sessions were excluded to avoid fatigue. For each of the three sessions, there were two trials per coordination mode, randomly ordered within and between subjects. Each trial lasted 10 s, during which the movement onset and offset of each index finger was recorded continuously. This permitted sufficient movement cycles to establish temporal regulation.

5.1.2 *Magnetic stimulation*

5 Hz stimulation was applied to the SMA for 10 seconds (50 stimuli) in six subjects while sitting at rest. The stimulation intensity was set at 90% of the average active motor threshold (AMT) for the left and right motor hand areas of each subject (located as described in section 2.5.2). The average AMT for the hand areas was used as it was found to be a more reproducible measure than the foot area. Stimulation intensity was kept low in order to induce a focal effect. The point of stimulation was located 2 cm in front of CZ. This position represents the hand area of SMA as established using fMRI (Lee et al., 1999). It was also anterior to the leg area in each subject, as determined from a motor response induced using TMS. The leg representation in the primary motor cortex is located directly adjacent and posterior to the SMA. Coil orientation over the SMA was with a backward-pointing handle and coil junction in the midline.

5.1.3 *EEG recording*

Nine subjects had EEG recordings performed in a separate experiment, on a different day, before and after stimulation (pre, post 0, post 25). Scalp electrodes C3 and C4 were positioned using TMS to locate the motor hand areas of the left and right motor cortex respectively. Electrodes were placed 2.5 cm anterior, 5 cm anterior and 2.5 cm posterior to C3, giving F3, FC3 and CP3 respectively. This was repeated for C4, giving F4, FC4 and CP4. FCZ and CZ were positioned according to the 10-20 system. The electrodes were referenced to linked ears. The point of stimulation (2 cm anterior to CZ) thus lay between FCZ and CZ. 5 Hz rTMS was given to the SMA with the subject at rest and with the eyes closed. Stimulation parameters were identical to those used for the behavioural recordings (90% AMT, 50 stimuli). EEG signals were amplified and band-pass filtered (0.53-300 Hz) with a sampling rate of 1000 Hz.

5.1.4 *Data analysis*

The measurement of interest represented the absolute temporal deviation between the taps produced by the finger movements on the response keys (figure 5.1), averaged per movement cycle. In particular, the asynchrony at tap onset and offset between the fingers was determined and taken as an accuracy index of temporal coordination. The values were normalized with respect to the pre-stimulation values. The normalized data were then analysed per coordination mode with a non-parametric (Friedman) ANOVA.

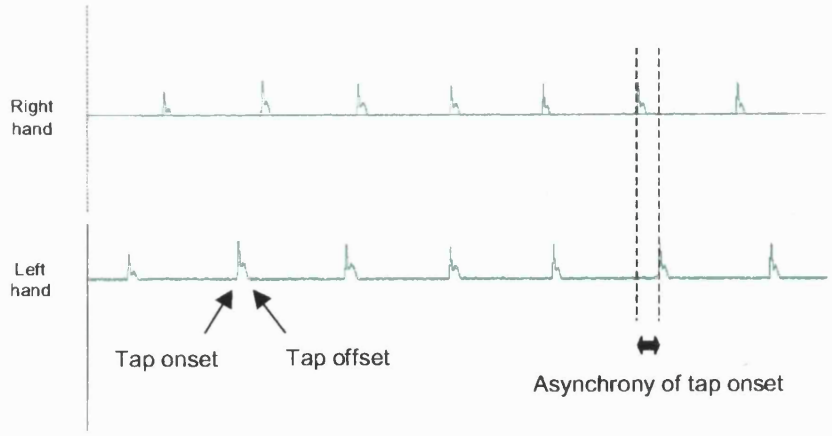


Figure 5.1. Schematic representation of the bimanual finger movements. Tap onset and offset were recorded and the temporal deviation between taps calculated.

Changes in the coupling between the primary motor cortices in the post-stimulation stage were determined through frequency analysis of scalp EEG as described in section 2.6. The variance of the spectral power estimates was stabilised by logarithmic transformation (Halliday et al., 1995) and the variance of the modulus of the coherency was normalised using a Fisher transform (Rosenberg et al, 1989). Transformed coherence was assessed over the alpha (7.8-13.6 Hz) and beta (14.6-30.3 Hz) bands by summing the peak value per band in each subject together with the bin either side, and thereafter averaging across subjects. Log power was measured over the same bins. The alpha and beta bands were chosen due to their distinct character as well as different roles in brain function (Salmelin et al., 1995). Also, activities in both frequency bands have been shown in earlier work to be involved in bimanual coordination (Andres et al., 1999). The transformed coherence measurements were normalized with respect to the pre-stimulation values and analysed per frequency band using non-parametric statistics (Friedman ANOVA). Separate ANOVAs were performed for different connections, as well as for log power at the various electrodes.

5.2 Results

The behavioural data for the in-phase and anti-phase modes are illustrated in figure 5.2. It can be observed that the temporal accuracy of both tasks transiently deteriorated following rTMS of the SMA. Despite the deterioration of both types of patterns, this effect only reached the level of significance for the anti-phase mode ($p = 0.003$).

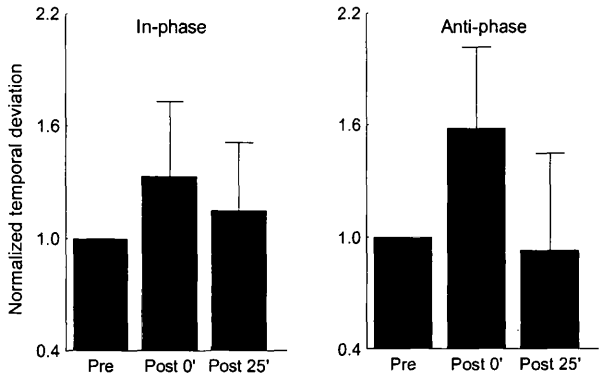


Figure 5.2. Changes in the temporal accuracy of in-phase and anti-phase movements in the different conditions; pre-stimulation (pre), immediately after (post 0) and 25 min after (post 25) 5 Hz rTMS of the SMA. Data are normalized to the pre-stimulation values. The error bars denote the SD around the means. An increased error score can be noticed in the immediate post-stimulation condition in comparison to the pre-stimulation and late post-stimulation conditions, which is most evident for the anti-phase mode.

The EEG data revealed that the functional connectivity between the primary motor areas (C3-C4) was significantly modified in the alpha and beta bands following rTMS of the SMA ($p = 0.015$ alpha; $p = 0.028$ beta). Figure 5.3 illustrates that the stimulation reduced the coherence between the hand areas of the primary motor cortices, suggesting that their functional coupling was less tight. Separate analyses for other interhemispheric connections (premotor: FC3-FC4; superior parietal: CP3-CP4) did not reveal significant effects ($p > 0.05$). EEG power did not change with the stimulation ($p > 0.05$).

The dominant peak frequency in the alpha band was around 8 Hz and did not differ as a function of the stimulation. The mean scores were 8.5, 8.5 and 8.4 Hz for pre, post 0 and post 25 stimulation conditions, respectively. The dominant peak frequency in the beta band was around 16 Hz and shifted slightly due to the stimulation. The mean scores were 16.6, 15.8 and 16.2 Hz for pre, post 0 and post 25 stimulation conditions, respectively.

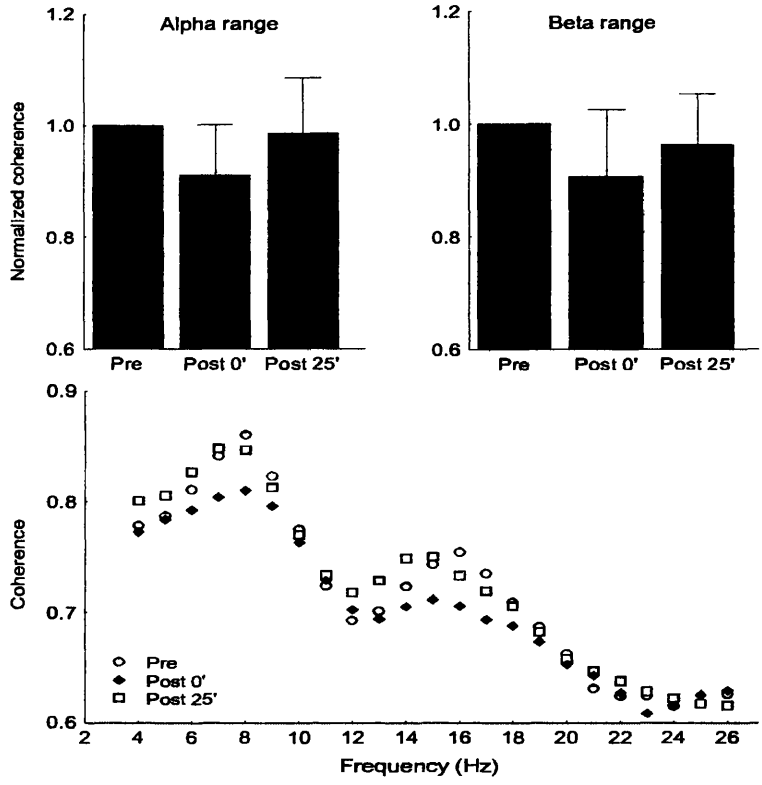


Figure 5.3. Upper panel: Changes in the transformed coherence in the alpha and beta bands for the interhemispheric C3-C4 connection in the different conditions; pre-stimulation (pre), immediately after (post 0) and 25 min after (post 25) 5 Hz rTMS of the SMA. Data are normalized to the pre-stimulation values. The error bars denote the SD around the means. A reduced degree of coherence can be observed in the immediate post-stimulation condition as compared to the pre-stimulation and late post-stimulation conditions. **Lower panel:** Untransformed coherence spectrum (data from a representative subject). The decrease in the alpha band (around 8 Hz) as well as the beta band (around 16 Hz) as a function of rTMS can be noticed.

5.3 Discussion

Notwithstanding a high degree of flexibility during coordinated behaviour, a strong tendency exists towards simultaneity of motion when executing bimanual patterns. In-phase and anti-phase coordination are preferred movement configurations when the upper limbs are moved with equal tempo. Behavioural, clinical and functional imaging studies have indicated that the in-phase pattern is the more basic and successful form of coordination (Fink et al., 1999; Kelso, 1995; Sadato et al., 1997; Serrien et al., 2001; Swinnen et al., 1998).

In this study, high frequency rTMS to the SMA caused a deterioration of temporal inter-limb regulation, immediately following stimulation. The effect was stronger for anti-phase than in-phase movements, supporting its more intricate coordinative requirements. Accordingly, these data are in line with earlier imaging and EEG studies that have shown the SMA to be particularly involved in the processing of complex, as compared to simple, sequences (Gerloff et al., 1997; Lang et al., 1989; Shibasaki et al., 1993). These results are also supported by more recent studies. Meyer-Lindberg et al. (2002) demonstrated that double-pulse TMS over the SMA could induce phase transitions from the anti-phase coordination mode to the more stable in-phase mode. Steyvers et al. (2003) recently showed that 20 Hz rTMS to the SMA during anti-phase movements transiently disturbed coordination by increasing the mean absolute error of the relative phase between hands. This effect was not seen during in-phase movements, did not affect cycle duration and occurred in the absence of phase transitions. Low frequency (1 Hz) rTMS to the rostral parts of the SMA, given immediately prior to performance of a goal-directed bimanual coordination task, increased the variability of certain bimanual timing intervals (Obhi et al., 2002).

In the present context, it is hypothesized that rTMS of the SMA modified the functional coupling between the primary motor cortices. In particular, the EEG recordings revealed a reduction of the alpha and beta band coherence in the post-stimulation stage, albeit recorded at rest. It has been suggested that high frequency rTMS increases cortical excitability and/or decreases cortical inhibition (Pascual-Leone et al., 1994). Changes in inhibitory control can be particularly associated with modulations in the alpha band (Hummel et al., 2002), and may partly underlie the effects seen here as the execution of the anti-phase mode necessitates the suppression of the more intrinsic in-phase mode. Hence, the deterioration in the interhemispheric coupling due to SMA stimulation might compromise the regulation of this accurate task. In this respect, the SMA is recognized as having an important integrative role in the coordination of bimanual activities. It operates bilaterally with interhemispheric interactions, adjusting the activity of both primary motor areas. Such a significant function is supported by neurophysiological work in monkeys, which has reinforced the hypothesis that unimanual and bimanual movements are differently represented in the SMA (Donchin et al., 2001).

It is necessary, however, to consider two alternative suggestions. The first is that rTMS might have exerted its effects through stimulation of premotor or primary motor cortices. This seems unlikely given that stimulation was performed over the midline at

low intensity and that hand motor area is about 6-7 cm lateral to this position. The second point is whether the effects of rTMS could have been due to non-specific attentional changes. However, rTMS was not delivered during movement and caused no adjustments in EEG power, which might have been expected with modulations in attention or arousal.

These data provide evidence that the SMA is critically involved in the temporal organisation of basic behaviour as a function of coordinative complexity. Previously, Manganotti et al. (1998) observed larger coherence increases in the alpha and beta bands during movement sequences that involved a high degree of complexity. This was suggestive of augmented interactions between brain areas when integrating difficult, as compared to easy, responses. Therefore, it can be hypothesised that a reduced degree of functional interhemispheric coupling as observed here would have a stronger impact on the more complex anti-phase than in-phase mode. Along a similar line, previous work has shown that patients with an acquired lesion of the corpus callosum and accordingly reduced interhemispheric interactions have difficulties in performing bimanual configurations, and in particular the anti-phase mode (Serrien et al., 2001). A recent task-related EEG coherence study (Serrien and Brown, 2002), has shown that more complex bimanual movements are associated with more demanding levels of interhemispheric interaction, when compared with movements of lower complexity. As movement rate increased, there was a progressive reduction of interhemispheric coupling in the beta frequency band, in line with decrements in behavioural performance. Again, this was particularly evident in the anti-phase movements.

In conclusion, the present findings support the key role of the SMA for the organization of bimanual configurations as a function of task complexity. In particular, rTMS of the SMA disrupted the fine-tuning of inter-limb movements. This effect was associated with a reduced degree of EEG coherence between the primary motor cortices, albeit recorded at rest. These data suggest that rTMS of the SMA may transiently modify the functional interhemispheric coupling and thereby influence bimanual behaviour in which combined sequences need to be implemented into a coherent timing plan. It highlights the importance of an optimal integration of SMA activity into the motor control network that is used for the realisation of coordinated movements.

5.4 Summary of key points

- Moving the upper limbs at a common tempo according to an in-phase or anti-phase mode represents elementary coordination dynamics.
- The role of the supplementary motor area (SMA) has been emphasized for successful production of these patterns.
- The objective of this study was to investigate whether rTMS of the SMA at 5 Hz can interfere with these isofrequency configurations in the post-stimulation stage.
- Following rTMS, there was a degradation of the temporal control of both isofrequencies of bimanual movements, most evident in the anti-phase mode. This effect was associated with a decrease in the functional coupling between the primary motor cortices, as measured by EEG coherence.
- These data suggest that rTMS of the SMA can modify interhemispheric communication and accordingly modulate inter-limb behaviour.

6 The ipsilateral human motor cortex can functionally compensate for acute contralateral motor cortex dysfunction

It remains unclear why some patients who have a motor stroke make a good functional recovery. Possibilities include unaffected cortical areas compensating for affected regions by taking over function, or more use being made of subcortical motor systems. Functional imaging studies have revealed that several cortical areas, including contralesional premotor and primary motor (M1) cortices, are activated during contraction of the affected hand in stroke patients (Chollet et al., 1991; Weiller et al., 1993; Cramer et al., 1997, 1999; Cao et al., 1998; Seitz et al., 1998; Calautti et al., 2001; Nelles et al., 2001; Carey et al., 2002; Ward et al., 2003a). Some evidence supports a compensatory role for the contralesional premotor region: increased activation is more likely after some motor recovery has taken place (Nelles et al., 1999) and disruption of premotor function can lead to impaired performance of the affected hand (Johansen-berg et al., 2002). Whether changes in contralesional M1 function are mechanistically related to motor recovery is even less clear (Carey et al., 2002; Netz et al., 1997; Small et al., 2002; Shimizu et al., 2002). Greater ipsilateral compared to contralateral M1 activation occurs early, prior to substantial motor recovery, while improvements in the affected hand's performance with intensive training are associated with reversal of this ratio (Carey et al., 2002; Marshall et al., 2000), although this remains controversial (Calautti et al., 2001). Similarly, although TMS shows that ipsilateral changes in M1 excitability are more frequent after stroke, these are reversed in concert with motor recovery (Manganotti et al., 2002; Butefisch et al., 2003). Overall, these studies suggest that either ipsilateral M1 activation represents a compensatory mechanism, later replaced by other processes, or it is spurious, perhaps represented by mirror movements (Weiller et al., 1993) or unmasking of circuitry that is normally suppressed and has nothing to do with motor recovery (Turton et al., 1996).

This study therefore seeks evidence that ipsilateral M1 can compensate for dysfunction of contralateral M1 by testing subjects' ability to control the force of a fractionated finger movement, the type of movement most impaired following motor stroke. However, rather than study stroke patients, 5 Hz rTMS was used to elicit controlled, temporary and partial disruption of M1 function in healthy humans. Such trains are known to increase cortical excitability for short periods beyond the duration of the stimulation (Siebner and Rothwell, 2003; Peinemann et al., 2000; Wu et al., 2000; Di Lazzaro et

al., 2002a). This is achieved through intracortical mechanisms (Wu et al., 2000) that may involve suppression of the effects of inhibitory cortico-cortical inputs. Thus, tapping force was expected to increase temporarily following 5 Hz stimulation. It was hypothesised that acute disruption of M1 function would have only a modest, short-lived behavioural consequence on contralateral limb performance because the unstimulated M1 helps compensate for the deficit. This hypothesis generates two important predictions. First, there should be physiological evidence of changes in function of the unstimulated ipsilateral M1 *following* rTMS. These changes should favour inhibitory mechanisms if they are to have compensatory potential. Second, the effect of bilateral stimulation should exceed the algebraic sum of the behavioural effects of separate right and left unilateral stimulation, as a normal functioning ipsilateral M1 is no longer available to compensate.

6.1 Methods

Seven healthy volunteers were studied, three men and four women (mean age 30.9, range 21-47 years). All subjects were right-handed according to the Edinburgh inventory (Oldfield, 1971).

6.1.1 Finger-tapping experimental design and recording

Subjects were seated comfortably at a table with their right arm resting in front of them. They were asked to tap gently and briefly on a strain gauge with the tip of the right index finger at a steady rate (1 Hz, metronome-paced) and with a steady force (Figure 6.1). The force level was displayed on an oscilloscope screen in front of them and subjects were required to aim for a level of 0.1 Newtons. The experimental session began with a period of learning the task with visual feedback. Once learned, the screen was switched off and the task performed from memory. Subjects were then required to perform the tapping task continuously before (100 s), during (30 s) and after (200 s) rTMS to various cortical sites as detailed below. Subjects rested in between stimulation sessions with the exception of one practice run with visual feedback lasting 100-200 s. Electromyographic (EMG) signals were recorded bilaterally from the first dorsal interossei (FDI) using pairs of surface Ag-AgCl electrodes. Tap force was also recorded.

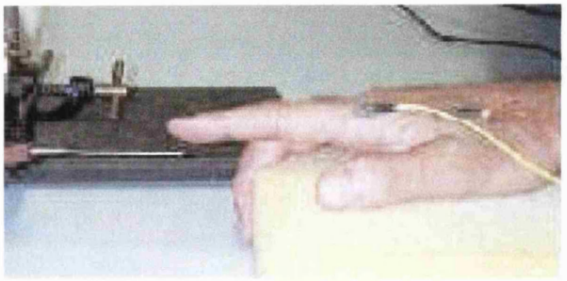


Figure 6.1. Tapping set-up.

EMG signals were amplified and band-pass filtered (53 to 300 Hz). After AD conversion using a 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) the data were stored on a personal computer (Spike 2, Cambridge Electronic Design, Cambridge, UK). All data were sampled at 1000 Hz.

6.1.2 Repetitive magnetic stimulation

RTMS was performed with a Magstim Rapid stimulator and figure of eight shaped-coils (Magstim company, Whitland, Dyfed, UK). The optimal positions for activation of the right and left FDI were marked as 'hot spots'. Active (AMT) and resting (RMT) motor thresholds for each M1 were established using previously described methods (chapter 2) and were determined at baseline immediately before rTMS.

Focal rTMS using a frequency of 5 Hz was applied at four sites (left motor cortex, right motor cortex, occipital cortex and simultaneous bilateral stimulation over both motor cortices). A 30 s train was given at each site at an intensity of 90 % AMT for the individual being tested and for the appropriate motor cortex. Lower intensity stimuli were used compared with previous authors (Siebner and Rothwell, 2003; Peinemann et al., 2000; Wu et al., 2000) to minimise direct activation of other brain regions by cortico-cortical and cortico-subcortical projections. Occipital cortex stimulation was given 2 cm left-lateral and 2 cm superior to the inion. The order of cortical stimulation was mixed pseudorandomly between subjects. When stimulating the motor cortex, coils were placed over the right and left motor cortices regardless of whether stimulation was unilateral or bilateral, so that conditions were as similar as possible between trains. The coil or coils being used were placed in position on each subject's head prior to the start of the recording and maintained in position for the whole recording so as not to introduce any added distraction. At least 20 minutes elapsed between stimulation sessions to allow for any after effects of rTMS to subside.

The low intensity of magnetic stimulation was confirmed in two ways. Firstly, FDI EMG was rectified and averaged across the stimuli in a given rTMS train. Direct EMG responses were defined as 15 ms to 25 ms post-stimulation increases greater than 2 SD of the EMG recorded over the 100ms before and after each shock. Secondly, three subjects received the standard train of 5 Hz 90 % AMT rTMS to the right motor cortex (RM1) while tonically contracting the right and left FDI muscles. EMG was rectified and averaged across stimuli. Transcallosal effects would be expected to appear as modulations in the EMG level in the right FDI (Ferbert et al., 1992; Kujirai et al., 1993; Ugawa et al., 1998; Di Lazzaro et al., 1999).

6.1.3 Paired-pulse experimental design

The same seven subjects each performed three further experiments, separated by at least a week, in which cortical excitability was tested (see section 2.5.5 and figure 2.5) using two Magstim Monopulse stimulators and a figure of eight shaped-coil connected to the stimulators by a Y-cable (Magstim company, Whitland, Dyfed, UK). Single-pulse (control) FDI MEPs were elicited from RM1 at baseline, and administered pseudorandomly with paired-pulse MEPs at known inhibitory (3ms) and facilitatory (10ms) interstimulus intervals (Kujirai et al., 1993; Ziemann, 1999). The stimulus intensities were set at 80% of AMT for the conditioning stimulus and 120% of RMT for the test stimulus. Each pair of pulses was 3.3 s apart. The subjects then received, as before, 5 Hz rTMS at 90% AMT to LM1 for 30 s. The MEP studies were repeated immediately following stimulation, and again at 5 and 20 minutes later. At each time point, recordings lasted 200 s, incorporating 20 trials each for the control, inhibitory and facilitatory MEPs (except baseline which was recorded twice).

Three conditions were tested in which MEPs were elicited from RM1. In the first, subjects sat at rest throughout the experiment (henceforward termed RRR). In the second, subjects performed the finger-tapping task with their right hand and tapped throughout the MEP recordings and during stimulation (TTT). In the third, subjects were required to tap only during stimulation and remain at rest during the MEP recordings (RTR). Finger tapping was paced with a 1 Hz auditory signal, with the left hand at rest. This signal was also given when the subjects were required to sit completely at rest.

6.1.4 Cortico-cortical coherence experimental design

On yet another day, the same seven subjects had EEG recordings performed, while finger tapping, before and after rTMS to the left motor cortex. The left and right motor hand areas were localised using single pulse TMS as described previously and surface

electrodes placed at these sites (C3 and C4 respectively). Additional electrodes were placed 2.5 cm anterior and 5 cm anterior of these first left and right electrodes (henceforward termed F3, FC3, F4 and FC4 respectively). Further electrodes were placed in sites corresponding to P3, P4 and CZ in the 10-20 system (see chapter 2). The EEG electrodes were referenced to linked ear electrodes. Active motor threshold for the left motor hand area was determined with the electrodes in situ. EMG data were recorded from right FDI using a pair of silver-silver chloride electrodes. EEG signals were amplified and band-pass filtered (0.53 to 300 Hz), AD converted and all data stored on a personal computer as above. Data were sampled at 1000 Hz.

Three minutes of data were recorded with the subjects at rest and with the 1 Hz auditory stimulus on. The subjects then practised the 1 Hz-paced finger-tapping task with their right index finger as before. When consistently tapping to 0.1 Newtons without visual feedback, two sessions were recorded. In the first, subjects tapped continuously before, during and after rTMS was given to the left motor cortex. In the second, this was repeated but with rTMS given to the occipital cortex. The order of cortical stimulation was mixed pseudorandomly between subjects and at least 20 min elapsed between stimulation sessions. 5 Hz rTMS was given for 30 s at 90% AMT of the left motor hand area for each subject.

EEG data were visually inspected for scalp EMG, eye movement artefact and mains spikes. Recordings were then divided into 90 s blocks before (pre), 0 to 90 s after (post 1) and 90 to 180 s after (post 2) the rTMS. Frequency analysis of the EEG data was performed using the discrete Fourier transform (Halliday et al., 1995), the results averaged and the power spectra and coherence estimated. The frequency resolution was 0.98 Hz and the recordings were Hanning-windowed to control spectral leakage. The variance of spectral power estimated was stabilised by logarithmic transformation (Halliday et al., 1995). To compare the coherence between EEG signals, the square root of the coherence was normalised using a Fisher transform (Rosenberg et al., 1989).

6.1.5 Statistical analysis

The pre-stimulation mean peak force of 1 Hz paced finger taps was calculated for each subject. For visualisation of the specific effects of rTMS over the left, right and both motor cortices, the percentage difference between the pre-stimulation mean peak force and peak forces of successive individual finger taps was determined. This was performed for all four stimulation sites. The results for the control occipital stimulation were then subtracted from the serial percentage differences for stimulation over the

left, right and both motor cortices. The results were averaged across subjects and the cumulative sums (cusums) of serial deviations from the record mean calculated. Thus, an increase in the cusum represents an increase in peak force with respect to a pre-stimulation control period.

The averaged serial percentage deviations with occipital effects subtracted were subjected to control charting and change-point analysis, using commercial software (Change-Point Analyser 2.0 shareware program, Taylor Enterprises, Illinois). Control charts consisted of plots of serial deviations from the mean deviation. Control limits were determined to give the maximum ranges over which values were expected to vary (with 99 % probability) assuming no change had occurred. Change-point analysis iteratively uses a combination of time varying cusums and bootstrapping to detect changes (Taylor WA, 2002). 10,000 bootstraps were performed in each test and only changes with probabilities of >99 % are reported. Confidence limits for change-point estimates were 95 %. The mean peak tapping force over the 90 s prior to rTMS was compared to the two candidate periods defined by change point analysis. The first time band was the period of significant effect following stimulation of LM1. The second time band was the remaining period over which change point analysis indicated that simultaneous bilateral stimulation was still effective.

For the paired-pulse experiments, results were obtained by averaging peak-to-peak MEP size across each time period for each subject. Seven time periods were defined with respect to rTMS; pre-stimulation, post 0-50 s, post 51-100 s, post 101-150 s, post 151-200 s, post 5-8 min and post 20-23 min. MEP size post-stimulation was expressed as a percentage of baseline control MEP size. This was performed for control, inhibitory and facilitatory MEPs. Percentage MEP data were analysed using non-parametric statistics (Friedman ANOVA) with post-hoc testing using the Wilcoxon 2-sample test.

For the coherence experiments, the EEG between 7.8-13.7 Hz (alpha) and 14.6-30.3 Hz (beta) was chosen for analysis. The average log power and transformed coherence values in each band were calculated across subjects for the rest condition and for the three time periods (pre, post 1, post 2) for both stimulation conditions (left M1 and occipital rTMS). Comparisons were made between rest and tapping pre-stimulation, and between left M1 and occipital stimulation during tapping. Both interhemispheric (C3-C4) and intrahemispheric (FC3-C3 and FC4-C4) coherence was examined for each frequency band. The results for each band were entered into separate factorial analysis of variance (ANOVA). Force and coherence data were analysed by separate

repeated measures general linear models, incorporating, where necessary, a Greenhouse-Geisser correction for non-sphericity. If main effects or interaction were significant, data were further compared post-hoc using the Student's 2-tailed t-test for paired samples.

6.2 Results

The 30 s train of 5 Hz rTMS applied at 90 % AMT to each cortical site evoked neither overt muscle twitches nor EMG responses in the contralateral finger in any of the stimulation sessions (figure 6.2), confirming its subthreshold nature (with the exception of one subject where there was a 47% increase in EMG at 21 ms post-stimulus during one of the rTMS trains). The low intensity of stimulation was further confirmed by the absence of transcallosal facilitation or inhibition of tonic ipsilateral EMG activity during rTMS (figure 6.2). There was also no change in mean tapping frequency or the variability (SD) of tapping frequency with stimulation and there was no evidence of mirror movements of the left hand either on visual inspection or in EMG records.

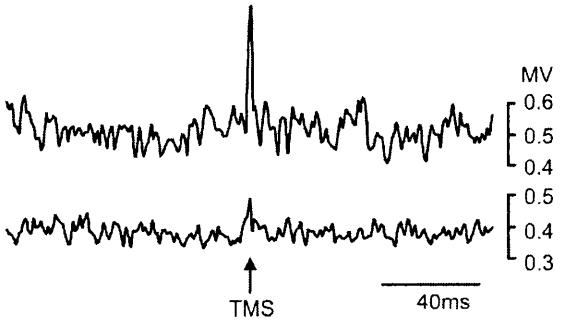


Figure 6.2. Top trace: rectified right FDI EMG in one subject averaged around each pulse in the rTMS train (30 s at 5 Hz and 90% active motor threshold) delivered over the left motor cortex confirming that stimulation was below motor threshold. **Bottom trace:** rectified right FDI EMG averaged in 3 subjects around the rTMS pulses for trains delivered over the right motor cortex. Subjects tonically contracted the right FDI during stimulation. There was no modulation in EMG in the 100ms after stimulation suggesting that rTMS over the right motor cortex did not induce transcallosal effects on the left motor cortex.

6.2.1 Behavioural effects of unilateral rTMS

Peak tapping force was modestly elevated after rTMS was delivered to the contralateral (left) M1. Figure 6.3 shows the effect of rTMS to different sites on the force of right-handed finger taps in a representative subject. Across subjects, there was a modest increase in tapping force with stimulation of the contralateral M1, and no significant change with control occipital rTMS. Cusums of the percentage difference

between the pre-stimulation mean peak force and consecutive peak forces of finger taps averaged across all seven subjects are plotted in figure 6.4. Stimulation had an effect that was greater when delivered to the contralateral (left) M1 than when delivered to the ipsilateral M1.

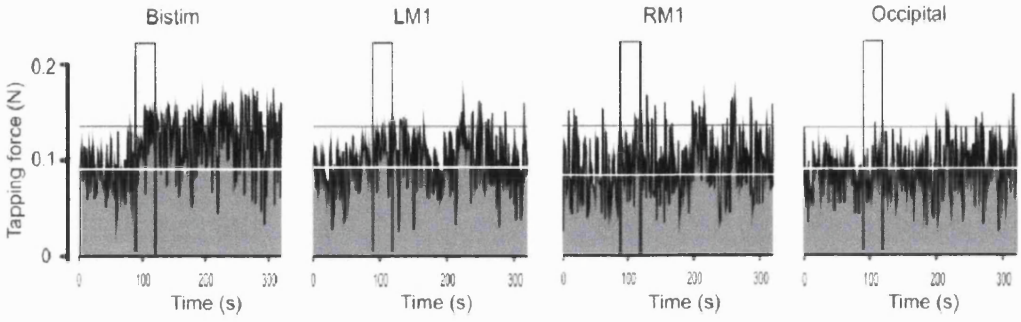


Figure 6.3. Tapping force raw data. Data from one subject showing the effect of rTMS on the force of successive finger taps made with the right forefinger. Stimulations over both motor cortices simultaneously (Bistim), left motor cortex (LM1), right motor cortex (RM1) and occipital cortex (occipital) are shown as boxed areas. The horizontal white line shows the prestimulation mean and the horizontal black line above this is twice the standard deviation. There is a clear increase in tapping force with simultaneous bilateral stimulation of the motor cortices, and no significant change with control occipital rTMS.

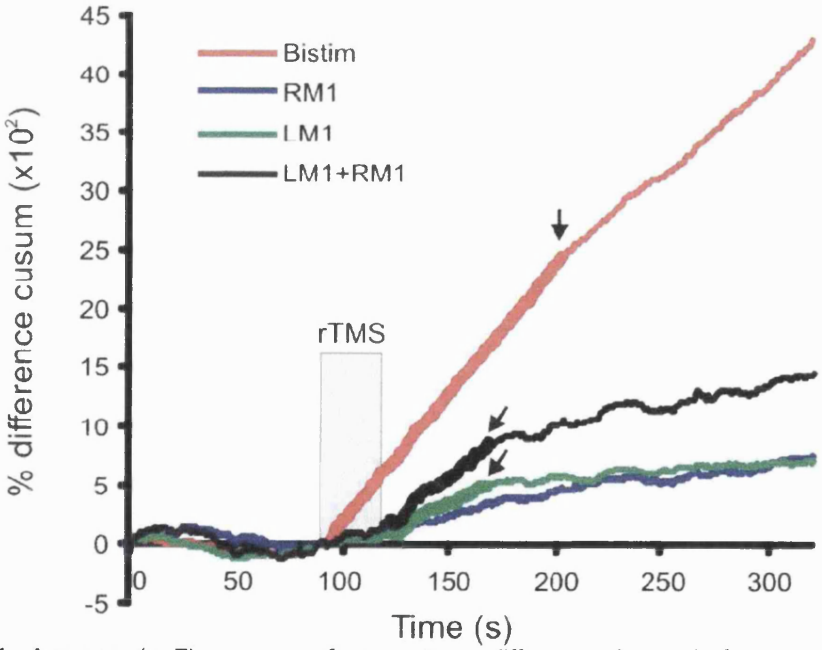
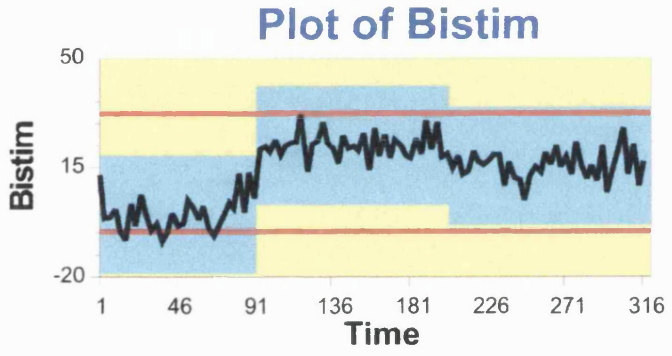


Figure 6.4. Average (n=7) cusums of percentage difference in peak force compared to baseline, with effects of occipital stimulation subtracted. Those periods of change identified by change point analysis have been plotted as broader lines and their terminations arrowed.

Change point analysis confirmed that tapping force became inappropriately elevated after rTMS was delivered to the contralateral primary motor cortex. This effect began 8 s (95% confidence limits 1-21 s) after the offset of stimulation and lasted until 51 s (43-63 s). Stimulation over the ipsilateral motor cortex did not elicit any significant change. Two subjects had rTMS to their LM1 repeated on three different days over the course of three weeks. Cusums of peak tapping force revealed an increase in force following the rTMS train on each occasion, indicating that the effect was reproducible within these subjects.



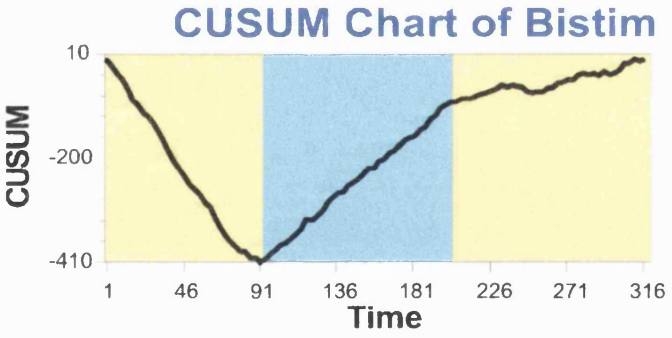


Table of Significant Changes for Bistim

Confidence Level = 99.5%, Confidence Interval = 95%, Bootstraps = 10000, Sampling Without Replacement

Time	Confidence Interva	Conf. Level	From	To	Level
94	(91, 94)	100%	0.17676	22.095	1
206	(194, 224)	100%	22.095	15.638	2

Figure 6.5. Change point analysis results for simultaneous bilateral stimulation of both motor cortices. **Top panel:** control chart showing serial percentage deviations from the mean deviation against time. RTMS started at 91 s. The effect of bilateral stimulation began during stimulation and continued until 85 s (95% confidence limits 74-104 s) after rTMS had ended. **Middle panel:** cusum of the serial percentage deviations from the mean deviation showing clearly the points of change. **Lower panel:** the times at which changes occurred, including the confidence limits.

6.2.2 Behavioural effects of bilateral rTMS

Strikingly, simultaneous bilateral stimulation of both motor cortices had a large effect on tapping force. Beginning during stimulation and lasting 110 s, this effect outlasted both contralateral (left) rTMS and the algebraic sum of separate stimulation of the right and left M1 (figures 6.4 and 6.5). Figure 6.6 confirms that the elevation in tapping force following bilateral rTMS was temporary and reversed without further training with visual feedback.

Change point analysis documented the duration of changes in tapping force (figure 6.5) but did not determine whether there were significant differences in the degree of change in tapping force between stimulation at different sites. This was analysed by a repeated measures general linear model concentrating on those periods before and after rTMS that were free from any distracting effects of stimulation. The mean peak tapping force prior to rTMS was compared with the two post-rTMS periods defined by change point analysis for the three stimulation conditions (left; right; simultaneous bilateral stimulation). The first of the post-rTMS candidate time periods was the 8 s to 51 s post-rTMS over which change point analysis indicated a significant effect from stimulation of the contralateral (left) M1. The second was the remaining period over which change point analysis indicated that only simultaneous bilateral stimulation was still effective (from 52 s to 85 s after the offset of stimulation). Significant main effects were confirmed for time ($F[2,12]=7.088$, $p=0.009$) and stimulation region ($F[2,12]=10.842$, $p=0.015$), and there was an additional interaction between time and stimulation region ($F[4,24]=9.410$, $p=0.007$). A second general linear model was performed using the factors time (pre, post 1, post 2 as before) and stimulation region (simultaneous bilateral stimulation; summed separate left and right stimulation). Again, there was a main effect of time ($F[2,12]=7.088$, $p=0.009$), of region ($F[1,6]=6.274$, $p=0.046$) and an interaction between time and region ($F[2,12]=6.036$, $p=0.015$). Post hoc tests demonstrated that over the first period post-rTMS, the effects of both left motor cortical stimulation and simultaneous bilateral stimulation were different to pre-rTMS ($p=0.022$ and $p=0.018$, respectively). However, this was only true of simultaneous bilateral stimulation for the second period post-rTMS ($p=0.018$), when the effects of simultaneous bilateral stimulation were also greater than those of left stimulation or summed separate left and right rTMS ($p=0.014$ and $p=0.023$, respectively). Mean peak tapping force increased by 9.6% after LM1 stimulation and by 22.1% after bilateral M1 stimulation. Right motor cortical stimulation did not have a significant effect on tapping force.

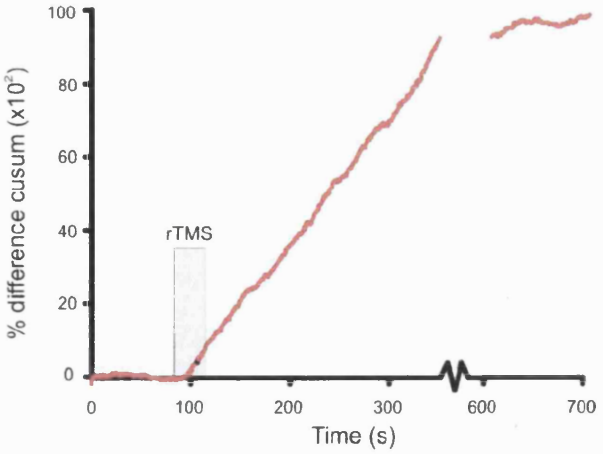


Figure 6.6. Cusum of percentage difference in peak force compared to baseline (without occipital effects removed) following simultaneous bilateral rTMS of both motor cortices in one subject. Recovery is delayed, but occurs without further training with visual feedback.

6.2.3 Effects of rTMS on cortical excitability

In the light of the above, the possibility was considered that the dramatic and prolonged behavioural effects of bilateral compared to contralateral rTMS arose because the ipsilateral M1 was blocked from compensating for the dysfunction of the contralateral cortex. In this formulation, compensatory change by the ipsilateral M1 would be expected to be maximal over the period beginning 50 s after the offset of rTMS, when compensation reverses the behavioural effects of contralateral stimulation alone. In addition, any compensatory change in the ipsilateral M1 would be expected to favour inhibition so as to counter the local excitatory effects of contralateral 5 Hz rTMS and resultant increase in tapping force.

Percentage MEP data for the seven subjects were entered into Friedman ANOVAs using factors of time (pre, post 0-50 s, post 51-100 s) and condition (RRR, TTT, RTR). Separate ANOVAs were performed for the control, inhibitory and facilitatory MEP data. Only the control data ANOVA was significant ($p = 0.003$). Post-hoc tests on the control MEP data confirmed that there was a significant reduction (of 37%) in mean control MEP size in the 51 to 100 s period (but not 0-50 s) following rTMS ($p=0.018$, Wilcoxon 2-sample test) when the subjects were tapping throughout (TTT) (figure 6.7). This effect disappeared at longer intervals. There was no significant change in MEP size when the subjects were at rest in the RRR and RTR conditions. The latter showed that delayed effects necessitated continuing task performance and were not a passive effect of rTMS, whether applied to the hemisphere when resting or active.

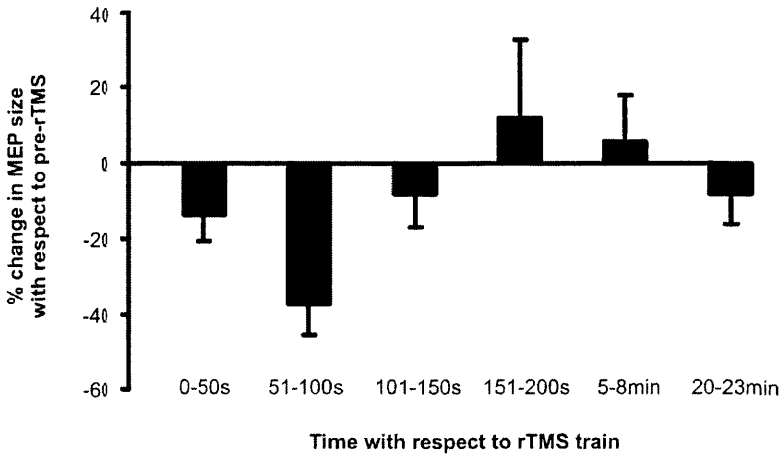


Figure 6.7. Effects of rTMS on cortical excitability. Percentage change in average (n=7) peak-to-peak control MEP sizes elicited from RM1 with the subjects performing the right-handed finger-tapping task after stimulation with 5 Hz rTMS to LM1. Percentage change is with respect to pre-rTMS MEP size. There is a significant reduction in mean MEP size (p=0.018) in the 51-100 s following rTMS. Bars are standard error of the mean.

6.2.4 Effects of tapping task on power and cortico-cortical coherence

EEG log power was assessed by separate ANOVAs for both alpha and beta frequencies. Four regions (C3, FC3, C4, FC4) and two conditions (rest, tapping) were entered into the ANOVAs. There were no main effects or interactions for either frequency band.

Interhemispheric (C3-C4) transformed coherence data were entered into an ANOVA with the within-subject factors of frequency (alpha, beta) and condition (rest, tapping). There was a significant main effect for frequency ($F[1,6] = 24.574$, $p = 0.003$) and for condition ($F[1,6] = 7.721$, $p = 0.032$), as well as a significant interaction between frequency and condition ($F[1,6] = 6.822$, $p = 0.040$). Post-hoc t-tests revealed that coherence in the alpha band increased 9.4% during finger tapping compared with rest ($p = 0.008$). There was no change in the beta frequency band.

Intrahemispheric (FC3-C3 and FC4-C4) transformed coherence data were assessed by ANOVA using within-subject factors of frequency (alpha, beta), region (left and right hemispheres) and condition (rest, tapping). There was a significant main effect for frequency ($F[1,6] = 8.084$, $p = 0.029$) but no other significant main effects or interactions.

6.2.5 Effects of rTMS on power and coherence during tapping task

Two stimulation conditions (left M1, occipital) and three time points (pre, post 1, post 2) were used as ANOVA within-subject factors to examine the effect of rTMS on EEG alpha and beta log power and transformed coherence during continuous finger-tapping. Due to the small sample size, separate ANOVAs were performed for each frequency band and for each EEG region. EEG power for C3, C4, FC3 and FC4 was assessed. There were no main effects or interactions for any region with the exception of a significant main effect of stimulation site ($F[1,6] = 7.344$, $p = 0.035$) for C4 power in the beta band. Interhemispheric (C3-C4) coherence and intrahemispheric (FC3-C3 and FC4-C4) coherence were also assessed separately. There were no significant main effects or interactions.

6.3 Discussion

These results show that independent stimulation of the contralateral motor cortex had a short-lived effect on the control of fine finger movements, whilst ipsilateral stimulation had no effect at all. In contrast, simultaneous bilateral stimulation of the primary motor cortex had a prolonged and stronger effect, which exceeded the algebraic sum of individual contralateral and ipsilateral stimulation. In addition, high frequency stimulation of the motor cortex contralateral to the tapping hand was associated with a delayed and temporary reduction in *ipsilateral* cortical excitability. These distant effects of rTMS are in keeping with evidence from positron emission tomography that trains of subthreshold rTMS at 5 Hz over the primary motor cortex lead to persisting increases in activity over both primary motor cortices (Siebner et al., 2000). Indeed, the results presented in chapter 4 reveal that 5 Hz rTMS given over the left motor cortex caused a bilateral decrease in intrahemispheric cortico-cortical coherence.

How could bilateral stimulation have produced a super-additive effect? One possibility is direct potentiation of the effects of bilateral rTMS through temporal summation following convergence on one structure. However, consideration of candidate sites for temporal summation makes this explanation unlikely. There was no evidence of transcallosal effects at the stimulation intensities used in this study so greater stimulation of the contralateral (left) M1 through additional direct activation of transcallosal inputs to this hemisphere seems unlikely. Of course, this is not to say that adaptive compensation did not involve transcallosal pathways (*vide infra* and Traversa et al., 1998). Temporal summation at a subcortical level seems equally unlikely. Low intensity shocks were deliberately used to minimise direct activation of other brain regions. Thus, shocks failed to elicit a direct response in activated muscle and were at

an intensity that does not evoke a descending volley in the corticospinal tract (Di Lazzaro et al., 1999) so non-linear interactions in the spinal cord seem improbable. The nature of the task, necessitating fine fractionated finger movements, also makes convergence at the level of the brainstem reticular formation and activation of reticulospinal projections unlikely (Lemon, 1993).

Alternatively, could bilateral stimulation have blocked any compensation that ordinarily dictates recovery from the effects of contralateral rTMS? The behavioural results were compatible with the ipsilateral M1 compensating for the effect of contralateral rTMS. Such an interpretation finds strong support in the cortical excitability studies. These demonstrated a reduction in ipsilateral cortical excitability during finger tapping in the period 51-100 s following contralateral M1 stimulation. Importantly, there was no reduction in cortical excitability ipsilaterally if the subject was at rest following rTMS. This, together with the delayed reduction in excitability when tapping following rTMS, indicates that acute dysfunction of the motor cortex contralateral to the tapping hand may have engendered secondary compensatory change within the ipsilateral motor cortex. This short-term use-dependent adaptational change (Rossi et al., 2000) only occurred when behaviourally relevant, i.e. when tapping. Despite the change in cortical excitability as measured with the MEP changes, the data did not reveal any change in cortico-cortical coherence with stimulation. There a several reasons why this might be so. Firstly, and the most likely explanation, is that the sample size was small. Secondly, the time periods used for calculating coherence (90 s) were necessarily longer than the time windows used for recording the MEP data (50 s). The change in peak tapping force after left M1 stimulation alone occurred in the first 50 s post-rTMS and the change in ipsilateral excitability occurred in the second 50 s post-rTMS. Thus, by looking at coherence data averaged over the 90 s post-rTMS, subtle changes may be missed. A third reason for lack of coherence change might be the task itself. This was a unimanual, phasic task, in contrast to the tonic bimanual contraction used in previous chapters. However, the data did reveal a significant increase in interhemispheric alpha coherence with tapping compared to rest. Fourthly, EEG was not recorded at rest after stimulation, so no comparison could be made between the effects of rTMS on rest coherence and the effects on coherence during the task.

Thus the evidence supports the hypothesis outlined in the introduction that the ipsilateral primary motor cortex may compensate for the acute dysfunction of the contralateral primary motor cortex. However, the focality of rTMS effects must also be considered. Low intensity shocks were deliberately used to minimise direct activation of

cortical regions other than the target primary motor cortex and it is noteworthy that effective stimulation field strength declines as the cubic function of distance from the centre of stimulation (Roth et al., 1991). Stimulation may have spread to the primary sensory cortex but this has little direct motor function. More importantly, given the evidence that activation of the ipsilateral premotor cortex is increased following chronic lesions of the motor cortex such as stroke (Seitz et al., 1998; Calautti et al., 2001; Nelles et al., 2001), could the effects of bilateral stimulation have arisen through direct stimulation of premotor cortex? This seems unlikely with shocks at the chosen intensity (Munchau et al., 2002). The nature of the behavioural deficit induced by 5Hz rTMS to the motor cortex also deserves further comment. It involved a selective increase in force without effects on mean movement frequency or the variability of movement frequency, in contrast to another rTMS study that used suprathreshold stimulation at 15 Hz over the motor cortex (Chen et al., 1997c). The effects in this study were temporary and reversed without further training with visual feedback. This suggests that the rTMS did not impair early motor consolidation (Muellbacher et al., 2002) and did not destroy the motor program or internal model related to the task. Rather, it temporarily disrupted how the motor cortex translated the program/model into desired forces, leading to an overestimate of the required force. This may have arisen through rTMS induced alterations in sensory feedback (Tsuji and Rothwell, 2002), changes in local cortical processing (Siebner and Rothwell, 2003; Wu et al., 2000; Pascual-Leone et al., 1998; Maeda et al., 2000a) or suppression of inhibitory inputs from other areas (chapter 4).

What is meant by compensation from ipsilateral (in this case right) motor cortex? There is substantial evidence that both motor cortices are activated in unilateral distal movements, particularly when these are fine or complex (Chen et al., 1997b; Kim et al., 1993; Kristeva et al., 1991; Sadato et al., 1997; Pfurtscheller and Lopes da Silva, 1999). One possibility is that compensation occurs through direct ipsilateral corticospinal and indirect descending ipsilateral projections to spinal motoneurones controlling the fingers. However, the anatomical and physiological evidence suggests that such pathways are relatively unimportant in the control of the distal upper limb muscles in primates, including humans (Nyberg-Hansen and Rinvik, 1963; Kuypers, 1964; Brinkman and Kuypers, 1973; Tanji et al., 1988; Marsden et al., 1999), particularly at low contraction strengths as utilised in the current paradigm (Wassermann et al., 1994; Ziemann et al., 1999b). Alternatively, both motor cortices may form a spatially distributed circuit linked through transcallosal and cortico-subcortico-cortical pathways that organises different aspects of movement, akin, for example, to the bilateral circuit subserving spatial attention (Oliveri et al., 1999).

Movement is then largely effected by the contralateral motor cortex. If this function is disturbed, the role of the ipsilateral motor cortex in this bilateral organisation may be increased (Siebner and Rothwell, 2003).

This is the first demonstration that the ipsilateral primary motor cortex is capable of functionally significant compensation for focal contralateral cortical dysfunction in the adult human. As such, it complements recent findings that the extent of topographical representation of swallowing muscles in ipsilateral motor cortex correlates with recovery of dysphagia following stroke (Siebner and Rothwell, 2003) and that the ipsilateral dorsal premotor cortex may also be capable of adaptive compensation after stroke, albeit as assessed by a selective reaction time task (Johansen-berg et al., 2002). The ability to induce reversible and controlled functional compensation in healthy subjects could provide an important model for testing therapeutic approaches for their potential to promote functional compensation in unaffected cortical areas following stroke.

6.4 Summary of key points

- Studies of recovery following stroke have shown alterations in function in various cortical areas, including the contralesional (unaffected) motor cortex (M1) but whether these changes contribute to recovery or are mere epiphenomena remains unclear.

- Evidence was sought that the ipsilateral primary motor cortex can compensate for dysfunction of the contralateral primary motor cortex.

- The change in force production during a finger-tapping task was recorded in response to acute disruption of M1 function by repetitive transcranial magnetic stimulation (rTMS).

- Neither control (occipital) nor ipsilateral M1 rTMS lead to a change in tapping force.

- RTMS over contralateral M1 had a short-lived effect and induced changes in *ipsilateral* M1 excitability around the time that these behavioural effects abated, consistent with delayed compensation by the ipsilateral M1.

- Simultaneous bilateral M1 stimulation, designed to prevent compensation by the ipsilateral M1, had a large and prolonged effect on tapping force.

- This is the first demonstration that the ipsilateral primary motor cortex is capable of functionally significant compensation for focal contralateral cortical dysfunction in the adult human.

7 Cortico-cortical coupling in chronic stroke: its relevance to recovery

Many imaging studies have shown that there is increased task-related, and invariably multifocal, brain activation in stroke patients during contraction of the affected hand compared to healthy subjects. In particular, the contralesional sensorimotor and premotor cortices, ipsilesional cerebellum, bilateral supplementary and cingulate motor areas and parietal cortex are over-activated (Chollet et al., 1991; Weiller et al., 1993; Cramer et al., 1997, 1999; Cao et al., 1998; Seitz et al., 1998; Nelles et al., 2001; Carey et al., 2002; Ward et al., 2003a). However, relatively few studies have specifically explored the relationship of such brain activation to motor recovery. Some evidence supports a compensatory role for the contralesional premotor region, particularly as increased activity is only present after some recovery has taken place (Nelles et al., 1999; Johansen-berg et al., 2002). Although other areas, particularly the contralesional primary motor cortex, may have the capacity to compensate for cortical dysfunction (see chapter 6), the extent to which activation changes relate to motor recovery is unclear (Carey et al., 2002; Johansen-berg et al., 2002; Netz et al., 1997, Shimizu et al., 2002; Mima et al., 2001b). Moreover, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have shown that increased cortical activity, whether compensatory or not, may subside in tandem with recovery (Marshall et al., 2000; Calautti et al., 2001; Feydy et al., 2002; Ward et al., 2003b). Transcranial magnetic stimulation studies have also shown that ipsilateral changes in primary motor cortex excitability are more frequent after stroke but that these changes resolve with motor recovery (Netz et al., 1997; Manganotti et al., 2002; Butefisch et al., 2003). Overall, it is likely that multiple areas are involved acutely following stroke and that this involvement is inversely correlated with recovery. In support of this schema, a recent study has demonstrated a negative correlation between outcome and the degree of task-related brain activation in the primary motor and sensory cortices, pre-supplementary, supplementary and cingulate motor areas, premotor cortex, prefrontal region, posterior parietal cortex and cerebellum (Ward et al., 2003a).

The above studies have focussed on how motor areas, albeit multiple, may individually contribute to compensation. In this chapter, the question of whether cortical areas also interact to form dynamic assemblies that may then compensate for disability is investigated. The pattern of interaction between different cortical areas may be evaluated using the coherence between scalp EEG signals (Thatcher et al., 1986;

Andrew and Pfurtscheller, 1996; Gerloff et al., 1998; Rappelsberger and Petsche, 1988; Andres et al., 1999). In contrast to EEG power, which provides a quantitative measure of the synchrony of relatively local sources within the range of an electrode, EEG coherence is a larger-scale measure, which captures dynamic functional interactions between cortical areas underlying electrodes separated by longer distances. There is considerable evidence to support the assumption that synchronized oscillations reflect a basic form of communication between cortical assemblies during task-related activity (see reviews by Bressler, 1995; Klimesch, 1996; Singer, 1994).

Cortico-cortical coherence was therefore investigated in patients with long-standing cortical and/or subcortical lesions that had initially resulted in a hemiparesis but with variable clinical outcome thereafter. Coherence was evaluated before and during the performance of a handgrip task. In choosing a handgrip task, patients could be selected who had a wider range of recovery since the performance of this task does not rely on the ability to perform fractionated finger movements. Handgrip function returns relatively early compared with fractionated finger movements in the recovery from motor stroke (Heller et al., 1987). Thus, patients with chronic stroke, and with varying degrees of motor recovery, were compared to healthy controls. First, differences in task-related cortico-cortical coherence between healthy subjects and stroke patients were looked for. Thereafter, correlations between any abnormal task-related coherence and the degree of motor recovery from stroke were sought.

7.1 Methods

7.1.1 Subjects

All patients and healthy subjects were right-handed according to the Edinburgh handedness scale (Oldfield, 1971). All subjects gave informed written consent prior to participating in the study, and the procedures approved by the joint ethics committee of the National Hospital for Neurology and Neurosurgery and of the Institute of Neurology, London.

The control group (11 males) comprised 16 healthy volunteers aged between 30 and 69 years [mean (SD) 51.3 (11.4) years]. Control subjects reported no history of neurological or psychiatric illness and had normal upper limb function bilaterally. Twenty-five stroke patients (20 males) were recruited from the outpatient services at the National Hospital for Neurology and Neurosurgery, London [range 25 to 69 years, mean (SD) 54.0 (13.1) years]. All patients had suffered a first stroke (ischaemic or haemorrhagic) at least 12 months previously, resulting in a hemiparesis with weakness

of at least the wrist and finger extensors (<4 on the MRC scale), for at least 48 hours after onset. All patients had brain imaging (CT or MRI) following their stroke. Exclusion criteria included: (i) more than one stroke clinically or on imaging; (ii) extensive small vessel disease on imaging; (iii) presence of other intracranial pathology; (iv) inability to perform the motor task or to cooperate with testing. Patient characteristics are listed in tables 7.1 and 7.2. Fifteen patients had suffered a left hemiparesis and ten a right hemiparesis. Four patients had a haemorrhagic stroke. Lesions involved cortical and subcortical structures but none involved brainstem structures.

7.1.2 Behavioural evaluation

A range of outcome measures was used to assess the functional recovery of patients after stroke. All patients were assessed on the same day as the EEG recordings. The following outcome measures were employed: (i) Barthel activities of daily living (ADL) index; (ii) Functional Independence Measure for motor (FIM-M) and (iii) cognitive abilities (FIM-C); (iv) Rankin disability scale; (v) nine hole peg test (9HPT); (vi) grip strength; (vii) modified Ashworth score (MAS); (vii) MRC power score for wrist extensors, wrist flexors, finger extensors and finger abductors.

The 9HPT was performed by measuring the time taken to place nine pegs with each hand. If patients failed to place all nine pegs within 60 seconds, the number of pegs successfully placed was recorded. Scores were recorded as pegs per second for each hand. The score for the impaired hand was then divided by the score for the unimpaired hand. Grip strength was recorded using the same manipulandum as for the EEG recording session. The strength of the impaired hand was divided by that of the unimpaired hand (Sunderland et al., 1989). Muscle power of the impaired hand was recorded for the wrist extensors, wrist flexors, finger extensors and finger abductors.

To compare scores between patients, the Rankin disability scale and MAS were converted such that increasing scores reflected improvement, by subtracting the measured score from the maximum score for that scale. Each of the eight outcome measures was then normalised. This created eight sets of outcome measures, each with 25 scores, one for each patient. A principal components analysis (PCA; see below) was performed on the whole data set to obtain representative vectors of outcome, or overall outcome scores.

7.1.3 Principal Components Analysis

The clinical outcome data x were sorted into an $i \times j$ matrix with the rows ($i = 8$) representing the eight different types of outcome measurements (variables) obtained

for each of the 25 subjects (j = 25). PCA was then employed to find a smaller set of variables with less redundancy (i.e. less correlation between the data elements), providing a similar representation of the original data matrix. In particular, after making each x(i) a zero mean variable, (i.e. 'centering' the data across subjects), x was linearly transformed to another matrix of equal size: $z = A \cdot x$, so that its rows z(i) (i.e. principal components, PCs) are uncorrelated and their variances equal unity, $E\{zz^T\} = 1$, where $E\{\}$ denotes expectation). This is equivalent to finding a rotated orthogonal coordinate system such that the elements of z in the new coordinates are uncorrelated (Bishop, 1998). At the same time, the variances of z projected back onto the original coordinate system are maximized so that the first axis (i.e. 1st PC) corresponds to the maximal variance. In this case, the transformation matrix A is calculated through eigen-value decomposition (Jackson, 1991) of the covariance matrix of x. After performing the PCA process described above, it was found that the first two PCs accounted for 82% of the total variance (65% and 17% for the 1st and 2nd PC, respectively). The remaining PCs were discarded as they individually accounted for <9% of the total variance. Hence, the original eight-dimensional (8D) data x were transformed (via projection to the 1st and 2nd PC axis), into a single 2D vector z. The 1st PC was evenly distributed across the eight disability and motor scales (figure 7.1), whereas the 2nd PC reflected upper limb motor scores (9HPT, MAS, grip strength and MRC power scale). Thus, the 1st PC reflected more global disability whereas the 2nd PC specifically described contralateral upper limb motor function.

7.1.4 EEG and EMG recording protocol

Subjects sat upright in a chair with their arms resting on a pillow on their laps. Scalp EEG was recorded from 64 electrodes mounted on a cap (FMS Easy Cap, Germany) using linked mastoid references, arranged according to the extended international 10-20 system and saved as continuous (CNT) files. Surface silver-silver chloride electrodes were used to record EMG from wrist extensor (WE), wrist flexor (WF), first dorsal interossei (FDI) and abductor pollicis brevis (APB) muscles bilaterally. Eye movements were recorded at the same time by extraoculography.

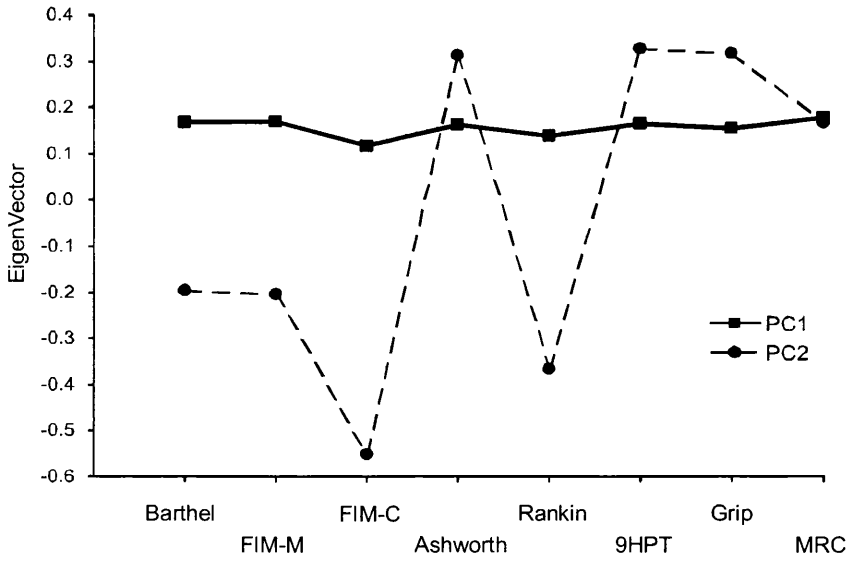


Figure 7.1. The distribution of the first and second principal components (PC1 and PC2) across the eight outcome measures. Barthel = Barthel activities of daily living (ADL) index; FIM-M = Functional Independence Measure for motor; FIM-C = Functional Independence Measure for cognitive abilities Rankin = Rankin disability scale; 9HPT = nine hole peg test; grip = maximal grip strength; Ashworth = modified Ashworth score; MRC = Cumulative MRC power scale for forearm and hand muscles.

Both control subjects and stroke patients followed the same protocol. Five minutes of baseline EEG and EMG were recorded with the subjects at rest and with their eyes open. Continuous data was then recorded while the subjects undertook a handgrip task. Handgrips were performed using a grip force transducer made from aluminium milled into a U-shape, with one arm of the U slightly thinner than the other. Strain gauges were mounted on the inner and outer sides of the thin arm to form a Wheatstone bridge circuit. This was then fed to a strain gauge amplifier. The output of the amplifier was recorded and also connected to an oscilloscope to give visual feedback to the subject. The maximum voluntary grip force for each hand was recorded for each subject. The handgrip task consisted of gripping the manipulandum twenty times at ~25% of the maximal grip force of that limb, with first one hand and then the other in a pseudorandomised order. Visual feedback from the oscilloscope screen ensured that subjects maintained a steady grip force. To time the individual handgrips, a red 'warning' LED signal lasting 4 s prepared the subject for a green 'go' LED signal. The green signal lasted 10 s during which time the subjects were required to maintain a steady grip on the manipulandum (figure 7.2). To avoid fatigue, there was a 10 to 20 s pause between handgrips during which the hand was at rest. No subjects displayed mirror movements during the grip task (as monitored visually and by EMG). All subjects

were able to perform the handgrip task and, in particular, none of the stroke subjects had difficulty with this task due to sensory impairment, although case 8 had difficulty in maintaining a constant grip force due to dyspraxia (see table 7.2).

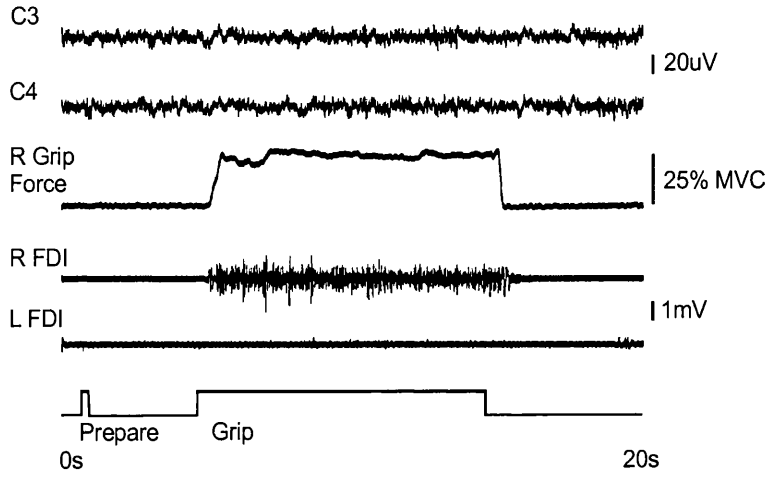


Figure 7.2. Example of data from a control subject collected in one prepare-grip cycle with the right hand. FDI = first dorsal interosseous. A red LED signal warned the subject to prepare to grip and a green LED signal indicated that the subject should maintain a steady grip on the strain gauge.

EEG and EMG signals were filtered (EEG band-pass filtered at 0.30 Hz to 70 Hz; EMG band-pass filtered at 5 Hz to 100 Hz) and digitally converted at a sampling rate of 500 Hz (Neuroscan, Neurosoft Inc. U.S.A.).

7.1.5 Data analysis

EEG data underwent ocular artefact reduction (Neuroscan software; see section 2.4) before the frequency analysis was performed. Nine EEG electrodes were selected for further analysis: F3, FC3, C3, FZ, FCZ, CZ, F4, FC4 and C4. These overlie cerebral motor areas, forming a grid across both hemispheres, and have been previously used as the electrodes of interest in studies of EEG-EEG coherence in other motor tasks (Gerloff et al., 1998; Andres et al., 1999). In particular, F3/4, FC3/4, C3/4, and FZ are likely to overlie the dorsolateral prefrontal and lateral premotor areas, primary sensorimotor cortices and medial prefrontal cortex, respectively, whereas FCZ and CZ were considered to overlie mesial motor areas, particularly the cingulate and supplementary motor areas (Homan et al., 1987; Steinmetz et al., 1989; Andres et al., 1999; Asada et al., 1999). Data was visually inspected for further artefact (scalp EMG, mains spikes) and segmented into artefact-free non-overlapping epochs of 4 s duration (mean per subject data set 22.3 epochs, range 14 to 25 epochs). Spectral power and

coherence were calculated with a fast Fourier transform algorithm (A. Pogosyan, Sobell) as described previously (section 2.6). Spectra were estimated by dividing the data epochs into a number of disjoint sections of 1 s duration using a segment length of 512 points, resulting in a frequency resolution of 1 Hz. The square root of the coherence was then normalised using a Fisher transform and the variance of spectral power estimates stabilised by logarithmic transformation (Halliday et al., 1995). Data were Hanning-windowed to control spectral leakage.

For each subject, data was analysed for three conditions: rest, preparing to grip ('prepare') and active handgrip ('grip'). The task-related changes in transformed coherence and spectral log power were calculated for each subject by subtracting transformed coherence or log power at rest from transformed coherence or log power during the other two conditions. The pattern of task-related coherence (TRCoh) during the prepare and grip phases of the task in healthy subjects were determined first. The TRCoh changes were assessed in the two frequency bands most associated with movement and its preparation, the alpha (9-12 Hz) and low beta (14-25 Hz) bands. This approach also limited the effects of ocular and movement artefact, manifest at low frequencies, and of electromyographic activity manifest at frequencies >25 Hz. To determine whether there were particular frequencies within the alpha and low beta bands that were most relevant to the task, hierarchical cluster analysis (Jambu M and Lebeaux M, 1983) was performed using Ward's method and Pearson correlation (software developed by A. Pogosyan, Sobell). This was executed on the TRCoh data for both sides in each subject in the control group (n=16). All the 1 Hz frequency bins within this band were equally able to discriminate the periods (figure 7.3) and, in particular, there was no difference between the alpha and beta bands (Fisher exact test, $p>0.05$). A mean of 18 bins/Hz and 17.9 bins/Hz (out of a maximum of 32 bins/Hz) were able to predict separate clusters with a minimum probability of 70% in the alpha and beta bands, respectively. The cluster analysis was repeated for the control TRCoh data from the prepare period. Again, all the bins within the 9-25 Hz band were able to discriminate between the rest and prepare periods and there was no difference between the alpha and beta bands (Fisher exact test $p > 0.05$; 19 bins/Hz for alpha; 20 bins/Hz for beta). Accordingly, the average of the alpha and low beta bands was used in further analyses and is henceforward termed the alpha-low beta band (although this is not to imply close correspondence between alpha and low beta activation in all paradigms).

In comparing strokes with healthy patients, the EEG electrodes in patients with left-sided strokes were inverted about the midline so that F4, FC4, etc overlay the affected hemisphere in all patients and the non-dominant hemisphere in all healthy subjects. Similarly, the left arm became the affected limb in all patients. Thereafter, the TRCoh in the alpha-low beta band was calculated and averaged to give six connectivity groupings of interest: left lateral frontal (LL; F3-FC3, FC3-C3, F3-C3), right lateral frontal (RL; F4-FC4, FC4-C4, F4-C4), left mesio-lateral (LML; F3-FZ, F3-FCZ, F3-CZ, FC3-FZ, FC3-FCZ, FC3-CZ, C3-FZ, C3-FCZ, C3-CZ), right mesio-lateral (RML; F4-FZ, F4-FCZ, F4-CZ, FC4-FZ, FC4-FCZ, FC4-CZ, C4-FZ, C4-FCZ, C4-CZ), mesial (M; FZ-FCZ, FCZ-CZ, FZ-CZ) and interhemispheric (IH; F3-F4, F3-FC4, F3-C4, FC3-F4, FC3-FC4, FC3-C4, C3-F4, C3-FC4, C3-C4). TRPow data were analysed using LL (F3, FC3, C3), RL (F4, FC4, C4) and M (FZ, FCZ, CZ) groupings. As preliminary analysis of TRCoh data in healthy subjects did not indicate a significant effect of dominance in our paradigm (see results section 7.2.1), a 'hand difference' was also calculated by subtracting TRCoh data when using one hand from TRCoh data when using the other hand (figure 7.4). For M and IH groupings, the mean TRCoh and task-related power (TRPow) during performance of the task with the right (unaffected in patients) hand was subtracted from that during performance of the task with the left (affected hand in patients). For the LL, RL, LML and RML groupings, the TRCoh and TRPow during performance of the contralateral hand were subtracted from that of the ipsilateral hand. A more negative score therefore represented increased TRCoh and TRPow during use of the dominant or unaffected hand for M and IH groupings and the contralateral hand for LL, RL, LML and RML groupings. Thus, among patients, a negative score for RL and RML connectivity groupings represented increased TRCoh and TRPow during use of the affected arm and a positive score for LL and LML represented increased TRCoh and TRPow during use of the affected arm. The above procedure was performed for both prepare and grasp periods.

Statistical analysis comprised two major stages. Firstly, in order to look for differences between strokes and controls, the hand difference scores for all subjects were entered into separate factorial analyses of variance (ANOVA) for the prepare and grip phases incorporating, where necessary, a Greenhouse-Geisser correction for non-sphericity. If main effects or interactions reached significance, data were further compared post-hoc using the Student's t-test (2-tailed). Bonferroni corrections were applied to account for multiple comparisons. Secondly, in order to see if connectivity changes were related to recovery, the hand difference scores in each stroke patient were linearly correlated with the respective recovery scores, as derived from the PCA.

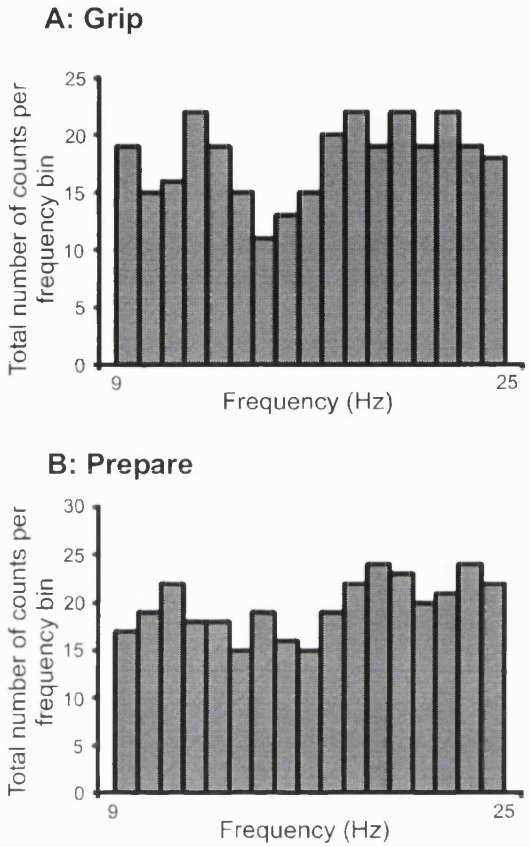


Figure 7.3. Cluster analysis of TRCoh data for both sides in each subject in the control group (n=16) within the 9-25 Hz band. All 1 Hz frequency bins are most equally discriminative in separating the rest and grip (A) and rest and prepare phases (B) of the task. Graphs depict the total number of counts per frequency bin (maximum = 32) that show >70% probability of predicting task-related coherence changes.

7.2 Results

7.2.1 Difference in TRCoh with hand used: control group

First, the pattern of coherence in the alpha-low beta band was established in healthy subjects. The link plots in figures 7.5 and 7.6 summarise the results in the grip and prepare phases and show no major lateralisation in healthy subjects. TRCoh was calculated for the six connectivity groupings of interest (LL, RL, LML, RML, M and IH) for both right and left hand use, during both the grip and prepare phases of movement. Grip data for each control subject were then entered into an ANOVA using the within-subject factors of hand (right, left) and connectivity grouping (LL, RL, LML, RML, IH, M). There was a significant main effect for connectivity grouping ($F[5,75] = 4.508$, $p =$

0.009) and an interaction between connectivity grouping and hand ($F[5,75] = 3.187$, $p = 0.032$). Post-hoc analysis revealed that there were no significant differences within a given connectivity grouping according to the hand used (the connectivity grouping x hand interaction was due to differences between connectivity groupings when a given hand was used). This was repeated for the prepare TRCoh data, using the same within-subject factors. There were no main effects or interactions ($p > 0.05$). TRPow grip and prepare control data were similarly analysed by ANOVA, using the within-subject factors of hand and electrode grouping (LL, RL, M). There were no main effects or interactions for either the grip or the prepare data. This therefore demonstrated that there were no major dominance or lateralisation effects within an electrode grouping in healthy subjects with hand use for either the grip or prepare tasks.

7.2.2 TRCoh and TRPow analysis: controls v strokes

To facilitate the comparison between healthy subjects and stroke patients, the measurements for hemispheres in patients with left-sided cerebrovascular lesions were reversed. The link plots in figures 7.5 and 7.6 show an increase in the number of connections with mean TRCoh > 0.05 in stroke patients gripping with their affected left hand relative to healthy subjects. There was no clear difference when patients gripped with their unaffected right hand or prepared to grip.

The next step was to calculate a hand difference score in the alpha-low beta band (see methods section 7.1.5 and figure 7.4) for each of the six connectivity groupings. TRCoh hand difference data for the prepare and grip phases in each subject were entered into separate ANOVAs using the within-subject factor of electrode grouping (LL, RL, LML, RML, IH, M) and the between-subject factor of group (control and stroke). For the prepare data, there was a main effect of region ($F[5,195] = 7.180$, $p = 0.002$) but no group interaction, so the pattern in healthy controls and patients with cerebral stroke did not differ during the prepare period (figure 7.7). However, in the grasp period there was an interaction between electrode grouping and subject group ($F[5,195] = 4.885$, $p = 0.011$). Post-hoc analysis revealed that, during the grip task, the hand differences for stroke subjects in the lateral frontal grouping over the unaffected hemisphere (mean $0.029 \pm$ SEM 0.018, $p = 0.018$), the right mesio-lateral grouping over the affected hemisphere (-0.082 ± 0.015, $p = 0.031$) and the mesial grouping (0.088 ± 0.018, $p = 0.010$) differed from those in healthy controls (-0.032 ± 0.021, -0.032 ± 0.016, and 0.013 ± 0.022, respectively). Thus, TRCoh was greater over left (unaffected) lateral frontal, right (affected) mesio-lateral and mesial areas during gripping with the affected compared to the unaffected hand in stroke patients (figure 7.7).

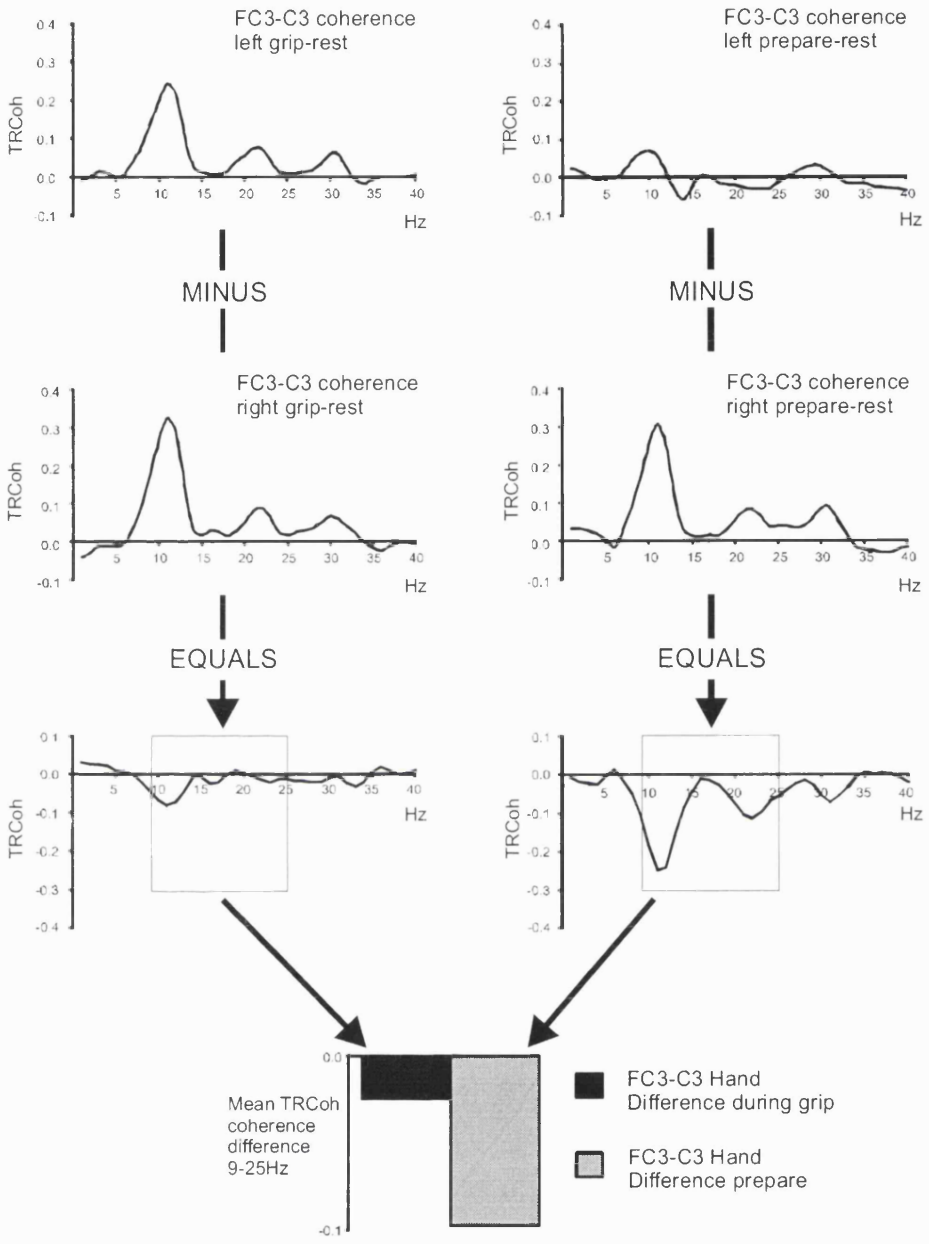


Figure 7.4. Calculation of hand difference score during grip and prepare to grip. Representative results for the transformed coherence between FC3 and C3 are shown in a healthy control.

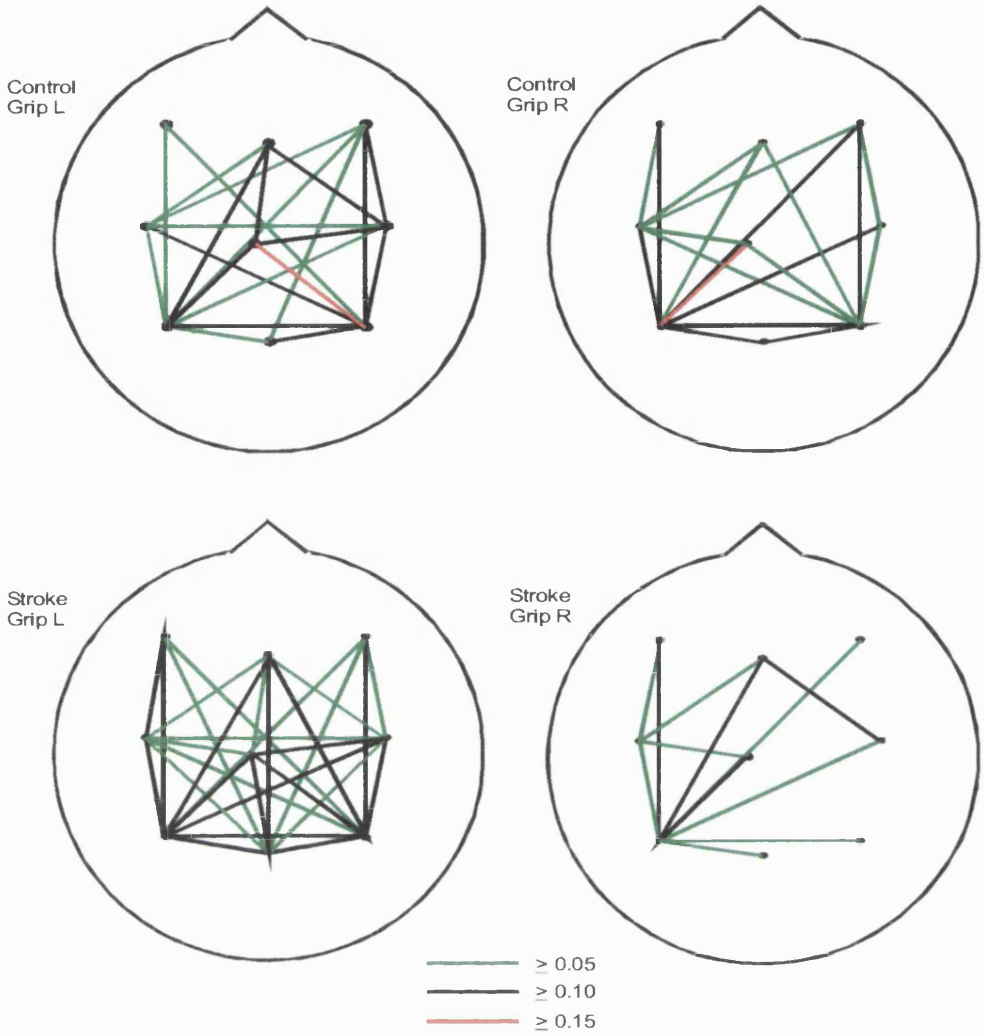


Figure 7.5. Schematic link plot of mean TRCoh in the alpha-low beta band during grip performed by healthy subjects (n =16) and stroke patients (n = 25, all right-handed and with hemispheres reversed in those patients with left-sided lesions, so the lesioned hemisphere is always on the right). TRCoh differences <0.05 are not shown. There were no TRCoh decreases larger than -0.05. The electrodes shown are F3, FC3, C3, FZ, FCZ, CZ, F4, FC4 and C4.

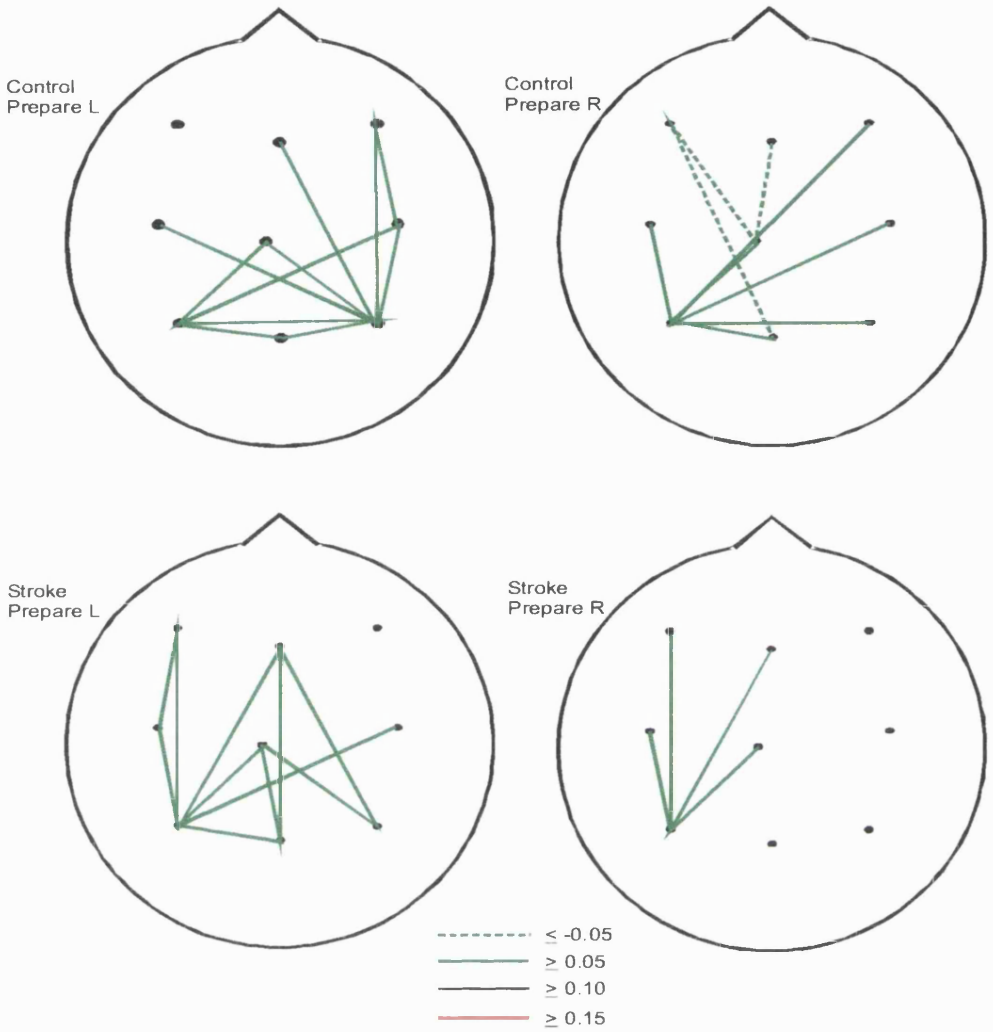


Figure 7.6. Schematic link plot of mean TRCoh in the alpha-low beta band during the prepare to grip phase performed by healthy subjects (n =16) and stroke patients (n = 25, all right-handed and with hemispheres reversed in those patients with left-sided lesions, so the lesioned hemisphere is always on the right). TRCoh differences <0.05 are not shown, except those TRCoh decreases larger than -0.05 (green dotted lines). The electrodes shown are F3, FC3, C3, FZ, FCZ, CZ, F4, FC4 and C4.

However, these differences could be accounted for by modulations of non-linearly related frequency components (Florian et al., 1998). Therefore, the mean log TRPow averaged over the 9-25 Hz band was calculated for the left lateral (F3/FC3/C3), right lateral (F4/FC4/C4) and mesial (FZ/FCZ/CZ) group electrodes and hand differences calculated as before. Subject data were entered into an ANOVA using the within-subject factor of region (LL, RL, M) and the between-subject factor of group. There was no difference ($p > 0.05$) between strokes and healthy subjects during the grasp (figure 7.8).

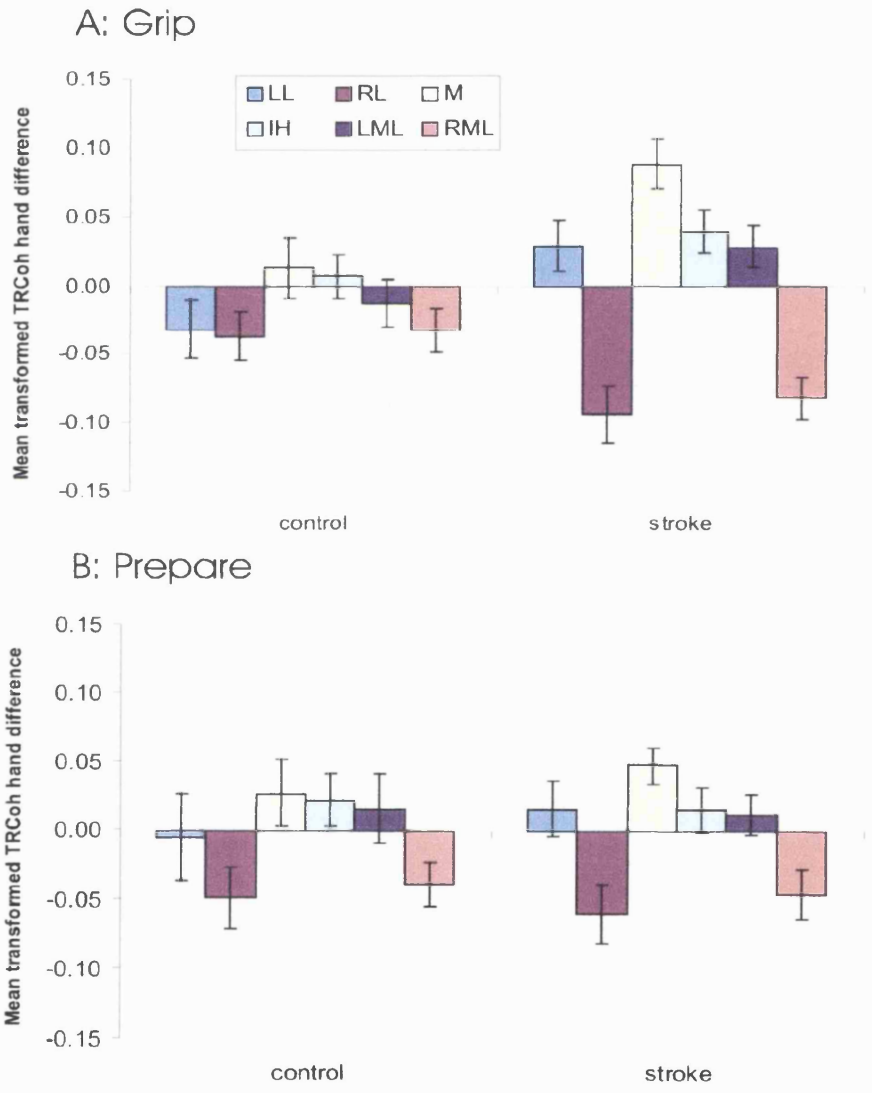


Figure 7.7. Bar chart of mean (± SEM) transformed TRCoh hand differences for controls (n = 16) and strokes (n = 25) during (A) grip and (B) prepare to grip for the six connectivity groupings.

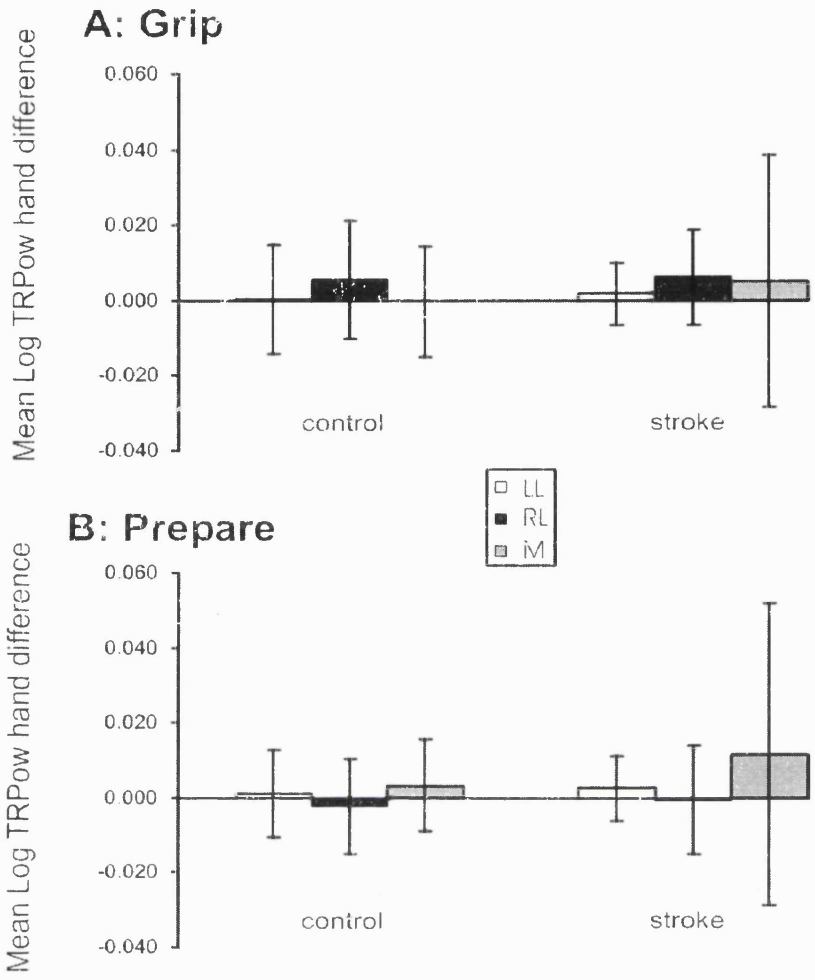


Figure 7.8. Bar chart of mean (± SEM) log TRPow hand differences for controls (n = 16) and strokes (n = 25) during (A) grip and (B) prepare to gip for the three electrode groupings.

7.2.3 Correlation of TRCoh and TRPow with recovery

The next question addressed was whether the TRCoh differences between healthy subjects and patients with cerebral strokes related to persisting disability among patients. Therefore, the hand differences for the contralesional lateral frontal, ipsilesional mesio-lateral and mesial groupings in the stroke patients were correlated with the first two principal components. These accounted for 65% and 17% of the total variance of the different outcome measures, respectively. Only the mesial hand difference was linearly related to disability and was limited to an inverse relationship with PC1, as shown in figure 7.9A ($r = -0.469$, $p = 0.018$). PC1 reflected both disability and motor impairment of the affected arm, whereas PC2 was a much more exclusive reflection of upper limb motor impairment (figure 7.1). Note that there was no

correlation between mesial TRCoh hand difference and PC2 and no correlation between mesial TRCoh and left or right grip strength alone.

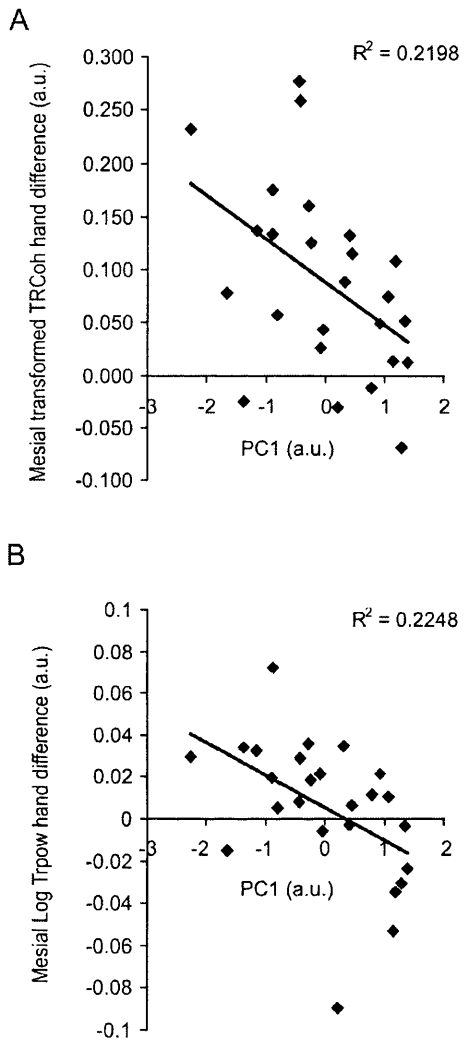


Figure 7.9. Correlation of (A) transformed TRCoh hand difference and (B) log TRPow hand difference with PC1 among 25 patients with cerebral stroke.

As before, the inverse relationship between mesial TRCoh and disability could have arisen through modulations of non-linearly related frequency components (Florian et al., 1998). To address this, the mean log TRPow hand difference averaged over the 9-25 Hz band for FZ, FCZ and CZ was correlated with disability. There was a negative correlation with PC1 (-0.474, p = 0.017) as shown in figure 7.9B. Therefore, over the mesial cortical areas, power and coherence are greater the worse the disability at

follow-up. In addition, the above demonstrates that the inverse correlation with coherence did not arise through a non-linear power interaction.

7.3 Discussion

This is the first study to investigate the changes in cortico-cortical connectivity after stroke and to relate them to recovery. The results demonstrated a difference in the connectivity pattern between healthy subjects and patients with cerebral stroke in the lateral frontal region of the unaffected hemisphere, the mesio-lateral region of the affected hemisphere and over the mesial motor area. The task-related EEG-EEG coherence in these areas increased in stroke patients during a grip task performed with the affected hand. Furthermore, the increase in mesial coherence was negatively correlated with recovery. In other words, as disability (or residual deficit) increased, task-related mesial coherence also increased.

Such differences in connectivity between controls and strokes may represent activities that are related to compensation for persisting impairment and disability or they may represent epiphenomena, such as the release of the unaffected hemisphere from transcallosal inhibition by the affected hemisphere (Traversa et al., 1998). Thus, a critical finding was the relationship between mesial task-related coherence increments upon use of the affected hand and disability, whereby higher levels of coherence were associated with greater disability. A similar relationship was evident for mesial power increments upon use of the affected hand. Indeed, both power and coherence accounted for over 20% of the variance in the first principal component describing disability. This relationship between spectral changes and disability would be compatible with a mechanistic role of increased cortico-cortical coupling in recovery from disability, at least over the mesial cortex.

There are a number of other potential confounds that need to be considered. Could the increased coherence between electrodes overlying mesial motor areas be a mere by-product of the increase in local EEG power over this region with increasing disability? This is unlikely because increases in the power of non-coupled activities would have been expected to decrease, rather than increase, local cortico-cortical coherence. Could the increase in coherence represent greater passive volume conduction between electrodes in tandem with the power increases? At worst, given the normalised nature of coherence, this would be expected to give a coherence that remained unchanged in the face of increasing local power. Thus, it can be concluded that the task-related power and coherence increases over the mesial motor areas inversely correlating with

disability reflect separate processes of regional activation (Pfurtscheller, 1992; Toro et al., 1994a, b; Stancak and Pfurtscheller, 1995; Gerloff et al., 1998) and inter and intra-regional coupling (Andres et al. 1999; Gerloff et al. 1998; Rappelsberger and Petsche 1988; Thatcher et al. 1986), respectively. Both these processes are more active in patients with greater disability. The increase in activation of the mesial motor cortical areas in more disabled patients was closely paralleled in a recent fMRI study (Ward et al., 2003a).

This is the first demonstration of an association between increased cortico-cortical connectivity and residual disability following stroke. The implication is that the multiple areas of increased activation identified following stroke may be working as functional networks to achieve compensation rather than as isolated units. However, similar TRCoh increases in the alpha and beta band are reported in healthy subjects during complex as opposed to simple movement sequences (Manganotti et al., 1998; Andres et al., 1999). This raises the possibility that the greater regional activation and cortico-cortical coupling, as indexed by power and coherence increases, were the product of a greater degree of effort or increased attentional demands in those patients that were poorly recovered. However, performance levels were matched across subjects by asking both patients and healthy subjects to grip at a fixed percentage of their own maximal grip strength, as indicated by visual feedback. This approach matches subjective sense of effort related to the grip task (Ward et al., 2003a). The alternative strategy of asking all subjects to grip with a fixed force would not have controlled for sense of effort and would have been likely to over-estimate changes associated with recovery (Ward et al., 2003b). A direct relationship between grip force per se and mesial changes also seems unlikely to account for the correlation between mesial power or coherence and PC1, given the absent correlation with PC2, which was more specifically related to motor impairment and hence grip strength.

Attentional demands are, however, difficult to control and one would not refute the possibility that some of the mesial frontal changes correlating with disability (PC1) were related to an increased attention to action in those patients with greater disability. Increases in task-related brain activation as a result of increased attention to a simple motor task have been observed in a number of motor regions, including SMA and cingulate cortex (Johansen-berg and Matthews, 2002). Indeed, increased attention to action would be an appealing strategy for compensation. It might explain why mesial changes correlated with PC1, a descriptor of a disability and impairment across a large range of measures not specifically related to the grip task. It might also explain the common clinical observation that stroke patients have greater difficulty when they are

distracted or focus on more than one task. It seems likely that increasing attention to action might enhance activity in motor areas and relevant cortico-cortical interactions (Rowe et al., 2002). In the sensory sphere, at least, there is general consensus that directing selective attention to a stimulus causes an increase in stimulus-related brain activity (Posner et al., 1982; Friston et al., 1995; Jancke et al., 1999; Mima et al., 1998).

One issue not addressed in the current study is the interaction of stroke and cerebral dominance. As in many other studies, the left and right hemisphere strokes were grouped together. This approach increased the power of the study to detect differences between healthy controls and stroke patients, focusing on changes that are independent of dominance and therefore may arguably be of more general significance. Nevertheless, although the results in healthy subjects did not demonstrate asymmetries related to use of the dominant as opposed to non-dominant hand, any exacerbation of differences due to dominance among the stroke subjects could have been overlooked. In particular, the importance of the left hemisphere for the representation of skilled behaviour has been emphasized (Haaland et al., 2000). Lesion studies have shown that left hemisphere damage produces bilateral deficits on a variety of motor tasks whereas right hemisphere damage is more prone to produce contralateral deficits (Haaland and Harrington, 1994). Using magnetic resonance morphometry, Amunts et al. (1996) estimated a larger hand motor area in the dominant than non-dominant hemisphere, implicating a richer interconnectivity and increased dispersion of movement representations.

In summary, these results are in line with those from several other studies using complementary techniques of TMS and functional imaging (Marshall et al., 2000; Calautti et al., 2001; Small et al., 2002; Turton et al., 1996; Liepert et al., 2000; Manganotti et al., 2002; Netz et al., 1997; Butefisch et al., 2003; Ward et al., 2003a,b). Broadly, these tend to suggest that differences from normality tend to be greater in patients with persisting disability. They may therefore reflect activities, such as increased attention to action, that dynamically but imperfectly compensate for brain damage and are unnecessary once damage or its effects have been reversed through other means such as delayed improvement in corticospinal function (Turton et al., 1996; Traversa et al., 1997; Pennisi et al., 2002) or use dependent cortical reorganisation (Carey et al., 2002; Jang et al., 2003). Such compensatory activities are expressed as functional cortical networks linking multiple cortical areas. In the future,

serial studies will be useful to explore further the relationship between task-related cortico-cortical coherence and disability after stroke.

7.4 Summary of key points

- Cortico-cortical coherence was investigated in healthy subjects and patients with chronic stroke involving one cerebral hemisphere and having varying degrees of motor recovery
- Scalp EEG was recorded at rest and while right-handed subjects performed a unimanual grip task.
- The degree of functional recovery following stroke was assessed using a range of outcome measures.
- Compared to healthy subjects, task-related EEG-EEG coherence was increased between mesial and lateral frontal regions of the affected hemisphere, over mesial frontal regions and over lateral frontal areas of the unaffected hemisphere when patients with stroke gripped with their affected hand.
- Mesial task-related power and coherence were negatively correlated with recovery.
- The results are consistent with the hypothesis that increases in the coupling between cortical areas may dynamically, albeit imperfectly, compensate for brain damage following stroke. Some of this coupling, particularly that over mesial frontal areas, diminishes as disability lessens.

Table 7.1. Patient characteristics. Initial severity refers to the cumulative MRC grade for wrist extension and flexion and finger extension and abduction of the affected arm as recorded in the medical records at the time of stroke. Cumulative MRC grade of the wrist and finger extensors alone was <4 for at least 48 hours after onset. IC = internal capsule; MCA = middle cerebral artery.

Case	Age (years)	Gender	Affected hand	Site of lesion	Time since stroke (months)	Initial severity	Other deficits at time of testing
1	63	M	R	L MCA	12	14	
2	27	F	L	R MCA	74	0	
3	69	M	L	R MCA	30	0	Homonymous hemianopia
4	53	M	L	R IC	17	0	Mild sensory impairment
5	45	M	R	L MCA	77	0	Expressive dysphasia
6	62	M	L	R MCA	29	0	
7	25	F	R	L IC	41	0	
8	51	M	L	R MCA	45	0	Moderate sensory impairment
9	33	M	R	L MCA	28	0	Mild expressive dysphasia
10	64	M	R	L MCA	25	0	Expressive dysphasia
11	60	M	R	L MCA	30	2	
12	69	M	L	R MCA	74	0	Mild sensory impairment
13	66	M	L	R thalamic haemorrhage	43	0	Mild dysarthria
14	37	F	L	R MCA	50	0	
15	64	M	R	L MCA haemorrhage	30	0	
16	61	F	L	R MCA	43	0	
17	64	M	R	L MCA	24	0	
18	55	M	L	R IC haemorrhage	30	0	
19	50	M	L	R MCA haemorrhage	21	0	Mild sensory impairment
20	63	M	L	R IC	32	12	
21	48	F	R	L MCA	32	0	Homonymous hemianopia
22	66	M	L	R IC	19	0	
23	42	M	R	L MCA	26	0	
24	66	M	L	R MCA	26	0	
25	48	M	L	R MCA	39	0	

Table 7.2. Patient outcome scores. *Cumulative MRC score for wrist extension and flexion and finger extension and abduction of the affected arm at time of EEG recording.

Case	Barthel (0-20)	FIM M (13-91)	FIM C (5-35)	Rankin (5-0)	Ashworth scale (5-0)	Grip strength of impaired hand (% contralateral side)	9HPT for impaired hand (% contralateral side)	MRC scale (0-20: total for 4 muscles)*	Recovery score (PC1)	Recovery score (PC2)
1	20	91	32	2	0	96.7	90	20	0.9207	1.0481
2	20	86	35	1	3	60.4	23.3	18	0.4142	-1.1805
3	18	83	31	3	2	24.2	0	15	-0.4327	0.4301
4	20	91	35	2	0	181.1	59.4	20	1.1413	0.6543
5	19	86	28	2	4	1.3	0	0	-0.8057	-0.4157
6	20	89	34	2	1	5.0	0	14	0.2135	-0.5959
7	20	91	31	2	0	70.2	88.5	19	0.7837	1.0481
8	20	86	34	2	3	7.9	0	17	-0.0433	-1.0272
9	20	88	33	2	3	12.5	0	5	-0.2796	-1.1078
10	13	58	21	4	3	0.7	0	0	-2.2660	3.0328
11	20	89	30	3	0	57.5	46.0	18	0.3223	1.2977
12	16	70	33	2	3	0.7	0	0	-1.1444	-0.7353
13	20	78	34	2	3	40.0	3.1	14	-0.0751	-0.6355
14	18	89	35	2	3	5.2	0	3	-0.4136	-1.3849
15	20	91	34	1	0	142.4	90.2	20	1.2820	0.4629
16	15	63	29	3	4	No data	0	0	-1.6556	0.6591
17	17	89	35	2	1	10.0	0	6	-0.2366	-0.8306
18	20	91	35	1	0	80.7	70.7	20	1.0671	-0.178
19	15	67	34	3	4	2.1	0	1	-1.3755	-0.3001
20	20	91	35	0	0	85.7	94.6	20	1.3378	-0.3962
21	17	76	33	2	4	1.1	0	3	-0.8963	-0.8811
22	18	76	32	2	4	12.5	0	0	-0.8813	-0.7999
23	20	89	33	1	0	132.7	90.6	20	1.1839	0.6449
24	20	91	35	1	0	170.3	88.7	20	1.3901	0.4049
25	20	84	34	3	0	92.6	38.0	18	0.4494	0.7668

8 Discussion

This thesis presents work investigating the oscillatory activity generated by ensembles of neurons in cortical areas involved in motor control. Such oscillatory activity was determined from surface electroencephalographic (EEG) recordings. Data were analysed in the frequency domain using techniques that allowed a statistical evaluation of any linear relationships between oscillatory components of the recorded signals. The effects of repetitive transcranial magnetic stimulation (rTMS) on this activity and also on motor behaviour were studied. In particular, the relationship of cortico-cortical interactions with motor function was explored.

8.1 Summary of experimental results

Previous studies have mainly tried to define rTMS effects by evaluating changes in motor cortex excitability using single and paired pulse magnetic stimulation (Pascual-Leone et al. 1994, 1998; Chen et al. 1997b; Berardelli et al. 1998; Maeda et al. 2000a; Peinemann et al. 2000; Gerschlager et al. 2001) or local glucose metabolism using positron emission tomography (Siebner et al. 2000, 2001). Repetitive TMS can modulate cortical function by enhancing or decreasing cortical excitability depending on the parameters of stimulation (Pascual-Leone et al., 1998). The mechanisms underlying these modulations following rTMS remain unclear. RTMS could have its effects through the modulation of local intracortical circuits or through the modulation of coupling with distant sites.

The pattern of interaction between different cortical areas and modulations of cortico-cortical coupling may be more directly investigated through the assessment of changes in the coherence between EEG signals simultaneously recorded over different cortical sites (Thatcher et al., 1986; Andrew and Pfurtscheller, 1996; Gerloff et al., 1998; Rappelsberger and Petsche, 1988; Andres et al., 1999). In contrast to EEG power, which provides a quantitative measure of the synchrony of relatively local sources within the range of an electrode, EEG coherence is a larger-scale measure. Coherence captures dynamic functional interactions between cortical areas underlying electrodes separated by longer distances. There is considerable evidence to support the assumption that synchronized oscillations reflect a basic form of communication between cortical assemblies during task-related activity (see reviews by Bressler, 1995; Klimesch, 1996; Singer, 1994).

Changes in regional EEG power in the alpha band tend to involve inhibitory mechanisms (Pfurtscheller 1992; Chen et al. 1998; Worden et al. 2000; Pfurtscheller et al. 2000; Hummel et al. 2002). If coherence within the alpha band were to have similar significance, then increased coupling between two areas could contribute to the reduction in excitability of the stimulated primary motor cortex that occurs with low frequency (stimulus rates of 1 Hz or less) rTMS (Chen et al. 1997b; Siebner et al., 1999a; Muellbacher et al., 2000; Maeda et al., 2000; Touge et al., 2001). In contrast, since higher frequencies (stimulus rates of more than 1 Hz) may promote a short-term increase in cortical excitability (Pascual-Leone et al. 1998; Maeda et al. 2000a; Peinemann et al. 2000; Di Lazzaro et al. 2002a; Wu et al. 2000), a reduction in cortico-cortical coupling in the alpha band might be expected.

8.1.1 Effects of 1 Hz and 5 Hz rTMS to motor cortex on coherence and reaction times

Accordingly, the effects on cortico-cortical coherence of both high (5 Hz) and low (1 Hz) frequency rTMS to the primary motor cortex were studied in healthy controls. RTMS given at 1 Hz caused a persistent increase in the alpha coherence between the ipsilateral stimulated primary motor and more anterior motor areas (intrahemispheric) and also in the alpha coherence (during tonic muscle contraction) between the ipsilateral stimulated and contralateral unstimulated motor areas (interhemispheric). There was no change in beta intra- or inter-hemispheric coherence. The effect of 1 Hz rTMS was relatively focal as there was no change in the coherence between primary motor cortex and more anterior motor areas over the cerebral hemisphere contralateral to stimulation. In contrast, 5 Hz rTMS caused a significant decrease in the upper alpha coherence between the primary motor and more anterior motor areas of both hemispheres. Interhemispheric alpha coherence was not affected by 5 Hz rTMS, although there was a decrease in interhemispheric beta coherence with muscle contraction compared with rest.

Although motor and premotor area alpha and beta power in both paradigms was reduced during voluntary muscle contraction compared with rest, there was no pre-stimulation variation in alpha coherence with rest versus contraction. This may be because sustained tonic contraction was examined, avoiding transients occurring during the first few seconds of contraction (Crone et al, 1998). Changes in coherence are, however, reported in phasic tasks (Gerloff et al., 1998; Manganotti et al., 1998). Independent changes in regional EEG power and EEG-EEG coherence have been noted before and it has been suggested that the two measures may relate to different

aspects of cortical function that might, to some extent, operate independently of each other (Gerloff et al. 1998).

5 Hz rTMS changed cortical coupling but the effect was only significant during the active contraction of muscles and in the upper alpha band. Other reports also support the notion that activities in the upper and lower alpha bands are likely to be functionally distinct (Manganotti et al. 1998; Mima et al. 2000b; Pfurtscheller et al. 2000). It is noteworthy that the effects of 1 Hz rTMS on alpha band coupling are not only opposite but also less specific. Thus they do not depend on functional state (rest compared to voluntary tonic activity) and are more generalised, affecting interhemispheric coupling as well. These differences may be a function of the different frequency of stimulation and/or the greater number of stimuli delivered.

The changes in coherence were unlikely to be due to a general alteration in arousal of subjects for several reasons. Firstly, opposite effects were achieved using different frequencies of stimulation. Secondly, the intrahemispheric changes following 5 Hz rTMS and the interhemispheric changes following 1 Hz rTMS were confined to periods of tonic contraction. An effect of general arousal would have been expected to involve both rest and active conditions. Thirdly, the lack of rTMS effects on regional power is also against any non-specific arousal changes during the paradigms. Lastly, the differences in non-linear components of the signals did not account for differences in coherence. There was no change in EEG power with rTMS so that the changes in EEG-EEG coherence in the alpha band reflected a change in the absolute degree of coupling between areas. The effects must have been purely central in origin because the intensity of the individual rTMS pulses was below resting motor threshold and rTMS was performed at rest. Therefore, no muscle twitches were evoked that could modify central processing through changed afferent input. On the other hand, such low intensities are used as conditioning pulses in paired-pulse testing of cortical inhibition and facilitation (Kujirai et al., 1993). Thus, the rTMS was probably capable of exciting cortical interneurones. Subthreshold 1 Hz rTMS has been shown to decrease local cerebral blood flow, consistent with TMS-induced activation of local inhibitory mechanisms, whereas subthreshold 20 Hz rTMS increased local cerebral blood flow (Speer et al., 2000).

Inhibitory connections between the primary motor cortex and more anterior areas have recently been reported in humans (Civardi et al., 2001). It is tempting to assume an overall inhibitory function for the coupling between premotor and motor cortex in the alpha band. RTMS might modify inhibitory coupling through effects on inhibitory

projections from anterior motor areas to primary motor cortex or through effects on local GABAergic interneurones that receive input from excitatory axons coming from the premotor cortex. The GABAergic interneurones might then be modulated by local TMS over the primary motor cortex (Kujirai et al. 1993; Ziemann et al. 1996a,b; Werhahn et al. 1999; Di Lazzaro et al. 2000). Either way, rTMS might alter the efficacy of inhibitory cortico-cortical inputs to the motor cortex, thereby contributing to the changes in excitability observed following this intervention (Pascual-Leone et al. 1998; Maeda et al. 2000a; Peinemann et al. 2000; Di Lazzaro et al. 2002b). Alternatively, the effects of rTMS on the motor cortex might be more indirect, involving remote after-effects on subcortical structures that then cause secondary changes in cortico-cortical coupling. The pharmacological basis of the short-term plasticity in cortico-cortical coupling induced by rTMS is unclear. Gamma-aminobutyric acid (GABA) transmission is implicated in the effects of TMS on the cortex (Kujirai et al., 1993; Ziemann et al., 1996a,b; Werhahn et al., 1999) and modulation of GABA is felt to be the key mechanism of short-term plasticity in the adult mammalian central nervous system (Jones, 1993; Donoghue et al., 1996).

Thus, rTMS, given to the left motor cortex at different frequencies, has different effects on cortico-cortical coupling. Although the sample size was small, the effects of left motor cortex rTMS on right hand reaction times also differed according to whether high or low frequency stimulation was given. Left hand reaction times were not affected. Reaction times were longer after 5 Hz stimulation than after 1 Hz rTMS. Premotor cortical areas are involved in responses to perceptual cues (Halsband and Freund 1990; Jeannerod et al, 1995) and are also implicated in the initiation and selection of movement (Deiber et al, 1991; Rao et al, 1997). It could be speculated that 5 Hz rTMS causes an imbalance in the net inhibitory connections between premotor cortex and motor cortex and that this modification in the flow of information between the two cortical structures is responsible for a delayed reaction. In this context, diminished inhibition seems functionally more important than increased inhibition.

8.1.2 Effects of 5 Hz rTMS to the SMA on coherence and bimanual coordination

High frequency rTMS to the SMA caused a deterioration of temporal inter-limb regulation, immediately following stimulation. The effect was stronger for anti-phase than in-phase movements, supporting the former's more intricate coordinative requirements. This effect was associated with a reduced degree of EEG coherence between the primary motor cortices, albeit recorded at rest. RTMS was unlikely to have exerted its effects through stimulation of premotor or primary motor cortices as

stimulation was performed over the midline at low intensity. Also, rTMS caused no modulations of EEG power, which might have been expected with alterations in attention or arousal. Accordingly, these data are in line with earlier imaging and EEG studies that have shown the SMA to be particularly involved in the processing of complex, as compared to simple, sequences (Gerloff et al., 1997; Lang et al., 1989; Shibasaki et al., 1993). These results are also supported by more recent studies showing that rTMS to the SMA can disturb aspects of bimanual coordination (Meyer-Lindberg et al., 2002; Steyvers et al.; 2003; Obhi et al., 2002). The present findings support the key role of the SMA for the organization of bimanual configurations as a function of task complexity. In particular, rTMS of the SMA disrupted the fine-tuning of inter-limb movements. These data suggest that rTMS of the SMA may transiently modify the functional interhemispheric coupling and thereby influence bimanual behaviour in which combined sequences need to be implemented into a coherent timing plan. They also highlight the importance of an optimal integration of SMA activity into the motor control network that is used for the realisation of coordinated movements.

8.1.3 Effects of 5 Hz rTMS to motor cortex on coherence, finger tapping and cortical excitability

It remains unclear why some patients who have had a motor stroke make a good functional recovery. Possibilities include unaffected cortical areas compensating for affected regions by taking over function, or more use being made of subcortical motor systems. Many imaging studies have shown that there is increased task-related, and invariably multifocal, brain activation in stroke patients during contraction of the affected hand compared to healthy subjects. In particular, there is increased activation of the motor cortex ipsilateral to the affected hand. Overall, these studies suggest that either ipsilateral M1 activation represents a compensatory mechanism, later replaced by other processes, or it is spurious, perhaps represented by mirror movements (Weiller et al., 1993) or unmasking of circuitry that is normally suppressed and has nothing to do with motor recovery (Turton et al., 1996).

5 Hz rTMS was used to elicit controlled, temporary and partial disruption of M1 function in healthy humans. Independent stimulation of the contralateral motor cortex had a short-lived effect on the control of fine finger movements, whilst ipsilateral stimulation had no effect at all. In contrast, simultaneous bilateral stimulation of the primary motor cortex had a prolonged and stronger effect, which exceeded the algebraic sum of individual contralateral and ipsilateral stimulation. In addition, high frequency

stimulation of the motor cortex contralateral to the tapping hand was associated with a delayed and temporary reduction in *ipsilateral* cortical excitability. These distant effects of rTMS are in keeping with evidence from positron emission tomography that trains of subthreshold rTMS at 5 Hz over the primary motor cortex lead to persisting increases in activity over both primary motor cortices (Siebner et al., 2000). Indeed, the results presented in chapter 4 revealed that 5 Hz rTMS given over the left motor cortex caused a bilateral decrease in intrahemispheric cortico-cortical coherence.

The behavioural results were compatible with the ipsilateral M1 compensating for the effect of contralateral rTMS. Such an interpretation finds strong support in the cortical excitability studies that demonstrated a reduction in ipsilateral cortical excitability during finger tapping in the period 51-100 s following contralateral M1 stimulation. Importantly, there was no reduction in cortical excitability ipsilaterally if the subject was at rest following rTMS. This, together with the delayed reduction in excitability when tapping following rTMS, indicates that acute dysfunction of the motor cortex contralateral to the tapping hand may have engendered secondary compensatory change within the ipsilateral motor cortex. This short-term use-dependent adaptational change (Rossi et al., 2000) only occurred when behaviourally relevant, i.e. when tapping.

The behavioural deficit induced by 5Hz rTMS to the motor cortex involved a selective increase in force without effects on mean movement frequency or the variability of movement frequency. The effects in this study were temporary and reversed without further training with visual feedback. This suggests that the rTMS did not impair early motor consolidation (Muellbacher et al., 2002) and did not destroy the motor program or internal model related to the task. Rather, it temporarily disrupted how the motor cortex translated the program/model into desired forces, leading to an overestimate of the required force. This may have arisen through rTMS induced alterations in sensory feedback (Tsuji and Rothwell, 2002), changes in local cortical processing (Siebner and Rothwell, 2003; Wu et al., 2000; Pascual-Leone et al., 1998; Maeda et al., 2000a) or suppression of inhibitory inputs from other areas (chapter 4).

There is substantial evidence that both motor cortices are activated in unilateral distal movements, particularly when these are fine or complex (Chen et al., 1997a; Kim et al., 1993; Kristeva et al., 1991; Sadato et al., 1997; Pfurtscheller and Lopes da Silva, 1999). The two motor cortices may form a spatially distributed circuit linked through transcallosal and cortico-subcortico-cortical pathways that organises different aspects of movement, akin, for example, to the bilateral circuit subserving spatial attention (Oliveri et al., 1999). Movement is then largely effected by the contralateral motor

cortex. If this function is disturbed, the role of the ipsilateral motor cortex in this bilateral organisation may be increased (Siebner and Rothwell, 2003). This is the first demonstration that the ipsilateral primary motor cortex is capable of functionally significant compensation for focal contralateral cortical dysfunction in the adult human.

8.1.4 Coherence changes after stroke and relationship to motor recovery

Thus, given the above findings, the contralesional primary motor cortex may have the capacity to compensate for cortical dysfunction after stroke. Many imaging studies have shown that there is increased task-related brain activation in stroke patients during contraction of the affected hand compared to healthy subjects, although the extent to which activation changes relate to motor recovery is unclear (Carey et al., 2002; Johansen-berg et al., 2002; Netz et al., 1997, Shimizu et al., 2002; Mima et al., 2001b). Some evidence supports a compensatory role for the contralesional premotor region, particularly as increased activity is only present after some recovery has taken place (Nelles et al., 1999; Johansen-berg et al., 2002). Overall, however, it is likely that multiple areas are involved acutely following stroke and that this involvement is inversely correlated with recovery. In support of this idea, a recent study has demonstrated a negative correlation between outcome and the degree of task-related brain activation in the primary motor and sensory cortices, pre-supplementary, supplementary and cingulate motor areas, premotor cortex, prefrontal region, posterior parietal cortex and cerebellum (Ward et al., 2003a)

With this in mind, the question of whether multiple cortical areas also interact to form dynamic assemblies that may then compensate for disability was investigated. The results demonstrated a difference in the connectivity pattern between healthy subjects and patients with cerebral stroke in the lateral frontal region of the unaffected hemisphere, the mesio-lateral region of the affected hemisphere and over the mesial motor area. The task-related EEG-EEG coherence in these areas increased in stroke patients during a grip task performed with the affected hand. Furthermore, the increase in mesial coherence was negatively correlated with recovery. In other words, as disability (or residual deficit) increased, task-related mesial coherence also increased. This increase in activation of the mesial motor cortical areas in more disabled patients was closely paralleled in a recent fMRI study (Ward et al., 2003a).

Such differences in connectivity between controls and strokes may represent activities that are related to compensation for persisting impairment and disability or they may represent epiphenomena, such as the release of the unaffected hemisphere from transcallosal inhibition by the affected hemisphere (Traversa et al., 1998). Thus, a

critical finding was the linear relationship between mesial task-related coherence increments upon use of the affected hand and disability, whereby higher levels of coherence were associated with greater disability. A similar relationship was evident for mesial power increments upon use of the affected hand. Indeed, both power and coherence accounted for over 20% of the variance in the first principal component describing disability. This relationship between spectral changes and disability would be compatible with a mechanistic role of increased cortico-cortical coupling in recovery from disability, at least over the mesial cortex.

This is the first demonstration of an association between increased cortico-cortical connectivity and residual disability following stroke. The implication is that the multiple areas of increased activation identified following stroke may be working as functional networks to achieve compensation rather than as isolated units. These results are in line with those from several other studies using complementary techniques of TMS and functional imaging (Marshall et al., 2000; Calautti et al., 2001; Small et al., 2002; Turton et al., 1996; Liepert et al., 2000; Manganotti et al., 2002; Netz et al., 1997; Butefisch et al., 2003; Ward et al., 2003a,b). Overall, these tend to suggest that differences from normality tend to be greater in patients with persisting disability. They may therefore reflect activities, such as increased attention to action, that dynamically but imperfectly compensate for brain damage and are unnecessary once damage or its effects have been reversed through other means such as use dependent cortical reorganisation (Carey et al., 2002; Jang et al., 2003). Such compensatory activities are expressed as functional cortical networks linking multiple cortical areas.

8.2 Overall conclusions

RTMS can be used to produce effects on cortical circuits that outlast the duration of stimulation. This provides an opportunity to provoke and study mechanisms of acute cortical reorganisation in the healthy human cortex. The findings presented in this thesis confirm that rTMS to motor areas of the healthy human brain causes effects that outlast the duration of the train of stimulation. In addition, rTMS to motor areas causes changes in cortico-cortical coherence, associated changes in motor behaviour and differential effects dependant on stimulation parameters. Disruption to one motor cortex with rTMS may be compensated for functionally by the opposite motor cortex in healthy humans. Lastly, changes in cortico-cortical connectivity after stroke were shown to relate to functional recovery. These results have led to several conclusions.

8.2.1 rTMS can acutely disrupt cortical function at a behavioural level

Previous studies have demonstrated the differing effects of high and low frequency rTMS on levels of corticospinal excitability as measured by MEP size. However, it has been more difficult to show behavioural effects of rTMS. The findings presented here have shown that rTMS can modulate performance in a motor task. 5 Hz rTMS to the primary motor cortex caused a small, transient increase in mean peak finger tapping force. 5 Hz rTMS to the SMA caused deterioration in the temporal accuracy during an anti-phase task. There was also a suggestion that high and low frequency rTMS may have differential effects on reaction times.

8.2.2 Effects of rTMS are partly mediated by changes in cortico-cortical coupling

Are the changes in motor behaviour induced by rTMS associated with cortical changes? The present findings have demonstrated that cortico-cortical coherence can be manipulated using rTMS. Previous studies have suggested that low frequency stimulation increases cortical inhibition whereas high frequency stimulation decreases inhibition. Here, low frequency rTMS to the primary motor cortex caused an increase in inter- and intra-hemispheric alpha coherence. In contrast, high frequency rTMS caused a reduction in intrahemispheric alpha coherence. Stimulating the SMA with high frequency rTMS decreased interhemispheric alpha and beta coherence. It was also possible to influence coupling in a network downstream of the stimulation.

8.2.3 Effects of rTMS are limited by acute plasticity at cortical level

Behavioural effects following rTMS may be difficult to demonstrate because the healthy human cortex is, in some way, able to compensate for acute disruption of function. The idea of cortical compensation is not new and has been postulated as a mechanism for recovery after stroke. High frequency rTMS to the primary motor cortex caused a small and short-lived effect on tapping force, whereas the effect with bilateral stimulation was much greater than expected. Cortical excitability studies indicated that acute dysfunction of the motor cortex contralateral to the tapping hand may have engendered secondary compensatory change within the ipsilateral motor cortex.

8.2.4 Chronic plasticity in stroke is partly mediated by changes in cortico-cortical coupling

Having demonstrated the functional relevance of cortico-cortical coupling in the motor system, what happens when the cortex is damaged by disease? This question was addressed by studying cortico-cortical coherence in patients with stroke in relation to

recovery. When gripping with the affected hand, the EEG-EEG coherence in stroke patients, compared to healthy controls, was increased in motor areas of the brain bilaterally. However, only the coherence changes in mesial areas correlated with recovery and this correlation was negative. In other words, as patients recovered motor function after the stroke, so the coherence increases reverted to normal.

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In conclusion, the series of experiments described in chapters 3 to 7 have shown that combined EEG and TMS techniques, which complement those of functional imaging, can prove useful in the investigation of cortical motor function. The ability of rTMS to cause lasting effects on cortical function gives it the potential to interfere actively with cortical plasticity in humans. Further experimentation may allow the possibility of predicting the modulatory effects of rTMS. This could have therapeutic implications for patients such that stimulation techniques could be used to manipulate cortical reorganisation. RTMS protocols might be used to suppress the mechanisms that mediate 'maladaptive' plasticity, leading to a deterioration of brain function (for example, dystonia), or rTMS might be used to enhance 'beneficial' plasticity (for example, motor recovery after stroke).

The ability to induce reversible and controlled functional compensation in healthy subjects provides an important model for the screening of treatments for their potential to promote functional compensation in unaffected cortical areas following stroke. In the future, serial studies will be useful to explore further the relationship between task-related cortico-cortical coherence and disability after stroke. Given the fact that coherence changes in mesial areas correlated with recovery of motor function after stroke, one might expect greater functional compromise in stroke patients than in healthy controls if rTMS was applied to these areas. Combining research techniques in this way has already proven to be useful. For example, TMS applied to the premotor cortex in stroke patients was associated with slower simple reaction times of finger movements (Johansen-berg et al., 2002). FMRI of the same patients performing the same task showed an inverse correlation between the relative hemispheric lateralisation of fMRI activation and the reaction times. This suggests that the increased activation in ipsilateral cortical motor areas during movements of a paretic hand represents a functionally relevant response to the brain injury.

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