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The Use Of Functional Neuroimaging To Study Reorganisation Of The Motor System During Task Performance Following Altered Corticospinal Excitability Caused By Repetitive Transcranial Magnetic Stimulation

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) in motor areas has been shown to induce transient and reversible changes in the corticospinal excitability of healthy individuals' brains. Furthermore, a number of studies have shown that this reorganisation can occur not only at the site of stimulation but also in other areas which might be anatomically or functionally connected to it. These effects are thought to depend on various parameters such as the protocol of stimulation, the site of stimulation and the behavioural paradigm chosen to study the effects.

The aim of this thesis was to use functional neuroimaging in order to explore how the effects of long-lasting rTMS protocols (30-60min stimulation) on the motor system depend on specific conditioning parameters such as the frequency, the site and the pattern of stimulation. We examined how the pattern of activity in the brain reorganises depending on the anatomical and *effective* connectivity of the stimulated area, and on the behavioural task.

Initial studies presented in this thesis used Positron Emission Tomography to compare the effects of high (5Hz) versus low (1Hz) frequency of rTMS on activity and motor network connectivity of the primary motor cortex (M1) during performance of a simple finger movement task or at rest. I found that task-related activity of motor area 4p within M1 and its connectivity with non-primary motor areas, such as the ipsilesional dorsal premotor (PMd) cortex could be modulated bidirectionally with low or high frequency rTMS over M1: compared to sham stimulation, 5Hz rTMS reduced task-related activity and network connectivity of that area. The opposite was true for 1Hz rTMS. A further study using the same task examined whether such reorganisation would be observed with 5Hz rTMS over the PMd. The effect of 5Hz rTMS on this site of stimulation revealed a different pattern of regional cerebral blood flow than rTMS over M1. However, a similar reorganisation in task-related activity and network connectivity was observed, particularly within the PMd area caudal to the stimulated site, which increased in activity after 5Hz rTMS. These two studies demonstrated that rTMS leads to widespread activity changes. However, changes in task-related activity and motor network connectivity occur in motor areas adjacent to the stimulated site. Given the lack of any behavioural effects in these studies, it can be hypothesised that these changes occur as compensatory mechanisms to the "virtual lesion" caused by rTMS.

A functional magnetic resonance imaging (fMRI) study of the effects of rTMS over the lateral prefrontal cortex showed task-related changes in activity during performance of a cued choice

reaction time task. Targeting the dorsolateral prefrontal cortex (DLPFC) with 5Hz rTMS led to task-related decreases in activity in the adjacent ventrolateral prefrontal cortex. This is reminiscent of the task-related decreases in activity of area 4p observed following 5Hz rTMS over M1 (described above). The side of lateral prefrontal conditioning affected behavioural performance in a further study which distinguished motor from spatial attention in the same behavioural paradigm. Left DLPFC stimulation led to a more prominent switch cost in the motor attention version of this task, confirming a left-lateralised dominance for switching motor responses, described in previous studies.

These results provide new evidence that reorganisation in the brain observed following rTMS conditioning is very similar to reorganisation observed following lesions in patients. This reorganisation seems to depend on the task that is required to be performed and determines the pattern of activity obtained in functional neuroimaging studies. In addition, whether this will lead to a behavioural effect depends on the role of that particular area during task performance which is a function of its *effective* connectivity. These factors seem to determine whether the brain can compensate for the "virtual lesion" induced by rTMS.

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ABBREVIATIONS

AC	Anterior Commisure
AMT	Active Motor Threshold
AP	Action Potential
BOLD	Blood Oxygen Level Dependent
CMA	Cingulate Motor Area
CS	Conditioning Stimulus
CSN	Corticospinal Neurons
CST	Corticospinal Tract
EEG	Electroencephalography
EMG	Electromyogaphy
EPI	Echo-planar Imaging
FDI	First Dorsal Interosseus
fMRI	Functional Magnetic Resonance Imaging
FWHM	Full Width at Half Maximum
HRF	Haemoynamic Response Function
GABA	Gamma-AminoButyric Acid
GLM	General Linear Model
ICI	Intracortical Inhibition
ICF	Intracortical Facilitation
ISI	Inter-Stimulus Interval
ITI	Inter-Train Interval
LFP	Local Field Potential
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MRI	Magentic Resonance Imaging
MS	Multiple Sclerosis
PC	Posterior Commisure
PET	Positron Emission Tomography
PMd	Premotor Cortex

rCBF	Regional Cerebral Blood Flow
RMT	Resting Motor Threshold
RF	Radiofrequency
rTMS	Repetitive Transcranial Magnetic Stimulation
SEP	Somatosensory Evoked Potential
SMA	Supplementary Motor Area
SNR	Signal to Noise Ratio
SPM	Statistical Parametric Mapping
TE	Echo Time
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TR	Repetition Time
VAC	vertical plane extending from AC perpendicular to AC-PC line
VPC	vertical plane extending from PC perpendicular to AC-PC line

1. Structure and Function of the Motor System

1.1 INTRODUCTION

The purpose of this thesis is to investigate reorganisation of the cortical motor and attention systems, following temporary disruption of function in one part induced by repetitive TMS (rTMS). The aim was to try to learn how interconnected systems in the brain are organised under normal circumstances, and get insight into how they may react after damage, such as stroke. I will study healthy brain and use rTMS to disrupt function temporarily. Brain imaging will be used to test how parts of the brain remote from the stimulation site react to the disruption and test how this relates to changes in behavioural performance.

This chapter reviews the functional neuroanatomy of the cortical systems underlying voluntary movement, in order to provide a framework for interpreting and understanding the results of the experiments presented in the subsequent chapters. Particular attention is paid to two sytems that have been studied in this thesis. The first is the motor system, comprising a well-characterised distributed network that includes cortical and subcortical subcomponents. The connections between these are well-known as are many of the functions of individual areas. The motor system has the advantage of having been studied extensively with TMS (see Section 3). This is because stimulation of the motor cortex with TMS leads to a well defined and easily measured muscle twitch, namely the motor evoked potential (MEP), which gives a very good measure of the effects of rTMS on the motor system making the interpretation of the results easier in terms of understanding whether the conditioning paradigm used led to facilitation or inhibition of the area stimulated. The second system was the prefrontal attentional network. This is a less well-characterised network and its reaction to rTMS is not as clear given that its function can only be tested in behavioural paradigms. A brief section on the anatomy and role of the lateral prefrontal cortex in motor control is provided to account for the experiments presented in Sections 6 and 7 of this thesis.

1.2 THE MOTOR SYSTEM

1.2.1 Background

Most of the work concerning the localisation of specific brain regions responsible for motor function came from animal studies (Porter and Lemon 1993). Attempts to localise specific brain regions for motor function accelerated with the work of Sherrington in the later part of the 19th century as well as Ferrier, Fritsch and Hitzig who demonstrated that motor areas were electrically excitable (Porter and Lemon 1993). Evidence showed that removal of cortex in the precentral region produced paralysis in certain animals, and that stimulation of the same cortex could elicit muscle responses. (Leyton and Sherrington 1917). Later, Sherrington and Leyton were able to localise physiologically excitable cortex to an area anterior to the central sulcus, the precentral gyrus.

Around the same time, Brodmann was performing histological studies to map out cortical cytoarchitecture (Figure 1.1). Suggestions were made that anatomical divisions would correspond to functional divisions.

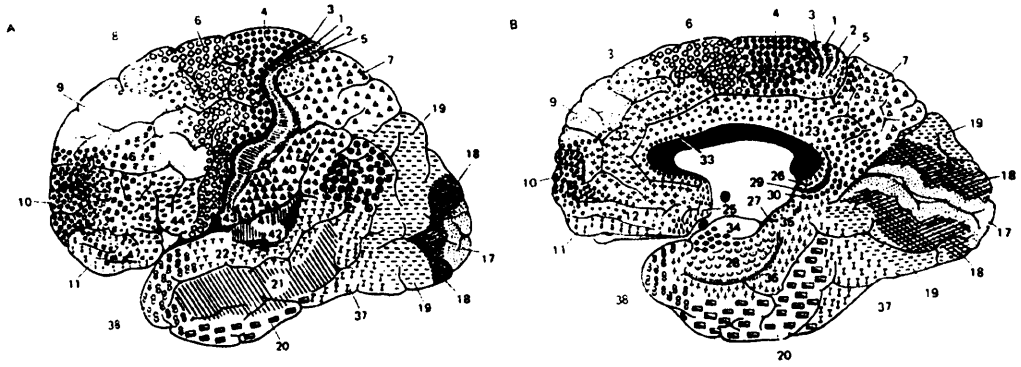

Figure 1.1: Brodman Areas. Lateral and mesial views of the human brain showing the cortical cytoarchitectonic regions (Adapted from Brodmann, 1909)

"There is an undisputed axiom: physiologically dissimilar elements have dissimilar structures. Reversing this statement, one may equally justifiably conclude that parts of organs that are structurally different must serve different purposes"

Brodmann, 1909

Direct evidence of a link between histology and physiology came with the work of Campbell, who observed a correlation of Brodmann's areas 4 and 6 with electrically excitable motor cortex. Just as Sherrington and Leyton had localised the physiologically excitable cortex to the precentral gyrus, so Campbell found the distinct histology of this region.

However, it soon became clear that this region comprised multiple motor areas, whose exact subdivisions in terms of movement control are still under debate (Graziano *et al.* 2002). Electrical stimulation studies revealed that stimulation of the primary motor cortex required lower thresholds of stimulation and evoked the simplest movements, whilst stimulation of the currently known premotor and SMA regions had a higher threshold and evoked more complex movement (Porter and Lemon 1993). Cingulate motor areas were not studied early on as they were not localised at the surface of the brain. The controversy over the number of cortical motor areas that exist has been confounded by disagreement over what criteria should be used to define a motor area. Cortical motor areas have been recently defined by Roland and Zilles as those areas having projections to spinal motor neurons, containing a representation of somatomotor apparatus and always being active during the planning and execution of voluntary movements (Roland and Zilles, 1996).

As a first approximation, the motor system is considered to include the agranular frontal cortex (Brodmann areas 4 and 6) and cingulate motor areas (BA 24). The "primary" motor cortex (area4) and "premotor" cortex (lateral area 6) were functionally divided based on lesion studies (Fulton 1935). Area 6 on the medial wall was also designated as a functionally distinct unit, the "supplementary" motor area (Woolsey *et al.* 1952, Fink et al. 1995). Subsequently, however, the model has become significantly more complex (Rizolatti *et al.* 1998, Roland and Zilles 1996, Geyer *et al.* 2000). Many subregions have been identified on the basis of functional and microscopic

characteristics looking at the cytoarchitecture, myeloarchitecture, metabolic architecture, connectivity and receptor mapping. Studies in the monkey using a number of these techniques in the same animals have allowed for more detailed structure-function relationships to be defined.

1.2.2 Primary Motor Areas

The primary motor cortex, M1, corresponds to Brodmann Area 4, which is classically characterised by a lack of granule cells in layer IV and a high density of giant Betz cells in layer V (Meyer 1987). The caudal border of M1 lies close to the fundus of the central sulcus. From there, M1 extends to the vertex of the precentral gyrus and from the Sylvian fissure anteriorly to the mesial wall of the frontal lobe. The rostral border of M1 is difficult to define, as the cytoarchitecural transition between areas 4 and 6 is gradual.

Although the cytoarchitectonic maps of Brodmann and Campbell both describe M1 as a single homogeneous region, recent studies by Zilles and colleagues have proposed that area 4 should be subdivided into an anterior (4a) and a posterior (4p) portion based on cyto- and myeloarchitecture and receptor density (Geyer *et al.*, 1996) – figure 1.2. It was observed that area 4p contained a higher density of Nissl-stained neurones than area 4a. Brain imaging evidence suggests that there are separate representations of some digits in the two regions and there are tentative suggestions of functional differences between them, though these are poorly defined (Geyer *et al.* 1996, Nakada *et al.* 2000).

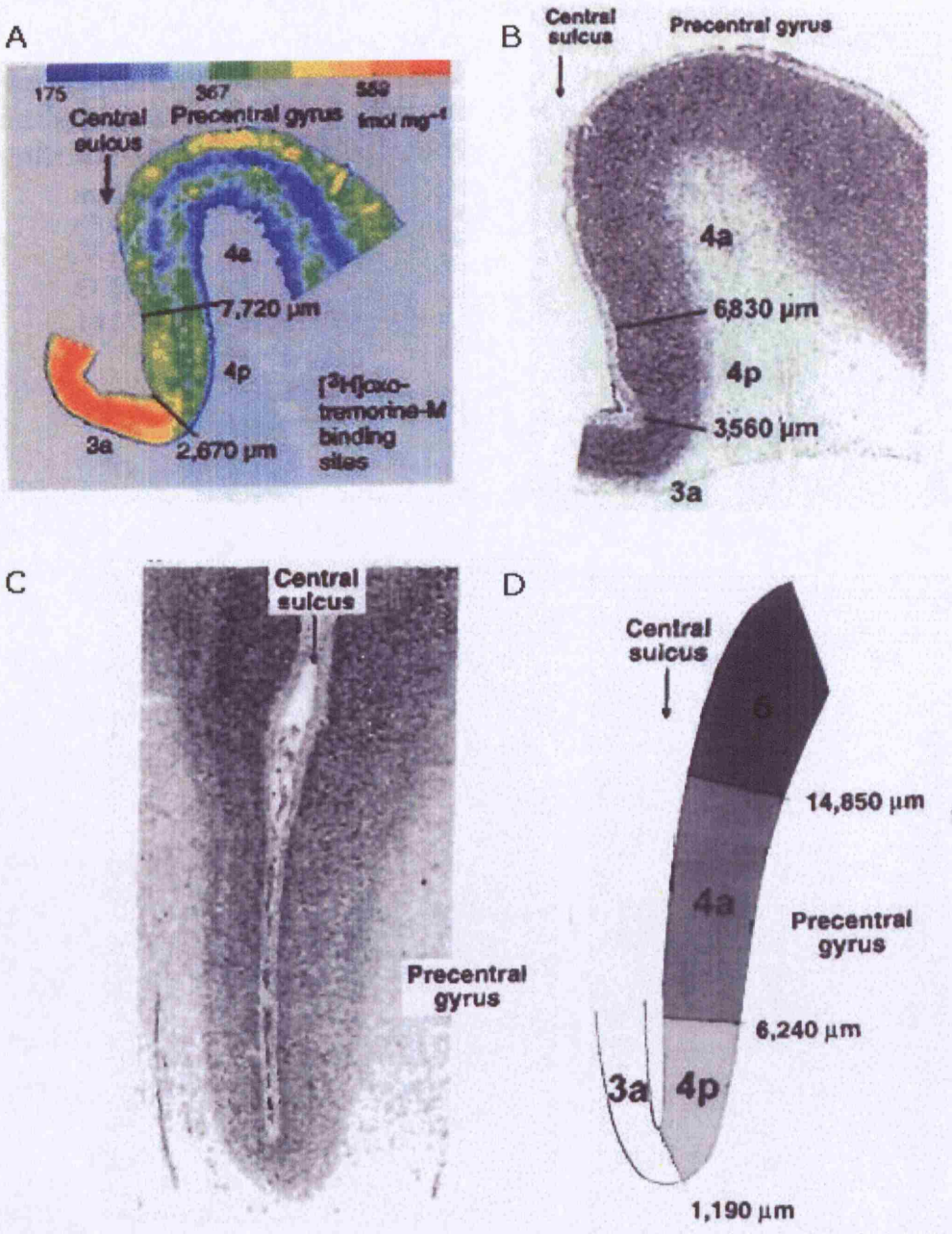

Figure 1.2: Areas 4a and 4p in the Primary Motor Cortex. Adapted from Geyer *et al.* (1996). Cytoarchitecture of area 4. (**A**) Contrast-enhanced, colour-coded autoradiograph of section through the precentral gyrus showing areas 4a and 4p according to the distribution of muscarinic M2 and M4 receptors. (**B**) Nissl-stained adjacent section: the dark lines represent positions of the borders between 3a/4p and 4p/4a, respectively. Border between 4a and 6 not shown. (**C**,**D**) Quantitative cytoarchitecture of area 4: **C** grey-level index image showing densities of neurons in a Nissl-stained section through pre- and postcentral gyrus, roughly half-way between interhemispheric and Sylvian fissures. (**D**) Corresponding borders between areas 3a/4p, 4p/4a and 4a/6.

The basic somatotopic organisation of M1 - different parts of the cerebral cortex control movements of different parts of the body, forming a motor map (Penfield and Boldrey 1937) as first proposed by Sherrington, Penfield and others – has been confirmed by functional neuroimaging studies (Grafton *et al.*1991, Rao *et al.* 1995). However, there is considerable overlap between the representations of different body parts, due to divergence of the cortical output of a particular area to multiple motor neuron pools and hence can activate multiple muscles and enable synergistic movements (Shinoda *et al.* 1981, Cheney *et al.* 1985). Sanes *et al.* (1995) were able to demonstrate the presence of overlapping and distributed activity during movement of fingers, wrist and arm, in healthy human subjects using functional magnetic resonance imaging (fMRI).

Information on the connectivity between motor areas comes mainly from studies of non-human primates, with little direct evidence to confirm whether the same patterns of connectivity hold in the human.

The major output of M1 is the corticospinal tract, consisting of descending projections from Betz cells and other layer V pyramidal cells (Ghosh and Porter, 1988). These cells are mainly excitatory and receive inputs from glutamatergic horizontal connection systems within M1 (Hess et al. 1994), the strength of which is probably influenced by GABA-ergic inhibitory interneurons (Hess and Donoghue 1994, Donoghue 1995, Hess *et al.* 1996).

In addition to these horizontal connections, M1 receives projections from areas of the primary somatosensory cortex (mostly from area 2), the secondary somatosensory cortex, the thalamus, as well as parietal areas 5 and 7b (Jones *et al.* 1978, Leichnetz 1986, Ghosh *et al.* 1987). Projections from the lateral and medial premotor areas (ventrolateral premotor, caudal dorsolateral premotor and supplementary motor areas) also terminate in M1 (Mukkassa & Strick 1987, Tokuno & Tanji 1993, Passingham 1993). Finally, there are transcallosal afferents from the contralateral M1 and premotor areas (Rouiller *et al.* 1994).

Single-neurone recordings in M1 in non-human primates have revealed the particular features and importance of this area in the execution and control of voluntary

movement. Within a particular corticospinal tract neurone, firing rate encodes various aspects of a motor task such as the amount of force generated in target muscles (Evarts 1968) or the direction of movement – with increased firing only occurring when movements are in a particular direction (Georgopoulos *et al.* 1982). However, as well as roughly representing movement features on an individual basis, these features are also more precisely encoded by population of neurons in M1 (Georgopoulos *et al.* 1984). Subsequent experiments suggest that the motor cortex represents movements in terms of the direction and velocity of the required movement, not on the basis of individual muscles (Ashe & Georgopoulos 1994, Kakei *et al* 1999).

1.2.3 Non-Primary Motor Areas

The original cytoarchitectonic maps of the human cortex differentiated between precentral and intermediate precentral cortex (Brodmann's areas 4 and 6 respectively). When Campbell identified that area (1905) he hypothesised that it was involved in higher aspects of motor control. Fulton proposed a functional distinction between 'primary' motor cortex (area 4) and 'premotor' cortex, lateral area 6, based on lesion studies (Fulton 1935). Area 6 on the medial wall was also designated a functionally distinct unit, the 'supplementary' motor area (Woolsey et al. 1952). Penfield and Rasmussen (1952) reported that they could elicit motor responses from:

"*an area from which bilaterally synergistic movements may be produced [...] called supplementary motor area to distinguish it from the classical sensorimotor and the second sensory and motor representation. This [...] area is comparatively small and is situated within the superior intermediate frontal regions within the longitudinal fissure.*"

Penfield & Rasmussen, 1952

A dominating idea at the time of Sherrington and Brodman was that the primary motor cortex constituted the main output of the motor system and other areas fed into this in order to produce the output to the spinal cord. The consequence of this arrangement would be that damage to any one of these areas would lead to a

permanent loss of that function. However, a lot of evidence, described in the next section on plasticity, suggests that this is not the case and that recovery of function can occur after damage. This and further physiological evidence have suggested a more distributed network for these areas with cortico-cortical connections and functional overlap which might provide the basis for functional reorganisation in the motor system after damage. Although the primary motor cortex has the majority of corticospinal output, there are also outputs from other motor areas such as the premotor cortex, the supplementary motor and cingulate motor areas (SMA and CMA). Their involvement following damage suggests a fair amount of degeneracy in the system: an ability of areas to take up other functions when required (Friston and Price 2003). Indeed, despite the fact that different subregions of the motor system contain neurons that tend to have particular firing patterns, the details of which are mentioned in the sections about each subregion, there are also neurones in each area with properties often found in other parts of the system. Thus, in primary motor areas there are visually sensitive neurones and in premotor areas there are force related ones (for a review, see Rizolatti et al. 2002). This mix of separate and overlapping functions might lead to a model where even transient damage to one area could be "compensated" by activity in other areas. The purpose of the work presented in this thesis is to examine that hypothesis with current techniques used in brain neurosciences.

The premotor area corresponds to area 6, but is divided into lateral and medial components, with the latter component being termed the supplementary motor area. Electrical stimulation of area 6 also produces motor effects, but these occur with higher intensities and/or longer duration stimulation than required to elicit responses via M1 (Wiesendanger 1981). Area 6 is also characterised by an agranular layer IV, as area 4, but lacks the high density of giant Betz cells in layer V, which are a specific landmark of area 4.

▪ 1.2.3.1 The Lateral Premotor Area

The lateral premotor area corresponds to the dorsal convexity of area 6 and is further subdivided into ventral and dorsal components each containing a dorsal and a rostral subdivision, according to studies in macaque brains (Barbas & Pandya 1987, Matelli *et al.* 1985). However, the homology between human and macaque premotor cortex is not clear (Figure 1.3 from Rizzolatti et al. 1998). In monkeys, the border between agranular premotor cortex and the granular prefrontal cortex is the arcuate sulcus. Based on studies of the neurodevelopmental branching of the precentral sulcus, the dorsal part of the premotor cortex can be divided into two parts: a caudal part, corresponding to monkey F2 and a dorsal part corresponding to monkey F7. Similarly the ventral part of human precentral gyrus is divided into a rostral part (BA44) and a caudal part (ventral area 6) corresponding to monkey F5 and F4 respectively. Human premotor cortex includes two further subdivisions: BA 45 and the dorsal agranular region caudal to the middle frontal gyrus (Rizzolatti *et al.* 1998).

The functional roles of the premotor areas were initially characterised in monkeys. Though these areas contribute fibres to the descending tracts and can be electrically stimulated to evoke motor responses, the neurons in premotor areas show different response profiles during the cueing and execution of movement than the majority of M1 neurons. By instructing a delay between the cueing and execution of movement in recording studies of monkeys, it has been possible to dissociate set-related activity that occurs during the delay, from the execution-related activity. Neurons that predominantly respond during the cue period are more commonly found in the premotor aeras than in M1. The converse is true for neurons responding mostly to the execution of the movement (Wise *et al.* 1986, Tanji & Kurata 1982, Weinrich *et al.* 1984).

The location, connectivity and functional specialisation of the dorsal and the ventral lateral premotor cortex are discussed below.

The dorsolateral premotor cortex (PMd) receives inputs from area 5 of the parietal cortex (Petrides & Pandya 1984). It is located within the rostral precentral gyrus and the caudal superior frontal gyrus (Rizzolatti et al. 1998). The PMd appears to be involved in action planning, response selection, movement preparation, and visual

guidance of motor responses, particularly when the actions are cued by arbitrary associations (Wise et al. 1996). Caudal PMd appears to be engaged in simpler, automatic motor tasks (Jueptner et al. 1997) whereas rostral PMd might be involved with more cognitive aspects of motor behaviour (Passingham 1997).

The ventrolateral premotor cortex (PMv) receives inputs from area 7 of the parietal cortex (Petrides & Pandya 1984). It is located ventral to the frontal eye fields and caudal to BA 44/45 although the extent of this area is not as well established (Grezes & Decety 2001). In the macaque this area has been implicated in visuomotor transformations during grasping and object-cued movements, as well as in action observation (Rizzolatti *et al.* 1998).

▪ 1.2.3.2 The Supplementary Motor Area

The supplementary motor area (SMA) is defined as mesial area 6. This area is subdivided into two further anatomically distinct regions on the basis of cytoarchitecture and neurochemistry (Zilles *et al.* 1995): the SMA-proper in the human corresponds to medial area F3 in macaque, and the pre-SMA in the human to medial area F6 in macaque. In the case of medial motor areas, there is a rough correspondence between cytoarchitectonic borders and gross anatomy. The border between M1 and SMA-proper is the VCP line (the vertical line passing the posterior commissure and perpendicular to the line between the anterior and posterior commissures (AC-PC)) and the border between SMA-proper and pre-SMA the VCA line (the line passing through the anterior commissure and perpendicular to the AC-PC line) (Vorobiev *et al.* 1998).

Through use of anterograde and retrograde tracing methods, the connectivity of this area has been well characterised (Wiesendanger *et al.* 1984). Inputs to the SMA originate in areas 5 and 7 of the parietal lobe, the secondary somatosensory cortex (SII), M1 and the lateral premotor area, as well as areas of the thalamus receiving inputs in turn from the basal ganglia (Weinrich *et al.* 1984). Efferents of the SMA project bilaterally to the lateral premotor area, M1 and the corresponding regions of SMA in the opposite hemisphere. The SMA also sends descending fibres to the corticospinal tract and to the red nucleus.

The SMA-proper appears to contain a somatotopic representation (Fink et al. 1997) and seems to be involved in simple motor tasks (Picard & Strick 2001), externally triggered movements (Jahanshahi *et al.* 1995, Jenkins *et al.* 1997), motor preparation and learnt sequences (Passingham 1996, Deiber *et al.* 1999).The pre-SMA appears to be involved in more cognitive aspects of motor control such as processing of movement cues, rather than response selection (Picard & Strick 2001).

- **1.2.3.3 The Cingulate Motor Areas**

The cingulate motor areas (CMA) consist of three subdivisions of BA24, located within the cingulate sulcus. In the monkey CMAs can be divided into rostral (CMAr, area24c), ventral (CMAv, area 23c) and dorsal (CMAd, area 24d) subregions (Picard & Strick). Although precise homologies between monkey and human CMAs have yet to be established, human CMAs can still be divided into a rostral (RCZ) and a caudal (CCZ) zone. Human imaging studies have shown that there may be a somatotopic representation of body parts along the cingulate sulcus and gyrus (Paus *et al.* 1993, Picard & Strick 1996). CCZ appears to be activated in simple motor tasks whereas more complex actions involving, for example, as conditional associations between cues and movements activated RCZ.

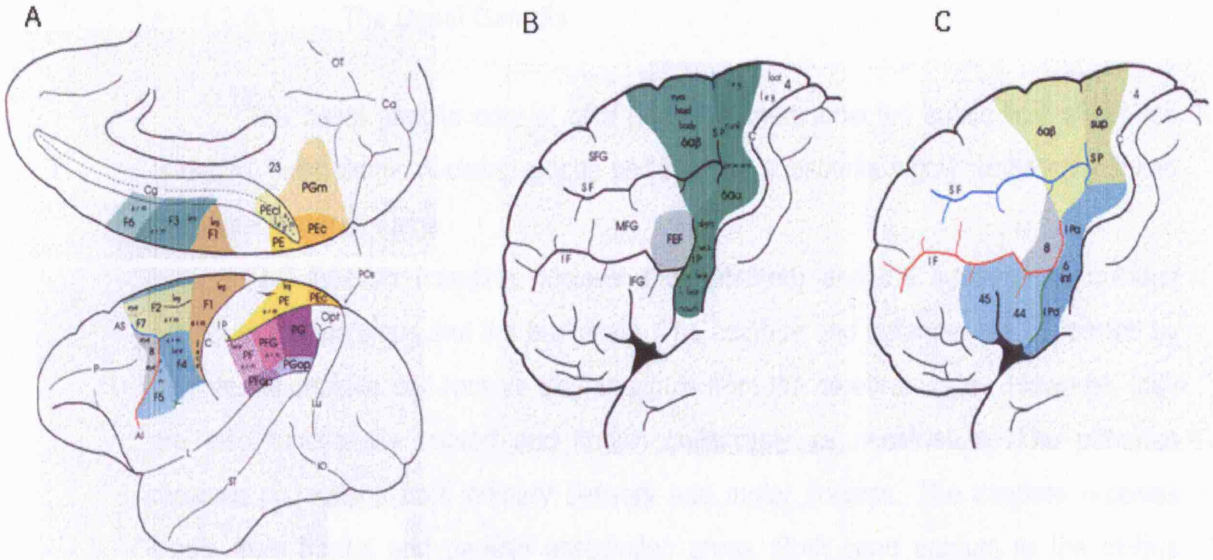

Figure 1.3: The Motor Network. Adapted from Rizzolatti *et al.* (1998) **(A)** Mesial and lateral views of the macaque brain showing the cytoarchitectonic parcellation of the agranular frontal cortex and of the posterior parietal cortex Rizzolatti *et al.* 1998). AG, annectant gyrus; C, central sulcus; Ca,, calcarine fissure; Cg, cingulate sulcus; IO, inferior occipital sulcus; L, lateral fissure; Lu, lunate sulcus; OT, occipitotemporal sulcus; P, principal sulcus; Pos, parieto-occipital sulcus; ST, superior temporal sulcus. (B) Lateral view of the human frontal lobe showing the cytoarchitectonic parcellation of Brodmann area 6 according to Vogt (Vogt & Vogt 1919) and the motor representations as determined by electrical surface stimulation by Foerster (1936). (C) Lateral view of human frontal lobe showing a parcellation of the motor cortex according to the proposed homologies with the monkey motor cortex (A). The homology is based on cytoarchitectonics, electrical stimulation and sulci embryology. The use of identical colours indicates homologous areas. SF, superior frontal sulcus (human); SP, superior precentral sulcus (human); AS, arcuate sulcus (monkey); IF, inferior frontal sulcus (human); IPa, acending branch of inferior precentral sulcus (human); IPd, descending branch of inferior precentral sulcus (human); AI, inferior limb of arcuate sulcus (monkey); IFG, inferior frontal gyrus; SFG, superior frontal gyrus.

1.2.4 Subcortical Motor Areas

▪ 1.2.4.1 The Basal Ganglia

The basal ganglia consist of a group of interconnected subcortical structures (striatum, subthalamic nucleus, globus pallidus and substantia nigra) reciprocally linked to cortical motor systems.

The striatum (caudate nucleus and putamen) and the subthalamic nucleus project to the thalamus and the brainstem. The caudate and putamen are separated by the internal capsule and receive distinct inputs from the cerebral cortex. However, they are also functionally related and known collectively as neostriatum. The putamen receives projections from primary sensory and motor cortices. The caudate receives inputs from frontal and parietal association areas. Both send outputs to the globus pallidus and the substantia nigra pars reticulata.

The globus pallidus and the substantia nigra pars reticulata are the main output areas of the basal ganglia. The globus pallidus is divided into an internal and an external segment. The internal segment projects to the thalamus. The external segment projects to the subthalamic nucleus.

Damage to the basal ganglia results in abnormalities of movement (e.g. bradykinesia and tremor) and muscle tone (Crossman & Neary 1995).

▪ 1.2.4.2 The Thalamus

The thalamus consists of a number of nuclei which relay information to and from the cerebral cortex. The thalamus is divided into three main groups: anterior, medial and lateral. Further subdivisions exist within these groups. The most important nuclei for the motor system are located in the lateral nuclear group and have highly organised connections with cortical and subcortical motor areas (Jones 1987). The ventral lateral nucleus receives inputs from the contralateral dentate nucleus of the cerebellum (see below) and projects to motor cortical areas (Matelli & Luppino 1996, Matelli *et al.* 1989). The ventral anterior nucleus also projects to motor cortical areas but

receives direct connections from parts of the ipsilateral basal ganglia including the globus pallidus and substantia nigra (Jones 1987).

- **1.2.4.3 The Cerebellum**

The cerebellum has a three-layered cortex (the outer molecular layer, the intermediate Purkinje cell layer and the inner granular layer) surrounding a white matter core, which contains the cerebellar nuclei. Movement-related sensory information from muscle spindles, tendon organs, joint and cutaneous receptors, as well as from spinal interneurons is conveyed via the spinocerebellar tracts to the medial parts of cerebellar hemispheres.

The cerebellar hemispheres and middle part of the vermis, also referred to as the cerebrocerebellum, receive inputs from the cerebral cortex via the pontine nuclei. The majority of cortico-pontine tracts originate in the sensorimotor areas, such as primary motor and sensory cortices, SMA and premotor cortex (Allen & Tsukahara 1974). The cerebellar nuclei influence movement via excitatory projections to the spinal cord and via the ventrolateral thalamus (Asanuma et al. 1983) to the primary, supplementary and premotor cortices (Matelli & Luppino 1996). Recent studies have revealed the presence of distinct cerebello-thalamocortical circuits for motor processing in monkeys comprising cerebellar lobules IV-VI, distinct regions of the dentate nucleus & ventrolateral thalamus and M1. The cerebellum in monkeys also receives inputs from and projects to prefrontal and parietal cortices (Dum *et al.* 2002, Kelly & Strick 2003, Clower *et al.* 2004), suggesting a role in cognitive and visuospatial functions. The human cerebellum may also receive distinct subdivisions for motor and non-motor processes (Desmond *et al.* 1997).

Damage to the cerebellum causes deficits of coordinated movement such as ataxia, tremor, nystagmus and poor balance, underlying the role of the cerebellum in fine-tuning motor behaviour. The cerebellum has been implicated in accurate timing of movements (Sakai et al. 2000) and in motor learning (Doyon *et al.* 2003). The unique cellular architecture provides a suitable substrate for error based Hebbian learning mechanisms. Long term potentiation (LTP) and depression (LTD) have already been demonstrated at parallel fibre-Purkinje cell synapses in vitro (Crepel and Jaillard 2002).

1.2.5 Dorsolateral Prefrontal Cortex and the Fronto-Parietal Network

In addition to the motor system studied in this thesis, another site, the dorso-lateral prefrontal cortex, was tested to examine whether the general lessons learnt on the motor system, whose connections and physiology are well known, could be applied equally to that system. A brief review of the anatomy and function of this area are provided below.

The dorsal (or dorso-lateral) prefrontal cortex (DPFC or DLPFC) refers to areas 46 and the neighbouring region 46/9. It is located in the upper part of lateral surface of the prefrontal cortex. The homologue area of the human DLPFC in the monkey brain lies in and around the principal sulcus (Petrides & Pandya 1999). As is the case in other associative cortical areas, the DLPFC is characterised cytoarchitecturally by the presence of a well-developed granular layer IV, which makes it easily distinguishable from area 9. In terms of its anatomical connections, the DLPFC receives inputs from all posterior regions of associative cortex (Barbas, 2000; Miller & Cohen, 2001) including the lateral parietal cortex (Petrides & Pandya 1999). It is the only area that receives information from all sensory modalities (Passingham et al. in press).

The role of the DLPFC has been hard to identify, as this structure lies at an anterior point of rich convergence of many pathways both ventral and dorsal, emanating from the sensory modality areas (Young 1992). These anatomical connections indicate how this area is operational on very high-level cognitive functions. Physiological data has shown, for example, that many cells in the DLPFC fire when monkeys remember spatial locations and they are also active when integrating information about spatial location and reward (Kobayashi et al. 2002, Wallis & Miller 2003). Although the DLPFC projects neither to the primary motor cortex nor directly to the spinal cord, it has been characterised as important for the endogenous generation of action (Frith et al. 1991, Playford et al. 1992).

The DPFC is also important for working memory and short-term memory-guided behaviour. Lesion to this area in macaque monkeys impairs the ability to produce

delayed responses (Goldman et al., 1971). This was tested by requiring the monkeys to remember either of two visually presented spatial locations, and to act accordingly after a delay, during which a black screen was presented such that the monkeys could not see the targets (Goldman et al., 1971). The monkeys tested made substantially more errors after lesion in the DPFC. Based on this finding, the DPFC has been proposed as the neural substrate for working memory in macaque monkeys (Goldman-Rakic, 1992; Goldman-Rakic, 1995). In humans, this has been confirmed by a number of functional imaging (Cohen et al., 1997; D'Esposito et al., 1998; D'Esposito et al., 2000; Owen, 1997; Petrides, 2000; Rowe and Passingham, 2001; Rowe et al., 2000) as well as transcranial magnetic stimulation studies (Mottaghy et al., 2003; Mull and Seyal, 2001). Rowe and Passingham (2001) specifically highlight the area's function in the selection of action based on working memory.

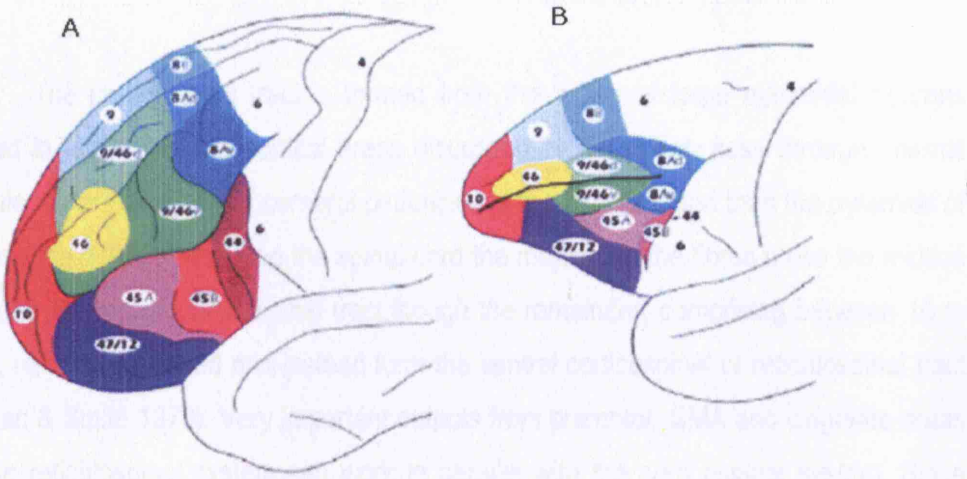

Figure 1.4: The Dorso-Lateral Prefrontal Areas. Adapted from Petrides & Pandya (1999) **(A)** The dorsal prefrontal cortex (DPFC) refers to areas 46 and the neighbouring region 46/9. **(B)** The homologue of this area in the macaque monkey brain lies in and around the principal sulcus.

1.2.6 Descending Tracts of the Motor System

The cortical components of the motor system are capable of influencing the activity of spinal motor neurons via direct (thought the corticospinal tract) or indirect

pathways (via brainstem structures). The tracts involved in voluntary control of the upper limb are outlined below.

- **1.2.6.1 The Corticospinal Tract**

The corticospinal projections form a direct pathway from cerebral cortex to the spinal motor neurons. The corticospinal fibres originate in motor and sensory regions of the cortex. However, the primary motor cortex contributes the majority of fibres contained in this tract, other portions of the fibres originating in the lateral premotor, SMA and cingulate gyrus. The distribution of corticospinal neurons in these areas has been studied in monkeys using retrograde labelling (Dum & Strick 1991). These studies have shown that almost 50% of these projections come from M1, while SMA and cingulate motor areas each accounted for 20% and the lateral premotor areas approximately 10%.

The corticospinal tract is formed from the axons of large pyramidal neurons located in layer V of the cortical areas discussed above, which pass through internal capsule, before forming the cerebral peduncles of the forebrain and then the pyramids of the medulla. Prior to reaching the spinal cord the majority of the fibres cross the midline and form the lateral corticospinal tract,though the remainder, comprising between 10 to 30 %, remain uncrossed and instead form the ventral corticospinal or reticulospinal tract (Nathan & Smith 1973). Very important outputs from premotor, SMA and cingulate areas via the reticulospinal system can work in parallel with the corticospinal system. Since they receive bilateral inputs and outputs, they are thought to play some contribution in the recruitment of contralateral PMd when M1 has been lesioned (see Section 3 and Johansen-Berg *et al.* 2002, Lee *et al.* 2003). Figure 1.5 shows the CST. Corticospinal axons terminate at all layers of the grey matter of the spinal cord. The axons from the lateral corticospinal tract tend to synapse with either spinal motorneurons in the lateral motor nuclei of the ventral horn or on interneurons of the intermediate zone, and exert their influence on distal musculature. John Eccles, a neurophysiologist from Autralia was amongst the first to discover inhibitory pathways in the CNS. His work revealed that the

CST selectively inhibits certain motor neurones in order to enable fine voluntary movements and better motor control.

Most of the work on the reticulospinal systems originated with the studies of Lawrence and Kuypers (1968) on the "ventral brainstem" pathways. They examined the effects of stimulation and selective destruction of specific descending upper motor neuron pathways in the brainstem of monkeys. The axons of the ventral corticospinal tract terminate in more ventromedial portions of the spinal gray matter and are associated with control of both axial and proximal muscles: these muscles are most often indirectly excited by axons which synapse onto interneurons (Schieber 1999). Direct monosynaptic projections are most commonly from axons originating in the hand area and may therefore have a role in dexterous movements of distal muscles (Muir and Lemon 1983).

The importance of the corticospinal tract to fine movements of the hand is illustrated by correlations between digital dexterity in different primates and with both the cross-sectional size of the pyramidal tract and the number of cortico-motoneuronal connections (Kuypers 1968, Heffner & Masterton 1983).

Damage to the corticospinal tract can result in initial hemiparesis, though depending on the location of the damage, almost complete recovery of motor function is possible due to the presence of intact parallel descending outputs of the motor system (Fries *et al.* 1993).

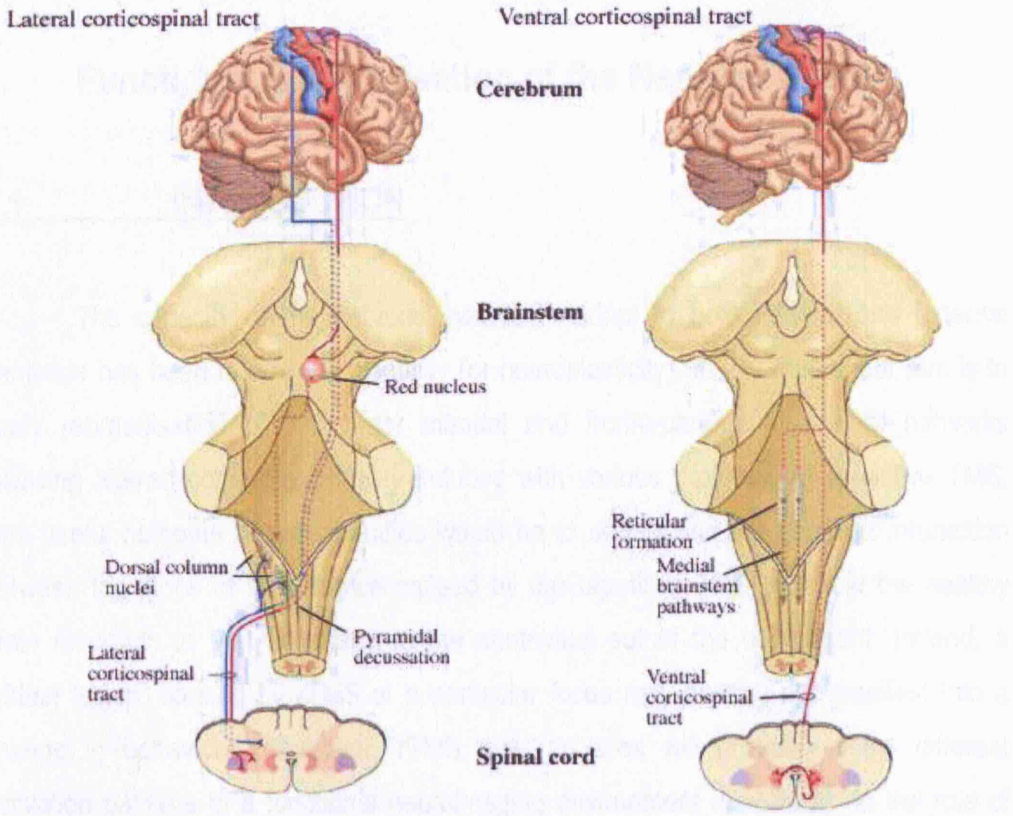

Figure 1.5: Decending Tracts of the Motor System. Diagram of the descending tracts of the motor system **(A)** The lateral corticospinal tract (CST) **(B)** The ventral CST (from http://www.utdallas.edu/~tres/integ).

1.3 CONCLUSION

This section provided a summary of the anatomy of the motor system and the key areas that were studied in the subsequent chapters presented in this thesis. Indeed the function of these areas cannot be fully understood without any knowledge about their structure and more importantly their connectivity. Anatomical connectivity between areas suggests that they might form part of a network sub serving a particular function. How activity in these areas is modified if their excitability is changed depends on which areas they are connected to.

2. Functional Reorganisation of the Nervous System

2.1 INTRODUCTION

The capacity of the nervous system to adapt to both internal and external demands has been referred to plasticity (or neuroplasticity). In this thesis, our aim is to study reorganisation of the motor manual and fronto-parietal attentional networks following altered cortical excitability induced with various protocols of repetitive TMS. One useful outcome of these studies would be to understand the complex interaction between the types of interference caused by the repetitive TMS and how the healthy brain responds to that according to the contextual set of the experiment. Indeed, a "virtual lesion" caused by rTMS at a particular focus may or may not manifest into a change in behaviour. Moreover, rTMS over an area will probably show different activation patterns in a functional neuroimaging environment depending on the role of that area and on its connectivity with other brain regions, which may or may not be co-activated in a given task.

This section gives a brief overview of the extensive literature on reorganisation with examples ranging from motor learning, to animal models of reorganisation in stroke recovery. Initial studies on plasticity have examined local changes in connectivity after peripheral or central damage. Work by Merzenich, Sanes and Donoghue has examined the mechanisms of such plasticity in animal brains (Recanzone et al. 1992, 1993, Donoghue et al. 1992). Following these studies, Randolph Nudo and colleagues have explored local reorganisation of the sensorimotor system and the effects of treatment in awake behaving non-human primates (Nudo and Milliken 1996). However, all this work has focused on local changes, hypothesised to be mediated by GABAergic interneuronal connections following focal ischaemic lesions (Qu et al. 1998a,b; Redecker et al. 2000). More and more attention is now turning into how can the findings from these experiments be extrapolated to the human brain. As a consequence, network responses to injury, involving areas remote from the initial site of damage, need to be studied. Although the exact mechanisms of rTMS are still poorly understood, reorganisation

following rTMS might share some common mechanisms with the studies that will be mentioned below. This overview will allow us to make some interesting parallels between the findings described in the experimental chapters of this thesis and the findings described previously in the "plasticity" literature.

2.2 PROCESSES MEDIATING MOTOR RECOVERY

The nervous system has an extremely flexible structural and functional organization. This flexibility allows it to transform itself as well as to analyse, store, and react to changes inside the body or to stimuli outside the body. This kind of complex adaptation, called plasticity, seems to occur not only during development but also in adult life, although the mechanisms may be different (Kemp *et al.* 2000, Bimonte *et al.* 2002, Crutcher, 2002).

Starting with studies on learning and memory, the mechanisms of how the nervous system is modulated to meet the requirements of a changing environment have been studied for over 50 years. However, the observation that patients with cerebrovascular accidents could spontaneously recover some brain function has led to the idea that similar mechanisms or processes as learning & memory might underlie recovery. Many pathways in the brain have the potential to reorganize ranging from the motor system, which we are giving examples of in this thesis to the very well studied visual system (Gilbert C 1996, 1998).

The capacity of the brain to reorganize through skill learning or following a lesion may be *adaptive*, and lead to an improvement of performance of the system, or *maladaptive*, which could result in poorer outcome following the change. This latter phenomenon is exemplified in task-specific dystonia, such as in "pianist's cramp". It is believed that increased demand of sensory-motor integration in professional musicians may harbour the risk of unwanted change. Thus, faulty practice may result in unwanted cortical rearrangement and set the stage for motor control problems such as overuse syndrome and focal dystonias (Pujol *et al.* 2000, Pascual-Leone *et al.* 2001). In this case, the obligatory link between plasticity and adaptation may be altogether incorrect. Instead it may be more appropriate to think of plasticity as the consequence of activation

of a given neural pathway. In this framework the potential separation between brain changes and behavioral desirability becomes clear.

Several processes may contribute to recovery of motor function after stroke. First, resolution of pathological changes may allow for recovery at the cellular level. Second, development of compensatory movement strategies may enable "recovery" of certain motor functions, although subtle differences in movement kinematics would then exist. Finally, undamaged regions in the sensorimotor network may take over function of damaged or disconnected areas.

2.3 EXAMPLES OF REORGANISATION IN HEALTH AND DISEASE

In recent years, it has become increasingly clear that representational networks in the adult brain are capable of extensive reorganization. The brain does not only undergo reorganization but it is constantly reorganizing (Fuster 1995, Kaas 1997). This is an intrinsic property of the human nervous system that allows it not only to grow and develop but also to adapt to changes in the environment. For example, in normal humans and animals, cortical reorganization occurs with learning new motor skills (Karni *et al.* 1995), as well as learning of auditory (Recanzone *et al.* 1993) and visual (Walsh *et al.* 1998) tasks. Similarly, recovery of motor function after a lesion is associated with changes in motor cortical representations (Cramer & Bastings 2000). Undamaged areas and pathways in the brain are thought to "take over" the functions of damaged regions (Chollet et al. 1991, Weiller et al. 1992).

Motor plasticity is possible as the motor system is organized as a distributed network. Although different motor areas are functionally specialized, a concept defined as functional segregation (Friston 2002) there is nevertheless substantial functional overlap between them. In addition, the dense interconnections between motor areas and the presence of parallel descending motor pathways (see anatomy section1) allow for the possibility of intact regions of the network compensating for the loss of damaged or disconnected areas.

A need to better characterize brain plasticity and reorganization has led to a number of observations, which have been described following cortical lesions both in animal experiments and in patients suffering from stroke. These have allowed tentative models of recovery to be drawn.

Reorganisation following a focal lesion can occur in regions adjacent to the lesion (or disrupted area), in undamaged areas of the lesioned hemisphere, or in areas in the intact hemisphere.

2.3.1 Reorganisation in Areas Adjacent to the Lesion

The primary sensory and motor areas are organized somatotopically: they can be thought of as containing representational "maps" of the sensory-motor apparatus of the body. Injury to an area may involve local reorganization of these maps.

The ability for local cortical reorganization to occur as a result of altered input has been extensively investigated in the somatosensory cortex after peripheral damage in monkeys (Kaas 1991, Merzenich & Jenkins 1993). Amputation or deafferentation of a digit or a limb results in immediate silencing of the area of somatosensory cortex that previously represented the affected body part. Over time, however, the cortical region begins to respond to sensory inputs from body parts represented by adjacent areas of cortex (Figure 2.1). Similar phenomena occur in humans after amputation (Kew et al. 1994) and may provide an explanation for some features of phantom limb sensations (Flor *et al.* 1995, Knecht *et al.* 1995).

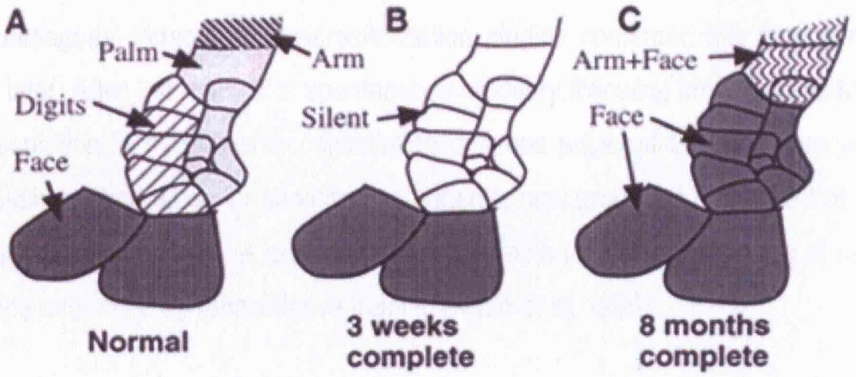

Figure 2.1: Somatosensory Reorganisation Following Deafferentation. Adapted from Jain et al. 1998: Processes mediating somatosensory reorganisation following deafferentation of digits in adult macaque brains **(A)** Normal somatotopy in hand and face region of area 3b **(B, C)** Reorganisation of hand region of cortex following different recovery periods.

This reorganization can occur very rapidly as shown in a study by Brasil-Neto and colleagues (1993). They studied the changes in excitability of the corticospinal system following an ischemic nerve block (Bier's block) at the human forearm. The authors found that ischemic nerve block led to increases in the amplitudes of MEPs in muscles immediately proximal to the anaesthetized forearm. Since they found no changes in H reflexes and peripheral M responses as well as no difference following transcranial electrical stimulation and spinal electrical stimulation they concluded that ischemic nerve block led to an increased excitability of the corticospinal system due to temporary removal of myelinated afferent input which led to disinhibition at the motor cortical level.

Local cortical reorganization can also occur after central damage. After lesions to the representation of a single digit in the somatosensory cortex of monkeys, adjacent areas of cortex begin responding to sensory stimulation of the affected digits (Doetsch *et al.* 1990)

Recovery of movement after damage to the motor system is also paralleled by local cortical reorganization in some cases. As early as 1950, a series of electrical stimulation studies by Glees & Cole demonstrated that following a lesion to the thumb area of M1, the motor representation of the thumb shifted to the adjacent intact cortex

(Glees & Cole 1950). Local remapping of the sensorimotor cortex had occurred. Nudo and colleagues' intractortical microstimulation studies confirmed this finding nearly 50 years later. After 3-4 months of spontaneous recovery following small lesions to the digit representation in motor cortex, stimulation of areas adjacent to the lesion, which had previously evoked elbow or shoulder movements, now produced movement of the digits (Nudo & Milliken, 1996). A subsequent study demonstrated that this local remapping could be enhanced by rehabilitative training (Nudo *et al.* 1996).

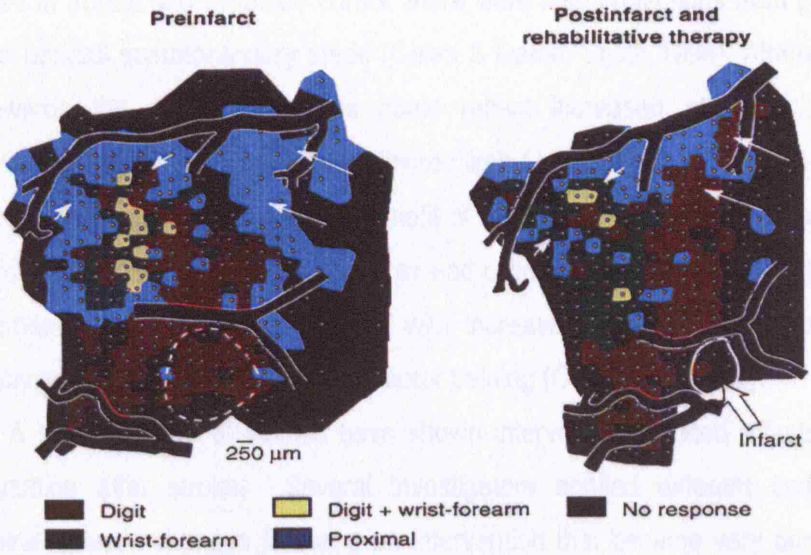

Figure 2.2: Sensorimotor Reorganisation Following M1 Lesion. Adapted from Nudo et al. 1996: Processes mediating reorganisation of hand representations in M1 in an adult squirrel monkey before infarct (left) and after focal ischemic infarct and rehabilitative training (right). Intra-cortical microelectrode stimulation techniques were used to define movements evoked by near-threshold electrical stimulation (<30μA). Small white circles represent each microelectrode penetration site. The dashed circle in the preinfarct map encompasses cortical territory targeted for ischemic infarct. The large white arrow in the postinfarct map indicates the infracted region. The reduction in size of the infracted zone is attributable to tissue necrosis during the rehabilitation period. Long thin arrows point to adjacent, undamaged cortex in which digit representations (red) appear to have invaded regions formerly occupied by representations of the elbow and shoulder (blue). Short thin arrows point to wrist-forearm representations (green) that appear to have invaded digit elbow and shoulder representations.

Evidence for local remapping in sensorimotor cortex has also been provided in humans using functional neuroimaging, with good examples coming from stroke patients. A PET study found a ventral shift of activation during hand movement into the face area of motor cortex in some patients after capsular stroke (Weiller *et al.* 1993). FMRI studies have found a posterior shift in the location of motor cortical activity following stroke (Pineiro *et al.* 2001) but also in multiple sclerosis (Lee et al. 2000).

A posterior shift could reflect increased recruitment of corticospinal projections from somatosensory areas. A study using retrograde transport of fluorescent tracers

from the spinal cord of the monkey found that although 70% of corticospinal projections originated in frontal and cingulate cortex, there were also projections from primary and posterior parietal somatosensory areas (Galea & Darian-Smith 1994). Alternatively, the shift towards the postcentral gyrus could reflect increased attention to sensory information to guide movement of the affected limb (Johansen-Berg & Matthews 2002). Further evidence about the functional benefit of reorganization of areas adjacent to the lesion comes from a recent study by Carey and colleagues, who were able to show an improvement in performance associated with increased activation of the peri-lesional area in chronic stroke patients following motor training (Carey et al. 2002).

A small number of studies have shown intervention-induced effects on motor reorganization after stroke. Several investigators applied different techniques to demonstrate plastic changes following an intervention that became very popular in the 1990s, such as constrained-induced movement therapy (CIMT) (Taub *et al.* 1993). Liepert and coworkers (1998, 2000) investigated the effects of this treatment intervention on a group of chronic stroke patients using TMS. Prior to CIMT, patients had high motor thresholds and small motor output maps in the lesioned hemisphere. After therapy, motor output maps in the affected hemisphere had increased by approximately 40% while those in the unaffected hemisphere did not change significantly. These changes located at the site of the lesion presumably reflect use-dependent changes probably mediated by GABA-dependent modulation of horizontal intracortical inhibitory circuits, which strongly influence the size of a representation area (Jacobs & Donoghue, 1991). The authors described similar findings concerning motor thresholds (they decreased in the affected hemisphere) and motor output maps following different physiotherapeutical interventions in early stroke patients (4-8 weeks after stroke) (Liepert *et al.* 2000). Their results suggest an important role of the motor areas adjacent to the lesioned site in mediating functional recovery following a lesion and hypothesized that these might be the sites targeted by physiotherapeutical interventions.

2.3.2 Reorganisation in Remote Areas

Plasticity can also occur over a wider spatial scale, with distinct intact brain regions seem to contribute to the functions lost by the lesioned cortex. This is made possible by the functional overlap within the sensorimotor network. An additional mechanism why this could take place is through the *functional integration* of different brain areas into a network of activity during the performance of a task (Friston 2002). As a consequence any change in activity within one area of the network will affect remote sites due to altered *effective connectivity.*

The role of non-primary motor areas in recovery of movement has been demonstrated in both human and animal studies. Premotor and supplementary motor areas have direct corticospinal projections (Section1). The altered role of SMA after primary motor cortex damage was demonstrated by a single cell recording study which recorded neuronal responses during a key pressing task before and after an M1 lesion in a monkey (Aizawa *et al.* 1991). Before the M1 lesion, SMA neurons were active during task learning but this activity disappeared once the task became overlearnt. However, after a lesion to M1 the SMA became active during task performance again. This is consistent with the finding from some brain imaging studies of differentially increased activation in the SMA in recovered stroke patients (Cramer *et al.* 1997, Weiller *et al.* 1993). Altered SMA activity could reflect increased recruitment of non-primary motor corticospinal projections, or it could reflect the need to "relearn" previously automatic or effortless movements after damage. An alternative hypothesis of this reorganization would be that in the healthy state, the SMA activity initially contributed to the movement, however, as learning progressed, its function was replaced by motor cortex activity. In the absence of the motor cortex due to a lesion, this can no longer occur.

A number of functional neuroimaging studies involving patients recovering from stroke have shown that recovery seems to be subserved not simply by one particular area but by a widespread network of areas which include activation of the motor areas contralateral to the lesioned site (Chollet *et al.* 1991). These areas include frontal and parietal cortices, as well as the basal ganglia and cerebellum suggesting multiple processes of reorganization ranging from physiological changes in activity and

disinhibition of brain areas following a lesion in the cortex, to involvement of cognitive processes of attention and learning in order to regain function. The additional areas recruited during recovery of motor cortical stroke are in accordance with the finding that various brain areas are devoted to motor control and motor activity (Rizzolatti *et al.* 1998). There is a convergence of results from many studies on stroke patients that have found ipsilesional premotor cortex, SMA, anterior parts of the insula/frontal operculum and bilateral posterior parietal cortices to be more active compared to age-matched controls (Weiller *et al.* 1992, Cao *et al.* 1998, Dettmers *et al.* 1997, Seitz *et al.* 1998). These results suggest that sensorimotor functions are represented in extended, variable, probably parallel processing, bilateral networks (Weiller & Rijntjes 1999).

Although stroke patients usually show stronger activations of their motor network during task performance, this might not represent recovery but might be an epiphenomenon reflecting a greater effort to perform the movement (Price *et al.* 1994) or a lack of disinhibition (Liepert *et al.* 2000, Witte 1998). The former concern was addressed in a study by Ward and colleagues (figure 2.3) (2003) in which patients' acitivity patterns relating to motor recovery were assessed on a task that they could perform independent of their amount of disability induced by the stroke. They were able to show that functional recovery over time was better associated with a decrease in the activity of the additional motor areas recruited at the first stages following the stroke (Ward et al. 2003). Further studies, examining the role of non-primary motor areas in the functional recovery from stroke, were performed with TMS. This technique was used in patients suffering from motor cortical strokes by Johansen-Berg and coworkers (2002) to induce transient inactivation the dorsal premotor cortex, believed to play an important role in recovery as shown in animal models of stroke (Liu & Rouiller 1999). The authors were able to show that disruption of that area lead to significant worsening of behavioral performance in a simple reaction time task in chronic stroke patients, thus confirming the critical role of that area in recovery.

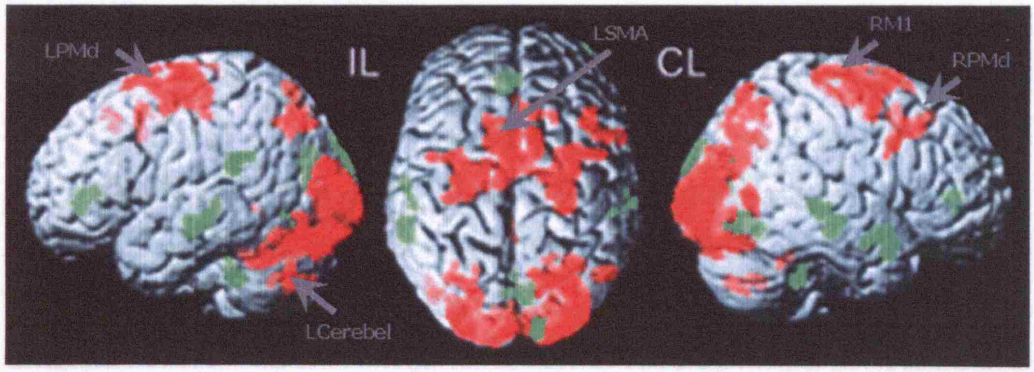

Figure 2.3: Reorganisation of the Human Motor System Following Stroke. Adapted from Ward *et al.* 2003: Results of single subject longitudinal analysis for linear changes in task-related brain activations over sessions as a function of recovery. The patient suffered a left-sided pontine infarct resulting in right hemiparesis. Results are surface rendered onto a canonical brain, with red areas representing recovery-related *decreases* in task-related activation across sessions, and green areas representing the equivalent recovery-related *increases*.

2.4 CONCLUSION

The aim of this section was to provide a very broad overview of the extensive literature on reorganization of cortical brain regions in health and disease, so as to provide the theoretical framework on which we can base our results from the experiments described in the present thesis. The ability of the human brain to reorganize, even in adulthood, has lead to a wealth of studies to understand the different mechanisms with the vision to interfere with the processes mediating recovery. The purpose of the subsequent chapters is to provide more insights into how the healthy brain reorganizes following and artificially induced change in excitability of particular brain regions with rTMS. With the theoretical frameworks set out in this section in mind, we will hopefully be able to draw some parallels with previous experience on reorganization from animal experiments and human studies in healthy individuals and patients.

3. Methods for Inducing and Studying Changes in Cortical Excitability of the Human Sensorimotor System

3.1 INTRODUCTION

Imaging techniques can provide powerful methods for mapping human brain function in vivo. They have been used extensively in the study of brain activation patterns associated with various types of tasks in healthy volunteers or in patients. In the same way as functional neuroimaging has been used to compare differences in brain activation patterns associated with motor tasks in healthy subjects versus stroke, we set out to compare how changing the excitability of specific regions in the motor system of healthy individuals using repetitive TMS affected the normal pattern of neuronal activity in that region.

All the experiments in this thesis use repetitive transcranial magnetic stimulation (rTMS) with various stimulation parameters to study changes in brain activation patterns or basic motor behaviour under different task conditions. The general principles of TMS and the methods of measuring and modulating cortical excitability are described in the first section of this chapter. In the second section, we provide a discussion of the physiological basis of neural activity followed by details of how various neuroimaging techniques detect these physiological markers of neuronal activity. The two neuroimaging techniques used in this thesis, namely positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are outlined and a summary of the neurimaging data analysis methods used in this thesis are provided.

3.2 TRANSCRANIAL MAGNETIC STIMULATION (TMS)

TMS has been successfully used to study cortical excitability, plasticity, connectivity and functional organisation of the human brain (see Siebner & Rothwell 2003 for a review). The main advantage of TMS is that it is a painless and non-invasive tool that stimulates the cortex via the intact scalp at a high temporal resolution (~1ms).

3.2.1 General Principles

Faraday demonstrated that an electrical current flowing through a coil of wire generates a time-varying magnetic field, that is proportional to the strength of the current. This in turn can induce an electric current in a second coil of wire. TMS is based on this principle, known as electromagnetic induction (see Walsh & Cowey for a review): it utilises a time-varying magnetic field to induce electrical currents in conducting tissue (Jalinous 1991). In a TMS experiment the second coil is replaced by the human cortex. A stimulating coil is held over the scalp and a large (8,000 A) very rapidly changing (200 μs) current is passed through the coil, generating a changing magnetic field perpendicular to the coil. This field can reach up to 2.5T under the surface of the coil but decays by the square of the distance from the coil (figure 3.1). The magnetic field penetrates through the skull and then induces eddy currents parallel to the coil in the brain (Mills 1999, Walsh & Rushworth 1999). It is therefore the induced current that stimulates cortical tissue and not the magnetic field.

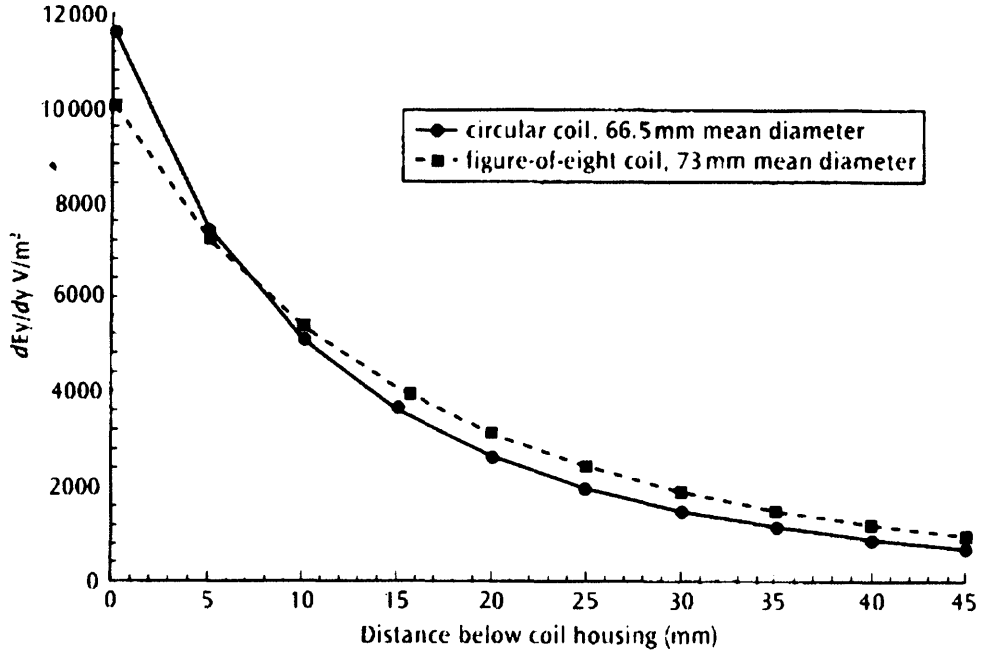

Figure 3.1: Decrease of Electric Field Strength With Distance From the TMS Coil.

Adapted from Barker (in Pascual-Leone *et al.* 2002): The decrease of the spatial derivative of electric field

with depth below the housing of the 66.5 mm mean diameter circular coil and 73 mm mean diameter figure-of-eight coil.

Unlike electrical fields, magnetic fields are hardly attenuated as they pass through the skull. The size of the induced tissue current is dependent on the rate of change of the current in the stimulating coil. Following Lenz's law, the induced current will flow in opposite direction to the current in the stimulating coil. If the right stimulation parameters are selected, the electrical current induced can depolarise cortical neurons and generate action potentials (AP) (Rothwell *et al.*1999).

3.2.2 Stimulation Area

The currents induced by TMS flow parallel to the surface of the brain (Tofts 1990) in the opposite direction to the electrical current in the stimulating coil. Thus, the underlying brain tissue stimulated by TMS will probably depend on the way the cortex is folded and the orientation of the coil. Since neurons are preferentially activated by longitudinal rather than transverse currents, TMS preferentially activates pyramidal tract neurones trans-synaptically, running parallel to the longitudinal axis of the TMS coil (Amassian *et al.* 1992b). Small alterations in the orientation of the TMS coil on the scalp can therefore alter the efficacy of stimulation and result in excitation of different populations of cortical neurons (Amassian *et al.* 1992a).

The accuracy of the site of TMS stimulation depends on a number of parameters that are discussed here. With a circular coil, the induced current of stimulation is maximal in an annulus under the coil. The larger the coil, the larger the annulus of current, and the less focal the site of stimulation. In addition, the size of the coil influences the depth of penetration of the field in the brain. The focality of stimulation can be changed by altering the geometry of the coil. For example, the electric current induced by coils wound in a figure-of-eight shape is approximately twice as strong under the junction region of the loop as it is at each edge (see figure 3.2). Fibres oriented parallel to the junction of the coil will therefore be stimulated best at the bifurcation points (Maccabee *et al.* 1993). This allows for a more focal activation of the brain. With a

standard figure-of-eight coil the stimulated region covers approximately 4 cm and is therefore likely to stimulate a corresponding region in the brain.

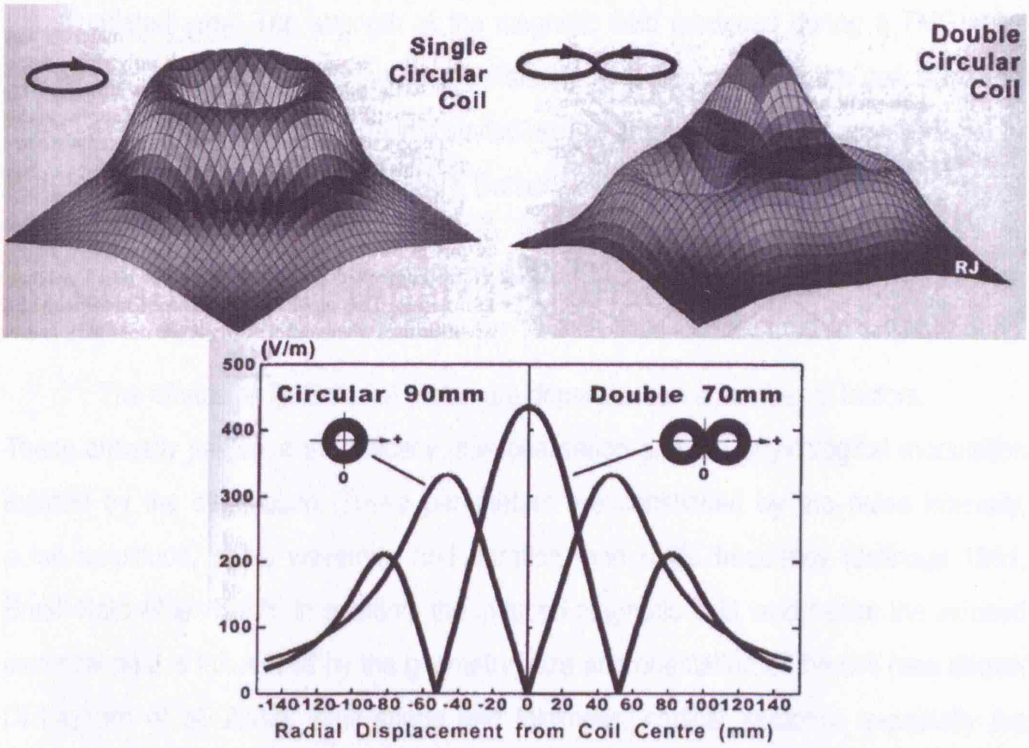

Figure 3.2: Magnetic Field Strengths with Different TMS Coils. Adapted from Barker (in Pascual-Leone *et al.* 2002): Three-dimensional representations of the induced electric fields are shown for two coil types: circular 90 mm and double 70 mm coils. The circular coil has a larger area of effect, with the induced field being zero directly under its centre. The double figure-of-eight coil has a more focal area of effect.

Whilst the extent of the stimulated tissue depends on the specifics of coil geometry and position, stimulation of cortical and subcortical tissue can in principle be achieved 2-3 cm away from the coil (Harmer *et al.* 2001). Stimulation at higher intensities and with larger coils may even be more effective, with the downside of possible activation of adjacent brain regions.

The spatial resolution of TMS had been estimated in the range of 1cm. This does not imply that TMS stimulates a 1cm region exclusively but rather that differential effects of stimulation of the cortex can occur with coil position changes of 0.5-1cm (Brasil-Neto *et al.* 1992, Wassermann *et al.* 1992). Under most circumstances, however, TMS is likely to stimulate a wider range of tissue. As mentioned earlier, stimulation

intensity is one factor determining the spread of stimulation. Other factors, which may be hard to measure, include the excitability of neighbouring tissue and the connectivity of the stimulated area. The strength of the magnetic field produced during a TMS pulse decreases progressively with increasing distance from the centre of the coil, such that effective direct stimulation occurs in a limited area of cortex close to the centre of the coil in a figure-of-eight coil (Roth et al. 1991, Barker 1999).

3.2.3 Studying the motor system with TMS

The effects of TMS on the cortex are dependent on a number of factors. These critically influence the efficacy, the localisation and the physiological modulation exerted by the stimulation. These parameters are constituted by the pulse intensity, pulse amplitude, pulse waveform and duration, and pulse frequency (Jalinous 1991, Brasil-Neto *et al.* 1992). In addition, the induced magnetic field, and hence the induced electrical field is influenced by the geometry, size and orientation of the coil (see above, Di Lazzaro *et al.* 2002), skull shape and thickness, cortical anatomy, especially the orientation of the stimulated neurons (Rothwell 1997), the coil-tissue distance (Jalinous 1991) and the temperature of the conducting medium (Maccabee *et al.* 1998).

TMS can be used to assess the excitability of the motor system directly by measuring motor evoked potentials (MEP) or indirectly by functional neuroimaging (such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), electroencephalography (EEG)) and measures of motor behaviour (e.g. reaction times, response or movement accuracy, motor learning, force generation, movement velocity). Electrophysiological techniques provide a direct, objective measure of cortical and/or corticospinal excitability. These methods are described below.

▪ 3.2.3.1 Generation of a Motor Evoked Potential (MEP) and Motor Threshold (MT)

In contrast to transcranial electric stimulation (TES), which predominantly activates vertical corticospinal axons directly (Rothwell 1997), TMS modulates corticospinal neurons (CSN) primarily via activation of their horizontally oriented afferents. Direct stimulation of CSN only occurs at very high intensities of stimulation (Rothwell 1997). This is explained by the direction of the cortically induced currents with respect to the orientation of cortical neurons. Stimulation is maximal in those neuronal elements located in the plane of the coil.

Effective stimulation of the motor cortex with TMS is prone to induce compound motor action potentials in the contralateral limb. These lead to the generation of motor evoked potentials (MEP), whose amplitude can be measured with surface electromyographic recordings (EMG) (figure 3.3). Furthermore TMS of the motor cortex elicits a series of descending positive waves with a periodicity of approximately 600Hz and a duration of 10ms.Given the order of their occurrence, the first wave, termed direct (D) wave, is followed by a series of indirect (I) waves which appear at regular intervals of about 1.5ms, starting at about 1.5ms after the earliest D wave. Work by Amassian and colleagues in animals and humans have provided most of the evidence concerning the nature of these waves. Their studies showed that the initial D wave is produced by direct excitation of corticospinal fibres, presumably at the axon hillock or in the first or second internode, while the later I waves are probably generated by interposed synapses as well as additional recruitment of corticospinal neurons.

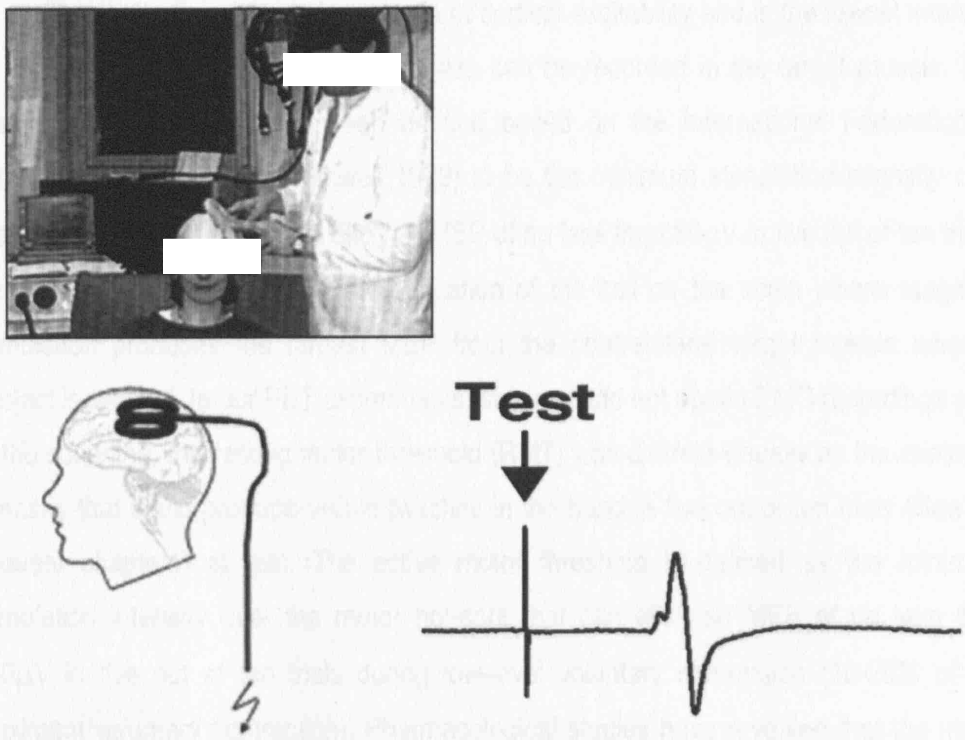

Figure 3.3: Generation of a Single Motor Evoked Potential. TMS of the primary motor area at a sufficient intensity leads to the generation of a motor evoked potential (right) whose amplitude can be quantified with electromyography (EMG).

Different orientations and intensities of TMS can recruit different proportions of D and I waves. Because D waves are the result of stimulation at the axon of pyramidal tract neurons, they are not greatly influenced by changes in the level of excitability within the grey matter of the cerebral cortex (Di Lazzaro *et al.* 1999). However, the situation is quite different for I waves, which are highly sensitive to the level of cortical excitability at the time of the stimulus is given because they are synaptically induced. The descending corticospinal volleys produced by TMS release excitatory postsynaptic potentials (EPSPs) at the motor neurone, and these develop over a period of several milliseconds, depending on the number of I waves that have been evoked. This explains why the onset latency, amplitude and threshold of an MEP can change according to whether subjects are relaxed or active, for example (Day *et al.* 1987, Thompson *et al.* 1987).

The motor threshold is a measure of cortical excitability and is the lowest intensity of TMS at which a MEP of a defined size can be recorded in the target muscle. The resting motor threshold has been defined based on the International Federation of Clinical Neurophysiology (Rothwell 1999) to be the minimum stimulation intensity over the "motor hot spot" that could elicit an MEP of no less than 50μV in five out of ten trials. The "motor hot spot" is the optimal location of the coil on the scalp where magnetic stimulation produces the largest MEP from the contralateral target muscle when a subject is relaxed. In our PET experiments, since we did not obtain EMG recordings prior to the scanning, the resting motor threshold (RMT) was defined visually as the minimum intensity that could produce visible twitches in the hand in five out of ten trials (See the relevant chapters) at rest. The active motor threshold is defined as the minimum stimulation intensity over the motor hot-spot that can elicit an MEP of no less than 200μV in five out of ten trials during low-level voluntary contraction (10-20% of the maximum voluntary contraction). Pharmacological studies have revealed that the motor threshold seems to be affected with the administration of drugs acting on the neuronal membrane excitability. Drugs acting on the synaptic transmission seem to have no effect on the motor threshold, suggesting that it is primarily affected by the membrane properties of the corticospinal system (Ziemann *et al.* 1995, 1996a 1996b).

▪ 3.2.3.2 Probing intracortical excitability

The intracortical inhibitory and facilitatory circuits can be tested by the paired pulse technique. This technique initially involved stimulating the primary motor cortex with two consecutive pulses at suprathreshold intensities separated by an interstimulus interval (ISI) ranging between 50 and 200 ms (Claus *et al.* 1992, Valls-Sole *et al.* 1992). These pulses are applied through the same stimulation coil placed over the motor "hot spot". This technique is now mainly used to test the long interval intracortical inhibition, which is a good measure of $GABA_B$ inhibitory activity as revealed in a study by Ziemann and colleagues (1996).

Kujirai *et al.* developed a new paired pulse stimulation method (Kujirai *et al.* 1993) in which a subthreshold conditioning pulse precedes a suprathreshold test pulse

(which produces a clear electromyographic response in a contralateral muscle) by only a couple of ms (1-16ms). This paradigm allows for the short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) to be tested by measuring the effect of the first, subthreshold, conditioning stimulus (CS) on the size of the myographic response of the second, suprathreshold, test stimulus. At ISIs of 1-5 ms the response of the TS is inhibited, whereas at ISIs above 6ms it is facilitated (figure 3.4). The amount of inhibition or facilitation induced using this paradigm depends on the intensity of the conditioning stimulus (Kujirai *et al.* 1993, Ziemann *et al.* 1996, Di Lazzaro *et al.* 1998). The intensity of the test pulse is usually adjusted to produce an MEP of 1mV in peak-to-peak amplitude in the target muscle, so that the test MEP is big enough to show good inhibition but not too big to have a ceiling effect after facilitation.

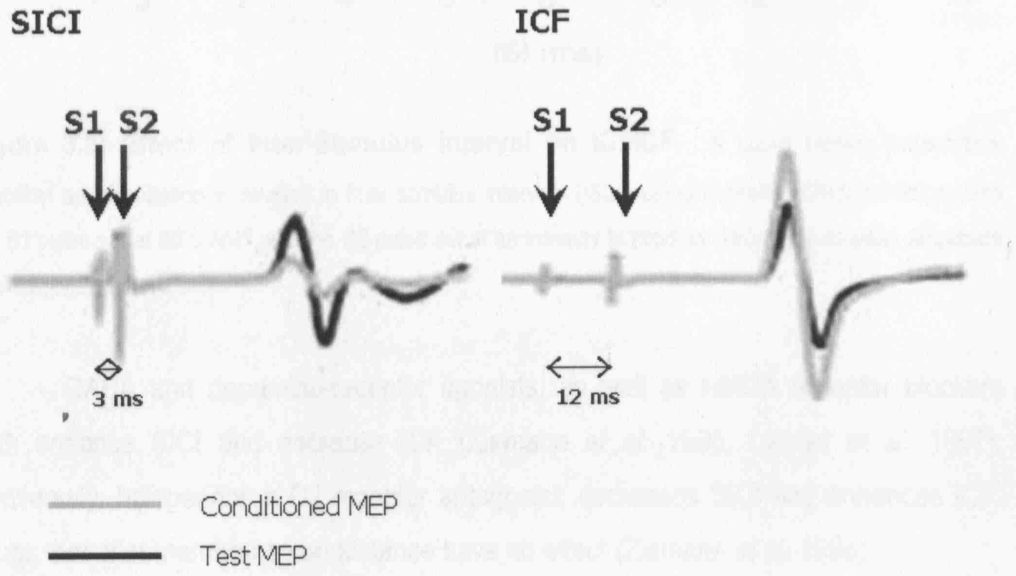

Figure 3.4: Intracortical Inhibition and Faciliatation Tested with Paired Pulse TMS.
Example of a paired-pulse conditioning paradigm over M1 with two inter-stimulus intervals (ISIs): 3ms leading to an inhibition of the conditioned MEP (testing short interval intracortical inhibition) and 12 ms leading to a facilitation of the conditioned MEP (testing intracortical facilitation). S1, the first TMS pulse is applied at various ISIs at *subthreshold* intensity (usually 80% active motor threshold AMT) to condition the muscle response elicited by S2, the second TMS pulse applied at an intensity to evoke a 1mV MEP.

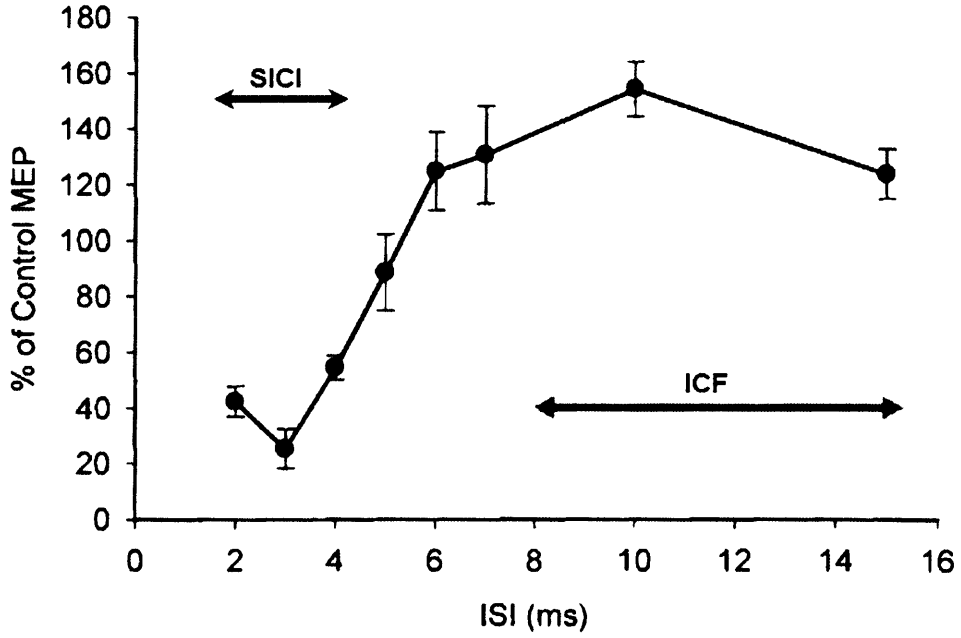

Figure 3.5: Effect of Inter-Stimulus Interval on ICI/ICF. A curve plotting intracortical inhibition and facilitation in relation to inter-stimulus intervals (ISIs), using Kujirai's (1993) paradigm with the S1 pulse set at 80% AMT and the S2 pulse set at an intensty to produce 1mV peak-to-peak amplitude when delivered on its own.

GABA and dopamine-receptor agonists, as well as NMDA receptor blockers both enhance SICI and decrease ICF (Ziemann *et al.* 1996, Liepert *et al.* 1997). Conversely, haloperidol, a D2 receptor antagonist, decreases SICI and enhances ICF. Drugs that alter membrane conductance have no effect (Ziemann *et al.* 1998).

Many studies have suggested that these TMS effects are cortical. There is evidence that the CS does not affect the size of the H-reflex (Kujirai *et al.* 1993) and that its effects are altered by cortical myoclonus (Brown *et al.* 1996). Further evidence came from epidural recordings from the cervical spinal cord in intractable pain patients (Di Lazzaro *et al.* 1998, 1999, 2002).The different dynamics between SICI and ICF imply that these effects are mediated by different intracortical structures within the motor system (Ziemann *et al.* 1996, Nakamura *et al.* 1997, Abbruzzese *et al.* 1999, Sanger *et al.* 2001).

▪ 3.2.3.3 Paired pulse and connectivity

Connectivity between interconnected areas of the motor system can be studied using a variation of the paired-pulse techniques described above. If one wants to study inputs into the motor cortex, the second stimulus S2 in paired pulse paradigm is applied over the motor hot spot for the hand area, for instance, at an intensity sufficient to evoke an MEP in the target muscle. The first stimulus S1 can then be applied over another (rather than the same) area of cortex at various time intervals beforehand. If S1 affects the amplitude of the response to S2, there is likely to be a functional connection between the two cortical sites. However, before this can be assumed, it is important to verify that the effect on S2 is at a cortical rather than at a spinal level. Cracco *et al.* (1989) first tested interhemispheric connectivity by demonstrating TMS-evoked transcallosal responses. Ferbert et al. showed that conditioning stimuli delivered to the motor cortex of one hemisphere using a figure-of-eight coil caused inhibition of MEPs elicited by a test stimulus delivered 5-6ms later using a separate figure-of-eight coil over the contralateral motor cortex. Other studies have also demonstrated the presence of inhibitory interactions between homologous primary motor areas by inducing silent periods in active muscles ipsilateral to the site of stimulation (Meyer *et al.* 1995, 1998). Ugawa *et al.* (1993) and Hanajima *et al.* (2001) reported the presence of subtle facilitatory interactions between motor cortices at short interstimulus intervals (4-5ms) followed by late inhibition. These studies demonstrated that stimulation of M1 led to immediate effects on distributed brain areas.

Civardi et al (2001) demonstrated intra-hemispheric effects of TMS by showing that at an ISI of 6ms stimulation of the premotor cortex (defined functionally to be 3cm anterior of the motor "hot spot") with sub- and suprathreshold conditioning stimuli lead to inhibition and facilitation of responses from the ipsilateral motor hot-spot respectively. Since then, Mochizuki *et al.* (2004) have demonstrated inhibition of test MEPs by applying a subthreshold conditioning stimulus some 8ms prior to the test stimulus over the contralateral PMd area.

▪ 3.2.3.4 Distinguishing cortical from spinal effects after rTMS of the motor cortex

In the chapters presented in this thesis, we did not perform any control experiments to check that the effects we are describing are cortical. There is, however, extensive literature which describes the physiological results obtained following rTMS conditioning of the motor cortex and that has been able to confirm that these rTMS effects are cortical. In this section, we briefly describe the methods used to reach that conclusion. Four main techniques are available to distinguish cortical from spinal effects; however, none of them are sufficient on their own to provide a definite answer.

1. The most direct method is to record the descending volleys evoked by TMS directly before and after a conditioning intervention, and has been performed mainly in studies by Di Lazzaro and colleagues (1998a,b, 1999, 2002, 2005)

2. Another method is to compare the motor responses evoked by TMS with the responses evoked by TES (Di Lazzaro *et al.* 1999): this is because TES at low intensities of stimulation can produce a relatively pure D wave that is unaffected by changes in cortical excitability. Therefore, if the response to TES is unchanged by an intervention while the response to TMS is, this suggests that there may have been a change of cortical excitability.

3. A third method, which is similar in its principle to the ones mentioned above, is to use transmastoid stimulation of the descending tracts in order to evoke a single descending volley that would be unaffected by changes in cortical excitability (Taylor et al. 2002).

4. Finally, a method that has been used extensively by the Hallett group in their studies of paired associative stimulation (which involves pairing peripheral median nerve stimulation with TMS of the motor cortex) involves the use of H reflexes or F waves to measure the excitability of spinal reflex pathways and motoneurones. Again,

the idea is that if these remain unchanged by an intervention whilst responses to TMS differ, it is likely that the intervention has altered cortical rather than spinal excitability.

▪ 3.2.3.5 Repetitive TMS: A mechanism to induce changes in excitability in the motor system

Instead of delivering single or paired pulse TMS in order to probe cortical excitability in the human brain, TMS can be delivered repetitively to produce repeated stimulation similar to that used in animal preparations for plasticity-induction (Froc *et al.* 2000, 2005). If applied repetitively, TMS at a frequency of 0.3Hz or more can produce changes in cortical excitability outlasting the stimulation period, depending on the intensity, duration, pattern and frequency of stimulation (Pascual-Leone *et al.* 1994, Chen *et al.* 1997, Muellbacher *et al.* 2000, Romeo *et al.* 2000, Fierro *et al.* 2001, Modugno *et al.* 2001, Tsuji & Rothwell 2002, Gilio *et al.* 2003, Schambra *et al.* 2003).

Repetitive TMS (rTMS) typically induces decreases or increases in MEP amplitude after low-frequency (<1Hz) and high-frequency (>1Hz) stimulation, respectively.

❖ Local effects of rTMS in the sensorimotor system:

The main interest in using rTMS is that it can produce effects that outlast the stimulation period for minutes or hours. Low-frequency stimulation of M1 at suprathreshold intensities has been shown to reduce corticospinal excitability as measured by an increased corticomotor resting thresholds (Muellbacher *et al.* 2000, Fitzgerald *et al.* 2002), decrease MEP size and reduce the slope of MEP recruitment curves in relaxed hand muscles (Wassermann *et al.* 1996, Chen *et al.* 1997, Muellbacher *et al.* 2000, Maeda *et al.* 2000, Touge *et al.* 2001). These effects are attenuated with the use of subthreshold intensities so that longer stimulation periods are required in order to produce the same after-effects (Maeda *et al.* 2000, Gerschlager *et al.* 2001, Munchau *et al.* 2002, Schambra *et al.* 2003). This dose dependency has been illustrated in several studies (Wassermann *et al.* 1998). For example, 15 min of 1Hz rTMS at an intensity of 105% RMT can only evoke subtle changes in corticospinal

excitability whilst higher intensities (110-150% RMT) can decrease contralateral MEPs for a period of up to 15min after the stimulation (Schambra *et al.* 2003).

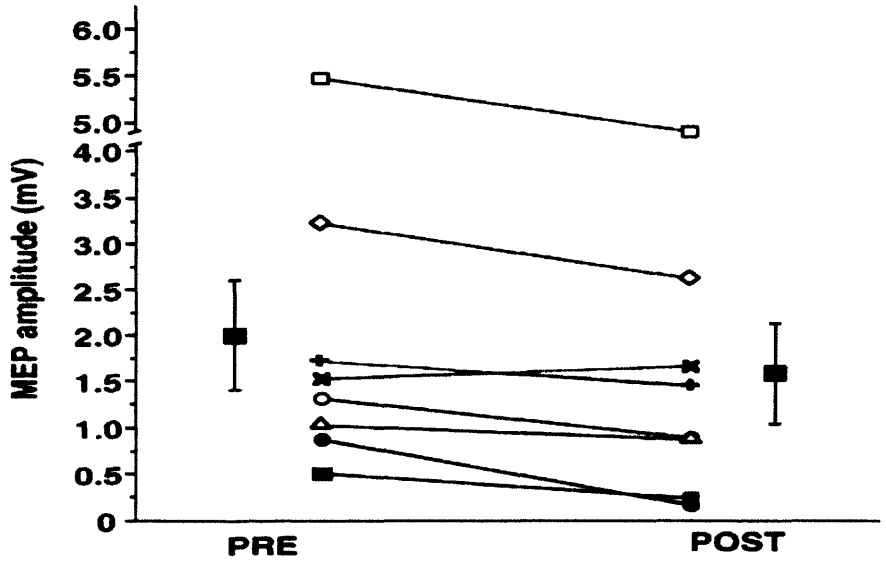

Figure 3.6: Effects of Low-Frequency rTMS on MEP Size. Depression of the motor cortex excitability as measured by single MEPs following low-frequency (0.9Hz) rTMS for 15 minutes (810 pulses) at suprathreshold (115%) intensity. The decrease in cortical excitability lasted for at least 15 minutes after the end of the stimulation. The authors postulated that a similar mechanism to long-term depression (LTD) might underlie this decerase (Chen *et al.* 1997).

Facilitatory effects have usually been induced with high-frequency repetitive stimulation protocols (>5-10Hz), which tend to increase resting excitability (Chen *et al.* 1997, Maeda *et al.* 2000). When applied at suprathreshold intensities, even relatively short trains of 500ms at 150% RMT can increase the MEP size for about 3 min after the stimulation (Pascual-Leone *et al.* 1994). Similarly suprathreshold 5Hz rTMS over the motor cortex and have shown that it increases corticospinal exitability as measured by an increase in MEP size (Berardelli *et al.* 1998). As with low-frequency rTMS, stimulation, these effects are attenuated with the use of subthreshold intensities, such that longer periods of stimulation are required. Peinemann *et al.* (2004) have shown that long trains of 5Hz rTMS could produce a facilitatory effect over the motor cortex that lasted up to 1hour after the end of the stimulation period (see figure 3.7).

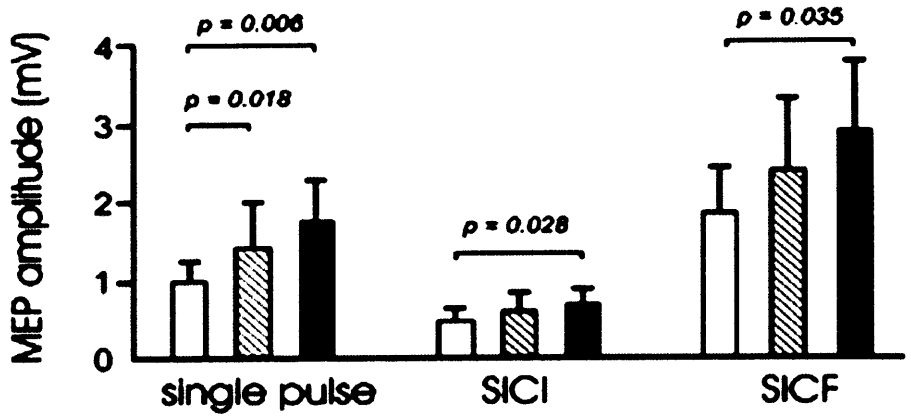

Figure 3.7: Effects of High-Frequency (5Hz) rTMS on MEP size and ICI/ICF. Effects of subthreshold 5Hz rTMS (1800 pulses) over the left M1 on corticospinal excitability as measured by the MEP amplitude of the right FDI muscle. Each column represents mean amplitude of MEPs for 8 healthy volunteers before (white columns) and after rTMS (hashed and black columns). MEP amplitudes are shown separately for single-pulse TMS, short-latency intracortical inhibition (SICI) and intracortical facilitation (SICF).

RTMS at subthreshold intensities has been used in the chapters presented in this thesis in order to keep the area stimulated as focal as possible by avoiding spread of stimulation to adjacent cortical areas and to rule out after-effects of rTMS caused by re-afferent sensory input that could be induced with suprathreshold intensities.

❖ **Remote effects of rTMS in the sensorimotor system:**

i. **Effects measured physiologically**

An interesting feature of rTMS is the induction of effects remote from the site of stimulation. In a study by Gilio *et al.* (2003), subthreshold low-frequency rTMS at 1Hz for 15 min over M1 reduced interhemispheric inhibition in the contralateral side with a concomitant increase in that side's corticospinal excitability. Using a series of control experiments, the authors suggest that these effects were probably mediated by cortico-cortical projections rather than cortico-spinal mechanisms or reafferent feedack, as might have been the case in a similar study performed with suprathreshold 1Hz rTMS

(Schambra *et al.* 2003). RTMS to the primary motor cortex results in a range of lasting changes in the excitability of distributed brain areas (measured in the contralateral motor cortex); the direction of the changes differs between the local and distributed effects. Although the exact mechanisms of interhemispheric effects of TMS are still poorly understood, rTMS to the primary motor cortex results in a range of lasting changes in the excitability of distributed brain areas mediated via direct cortico-cortical (Di Lazarro *et al.* 1999) or cortico-subcortico-cortical pathways (Gerloff *et al.* 1998).

RTMS over other regions within the motor system can produce after effects that can be measured in M1. Studies have shown that modulating the excitability of the premotor cortex with rTMS can induce more enduring changes in the excitability of the ipsilateral M1. Subthreshold 1Hz rTMS over the left PMd can decrease corticospinal excitability of M1 as measured by a reduction of MEP size, and alter the time course of SICI and ICF (Gerschlagter *et al.* 2001, Munchau *et al.* 2002). Conversely, subthreshold 5Hz TMS over the left PMd has been shown to increase corticospinal excitability for a period that outlasts the stimulation period (Rizzo *et al.* 2003). The direction of distributed changes in cortical excitability therefore appears to be frequency-dependent and the direction of effects on MEP amplitude appears to be similar for PMd and M1 stimulation. However, the mechanisms mediating these effects may not be similar, as will be shown in the subsequent chapters of this thesis. This is exemplified with studies looking at other measures of cortico-cortical excitability such as the paired-pulse technique. Using this technique, studies have shown that subthreshold 1Hz rTMS to PMd can increase ICF at an ISI of 7ms for up to one hour (Munchau *et al.* 2002), whereas subthreshold 5Hz rTMS decreases it (Rizzo *et al.* 2003). However, 1Hz rTMS over M1 has been shown to only decrease ICF and have no effect on ICI (Romero *et al.* 2002), whereas a recent study showed that 5Hz rTMS over M1 induced an increase in ICF and a decrease in SICI (Peinemann *et al.* 2004).

When two sessions of 1Hz rTMS are delivered to the left PMd on consecutive days, the effects on motor excitability have a longer duration (Baumer *et al.* 2003) suggesting that the distributed effects of premotor stimulation are not restricted to an immediate modulation of motor excitability.

In addition to the local and remote changes in corticospinal excitability induced by rTMS, studies have also reported changes in the responsiveness of the stimulated area to inputs coming from interconnected sensory afferents. For example, 1Hz rTMS reduces the amplitude of the Long Latency Stretch Reflex (LLSR) (Tsuji & Rothwell 2002). This may reflect decreased excitability of corticospinal projections or reduced sensitivity of M1 to sensory afferents i.e. to cortico-cortical inputs. Enomoto *et al.* (2001) have reported a decrease in the peripherally evoked SEP after 1Hz rTMS over M1. Knecht *et al.* (2003) have even reported a decrease in the performance of a tactile discrimination task with 1Hz rTMS to M1. Finally, Satow *et al.* (2003) found that 0.9Hz rTMS over the primary somatosensory cortex (S1) increased the thresholds for detecting tactile stimuli but did not change SEP amplitude or performance on a two point discrimination task. These studies suggest that 1Hz rTMS reduces the responsiveness of the somatosensory system to inputs coming from other areas. Conversely, high frequency (5Hz) rTMS seems to have the opposite effect on SEPs and tactile discrimination when delivered to S1 (Ragert et al. 2004). Ragert et al. (2004) showed that 5Hz rTMS to S1 increases the excitability of M1 and improves tactile discrimination suggesting that this frequency leads to an increase in the responsiveness to inputs of the area stimulated.

ii. Effects of rTMS on behaviour

TMS and rTMS can be used to improve or worsen motor performance in one of two ways: acutely (with single TMS puses or short trains of high-frequency rTMS) or by producing a transient conditioning (with prolonged trains of rTMS). All the experiments described in this these have used the latter technique, which is known to alter the excitability of the stimulated area for a period of time (see above) and can provide a useful model of recovery (see Siebner & Rothwell 2003 for a review). Day et al. (1989) first demonstrated a delay in the reaction times (RTs) of a simple cued wrist flexion paradigm of up to 150ms with no change in the pattern of muscle activity following stimulation of M1 with TMS.

Acute disruptive effects of single pulse suprathreshold TMS over the premotor cortex were demonstrated in a series of simple and choice reaction time tasks by

Schluter *et al.* (1998). The authors showed that TMS did not affect performance in simple RT tasks. However, TMS to the left premotor cortex at short, but not long, cue-stimulus intervals increased RTs in both right and left-handed responses in a choice RT task whereas TMS to the right PMd delayed RTs for left handed responses. This study revealed an asymmetry in premotor contribution to task performance and showed that disruption of performance might be dependent on the level of task-difficulty. Using a similar paradigm, Johansen-Berg et al. (2002) revealed the functional relevance of the right PMd in motor recovery following left motor cortical lesions by showing a delay in simple reaction times following TMS over the right premotor area in patients suffering from stroke but not in age-matched control subjects.

Prolonged trains of rTMS can be used to alter motor behaviour by inducing lasting changes in the responsiveness of the stimulated cortex and connected areas. However, task performance may fail to be affected by TMS or rTMS for various resons. These include: a) the stimulated area may not be uniquely involved in a particular aspect of the task being tested; b) other areas may compensate for the TMS induced disruption; c) the rTMS may not be a large enough insult to disrupt the function of that area in the task. These factors complicate the interpretation of null results. Despite the well-documented effects of 1Hz rTMS on the excitability of the motor cortex (see above), no impairment of manual motor control by 1Hz rTMS has been convincingly demonstrated during simple motor tasks such as paced fist clench (Pascual-Leone *et al.* 1998), finger tapping (Wassermann *et al.* 1996, Chen *et al.* 1997), maintenance of tonic contraction (Strens *et al.* 2002), peak force and acceleration during finger pinch (Muellbacher *et al.* 2000).

Recent work has revealed effects of rTMS on more demanding motor behaviour or in motor learning. Subthreshold 1Hz rTMS to M1 decreased contralateral finger tapping rates (Jancke *et al.* 2003). Similarly, Schlaghecken *et al.* (2003) showed that subthreshold 1Hz rTMS to left motor and premotor cortices increased RTs in a masked priming task. These studies suggest that the motor system may be able to compensate more easily for changes in cortical excitability during simple tasks than during more demanding motor behaviour. Studies have shown that suprathresold 1Hz rTMS can impair early consolidation of task-specific motor learning (Muellbacher *et al.* 2002,

Baraduc *et al.* 2004). Baraduc *et al.* (2004) showed that although motor learning of a simple ballistic movement task could be impaired with rTMS, learning of a dynamic force field was not affected.

In a study by Chouinard et al. (2005), rTMS showed disruption in more subtle measures of motor and premotor functions, in the anticipation of forces during a precision grip task. The authors used two experiments, in which subjects used precision grip to lift different weights in a series of trials both before and after a 15min session of 1Hz rTMS. When rTMS was given to M1, subjects were disrupted in their ability to scale forces based on information acquired through previous lifts. However, in the experiment in which cues could predict which of two weights subjects had to lift, disruption in performance was obtained with stimulation of the PMd.

Additional evidence of disruptive rTMS effects on the motor system comes from the study by Strens *et al.* (2003). In that study, 5Hz rTMS was used to disrupt M1 ipsilateral and contralateral to the moving index finger during the performance of a finger force-tapping task. This study revealed the functional relevance of the ipsilateral M1 in maintaining task performance following altered cortical excitability of contralateral left M1 in healthy individuals during right index finger movmement.

Improvements in task performance have come from experiments revealing "paradoxical facilitation" of the motor cortex opposite to the one stimulated by rTMS (Kobayashi *et al.* 2004). 1Hz rTMS over the left M1 led to an improvement in performance of a sequential key-pressing task with the hand ipsilateral to the stimulated hemisphere, and no change in performance with the hand contralateral to the site of stimulation. The authors suggest that this may be due to a release in transcallosal inhibition imposed by the stimulated hemisphere. There is evidence of increased excitability of the motor cortex contralateral to left M1 stimulated with 1Hz rTMS (Gilio et al. 2003), which might be a mechanism by which this paradoxical facilitation of performance of the left hand could occur.

iii. Effects of rTMS measured with functional neuroimaging

Functional neuroimaging allows the visualisation of TMS effects on

synaptic activity throughout the brain, both at rest and during task performance. The strength of combining these techniques is that they allow us to see the remote changes induced when rTMS is applied over an area during various types of tasks in which one can vary the cognitive demand in a measurable way. However, the regional cerebral blood flow (rCBF) acquired with positron emission tomography (PET) and the blood oxygen level dependent (BOLD) signal obtained with functional magnetic resonance imaging (fMRI) provide only indirect measures of neuronal activity.

RTMS delivered to the premotor and motor cortices can alter the excitability of distributed brain areas as mentioned in previous sections. One of the earliest PET imaging experiments to study the effects of rTMS over the motor cortex showed increased rCBF in the left M1 (the site of stimulation) following suprathreshold 1Hz rTMS that slowly decreased in magnitude after cessation of stimulation (Fox *et al.* 1997). Remote effects of 1Hz rTMS included increases in rCBF in the ipsilateral sensory and premotor areas and contralateral SMA that correlated positively with the rCBF changes at the site of stimulation. Negative correlations were seen in the contralateral M1. As with the effects on corticospinal excitability measured electrophysiologically, these results may have reflected a mixture of an rTMS effect and an effect coming from re-afferent feedback due to repeated hand movements induced by suprathreshold stimuli. Later studies set out to investigate the neural correlates of increased or decreased cortical excitability induced by paired-pulse TMS using PET (Strafella & Paus, 2001). They showed that decreased MEP size following inhibitory ISIs (3ms) of paired-pulse stimulation correlated with rCBF increases in the stimulated M1 area, the ipsilateral premotor cortex and contralateral M1. Conversely, increased MEP size caused by facilitatory ISIs (12ms) correlated positively with an increase in rCBF at the stimulation site only (M1), thus revealing possible separate networks for SICI and ICF in the motor cortex. However, it might be argued that the correlations between rCBF and physiology tested in that experiment might have told us more about the effects on the test pulse than on whether excitatory or inhibitory connections had been stimulated. Indeed, the networks activated by the conditioning pulse are always the same. What is varying is the timing of the test pulse which determines which effects are assessed. Examining the effects of intensity (sub- or suprathreshold) following 1Hz rTMS to M1, Speer et al.

(2003) reported increases in rCBF at the site of stimulation (M1), contralateral cerebellum and bilateral subcortical structures. In their study, subthreshold stimulation at 1Hz did not evoke a significant increase in rCBF whereas suprathreshold rTMS did. Similarly, Okabe et al. (2003) failed to reveal any changes in rCBF at the site of stimulation (M1) during subthreshold 1Hz rTMS but reported remote effects such as increased synaptic activity in the cerebellum and decreased synaptic activity in the contralateral M1, SMA, premotor and parietal regions. Using short trains of subthreshold rTMS at 1-5Hz, Siebner *et al.* (2001) showed frequency-dependent changes in rCBF at the site of stimulation (M1). Chouinard *et al.* (2003) examined site-specific effects of subthreshold rTMS at 1Hz to M1 and PMd. Simultaneous TMS/PET scanning allowed for changes in rCBF to be corelated to the amount of inhibition, measured by a decrease in MEP size, induced by 1Hz rTMS. Both sites of stimulation led to widespread changes in rCBF. 1Hz rTMS over M1 led to positive correlations with decreased MEP size in the contralateral M1, ipsilateral cerebellum, cingulate motor area and subcortical structures. A simple comparison of rCBF before and after rTMS demonstrated a trend towards increased rCBF at the site of stimulation and the ipsilateral premotor cortex. Following subthreshold 1Hz rTMS to the left PMd, widespread positive correlations with decreased MEP amplitude were seen bilaterally in the ventral premotor areas, cingulate motor areas, subcortical structures and a range of prefrontal and parietal regions. The stimulated area showed no change whereas decreased correlations with decreased MEP amplitude were found in the left M1 and PMd. The authors infer that areas showing parallel changes in rCBF with MEP amplitude are likely to be anatomically connected to the site of stimulation, as suggested by the macaque literature. Interestingly, although 1Hz rTMS to the motor and premotor areas had similar effects on MEP amplitudes, there was very little overlap in the areas where rCBF changes were observed. This observation will be discussed in the subsequent chapters.

▪ 3.2.3.6 Possible mechanisms of action of 5Hz rTMS

In most of the studies presented in this thesis,5Hz rTMS was used to condition areas of the motor system prior to any scanning and performance of motor tasks. Although there is little evidence of the exact effect of 5Hz rTMS on the brain, Di Lazarro and colleagues provided evidence for the cortical origin of the effects of that particular frequency of rTMS conditioning by direct recording of the corticospinal volleys evoked by single pulse TMS in conscious human subjects who had received an implanted epidural stimulator for the control of pain (Di Lazzaro et al. 2002a,b). They found that suprathreshold 5Hz stimulation of the motor cortex is accompanied by a gradual increase in the size and number of descending corticospinal volleys evoked by each TMS pulse that parallels the increase in the MEP (Di Lazzaro et al. 2002a). Subthreshold 5Hz rTMS (50 total stimuli at AMT) had no effect on MEPs but reduced SICI (Di Lazzaro et al. 2002b). Previous studies with epidural recordings had shown that this MEP suppression was accompanied by a reduction in the size and number of the later (I3, I4) I waves (Di Lazzaro et al. 1998). After 5Hz rTMS this effect was abolished consistent with the hypothesis that reduced SICI was the cause of the effects observed in the motor cortex (Di Lazzaro et al. 2002b).

A study by Touge *et al.* (2001) has hypothesised that rTMS conditioning effects might be due to altered synaptic efficacy following a change in the excitability of cortical neurones rather than being due to a change in synaptic connections to pyramidal cells. This hypothesis came from the observation that the conditioning effects of rTMS were only apparent subjects were tested at rest and they disappeared when MEPs recorded from test stimuli were given during voluntary contraction.

3.3 INTRODUCTION TO FUNCTIONAL NEUROIMAGING METHODS

The purpose of this section is to provide a suitable background to the neuroimaging methods used and discussed in this thesis. The physiological basis of neural activity is discussed followed by details of how various neuroimaging techniques detect these physiological markers of neuronal activity.

3.3.1 Physiological Processes Involved In Brain Activation

Brain activation relies on two major processes: neuronal firing and synaptic transmission. These involve various electrical and metabolic changes that are discussed below.

▪ 3.3.1.1 Neuronal firing

In a resting state, i.e. when no impulses are conducted, neurons' axon membrane is relatively impermeable to ions. The balance of intracellular and extracellular ionic concentrations leads to the generation of the resting membrane potential. In this state, there is a preponderance of potassium ions (K+) in the intracellular environment and a higher concentration of sodium ions (Na+) in the extracellular environment. The resting membrane is relatively permeable to K+ ions; hence, there will be an outward flux of these ions down their concentration gradient, limited by the electrical potential difference generated by this efflux of positive charge. The resting membrane is relatively impermeable to Na+ ions, so only a small influx of Na+ can occur. Both the K+ and Na+ fluxes are counter-balanced by an ATPdriven Na+-K+ exchange pump. This energy-dependent pump exchanges three Na+ ions from intracellular solution for two K+ ions from the extracellular solution, hence maintaining the ionic gradients.

Conduction of an electrical impulse along the nerve axon requires generation of an *action potential.* Arrival of a wave of depolarising voltage causes voltage-gated Na+ channels in the axon membrane to open, allowing Na+ to enter the neuron down its concentration gradient. (See Figure 3.10) This influx of positive charge

causes further depolarization and, consequently, the activation of additional voltage-gated Na+ channels in a positive feedback loop. This feedback mechanism causes a massive influx of Na+. However, this influx is gradually halted by the inactivation of the voltage-gated Na+ channels; this reconfiguration of the protein structure of the channel blocks the passage of Na+ through the channel pore. From this stage, voltage-gated K+ fluxes predominate. The associated K+ channels have slower kinetics than the Na+ channels, leading to a delayed period of increased potassium conductance. This reverses the depolarization of the axon membrane, leading to a brief period of hyper-polarisation before these channels also deactivate, returning the axon to the resting membrane potential.

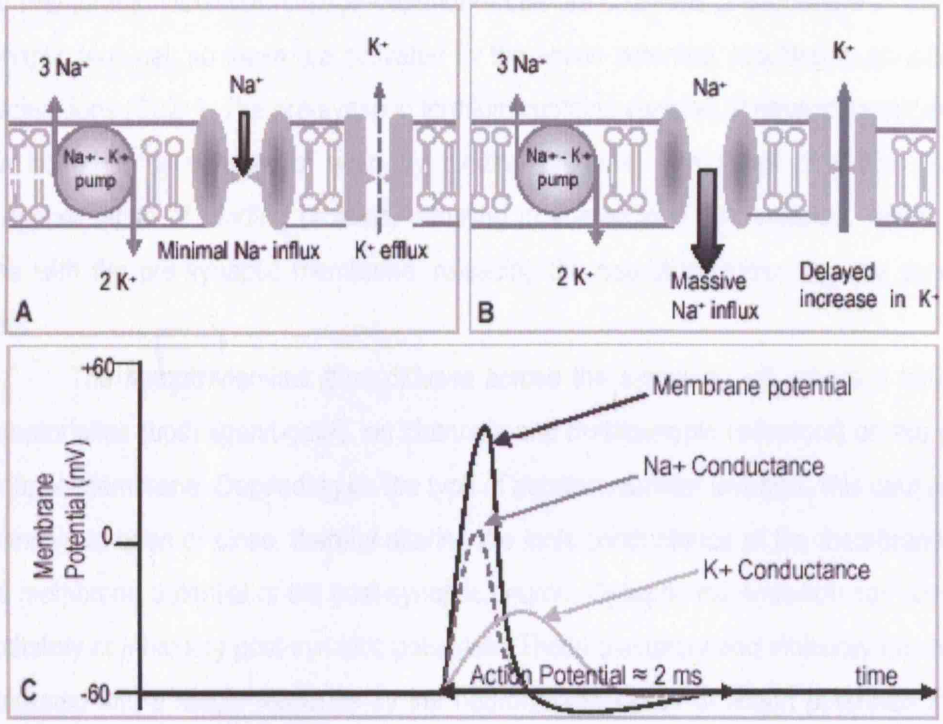

Figure 3.10: Illustration of ionic fluxes that generate (A) the resting membrane potential, (B) the action potential and (C) the temporal evolution of these fluxes and the associated membrane potential changes.

Following the propagation of the action potential, the axon membrane enters a *refractory period*, during which it is unexcitable; this phenomenon limits the frequency at which action potentials can be generated. This limitation is important since the action

potential is an all-or-nothing phenomenon: information is encoded by frequency of action potentials, and not by their amplitude, as this is held constant by the positive feedback loop of Na+ channel activation.

▪ 3.3.1.2 Synaptic Transmission

Synapses are the junctions between two neurons and are the site of information transfer between them. This transfer is most commonly mediated by chemical processes rather than by electrical processes. Synaptic transmission is initiated by the arrival of the action potential at the pre-synaptic terminal. As in the axon, this causes depolarization of the membrane. However, voltage-dependent calcium channels predominate in the pre-synaptic terminal, so these are activated by the action potential, resulting in an influx of calcium ions (Ca2+). The pre-synaptic terminal contains vesicles of neurotransmitter that are bound to a network of actin by binding proteins. The influx of Ca2+ causes phosphorylation of binding proteins, resulting in liberation of the vesicles, which then fuse with the pre-synaptic membrane, releasing the neurotransmitter into the synaptic cleft.

The neurotransmitter then diffuses across the synaptic cleft, where it binds to receptor sites (both ligand-gated ion channels and metabotropic receptors) on the post-synaptic membrane. Depending on the type of neurotransmitter involved, this causes ion channels to open or close, thereby altering the ionic conductance of the membrane and the membrane potential of the post-synaptic neuron. Synaptic transmission can result in excitatory or inhibitory post-synaptic potentials. These excitatory and inhibitory inputs are integrated into a single response by the neuron. Generation of action potentials in the post-synaptic cell usually occurs at the axon hillock, the initial segment of the axon, where the highest density of voltage-gated Na+ channels is situated. Generation of an action potential at this site is dependent on the summation of synaptic potentials that reach the axon hillock from synapses upstream in the neuron, at the level of the soma and the dendrites.

Removal of neurotransmitters from the synaptic cleft is critical for efficient chemical transmission. Timely removal of these molecules prevents desensitisation of

the post-synaptic membrane receptors and allows rapid transmission of successive pre-synaptic signals. Removal of the neurotransmitter can occur in several ways: by diffusion, by enzymatic hydrolysis or by uptake into surrounding cells. The actions of the major neurotransmitters in the cerebral cortex, glutamate and GABA, are terminated by uptake into pre-synaptic nerve terminals, postsynaptic cells and glial cells, which appear to be most important for these transmitters. This uptake is mediated by specific transport proteins that are driven by the energy generated by the movement of Na+ down its electrochemical gradient, resulting in tightly coupled uptake of neurotransmitter and Na+. The resulting increase in intraglial Na+ is counterbalanced by activation of the ATP-driven Na+-K+ pump. Following re-uptake into glial cells, the neurotransmitter is converted into an inactive form, allowing it to be released from glia, without active properties, into the intracellular space. The inactivated molecule is transported back into the pre-synaptic terminal where it is repackaged into vesicles.

▪ 3.3.1.3 The Energetic Demands of Brain Activation

The vascular and metabolic changes associated with brain activation are important issues to consider before outlining individual functional neuroimaging methods. Although the human brain makes up approximately 2 % of total body weight, it accounts for nearly 20 % of oxygen utilisation and 25 % of total body glucose utilisation in a resting state (Clarke & Sokoloff in Siegel book 1998). The preceding description of neuronal physiology outlined several energy-consuming changes in nerve signalling. Even before localised changes in neuronal activity are introduced, there is still a high metabolic demand in the brain, as suggested by the large proportion of glucose and oxygen supplied to it. Maintenance of the resting membrane potential alone requires ATP to drive the Na+-K+ exchange pump. In addition, many neurons exhibit tonic firing rates in the 'resting' state; hence there will be a background level of synaptic activity requiring the associated neurotransmitter release, re-uptake and repackaging. Both neuronal and glial cells also require energy for general cell maintenance, such as membrane renewal, protein synthesis, gene expression, etc.

Increased neuronal activation is invariably accompanied by an increase in blood flow to the activated region, resulting in an increased supply of oxygen and glucose to these tissues. The coupling of neuronal activation to blood flow is
supposed to be mediated by K+ ions via *astrocytes*, a class of glial cell. Golgi was the first to suggest that these cells were involved in the provision of metabolites to neurons, in view of their cytological characteristics; astrocytes are star-shaped (*stellate*) cells with relatively long processes that form *end-feet*, which can contact with both neurons and blood vessels. These processes cover the entire surface of intraparenchymal capillaries (Peters et al. 1991), hence forming part of the blood-brain barrier, and are a major site of glucose uptake from the blood. Via end-feet wrapped around synaptic contacts, these cells are also involved in the re-uptake of neurotransmitters from the intracellular environment, so they can 'sense' the level of neuronal/synaptic activity in its locality and couple this level to blood flow via localised vasodilation (Paulson & Newman 1987). It is speculated that release of K+, H+ or nitric oxide (NO) from glial end-feet could influence capillary endothelial cells.

Current evidence suggests that any increase in oxygen consumption by the activated tissues is far outweighed by the increase in oxygen supply to the local area, leading to an increase in the proportion of oxyhaemoglobin present in the tissue (Fox & Raichle 1986, Malonek & Grinvald 1996). In addition, these changes in blood flow are accompanied by changes in glucose utilization that also exceed the increase in oxygen consumption in some studies (Madsen et al. 1995). This suggests that the transient energy demands during brain activation may not be primarily met by oxidative metabolism of glucose. Recent studies suggest that anaerobic glycolysis is a major source of energy for neuronal activation and that this process occurs in astrocytes (Magistretti & Pellerin 1999).

As discussed earlier, following uptake into astrocytes neurotransmitters must be transformed into an inactive form. For example, glutamate is converted to glutamine, a process requiring energy. This energy is provided by glycolysis, using either glucose from the blood or glycogen stored in the astrocytes. The conversion of neurotransmitters in the astrocytes appears to be the major energy consuming process accompanying

increases in neuronal activity Magistretti & Pellerin 1999, Shulman & Rothman 1993, Rothman *et al.* 1999).

Similarly, synaptic release of an inhibitory neurotransmitter, such as GABA, would be expected to induce a positive change in metabolic rate Jueptner & Weiller, 1995). Evidence that the rate of synaptic activity, or more specifically rate of neurotransmitter recycling, rather than the increased rate of action potential firing, determines increases in metabolism has been provided by a recent study by Logothetis et al. (2001) In this study functional MRI and electrical measures of brain activity were acquired simultaneously. Hence, the authors were able to distinguish the action potentials and local field potentials, which are more slowly varying electrical potentials that are determined by the synaptic input and integrative processes occurring in the dendrites. They found that the major correlate of the functional MRI signal was the local field potentials, confirming the importance of synaptic activity. (For review see Logothetis (2002)).

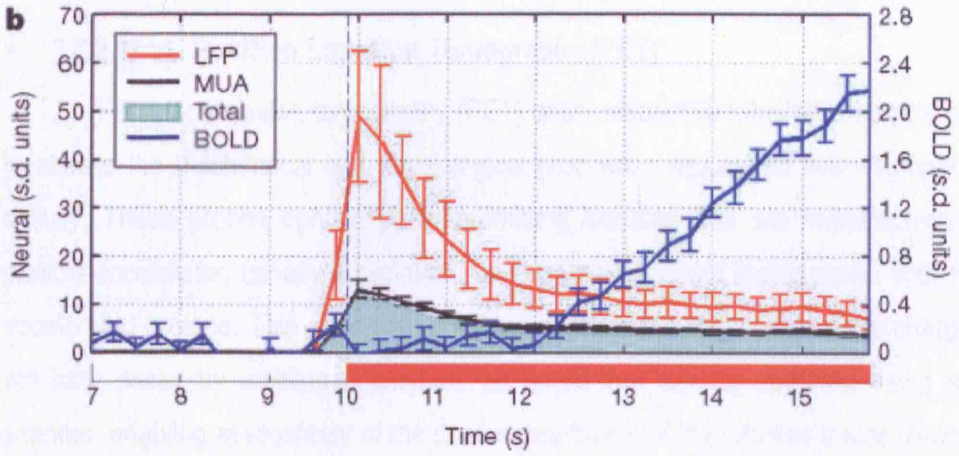

Figure 3.11: Coupling of BOLD Response With Electrophysiological Measures of Brain Activity. Mean LFP (red), MUA (black) and total (green surface) neural response together with the BOLD signal (blue). This figure is taken from Logothetis et al. (2001), who provided evidence that the fMRI BOLD signal correlated best with LFP activity. LFP = local field potentials, MUA = multi-unit activity

3.3.2 Functional Neuroimaging: Methods for In-Vivo Studies of Human Brain Function

Haemodynamic functional neuroimaging methods such as PET and fMRI provide indirect measures of neuronal activity. They are predicated on the relationship between increased synaptic activity leading to an increase in oxygen and glucose consumption and subsequent increases in cerebral blood flow (CBF) (Raichle 1998). As described in the section above, neuronal activity is metabolically demanding. Increases in neuronal activity cause an increase in oxygen & glucose consumption, which can be measured in humans with PET using a variety of isotopes (Frackowiak *et al.* 1980a&b, Fox *et al.* 1988). This leads to a subsequent increase in blood flow, which may be triggered by a variety of mechanisms including changes in pH (Kuschinsky & Wahl 1978), astrocyte function (Bonvento *et al.* 2002) and nitric oxide release (Dirnagl *et al.* 1993).

▪ 3.3.2.1 Positron Emission Tomography (PET)

Positron emission tomography (PET) uses radioactive labelled biological probes to assess the biochemical and physiological processes associated with regional brain activity. These probes contain positron-emitting isotopes that are manufactured in a particle accelerator, usually a cyclotron, by bombarding target stable nuclei with rapidly accelerated protons. This generates unstable nuclei with excessive positive charge that will later decay by emitting a positron; an event that can be detected using a PET scanner, enabling assessment of the spatial distribution of the labelled tracer (Reiman *et al.* 2002).

Once the labelled molecules have been synthesised, they can be either intravenously injected or inhaled, and the distribution of these molecules in brain tissue can be measured by making regional measurements of PET counts in the brain. The radiotracer will decay by emitting a positron, a sub-atomic particle with a positive charge and an equivalent mass to an electron, which will travel a short distance before colliding with an electron in its local environment (Cherry & Phelps in Toga & Mazziota (Eds)1996).

This positron-electron pair undergoes annihilation, creating a pair of high-energy photons, or gamma rays, with equal and opposite velocities. Due to their high energy, a proportion of these photons will not be attenuated by the brain tissues or skull and will be detected by the array of scintillation detectors around the head, which constitute the recording apparatus of the PET scanner. These detectors precisely time and count the arrival of the photons, allowing identification of 'coincidence' events, where two photons are detected almost simultaneously on opposite sides of the head (figure 3.11). Detection of both photons of the original pair enables localisation of the annihilation event. Once data correction techniques are applied to the resulting count density across the brain, one has a measure of the concentration of the positron-emitting molecule in the tissue.

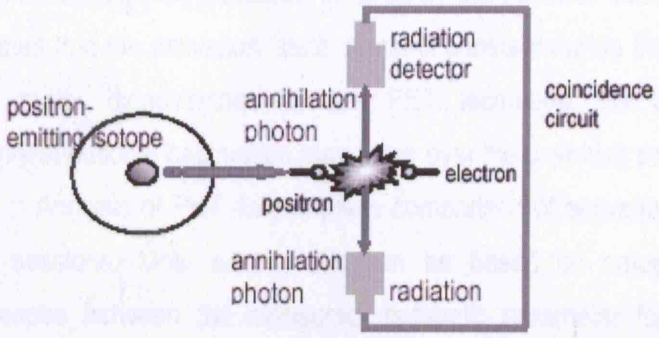

Figure 3.12: Positron Emission Tomography. Adapted from Reiman *et al.* (2000). Diagram of positron emission and coincidence detection.

An important feature of PET is the possibility of incorporating positron-emitting isotopes into many molecules of interest, such as metabolites and drugs. Carbon, nitrogen and oxygen are the major elements of organic molecules; hence the positron-emitting radioisotopes of these elements (^{11}C, ^{13}N and ^{15}O, respectively) are extremely useful markers. Flourine-18 ($_{18}F$) is also a useful positron-emitting tag, as it can be used as a hydrogen or hydroxyl analogue. These radioisotopes can be used to label a wide variety of biological substrates without compromising their biological activity. Choice of particular labelled compounds enables measurement of a local physiological or metabolic parameter in the brain: $H_2{}^{15}O$ or [^{15}O]-butanol can assess rCBF, [^{18}F]-fluorodeoxyglucose can evaluate rCMRglc and $^{15}O_2$ can measure $rCMRO_2$.

Most functional PET studies use $H_2{}^{15}O$, and hence rCBF, to assess relative brain activity. This tracer has a half-life of 2.07 minutes, allowing multiple scans to be performed in the same scanning session with intervals of 10-15 minutes between successive scans. This technique involves scans up to 1 minute in length, with each scan representing a block of a particular experimental condition (Vlokow *et al.* 1991).
The need to collect as many PET counts as possible limits the temporal resolution of the technique; more counts will be acquired during longer scanning periods, so scan times are maximised to allow optimal sampling of radiotracer decay. Hence, the behavioural condition of interest in a PET study must be studied in 'blocks' of stimulation, consisting of multiple events, e.g. finger taps, or continuous sensory stimulation, that continue for at least the duration of the PET scan. The signal in the resulting PET images is proportional to the time-integrated activation induced by the stimulus over the scanning period, and assumes that the activation 'state' remains constant during this period. This assumption is a major disadvantage of the PET technique, as considerable learning or sensory/attentional habituation may occur over the scanning period.

Analysis of PET data requires comparison of activation levels between different scan sessions. Most simply, this can be based on categorical comparisons; any differences between the measured metabolic parameter for the two conditions are attributed to the difference in task demands, as shown in Figure 3.12. Alternatively *factorial designs* can be used as they allow assessment of interactions between the different conditions (Friston *et al.* 1996).

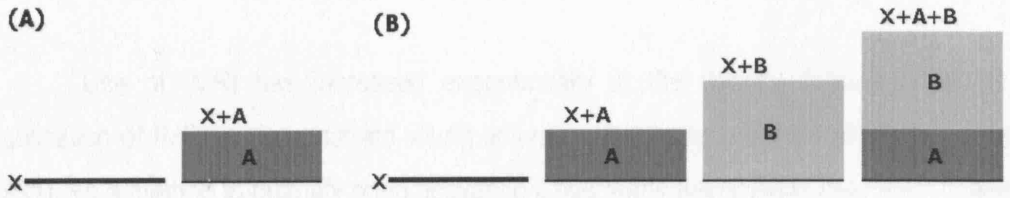

Figure 3.13: Epoch Designs And Subtraction in PET. Diagram illustrating (A) subtraction and (B) factorial PET experiments with a baseline condition X and two tasks A and B.

The spatial resolution of the PET technique is limited by several factors. Theoretically, the spatial resolution of PET is limited to 2 - 3 mm FWHM; this is due to, firstly, the distance that the positron travels before annihilation occurs and also any subsequent deviation of the resulting gamma rays from the expected 180° angle. The former issue is particularly influential for ^{15}O-labelled tracer techniques as the emitted positron has a relatively high energy level and can thus travel a longer distance before annihilation. PET studies that use rapidly decaying tracers are also subject to additional loss of spatial resolution; the acquired images contain relatively few PET counts per voxel. This is a consequence of both the brevity of the scans and the increased likelihood of saturation of the coincidence detectors, resulting in an under-estimation of PET counts. The limited number of counts per voxel results in relatively 'noisy' images; a problem that can be counteracted by smoothing the data to a lower spatial resolution, effectively increasing the number of counts contributing to each voxel and hence increasing the signal-to-noise ratio[37], resulting in a resolution of approximately 10-20 mm FWHM.

In conclusion, the temporal and spatial resolution of the PET technique is quite poor. However, the possibility of quantifying the physiological and metabolic processes associated with brain activation during movement and at rest is a distinct advantage of the PET technique over all other neuroimaging methods.

▪ 3.3.2.2 Functional Magnetic Resonance Imaging (fMRI)

Use of fMRI has increased exponentially in the decade following the first publication of fMRI to demonstrate visual activation in human subjects (Belliveau *et al.* 1991). As a method to quantify brain activation it has many advantages over PET. These include improved temporal and spatial resolution and the ability to perform repeated measurements safely on the same subjects.

• 3.3.2.2.1 Physical Principles of Magnetic Resonance

Nuclei with an odd total number of protons and neutrons, such as the hydrogen nuclei in water, possess a magnetic property called spin, which refers to the rotation of the nucleus along an axis. When the brain is put into an MRI scanner where there is a static and strong (e.g. 3 Tesla) magnetic field, the spinning nuclei in the brain tissue will align to the direction of the magnetic field, in the sense that axis of spin will roughly shift to the same direction as the magnetic field. Such alignment is however not perfect, and the axis of spin will precess around the direction of the magnetic field at a frequency determined by the strength of the static magnetic field and the type of nucleus in question. This precession frequency is known as the Larmor frequency. When a radiofrequency pulse that matches with the Larmor frequency is applied to the nuclei, the axis of spin of the nuclei deviates from the static position, and there is said to be an excitation, which reflects the fact that the deviated nuclei carry energy as transferred from the pulse. The degree to which the axis of spin deviates is also called the *flip angle,* which can be controlled by varying the strength and the duration of the radiofrequency pulse. Thus, when the flip angle is set at 90 degrees as in all fMRI experiments described in this thesis, the radiofrequency pulses have the capacity to cause the axis of spinning nuclei to change from the direction of the static magnetic field to a perpendicular direction. Since the radiofrequency pulse is only transient, the orientation of the axis of spin will eventually resume to the direction of the static magnetic field. The rate at which the axis of spin resume to the original position reflects the rate at which the

nuclei lose the energy gained from the radiofrequency pulse. There are two ways by which the nuclei can lose their energy gained from the pulse: the first is by giving it off to neighbouring molecules (*spin-lattice relaxation*); the second is by giving energy off to neighbouring spinning nuclei and thus desynchronising them (*spin-spin relaxation*).

For a given medium, the rate of spin-lattice relaxation is determined by a constant called T1. T1 differs for different materials, because different molecules have different mobilities and thus different rates of taking away the energy from the excited spinning nuclei. In particular, when compared to small water molecules and large protein molecules, mid-sized fat molecules move at frequencies close to the Larmor frequencies of the excited nuclei, and therefore can absorb the energy quickly. Therefore by measuring the different T1 constants in different parts of the brain, one can distinguish different brain tissues such as grey and white matter which have different fat contents. This is the basis for structural MRI imaging, and is also the reason why the structural images are often called T1-weighted images (or simply T1 images).

For a given medium, the rate of spin-spin relaxation is in principal governed by another constant called T2. However, when the local magnetic field is not homogeneous, the Larmor frequencies of spinning nuclei in a region will vary, which increases the rate at which the precessions loose coherence and thus accelerates spin-spin relaxation. Since the local magnetic field is never perfectly homogenous, the effective T2, called T2*, is always different from the theoretical constant. A change of T2* in a certain tissue also reflects a change of homogeneity of the local magnetic field.

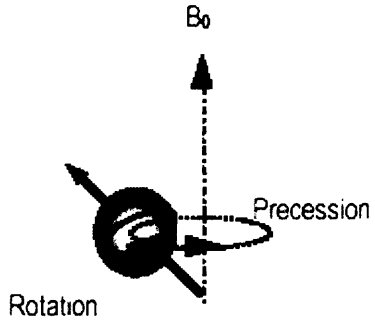

Figure 3.14: Magnetic Field and Nuclear Spin of an Atom. The effect of B0 on an atom showing both the precession and nuclear spin.

- **3.3.2.2.2 Physics and Physiology of BOLD-fMRI**

Functional MRI uses rapid MR imaging techniques to assess contrast changes, induced by localised neuronal activity. These contrast changes are the result of alterations in the local magnetic susceptibility caused by physiological events in the area of increased activity.

➢ **Susceptibility contrast agents**

Deoxyhaemoglobin is an endogenous paramagnetic contrast agent that can be used as a marker of regional cerebral activation. Haemoglobin is the primary carrier of oxygen in the blood and contains iron in the form of Fe^{2+}. Deoxyhaemoglobin (deoxy-Hb) is the name used to refer to haemoglobin that is not bound to oxygen. This form of the molecule contains paramagnetic iron. In the oxygenated form, oxyhaemoglobin (oxy-Hb), the iron becomes diamagnetic (Pauling & Coryell 1936). Hence, blood susceptibility is negatively correlated to blood oxygenation. Decreases in blood oxygenation cause a decrease in MR signals in the vicinity of vessels in the brain. The ability of changes in oxygenation to modulate magnetic susceptibility, and hence MR signal, is the basis for blood oxygenation level dependent (BOLD) contrast which is the most commonly used contrast mechanism in functional MRI. The BOLD signal in brain tissues (Ogawa et al.,

1990) depends on the T2* constant, which in turn depends on the homogeneity of the local magnetic field, so that an increase in the ratio of oxyhaemoglobin to deoxyhaemoglobin following neural activity is reflected by an increase in the BOLD signal. The more detailed aspects of the physiology underlying the BOLD signal which are still incompletely understood, are described below.

➢ Principles of the Blood Oxygenation Level Dependent (BOLD) Effect

The first papers to show MR signal changes due to regional cerebral activation were published in 1992 (Bandettini *et al.* 1992, Kwong *et al.* 1992, Ogawa *et al.* 1992). Though the increase in metabolic demand in the activated region would predict an increase in local oxygen consumption, and hence a local increase in deoxy-Hb concentration and therefore a decrease in MR signal, this does not occur. All reported small local signal *increases* in the activated cortical region. Instead, the local vasodilation accompanying the increased neural activity provides a supply of oxygenated blood that exceeds the demands of the tissue, resulting in a local increase in oxy-Hb and, hence, a decrease in the proportion of paramagnetic deoxy-Hb. This causes an increase in the T2* value, and hence a decrease in the rate of T2* relaxation. This working model can account for the observed increase in MR signal from activated regions and is illustrated in Figure 3.15.

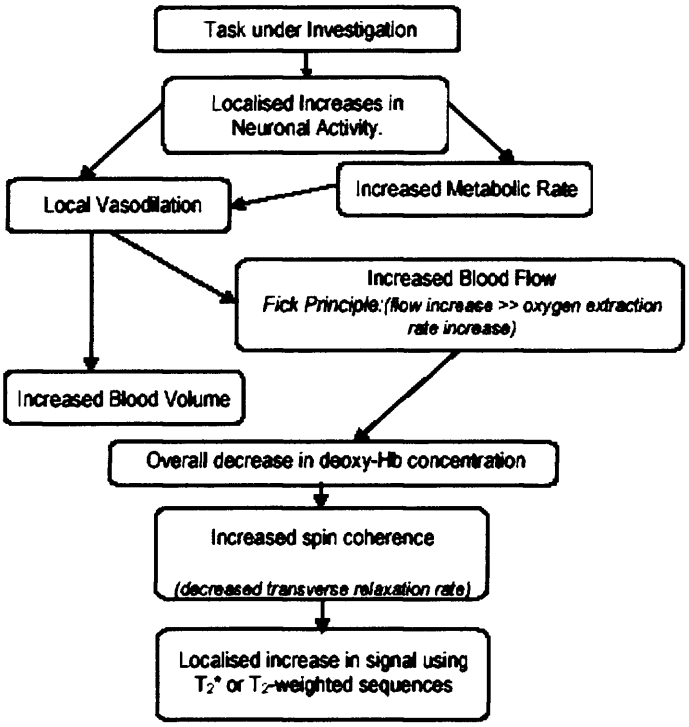

Figure 3.15: BOLD Signal Generation in fMRI. Adapted from Bandettini *et al.* (2000): Flow chart of the sequence of events that generate the BOLD signal.

BOLD signal changes occur due to an increase in blood flow to activated areas, that exceeds the requirements dictated by the local increase in oxygen metabolism, $CMRO_2$. Many studies have attempted to determine the interplay between CBF, CBV and $CMRO_2$ by modelling the sequence of physiological events that results in the BOLD effect.

Explanations for the mismatch between oxygen consumption and blood flow include the oxygen limitation model (Buxton *et al.* 1997). This model holds that oxygen transfer into brain tissue is limited by diffusion from capillaries and depends largely on the oxygen concentration gradient between the vasculature and the tissue. The gradient can be made steeper by increasing blood flow over and above oxygen consumption. Thus the mismatch between blood flow and oxygen extraction would meet the increased oxygen transport demand. An alternative explanation is the suggestion that there is a transient switch to non-oxidative metabolism during neuronal activity (Magistretti &

Pellerin 1999). As discussed in previous sections, there is evidence that glutamate released at the synapse is taken up by astrocytes triggering increased glucose uptake from capillaries. The glucose is metabolized glycolytically into lactate, which is then oxidized by neurons. This series of events would therefore be reflected by an initial mismatch between blood flow and oxygen consumption followed by later recoupling of the two. The initial mismatch would result in the increase in deoxy-Hb detected by BOLD imaging. However, there are suggestions that the existence of the mismatch varies between different cortical areas. For example, certain types of stimulation (e.g. flashing visual checkerboards) have been shown to produce similar increases in blood flow and oxygen consumption in visual cortex (Marrett & Gjedde 1997).

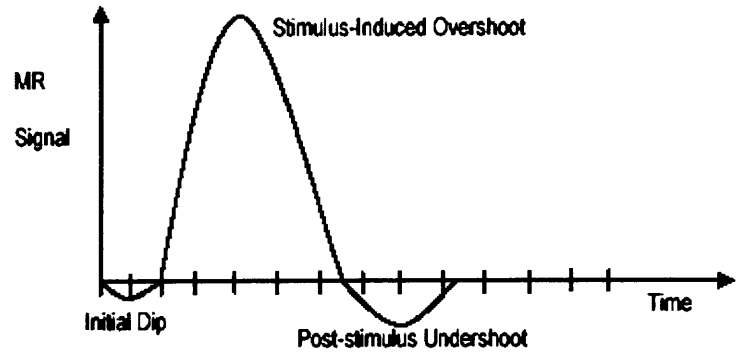

Figure 3.16: The Haemodynamic Response. Diagram showing the haemodynamic response of the BOLD signal, with its three components: the initial dip, the stimulus-induced overshoot and post-stimulus undershoot. Buxton and colleagues (1998) came up with a model, which would explain why the haemodynamic response following activation does not always consist of a simple rise to a peak positive MR signal and a subsequent return to the baseline signal. Instead the BOLD signal often undershoots the baseline, particularly when there are long periods between each stimulus event. The *balloon model* proposed by Buxton *et al.* treats the veins as an expandable compartment, which is inflated by outflow from the capillaries. However, the outflow from the actual compartment will depend on pressure against the balloon wall that is determined by the volume of blood present within it. If the stiffness of the compartment increases as it inflates then drainage will occur slowly after activation, resulting in the accumulation of deoxy-Hb in the venous compartment and the generation of a post-stimulus undershoot. This model can also explain the initial dip, which can be viewed as an early increase in oxygen extraction from capillaries causing an increase in deoxy-Hb concentration in the venous compartment, resulting in an initial decrease in signal intensity prior to the dilution effect of the increase in CBF. The haemodynamic response will then compensate for this initial surge in oxygen extraction, generating the stimulus-correlated overshoot.

➢ **Spatial Resolution of fMRI**

The local increase in oxy-Hb concentration that underlies the BOLD effect varies for different vessels surrounding the area of neuronal activation. After vessel dilation has been triggered, the blood flow rate increases leading to an increase in the delivery of oxygenated blood. The times at which these changes in blood oxygenation occur in each portion of the vascular bed are determined by the transit time through the system. The transit time from the arterial sphincters via the capillaries to the veins is approximately 2 – 3 s (Bandettini 1999). Whereas arteries exhibit virtually no change in haemoglobin oxygen saturation and capillaries increase saturation by ~10%, the largest activation induced change in saturation is seen in the veins, an increase of approximately 30% (Bandettini 1999). As veins have the largest diameter of the components of the vascular tree, they can contribute a large blood volume to a single imaging voxel, compared with smaller vessels, such as capillaries, which are also well spaced in the tissue and therefore contain a smaller blood volume. Hence, the strongest BOLD signal changes are usually observed in the veins.

This dominance of venous signal change means that imaging voxels may be located 'downstream' of the neurons that are responsible for the change in oxygenation. However, only vessels near to the site of the oxygenation changes will contribute to the BOLD effect, as dilution of these changes will occur at more distant locations. Studies comparing activation-induced signal changes measured using BOLD and perfusion-ASL techniques show general spatial overlap, but activation is more diffuse and includes large veins with the BOLD technique (Detre & Wang 2002).

Recently, it has been suggested that mapping of the initial dip may provide a more localised measure of neural activity, as the postulated initial increase in deoxyhaemoglobin would occur nearer the capillary bed, the site of oxygen extraction (Zarahn 2001). Studies have shown that fewer voxels show the initial dip than show the positive BOLD response. In addition, only the positive BOLD response is seen (and not the initial dip) in venous regions (Menon et al. 1995).However, the initial dip is not always

observed, particularly at lower field strengths, and may therefore not be a robust marker of localized activity.

- **Temporal resolution of fMRI**

The transit time from the arteries through to the veins is of similar length to the time delay before positive fMRI signal changes are observed following the start of the neural activity, approximately 2 - 3 s. The positive signal change peaks approximately 6 – 9 s after the onset of the activation (DeYoe *et al.* 1994). Return to the baseline signal occurs more slowly than the rise time, taking about 7 – 11 s from cessation of activation. Temporal resolution of fMRI is dependent on the repetition time (TR) between acquisitions of signal from the same slice. The choice of TR depends somewhat on T1 recovery time as it is preferable to allow long enough for sufficient recovery of longitudinal magnetization to take place before the next RF pulse.

The relationship between the duration of the haemodynamic response and the duration of the stimulus is roughly linear, until very short stimulus durations are used; below a specific duration, the resulting haemodynamic response stays constant. The shape and timing of the responses to such short stimuli demonstrates an important temporal feature of the BOLD response. The temporal evolution of the BOLD signal change is delayed relative to the neuronal activity and the duration of this activity is far less than the temporal width of the BOLD response. Hence, the hemodynamic response can be likened to a low-pass filter that temporally blurs the neuronal events responsible for the generation of BOLD signal changes (Friston *et al.* 1994). Linear deconvolution can be used to determine the characteristics of this filter. This involves removing the effect of the input, the stimulus duration and amplitude, from the measured BOLD response to give a haemodynamic impulse response. The resulting filter closely resembles the actual haemodynamic response elicited by even very brief stimuli (Bandettini 1999). The ability to detect responses to short duration stimuli with fMRI has led to the use of event based experimental designs.

- **3.3.2.2.3 Experimental Design for Functional MRI**

Unlike in PET studies, the measured signal intensity for BOLD fMRI cannot be expressed as a physical quantity such as rate of oxygen consumption or blood flow. Though the images acquired are weighted by the concentration of deoxyhaemoglobin, they are not a precise measure of this concentration. For example, comparison of the resting BOLD signal in a particular brain region across subjects would not yield interpretable results, as the measured signal can be influenced by non-functional differences such as between-session differences in scanner set-up, variation in location of the subject relative to the scanner, etc. Instead, functional information is derived by identifying changes in the MR signal that relate to different experimental conditions that are varied over the time course of the scanning session.

The importance of temporal resolution of fMRI depends somewhat on the paradigm chosen. For example, a conventional block design where blocks of 20-40 seconds duration alternate with rest blocks assumes a constant response across the whole block (once the box car has been convolved with the HRF).

However, event-related designs can detect haemodynamic responses to very transient stimuli. By presenting stimuli individually one can assess characteristics of functional activation that cannot be observed with block designs. Indeed, the grouping of trials of the same type with each other in block designs prevents the randomisation of trials, as a block can only consist of like trials, and hence makes block designs undesirable for detection of responses to novel or oddball stimuli, as such stimuli will lose their novelty further into the block of trials.

Unlike with block designs, event-related designs allow for randomisation of trial order. Further randomness can be introduced into the experimental design by 'jittering' the length of the intervals between subsequent trials (or the stimulus onset asynchrony, SOA), removing the temporal predictability of trial onset and adding lower frequency components to the task-induced variance (Aguirre & D'Esposito in Bandettini 1999).

Another major benefit of event-related designs is that the BOLD response to an individual stimulus can be measured. Use of long SOAs between trials results in minimal overlap between haemodynamic responses, enabling good numerical fitting of the

response function. Such fitting procedures allow assessment of inter-regional, age- or disease related and task-specific variability in the haemodynamic response.

Because the frequency structure of a series of brief events is dominated by higher frequencies, which are diminished by the low-pass filter of the haemodynamic response, event-related designs are less sensitive than block designs (Friston *et al.* 1999). However, the flexibility of the event-related approach for investigation of the response to randomised events outweighs this concern.

3.3.3 Neurovascular Coupling: Excitation or Inhibition?

Interpretation of most fMRI and PET studies rests on the assumption that the BOLD or rCBF responses are an indirect marker of excitatory neuronal activity. However, BOLD signal change is a summary value that could reflect a number of different processes. A discussion on the metabolic demands of neuronal activation has been provided in section 3.3.2.2.2.

The mechanisms by which rTMS reduces or increases cortical excitability remains unclear. It may involve a mixture of processes such as reduced efficacy of excitatory inputs or increased activity of inhibitory interneurons in the case of increased inhibition. It is therefore important to consider the relationship between CBF, BOLD and inhibitory activity before examining the effects of rTMS on the heamodynamic response with functional neuroimaging techniques.

Studies using 2-deoxyglucose have shown that even purely inhibitory synaptic activity in cat superior olive may result in increased local metabolism (Nudo & Masterton 1986). However, a theoretical argument can be made that inhibitory synaptic activity should have little direct impact on the BOLD signal. The argument rests on the following facts: only 15-30% of neurons in the CNS are inhibitory; inhibitory neurons make fewer synapses than excitatory neurons; inhibitory synapses operate more efficiently and the chloride transport uses less energy than the metabolically-demanding sodium pump (Waldvogel 2000). Indirect evidence to support this argument comes from a study by Waldvogel *et al.* (2000). They used a go-nogo task to study inhibition with TMS and compare this with the BOLD response. They were able to show that during tasks for

which TMS investigation would suggest the occurrence of inhibitory activity in M1 (the "no-go" periods), no increase in BOLD signal was detected in M1. These results have been debated by further studies from Gerloff *et al.* (2004) who have argued that negative BOLD responses could be induced in the 'no-go' periods of a 'go-nogo' task when the stimulus-response "programs" had been learnt which involved finger sequences being associated with particular symbols which instructed subjects to move or not to move. They argued that contextual modulation of learned behavioral programs depends on an interplay of focal increases and decreases of neural activity and that they could find inhibitory changes in a sensorimotor-cerebellar network reflected by a clear negative BOLD signal in that task.

Studies using rat cerebellum have demonstrated that increases in both inhibitory and excitatory synaptic activity lead to increases in CBF. When inhibitory and excitatory pathways were stimulated simultaneously greater increases in CBF were observed than during stimulation of either pathway alone (Caesar et al., 2003). These findings suggest that active synaptic inhibition (increased activity of inhibitory interneurons) results in increased CBF. Decreased excitability of a brain region may also represent deactivation: reduced intrinsic activity or loss of excitatory input from connecting areas. (Gold and Lauritzen, 2002) have demonstrated that baseline CBF levels are relatively insensitive to decreases in synaptic activity especially when compared to the robust increases in CBF seen with increased excitatory activity. The implication of this finding is that detecting decreases in synaptic activity via decreases in rCBF may be problematic.

As discussed in section 3.3.1.3, Logothetis and colleagues were able to provide direct evidence of the relationship between electrical activity and BOLD signal in a series of technically impressive studies. They performed simultaneous electrophysiological recording and fMRI scanning during visual simulation in anaesthetized macaque monkeys (Logothetis *et al.* 2001). They compared the haemodynamic response to electrode measurements that were filtered to differentiate high and low frequency components. High frequency (300-1500Hz) components represented single- and multi-unit activity (MUA). Low frequency (40-130Hz) components represented local field potentials (LFPs). LFPs reflect spiking and subthreshold integrative processes whereas MUA reflects only spiking. Although both MUA and LFPs correlated with BOLD signal,

LFPs were a significantly better predictor of BOLD response. This suggests that the signal measured in fMRI reflects input and local processing of an area rather than its spiking output. These findings are consistent with the fact that wheras spiking activity itself is not very metabolically demanding, events at the synapse are highly metabolically demanding (Arthurs & Boniface 2002).

However, it is important to note that although in the Logothetis et al. study LFPs provided a better predictor of BOLD signal in certain cases, there was nevertheless a significant correlation between spiking activity and the haemodynamic response (Logothetis *et al.* 2001). In addition, LFP includes contributions both from subthreshold potentials and from spiking activity. Therefore spiking activity is reflected in the BOLD signal. Consistent with the correlation between BOLD activity and LFPs (Logothetis *et al.* 2001), BOLD is also positively correlated with population level electrical activity, as measured by somatosensory evoked potentials (SEP) after electrical stimulation of the median nerve (Arthurs et al. 2000). Increases in stimulation intensity led to correlated increases in BOLD and SEP amplitude.

Recent work by Shmuel et al. (2002) has set out to investigate the origins of the negative BOLD responses observed in the initial dip and post stimulus haemodynamic response. They have formulated the hypothesis that negative BOLD may be elicited by neuronal de-activation in the same way as positive BOLD response is an indirect measure of neuronal activation (Shmuel et al. 2003).

3.3.4 Analysis of Functional Neuroimaging Data

This section provides a brief overview of the image analysis used to analyse the PET and fMRI studies described in the subsequent chapters. All image analysis was performed using Statistical Parametric Mapping software, SPM (Wellcome Department of Imaging Neuroscience, UCL, UK. http://www.fil.ion.ucl.ac.uk/spm). The PET experiments in Chapters 4 and 5 were analysed using SPM99, and the fMRI experiments in Chapter 6 and 7 were analysed using SPM2.

- **3.3.4.1 Preprocessing**

The analysis of functional imaging data is based on the assumption that the data acquired from a particular voxel over the course of an experiment are derived from the same anatomical location. In order to ensure that this assump\tion is not violated the initial step in the data analysis process involves realigning the data to remove the effects of subject movement. Following realignment the data are normalised: transformed into standardised anatomical space and spatially smoothed. Data from individual subjects were pre-processed independently.

- **Image Reconstruction:**

As discussed in previous sections, the acquired MR signal is represented in a spatial frequency space, the k-space. Reconstruction of this data into image space requires two-dimensional fast Fourier transformation (FFT) of the data corresponding to each acquired slice. Nyquist ghost correction techniques may be applied at this stage.

- **Motion Correction – Realignment & Unwarping**

During the course of an fMRI experiment, there will be movement of the brain within the imaged space due to physiological factors such as cardio-respiratory cycle, and to subject motion. The result of this is that the signal from an individual voxel may not correspond to the same point in the brain throughout the experiment. **Realignment** of PET and fMRI data involved estimating six parameters describing a rigid-body affine transformation that minimised the sum-of-squared differences between successive scans. These transformations (estimated relative to the first scan of the time-series) were then applied to the entire data-set, following which the images were re-sampled using spline interpolation (Friston et al., 1995a).

However, realignment does not remove all variance within the data caused by movement e.g. EPI data are very susceptible to distortions which result in movement-by-susceptibility interactions which cannot be removed by realignment.

Movement parameters can be included as nuisance variables in the statistical model for fMRI data. However, this may be problematic for motor paradigms, as experimental variance can be explained away. To deal with this residual variance, an **unwarping** procedure (implemented in SPM2) was applied to the fMRI data whereby the rate of change of deformation in the EPI images was estimated. These derivative fields

were then applied to the data to remove susceptibility-by-movement interactions (Andersson et al., 2001).

Structural MR images from individual subjects were coregistered to the mean functional image using the same procedure as realignment and normalized to a standard anatomical space.

- **Spatial Normalisation**

Spatial normalisation involves the transformation of individual brains to fit to a 'standard' brain template, most commonly the Montreal Neurological Institute (MNI) template. Functional images in this thesis were normalised to a standardised anatomic space (Talairach & Tournoux 1988) by matching to a standardised PET or EPI template using linear (twelve parameter affine transformation) and non-linear (discrete cosine basis functions) spatial deformations (Friston et al. 1995a). The aim of this pre-processing stage is to minimise anatomical differences between individuals so that data can be pooled and a standard coordinate system can be used to report activations.

- **Spatial smoothing**

The main reason for spatially blurring fMRI data is to increase the signal-to-noise ratio. If noise varies randomly from voxel to voxel then spatially smoothing could cancel out the noise. By contrast, if activation is larger than the size of the chosen smoothing kernel then activation should not be cancelled out to the same extent as noise. A second reason for spatial smoothing is that later clustering steps use Gaussian random field theory which assumes that the data is spatially smooth. Each image was smoothed with an isotropic Gaussian kernel of 12 mm full-width at half-maximum for PET data and 8mm for fMRI data.

▪ 3.3.4.2 Anatomical localization

The anatomic locations presented in this thesis have been identified in two ways. For brain areas where a probabilistic cytoarchitectonic atlas has been published the probability of the co-ordinates under investigation being in a particular area in more than 3/10 subjects has been used to define the extent of a region (Geyer *et al.* 1996, Geyer *et al.* 1999, Geyer *et al.* 2000, Grefkes *et al.* 2001). For those areas where such probabilistic data are not available, the coordinates were displayed on single subject or

canonical structural images, and the sulcal and gyral anatomy was identified with the aid of an atlas (Duvernoy, 1999) and reviews about anatomical localisations (Picard & Strick 2001).

▪ 3.3.4.3 Statistical Analysis of functional neuroimaging data

• 3.3.4.3.1 The General Linear Model

Once the data has been preprocessed, the next step is to assess the degree to which the signal from different voxels corresponds to the task paradigm. The experiments presented here use the General Linear Model, which is the basis of the Statistical Parametric Mapping fMRI analysis package (Wellcome Department of Imaging Neuroscience, London, UK). The General Linear Model (GLM) describes the varying intensities of the time course of each voxel as a linear combination of explanatory variables and an error term, corresponding to the residual variability. The GLM can be described with the

$$y = \beta x + \varepsilon$$

The observed response variable (voxel-specific rCBF or BOLD responses) y is modelled in terms of a linear combination of explanatory variables in the design matrix X plus an independently and identically distributed Gaussian error term ε. The same equation can be modified if there are more observations:

$$y_i = \beta_1 x_{i1} + \ldots\ldots + \beta_m x_{im} + \varepsilon_i$$

where β_m is an unknown parameter for each voxel that defines the relative contribution of each of the explanatory variables, x_{im}. The values of β_m are estimated by use of least-squares minimisation on voxel-wise basis The explanatory variables usually correspond to haemodynamic models of the effect of particular task demands or other possible determinants of signal changes such as variations in performance of the subject or motion realignment parameters.

Regionally specific effects can be identified by generating SPMs based on differences between the parameter estimates (βm) for different conditions described by the corresponding explanatory variables, x_m. This requires the specification of linear

contrasts between the variables of interest, which are tested for significant differences by calculation of a statistical variable, either t- or F-values for each voxel.

T-tests are used to test differences between particular β parameters that form the effect of interest as specified by an individual contrast. The most simple contrasts test the statistical significance of signal changes modelled by one of the explanatory variables compared with a rest condition. T-tests can also be used to test the differences between the signal changes described by one explanatory variable relative to others. Parameter estimates therefore give an estimate of the amplitude of the response in a particular condition (and can be converted to values such as percentage signal change).

F-tests, on the other hand, are used for simultaneously testing several contrasts at once. This approach is particularly useful for detecting effects when a set of basis functions is used to model the haemodynamic response function – an F-test can simultaneously test the effect of each of the basis functions and any linear combination of these.

- **3.3.4.3.2 Statistical Inference**

The parametric tests discussed in the previous two sections involve generation of statistical variables – *t*- and *F*-statistics. All of these statistical variables can be transformed to Z-scores – the distribution of which forms a Gaussian, Normal distribution. This allows determination of the statistical significance of each transformed variable in the form of probability values that signify the confidence that the null hypothesis is true.

Our SPM analyses use a mass-univariate approach whereby standard univariate statistical tests are applied to each voxel. The results are interpreted as spatially extended process by referring to Gaussian random field theory, a methodological approach that models the probabilistic characteristics of continuous, spatially extended statistical fields (Worsley et al., 1992;Worsley et al., 1996). The adjustment of p values based on GRF theory depends critically on the class of inference made using SPMs. In the case that no prior anatomical hypothesis exists about the regional specificity of an experimental effect, it is necessary to correct for multiple comparisons across the entire brain. In some cases a more constrained anatomical

hypothesis may exist, in which case it is possible to restrict the correction for multiple comparisons to either a single voxel (in which case an uncorrected p-value can be used) or a restricted volume of interest.

If one has an anatomically constrained hypothesis about an *a priori* effect of an experimental manipulation then the resulting transformed z-values and their associated probabilities can be used for the purpose of statistical inference, using small volume correction, without the need for correction of the statistics for multiple comparisons. Corrections for multiple comparisons are necessary when one does not have an anatomically constrained hypothesis for an effect, as large numbers of non-independent statistical comparisons have been performed – one for each voxel within the brain. The use of Gaussian random field theory to estimate the smoothness of the statistical map in terms of resolution elements or "resels" allows for a more lenient correction than voxel-based thresholding. If size of activated clusters is also taken into account, also using Gaussian random field theory can also be more sensitive and might be more physiologically informed since activated regions are expected to extend over multiple voxels. Unless stated otherwise, activations in this thesis are reported when surviving a threshold of $p<0.05$ corrected for the entire brain or the search volume of interest based on a priori hypotheses.

- **3.3.4.3.3 Group Analyses**

Most fMRI experiments aim to characterize brain activation patterns for a particular population or differences between populations. Statistical methods for assessing group activation include "fixed effects" and "random effects" analyses. Fixed effects analyses only consider within subject variance whereas random effects analyses additionally consider between subject variances. Random effects analyses are therefore more conservative, but present a more valid representation of results if conclusions are to be made about the population. The groups analyses carried out in the PET experiments in this thesis employed fixed effects analyses whereas the fMRI experiements have used random effects analyses.

- **3.3.4.3.4 Analyses of Effective Connectivity**

Traditionally, the high spatial resolution available with functional neuroimaging data has lent itself to analyses of functional specialization, which describes the role of anatomically distinct areas as a set of units specialized to perform particular aspects of a task.

Analyses of effective connectivity test the influence that one neural system exerts over another (Friston *et al.* 1993). They study the functional integration of cortical areas, which describes the pattern of connections established between cortical areas, that is how these anatomically distinct functionally specialised areas unite, via extrinsic connections in order to subserve particular functions. Studies using multiunit microelectrode recordings of separable spike trains (Gerstein and Perkel, 1969;Aertsen and Preissl, 1991) have defined *functional connectivity* as the temporal correlations between spatially remote neurophysiological events (Friston *et al.* 1993b). *Effective connectivity* is defined as the influence that one neural system exerts over another either directly or indirectly (Friston et al., 1993a).

- **Psychophysiological Interactions**

Psychophysiological Interactions (PPIs) aim to explain responses in one cortical area in terms of an interaction between activity in another cortical area (index area) and the influence of an experimental parameter.

The analysis is constructed to test for differences in the regression slope of the activity in the index area on the activity in all remaining areas under the different experimental conditions. As these regression slopes are a metric of the coupling between areas, the PPI identifies areas where the degree of correlation with the index region is modulated significantly by the experimental variable. The presence of a significant change in coupling between the index region and other brain areas can be interpreted in two distinct ways: either as a change in the influence of the index area on other brain regions, or as a change in the responsiveness of the index area to inputs from other brain regions (Figure 3.17 PPI regression lines). A significant PPI cannot be used to disambiguate these interpretations post-hoc. A PPI is used to test an a priori hypothesis about decreased responsiveness or increased influence of the index region. In Chapters 4 and 5 PPIs were constructed to identify differences in the effect of rTMS

on movement-related activity. While the rTMS variable (Real versus Sham) appears explicitly in the model, the effect of task (for example move vs baseline) enters vicariously as the principle cause of variance in the index region.

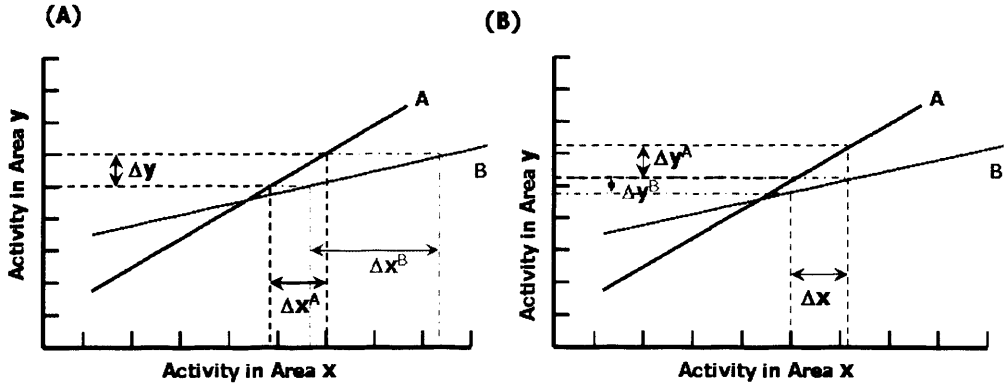

Figure 3.17: Psycho-Physiological Interactions. The importance of devising a priori hypotheses to motivate a PPI is illustrated here. This diagram shows two mathematically equivalent but biologically different interpretations of PPI results. In both graphs, x (abscissa) represents activity in an index area subtending the physiological variance in the PPI analysis. Conditions A and B represent some psychological or experimental (contextual) manipulation. (A) During A, a unit increase in activity in area y is associated with a small increase in activity in area x: Δx^A(red dashed line). During B, y is associated with a larger increase in activity in area x: Δx^B(gray dashed line). Consequently, this could be interpreted as a decrease in the sensitivity of area y to inputs from area x following intervention B. (B) In this case, during A, a unit increase in activity in area x is associated with a large increase in activity in area y: Δy^A(red dashed line). During B, x is associated with a smaller change in activity in area y: Δy^B (gray dashed line). Therefore, area x seems to be more responsive to inputs coming from area y following intervention B. Furthermore, these PPI plots can only tell us about the relative changes in coupling between conditions following a physiological manipulation. With no prior information from the main GLM analysis we would not be able to distinguish whether a change in the regression slope from condition A to condition B signifies an increase in coupling between the two areas from negative coupling to positive coupling or from positive coupling to even more positive coupling.

3.4 CONCLUSIONS

The aim of this chapter was to discuss the physiological and technical bases of the various TMS and neuroimaging methods used and discussed in this thesis. Although TMS can be used as a technique to measure cortical excitability changes in the motor system, when applied repetitively, it can induce such changes in the area stimulated and in anatomically connected regions, which can be measured with functional neuroimaging techniques. The aim of the subsequent chapters is therefore to use these already established methodologies along with their respective technical implications and statistical methods in order to study reorganization of the motor system following periods of altered cortical excitability induced by various parameters of rTMS.

4. Frequency-specific changes in regional cerebral blood flow and motor system connectivity following rTMS to the primary motor cortex

4.1 INTRODUCTION

A number of studies have used functional brain imaging techniques to explore patterns of activity related to reorganisation of the motor system following brain injury (Section 2). The aim of this thesis was to examine how the brain responds to alterations in cortical excitability induced with rTMS conditioning on the motor system. This study explores how frequency of rTMS stimulation over the motor cortex in healthy individuals affects (1) the pattern of rCBF at rest and during movement, (2) task-related activity during performance of a simple motor task and (3) task-related coupling between areas of the motor system.

4.2 BACKGROUND AND RATIONALE

RTMS to the human primary motor cortex can induce bi-directional changes in corticospinal excitability depending on the stimulation frequency used (Pascual-Leone *et al.* 1994, Maeda *et al.* 2000). These changes in excitability can outlast the stimulation period for several minutes (Pascual-Leone *et al.* 1998). In healthy volunteers, long periods of rTMS at frequencies around 1Hz produce robust and long-lasting depression of motor evoked potentials (MEPs) (Chen *et al.*, 1997;Touge *et al.*, 2001;Wassermann, 1998). Prolonged high-frequency rTMS (at frequencies of 5Hz or more) causes lasting increases of corticospinal excitability (Berardelli *et al.*, 1998;Peinemann *et al.*, 2004). These bidirectional effects on the excitability of M1 with 1Hz and 5Hz-rTMS have also been observed when stimulating non-primary motor areas, such as the dorsal premotor cortex, (Gerschlarger *et al.* 2001, Rizzo *et al.*, 2004).

Measurements of MEPs provide limited insight into the conditioning effects of rTMS on the motor system. The amplitude of the MEP reflects changes in the

corticospinal output from M1 and intracortical excitability of the stimulated M1. It is unclear if the opposite effects of high- and low-frequency rTMS on corticospinal excitability translate into changes in local cortico-cortical and inter-regional connectivity within the motor system. Using positron emission tomography (PET) of normalised regional cerebral blood flow (rCBF) Lee *et al.* (2003) have shown that subthreshold 1Hz rTMS to left M1 causes reorganisation in the motor system without affecting task performance. Changes in effective connectivity were seen within the stimulated M1 and between premotor, supplementary motor and primary motor areas (Lee *et al.*, 2003). The experiment presented in this section aimed to characterise the effects of subthreshold 5Hz rTMS to the motor cortex and compare the effects of low-frequency (1Hz) and high-frequency (5Hz) rTMS to left M1 on finger movements and regional neuronal activity (indexed by rCBF) at baseline and during movement.

The data published by Lee et al. (2003) were supplemented with new data in which healthy volunteers were stimulated with high-frequency 5 Hz rTMS and scanned using the same protocol as in the previous study.

4.3 MATERIALS AND METHODS

4.3.1 Subjects

Sixteen healthy, right-handed volunteers (3 females) aged between 20 and 68 (mean age: 36.8), with no history of neurological disorder or head injury were recruited from the database of volunteers at the Functional Imaging Laboratory, Institute of Neurology, University College London, UK. Data from the eight subjects receiving low-frequency stimulation (group 1; 2 females, mean age: 37, age range: 20-68) have been published in a separate paper (Lee et al., 2003). Eight further subjects (group 2; 2 females, mean age: 37.7, age range: 23-54) were recruited for the high-frequency rTMS study. Written informed consent was obtained from all participants. The study was approved by the joint ethics committee for the National Hospital for Neurology and Neurosurgery (UCLH NHS Trust) and the Institute of Neurology (UCL). The

administration of radioactivity was approved by the Administration of Radioactive Substances Advisory Committee (RPC528-890 (14364)).

4.3.2 Study Design

The study comprised a 2x2x2 factorial design, with two levels per factor: "frequency" (1Hz rTMS versus 5Hz rTMS), "intervention" (real-rTMS versus sham-rTMS) and "task" (movement versus baseline). Figure 4.1 illustrates the study design. Subjects in each group received real and sham-rTMS on two separate days, at least one week apart. The order of intervention was counterbalanced across subjects. Group 1 were given low-frequency (1Hz) conditioning rTMS (real and sham 1Hz rTMS), and Group 2 were given high-frequency (5Hz) conditioning rTMS (real and sham 5Hz rTMS). The effects of rTMS were assessed by consecutive PET measurements of rCBF in the first hour after rTMS. Within each scanning session the baseline and movement tasks were alternated. The order of tasks was counterbalanced across subjects, but was kept constant within subjects between sessions.

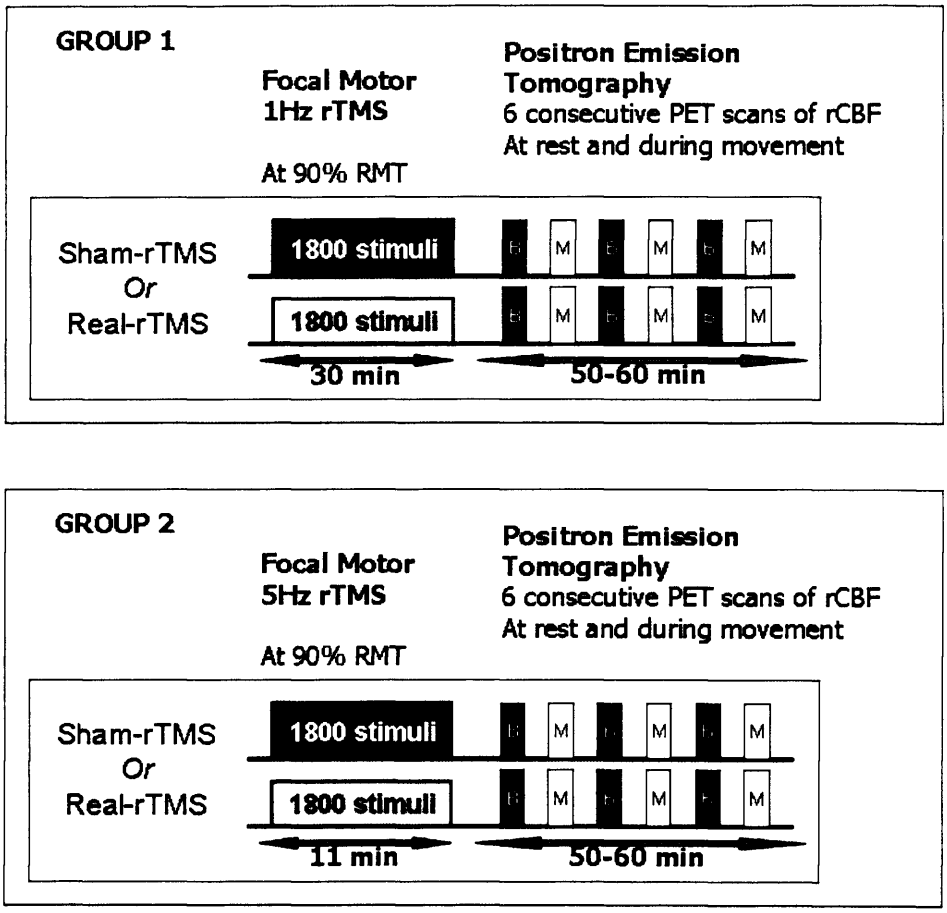

Figure 4.1: Experimental Design. 16 Healthy volunteers were divided into two groups of 8. All participants received 1800 biphasic stimuli of real rTMS or sham rTMS at a frequency of 1Hz for group1 and 5Hz for group 2 on two separate days. The site of stimulation was the motor "hot spot", a reliable functional marker for the primary motor hand area. Participants in group 1 received 1Hz real- or sham-rTMS at 90% resting motor threshold (RMT) in two continuous blocks of 15 minutes, whereas in group 2 received 5Hz rTMS at 90% RMT in six one-minute blocks separated by one-minute intervals. Changes in regional cerebral blood flow were mapped using PET. Six sequential H_2 ^{15}O-PET scans were acquired at baseline (B) or during a paced free selection of finger movements task (M) in alternation during the hour after the end of rTMS. The order of intervention (real-rTMS vs sham-rTMS) and the experimental conditions (B vs M) were counterbalanced across subjects.

4.3.3 Repetitive Transcranial Magnetic Stimulation (rTMS)

In each rTMS session 1800 biphasic stimuli, at a stimulation intensity of 90% of resting motor threshold (RMT) for the right first dorsal interosseous (FDI) muscle, were given over left M1 area using a MagStim-rapid stimulator connected to four booster modules (MagStim Company, Whitland, Wales, UK; www.magstim.com). The subjects in group 1 received two 15-minutes trains of 1Hz rTMS separated by an inter-train interval of one minute. Subjects in group 2 received six 1-minute trains of 5Hz rTMS with a one-minute inter-train interval. A standard figure-of-eight shaped coil (Double 70mm - Coil Type P/N 9925, MagStim Company, Whitland, Wales, UK) was used for real-rTMS. For sham-rTMS a specially designed sham coil that induced no magnetic field but provided a comparable acoustic stimulus was used (MagStim Company, Whitland, Wales, UK). The coil was positioned with the handle at 45° to the sagittal plane. The current flow of the initial rising phase of the biphasic pulse in the TMS coil induced a current flowing from posterior-to-anterior in the underlying motor cortex.

The site of rTMS stimulation was located at the "motor hot spot" definedfunctionally as the point of maximum evoked motor response in the relaxed right first dorsal interosseous (FDI). The resting motor threshold was defined as the lowest stimulus intensity that elicited at least five twitches in ten consecutive stimuli given over the "motor hot spot". The FDI was used to define the motor threshold because TMS-evoked twitches are clearly visible and it has a threshold below other intrinsic hand muscles. This ensured that the intensity used for rTMS was below motor threshold for all hand muscles. The use of sub-threshold intensity (i) avoided muscle twitches during rTMS that could modulate central processing via sensory afferents and (ii) reduced the spread of stimulation away from the targeted site. An intensity of 90% RMT was used because this is above the threshold for activating corticospinal output projections. This threshold is usually assessed by measuring the active motor threshold (the intensity needed to produce EMG activation in pre-contracting muscles) and is equivalent to approximately 80% RMT. Thus, we could be certain that the rTMS pulses would produce

synaptic activation in at least some of the anatomical targets of M1. Most of the assumptions and interpretation of the data relating to changes within M1 caused by rTMS was based on the following physical principle of TMS. The magnetic electric fields of transcranial magnetic stimulators decrease quickly with distance from the coil (Ruohonen & Ilmoniemi, 2002). It is therefore more likely that any rTMS effect would be strongest in brain regions located closest to the scalp, where the TMS is positioned. In M1, this would be in area 4a.

4.3.4 Motor Task

The scanning and task protocols used in this study are identical to those described in Siebner *et al.* (2003) and in Lee *et al.* (2003). Briefly, subjects underwent six sequential $H_2{}^{15}O$-PET scans on each of the two days. All scans were acquired within the first hour after conditioning the motor hand area with rTMS. Normalized rCBF-dependent ^{15}O uptake (referred to hereafter as rCBF) was used as an index of regional synaptic activity during two experimental conditions: baseline (B) and random selection of finger movements (M). Three PET scans were acquired for each experimental condition in alternating order (B-M-B-M-B-M or M-B-M-B-M-B). Subjects were required to keep their eyes open and fixate a cross on the centre of a screen located 0.7m in front of their face. A pacing tone sounded every 2 seconds during both conditions. During the movement task, subjects were required to freely select and execute brisk flexion movements with the index, middle, ring or little finger of their right hand. All responses were recorded by a computer (Apple Macintosh 7300) using COGENT Cognitive Interface Software (Wellcome Dept. of Imaging Neuroscience, London, UK). The data were subsequently analysed using Matlab 6.0 (Mathworks, Sherborn, MA) and SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA). To ensure a stable level of task performance, the movement task started approximately 20 seconds prior to the onset of the PET scan and lasted for the entire 90-second period of data acquisition. During the baseline condition, subjects were instructed to watch the fixation point and listen to the tones.

❖ **Behavioural assessment**

Subjects performed two additional finger-tapping tasks with their right hand after the first, third, and fifth PET scans. In the 'simple tapping task' subjects tapped their right index finger as many times as possible during a ten second interval. In the 'sequential tapping task' subjects were asked to repeat an ascending sequence (index, middle, ring, little finger) as quickly as possible for ten seconds. Subjects performed each of the three tasks twice in the PET scanner prior to rTMS on both scanning sessions.

The mean interval between responses and duration of button presses were calculated as indices of motor performance. A two-way ANOVA was performed to compare the two groups, with the factors group (1Hz rTMS versus 5Hz rTMS) and intervention (post-real rTMS versus post-sham rTMS). A paired-samples t-test was used for within-group comparisons of differences after real-rTMS and sham-rTMS. The mean duration of button presses during the scanned movement task were analysed using the procedure described above. Simpson's equitability index (Simpson EH, 1949) was calculated for sequential response pairs and taken as a measure of the randomness of the sequence. This index varies between 0 and 1. A value of 1 indicates that over a series of responses any given response was equally likely to be followed by any other response. Data from the three repetitions of this task, during each scan, were analysed to provide two values of randomness for each subject: one after sham-rTMS and one after real-rTMS. These data were analysed in the manner described above.

4.3.5 Functional Neuroimaging

❖ **PET data acquisition**

PET was performed using a CTI ECAT HR+ scanner (CTI, Knoxville, TN) in three-dimensional mode with inter-detector collimating septa removed. The axial field of view was 155 mm providing whole brain coverage including cerebellum. The subjects lay supine in the scanner. A padded helmet with a chinstrap, fixed to the headrest, reduced head movement. A TV monitor was adjusted to give subjects an unrestricted view of the instructions and fixation point.

Regional cerebral blood flow was assessed using $H_2{}^{15}O$. Six to ten mCi (mean 8.9 mCi) were delivered intravenously over 20s to the left arm. Image acquisition began 5s before the rising phase of the count curve, approximately 25-35s after injection, and continued for 90s. Correction for tissue and helmet attenuation was made using a transmission scan from $^{68}Ga/^{68}Ge$ sources at the start of each scanning session. The interscan interval was approximately 8 minutes. Corrected data were reconstructed by three dimensional filtered back-projection (Hanning filter, cut off frequency 0.5 cycles/pixel) and scatter correction. Sixty-three transverse planes were obtained with 128 x 128 pixel image matrix, with a pixel size of 2.4 x 2.1 x 2.1 mm, and a resolution of about 6 mm at full width half maximum.

Anatomic structural images were acquired prior to rTMS stimulation, with the TMS surface markers in place, using a SONATA MR scanner at 1.5 Tesla (Siemens, Erlangen, Germany) with a T1-weighted 3D MDEFT sequence. The isotropic spatial resolution was 1 mm. Relevant imaging parameters were TR/TE/TI = 12.24/3.56/530 ms, BW = 106 Hz/Pixel, flip angle =23deg (Deichmann et al., 2004). This structural image was also used to exclude asymptomatic structural brain abnormalities.

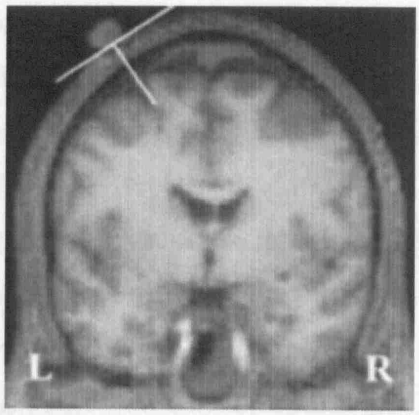

Figure 4.2: Location of the TMS Coil. Position of TMS coil shown on a coronal T1-weighted structural scan from one single subject.

❖ **Image analysis**

All image analysis was performed using Statistical Parametric Mapping software, SPM99 (Wellcome Department of Imaging Neuroscience, UCL, UK. http://www.fil.ion.ucl.ac.uk/spm). For each subject, images were realigned to the first image by rigid body correction for head movements between scans and change of

position between sessions (Friston et al., 1995a). All images were then normalised to a standardised anatomic space (Talairach J. and Tournoux P, 1998), by matching to a standardised PET template using linear and non-linear spatial transformations (Friston et al., 1995a). Each image was smoothed with an isotropic Gaussian kernel of 12 mm full-width at half-maximum, to accommodate inter-subject differences in anatomy and enable the application of Gaussian Field corrections during inference (Friston et al., 1995a).

The primary analysis employed a general linear model that included two groups (1Hz rTMS and 5Hz rTMS) of twelve regressors as covariates modelling the task (movement selection versus baseline) separately for each consecutive scan pair (first to third) under each condition of treatment (rTMS versus sham). The effect of global differences in cerebral blood flow among scans was removed by treating global activity as a confound and scaling to a nominal grand mean global activity of 50 ml/100g/min (Friston et al., 1995b). This statistical model enabled characterisation of the main effects of rTMS (rTMS versus sham), task (movement versus rest) and frequency (1Hz versus 5Hz). In addition, interactions between frequency, rTMS and movement were examined within and between groups. For the main effect of movement the reporting criterion was set at $P < 0.05$ corrected for multiple non-independent comparisons over the whole brain. Results for the main effects of rTMS and all interactions are reported at $P < 0.05$ using a small volume correction (16mm radius sphere centred on those maxima from the main effect of movement in table 2).

Changes in effective connectivity within the motor network were assessed using the 'Physio-physiological Interaction' (PPI) method described by (Friston et al., 1997) and mention in chapter 3. The PPI analysis explains responses in one cortical area in terms of an interaction between activity in another cortical area (index area) and the influence of an experimental parameter. The analysis is constructed to test for differences in the regression slope of the activity in all areas on the index area, under the different experimental conditions. As these regression slopes are a metric of the coupling between areas, the PPI identifies areas where the degree of correlation with the index region is modulated significantly by the experimental variable. In this study the model for the PPI analysis was constructed to identify differences in the effect of rTMS on movement related activity depending on the frequency used (1Hz versus 5Hz). While the

rTMS variable (1Hz rTMS versus sham and 5Hz rTMS versus sham) appears explicitly in the model, the effect of task (move versus baseline) enters vicariously as the principle cause of variance in the index region. Separate PPIs were used to test a priori hypotheses about the differential effects of 1Hz and 5Hz rTMS on cortical excitability at the site of stimulation, and on the connectivity on the motor areas engaged in task performance. The a priori hypotheses used in this study relied mainly on previous physiological evidence of the effects of 1 & 5 Hz rTMS (see below). Electrophysiological measures of changes in excitability were not acquired in this study due to time constraints and limitations in the use of subjects coming from the departmental volunteers' database.

The first PPI was constructed to test the hypothesis that the two frequencies of rTMS differentially modulate the excitability or responsiveness of the site of stimulation to activity in other motor areas. This hypothesis is based on neurophysiological data suggesting that 1Hz rTMS (Chen, 2004;Maeda et al., 2000;Touge et al., 2001) and 5Hz rTMS (Berardelli et al., 1998;Peinemann et al., 2000;Peinemann et al., 2004) have different effects on the responsiveness of M1 to exogenous inputs and the responsiveness of S1 to endogenous sensory inputs (Ragert et al., 2004;Knecht et al., 2003;Satow et al., 2003). The index area used to construct the regressors consisted of the first eigenvariate of the rCBF signal from a sphere (radius 8mm) centred on a voxel in M1 showing maximally increased activity after real-rTMS, closest to the average fiducial location from the structural scans (table 4.4) (Lee et al., 2003). The eigenvariate was adjusted for subject-specific effects. Two PPIs (regressors representing the interaction between physiological activity and experimental manipulation) were obtained by multiplying the eigenvariate by the rTMS specific effects. This was done separately for the two rTMS frequencies. Having included the effects of the physiological component (activity in the index region) and the two rTMS components (1Hz and 5Hz) in the same model, SPM was used to test for the within and between group differences in the PPIs. The SPM [t] testing the significance of each PPI indicates a difference in the regression slopes linking the activity in the index area to activity in significant brain areas, depending on the type of rTMS (real versus sham). Contrasts were used to identify significant differences in the PPIs at the two different frequencies. In order to

quantify the effects of rTMS on the motor network identified by the primary analysis, regression slopes were plotted for areas where $P < 0.05$ (corrected for a 16mm radius sphere centred on the maxima of the main effect of movement). In these analyses, a significant increase in the regression slope between two areas can be interpreted as a reduction in the magnitude of the response of the index area (the site of rTMS) to putative input from another brain area. Therefore, positive interactions identify regions whose activity during movement is associated with a smaller response at the site of rTMS after stimulation (i.e. decreased excitability).

The second analyses of effective connectivity were designed to test for changes in the coupling within the motor areas involved in the movement task that were differently affected by rTMS at low and high-frequencies. Three areas involved in action preparation and execution were chosen, based on known anatomical connections with the site of stimulation: left M1, left dorsal premotor cortex and left SMA from the main effects of movement (table 4.2). Separate PPI analyses were performed for each area. In each case the physiological explanatory variable was the first eigenvariate of the rCBF signal from a region of interest (sphere 8mm radius) identified previously by the main effect of movement. The PPIs were constructed and tested as described above. In these PPI analyses a significant increase in the regression slope between two areas (a positive interaction) tested for an expected increase in movement-related coupling between the index area and other significant sites after real-rTMS. Contrasts were used to identify significant frequency-dependent differences in the effect of rTMS on movement-related coupling.

The location of local peaks of activation in the sensorimotor areas 4a and 4p were related to the probabilistic cytoarchitectural population maps provided online (Brain Mapping Group, http://www.bic.mni.mcgill.ca/cytoarchitectonics/), (for further discussion of this technique, see Roland and Zilles 1998

4.4 RESULTS

No subjects reported adverse side effects during the course of the study, nor were any visible motor responses evoked during rTMS. Mean resting motor threshold was 57%, ranging from 44 to 72% of maximum output of the Magstim rapid stimulator.

4.4.1 Behavioural Data

All sixteen participants found the motor tasks easy to perform. One subject was excluded from the 1Hz-rTMS paired sample t-test in the sequential tapping task because she failed to make any presses with the ring or little fingers.

A two-way repeated measures ANOVA with stimulation frequency (1Hz versus 5Hz rTMS) and type of rTMS (real versus sham) as main factors revealed no significant main effects or interactions, indicating that both groups performed the three tasks similarly and that motor performance during PET scanning was unaffected by rTMS.

Table 4.1 shows the averaged group values (mean +/- SD) of the variables used to assess behaviour after rTMS in both groups of subjects in terms of motor performance and free selection of movement measured by Simpson's equitability index. The results of the individual paired sample t-tests performed for the 1Hz and 5Hz rTMS groups are also shown. Statistical analysis of these data showed that neither 1Hz nor 5Hz rTMS had any significant effect on motor performance or free selection of movement. In the index tapping task and the sequential tapping task there was no effect of either frequency of rTMS on the duration of button presses or on the interval between button presses. During freely selected finger movements rTMS had no effect on the duration of button presses or on the randomness of the responses.

		Post-Sham	Post-TMS	t	P
Index tapping					
(n =16)					
Duration (ms)	**1Hz**	111.73(+/-21.296)	119.79(+/-22.36)	-1.49(df=7)	0.18
	5Hz	119.68(+/-17.82)	115.43(+/-20.02)	0.67(df=7)	0.53
Interval (msec)	**1Hz**	198.16(+/-16.19)	199.48(+/-18.39)	-0.38(df=7)	0.71
	5Hz	210.65(+/-27.71)	202.64(+/-18.91)	0.9(df=7)	0.4
Sequential tapping					
(n=15)					
Duration (ms)	**1Hz**	242.22(+/-180.98)	244.61(+/-154.68)	-0.12(df=6)	0.91
	5Hz	186.53(+/-40.69)	189.79(+/-53.72)	-0.2(df=7)	0.85
Interval (ms)	**1Hz**	310.68(+/-80.53)	324.26(+/-67.6)	-0.73(df=6)	0.5
	5Hz	210.65(+/- 27.71)	202.64(+/-18.91)	0.89(df=7)	0.4
Random selection task					
(n=16)					
Duration (ms)	**1Hz**	234.89(+/-50.89)	224.5(+/-57.87)	0.83(df=7)	0.43
	5Hz	221.02(+/-34.7)	207.77(+/-25.42)	-0.42(df=7)	0.69
Simpson's equitability index	**1Hz**	0.77(+/-0.13)	0.76(+/-0.13)	0.59(df=7)	0.57
	5Hz	0.68(+/-0.05)	0.7(+/-0.08)	-0.56(df=7)	0.6

Table 4.1: Frequency-dependent changes in behaviour following rTMS to M1. Mean group data (+/- SD) for each group of kinematic measures: mean duration of key presses and interval between key presses for index tapping and sequential motor tasks and mean duration of key presses for the paced free selection task.

4.4.2 Imaging Data

▪ 4.4.2.1 Movement-related changes in rCBF (main effect of task)

The main effect of movement was associated with increased rCBF in a well-defined network of areas engaged in freely selected right-hand movements (Table 4.2) (reported at $p<0.05$ corrected for multiple comparisons). These comprised left M1, extending to the left dorsal and ventral premotor cortices, left SMA and left rostral cingulate motor area. Other areas included the lateral and medial prefrontal cortices bilaterally, the left insula, the right and left secondary somatosensory areas and cerebellum. There were no significant differences in movement-related activation between the two groups of subjects (i.e. no movement-by-group interactions).

Anatomical Location		MNI Coordinates of Peak activation			P value whole volume Corrected	Z value of peak activation
		X	y	z		
Primary Sensorimotor	Left	-42	-28	56	<0.001	>8
Premotor (PMd)	Left	-34	-16	64	<0.001	>8
Dorso-lateral Prefrontal area	Right	34	4	54	0.002	5.4
Premotor (PMv)	Left	-58	0	32	<0.001	>8
Cingulate motor (caudal)	Left	-8	-8	56	<0.001	6.89
	Left	-4	6	46	<0.001	6.5
Cerebellum	Left	-24	-60	-26	<0.001	5.9
Secondary Somatosensory	Left	-58	-24	44	<0.001	>8
	Right	58	-28	44	<0.001	5.73
	Right	66	-20	18	0.001	5.48
Superior Frontal Sulcus	Left	-30	38	26	0.008	5.09
	Right	16	2	58	0.004	5.42
	Right	22	-52	-24	<0.001	>8
Middle frontal Gyrus	Right	38	42	26	0.001	5.53
Inferior Frontal Gyrus	Right	64	8	20	0.057	4.67
Superior Temporal Gyrus	Left	-40	-32	12	0.001	5.01
Insula	Left	-44	-4	4	<0.001	6.29

Table 4.2: Main effects of movement. Maxima of regional increases in normalized rCBF during movement compared to baseline.

- **4.4.2.2 Changes in rCBF induced by rTMS**

The changes in rCBF induced by low-frequency, 1Hz, rTMS have already been described in the study by Lee *et al.* (2003). Therefore we will focus on the main effects of 5Hz rTMS and the comparison of effects of 1Hz and 5Hz rTMS.

- **4.4.2.2.1 Changes in rCBF induced by high-frequency (5Hz) rTMS**

5Hz rTMS caused widespread increases in rCBF at the site of stimulation (M1: x = -22, y = -18, z = 72; Z = 7.23; $p<0.001$), the left ventral premotor area, bilaterally in prefrontal and somatosensory cortices, anterior cingulate motor, intraparietal and superior parietal areas. Decreases in rCBF were seen in the superior cerebellum and superior temporal gyri bilaterally. The relative increases and decreases in rCBF following 5Hz rTMS are listed in table 4.3.

Anatomical Location		MNI Coordinates of peak activation			P value whole volume corrected	Z value of peak activation
		X	Y	z		
3a) Increased rCBF						
Primary sensorimotor	Left	-22	-18	72	<0.001	7.23
	Left	-24	-44	48	0.04	4.71
Premotor (PMd)	Left	-34	2	52	<0.001	>8
	Right	40	2	48	<0.001	7.12
Premotor (PMv)	Right	62	12	36	0.002	5.39
	Right	64	10	32	0.02	4.9
Cingulate Motor (caudal)	Left	-6	-18	46	0.014	4.98
	Right	16	-28	42	<0.001	5.91
Primary somatosensory	Right	62	-18	44	<0.001	>8
	Right	52	-22	42	<0.001	5.93
	Left	-32	-54	60	<0.001	5.72
Prefrontal (Dorsolateral)	Right	38	40	28	<0.001	6.48
	Left	-36	34	46	<0.001	6.07
	Left	-30	44	22	0.003	5.34
Intra-Parietal Sulcus	Right	40	-52	46	<0.001	6.17
	Left	-46	-48	46	0.006	5.18
Superior Parietal cortex	Right	12	-60	56	0.031	4.81
Decreased rCBF						
Superior cerebellum (Anterior lobe)	Right	14	-40	-22	<0.001	>8
Superior Cerebellum (Posterior lobe)	Right	46	-56	-40	0.016	4.96
	Left	-32	-66	-22	<0.001	6.87
Cerebellum (Uvula)	Right	12	-70	-32	0.001	5.77
Superior Temporal Gyrus	Left	-48	-8	-4	0.002	5.35
Superior Temporal Gyrus	Right	58	16	-24	0.022	4.88

Anatomical Location		*MNI Coordinates*			*P value*	*Z value*
		of peak activation			**whole volume**	**of peak**
		X	**y**	**z**	**corrected**	**activation**
Superior Temporal Gyrus	Right	34	-68	10	<0.001	6.05
	Right	58	-46	-28	0.001	5.5
Claustrum	Left	-34	0	-6	0.002	5.42
Fusiform gyrus	Left	-24	-56	-8	<0.001	7.62
Orbitofrontal cortex	Left	-30	10	-16	<0.001	>8

Table 4.3: Main effects of 5Hz rTMS masked by the main effects of movement (table 4.2). 3a) Main effect of rTMS – increases: maxima of regional increases in normalized rCBF after real-rTMS compared to sham. 3b) Main effect of rTMS – decreases: maxima of regional decreases in normalized rCBF after real-rTMS compared to sham.

- **4.4.2.2.2 Changes in rTMS common to both rTMS frequencies (Conjunction of the simple main effects of rTMS)**

A conjunction analysis of the main effects of rTMS between the two groups was used to identify areas of increased and decreased rCBF common to both frequencies. The results are reported in Table 4.4 at $p < 0.05$ corrected for multiple comparisons (see also figure 4.3).

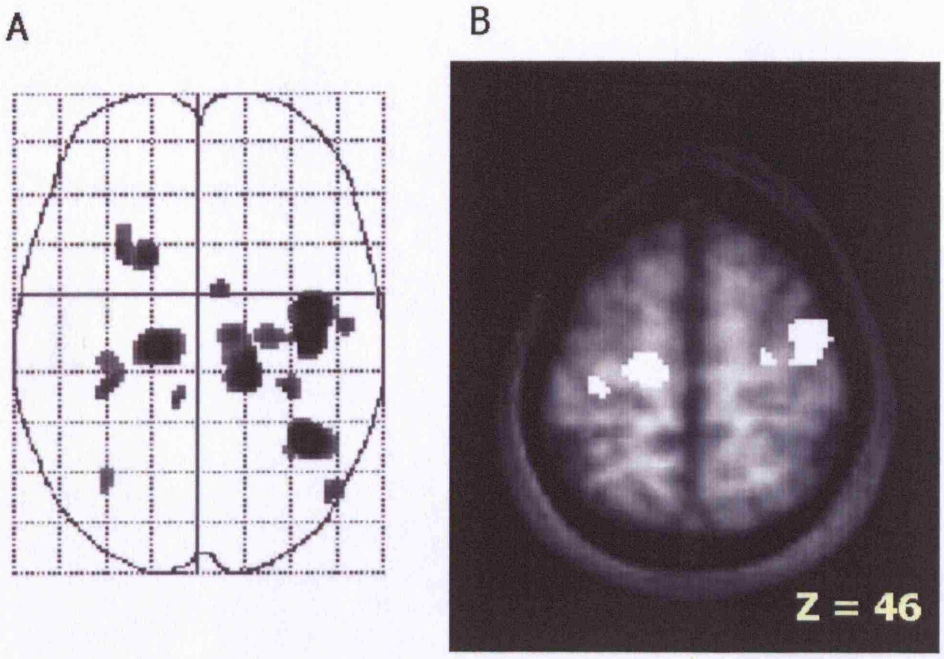

Figure 4.3: Conjunction of Main Effects: Increases. Increases in regional cerebral blood flow following 1 and 5Hz rTMS to M1 (conjunction of the main effect of rTMS). Results are displayed A) as a statistical parametric map on transverse section in stereotactic space and B) on a transverse section of the averaged T1-weighted structural brain scans of 15 subjects.

Common increases in rCBF after 1Hz and 5Hz rTMS were seen in the left and right M1, extending to left SMA, right dorsal and ventral premotor areas, left ventromedial prefrontal cortex, bilateral caudal and rostral cingulate motor areas. Areas showing conjoint decreases in rCBF following rTMS included the right putamen, left head of caudate and the right cerebellar vermis.

Anatomical Location		MNI Coordinates of peak activation			P value whole volume corrected	Z value of peak activation
		x	y	Z		
4a) Increased rCBF						
Sensorimotor cortex	Right	40	-16	46	<0.001	>8
	Right	36	-34	60	0.047	4.82
	Left	-32	-24	62	0.014	5.08
Premotor (PMd)	Right	44	-12	66	<0.001	6.84
	Right	40	-4	62	<0.001	6.53
	Right	42	-10	56	<0.001	6.6
	Right	26	-16	66	<0.001	5.92
Premotor (PMv)	Right	54	-10	18	0.002	5.51
SMA	Left	-14	-20	64	<0.001	7.12
Cingulate Motor (caudal)	Left	-10	-20	44	0.035	4.88
	Right	18	-28	38	<0.001	7.36
	Right	12	-14	38	0.001	5.59
Cingulate Motor (rostral)	Left	-18	12	44	<0.001	6.05
Caudate	Right	8	2	8	0.001	5.63
Temporo-Parietal Junction	Right	50	-70	22	0.002	5.54
Inferior Frontal Sulcus	Left	-28	16	32	0.001	5.72
	Left	-28	22	22	0.007	5.23
Insula	Right	32	-34	18	<0.001	5.88
	Left	-32	-32	22	0.001	5.72
Posterior Temporal Cortex	Left	-34	-66	18	0.007	5.22
4b) Decreased rCBF						
Cerebellum	Right	4	-64	-26	0.006	5.25
Putamen	Right	32	8	0	0.007	5.23
Head of Caudate	Left	-12	6	6	<0.001	6.51
Insula	Left	-40	12	4	0.046	4.82

Anatomical Location		*MNI Coordinates*			*P value*	*Z value*
		of peak activation			**whole volume**	**of peak**
		x	**y**	**z**	**corrected**	**activation**
Pars Opercularis	Left	-50	20	-4	0.001	5.66
	Left	-44	12	22	0.02	5.01
Superior Temporal Gyrus	Left	-52	-10	-6	<0.001	>8
	Right	40	-30	2	<0.001	6.28
	Right	58	14	-28	0.034	4.89
Middle Temporal Gyrus	Left	-54	-40	-8	<0.001	>8
Inferior Temporal Gyrus	Right	42	-50	-16	0.005	5.31
Frontal Pole	Left	-12	70	4	0.01	5.16
	Left	-10	66	-6	0.025	4.96
	Left	-20	66	12	0.021	5
	Right	10	64	-8	0.043	4.84
Pons	Right	2	-26	-26	<0.001	>8
Parahippocampal gyrus	Left	-20	-36	-10	<0.001	6.39
	Left	-20	0	-28	<0.001	6.54
	Right	24	0	-38	0.033	4.9
Hypothalamus	Left	-4	-10	-8	0.001	5.58

Table 4.4: Conjunction of main effect of 1 and 5Hz-rTMS. 4a) Increases: Maxima of regional increases in normalized rCBF after real-rTMS. 4b) Decreases: Maxima of regional rCBF decreases after rTMS. $P < 0.05$ (whole volume corrected).

- **4.4.2.2.3 Frequency-dependent differences in rCBF changes (rTMS-by-group interactions)**

Areas which showed significant differences in rCBF following 1Hz rTMS compared to 5Hz rTMS are shown in table 4.5. In the motor system, 1Hz rTMS caused greater increases in rCBF in the right and left cerebellar cortices than 5Hz rTMS. Conversely, 5Hz rTMS caused greater increases in rCBF than 1Hz bilaterally in rostral PMd, dorsolateral and medial prefrontal cortex.

Anatomical Location		MNI Coordinates of peak activation			P value whole volume corrected	Z value of peak activation
		x	y	Z		
1Hz-rTMS>5Hz-rTMS						
Cerebellum	Right	22	-50	-16	<0.001	>8
	Right	10	-36	-22	<0.001	6.24
	Left	-26	-62	-20	<0.001	7.21
	Left	-20	-58	-32	<0.001	5.68
	Left	-10	-68	-38	0.005	5.21
	Left	-32	-54	-40	0.021	4.9
Globus Pallidus	Right	22	-2	-6	0.006	5.16
Prefrontal cortex	Left	-54	38	-2	0.015	4.97
Insula	Left	-30	16	-11	<0.001	5.69
Inferior Temporal cortex	Left	-36	4	-30	0.002	5.42
Precuneus	Left	-24	-56	26	0.018	4.93
Inferior Occipital cortex	Right	18	-84	-8	0.035	4.78
Hippocampus	Left	-36	-26	-6	0.02	4.91
Amygdala	Left	-30	0	-14	<0.001	5.71
5Hz-rTMS>1Hz-rTMS						
Premotor (PMd)	Right	40	8	52	<0.001	7.39
	Left	-34	0	52	<0.001	6.91
Anterior Cingulate (rostral)	Right	4	30	40	<0.001	6.92
Cingulate Motor (Caudal)	Right	6	0	38	<0.001	5.78
Cerebellum	Right	34	-36	-28	0.04	4.75
	Left	-2	-42	-6	0.003	5.32
Primary Somatosensory	Right	60	-16	40	0.002	5.37
Secondary Somatosensory	Right	46	-20	28	0.001	5.54
	Left	-40	-24	36	0.034	4.79
Superior Frontal Gyrus	Right	4	22	56	<0.001	7.78
Superior Frontal Sulcus	Left	-28	44	24	0.002	5.44

Anatomical Location		*MNI Coordinates*			*P value*	*Z value*
		of peak activation			**whole volume**	**of peak**
		x	**y**	**Z**	**corrected**	**activation**
Inferior Frontal Gyrus	Right	46	30	26	<0.001	5.73
Superior Temporal Gyrus	Right	50	-14	-8	<0.001	7.04
Orbitofrontal Cortex	Right	32	26	-12	0.014	4.98

Table 4.5: Frequency-by-rTMS interactions masked by the main effects of movement.

- **4.4.2.2.4 Frequency-dependent changes in task-related activations (Movement-by-rTMS-by-group)**

A difference in task-related changes in rCBF after rTMS at 1Hz and 5Hz was seen in left Brodmann area 4p, deep in the central sulcus (figure 4.4) (x = -28, y = -24, z = 46; Z = 3.38; p = 0.044). Figure 4.4 shows the location and contrasts of parameter estimates for this site. Task-related response in this area increased after 1Hz-rTMS for the duration of the scanning session. After 5Hz-rTMS there is a decrease in task-related response, especially marked in the first pair of scans following. No task-related changes in rCBF were seen in motor areas following 5Hz TMS.

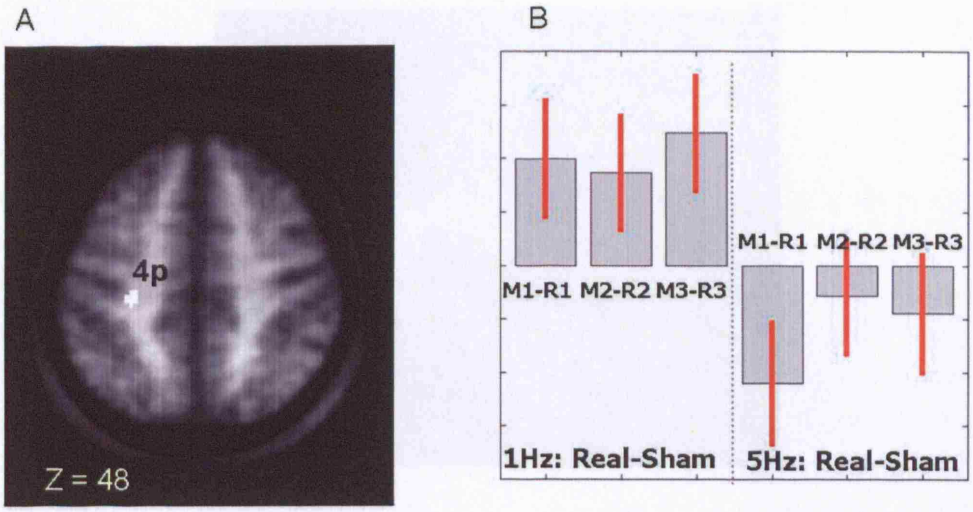

Figure 4.4: Task by frequency by intervention interaction. A) Location frequency-by-intervention-by-task interaction: area 4p (x = -28, y = -26, z = 48) displayed on a transverse section of the averaged T1-weighted structural brain scans (average of 15 subjects. B) Parameter estimates of the difference in mean (+/-SE) task-related activations between real and sham conditions in each of the 3 pairs of move-baseline (M-B) scans for i) 1Hz-rTMS and ii) 5Hz-rTMS at the interaction site. As can be seen in figure 1, all participants underwent 6 consecutive PET scans at Baseline (B) or during movement (M). These have been grouped into 3 pairs of scans. M1-B1: difference of the average of the first move and rest scans. M2-B2: difference of the average of the second move and rest scans. M3-B3: difference of the average of the third move and rest scans.

- **4.4.2.2.5 Frequency-specific PPIs between site of rTMS and other areas**

There were significant differences in frequency-specific modulation of the coupling between the site of rTMS and activity in left area 4p (x = -38, y = -20, z = 42; Z = 3.27, p = 0.048). Previous results (Lee *et al.*, 2003) suggest that 1Hz-rTMS reduced the responsiveness of the site of stimulation to inputs from distant areas. The difference in regression slopes between the two areas (site of stimulation and area 4p) after real and sham 5Hz-rTMS extend this finding and suggests that the site of rTMS stimulation is more responsive to inputs from area 4p following 5Hz-rTMS relative to 1zrTMS (see figure 4.5).

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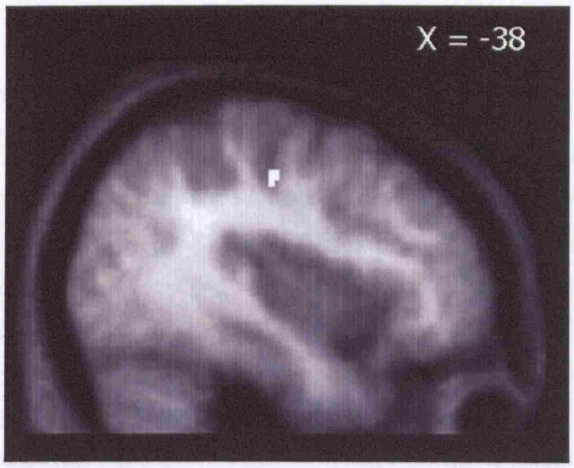

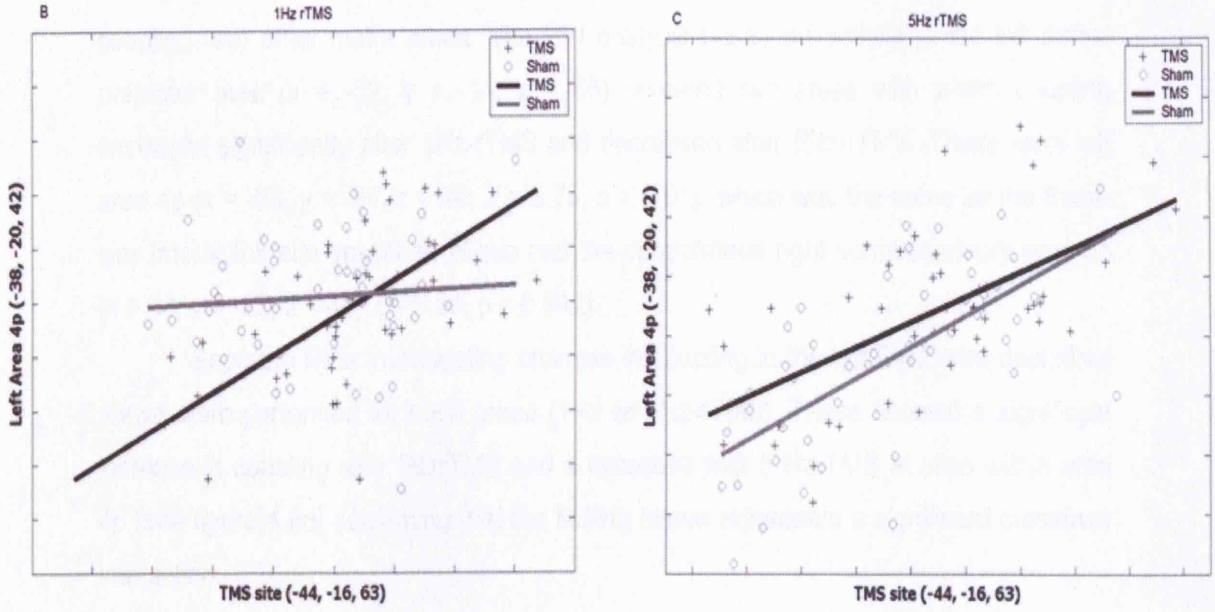

Figure 4.5: Frequency-dependent changes in effective connectivity (physiophysiological interaction) with the site of rTMS stimulation. a) Anatomical location on a sagittal slice (average T1-weighted structural scans) of the significant area obtained from the first PPI analysis (x = - 38, y = -20, z = 42; = 3.27, p = 0.048). Left area 4p showed an increase in coupling with the TMS-site following 1Hz-rTMS and a decrease in coupling, albeit with a smaller amplitude following 5Hz-rTMS. Graphical representations illustrating the physiophysiological interactions between the site of rTMS region of interest (x = -44, y = -16, z = 63) (abscissa) and area 4p revealed from the analysis (x = -38, y = -20, z = 42) (ordinate). PPI plots for b) 1Hz and c) 5Hz-rTMS respectively between the activities in the two regions. TMS=Real-rTMS (crosses), SHAM=Sham-rTMS (circles) session. Group1: 1Hz Sham-rTMS: r^2 = 0.00, F = 0.26, gradient of the slope = 0.06; 1Hz Real-rTMS: r^2 = 0.45, F = 37.01, gradient = 0.69. Group2: 5Hz Sham-rTMS: r^2 = 0.43, F = 35.03, gradient = 0.52; 5Hz Real-rTMS: r^2 = 0.34, F = 23.99, gradient = 0.42. Although 5Hz rTMS leads to a smaller gradient change, it is still statistically significant and in the opposite direction to the change induced with 1Hz rTMS. (Least square statistics using the matlab statistic toolbox)

- **4.4.2.2.6 Frequency-specific PPIs between motor areas engaged in the task**

Figure 4.6 summarizes the results obtained from the analyses of effective connectivity looking for frequency-specific changes in coupling between components of the motor system activated during the movement task. The PPI analyses based on

activity in left M1 area and left SMA did not reveal frequency-specific differences in coupling with other motor areas. The PPI analysis based on activity in the left dorsal premotor area (x = -32, y = -14, z = 66) revealed two areas with which coupling increased significantly after 1Hz-rTMS and decreased after 5Hz-rTMS. These were left area 4p (x = -28, y = -24, z = 46; Z = 3.76, p = 0.01), which was the same as the three-way interaction site described above and the contralateral right somatosensory area 3b (x = 48, y = -22, z = 44; Z = 3.39, p = 0.042).

Separate PPIs investigating changes in coupling in the left PMd area described above were performed for each group (1Hz or 5Hz-rTMS). These showed a significant increase in coupling with 1HzrTMS and a decrease with 5 HzrTMS at sites within area 4p (see figure 4.6d) confirming that the finding above represents a significant crossover interaction.

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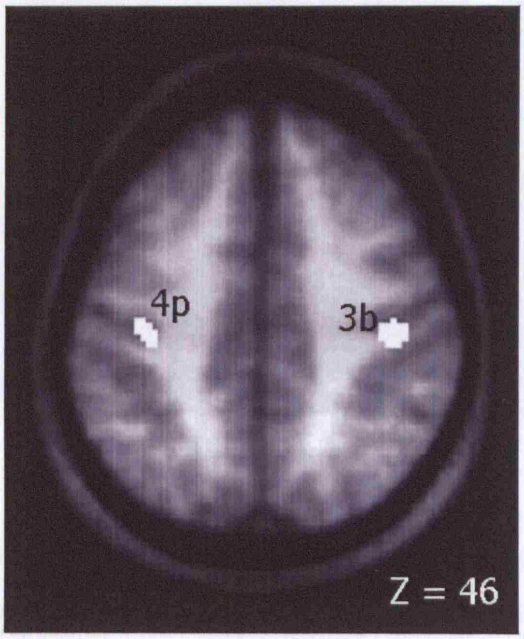


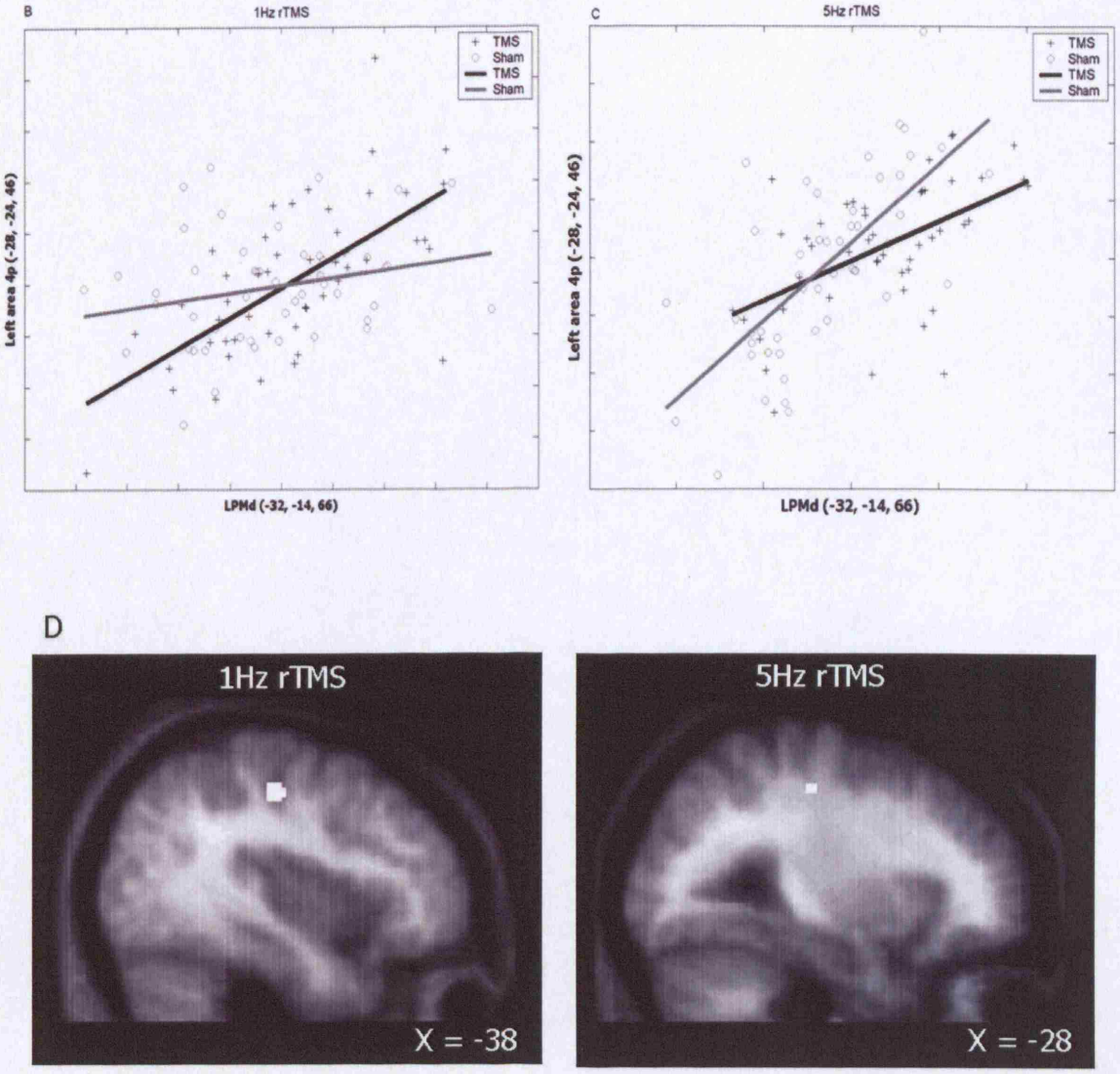


Figure 4.6: Frequency-dependent changes in effective connectivity (physiophysiological interaction) with the left PMd (x = -32, y = -14, z = 66) from the main effect of movement (table 4.2). A) Anatomical location on a transverse slice (average T1-weighted structural scans) of the significant areas obtained from the second PPI analysis. Left area 4p (x = -28, y = -24, z = 46; Z = 3.76, p = 0.01) shows a positive PPI with the left PMd following 1Hz-rTMS and a negative PPI following 5Hz-rTMS. B) Graphical representations illustrating the physiophysiological interactions between the left PMd region of interest (x = -32, y = -14, z = 66) (abscissa) and area 4p revealed from the analysis (x = -28, y = -24, z = 46) (ordinate) for 1Hz and C) 5Hz-rTMS respectively. TMS=Real-rTMS (crosses), SHAM=Sham-rTMS (circles) session. Group1: 1Hz Sham-rTMS: r^2 = 0.06, F =

2.72, gradient = 0.11; 1Hz Real-rTMS: r^2 = 0.42, F = 33.42, gradient = 0.00. Group2: 5Hz Sham-rTMS: r^2 = 0.47, F = 41.43, gradient = 0.00; 5Hz Real-rTMS: r^2 = 0.28, F = 18.35, gradient = 0.00. D) Anatomical location on a sagittal slice (average T1-weighted structural scans) of the significant sites within area 4p found in the interaction of PPIs described above, when the coupling with left PMd following 1HzrTMS (top – area showing increase in coupling) and 5HzrTMS (bottom – area showing decrease in coupling) were analysed separately, confirming the crossover interaction shown in graphs B and C. (Least square statistics using the matlab statistic toolbox)

4.5 DISCUSSION

4.5.1 Frequency-Dependent Changes in rCBF following rTMS

Previous PET studies have shown that high frequency rTMS to M1 (5Hz) (Siebner et al., 2000) and prefrontal cortex (10Hz) (Paus et al., 2001) produces lasting increases in regional glucose metabolism and rCBF respectively at the site of stimulation. Low-frequency sub-threshold rTMS to M1 produces lasting increases in rCBF locally and in adjacent and contralateral motor areas (Lee et al., 2003d).

These results extend previous imaging studies of motor cortex rTMS (Fox et al., 1997;Siebner et al., 2000;Siebner et al., 2001). Stimulation of M1 with 1Hz and 5Hz-rTMS induces similar changes in rCBF at the site of stimulation and in remote areas, despite neurophysiological evidence that these frequencies do not have the same effect on cortical excitability. 1Hz-rTMS produces lasting inhibition of cortico-cortical and corticospinal excitability (Chen et al., 1997;Muellbacher et al., 2000;Tsuji and Rothwell, 2002) whereas 5Hz-rTMS produces increased cortical excitability (Berardelli et al., 1998;Di Lazzaro et al., 2002;Gangitano et al., 2002) that can outlast the period of stimulation by up to 1 hour (Peinemann et al., 2004).

Common increases in rCBF following stimulation with the different frequencies suggests that the direction of rCBF changes observed following conditioning with rTMS may depend more on the site of stimulation than the frequency used. This is supported by the findings of Siebner et al., (2003). Using the same experimental protocol as this study the authors showed that stimulation of the left dorsal premotor cortex at 1Hz-rTMS

led to widespread bilateral decreases in rCBF at the site of stimulation and connected areas.

In this study, some frequency-specific differences in rCBF are seen in areas remote from the site of stimulation. In the absence of neurophysiological data we do not have any specific hypothesis about differential remote effects on cortical or sub-cortical excitability of stimulating at these two frequencies. Any interpretation of differences in rCBF changes with respect to cortical / sub-cortical excitability is therefore problematic.

Taken together, these data and those of Siebner et al. (2001, 2003, (Takano et al., 2004)) emphasise the difficulty of interpreting increases and decreases in rCBF as the neural correlates of increased and decreased cortical excitability. Changes in rCBF reflect changes in synaptic activity of both inhibitory and excitatory neurons; inferences about the effects of rTMS on cortical excitability require additional information from motor threshold, MEP recruitment curves, silent period duration and intracortical inhibition / facilitation curves. In non-primary motor areas this information is more difficult to ascertain. A strict dichotomy between the corticospinal effects obtained with high and low-frequency rTMS may be an oversimplification. The effects of rTMS appear to depend on the excitability of the stimulated cortex prior to conditioning. (Ziemann et al., 1998) demonstrated that the response to rTMS is influenced by changes in cortical excitability induced by acute deafferentation. (Siebner et al., 2004) have shown that preconditioning M1 with cathodal transcranial direct current stimulation (TDCS) reverses the inhibitory effects of 1Hz rTMS, resulting in a paradoxical facilitation.

4.5.2 Frequency-Dependent Changes in Responsiveness of the Site of Stimulation Following rTMS

The first PPI analysis suggests that 1Hz and 5Hz rTMS have opposite effects on the synaptic efficacy of inputs to the site of stimulation. Following 5Hz-rTMS the site of stimulation appears to become more responsive to inputs from area 4p whereas the opposite occurs after 1Hz-rTMS. These results confirm the initial hypothesis that increased cortical excitability induced by 5Hz-rTMS has an opposite effect on the connectivity of the motor network from the decreased cortical excitability induced by

1Hz-rTMS. This interpretation is supported by previous studies (Ragert et al., 2004) showing that 5Hz-rTMS to S1 increases excitability and improves tactile discrimination, suggesting that responsiveness to inputs increases following stimulation at 5Hz. In contrast, Satow et al (2003) showed increased tactile thresholds following 1Hz rTMS to sensory cortex (S1) and Knecht et al (2003) showed decreased tactile discrimination following 1Hz rTMS to S1, suggesting 1Hz rTMS reduces responsiveness to inputs (Knecht et al., 2003;Satow et al., 2003).

4.5.3 Role of Primary And Non-Primary Motor Areas Following Altered Excitability Of The Motor Cortex

The two frequencies of stimulation resulted in task-specific differences in rCBF, while motor behaviour remained unchanged. This frequency-dependent difference in the effect of rTMS on movement-related activation suggests that maintenance of motor performance may involve different mechanisms depending on the specific alterations in cortical excitability. Following 1Hz-rTMS, Area 4p is more active during movement than baseline whereas movement related activity in the same location is reduced following perturbation of cortical excitability by 5Hz-rTMS. It may be the case that increased motor cortex excitability induced by 5Hz-rTMS facilitates the contribution of superficial motor areas (preferentially stimulated by TMS) to motor output. By contrast, inhibition of superficial motor areas by 1Hz rTMS may necessitate greater involvement of inferomedial motor cortex to maintain motor performance (Binkofski et al., 2002;Lee et al., 2003a).

The second PPI analysis suggests that movement-related coupling between the left PMd area engaged in task performance and left Area 4p is differentially affected by the two rTMS frequencies: coupling between these two areas decreased after 5Hz-rTMS and increased after 1Hz-rTMS. This relative decrease in coupling between left PMd and left area 4p following 5Hz rTMS may reflect a shift in activity towards an area of enhanced excitability (and hence responsiveness to cortical inputs) at the site of

stimulation with rTMS. This is the opposite effect to that described following 1Hz rTMS by Lee et al (2003).

These results suggest that rapid reorganisation of the motor system occurs to maintain task performance during periods of altered cortical excitability. This reorganisation differs according to the modulation of excitability which is a function of rTMS frequency. Our hypothesis that changes in the activity and connectivity of left motor and premotor cortices may represent compensatory activity is supported by the reorganisation observed in animal studies where motor cortical excitability is modulated by pharmacological inactivation (Nudo and Milliken, 1996;Nudo, 1999;Nudo et al., 2001) or use-dependent plasticity (Nudo et al., 1996;Nudo and Friel, 1999). These manipulations have opposite effects on cortical excitability that result in different effects on the connectivity of motor networks. This formulation has interesting parallels with the findings of cortical hyperexcitability in perilesional areas in animal models of stroke (Buonomano and Merzenich, 1998;Donoghue et al., 1990;Hess and Donoghue, 1996;Jacobs and Donoghue, 1991) in that increasing excitability by whatever mechanism may increase responsiveness to inputs, thus facilitating plasticity.

The hypothesis that ipsilesional motor and premotor areas are functionally relevant in maintaining motor performance during periods of abnormal cortical motor excitability is supported by studies examining the involvement of the ipsilesional motor (Werhahn et al., 2003) and premotor (Fridman et al., 2004) cortices in recovery from stroke in humans Fridman *et al.* 2004 tested the functional contribution of ipsilesional dorsal premotor cortex in chronic stroke patients with good motor recovery by using TMS to delay reaction times in the paretic hand in a simple reaction time task. Werhahn *et al.* (2003) showed that TMS to the lesioned M1 caused delayed reaction times in the paretic hand that correlated with the degree of functional recovery. Further support for the role of ipsilesional premotor cortex comes from animal lesion studies – see chapter 2 in this thesis (Frost et al., 2003;Liu and Rouiller, 1999), which suggest that the integrity of this area is vital for functional recovery following unilateral lesions of the sensorimotor cortex in adult monkeys. Studies on patients recovering from middle cerebral artery infarctions (Seitz et al., 1998;Weiller et al., 1992) have also described the role of this area in

recovery. This study extends these findings by suggesting that depending on the excitability of M1, its coupling with the PMd can be bidirectionally modulated.

4.6 CONCLUSION

This experiment revealed that high-frequency (5Hz) rTMS over M1 induces changes in activity in local and remote areas of the motor system, similar to those observed when stimulating with 1Hz rTMS (Lee *et al.* 2003), despite evidence that these frequencies of stimulation have opposite physiological effets. This is a powerful demonstration of the dissociation between changes in rCBF and changes in excitability. Bi-directional effects, induced when stimulating with1Hz and 5Hz as shown in physiological studies, *were* found when looking at the movement-related activity and connectivity during the performance of a simple motor task. 5Hz rTMS induced *decreases* in the activity of area 4p and its coupling with the left PMd. The opposite was true with 1Hz rTMS. These results suggest that these areas are crucial for maintaining task performance during periods of altered excitability and extends previous work on the reorganization of the motor system by demonstrating that similar changes as observed in various cases such as lesions to motor areas or motor learning can be induced with rTMS as well. Moreover, the way the motor system compensates for these changes is very similar. RTMS could therefore provide a useful tool for creating models of motor recovery under various task conditions.

5. Site-specific changes in regional cerebral blood flow and motor system connectivity following rTMS to the dorsal premotor cortex

5.1 INTRODUCTION

Most studies of the effects of rTMS focused on the primary motor hand area (M1) where there is neurophysiological evidence that they lead to lasting effects on the excitability of corticospinal projections (Pascual-Leone *et al.* 1994, Chen *et al.* 1997) without affecting basic motor behaviour (Muellbacher *et al.* 2000). Functional neuroimaging have recently provided evidence of much more widespread effects both at the site of stimulation and in distant connected structures (chapter 3, Fox *et al.* 1997, Siebner *et al.* 2001).

In the previous section on M1, we saw that rTMS led to changes in the pattern of task-related activity and connectivity, suggesting that the brain reorganizes in response to the disruption caused by stimulation, perhaps reducing the impact on task performance (Lee *et al.* 2003, Rounis *et al.* 2005). The primary aim of the study in the present chapter was to test if similar reorganization occurs after rTMS to a different frontal cortical area, the left PMd.

A study by Siebner *et al.* (2003) used PET to examine the effects of 1Hz rTMS over the left PMd on the pattern of cortical activity using the same paradigm as described in the previous chapter. However, it was designed to compare results in a group of healthy volunteers with a group of patients with focal hand/arm dystonia (Siebner *et al.* 2003). rTMS led to a generalized decrease in rCBF in PMd and connected cortical and subcortical motor structures, but there was no effect on task-related activation; connectivity was not investigated. In the present study we have explored these effects in more detail in a total of 30 healthy subjects. In this chapter, I used 5 Hz rTMS rather than 1 Hz rTMS. This is because physiological evidence

suggests that bidirectional effects on the excitability of M1 similar to those reported following M1 conditioning, were found following 1Hz (Gerschlager *et al* 2001) and 5Hz (Rizzo *et al* 2004) rTMS over left PMd.

One feature common to the design of many rTMS interventions for therapy is that the stimulation is given in several short blocks of rTMS, each separated from the next by a rest period of seconds or minutes. This makes the procedure more comfortable for patients as well as reducing coil heating problems in long rTMS sessions. Depending on the frequency of stimulation it may also be a safety requirement to limit the number of high frequency pulses given in a certain period of time. However, it is not clear how the duration of this rest period may change the long term response of the brain to rTMS. In a preliminary series of physiological experiments to address the question on the premotor cortex i found that increasing the interval between short trains of 5 Hz rTMS from 1 min to 5 min reduced or abolished the after effects on excitability of motor cortex. In view of this the experiments were extended and PET methods used to document the extent of these changes in all of the circuits affected by 5 Hz stimulation. The data show that the duration of the rest period has a surprisingly strong effect on the final pattern of response to rTMS. Two patterns of stimulation were chosen. The first pattern of stimulation, with a 1 minute inter-train interval (ITI) between the stimulation blocks, corresponds to the pattern known to produce strong physiological effects on the excitability of M1 when applied to PMd (Rizzo *et al.* 2004). The second pattern of stimulation, using a 5min ITI, aimed to match the duration of stimulation with previous studies in our group that used 1Hz rTMS to stimulate M1 and PMd (Lee et al. 2003, Siebner et al. 2003). In both sets of experiments the total number of TMS pulses was the same, 1800, administered in 6 trains each lasting 1min.

5.2 MATERIALS AND METHODS

5.2.1 Subjects

Right-handed volunteers, with no history of neurological disorder or head injury were recruited from the database of volunteers at the Functional Imaging Laboratory, Institute of Neurology, University College London, UK. Sixteen volunteers (1 female; age range: 21 to 37) and 14 volunteers (5 females; age range: 24 to 37) participated in a neuroimaging experiment and an electrophysiological study respectively (3 in both). Written informed consent was obtained from all participants. The study was approved by the joint ethics committee for the National Hospital for Neurology and Neurosurgery (UCLH NHS Trust) and the Institute of Neurology (UCL). The administration of radioactivity was approved by the Administration of Radioactive Substances Advisory Committee (RPC528-890 (14364)).

5.2.2 RTMS Conditioning Of LPMd: A Physiological Study

- **5.2.2.1 Conditioning effects of premotor 5Hz rTMS on M1 excitability**

The electrophysiological experiment was designed (a) to examine whether the effect of short ITI (1min) subthreshold 5Hz rTMS over PMd (1800 pulses) on motor cortical excitability can also be produced at an intensity of 90% resting motor threshold (RMT) (Rizzo *et al.* 2004, Peinemann *et al.* 2004) and (b) to investigate how lengthening the ITI influences the late effects of 5Hz rTMS conditioning. The short ITI (1min) matched the ITI used in a previous rTMS study (Rizzo *et al.* 2004). The long ITI (5min) was chosen to match the duration of the 5Hz rTMS session to that of continuous 1Hz rTMS (30min), so that the total duration of stimulation would be the same as that used in Siebner *et al*, 2003.

Physiological excitability of the left M1 hand area was assessed with single and paired-pulse TMS before and after rTMS conditioning in 10 healthy volunteers (three of

whom also participated in the PET experiment). The rTMS parameters are described below. Measurements were performed with a High Power Magstim 200 machine and a figure-of-eight shaped coil with a mean diameter of 9cm (Magstim Co., Whiteland, Dyfed, UK). The coil was placed tangentially to the scalp with the junctional region pointing backwards and laterally at a 45° angle away from the midline, approximately perpendicular to the line of the central sulcus thus inducing a posterior-anterior current in the brain.

Motor evoked potentials (MEPs) were recorded from Ag-AgCl surface electrodes over the right first dorsal interosseous (FDI) muscle, using a belly-tendon montage. The signal was amplified (gain: x 5000) and band-pass filtered (10Hz to 3000Hz) by a Digitimer D150 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK) and recorded at a sampling rate of 5KHz on a personal computer for off-line analysis (NUCURSOR, Sobell Dept., Institute of Neurology, UCL, UK).

Short interval intracortical inhibition (SICI) and facilitation (ICF) were studied using the conditioning-test paradigm introduced by Kujirai *et al.* (1993). Two monophasic magnetic stimuli were given through the same stimulating coil over the left motor hand area. The intensity of the conditioning stimulus was set at 80% active motor threshold (AMT). The test stimulus was adjusted to an intensity that evoked an EMG response of ~1mV peak to peak (about 115-125% RMT) before rTMS conditioning. The following four interstimulus intervals (ISIs) were tested: 2, 3, 10 and 15ms. In every block the control condition was tested 20 times and each of the conditioning-test stimuli 10 times. The order of conditions was randomized. Measurements were made on each individual trial. The mean peak-to-peak amplitude of the conditioned MEP at each ISI was expressed as a percentage of the mean peak-to-peak size of the unconditioned test pulse in that block. A block of 60 trials before rTMS conditioning was tested and compared to a similar block recorded 5minutes after conditioning. The inter-trial interval within each block was 5 s (+/-10%). The time course of the rTMS effect was not studied in this experiment.

- **5.2.2.2 Statistical Analysis**

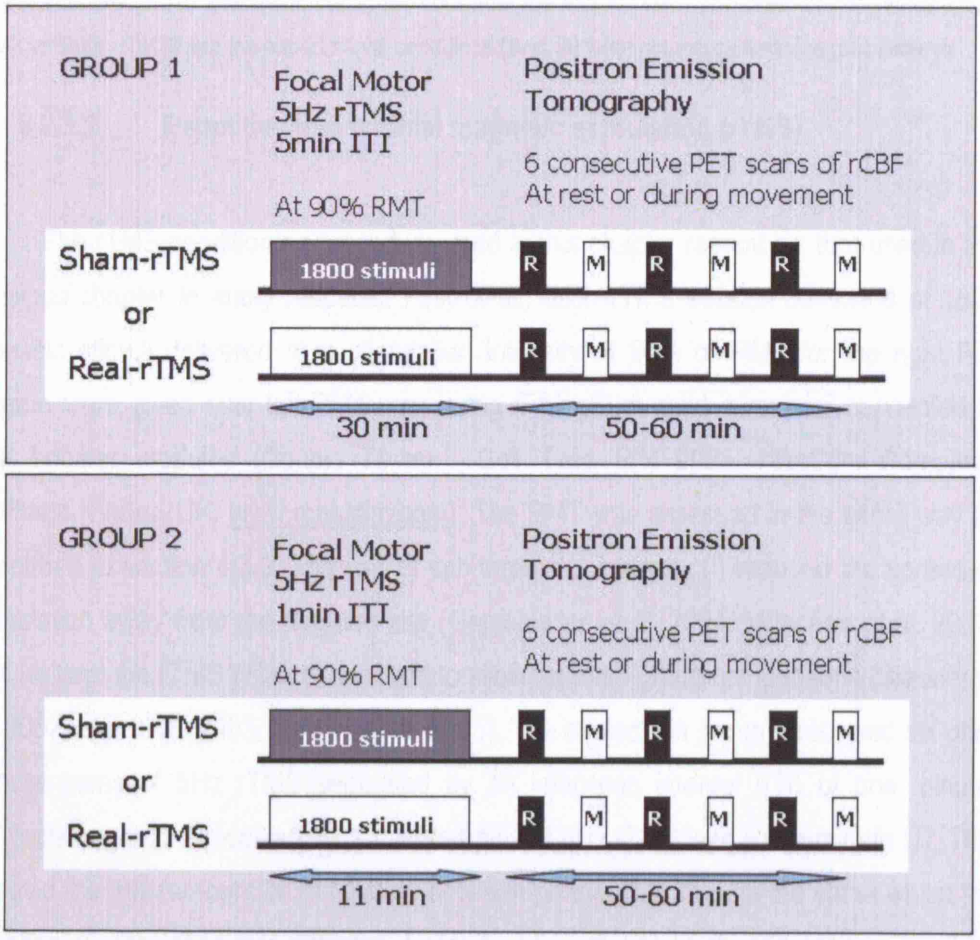
The effects of premotor 5Hz rTMS at 1min versus 5min ITI on MEP amplitude were evaluated by separate repeated-measures analyses of variance (ANOVA) for the

single and each pair of inhibitory and facilitatory paired-pulse conditions combined (i.e. 2 & 3ms and 10 & 15 ms respectively). Three two-way ANOVAs with TIME (pre vs post rTMS) and ITI (1min vs 5min ITI) were computed. When an F-value was significant, post hoc paired-sample t tests were performed. Data are presented as means +/- S.D. A p-value of <0.05 was considered significant.

5.2.3 RTMS Conditioning Of LPMd: Positron Emission Tomography

▪ 5.2.3.1 Study Design

This study uses essentially the same design as in the previous chapter, the only difference being that the two groups of subjects were allocated to the same frequency of rTMS stimulation, the difference between the groups being the pattern of rTMS conditioning. It comprised a 2x2x2 factorial design, with two levels per factor: "pattern of rTMS" (1min versus 5min ITI), "intervention" (real-rTMS versus sham-rTMS) and "task" (movement versus baseline). Figure 1 illustrates the study design. Subjects in each group received real and sham-rTMS on two separate days, at least a week apart. The order of interventions was counterbalanced across subjects. Group 1 was given high-frequency conditioning rTMS (real and sham 5Hz rTMS) with a short ITI (1min), and Group 2 was given high-frequency conditioning rTMS (real and sham 5Hz rTMS) with a long ITI (5min). The effects of rTMS were assessed by consecutive PET measurements of rCBF in the first hour after rTMS. Within each scanning session the baseline and movement tasks were alternated. The order of tasks was counterbalanced across subjects, but was kept constant within subjects between sessions.



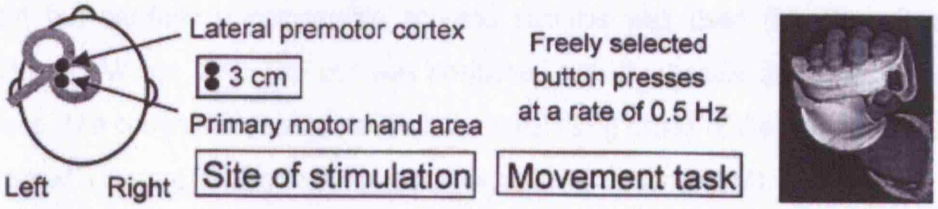


Figure 5.1: Experimental Design: 16 Healthy volunteers were divided into two groups of 8. All participants received 1800 biphasic stimuli of real rTMS or sham rTMS at a frequency of 5Hz rTMS on two separate days. The site of stimulation was 3cm anterior to the motor "hot spot", a reliable functional marker for the rostral dorsal premotor area. In both groups, participants received 5Hz real- or sham- rTMS at 90% resting motor threshold (RMT) in six one-minute blocks. In group1, the rTMS blocks were interspersed by a 5 min gap (ITI), whereas in group 2 they received 5Hz rTMS at 90% RMT in six one-minute blocks separated by one-minute intervals. Changes in regional cerebral blood flow were mapped using PET. Six sequential H2 15O-PET scans were acquired at baseline (B) or during a paced free selection of finger

movements task (M) in alternation during the hour after the end of rTMS. The order of intervention (real-rTMS vs sham-rTMS) and the experimental conditions (B vs M) were counterbalanced across subjects.

▪ 5.2.3.2 Repetitive transcranial magnetic stimulation (rTMS)

The rTMS conditioning procedure used in this chapter resembles that used in the previous chapter in many respects. First of all, each rTMS session consisted of 1800 biphasic stimuli delivered at a stimulation intensity of 90% of RMT for the right FDI muscle were given over left PMd area using a MagStim-rapid stimulator connected to four booster modules (Double 70mm - Coil Type P/N 9925, MagStim Company, Whitland, Wales, UK; www.magstim.com). The RMT was assessed in the same way as described in section 4.3.3. The use of sub-threshold intensity (i) reduced the spread of stimulation away from the targeted site (Gerschlager *et al.* 2001, Munchau *et al.* 2002) and (ii) kept the rTMS protocol constant to allow for multi-group comparisons (Siebner *et al.* 2003, Lee *et al.* 2003, Rounis *et al.* 2005). The subjects in group 1 received six one-minute trains of 5Hz rTMS separated by an inter-train interval (ITI) of one minute. Subjects in group 2 received six 1-minute trains of 5Hz rTMS with a five-minute ITI. This allowed the total amount of stimulation between protocols to remain the same whilst the duration of stimulation was different. A standard figure-of-eight shaped coil was used for real-rTMS. For sham-rTMS a specially designed sham coil that induced no magnetic field but provided a comparable acoustic stimulus was used (MagStim Company, Whitland, Wales, UK). The coil was positioned with the handle at 45° to the sagittal plane. The current flow associated with the initial rising phase of the biphasic TMS pulse induced a current flowing from caudal to rostral in the underlying M1.

The site of rTMS stimulation was located 3cm anterior to the "motor hot spot" defined functionally as the point of maximum evoked motor response in the relaxed right FDI, which is the same definition as in the study by Siebner *et al.* (2003). In that study the average site of PMd stimulation was located at -39, 5, 66 using fiducial markers on structural T1-weighted scans (Rowe et al. 2006 submitted). Most of the assumptions and interpretation of the data relating to changes within M1 caused by PMd rTMS are based on the following physical principle underlying TMS. The electric fields of transcranial magnetic stimulators decrease rapidly with distance from the coil (Ruohonen &

Ilmoniemi, 2002). It is therefore likely that any rTMS effect will be strongest in brain regions located closest to the scalp where the TMS is positioned. In this experiment, the PMd area we chose to stimulate is most likely the rostral PMd region in Brodmann area 6.

▪ 5.2.3.3 Motor Task

The scanning and task protocols we used are identical to those described in the previous chapter and in the studies by Siebner *et al.* (2003), Lee *et al.* (2003) and Rounis *et al.* (2005). Subjects underwent six sequential $H_2{}^{15}O$-PET scans on each of two days. Normalized rCBF-dependent ^{15}O uptake (referred to hereafter as rCBF) was used as an index of regional synaptic activity during two experimental conditions: baseline (B) and random selection of finger movements (M). Three PET scans were acquired for each experimental condition in alternating order (B-M-B-M-B-M or M-B-M-B-M-B). Subjects were required to keep their eyes open and fixate a cross on the centre of a screen located 0.7m in front of them. A pacing tone sounded every 2 seconds in both conditions. During the movement task, subjects were required to freely select and execute brisk flexion movements with the index, middle, ring or little finger of their right hand. All responses were recorded by a computer (Apple Macintosh 7300) using COGENT Cognitive Interface Software (Wellcome Dept. of Imaging Neuroscience, London, UK). The data were subsequently analysed using Matlab 6.0 (Mathworks, Sherborn, MA) and SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA). To ensure a stable level of task performance, the movement task started approximately 20 seconds prior to the onset of the PET scan and lasted for the entire 90-second period of data acquisition. During the baseline condition, subjects were instructed to watch the fixation point and listen to the tones.

❖ Behavioural Assessment

As described in the previous chapter, subjects performed two additional finger-tapping tasks with their right hand after the first, third, and fifth PET scans. In this 'simple tapping task' subjects tapped their right index finger as many times as possible in a ten

second interval. In a 'sequential tapping task' subjects were asked to repeat an ascending sequence (index, middle, ring, little finger) as quickly as possible for ten seconds. Subjects performed each of the three tasks twice in the PET scanner prior to rTMS in both scanning sessions.

The mean interval between response and duration of button presses were calculated as indices of motor performance. A two-way ANOVA was performed to compare the two groups with the stimulation factors (1min versus 5min ITI) and intervention (post-real rTMS versus post-sham rTMS). A paired-sample t-test was used for within-group comparisons of differences after real-rTMS and sham-rTMS. The mean duration of button presses during scanned movement tasks was analysed using the procedure described above. Simpson's equitability index (Simpson EH, 1949) was calculated for sequential response pairs and taken as a measure of randomness of sequences. This index varies between 0 and 1. A value of 1 indicates that over a series of responses any given response is equally likely to be followed by any other response. Data from three repetitions of this task during each scan were analysed to provide two values of randomness for each subject: one after sham-rTMS and one after real-rTMS. These data were also analysed in the manner described above.

▪ 5.2.3.4 PET data acquisition

PET was performed with a CTI ECAT HR+ scanner (CTI, Knoxville, TN) in three-dimensional mode with inter-detector collimating septa removed. The axial field of view was 155 mm providing whole brain coverage including cerebellum. The subjects lay supine in the scanner. A padded helmet with a chinstrap fixed to the headrest reduced head movement. A TV monitor was adjusted to give subjects an unrestricted view of instructions and the fixation point.

Normalized regional cerebral blood flow (rCBF) was assessed using $H_2{}^{15}O$. Six to ten mCi (mean 8.9 mCi) were delivered intravenously over 20s to the left arm. Image acquisition began 5s before the rising phase of the count curve, approximately 25-35s after injection, and continued for 90s. Correction for tissue and helmet attenuation was made using a transmission scan from $^{68}Ga/^{68}Ge$ sources at the start of each scanning session. The interscan interval was approximately 8 minutes. Corrected data were

reconstructed by three dimensional filtered back-projection (Hanning filter, cut off frequency 0.5 cycles/pixel) and scatter correction. Sixty-three transverse planes were obtained with a 128 x 128 pixel image matrix, with a pixel size of 2.4 x 2.1 x 2.1 mm, and a resolution of about 6 mm at full width half maximum.

No MRI scans of the subjects were obtained due to the fact that MRI scanners in the department were being dismantled. However, two previous studies, one of which has been described in the previous chapter, used the same TMS localisation technique (Siebner H *et al.* 2003, Rounis E. *et al.* 2005) and have shown consistent localisation of PMd. The mean nearest cortical contact to the centre of the TMS coil was x = -32, y = -14, z = 66 in MNI space (SD x = 3.7, y = 2.1, z = 3.6).

❖ Image Analysis

As in the previous chapter, all image analysis was performed using Statistical Parametric Mapping software, SPM99 (Wellcome Department of Imaging Neuroscience, UCL, UK. http://www.fil.ion.ucl.ac.uk/spm). For each subject, images were realigned to the first image by rigid body correction to correct artifacts due to head movements between scans and change of position between sessions (Friston *et al.*, 1995a). All images were then normalised to a standardised anatomic space (Talairach and Tournoux, 1998), by matching to a PET template using linear and non-linear spatial transformations (Friston *et al.*, 1995a). Each image was smoothed with an isotropic Gaussian kernel of 12 mm full-width at half-maximum to accommodate inter-subject differences in anatomy and enable the application of Random field corrections for inference (Friston *et al.*, 1995a).

The primary analysis employed a general linear model that included two groups (1min and 5min ITI 5Hz rTMS) of twelve regressors as covariates modeling the task (movement selection versus baseline) separately for each consecutive scan pair (first to third) under each condition of treatment (rTMS versus sham). The effect of global differences in cerebral blood flow was removed by treating global activity as a confound and scaling to a nominal grand mean global activity of 50 ml/100g/min (Friston *et al.*, 1995b). This statistical model enabled characterisation of the main effects of rTMS (rTMS versus sham), task (movement versus rest) and pattern of stimulation (1min

versus 5min). In addition, interactions between frequency, rTMS and movement were examined within and between groups. For the main effect of movement the reporting criterion was set at $p < 0.05$ corrected for multiple non-independent comparisons over the whole brain. Results for the main effects of rTMS and all interactions are reported at $p < 0.05$ using a small volume correction (16mm radius sphere centred on maxima from the analysis of main effects of movement in table 5.2).

Changes in effective connectivity within the motor network were also assessed using the 'physio-physiological interaction' (PPI) method described by (Friston *et al.*, 1997).

The analyses of effective connectivity presented here were designed to test for left PMd conditioning-dependent changes in coupling among motor areas engaged in movement. Five areas involved in action preparation and execution were chosen, based on known anatomical connections with the site of conditioning: left M1, left and right dorsal premotor cortex, left CMA and left SMA. These areas were selected according to known anatomical and 'effective' connectivity with the conditioned left PMd in this type of task (Picard & Strick 2001, Jahanshahi *et al* 1995, Jenkins *et al.* 2000, Okabe *et al* 2003, Oliviero *et al* 2003). Previous TMS studies using a conditioning-test paradigm have studied the connectivity of ipsilateral premotor-to-motor (Civardi *et al.* 2001) and contralateral premotor-to-motor (Mochizuki *et al.* 2004) connectivity. The role of the left SMA and its tight coupling to left PMd in this type of task has also been demonstrated previously (Jenkins *et al* 2000). Separate PPI analyses were performed for each area. In each case the first eigenvariate of the rCBF signal from a region of interest (sphere 8mm radius) identified by the main effect of movement constituted the physiological explanatory variable. A significant difference in the regression slope between two areas (a positive interaction) reflects a change in movement-related coupling from the index area to the significant site after real-rTMS.

The location of effects in sensorimotor area 4a and in dorsal premotor cortex were identified with reference to probabilistic cytoarchitectural population maps online (Brain Mapping Group, http://www.bic.mni.mcgill.ca/cytoarchitectonics/), (for further discussion of this technique, see Roland and Zilles 1998).

Electrophysiological measures of changes in excitability were acquired in only 3 of the 16 subjects studied due to time constraints and practical limitations. However, an additional 7 subjects were studied in a control physiological experiment to test for differential effects on M1 excitability following 5Hz rTMS conditioning with the two patterns of stimulation. The sizes of MEP amplitudes in response to single or paired TMS pulses were used to measure these changes. The latter were used to specifically probe changes in short intracortical inhibition and facilitation (Kujirai *et al.* 1993).

5.3 RESULTS

5.3.1 Physiological Effects of Premotor 5Hz rTMS

Previous studies have shown that 5 Hz rTMS to PMd (1500 stimuli at 90% AMT, 1 min ITI) increases the amplitude of MEPs evoked from the M1 for about 60 min. We confirmed that the slightly higher intensity 5 Hz rTMS in the present experiments (90% RMT) had the same effect, at least when given with a 1min ITI; however, this effect was not present with a 5 min ITI. This was confirmed in the statistical analysis of MEP data, shown in figure 5. A two-factor repeated measures ANOVA, with TIME (before versus after rTMS) and ITI (1min versus 5min) revealed a significant main effects of TIME on the mean MEP amplitude (TIME:$F_{1,13}$= 9.579, P=0.013) as well as a significant ITI-by-TIME interaction (interaction: $F_{1,13}$= 6.047, P=0.036). Post-hoc t-tests revealed that whereas the 5min ITI protocol had no significant effect on MEP size (t=-1.18, P=0.273), the 1min ITI paradigm led to a significant increase in MEP amplitude (t=-2.758, P=0.028). Figures 5.2b and 5.2c show the effects of 5Hz rTMS on SICI and ICF, respectively. Once again, a significant effect of TIME was found in the ICF condition (ICF - TIME: $F_{1,27}$= 6.763, P=0.02), but not in the SICI (SICI - TIME: $F_{1,27}$= 2.879, P=0.11). No ITI-by-TIME interaction was found in either SICI or ICF conditions.

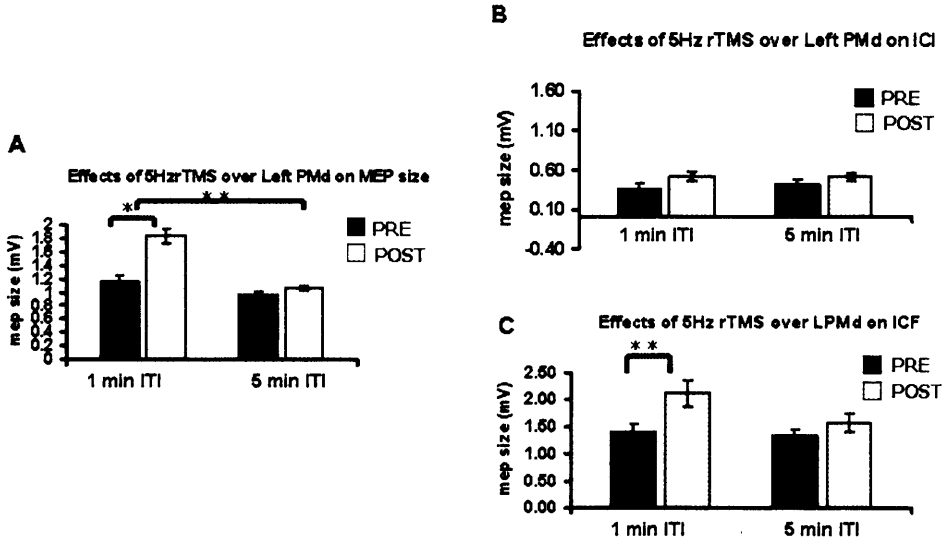


Figure 5.2: Physiological Effects of 5Hz rTMS with 1 and 5 min ITI on MEP. Conditioning effects of premotor 5Hz rTMS at 90% RMT at short (1min) and long (5min) inter-train intervals on MEP amplitude (A) and paired-pulse excitability (B&C). Intracortical inhibition (SICI) was assessed using interstimulus-intervals (ISIs) of 2 and 3 ms (B). Intracortical facilitation (ICF) was estimated using ISIs of 10 and 15 ms (C). Error bars are standard error of the mean (S.E.M.). Asterisks denote a significant change relative to baseline.

5.3.2 Imaging Results: rTMS Conditioning Effects on rCBF

No subject reported adverse side effects during the course of study other than mild discomfort at the site of rTMS. No visible motor responses were evoked during rTMS. Mean RMT was 57%, ranging from 46 to 83%.

▪ 5.3.2.1 Behavioural data

All sixteen participants found the motor tasks easy to perform. A two-way repeated measures ANOVA with ITI (1min versus 5min ITI) and type of rTMS (real versus sham) as main factors revealed no significant main effects or interactions, indicating that both groups performed the three tasks similarly and that motor performance during PET scanning was unaffected by rTMS.

Table 5.1 shows the average group values from both groups of subjects measured by Simpson's equitability index (mean +/- SD) of the variables used to assess motor performance and free selection of movement after rTMS. The results of individual paired sample t-tests performed for the 1min and 5min ITI groups are also shown. Statistical analysis of these data showed that neither 1min nor 5min ITI 5Hz- rTMS over the dorsal premotor cortex had any significant effect on motor performance or free selection of movement. In the index tapping task and the sequential tapping task there was no effect of rTMS on the duration of button presses or on the interval between button presses. During freely selected finger movements rTMS had no effect on the duration of button presses or on the randomness of responses.

Measure	ITI	Post-sham rTMS	Post-real rTMS	t-value	P-value
Index finger tapping task (n =16)					
Duration (ms)	**1min**	116.27 (± 19.12)	112.42 (± 20.39)	0.7	0.5
	5min	110.80 (± 22.63)	107.26 (± 23.21)	0.75	0.48
Interval (ms)	**1min**	210.65 (± 27.71)	202.64 (± 18.91)	0.97	0.36
	5min	198.73 (± 38.47)	200.38 (± 37.07)	-0.27	0.79
Sequential tapping task (n=16)					
Duration (ms)	**1min**	188.21 (± 57.73)	177.63 (± 61.77)	0.78	0.46
	5min	187.09 (±76.16)	170.72 (± 63.92)	0.51	0.62
Interval (ms)	**1min**	194.28 (± 42.39)	194.9 (± 50.64)	-0.14	0.98
	5min	308.71 (± 70.38)	310.98 (± 68.8)	-0.15	0.89
Random selection task (n=16)					
Duration (ms)	**1min**	235.98 (± 33.56)	248.61 (± 46.41)	-0.81	0.44
	5min	214.69 (± 40.42)	206.91 (± 44.27)	1.36	0.21
Reaction Times (ms)	**1min**	455.27 (± 139.07)	384.25 (± 173.22)	1.8	0.11
	5min	483.16 (± 132.64)	533.22 (± 191.4)	-1.3	0.23
Simpson's equitability index	**1min**	0.68 (± 0.05)	0.69 (± 0.08)	-0.16	0.87
	5min	0.77 (± 0.1)	0.74 (± 0.09)	0.9	0.4

Table 5.1: Pattern-dependent changes in behaviour following 5Hz rTMS to PMd. Mean group data (± SD) for each group of kinematic measures: mean duration of key presses and interval between key presses for index tapping and sequential motor tasks and mean duration of key presses for the paced free selection task

▪ 5.3.2.2 Imaging data

• 5.3.2.2.1 Movement-related changes in rCBF

Performance of the task (main effects of movement versus baseline) led to an increase in brain activity in a well-defined network of areas engaged in freely selected right-hand movements (Table 5.2) (reported at $p<0.05$ corrected for multiple comparisons). These included left M1, extending to the left dorsal and ventral premotor cortices, left SMA and left rostral CMA. Other areas included the lateral and medial prefrontal cortices bilaterally, the insula in both hemispheres, the right and left secondary somatosensory areas and superior cerebellum. There were no significant differences in movement-related activation between the two groups of subjects (*i.e.* no movement-by-group interactions).

Anatomical Location	Site				Peak	
		MNI Coordinates			P value	Z value
		x	y	Z		
Primary Sensorimotor Cortex	Left	-38	-18	62	<0.001	>8
Premotor (PMd)	Left	-38	-6	50	0	6.78
	Left	-26	-14	68	<0.001	7.12
	Right	30	-2	58	0.001	5.3
Premotor (PMv)	Left	-54	-2	26	0.001	6.05
Insula	Left	-42	0	6	<0.001	6.84
	Right	46	14	2	<0.001	5.67
Rostral cingulate motor area	Left	-10	2	48	<0.001	6.96
Caudal SMA	Left	-8	-8	56	<0.001	7.63
Superior Cerebellum	Left	-28	-56	-28	<0.001	6.35
	Right	26	-56	-24	<0.001	7.55
	Right	6	-68	-20	<0.001	5.48
Primary Somatosensory	Left	-46	-30	50	<0.001	>8
Secondary Somatosensory	Right	58	-26	40	<0.001	6.02
	Right	66	-34	40	<0.001	5.89
Superior Frontal Gyrus	Left	40	40	28	0.003	5.14
Inferior Frontal Gyrus	Right	54	10	14	<0.001	5.05
Intra-Parietal Sulcus	Right	36	-40	42	<0.001	5.3
Globus Pallidus externus	Left	-32	-4	0	<0.001	5.61

Table 5.2: Main effects of movement. Maxima of regional increases in normalized rCBF during freely selected finger movements compared to baseline (corrected at $P < 0.05$ for multiple comparisons).

- **5.3.2.2.2 Changes in rCBF induced by 5Hz rTMS over left PMd**

❖ **Effects of 5Hz with 1min ITI**

Compared with sham-rTMS, real 5Hz rTMS with 1min ITI caused widespread decreases of rCBF particularly in the frontal cortex bilaterally, with a left hemispheric preponderance. The maximal reductions of rCBF were found in the left primary motor, PMd (peaks at x = -38, y = -6, z = 50 and x = -16, y = -6, z = 50) and supplementary motor areas (SMA), the right anterior CMA, the right ventrolateral prefrontal area (VLPFC) and the dorsolateral prefrontal cortex (DLPFC) bilaterally. Figure 5.3 shows a few of the sites with decreased rCBF following rTMS compared to sham and the parameter estimates between real and sham rTMS in all conditions for the 5 and 1min ITI protocols respectively ($p<0.05$ corrected). Areas outside the frontal lobes that showed similar decreases in brain activity include the right somatosensory area in the postcentral gyrus as well as the left hippocampus and the right superior temporal cortex.

Increases in rCBF were mainly seen in limbic regions and basal ganglia (figure 5.4). These included the right hippocampus, left amygdala, left pars orbicularis and the left internal globus pallidus. Activity in the left superior cerebellum also increased following 5Hz rTMS with 1min ITI.

Anatomical Location	Site	MNI Coordinates			Peak	
		x	y	Z	P value	Z value
3a) Decrease in rCBF						
Primary sensorimotor cortex	Left	-22	-18	72	<0.001	7.23
	Left	-26	-34	60	0.006	5.16
Premotor (PMd)	Left	-38	-6	50	<0.001	5.84
	Left	-16	-6	50	0.001	5.6
	Right	22	8	42	<0.001	>8
Supplementary Motor Area	Left	-6	-12	68	<0.001	5.77
	Right	6	0	68	0.002	5.4
Pre-SMA	Left	-2	16	40	0.025	4.87
Primary somatosensory	Right	56	-24	50	0.001	5.46
Prefrontal (Ventrolateral)	Right	42	38	4	0.003	5.32
	Right	50	18	8	0.046	4.72
	Right	50	10	4	0.017	4.95
Prefrontal (Dorsolateral)	Left	-30	36	32	0.001	5.45
Inferior Frontal Gyrus	Right	64	10	32	0.02	4.9
Intra-Parietal Sulcus	Left	-40	-48	46	<0.001	7.09
Thalamus	Left	-18	-28	-2	<0.001	6.11
3b) Increase in rCBF						
Superior cerebellum	Right	12	-38	-22	0.008	5.1
	Left	-32	-46	-28	0.001	5.61
Insula	Left	-54	-14	12	<0.001	6.67
Hippocampus	Left	-34	-16	-10	0.002	5.43
Thalamus	Right	18	-14	6	0.021	4.9

Table 5.3: Main effects of 5Hz-rTMS with 1min ITI over PMd masked by the main effects of movement ($p<0.05$ corrected). 3a: Maxima of regional decreases in normalized rCBF after real-rTMS; 3b) Maxima of regional rCBF increases after rTMS.

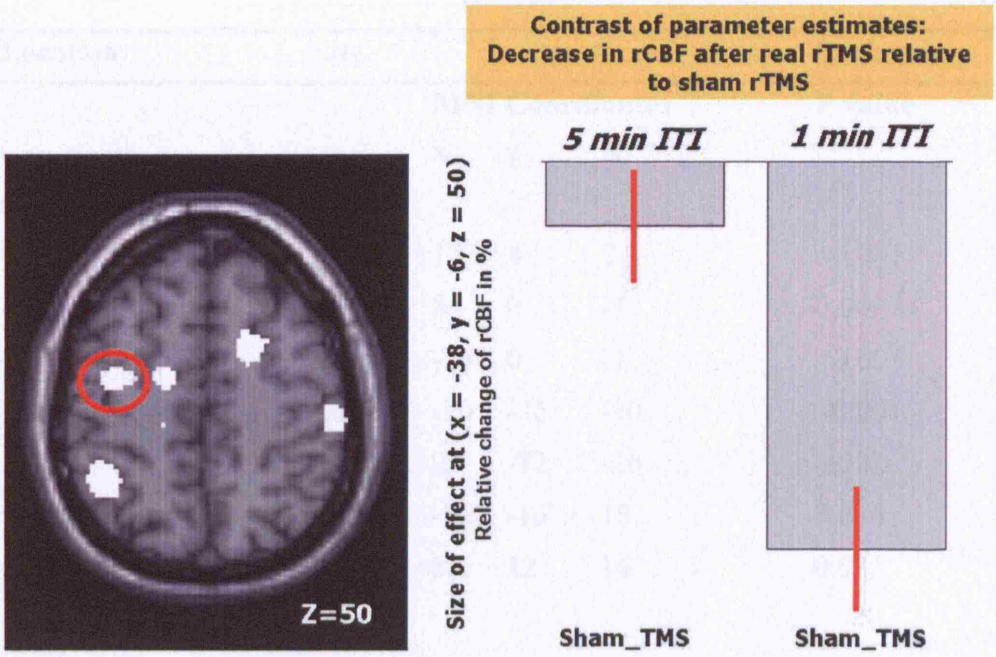


Figure 5.3: Decreases in regional cerebral blood flow following 5Hz rTMS with 1min ITI to left PMd.

Results are displayed on a transverse section of the canonical T1-weighted structural brain template from SPM99. The contrast of parameter estimates at the left PMd site show the total amount of rCBF decrease following rTMS conditioning in the 5min and the 1min ITI groups respectively. Note that although the main effect of rTMS is in the same direction for both groups, 1min ITI shows a stronger reduction than 5min ITI.

❖ **Effects of 5Hz with 5min ITI**

5Hz rTMS with longer ITI (5min) led to decreases of rCBF in the left head of caudate, left superior cerebellum, left and right hippocampus, external globus pallidus and insula. Increases of rCBF were found in the DLPFC bilaterally. The relative increases and decreases of rCBF following 5Hz rTMS with 1min and 5min ITI are listed in tables 5.3 and 5.4 at $p<0.05$ corrected for multiple comparisons.

Anatomical Location	Site				Peak	
		MNI Coordinates			P value	Z value
		x	y	Z		
4a) Decrease in rCBF						
Globus pallidus internus	Right	12	4	2	<0.001	>8
Putamen / Globus	Right	34	0	-4	0.009	5.08
pallidus externus	Left	-24	0	-4	<0.001	6.22
Superior Cerebellum (anterior)	Left	-20	-36	-30	<0.001	6.3
(posterior)	Right	20	-72	-26	0.002	5.4
Secondary Somatosensory	Left	-56	-16	18	0.034	4.79
Insula	Right	50	12	14	0.035	4.79
4b) Increase in rCBF						
Thalamus	Left	-16	-24	-4	0.001	6.69

Table 5.4: Main effects of 5Hz-rTMS with 5min ITI over PMd masked by the main effects of movement ($p<0.05$ corrected). 4a: Maxima of regional decreases in normalized rCBF after real-rTMS. 4b: Maxima of regional rCBF increases after real-rTMS.

Right panel: Conjunction of the main effects of TMS_SHAM (increases) following 5Hz rTMS on a rendered T1-weighted canonical brain. 1min ITI is represented in green, 5min ITI is represented in red. Results are displayed at $p<0.001$ uncorrected.

❖ Changes in rCBF common to both 5Hz protocols (conjunction of main effects)

In order to identify areas of increased and decreased rCBF common to both patterns of high frequency rTMS, a conjunction of the main effects of rTMS between the two groups was performed. The results are reported in table 5.5 at $p<0.05$ corrected for multiple comparisons and in figure 5.4 at $p<0.001$ uncorrected.

Anatomical Location	Site				Peak	
		MNI Coordinates			P value	Z value
		x	y	z		
5a) Common decreases in rCBF after5Hz rTMS at different ITIs						
Sensorimotor cortex	Right	30	-28	70	0.003	5.44
Inferior Frontal Gyrus	Right	26	12	36	0.001	5.72
Caudal SMA	Left	-4	-20	62	<0.001	5.9
Primary Somatosensory	Left	-24	-34	66	<0.001	5.81
Intraparietal Sulcus	Left	-34	-48	44	0.002	5.47
	Left	-28	-58	50	0.002	5.49
	Right	46	-42	54	0.006	5.28
Insula	Right	40	-18	2	0.014	5.09
Middle Temporal Gyrus	Right	58	-44	0	0.002	5.48
Inferior Temporal Gyrus	Right	46	-4	-32	0.001	5.58
Superior Cerebellum	Left	-14	-36	-28	0.008	5.21
Pons	Left	-2	-24	-28	0.009	5.19
Precuneus	Right	4	-40	50	0.027	4.95
5b) Common increases in rCBF after 5HzrTMS at two different ITIs						
Anterior Cingulate Gyrus	Left	-4	34	8	0.049	4.81
Superior Frontal Gyrus	Left	-12	60	18	0.028	4.94
Caudate (Head)	Left	-10	6	6	<0.001	5.93
Caudate (Body)	Left	-16	6	22	0.037	4.88
Hippocampus	Right	18	-32	-6	<0.001	6.36
Thalamus	Right	8	-20	16	<0.001	5.41

Table 5.5: Conjunction of main effect of 1 and 5 min ITI 5Hz-rTMS

5a: Decreases: Maxima of regional decreases in normalized rCBF after real-rTMS compared to sham.

5b: Increases: Maxima of regional rCBF increases after real-rTMS compared to sham.

$P < 0.05$ (whole volume corrected).

Activity in motor areas decreased following rTMS. Decreases of rCBF occurred in the right primary motor, supplementary motor and somatosensory areas. Increases in rCBF were found in the right parahippocampal gyrus, the left head and body of caudate nucleus and the left cingulate motor area.

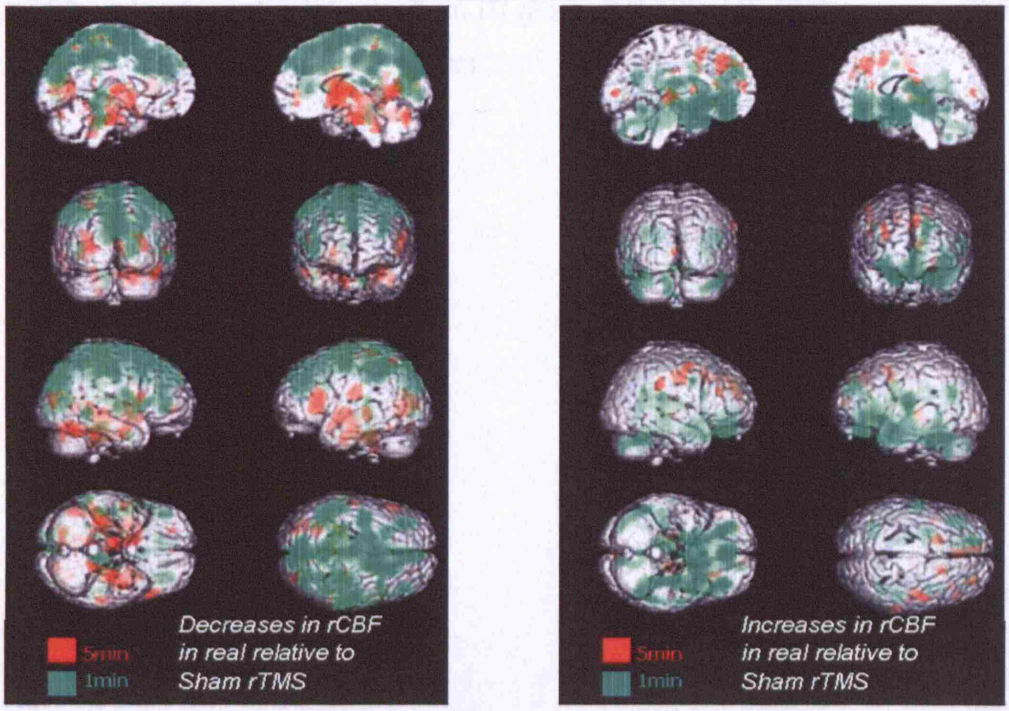


Figure 5.4: Conjunction of the main effects of subthreshold 5Hz rTMS at short and long ITI.

Left panel: Conjunction of the main effects of SHAM_TMS (decreases) following 5Hz rTMS on the rendered T1-weighted canonical template from SPM99. 1min ITI is represented in green, 5min ITI is represented in red. Results are displayed at $p<0.001$ uncorrected to show the more widespread nature of the effect following short (1min) ITI 5Hz rTMS conditioning.

❖ **Pattern-dependent differences in rCBF (Pattern-by-rTMS interaction)**

Areas which showed significant differences in rCBF change following 5Hz rTMS with 1min ITI compared with 5min ITI are shown in table 5.6. In the motor system, 5Hz rTMS with 1min ITI caused greater decreases of rCBF in right PMd, left caudal CMA and DLPFC bilaterally. Conversely, the 5min ITI group showed greater decreases of rCBF bilaterally in hippocampus and cerebellum.

Anatomical Location	Site				Peak activation	
		MNI Coordinates			P value	Z value
		x	y	z		
a) 1min ITI > 5min ITI						
PMd (rostroventral)	Right	22	12	44	<0.001	7.82
	Left	-16	-4	50	<0.001	5.29
Cingulate Motor Area (caudal)	Left	-12	-32	46	0.001	5.51
(rostral)	Left	-14	10	48	<0.001	5.33
	Right	8	-18	40	0.004	5.21
Anterior Cingulate Gyrus	Right	2	38	34	<0.001	5.87
Dorsolateral Prefrontal (DLPFC)	Right	44	34	24	0.006	4.99
	Left	-32	36	34	0.022	4.91
Primary Somatosensory	Right	56	-22	48	<0.001	5.89
	Right	46	-14	40	<0.001	5.83
Thalamus (ventral) posterolateral	Left	-18	-26	-4	<0.001	7.8
b) 5min ITI > 1min ITI						
Hippocampus	Right	36	-30	-4	<0.001	6.48
	Left	-32	-16	-16	<0.001	>8
Superior Cerebellum	Left	-24	-34	-30	0.001	5.65
	Right	46	-56	-26	<0.001	5.66
Globus pallidus internus	Right	12	4	-2	<0.001	6.12
Frontomedial cortex (IFG)	Right	26	48	-2	0.021	4.9

Table 5.6: Pattern-by-rTMS Interactions masked by the main effects of movement ($p<0.05$ whole volume corrected)

❖ **Movement-related changes in rCBF following 5Hz rTMS**

5Hz rTMS with 1min ITI lead to an increase in task-related rCBF in a region within the caudal part of the left PMd, at the border with Brodmann area 4a (figure 3a) (x = -16, y = -22, z = 68; Z = 3.2 ; p = 0.017). The Brain Mapping Group cytoarchitectonic map (www.bic.mni.mcgill.ca/cytoarchitectonics) gives a location probability of 0.4 for left area 4a and 0.7 for left area 6. Figure 5.5 shows the location and parameter estimates for this site. Task-related responses in this area increased significantly following conditioning with 5Hz-rTMS with 1min ITI for the duration of the scanning session. No task-related changes of rCBF were seen in the group stimulated with 5Hz-rTMS using the 5min ITI protocol. A task-by-rTMS-by-protocol interaction failed to reveal any changes in movement-related activity dependent on the rTMS protocol.

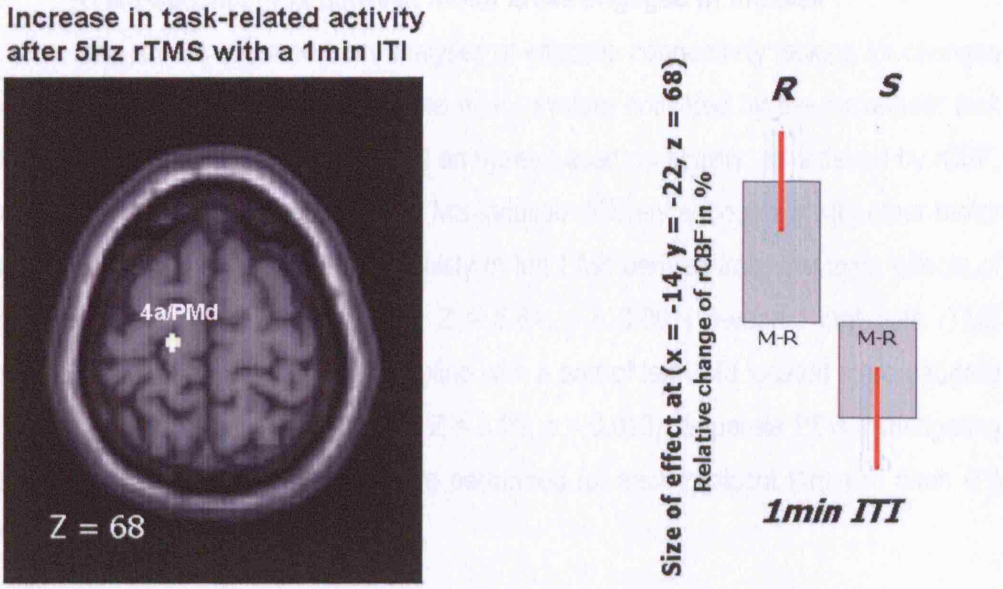


Figure 5.5: Task by intervention interaction following 5HzrTMS at short (1min) ITI.

Left panel: Location of the task-by-intervention interaction: area 4a) (x = -16, y = -22, z = 68; Z = 3.2 ; p = 0.017) displayed on a transverse section of the canonical T1-weighted structural template from SPM99. Right Panel: Parameter estimates of the difference in mean (+/-SE) task-related activations between real and sham conditions in all the pairs of move-baseline (M-B) scans 1min (short) ITI 5Hz-rTMS at the interaction site. As can be seen in figure 1, all participants underwent 6 consecutive PET scans: 3 at Baseline (B) and 3 during movement (M). All the M and B scans have been averaged together respectively. R = Real TMS session; S = Sham TMS session. The two columns represent the Real and Sham rCBF difference between the average of move and rest (M_R) scans for the short (1min) ITI group.

❖ **RTMS-specific PPIs between motor areas engaged in the task**

The results obtained from analyses of effective connectivity looking for changes in coupling between components of the motor system activated by the movement task are shown in figures 6 and 7. The PPI analyses based on activity, as indexed by rCBF, in left M1 and left SMA revealed no rTMS-induced differential coupling with other motor areas. The PPI analysis based on activity in left PMd derived from the main effects of movement ($x = -38$, $y = -6$, $z = 50$; $Z = 5.84$; $p < 0.001$) revealed that both rTMS protocols increased intra-regional coupling with a part of left PMd located more caudally and dorsally ($x = -34$, $y = -16$, $z = 66$; $Z = 3.29$; $p = 0.013$). Separate PPIs investigating coupling changes within left PMd were performed for each protocol (1min or 5min ITI) separately and are shown in figure 5.6.

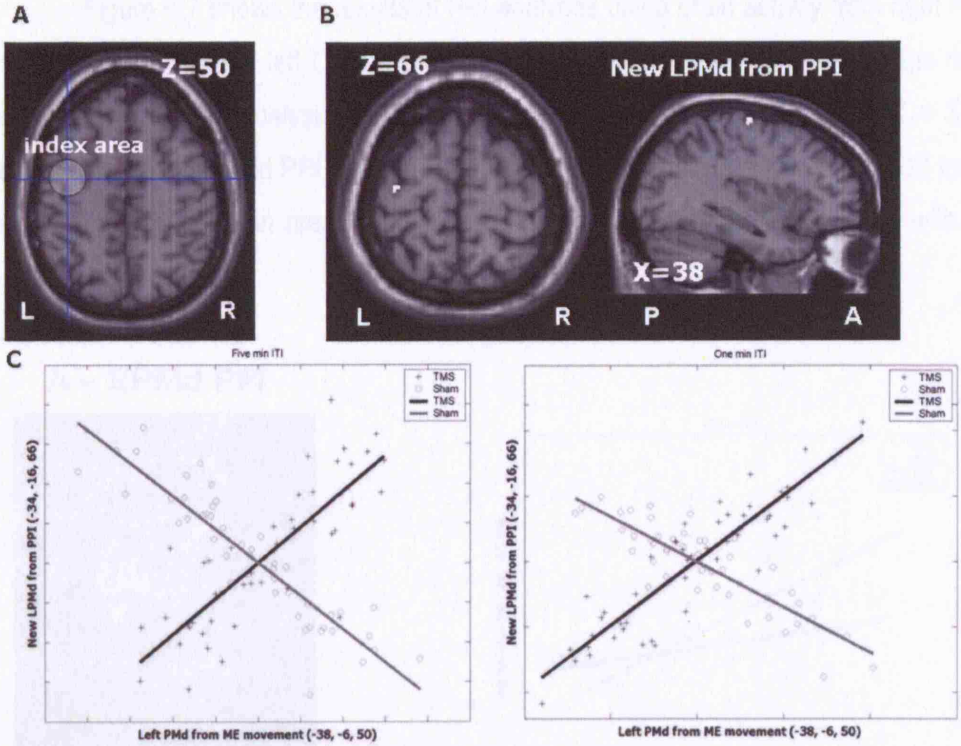


Figure 5.6: rTMS-dependent changes in effective connectivity (physiophysiological interaction) with the left PMd (x = -38, y = -6, z = 50) from the main effect of movement (table2).

A) Anatomical location on a transverse slice of the canonical template from SPM99 showing the left PMd region from the main effects of movement contrast, used as the index area in this PPI (x = -38, y = -6, z = 50). B) Anatomical location on transverse and axial slices of the significant PMd area obtained from the PPI analysis, which is located more dorso-laterally than the index region. The left PMd area from the PPI (x = -34, y = -16, z = 66; Z = 3.29; p = 0.013) shows a positive PPI with the left PMd from the main effects of movement following 5Hz-rTMS at both inter-train intervals

C) Graphical representations illustrating the physiophysiological interactions between the left PMd area from the main effects of movement (x = -38, y = -6, z = 50) (abscissa) and the left PMd area revealed from the PPI analysis (x = -34, y = -16, z = 66) (ordinate) for 5Hz at 5min and c) 1min ITI respectively. TMS=Real-rTMS (crosses), SHAM=Sham-rTMS (circles) session. Group1: 5Hz, 5min ITI Sham-rTMS: r^2 = 0.68, F = 100.06, gradient = 0.00; 5Hz 5min ITI Real-rTMS: r^2 = 0.84, F = 248.84, gradient = 0.00. Group2: 5Hz 1minITI Sham-rTMS: r^2 = 0.82, F = 215.82, gradient = 0.00; 5Hz 1min ITI Real-rTMS: r^2 = 0.71, F = 111.18, gradient = 0.00. (Least square statistics using the matlab statistic toolbox)

Figure 5.7 shows the results of PPI analyses using brain activity from right PMd (x=30, y=-2, z=58) and left CMA (x=-10, y=2, z=48) and at sites identified by the main effects of movement analysis. Both identified left M1 (x = -24, y=-28, z=56; Z = 3.63; p=0.004 in the right PMd PPI analysis; and x=-28, y=-26, z=58; Z = 3.29; p= 0.013 in the left CMA analysis) as an area with significantly lesser coupling after 5Hz rTMS with the 1min ITI protocol.

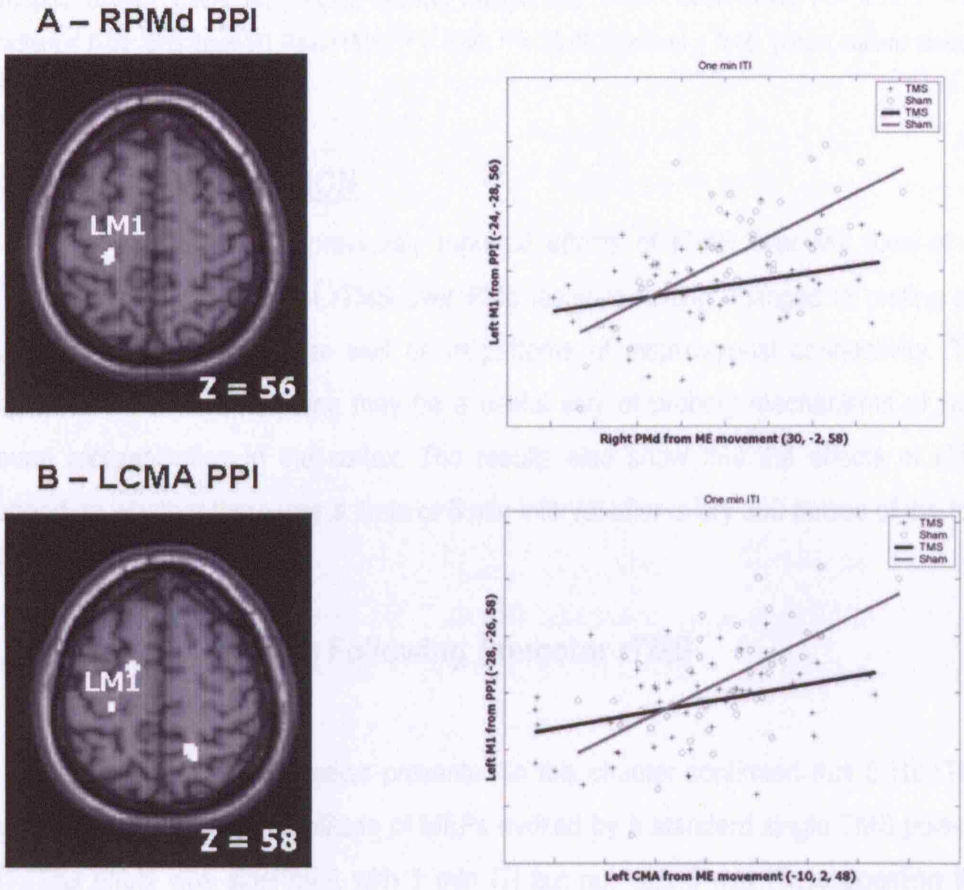


Figure 5.7: rTMS-dependent changes in effective connectivity (physiophysiological interaction) with the right PMd (x = 30, y = -2, z = 58) and the left cingulate motor area (x=-10, y=2, z=48) from the main effect of movement (table5.2).

A) Anatomical location on a transverse slice of the canonical template from SPM99 showing the significant left M1 area obtained from the PPI analysis using right PMd as the index area (x = -24, y = -28, z = 56; Z = 3.63; p=0.004), followed by graphical representations illustrating the physiophysiological interactions

between the right PMd area from the main effects of movement (x = 30, y = -2, z = 58) (abscissa) and the left M1 area revealed from the PPI analysis (x = -24, y =-28, z = 56) (ordinate) for 5Hz at 1min ITI. TMS=Real-rTMS (crosses), SHAM=Sham-rTMS (circles) session. Group2: 5Hz 1minITI Sham-rTMS: r^2 = 0.08, F = 3.91, gradient = 0.05; 5Hz 1min ITI Real-rTMS: r^2 = 0.47, F = 40.51, gradient = 0.00.

B) Anatomical location on a transverse slice (canonical T1-weighted template from SPM99) of the significant left M1 area obtained from the PPI analysis using left CMA (x=-28, y=-26, z=58; Z = 3.29; p= 0.013), followed by graphical representations illustrating the physiophysiological interactions between the left CMA area from the main effects of movement (x = -10, y = 2, z = 48) (abscissa) and the left M1 area revealed from the PPI analysis (x = -28, y = -26, z = 58) (ordinate) for 5Hz at 1min ITI. TMS=Real-rTMS (crosses), SHAM=Sham-rTMS (circles) session. Group2: 5Hz 1minITI Sham-rTMS: r^2 = 0.12, F = 6.3, gradient = 0.01; 5Hz 1min ITI Real-rTMS: r^2 = 0.36, F = 25.90, gradient = 0.00. (Least square statistics using the matlab statistic toolbox)

5.4 DISCUSSION

In agreement with previously reported effects of rTMS over M1 (Lee *et al.*, 2003), this study shows that rTMS over PMd leads to lasting changes in resting and movement related activity as well as in patterns of inter-regional connectivity. This implies that rTMS conditioning may be a useful way of probing mechanisms of rapid neural reorganisation in the cortex. The results also show that the effects of rTMS depend on whether there was a 1min or 5 min interval after every 300 pulses of the total 1800 applied.

5.4.1 Lasting Changes Following Premotor rTMS

The physiological studies presented in this chapter confirmed that 5 Hz rTMS over PMd increases the amplitude of MEPs evoked by a standard single TMS pulse to M1. The effect was significant with 1 min ITI but not with 5 min ITI, suggesting that longer pauses in the rTMS protocol reduce its efficacy to promote long term changes in excitability. This has significant implications in studies using high-frequency stimulation over the frontal areas. Indeed, many authors have been using various ITIs in their protocols of stimulation (Nahas *et al.* 2001). However, there was no account of the possibility that the effectiveness of stimulation might differ. Speers and colleagues have already suggested this might be the case with intensity of stimulation when low-

frequency, 1Hz rTMS, is given to the prefrontal cortex (Speers et al. 2003). Here i present evidence of a similar case with different ITIs for high frequency stimulation. We did not use a strictly parametric change in ITI because we were interested in comparing the 1min condition, known to produce robust physiological effects (Rizzo *et al.* 2004), to a protocol that would match the duration of 1Hz rTMS that was used in a similar experimental set-up by previous studies in our group (Siebner *et al.* 2003, Lee *et al.* 2003). Reduced effectiveness of 5 min compared with 1 min ITI was also evident in measures of rCBF, leading us to recommend short ITIs for future studies.

5 Hz rTMS with 1 min ITI lead to widespread decreases in rCBF in the PMd and M1 and a number of other connected sites within the frontal lobes. Conversely, rCBF in parts of the basal ganglia, limbic regions and cerebellum increased, perhaps reflecting a reciprocal pattern of connectivity (Siebner *et al*, 2003). 1 Hz rTMS to PMd led to similar widespread decreases in rCBF (Siebner *et al* 2003). This was unexpected since physiological studies show opposite effects of 1 Hz (Gerschlager *et al.* 2001) and 5 Hz rTMS (Rizzo *et al.* 2003) over the same area. However, as noted by others, rCBF is likely to reflect overall levels of synaptic activity, whether excitatory or inhibitory, whereas physiological measures reflect the net effect on neural excitability (Gold & Lauritzen, 2002). We have recently reported a similar lack of difference between rCBF effects of 1 Hz and 5 Hz rTMS over M1 (Rounis *et al.* 2005). However, we reported an overall increase in rCBF compared with the decrease seen here. It seems that the metabolic consequences of rTMS at rest may depend more on the region of cortex stimulated than on the frequency of the stimulus, reflecting possible differences in "resting" activity levels in the rostral PMd and M1 (Speer et al. 2003b).

The changes in rCBF following 5 Hz rTMS with a 5 min ITI were less widespread, with significant effects being limited to subcortical structures in basal ganglia and cerebellum. Whether this is due to the fact that these structures are more sensitive to the conditioning effects of rTMS than cortical areas in the frontal lobes is unclear from this study.

To my knowledge, Chouinard et al. (2003) are the only other group that has studied the effects of (1Hz) rTMS to PMd with PET. Imaging results in that study were analyzed in terms of the correlations between changes in rCBF and changes in the

amplitude of MEPs evoked from the M1. We set out to investigate whether the nature of rTMS insult delivered to the motor system affects net rCBF activity and reorganisation during movement whereas the aim of Chouinard's study was to investigate the neural correlate of excitability changes caused by rTMS over the left PMd, so that it is difficult to compare the data directly. Nevertheless, it is reassuring to note that like us, they found widespread changes in activation after 1Hz rTMS in many of the same cortical areas.

5.4.2 Effects of 5Hz rTMS over PMd on Task-Related Activity

5Hz rTMS with 1min ITI led to task-specific differences in rCBF, while motor behaviour remained unchanged. Evidence, from the physiological study, showed that this protocol of stimulation does lead to increased excitability of M1, as evidenced by an increase in MEP size, a decrease in SICI and an increase in ICF. Hence, the increase in task-related rCBF in a region of the caudal PMd at the border with area 4a, that is posterior and lateral to the main site of movement related activation in PMd, suggests a form of reorganization in order to maintain task performance. The exact position of the rTMS coil over PMd was not localized in the scanner. However, its location, 3cm anterior to the hand area of M1 is consistent with the idea that the area showing increased movement related activity might be posterior to the site of stimulation (Picard & Strick, 2001). This caudal PMd area has been implicated in movement preparation and generation (Gerardin *et al.* 2000, Jenkins *et al.* 2000, Ehrsson *et al.* 2000) whereas the rostral PMd's role has been associated with more cognitive processes (Toni *et al.* 1999, Sakai *et al.* 2000). The increase in movement-related activity in caudal PMd found here may account for an increased "preparatory" activity, which might compensate for the altered excitability induced by 5Hz rTMS in the stimulated rostral PMd.

This effect is reminiscent of the changes in task related activity after rTMS to M1. In those studies rTMS (at 1 Hz or 5 Hz) was given over primary M1 and changes in movement related activity (increase or decrease respectively) occurred in an adjacent area of sensorimotor cortex deep in the central sulcus. We suggest that rTMS over either PMd or M1 disrupts the normal movement related activity pattern in these areas. If so,

then the TMS-by-task interaction seen in the analysis of rCBF may well represent a reorganization the function of which is to maintain task performance.

In this experiment it may be that the increased movement related activation in caudal PMd is related to the increase in MEP amplitudes that occurred in the physiological studies after 5 Hz rTMS. For example, the overall reduction in rCBF caused by 5 Hz rTMS over PMd could be due to a decrease in ongoing inhibitory activity, at least in caudal PMd. If so, this area would be activated more by the volitional input associated with movement and show enhanced movement related rCBF. Similarly, if the MEP produced by single pulse TMS over M1 contains a contribution from activation of corticospinal output from this caudal region of PMd, then it too will be enhanced. As mentioned in the first chapter, a clear-cut functional distinction between caudal PMd and M1 is a subject of current debate. Graziano *et al.* (2002a, 2002b) do not support a definitive division between these two areas. Their electrical microstimulation results in primary motor and premotor cortex of awake monkeys suggest that some of the subdivisions previously made within motor and premotor areas may represent a larger map of manual space encoding movement. Further support for this hypothesis comes from anatomical studies in non-human primates and functional neuroimaging studies in humans (for a review, see Geyer S. 2004). This hypothesis is also consistent with data presented by Speer et al. (2003a) in which simultaneous TMS/PET was used in order to study the effects of low-frequency (1Hz) over M1. The authors found that the voxel of maximal M1 responsiveness to be located in the posterior premotor cortex and postulated that this position might be optimal for exciting M1 given that the cortical horizontal fibers run in the plane of the stimulating current (Speer et al. 2003).

5.4.3 Acute Reorganization of Frontal Lobe Areas Following rTMS to PMd

A decrease in coupling from right PMd and CMA to an area of left M1 was found during the movement task following left PMd conditioning with 5Hz rTMS with 1min ITI. In addition, coupling increased between the main site of movement related activity in PMd and a more dorso-lateral region close to the site of stimulation. Such effects are reminiscent of the changes in coupling of motor related cortical areas after rTMS to M1

(Rounis *et al.* 2005; Lee *et al.* 2003). In both cases, the reorganization of the pattern of connectivity after rTMS, sometimes involving areas that are not direct targets of rTMS, may be compensatory and maintain task performance in the face of disruption of the pattern of activity that normally accompanies movement. This is because these task-related changes in coupling are accompanied by no change in the behavioural performance of the subjects either in the free selection of movement task or in the simple and sequential tapping tasks. In this experiment, it may be that the contribution from connections between CMA and the right PMd to the left M1 was effectively replaced by increased connections between rostral and caudal left PMd. This decrease in movement-related coupling to M1 parallels the increased M1 excitability measured by single and paired pulse TMS following 5Hz rTMS with 1min ITI to the left PMd. I tentatively suggest that a decrease in coupling in this instance might reflect a shift of activity towards areas of increased excitability such that some of the activity required to perform this task has shifted intra-hemispherically. Several studies examining self-initiated versus externally triggered movements have suggested a crucial role for non-primary motor areas, particularly premotor and supplementary motor areas in the performance of this task in monkeys (Passingham 1996, 1993) and humans (Jahanshahi *et al* 1995, Jenkins *et al.* 2000, Grafton *et al.* 1998).

5.5 CONCLUSION

The present chapter provides yet another example that demonstrated acute reorganization of the motor manual network following 5Hz rTMS over the dorsal premotor area, this time. This study extends previous work by providing evidence of shifts of brain activity towards areas of increased excitability following a protocol of rTMS conditioning known to increased the excitability of the stimulated and neighbouring areas. A pattern of acute intra- and inter-regional reorganisation with and within the premotor area conditioned by high frequency rTMS was observed. These results also show that the nature of the effects depends crucially on the configuration of the rTMS pulses (short v. long ITI). The implication is that it may be possible, by changing the parameters of rTMS (frequency, configuration, number of pulses, intensity of each pulse), to manipulate at will patterns of task-related activity in any part of the cortex.

6. Acute changes in fronto-parietal activity following 5Hz rTMS over the dorso-lateral prefrontal cortex in a cued reaction time task

6.1 INTRODUCTION

The neuroimaging experiments presented in the previous sections, where rTMS was delivered to M1 and PMd, have revealed changes in the pattern of task-related activity and connectivity, suggesting that the brain reorganizes in response to the disruption caused by stimulation, perhaps reducing the impact on task performance. The primary aim of this study was to test if similar reorganization occurs when rTMS is delivered to the lateral prefrontal cortex. This area is involved in many complex cognitive motor behaviour. I hypothesized that changes in local cortical activity induced by rTMS might be associated with deficits in task performance due to evidence from a previous behavioural study (Siebner et al. 2003).

Many studies investigating attentional selection have identified specific cortical networks relating to spatial orienting attention (Steinmetz and Constantinidis 1995, Coull and Nobre 1998, Corbetta and Shulman 2002). These seem to show that similar cortical areas are involved in both overt and covert shifts in attention, in which attention is oriented to different locations in the visual field in the absence of eye movements (Schneider and Deubel 1995, Nobre et al. 1997, Corbetta et al. 1998). However, overt shifts of visual attention involving eye movements have been associated with frontal eye field (FEF) activity. A previous study in which the FEFs were stimulated with on-line single pulse TMS during a reaction time task with covert shifts of attention, showed very strong behavioural effects (Grosbras and Paus 2002). TMS over left FEF delivered prior to target onset led to a decrease in RTs for targets presented in the right hemifield only. TMS over the right FEF had a bilateral effect on RTs, however the effect was opposite in invalidly cued trials. In this experiment, I wanted to explore possible behavioural effects

of off-line rTMS delivered to the DLPFC, known to be more involved in covert attentional shifts (Corbetta et al. 1993, Nobre et al. 1997).

The "Posner task", a widely used attentional shifts' paradigm, consists of trials in which cues indicate the most likely position of a subsequent target to which the subject has to react as fast as possible. Typically, longer reaction times are found for incorrectly cued targets (invalid trials) as compared to correctly cued targets (valid trials) (Posner, 1980). This so-called invalidity effect has been interpreted as an indicator for reorienting of attention (Posner *et al.* 1984). Recent neuroimaging studies have successfully identified a sub-set of areas involved in reorienting attention in this context (Thiel *et al.* 2004, Giessing *et al.* 2004), which include the middle frontal gyri bilaterally, and the IPS. Neuropsychological studies in patients with frontal and parietal lesions have shown that damage to the parietal area leads to impaired stimulus detection, particularly when misleading advance information is provided, such that targets appear at unattended locations as in invalidly cued trials (Losier & Klein 2001, Petersen et al. 1989, Posner *et al.* 1984). Patients with prefrontal damage are very slow in such tasks (Petersen et al. 1989, Posner et al. 1987). However, there are wide discrepancies in the results obtained from patients with prefrontal lesions in various tasks (D'Esposito & Postle 1999).

In this study, the conditioning effect of high-frequency (5Hz) rTMS on the DLPFC was tested in a Posner-type cued choice reaction time task. Subjects had to press their left or right index finger as soon as they saw a target appearing to the corresponding left or right side of a central fixation cross. A cue preceding the target indicated in which direction the target would appear. In most cases (80% of the trials), the direction of the cue was correct (valid trials). In the remaining 20% of all trials, however, the direction of the cue was incorrect (invalid trials), leading to a conflict between the prepared response to move to one side and the realization that the target was on the opposite side. A consequence of this conflict is that reaction times (RT) in invalid trials are delayed, leading to a switch cost. The lateral prefrontal cortex has been shown to play a crucial role in the process of reorienting (Asaad *et al.* 2000, Wallis *et al.* 2001, Wallis and Miller 2003), as has the parietal cortex (Corbetta & Shulman 2002).

The aim of this study was to investigate whether altering the excitability of the DLPFC with rTMS would affect 1) behavioural performance in this task and 2) the

pattern of synaptic activity as observed with functional magnetic resonance imaging (fMRI). 5Hz rTMS in an "off-line" conditioning paradigm can induce efficient and long-lasting increases in neuronal excitability when applied to motor (Peinemann *et al.* 2004) and dorsal premotor (Rizzo *et al.* 2004) cortices after only 10 minutes of stimulation. The hypothesis is that, as in the case of online virtual lesions caused by single pulse TMS applied during the performance of a task (Jahanshahi and Rothwell 2000), the persistent effects of 5Hz rTMS on reorienting attention would probably be disruptive. A preliminary study using the same Posner-type task as the one in the present experiment, in which RTs were compared before and after 5Hz rTMS to test for behavioural effects on reorienting attention suggested that this conditioning paradigm might compromise performance in invalidly cued trials when applied over the right DLPFC (Siebner *et al.* 2003). Using the same task we wanted to compare the effects of right and left DLPFC stimulation on task performance and network connectivity following 5Hz rTMS.

6.2 METHODS

6.2.1 Subjects

Twelve healthy, right-handed volunteers (three females, nine males, aged between 24 and 48 (mean age: 31.1)), with normal vision and no history of neurological disorder or head injury were recruited from the database of volunteers at the Functional Imaging Laboratory, Institute of Neurology, University College London, UK, for the neuroimaging study. Written informed consent was obtained from all participants. The study was approved by the joint ethics committee for the National Hospital for Neurology and Neurosurgery (UCLH NHS Trust) and the Institute of Neurology (UCL).

6.2.2 Experimental Design

Subjects were required to attend four separate 20min event-related fMRI scanning sessions. On two scanning sessions they received rTMS conditioning to their

left (or right) DLPFC with either Real or Sham rTMS. On the subsequent two scanning sessions they received Real or Sham rTMS on the other side (left DLPFC if they had started with right or right DLPFC if they had started with left). The session order relating to the side (right or left DLPFC) and type (Real or Sham) of rTMS conditioning was counterbalanced across subjects. Thus we had two between-session factors, namely side and type of rTMS conditioning over the DLPFC in addition to the within-session factors in the experimental task described below. The use of separate sham conditions for each side of DLPFC conditioning was motivated by the knowledge that there might be lateralised task-related changes in BOLD activity in this type of task (Corbetta & Shulman 2002). I was interested in the effects of rTMS on performance of this task in all types of trials (main effects of rTMS), in the changes in synaptic activity associated with valid and invalid trials separately (simple main effect of task) as well as in the interactions with validity and side of hand response (rTMS-by-validity interactions and rTMS-by-validity-by-hand interaction). Given that the right and left DLPFC were targeted in separate sessions in this study, and previous studies which postulate lateralization in reorienting attention tasks (Corbetta and Shulman 2002, Liu *et al.* 2003, Rushworth *et al.* 1997, 2001a,b), i hypothesized that we would see effects of rTMS which might be different in both hemispheres.

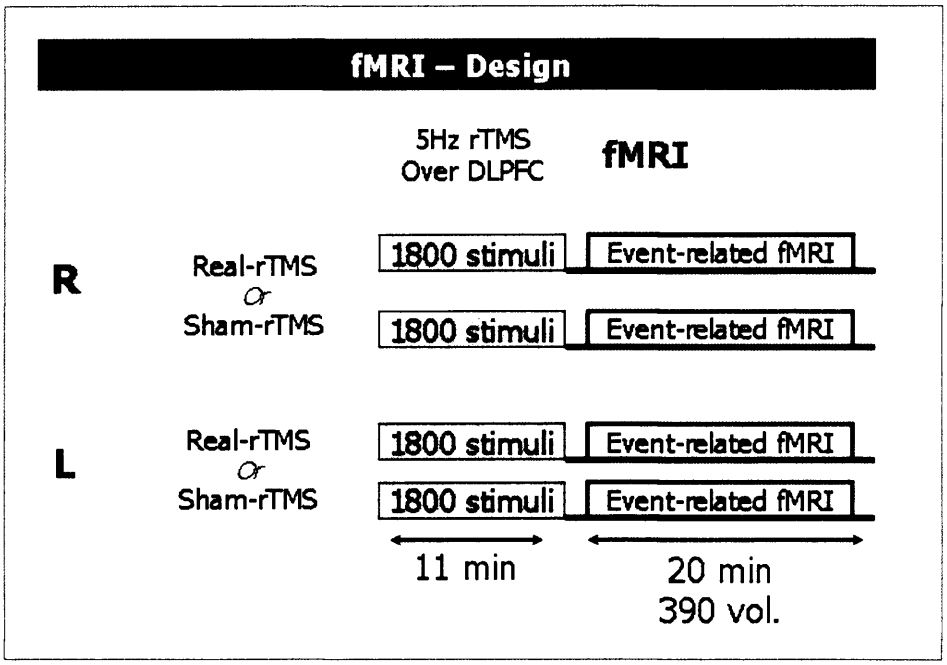


Figure 6.1: Experimental Design Subjects had to come on four separate sessions in which they received real and sham 5Hz rTMS to their right and their left DLPFC followed by a 20min fMRI scanning session. Subjects were allocated to each session in a counterbalanced order.

- **6.2.2.1 Experimental Task**

A cued choice reaction time task was chosen for this experiment (figure 6.2). Stimuli were presented against the gray background of a LCD monitor projected onto a screen via a mirror at a distance of 105 cm from the subjects' eyes in the scanner (with a NEC LT 157 projector). Responses were made on the key corresponding to the index finger in two separate four-key MRI-compatible response pads (one for each hand). Subjects were instructed to fixate a central black cross subtending 1° of visual angle. Each trial began with the 250ms presentation of a cue in the form of two directional arrows placed on either side of the fixation cross subtending 3.5° of visual angle and pointing to the right or the left. The side of the cue indicated whether subjects should prepare a response with their left or their right index finger. After the cue disappeared, the fixation cross was presented alone for 750 ms, followed by the presentation of a target stimulus (on screen for 250ms). The target stimulus was a "0" centered 4° to either

the left or right of the fixation cross. Subjects were required to respond as rapidly and accurately as possible, by pressing the index finger corresponding to the side where the target stimulus appeared. In 80% of the trials, the target location was correctly indicated by the cue (valid trials). In 20% of the trials, however, subjects were cued towards the wrong location (invalid trials). In this case the subjects had to move the index finger *opposite* to the one they had prepared to move, given the cue. Following the disappearance of the target, the fixation cross was presented alone for 1550ms before the next trial began; hence each trial was 2.8 seconds in duration. Validly and invalidly-cued trials were presented randomly and were interspersed by a small proportion of null events where no cues were presented other than the central fixation cross which remained constant on the screen. In each scanning sessions, subjects were presented with a total of 475 trials with the following proportions of events: 20% null events, 80% cued response-trials out of which 20% were invalidly cued and 80% were validly cued. The presence of null events and the random presentation of stimuli in each scanning session controlled for any order effects of stimulus presentations. Prior to scanning, subjects practiced the task for 10min in order to familiarize themselves with the task.

All stimuli were generated using COGENT 2000 graphics (available at www.fil.ion.ucl.ac.uk/cogent2000/) and behavioural responses were fed back onto a stimulus PC in order to record reaction times to target stimuli. The data were subsequently analyzed using Matlab 6.5 (Mathworks, Sherborn, MA) and SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA).

The task chosen in the present study is a variant of the Posner paradigm (Posner et al. 1984) for testing orienting attention. Previous neuroimaging studies have shown that invalid trials introduce consistent behavioural effects and changes in synaptic activity in consistent brain areas (Corbetta *et al.* 1993, Nobre *et al.* 1997, Nobre *et al.* 2001, Thiel *et al.* 2004).

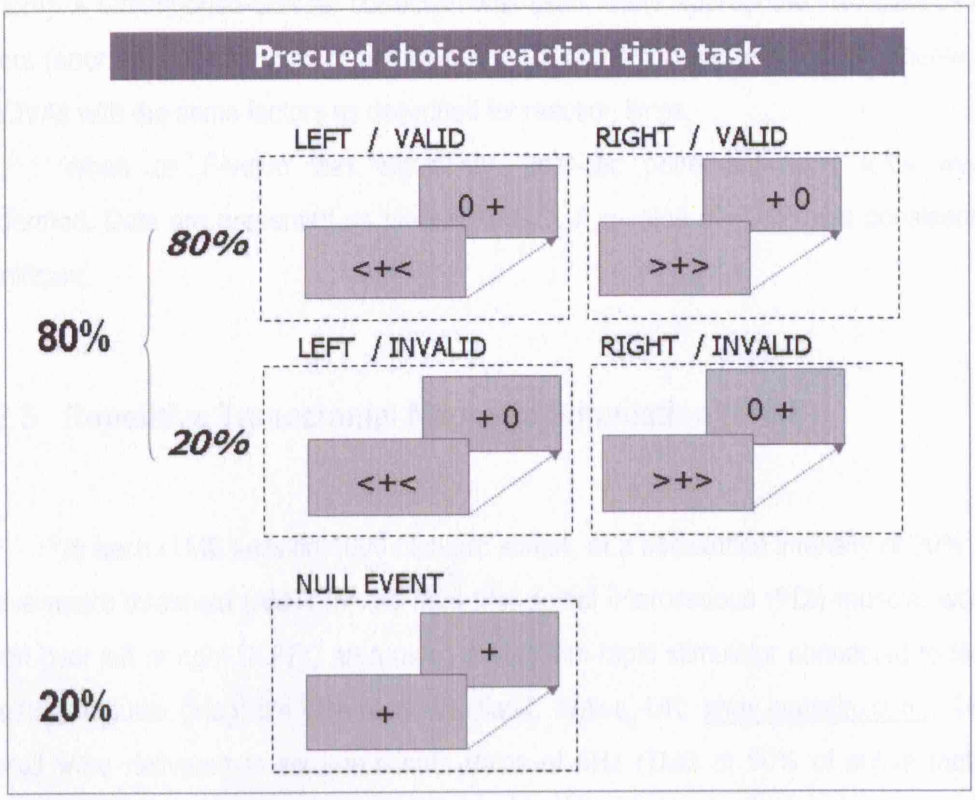


Figure 6.2: Task. The task was a Posner-type cued choice reaction time task. Subjects maintained fixation of a central black cross against a grey background. A warning cue in the form of two directional arrows placed on either side of the fixation cross indicated whether the subjects should prepare a response with their left or their right index finger. After the disappearance of the cue a target stimulus was presented to the left or the right of the fixation cross. Subjects were required to respond as rapidly and accurately as possible, by pressing the index finger corresponding to the side where the target stimulus appeared. In 80% of the trials, the target location was correctly indicated by the cue (valid trials). In 20% of the trials, the cue was incorrect and the target appeared in the opposite direction to what had been indicated by the cue (invalid trials). The proportion of trials and null events was 80% and 20% respectively.

▪ 6.2.2.2 Statistical analysis

For each subject, the mean reaction times in each condition were calculated and entered into a 4-way ANOVA with the following factors: the between-session factors of 1) *type* and 2) *side* of rTMS conditioning had two levels each (Real vs Sham for factor1; Right vs Left for factor2); the within-session factors of 3) *cue type* and 4) *side of motor response* also had two levels each (invalid vs valid for factor3 and right vs left for

factor4). A Greenhouse-Geisser correction was used where appropriate.Two classes of errors (errors of omission and of comission), were combined and entered into four-way ANOVAs with the same factors as described for reaction times.

When an F-value was significant, post-hoc paired-sample t tests were performed. Data are presented as means +/- S.D. A p-value of <0.05 was considered significant.

6.2.3 Repetitive Transcranial Magnetic Stimulation (rTMS)

In each rTMS session 1800 biphasic stimuli, at a stimulation intensity of 90% of active motor threshold (AMT) for the right first dorsal interosseous (FDI) muscle, were given over left or right DLPFC area using a MagStim-rapid stimulator connected to four booster modules (MagStim Company, Whitland, Wales, UK; www.magstim.com). The stimuli were delivered in six one-minute trains of 5Hz rTMS at 90% of active motor threshold (AMT) separated by an inter-train interval (ITI) of one minute. A standard figure-of-eight-shaped coil (Double 70-mm Coil Type P/N 9925, MagStim Company, Whitland, Wales, UK) was used for real-rTMS. For sham-rTMS, a specially designed sham coil that induced no magnetic field but provided a comparable acoustic stimulus was used (MagStim Company, Whitland, Wales, UK). The coil was positioned with the handle at 45° to the sagittal plane. The current flow of the initial rising phase of the biphasic pulse in the TMS coil induced a current flowing from posterior-to-anterior in the underlying cortex.

The site of rTMS stimulation was located 5cm anterior to the "motor hot spot" on a line parallel to the mid-sagittal line. This is the same location of DLPFC stimulation as in the study by Mottaghy *et al.* (2002) and Siebner et al (2003). The "motor hot spot" was defined functionally as the point of maximum evoked motor response in the slightly contracted right first dorsal interosseous (FDI). The active motor threshold was defined as the lowest stimulus intensity that elicited at least five twitches in ten consecutive stimuli given over the "motor hot spot". The contracted FDI was used to define the active motor threshold because TMS-evoked twitches are clearly visible and it has a threshold

below other intrinsic hand muscles (Wassermann et al. 1996). This ensured that the intensity used for rTMS was below motor threshold for all hand muscles. The use of sub-threshold intensity reduced the spread of stimulation away from the targeted site (Gershlager et al. 2001, Munchau et al. 2002, Pascual-Leone 1994). An intensity of 90% AMT was used according to the protocol of stimulation known to produce robust increases in corticospinal excitability when delivered to the PMd (Rizzo *et al.* 2004).

rTMS conditioning was performed "off-line" (Pascual-Leone et al. 1994, Jahanshahi and Rothwell 2000) *prior* to the scanning sessions, and following the practice session. The total duration of stimulation was 11 min and scanning was initiated at approximately 6min (±2.4min) after the end of the stimulation period. Previous studies have shown that the 5Hz rTMS effects can last for up to 1 hour after the end of the stimulation (Peinemann *et al.* 2004, Rounis *et al.* 2005, Rounis *et al.* submitted).

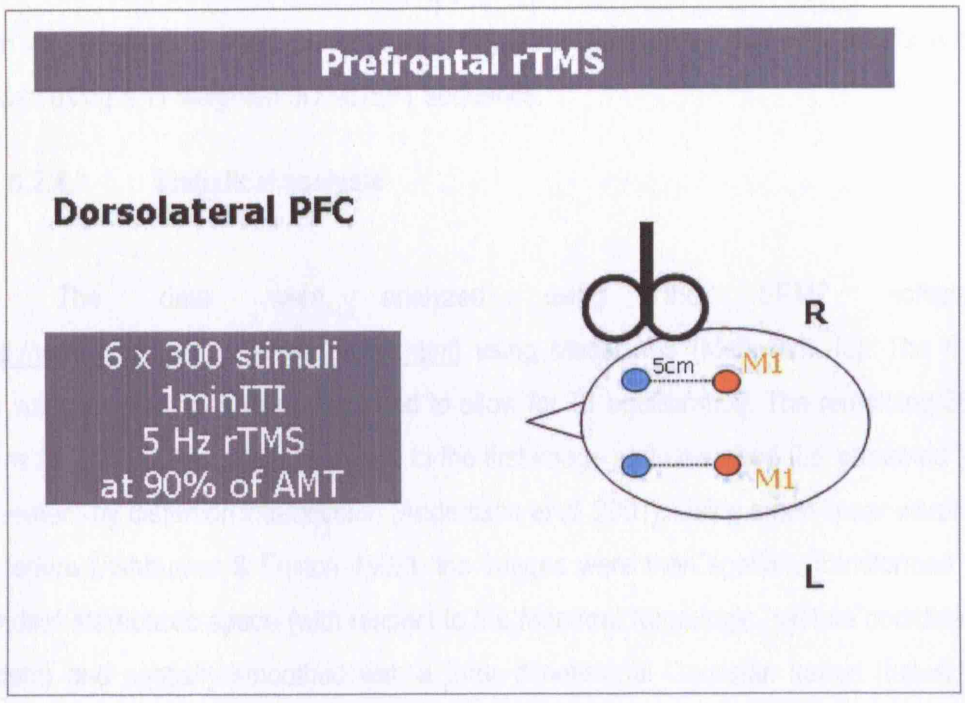


Figure 6.3: rTMS Protocol. rTMS conditioning protocol for the dorso-lateral prefrontal cortex (DLPFC). 5Hz rTMS was delivered in six 1min blocks (300 pulses) at 90% active motor threshold (AMT) separated by an inter-train interval (ITI) of 1min (Rizzo *et al.* 2004). The site of stimulation was determined functionally to be 5cm anterior to the motor hot spot (Mottaghy *et al.* 2002).

6.2.4 Functional Magnetic Resonance Imaging (fMRI)

- **6.2.4.1 Scanning procedure**

The experiment was performed on a 1.5T Sonata scanner (Siemens) with a head volume coil. A gradient EPI sequence was used (*TE* = 50ms, repeat time 3.6s). Each brain image was acquired in a descending sequence comprising 40 slices, each 2mm thick with 1mm gaps in-between for whole brain coverage, and consisting of 64x64 voxels. Head movement was minimised during scanning by comfortable external head restraint and subsequently corrected for using realignment and unwarping functions in the preprocessing prior to data analyses (see below). 390 whole brain images were obtained over

23.4min, with each subject taking part in four sessions. A T_1-weighted structural scan with fiducials marking the location of rTMS stimulation was also acquired for each subject using a T_1-weighted 3D MDEFT sequence.

- **6.2.4.2 Statistical analysis**

The data were analyzed using the SPM2 software (http://www.fil.ion.ucl.ac.uk/spm/spm2.html) using Matlab 6.5 (Mathwork, IL). The first five volumes of images were discarded to allow for T1 equilibration. The remaining 385 scans for each subject were realigned to the first image and unwarped (i.e. corrected for movement-by-distortion interactions) (Andersson *et al.* 2001). Using a non-linear warping procedure (Ashburner & Friston 1999), the images were then spatially transformed to standard stereotaxic space (with respect to the Montreal Neurologic Institute coordinate system) and spatially smoothed with a three-dimensional Gaussian kernel (full-width half-maximum: 8 mm).

In the subsequent statistical analysis, statistical parametric maps based on the *t*-statistics were calculated for condition-specific effects using a general linear model (Friston et al. 1995). Within each session, the four conditions of the 2x2 factorial experimental design (valid versus invalid cues x left versus right side targets) were modelled by representing each correct response as a delta function, convolved with two

basis functions: a canonical haemodynamic response function and its temporal derivative. Incorrect and missing responses (errors) were modelled separately in the same fashion. Effects of interest were examined in each subject using linear contrasts. To control for low-frequency drifts, high-pass filter was used with a 1/128 s cut-off; to control for serial autocorrelation in the data, the data were whitened using an AR(1) model (Friston et al. 2002).

The resulting subject-specific contrast images were then entered into second-level random effects analyses that were implemented by one-sample *t*-tests. All reported results are significant at a threshold of $p<0.05$ whole-brain corrected at the cluster-level (using a standard $p<0.001$ at the voxel-level).

6.3 RESULTS

No subjects reported adverse side effects during the course of the study. Mean active motor threshold for right FDI was 53% ranging from 46% to 63% of maximum stimulator output. Mean active motor threshold for left FDI was 49% ranging from 39% to 67 % of maximum stimulator output.

6.3.1 Behavioural Data

A four-way ANOVA with stimulation TYPE (Real versus Sham rTMS), stimulation SIDE (Right versus Left DLPFC), CUE (valid versus invalid) and HAND (Right versus Left) revealed a significant main effect of CUE ($F_{1,11}$ = 52.89, P=0.000). Reaction times on invalid trials were slower than on valid trials. No other significant main effects or interactions were revealed (table 6.1). Subsequent 3-way ANOVAS analyzing the effects of stimulation TYPE and SIDE as well as HAND on either valid or invalidly cued trials separately did not reveal any significant main effects or interactions except for a trend for stimulation TYPE-by-SIDE interaction ($F_{2,22}$= 6.27, P=0.054) for invalidly cued trials only, which suggested a slight difference in invalidly-cued RTs if subjects were stimulated with real rTMS compared to sham on the right versus the left DLPFC. However, post-hoc t-tests looking at whether the type of rTMS stimulation (real- or sham) affected the ratio of invalid versus valid response times for each side (left vs right) of

stimulation separately did not reveal any significant results. Table 6.1 and Figure 6.4 summarize the RT results.

Subjects made two types of errors in this task: errors of omission accounting for 10% of the total errors (missed responses) and errors of commission (responding to the opposite side of the target) accounting for 3%, making the total average of wrong responses 13%. Error rates were not significantly different either in validly or in invalidly cued trials after 5Hz rTMS to either hemisphere.

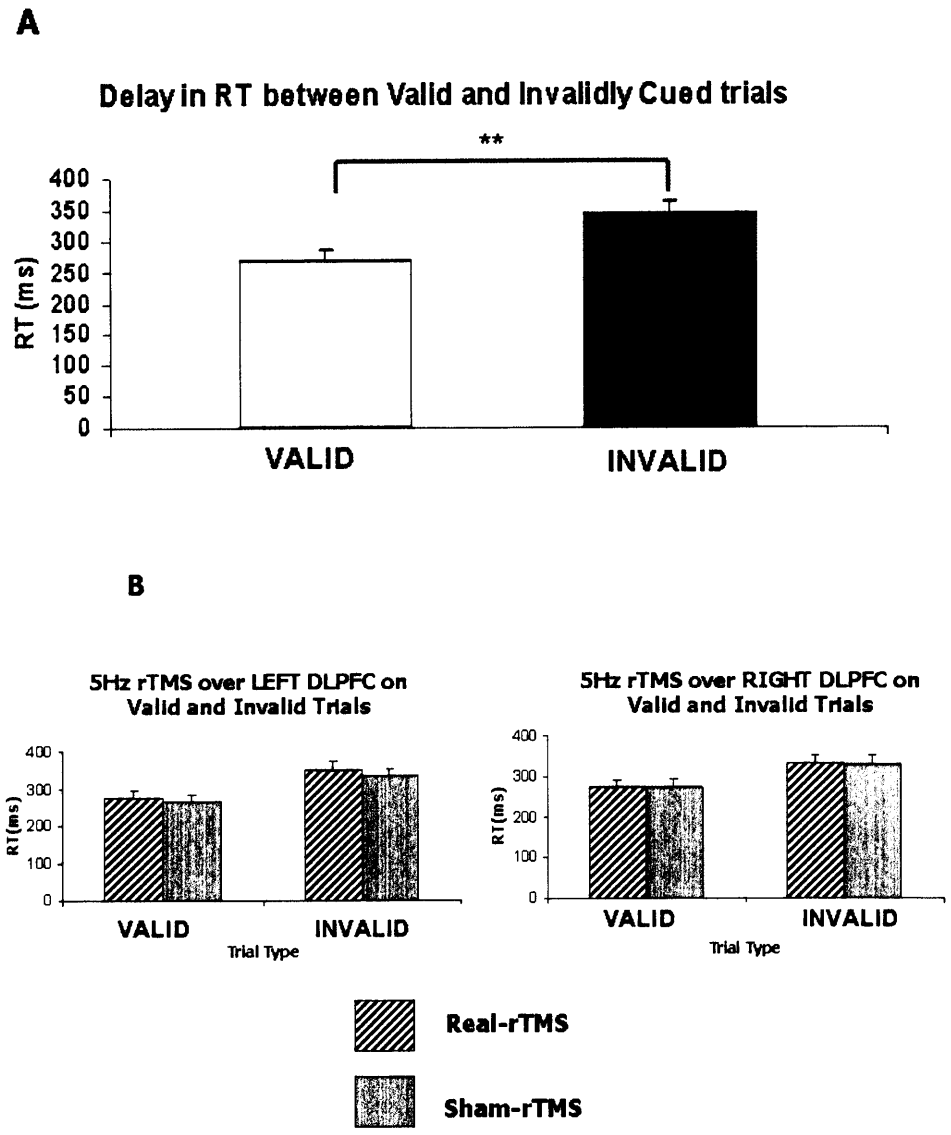


Figure 6.4: Behavioural Effects of Validity and 5Hz rTMS over DLPFC on RTs. A A significant main effect of cue revealed an effect of validity on response times (RTs) pooled across all levels of stimulation type, stimulation site and hand response. RTs in invalidly-cued trials were slower (352ms +/- 12ms) than in validly cued trials (267 ms +/- 7ms). **B** 5Hz rTMS did not lead to any significant changes in RTs either in valid or in invalid trials whether applied over the left or the right DLPFC.

	Post-Real rTMS	Post-Sham rTMS	t-value (df=11)	P-value
Invalid/Valid RT*100				
RIGHT SIDE	129.54(±25.02)	132.99(±22.64)	-0.92	0.377
LEFT SIDE	127.19(±18.90)	130.89(±12.55)	-1.15	0.275

Table 6.1: Behavioural effects of 5Hz rTMS over the DLPFC: Mean group data (± SD) for the ratio between invalidly versus validly cued reaction times in each condition in each session.

6.3.2 Imaging Data

▪ 6.3.2.1 Main effects and Interactions

The fMRI results in this study, a 2x2x2x2 factorial design with the factors side of stimulation, type of stimulation, validity of the cue and hand response, were analysed in a similar way to the behavioural results. All main effects, two, three and four way interactions and simple main effects were analysed. However, only those results that were significant, all brain corrected, are reported below.

▪ 6.3.2.2 The effect of cue validity on synaptic activity

As shown in figure 6.5 and table 6.2, the detection of invalidly cued trials, pooled across all levels of stimulation type, stimulation site and hand responses, was associated with increased synaptic activity in the right frontal and parietal lobes. The main effect of cue revealed significantly increased activity in the right dorso- and ventro-lateral prefrontal cortices (DLPFC and VLPFC), right inferior frontal gyrus (IFG) and insula, as well as the intraparietal sulci (IPS) bilaterally.

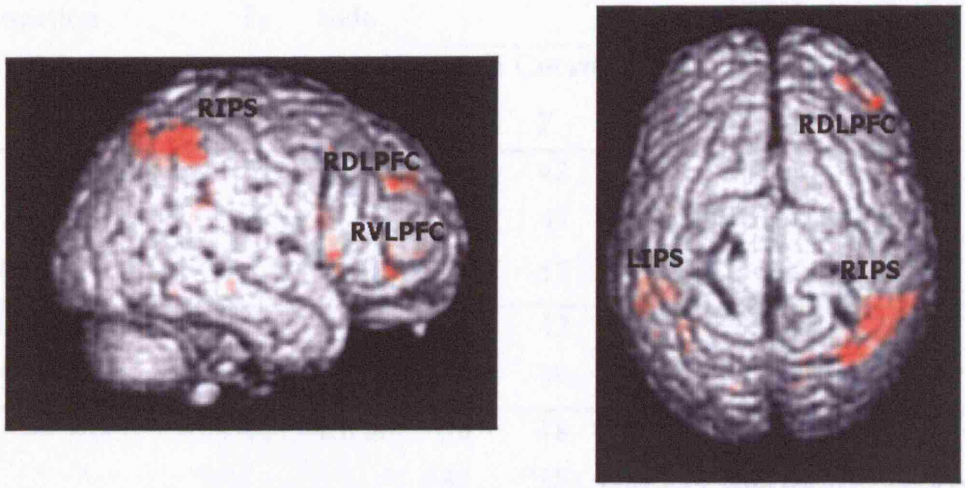


Figure 6.5: BOLD signal Increases in Invalid compared to Valid Trials. The effect of validity of cues on the pattern of BOLD activity. A direct contrast of invalid versus valid trials pooled across all levels of stimulation site, stimulation type and hand response is displayed on the rendered T1-weighted canonical template from SPM2. This contrast revealed a mainly right-lateralised fronto-parietal network for reorienting attention which included clusters in the right DLPFC and VLPFC and bilateral IPS.

Anatomical Location	k_E	Side	MNI Coordinates			Cluster P value	Cluster Z value
			x	y	Z		
Dorso-Lateral Prefrontal	21	Right	45	42	33	<0.001	5.87
			33	42	42	<0.001	4.06
			27	57	30	<0.001	3.57
Ventro-Lateral Prefrontal	15	Right	48	42	-6	0.004	4.22
			42	39	0	0.004	3.54
Insula	10	Right	36	18	-3	0.043	3.61
			42	15	3	0.043	3.35
Inferior Frontal Gyrus (IFG)	13	Right	51	9	24	0.01	3.79
			48	9	15	0.01	3.73
Intraparietal Sulcus (IPS)	31	Left	-39	-39	42	<0.001	4.99
			-51	-45	45	<0.001	3.71
			-33	-54	45	0.003	3.96
IPS / Angular gyrus	136	Right	33	-66	57	<0.001	4.44
			45	-57	51	<0.001	4.27
			54	-48	48	<0.001	4.27

Table 6.2: Effects of Validity: Areas of activation associated with presentation of the invalid trials compared to valid trials (k_E: cluster size)

▪ 6.3.2.3 The effects of 5Hz rTMS over DLPFC:

Inspection of structural T1-weighted scans with the TMS surface markers was used to obtain fiducial locations for all 12 subjects. Tthe average location of the fiducials adjacent to the DLPFC were: x = 37, y = 46, z = 51 (Right) and x = -34, y = 44, z = 55 (Left). These coordinates are more dorsal than the DLPFC sites reported in the literature (Boettiger and D'Esposito 2005, Corbetta and Shulman 2002). The location of the right fiducial is very close to a cluster in the right DLPFC we obtained in the present study, with significant activation in the invalid versus valid contrast for the main effect of cue (described above and in table2) located just below the stimulation site with the following coordinates: x = 33, y = 42, z = 42 (local maximum: $P<0.001$, Z = 4.06).

The data on the effects of rTMS over DLPFC is presented separately for the two hemispheres separately.

• 6.3.2.3.1 Left-sided DLPFC conditioning:

(a) Main effects of 5Hz rTMS across all conditions

5Hz rTMS over the left DLPFC caused widespread left-lateralised decreases in task-related activity as measured by the BOLD signal in areas remote from the site of rTMS. These included the superior parietal gyrus bilaterally; the left intraparietal sulcus (IPS), left ventrolateral prefrontal cortex (VLPFC), left superior temporal gyrus and lateral occipital cortex as well as the right primary sensorimotor area, right insula and right cerebellum (see table 6.3). No increases in BOLD were found in the main effects of 5Hz rTMS on the left side, nor were there any validity-by-rTMS interactions.

Anatomical Location	k_E	Side	MNI Coordinates			Cluster	
			x	y	z	P value	Z value
Decreases in BOLD following LEFT DLPFC conditioning with rTMS (SHAM_TMS)							
Ventrolateral Prefrontal Cortex	11	Left	-39	42	3	0.004	3.87
Superior Parietal Gyrus	47	Right	6	-60	60	<0.001	4.08
		Left	-3	-48	66	<0.001	3.92
Intraparietal Sulcus (IPS)	27	Left	-18	-69	51	0.006	4.15
			-21	-60	48	0.006	3.65
Superior Cerebellum	20	Right	21	-60	-21	0.031	4.11
Primary Sensorimotor Area	24	Right	45	-21	57	0.012	4.07
Insula	35	Right	45	15	-3	0.001	3.99
			48	6	3	0.001	3.45
Superior Temporal Gyrus	28	Left	-57	-15	3	0.005	4.00
			-45	-18	12	0.005	3.75
Lateral Occipital Cortex	32	Left	-51	-72	-9	0.002	4.00
			-42	-69	-9	0.002	3.5

Table 6.3: Main Effects of 5Hz rTMS over LEFT DLPFC in all conditions: Areas of de-activation associated with 5Hz rTMS over the LEFT DLPFC preconditioning with Real rTMS compared to Sham on all trials (k_E: cluster size)

(b) Simple main effects of 5Hz rTMS

Decreases in BOLD response were found during performance of invalid trials in the real-rTMS session compared to the sham when these were examined separately to validly cued trials (table 4A). The simple main effects of 5Hz rTMS on invalidly cued trials revealed a prominent decrease in BOLD activity over the left VLPFC (local maximum: x = -39,y = 42, z = 3; Z = 4.08; P = 0.012) and a decrease in the right anterior calcarine sulcus (local maxima: x = 27,y = -54, z = 9; Z = 4.18; P = 0.02; x = 21,y = -60, z = 12; Z = 3.77; P = 0.02). No significant increases in activity were found when the simple main effects of invalid and valid conditions were examined separately. In addition, there were no TMS-by-validity interactions following left sided conditioning, a result that might have been expected given the strong right-lateralised validity effect found in the lateral prefrontal cortex with a lack of left DLPFC activity as described above, in the invalid-vs-valid interaction.

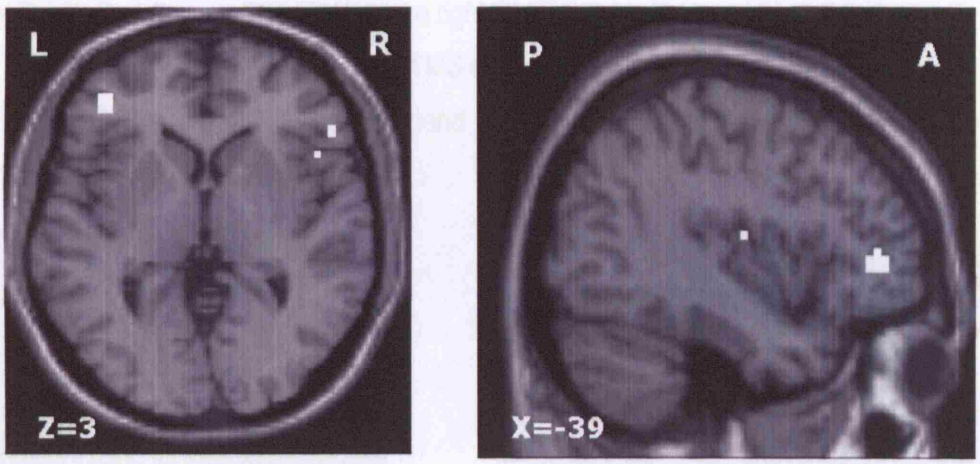


Figure 6.6: Simple main effect of left DLPFC conditioning on BOLD activity during the performance of invalidly cued trials. BOLD activity decreases in the VLPFC (local maximum: x = -39,y = 42, z = 3; Z = 4.08; P = 0.012) are displayed on transverse (left panel) and sagittal sections of the canonical T1-weighted structural template from SPM2.

- **6.3.2.3.2 Right-sided DLPFC conditioning:**

(a) Simple main effects of 5Hz rTMS

5Hz rTMS over the right DLPFC revealed a decrease in BOLD activity in the right ventromedial prefrontal cortex (local maximum: x = 18,y = 48, z = -3; Z = 4.57; P = 0.014) during performance of invalidly cued trials only. There were no significant main effects on all conditions or simple main effects of 5Hz rTMS when valid trials were tested separately.

(b) TMS-by-validity interaction

A two-way interaction (right 5Hz rTMS-by-validity) revealed a validity-specific decrease in BOLD activity of the right VLPFC following 5Hz rTMS over the right DLPFC compared to sham (local maximum: x = 36,y = 42, z = 12; Z = 4.31; P = 0.013). Activity in this area, located at the border of ventral area 9/46 and rostral area 47/12 (Duvernoy 1999, Talairach and Tournoux 1988, Petrides and Pandya 2002), showed a decrease in BOLD activity following 5Hz rTMS to the right DLPFC in invalid compared to valid trials.

Finally, three-way (side of rTMS-by-rTMS-by-validity) and four-way (side of rTMS-by-rTMS-by-validity-by-side of hand response) interactions did not reveal any significant changes in BOLD activity.

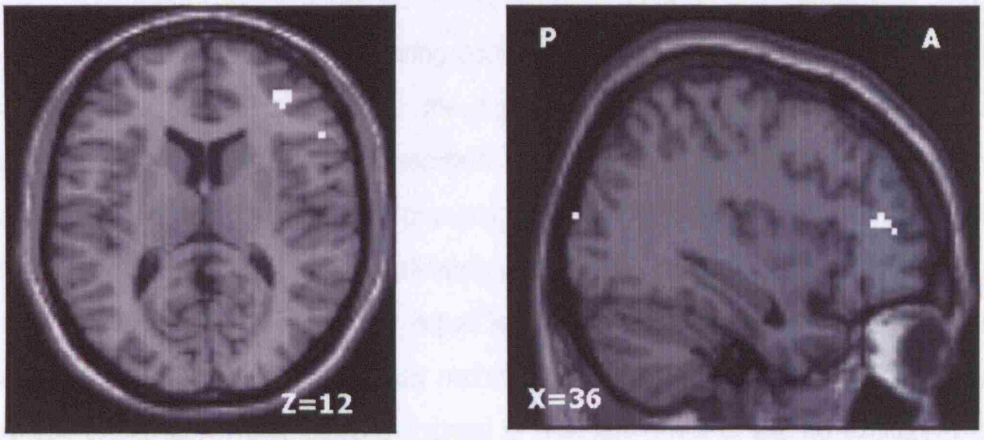


Figure 6.7: Validity-by-rTMS interaction following 5Hz rTMS to the right DLPFC: This figure shows the effect of right DLPFC conditioning on BOLD activity during the performance of invalidly cued trials. A significant decrease in BOLD was revealed in the right VLPFC (local maximum: x = 36,y = 42, z = 12; Z = 4.31; P = 0.013) displayed on transverse (left panel) and sagittal sections of the canonical T1-weighted structural template from SPM2.

6.4 DISCUSSION

The goal of the present study was to investigate if the lateral prefrontal cortex compensates for changes in excitability induced by a preceding rapid "off-line" rTMS conditioning during the performance of a cued choice reaction time task. Conditioning of neither the left nor the right DLPFC resulted in significant changes in behaviour or synaptic activity at the site of stimulation. Changes in synaptic activity were seen in remote areas that have previously been implicated in attentional reorienting tasks. The discussion will concentrate on these findings.

6.4.1 rTMS Conditioning of the DLPFC Did Not Affect Behaviour In This Task

In this study, rTMS conditioning of DLPFC did not affect behavior, despite evidence showing that DLPFC is a critical area involved in the task (Corbetta and Shulman 2002 as well as the BOLD signal changes observed in the present experiment when invalidly cued triasl were compared to validly cued trials pooled across all levels of stimulation type, stimulation side and hand).

A behavioral study, published in abstract form by our group (Siebner *et al.* 2003) showed increased reaction times during performance of invalidly cued trials in healthy volunteers following 5Hz rTMS to the right DLPFC using the same stimulation parameters as in the experiment described in this section. Interestingly, although the conditioning effects of 5Hz rTMS on physiological measures of motor cortical excitability have been reported to last for approximately one hour (Peinemann *et al.* 2004, Rizzo *et al.* 2004), this behavioral effect only lasted for 10 minutes. It is possible that the effect Siebner and colleagues observed may represent an interaction between rTMS induced changes in excitability and learning, instead of a simple effect of altered excitability on performance as the order of intervention was always task-rTMS-task. In the current study, a between-session comparison of motor behavior was made, which did not reveal any behavioural effect.

A possible explanation for the absence of any behavioural effects following 5Hz rTMS over the DLPFC may be that of rapid reorganisation of the areas involved in orienting enabling performance of this task to be maintained. The DLPFC or area 46 lies at an anterior point of rich convergence of many pathways both ventral and dorsal, emanating from the sensory modality areas (Young 1992). These anatomical connections indicate how this area is operational on very high-level cognitive functions. Its interaction with all other association networks might be so complex so that disruption of that single area on its own may not show any clear deficits. This is exemplified by a recent human neuroimaging study, which suggested a role for area 46 in visual awareness (Sahraie *et al.* 1997). In that study, the authors discussed about the difficulty previous investigators had had in finding any behavioural effects following lesions to the DLPFC and mentioned in a study by Latto and Cowey (1971) in which lesions to the frontal eye fields in monkeys could lead to visual field defects similar to those of lesioning V1 itself. The authors concluded that in order to produce such an effect in humans, it might be necessary for the lesions to be both large and bilateral.

In the present experiment, changes in the activity of adjacent and remote areas were observed as a result of the conditioning, which might have reflected compensatory adaptation in response to the rTMS.

6.4.2 rTMS To The DLPFC Did Not Alter BOLD Activity In The Stimulated Area

In addition to the lack of behavioural effect we found no change in synaptic activity at the site of stimulation in the present study. It is important to note here that unlike the studies presented in the previous chapters, the use of fMRI rather than PET scanning to look at the effects of rTMS on synaptic activity does not allow the effect of rTMS to be examined at rest. Indeed, PET scanning can measure brain activity levels at rest and these could be compared between the real rTMS and sham sessions. The comparisons in the present chapter, however, describe task-related activity following real and sham rTMS. The fact that we cannot examine the effect of rTMS at rest may in itself explain why we cannot see any BOLD signal changes at the site of stimulation.

However, this finding is not unique. Despite extensive physiological evidence that 5Hz rTMS to primary motor (Peinemann *et al.* 2004) and dorsal premotor areas (Rizzo *et al.* 2004) leads to lasting changes in the excitability of the motor cortex the PET studies described in Chapters 4 and 5, where 5Hz rTMS was delivered to the motor and premotor cortex, also failed to demonstrate a change in task-related synaptic activity at the site of rTMS. These results do not imply that 5Hz rTMS over the DLPFC did not lead to any changes in the excitability of the stimulated area. A number of PET and fMRI studies suggest that stimulation of an area with rTMS, particularly at subthreshold intensities, does not necessarily lead to changes in BOLD signal or rCBF at the site of stimulation in "on-line" rTMS studies(Bohning *et al.* 1999, Baudewig *et al.* 2001, Bestmann *et al.* 2003); activity changes have been reported in anatomically connected areas. Speer et al (2003) report a non-linear relationship between intensity of stimulation and rCBF in the stimulated M1 following 1Hz rTMS: subthreshold stimulation did not evoke a significant increase in rCBF, whereas suprathreshold stimulation did. The work presented in Chapter 4 has suggested that similar effects might occur with higher frequencies of stimulation (5Hz). In an "on-line" fMRI study by Bestmann et al. (2003) report no changes in BOLD signal during stimulation of M1 with subthreshold 4Hz rTMS. There is ample electrophysiological evidence that 5Hz rTMS does lead to an increase in the excitability of the stimulated region (Berardelli et al. 1998, Di Lazzarro et al. 2002) confirming that there appears to be a dissociation between excitability changes and changes in rCBF or BOLD signal. This is in line with observations that the absence of changes in rCBF does not rule out the presence of significant neuronal activity (Nielsen and Lauritzen 2001). Changes in BOLD signal reflect changes in synaptic activity of both inhibitory and excitatory neurons: if these changes in excitatory and inhibitory circuits occur simultaneously, they might cancel out and show no change in BOLD.

6.4.3 rTMS To The DLPFC Led To BOLD Activity Changes In Remote Areas

Changes in synaptic activity were detected in cortical areas that are anatomically and functionally connected to the DLPFC. Decreases in synaptic activity were seen in the VLPFC, VMPFC and IPS following DLPFC conditioning with 5Hz rTMS

during the performance of a reorienting task. Since it has been demonstrated that the fMRI BOLD signal is slightly better correlated to synaptically evoked local field potentials than to the spiking output of the area (Logothetis et al. 2001) and that BOLD deactivations might in fact reflect a decrease in neuronal activity (Shmuel et al 2002), remote BOLD signal changes might be explained by the connectivity of the DLPFC area to other sites. Excitability changes in DLPFC might induced by rTMS may lead to changes in postsynaptic activity in anatomically connected regions via direct activation of projecting fibres or indirectly by modulation of intermediate regions, which could result in BOLD signal increases or decreases at remote sites. Changes in synaptic activity in VLPFC and IPS mediated by direct activation of projecting fibres are in this study rendered more plausible by ample evidence from non-human primates about the connectivity of these areas with the DLPFC (Petrides and Pandya 1999, 2002). The region of DLPFC more activate during invalid than validly cued trials in our study lies at the border of the middle and superior frontal gyri, in area 46 (Petrides and Pandya 1999). The site of significant three-way interaction (x=36, y=42, z=12) was located ventrally in rostral area 47/12 (at the border with ventral area 9/46). In the macaque, this part of VLPFC is connected with area 46 (Petrides and Pandya 1999). Connections are also reported between rostral 47/12 and dorsal areas 46 and 9/46 (Petrides and Pandya 2002). There are also clear parietal-VLPFC connections (Goldman-Rakic and Cavada 1989, Petrides and Pandya 2002). The changes in synaptic activity in VLPFC may therefore have been mediated by direct connections from the conditioned DLPFC or may result from changes in the sensitivity of that area to inputs from the parietal areas activated during normal task performance in the invalidly cued trials. Since both prefrontal areas receive inputs from their parietal homologues (Petrides and Pandya 2002), changes in activity in the parietal cortex may reflect the altered excitability of the lateral prefrontal cortex to which the parietal areas are projecting.

6.4.4 Task-Related Changes in Synaptic Activity following rTMS Conditioning

Task-related activity in this experiment was primarily located in the right frontal and parietal regions, including the right dorso and ventro-lateral prefrontal cortices and the IPS bilaterally. This pattern is very similar to that observed in studies investigating attentional selection in the context of visual attention (Corbetta *et al.* 2000, Liu *et al.* 2003). Conditioning the *left* or the *right* DLPFC with rTMS resulted in slightly different changes in the activity pattern observed in remote areas. The results in this study mainly indicate a decrease in task-related activity in ventro-medial and ventro-lateral prefrontal cortices confirming previous data that rTMS to the DLPFC reduces synaptic activity on the stimulated site (Mottaghy et al. 2000). Mottaghy et al. (2000) studied the effects of a continuous train of 4hz rTMS to DLPFC at 110% of resting motor threshold while healthy subjects performed a 2-back working memory task. Focal rTMS to right and left DLPFC impaired task performance, an effect that might be attributed to the use of a higher intensity of stimulation which might have lead to a more prominent conditioning effect of the rTMS. As in the present study, rTMS-induced effects on the task-related activity pattern were different depending on the site of rTMS. 4Hz rTMS to left DLPFC reduced task-related increases in rCBF in the left DLPFC alone, whereas rTMS to the right DLPFC reduced task-related rCBF increases in right DLPFC and the parietal cortex bilaterally (Mottaghy et al. 2000). Previous studies report different patterns of cortical activity in the context of selecting task-relevant information when manipulating the cognitive demands of the tasks (Banich *et al.* 2000, Dove *et al.* 2000, MacDonald *et al.* 2000, Brass & von Cramon 2004). This led to the suggestion that the neuronal mechanisms involved in attentional selection per se and attentional selection in the context of task preparation might differ (Liu *et al.* 2003). Rushworth et al (2001a) distinguish between visual switching and response switching by asking subjects to attend either to different visual dimensions (visual switching task) or to alternate between different stimulus-response associations (response-switching condition). They observed strongly left-lateralised increases in rCBF in the parietal cortex in the latter condition. These results were confirmed in a subsequent study (Rushworth et al. 2001b) in which

disruption of the left anterior parietal cortex with rTMS in a "virtual lesion" paradigm led to a compromised performance in a motor attention task in healthy human volunteers.

This task does not distinguish between the two types of attention (motor or visual). Subjects not only had to perform covert shifts of attention to the target sites and may need to reorient their attention in invalidly cued trials, they were also required to press either their left or right index fingers in response to the target. Despite the prominent activity in right-lateralised fronto-parietal areas, suggesting a bias for visual attention, the left IPS activity observed in our study may reflect motor attention or selection of task-relevant information (Brass and von Cramon 2004). The main effect of rTMS observed when the left DLPFC was stimulated revealed a *decrease* in BOLD activity of the left VLPFC, adjacent to the stimulated site, the left IPS as well as the right primary motor cortex. These results are reminiscent of previous evidence that changes in DLPFC excitability affect other areas, including those at earlier levels of stimulus processing (such as the IPS, or the calcarine sulcus). Moore & Armstrong revealed a similar effect in area V4 following FEF microstimulation in monkeys (Moore & Atmstrong 2003) . Electrical stimulation at intensities insufficient to induce any saccades still led to an increase in V4 neurons' activity when a stimulus was presented in their receptive fields.

The lack of a validity-by-rTMS interactions following left DLPFC conditioning is likely to be due to the absence of left DLPFC activity in the initial comparison of invalid versus valid trials (the validity effect presented in the first part of the results section, pooled across real and sham rTMS sessions). Such an interaction was found with right DLPFC conditioning in the right VLPFC (see below) and it might have also been found had we targeted the right and left IPS which show very strong activity in the validity effect.

rTMS conditioning of the right DLPFC led to decreased task-related activity in the right ventromedial prefrontal cortex, and did not cause such widespread changes in activity as left DLPFC conditioning. The discrepancy between these results and the Mottaghy et al (2000) study might relate to differences in the tasks used. The exact functional role of the VMPFC revealed in our study is unclear. It is hypothesised to play a role in reward and decision-making, however, its exact contribution following rTMS to

DLPFC cannot be determined based on these results (Bechara *et al.* 1994, Hornak *et al.* 2004, Rolls *et al.* 1994, O'Doherty 2003).

Right-sided DLPFC conditioning with real 5Hz rTMS compared to sham decreased synaptic activity in right VLPFC in invalid compared to validly cued trials as revealed in the validity-by-rTMS interaction. This area was revealed in the contrast of invalid-valid trials collapsed across rTMS sessions and its activity was decreased as a consequence of right DLPFC conditioning. The functional significance of this finding is unclear, but points towards the possibility of a degenerative network in the prefrontal cortex for cued choice reaction tasks.

The exact function of the DLPFC and VLPFC within the lateral prefrontal cortex and their interaction in information processing has been a matter of debate, which led to the development of two models describing their respective roles. The "domain specificity" model (Goldman-Rakic 1987, Funahashi *et al.* 1993, Wilson *et al.* 1993, Scalaidhe *et al.* 1999) postulates a modular organization of working memory based on the domain of information processing: in this view, the DLPFC (corresponding to Walker's area 46 in the macaque brain) is specialized for "on-line" processing of information concerning the location of objects whereas the VLPFC is involved in processing the features and identity of objects within working memory. In contrast, the "operation-segregation" model (Petrides 1994, Owen *et al.* 1996) proposes two executive processing systems within the lateral frontal cortex: the VLPFC (Brodmann areas 45 and 47) (Brodmann 1909) serves as one level of interaction between short-term and long-term memory systems and executive processing, while the DLPFC constitutes another, "higher-order" level of interaction of executive processes with memory, and is recruited only when active manipulation and monitoring of information within working memory is required. Implicit in this model is the notion of applicability across stimulus type.

The finding that VLPFC activity changes according to the excitability state of the DLPFC during performance of a Posner-type reorienting task favors the 'operation-segregation' model because it allows for more flexibility in the way activity in the two regions changes following physiological changes, while the cognitive demands of the reorienting task do not changed, which would be hard to explain with a model that describes activity in these two areas based on stimulus modality. The domain specificity

model has recently been challenged with evidence from electrophysiological recording of prefrontal cells activity in monkeys. A study by Rao et al (1997) used electrophysiological recordings of neurons in primates' principal sulcus to show that they encoded either, or both, the location and the identity of stimuli presented in a novel delayed response procedure. In addition neurons located both dorsal and ventral to the principal sulcus were shown to be flexible, coding different stimulus attributes at different times according to task demands, therefore supporting the possibility of a more flexible organization between these areas. In the absence of any cognitive manipulations in the present task and any behavioural effects of conditioning, we hypothesize that the activity changes observed in this study might constitute rapid compensatory mechanisms to maintain performance.

6.5 CONCLUSION

This data introduces the possibility that changes in dorso- and ventro-lateral prefrontal task-related activity may occur in response to transient changes in excitability caused by rTMS conditioning as well as in response to changes in cognitive demand and different attentional tasks. However, the task used in this experiment failed to reveal whether the behavioural effects described by Siebner et al. (2003) and the neuroimaging effects found here relate to a disruption of the visual or the motor attentional network. This issue is dealt with in the next chapter.

7. Effects of rTMS over the DLPFC on motor versus visual attention: A behavioural study

7.1 INTRODUCTION

Many studies investigating attentional selection have identified specific cortical networks relating to spatial (Steinmetz and Constantinidis 1995, Coull and Nobre 1998, Corbetta and Shulman 2002) and feature-based attention (Liu *et al.* 2003). There is evidence that patients with lesions in the parietal cortex have a difficulty in "disengaging" covert attention from one focus to another (Posner *et al.* 1984). Many studies have found evidence for a right hemispheric dominance in controlling visuospatial attention (Mesulam 1981, Gitelman *et al.* 1999, Corbetta and Shulman 1998, Nobre *et al.* 1997). In the previous section, the task used in order to measure the effects of 5Hz rTMS over the DLPFC on reorienting attention could not have distinguished whether these effects were on motor or spatial attention.

The Posner task was mainly designed to test whether subjects could direct their covert attention to the target's location before its appearance, with the use of directional pre-cues. However, it has long been known that reaction times (RTs) can be shortened if subjects covertly attend to the motor response that they prepare to make (LaBerge *et al.* 1969). In the 1980s, Rosenbaum used precues to specify specific parameters of aimed hand movements such as their direction and extent (Rosenbaum 1980). He showed that the use of precues could reduce reaction times in a manner consistent with the partial programming of motor responses. Twenty years later, Rushworth and colleagues were able to use tasks in which precues prepared subjects to move in a particular direction. They referred to the process the brain uses to perform these tasks as "motor attention" to distinguish it from visual attention (Rushworth *et al.* 1997, 2001a, 2001b). They have shown that precues can be used to covertly attend to or prepare for particular movements (Rushworth *et al.* 1997). As in visual attentional tasks, when the precue is valid, there is a RT shortening, but when a precue is invalid responses are slower,

presumably because a new response must be prepared (see also Stelmach and Teulings, 1984). The parietal lobe has recently been implicated in the ability to perform invalidly precued trials in motor attention tasks (Rushworth *et al.* 2001b).

Most studies performed so far have investigated the role of the left parietal cortex in motor attention and the right parietal cortex in visual attention (Rushworth *et al.* 2001a). However, fMRI evidence has suggested that the lateral prefrontal cortex also plays a crucial role in shifting attention, particularly relating to selecting task-relevant information (see previous section and Brass *et al.* 2004). The aim of this study was to investigate the roles of the right and left dorso-lateral prefrontal cortices (DLPFC) in the control of motor versus spatial attention during performance of two cued reaction time tasks. I chose to disrupt these areas on separate sessions using the same stimulation protocol (high-frequency (5Hz) rTMS conditioning) as in the previous section.

Two tasks were designed which separately tested performance when cues directed either motor attention or spatial attention prior to a go signal. Both tasks consisted of a random presentation of frequent validly and infrequent invalidly cued trials in which subjects were asked to respond with a finger press as soon as they saw a subsequent target (see Figure 7.2 and Methods for more detail). In the spatial attention task, subjects had to press their right index finger in response to a lateralised target. In the motor attention task, they had to press either their right or left index fingers depending on the direction instructed by a central target. The proportion of valid (80%) and invalid (20%) trials was the same as in the previous experiment in order to ensure that subjects actually made use of the cues to direct attention or prepare a motor response.

The differences in response times, standard deviations of response times, and error rates between pre- and post-conditioning sessions were assessed. In addition, I wanted to get some idea about the time course of the conditioning effect. To this end, subjects performed an initial block of trials prior to any rTMS conditioning which was compared to two subsequent blocks of trials measured immediately after and 10 minutes after the conditioning, based on the study by Siebner et al. (2003).

7.2 METHODS

7.2.1 Subjects

Seventeen right-handed healthy volunteers, with no history of neurological disorders or head injury were recruited from the database of volunteers at the Functional Imaging Laboratory, Institute of Neurology, University College London, UK. They were divided into two groups of subjects allocated to one of two separate experiments: experiment 1 (motor attention task) included nine volunteers (1 female, 8 male; mean age = 35.6±6.7 years); experiment 2 included a further 8 volunteers (2 female, 6 male; mean age = 32±4.7 years). Written informed consent was obtained from all participants. The study was approved by the joint ethics committee for the National Hospital for Neurology and Neurosurgery (UCLH NHS Trust) and the Institute of Neurology (UCL).

7.2.2 RTMS Conditioning

Subjects came on two sessions on separate days for each experiment, in which they received rTMS either on their right or on their left DLPFC. The order in which they received these sessions was counterbalanced across subjects. In each rTMS session 1800 biphasic stimuli, at a stimulation intensity of 90% of active motor threshold (AMT) for the right first dorsal interosseous (FDI) muscle, were given over left or right DLPFC area using a MagStim-rapid stimulator connected to four booster modules (MagStim Company, Whitland, Wales, UK; www.magstim.com). The subjects were delivered with six one-minute trains of 5Hz rTMS separated by an inter-train interval (ITI) of one minute. A standard figure-of-eight-shaped coil (Double 70-mm Coil Type P/N 9925, MagStim Company, Whitland, Wales, UK) was used for real-rTMS. The coil was positioned with the handle at 45^{o} to the sagittal plane. The current flow of the initial rising phase of the biphasic pulse in the TMS coil induced a current flowing from posterior-to-anterior in the underlying cortex.

The site of rTMS stimulation was located 5cm anterior to the "motor hot spot" on a line parallel to the mid-sagittal line. This is the same location of DLPFC stimulation as in the study by Mottaghy *et al.* (2002) and in the study described in the previous section.

rTMS conditioning was performed "off-line" (Mottaghy *et al.* 2003, Jahanshahi and Rothwell 2000) *between* the behavioral testing sessions. The total duration of stimulation was 11 min and behavioral testing was initiated immediately after the end of the stimulation period. The experimental design is outlined in figure 7.1.

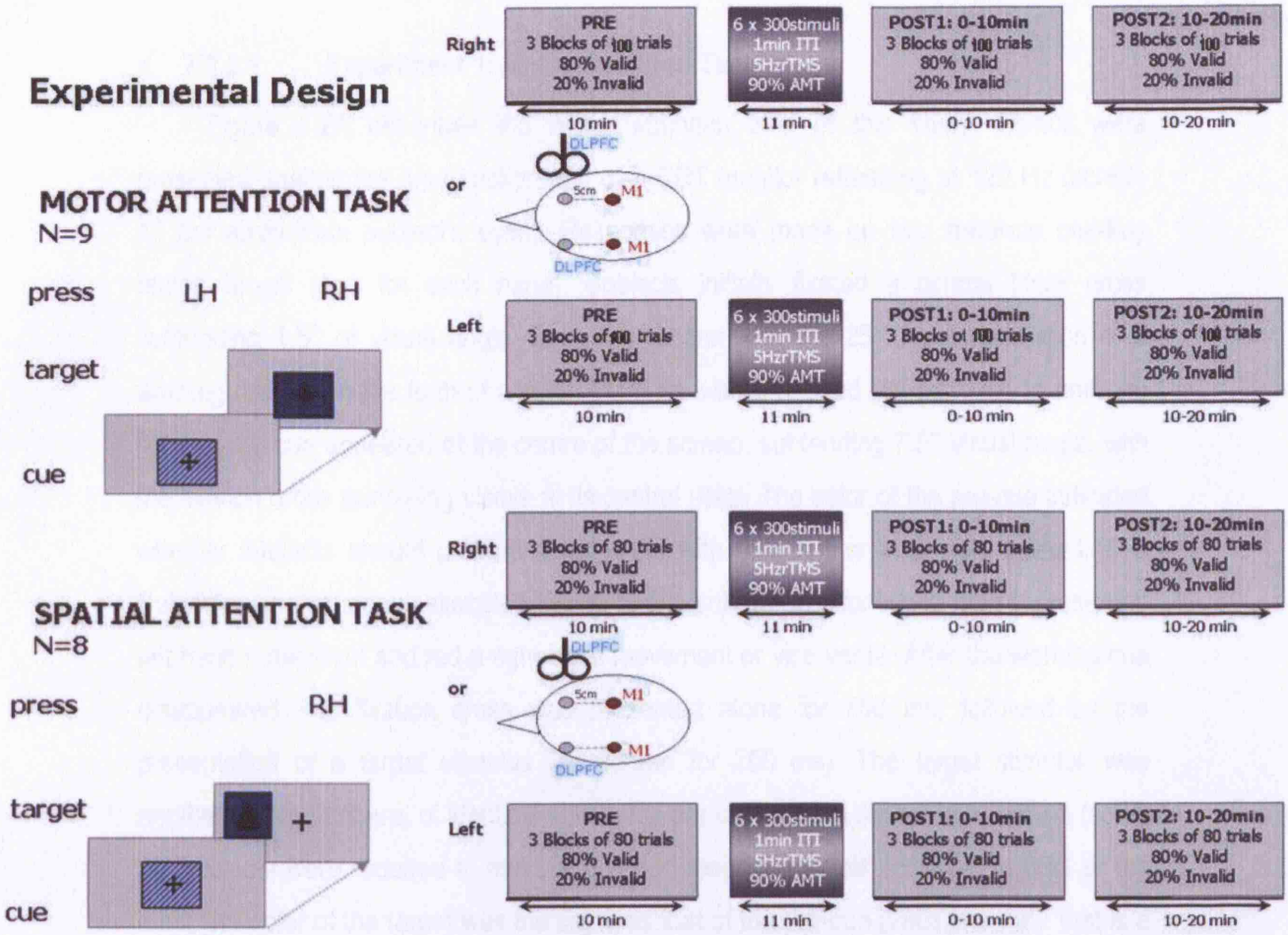


Figure 7.1: Experimental Design Two groups of subjects were tested separately on a spatial (n=8) or a motor attention task (n=9) before versus after 1800 pulses of subthreshold (90% active motor threshold) 5Hz rTMS over the dorso-lateral prefrontal cortex. The site of stimulation was located 5cm anterior to the motor "hot spot". Subjects in each group received rTMS either to their right or left side, in a counterbalanced order across subjects. A preliminary practice period of approximately 10 min preceded the first "pre" block of trials. Subjects then performed two blocks of "post" trials immediately after and 10 minutes after the end of the stimulation. In both tasks, subjects were randomly presented with valid or invalidly precued trials in which they were asked to respond with a finger press as soon as they saw a subsequent target (see Figure 7.2 for more detail). In the spatial attention task, subjects only had to press their right index finger in response to a lateralised target. In the motor attention task, they had to either press either their right or left index fingers depending on the direction instructed by a central target. The proportion of valid trials (80%) exceeded that of the invalid trials (20%).

7.2.3 Task

▪ 7.2.3.1 Experiment 1: Motor Attention Task

Figure 7.2A describes the motor attention task of this study. Stimuli were presented against the grey background of a CRT monitor refreshing at 120 Hz (screen 40 cm away from subject's eyes). Responses were made on two separate one-key button boxes (one for each hand). Subjects initially fixated a central black cross subtending 1.5° of visual angle. Each trial began with the 250 ms presentation of a warning pre-cue in the form of a colored square with a hashed (as opposed to uniform) fill. The pre-cue appeared at the centre of the screen, subtending 7.5° visual angle, with the fixation cross remaining visible at its central point. The color of the pre-cue indicated whether subjects should prepare a response with their left or their right index finger. Subjects were randomly allocated to one of two sub-groups, for which blue represented left hand movement and red a right hand movement or vice versa. After the warning cue disappeared, the fixation cross was presented alone for 750 ms, followed by the presentation of a target stimulus (on screen for 250 ms). The target stimulus was another colored square, of identical size to the pre-cue but this time with a uniform (solid) fill. Subjects were required to make a speeded response to this stimulus. In 80% of the trials, the color of the target was the same as that of the pre-cue (valid pre-cue): that is a blue-hashed rectangle was followed by a blue rectangle or a red-hashed rectangle was followed by a red rectangle. In 20% of the trials, however, the color of the pre-cue was different from that of the target (invalid trials). In this case, the subjects had to move *the opposite* index finger to the one they had prepared to move given the warning pre-cue. Subjects were asked to respond with the finger press corresponding to the color of the target as rapidly as possible and without making mistakes. Following the disappearance of the target, the fixation cross was presented alone for 750 ms before the next trial began; hence each trial was two seconds in duration. If subjects failed to respond in the one second period between the onset of the target and the beginning of the subsequent trial, they were considered to have made an error of omission. Trials were presented in a pseudorandom order, with 80 trials per block. In addition, 20 two-second long pauses were inserted at random places in this sequence, giving rise to unpredictable pauses

between trials. Subjects performed an initial practice block of 80 trials prior to each session. They then performed three blocks of 80 trials before rTMS conditioning, three blocks of 80 trials immediately after the conditioning and another three blocks of 80 trials ten minutes after the conditioning.

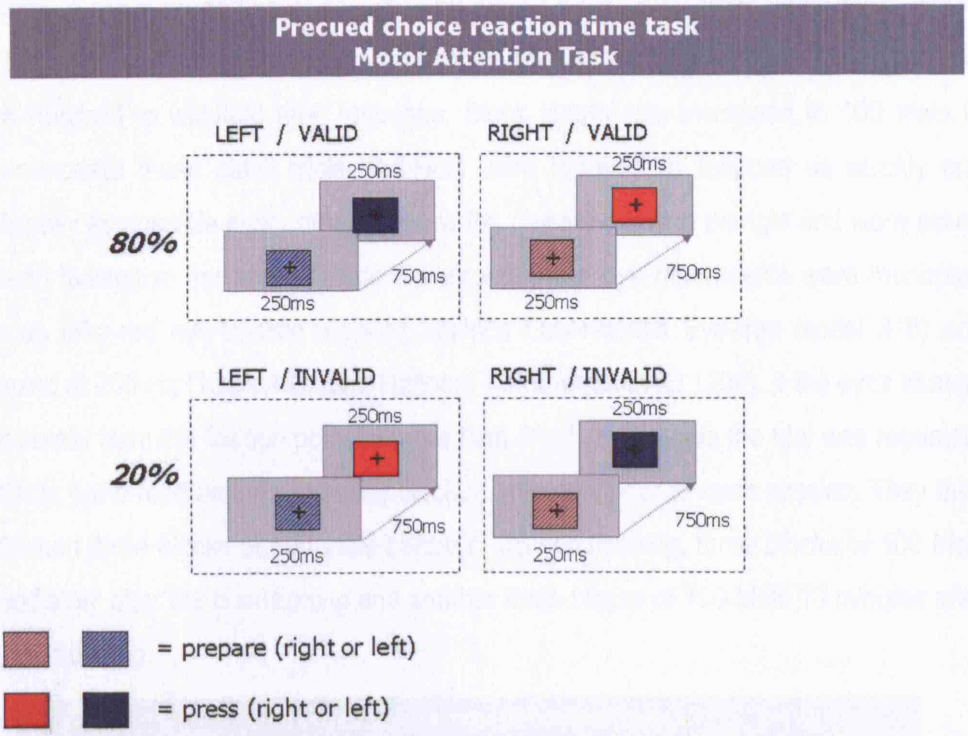


Figure 7.2A: Motor Attention Task

- **7.2.3.2 Experiment 2: Visual Attention Task**

The spatial attention task (figure 2B) retained the stimulus timings and the color-direction mappings of the motor attention task, but differed from it in the following respects. The central fixation cross now subtended only 1° of visual angle. The centrally-presented square-colored (hatched) pre-cue was also reduced in size, subtending 4° of visual angle. The color of the pre-cue now indicated the location to which subjects should direct their spatial attention. For this task, the subsequent target stimulus consisted of a black outline triangle (subtending 2°) presented within a solid-colored square (subtending 4°) centered 10° to either the left or right of the fixation cross. The color of the target square was consistent with its side of presentation, reflecting the color-direction mapping assigned to the current subject. Subjects were required to respond with a right index finger press on a single one-key button box whenever they detected an upward-facing triangle. The pre-cue indicated the correct direction of the target's appearance in 80% of the trials (valid trials). In 20% of the trials, the pre-cue

indicated the opposite direction (left when the target appeared right or vice versa). In addition, a small proportion of all valid and invalid trials (20%) were catch trials, where the target presented consisted of a *downward*-pointing triangle. In these trials, subjects were required to withhold their response. Block length was increased to 100 trials to accommodate these catch trials. Subjects were required to respond as quickly and accurately as possible every time they saw the *upward*-pointing triangle and were asked to keep fixating to the central fixation diamond. Their eye movements were monitored with an infra-red eye-tracker (Applied Science Laboratories Eye-trac model 310) and sampled at 200 Hz (12 bit A/D card; National Instruments DAQ 1200). If the eyes strayed horizontally from the fixation point by more than 3° of visual angle the trial was repeated. Subjects performed an initial practice block of 100 trials prior to each session. They then performed three blocks of 100 trials before rTMS conditioning, three blocks of 100 trials immediately after the conditioning and another three blocks of 100 trials 10 minutes after the conditioning.

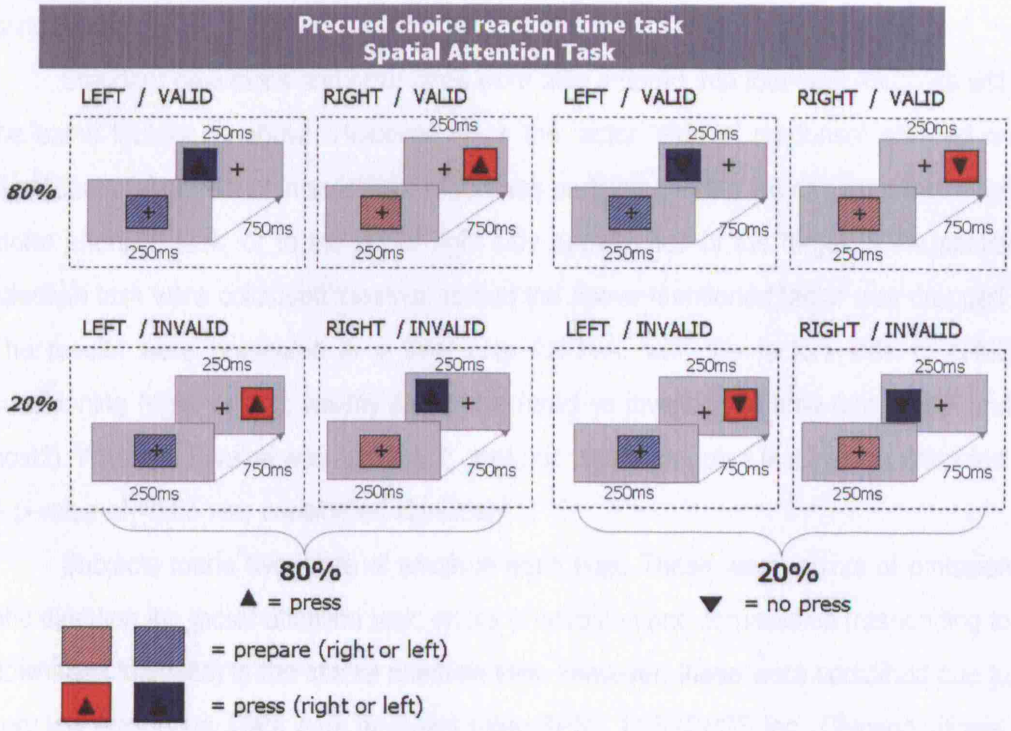


Figure 7.2B: Spatial Attention Task

▪ 7.2.3.3 Statistical Analysis

For each subject, the mean reaction time, standard deviation and error rates were calculated in each condition for each task. Trials were separated according to: 1) whether the response had been validly and invalidly cued; 2) either the side of stimulus presentation (spatial attention task) or side of response (motor attention task); 3) the side of rTMS conditioning; and 4) the time at which testing occurred relative to when the conditioning was performed (before, immediately after, or 10min after rTMS). Hence for each task we analysed four factors and their interactions with four-way repeated-measures ANOVAs, using the Greenhouse-Geisser correction where appropriate. The factor, side of rTMS conditioning had two levels (right vs left), the factor side of motor response (for the motor attention task) or side of stimulus appearance (for the spatial attention task) had two levels (right versus left), the factor cue validity had two levels (valid vs invalid) and the factor *time* had three levels (pre (i.e. *prior* to rTMS conditioning), post1 (0-10min *after* rTMS conditioning) and post2(10-20min *after* rTMS conditioning).

Standard deviations and error rates were also entered into four-way ANOVAs with the same factors as above. However, since the factor "side of response" showed no significant main effect or interactions, responses pertaining to the left or right hand in the motor attention task, or to the left or right side appearance of the target in the spatial attention task were collapsed together so that the above-mentioned factor was dropped. The results were presented in a three-way ANOVA, with the factors side of rTMS conditioning (right vs left), validity of the cue (valid vs invalid) and time (pre, post1 and post2). When an F-value was significant, post-hoc paired-sample t tests were performed. A p-value of <0.05 was considered significant.

Subjects made two kinds of errors in each task. These were: errors of omission and direction the motor attention task; errors of omission and commission (responding to downward triangles) in the spatial attention task. However, these were combined due to very low error rates. Data were analysed using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA). Results were considered significant if the p-value was <0.05.

7.3 RESULTS

No subject reported adverse side effects during the course of the study. Mean active motor threshold for rTMS over the right FDI was 49% ranging from 40% to 59% of maximum stimulator output and over the left FDI was 53% ranging from 37% to 61% of maximum stimulator output.

The numbers of errors in these tasks were small (>95% accuracy in both tasks). Error rates in the motor task did not differ significantly either in validly or in invalidly cued trials after 5Hz rTMS to either hemisphere, however there was a trend for a difference in error rates between validly and invalidly cued trials ($F_{1,8}$ = 4.914, P = 0.052). The same was true for errors in the spatial attention task (with a trend for errors in valid versus invalidly cued trials $F_{1,7}$ = 4.567, P = 0.059).

7.3.1 Experiment 1: Motor Attention Task

The effect of 5Hz rTMS over the right or left DLPFC on response times (Figure 7.3A) was examined with a four-way repeated-measures ANOVA. A significant main effect of CUE revealed that reaction times (RTs) on invalid trials were slower than on valid trials (main effect of CUE, $F_{1,8}$ = 32.557, P = 0.000). The main effect of *time* showed a learning trend with decreasing RTs in the post1 and post2 blocks of trials ($F_{2,16,}$ = 3.04, P = 0.063). The only significant interaction between factors induced by 5Hz rTMS was revealed by a 3-way interaction (side of stimulation-by-cue-by-*time* interaction: $F_{2,16}$ = 6.778, P = 0.023). RTs in both hands were slower in invalidly-cued trials compared to validly cued trials *after* 5Hz rTMS to the LEFT DLPFC (see Figure 3A). This was confirmed by post-hoc t-tests showing that RTs in invalidly cued trials were slowed in the first 10 minutes following 5Hz rTMS to the LEFT DLPFC (t_8 = -2.414, P = 0.027) compared to RTs in invalidly cued trials measured *prior* to the conditioning (figure 7.3A). Conversely, RTs in invalidly cued trials decreased with time following 5Hz rTMS to the RIGHT DLPFC (t_8 = 2.588, P = 0.019). It seems that left-sided rTMS affected left and right-handed responses equally, as no significant main effects or interactions were revealed for the factor "side of motor response".

I also looked for differences in the standard deviations (SDs) of response times (see Figure 7.4). We found a significant main effect of cue in the 3-way ANOVA performed for the SDs (CUE: $F_{1,8}$ = 5.03, P = 0.047;), and a significant two-way interaction of side of stimulation-by-time on SDs ($F_{2,16}$ = 6.706, P = 0.024). However, the three-way interaction of side of stimulation-by-time-by-*cue*, was not significant. This is despite the apparently opposite effect of left DLPFC stimulation on the SD of invalidly-cued trials compared to validly cued trials. As can be seen in Figure 4, the SD of invalidly cued trials was increased in the first 10min following 5Hz rTMS compared to pre-conditioning only when left DLPFC was targeted whereas the opposite was true for validly cued trials and for trials that followed right DLPFC conditioning. Since the mean RT data did reveal a significant three-way interaction (see above), we felt justified to perform follow up t-tests to confirm the effect of left DLPFC stimulation on the SD of invalidly-cued trials (post-hoc t test for invalid trials: post1 vs pre: t_8 = -2.992, P = 0.017, post2 vs pre t_8 = -0.567, P = 0.588; post-hoc t test for valid trials: post1 vs pre: t_8 = 1.135, P = 0.225, post2 vs pre t_8 = 1.521, P = 0.125). In short, rTMS to the left DLPFC induced an increase in invalidly cued trials' RTs and the standard deviation of these RTs in the first 10 minutes following the end of stimulation in this motor attention task.

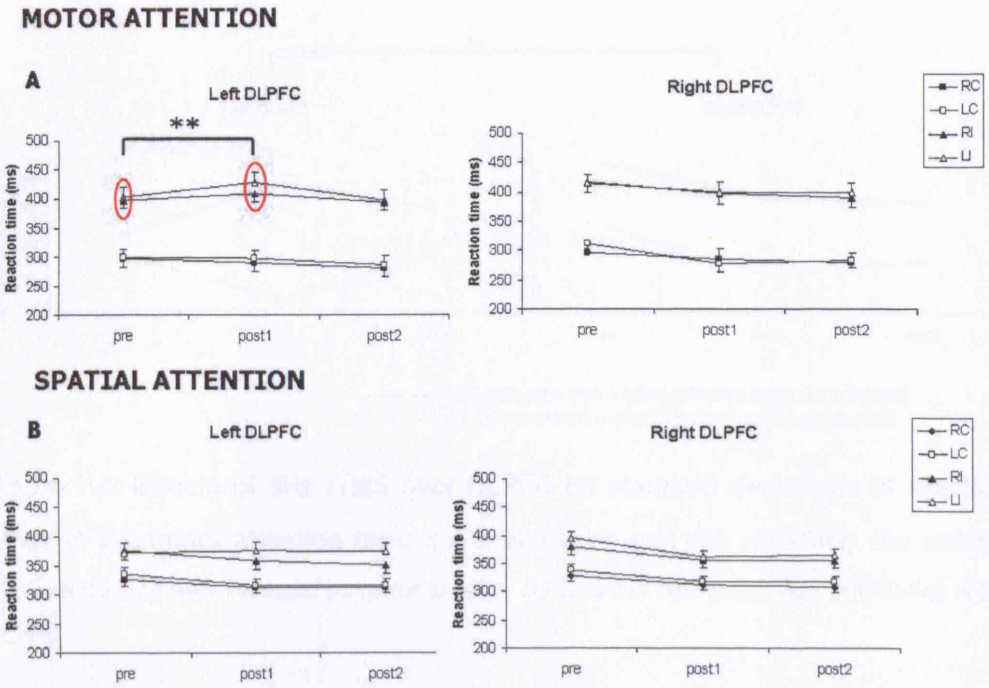


Figure 7.3: Effects of 5Hz rTMS over DLPFC on mean reaction times (with error bars showing standard deviations).

(A) **Motor attention task:** Left DLPFC conditioning led to *slower* RTs in invalidly cued trials in the first 10 minutes after stimulation compared to the pre-stimulation baseline, regardless of whether the right (12ms) or left (31ms) hand was used. This contrasts with the faster RTs found in other conditions at this time point (also following right DLPFC stimulation). RTs came back to baseline levels of performance as in the "pre" test block in the next 10-20 minutes after left 5Hz rTMS conditioning.

(B) **Spatial attention task:** RTs are lower in validly versus invalidly cued trials. However, 5Hz rTMS to the right *or* left DLPFC did not affect performance in either condition.

MOTOR ATTENTION

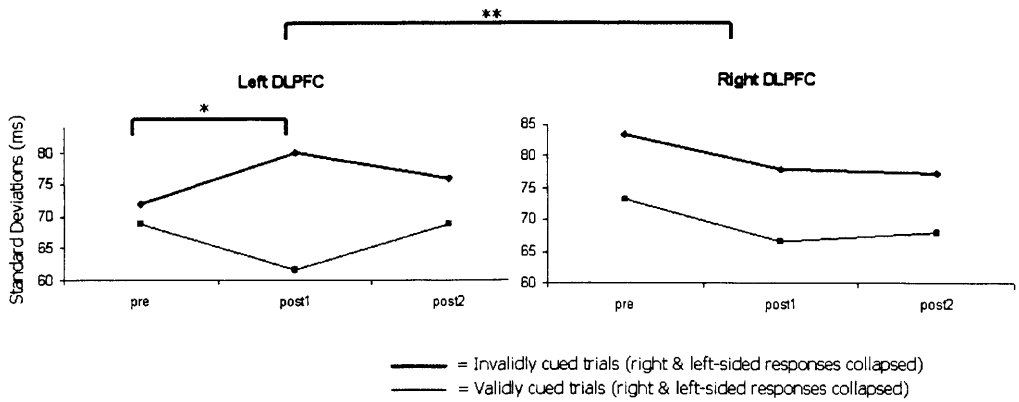


Figure 7.4: Effects of 5Hz rTMS over DLPFC on standard deviations of reaction times in the motor attention task: SDs in the invalidly cued trials immediately after left-sided rTMS conditioning were increased compared to validly cued trials or right-sided rTMS conditioning of the DLPFC.

7.3.2 Experiment 2: Spatial Attention Task

5Hz rTMS over either the left or the right DLPFC did not lead to any behavioral changes (Figure 3B). A four-way ANOVA revealed a simple main effect of validity, suggesting that RTs in invalidly cued trials were longer than in validly cued trials ($F_{1,7}$ = 81.872, P = 0.000) and a simple main effect of time (pre vs post1 vs post2) suggesting that some learning occurred as the RTs decreased over the blocks of trials ($F_{2,14}$ = 7.527, P = 0.031). No other significant main effects or interactions were found in RTs or standard deviations. Therefore, rTMS to either the left or the right DLPFC did not lead to any disruptive effects in the spatial attention task either in validly or in invalidly cued trials.

7.4 DISCUSSION

In this study, conditioning the dorso-lateral prefrontal cortex with "off-line" rTMS can induce task-specific behavioural changes. In particular, when comparing rTMS conditioning over the same areas in a paradigm involving motor attention and another involving spatial attention, there was an increase in response times for incorrectly cued responses when the left DLPFC was transiently disrupted during performance of the motor attention task. This suggests a crucial role for that area in shifting a prepared response from one hand to another when a precue had specified the wrong response. The fact that no effect was revealed when testing the same conditioning paradigm in the spatial attention task either suggests that this area may not be involved in the task or that compensatory mechanisms might operate which allow performance of the task to be unaltered. There is ample evidence in the literature that the former possibility cannot be true: The fronto-parietal network has been revealed in many neuroimaging studies investigating attentional selection in the context of visual attention (Corbetta *et al.* 2000, Pollmann *et al.* 2000, Corbetta & Shulman 2002, Liu *et al.* 2003).

7.4.1 Visual Attention And The Selection of Task-Relevant Information

The fact that 5Hz rTMS conditioning over DLPFC only led to an effect in the motor attention task suggests that different networks operate in spatial versus motor attention; otherwise one would expect that the spatial attention task would yield a similar result. Many studies have provided evidence in favour of this hypothesis (see previous section), namely, that different cortical networks might be involved in reorienting attention (Corbetta et al. 2000, Pollmann et al. 2000, Corbetta & Shulman, 2002) compared to the context of selecting task-relevant information (Banich *et al.* 2000, Dove *et al.* 2000, MacDonald *et al.* 2000, Brass & von Cramon 2004). However, this difference has been re-emphasised most recently by work from Rushworth et al. who have provided evidence for this dissociation by distinguishing between visual switching and

response switching. They tested parietal lesion patients in a task in which precues allowed subjects to covertly prepare subsequent cued hand movements as opposed to orienting eye movement. They were able to show that patients with left parietal lesions, as opposed to patients with right parietal lesions or control subjects, were impaired in their ability to disengage the focus of motor attention from one movement to another (Rushworth *et al.* 1997). A functional neuroimaging study, allowed them to identify the exact locus of motor attention to be in the left supramarginal gyrus and adjacent intraparietal sulcus areas (Rushworth et al. 2001a). The role of the left supramarginal gyrus/IPS in motor attention in healthy subjects was confirmed in a subsequent study using a Posner-type task (Rushworth et al. 2001b), in which disruption of the left anterior parietal cortex with rTMS in a "virtual lesion" paradigm led to a compromised performance in a motor attention task, whereas disruption of the right intra-parietal sulcus impaired spatial reorienting to invalidly cued trials.

In this experiment, subjects were asked to either direct their covert attention towards a particular side specified by the precue or prepare a motor response on a particular side also specified by the same precue, thus matching the working memory requirements of the two tasks. The motor attention paradigm created for the purposes of this study might have allowed better differentiation from the spatial attention paradigm, since the cues and targets to which subjects had to respond to were all centrally located. The process by which subjects responded to invalidly cued trials in the motor attention task probably involved canceling the previously prepared response in order to move in the opposite direction. In the spatial attention task, subjects always responded with the same hand, but they had to orient their attention to the opposite side following an invalid cue. Both instances lead to a switch, albeit in different modalities.

The present study extends results described by Rushworth and colleagues by suggesting a role for the left lateral prefrontal cortex in motor attention. We propose that this area forms a dynamic network with left parietal regions in the control of response switching.

7.4.2 Effects Of 5Hz rTMS Over DLPFC On Task Performance

The fact that an effect was obtained following left-sided stimulation of DLPFC, while Siebner et al obtained a similar effect following right-sided stimulation, may seem puzzling. In Siebner et al's study, it was unclear whether rTMS had interfered with the process of shifting spatial attention or the process of shifting motor attention (i.e. preparing a new response) on incorrectly cued trials. The current results suggest that the ability to shift spatial attention is not impaired following 5 Hz rTMS to the right DLPFC, so the deficit found in Siebner et al's study is instead likely to have reflected the task's motor demands (although note that subtle differences existed in the nature of the attentional precues used in the two studies: they were endogenous in the current research and exogenous in Siebner et al's study). It is possible that the nature of the stimulus-response associations used in the two tasks may explain the hemispheric discrepancy. In the current motor attention task a rather abstract S-R link was used requiring a complex process of translation (color to hand of response). By contrast, Siebner et al used a very direct S-R association (position to hand of response) that was very spatial in nature. These kinds of S-R associations are so automatic that they often interfere with responses when the spatial position of the response is irrelevant to the task that is being performed (see Cho & Proctor 2004 for the "Simon effect"). The left and right DLPFC may therefore be differentially involved in the selection of motor responses in tasks with different stimulus-response demands, with the right DLPFC playing a greater role when the movement is specified in a spatially congruent manner.

The behavioral effect of rTMS conditioning found in this study only lasted 10min after the end of the stimulation period. This is at odds with previous studies of the conditioning effects of the same 5Hz rTMS protocol (subthreshold, 1800 pulses) on physiological measures of motor cortical excitability which have reported the physiological effects to last for approximately one hour (Peinemann et al. 2004, Rizzo et al. 2004). One explanation of this discrepancy could be that we do not know how long the excitability in the DLPFC is altered with this particular frequency and intensity of rTMS: all the evidence for the duration of the effect is extrapolated from data on the premotor and motor cortices. It may be that this 5Hz rTMS protocol lasts for a shorter period when delivered to DLPFC. Another possible explanation might be that the effect we have observed in this study could be the result of rTMS interacting with a possibly

still persistent learning effect, a hypothesis postulated for the effect obtained by Siebner *et al* (2003) in the previous section. However, it must be noted that the experiment was preceded by a practice phase as well as the pre-rTMS block. Taken together, these results suggest that the left lateral prefrontal cortex part of a network responsible for selecting task-relevant responses. It is possible that its contribution would relate to its known role in working memory, allowing the "on-line" manipulation of information for accurate performance of the task (Goldman-Rakic *et al.* 1989, Petrides 1994, Owen *et al.* 1996). This is consistent with recent evidence by Sakai et al. (2002) who suggested that the fronto-parietal network might mediate the formation of distractor-resistant memory representations during a period of active maintenance within working memory. Future studies using functional neuroimaging and rTMS combined with analyses of effective connectivity might help to gain a better understanding of the exact role and interactions of the lateral prefrontal and parietal areas in processes involved in motor attention.

7.5 CONCLUSION

In summary, the effects of conditioning the right or the left DLPFC with 5Hz rTMS on a motor versus a spatial attention task was studied in this experiment. The finding of an increase in RTs following left DLPFC conditioning occurring only in the motor attention task suggests an irreplaceable role of this area in the rapid adjustment of prepared responses, and confirms that the type of "off-line" rTMS conditioning used in this study was a useful "virtual lesion" technique. It also suggests that future neuroimaging studies might need to include a "pre vs post" measurement even when comparing between real and sham sessions as there might be learning effects that would not be picked up in a simple between-session comparison.

8. GENERAL DISCUSSION

8.1 SUMMARY OF FINDINGS

The foregoing chapters (4 to 7) describe 2 PET, 1 fMRI and 1 behavioural experiments on how activity in the motor system and behaviour change following rTMS delivered to specific areas which are involved in performance of a task. RTMS was delivered in a "virtual lesion" paradigm and behavioural and neuroimaging techniques provided the way to observe whether the motor system managed to perform paced freely selected right finger movements (Chapters 4 & 5), or cued choice reaction time tasks (Chapters 6 & 7) in the same way as normal or whether it showed any worsening following the period of altered cortical excitability induced by the rTMS.

The PET experiment described in Chapter 4 was set up to test if high-frequency rTMS over M1, known to be physiologically excitatory, would lead to changes in behavour or in the pattern of rCBF at rest or during performance of a paced free selection of finger movement task. A previous study by Lee *et al.* (2003) had shown that following inhibitory 1Hz rTMS over M1, there were changes in movement-related activity and motor network connectivity, coupled with no difference in behavioural performance. They interpreted these results as resembling a rapid reorganisation of the motor system presumably in order to compensate for the excitability changes induced with 1Hz rTMS. Using the same experimental protocol, we acquired a new data set with 5Hz rTMS in which we were able to show that this frequency of stimulation revealed similar main effects of TMS as 1Hz rTMS, but showed opposite movement-related activity changes in area 4p and opposite effects on coupling between the site of stimulation (i.e. M1) and the LPMd area from the main effects of movement with area 4p. 5Hz rTMS, which physiologically is known to induce increases in the excitability of the stimulated site when applied to left M1 (Berardelli et al. 1998, Peinemann et al. 2004) or left PMd (Rizzo et al. 2004), led to a *decrease* in movement related activity in area 4p as well as a *decrease* its coupling with the stimulation site and LPMd whilst motor performance remained the same. Given the lack of change in behaviour observed with that frequency of stimulation,

we hypothesise that, as in the study by Lee et al. (2003), these changes might constitute some form of compensatory reorganisation following the period of altered excitability induced by rTMS. Interestingly, the changes we observed were located intra-hemispherically in regions adjacent to the site of stimulation (area 4p and LPMd). These areas have been implicated in functional recovery following M1 lesions in patients (Seitz *et al.* 1998, Werhahn *et al.* 2003, Fridman *et al.* 2004). However, the changes observed with 5Hz rTMS are interesting in two ways: 1) they led to decreases in rCBF rather than increases (in 1Hz rTMS), which, given the excitatory effect of this frequency of stimulation, could be interpreted as rendering the stimulated site more efficient in performing the same task and 2) movement-related activity or coupling with the contralateral, right PMd was not changed as in the study by Lee et al. (2003). In their study, Lee et al. showed a task-related interaction in that area, which has also been functionally implicated in recovery from stroke (Johansen-Berg *et al.* 2002). In the study by Fridman et al. (2004), the authors hypothesised that the implication of the right PMd in functional recovery from M1 lesions might depend on the extent of damage in the left hemisphere, such that if the lesion is so large as to involve the left PMd, right PMd might then be recruited for performing a task. The reason why the right PMd might not have been affected by 5Hz rTMS conditioning of left M1 might be due to the fact that this area would not normally be activated in this task but can be recruited to functionally comensate reduced activation following 1Hz rTMS. This is not the case with 5Hz rTMS.

The experiment described in Chapter 5 was set up to investigate further the nature of the reorganisation observed in this task following 5Hz rTMS, particularly the movement-related deactivations and decrease in coupling, by targeting another site: this time the left PMd. Previous studies have revealed an important role for this area in free selection of finger movements (Jahanshahi *et al.* 1995, Jenkins *et al.* 2000). 5Hz rTMS over PMd has also shown very strong and consistent effects on the excitability of M1 (Rizzo *et al.* 2004). The effects on rCBF in this study also suggested a reorganisation to maintain performance with an increase in movement-related activity in caudal PMd and changes in movement-related coupling such that areas within the left PMd itself increased their coupling together after 5Hz rTMS and areas such as the right PMd and CMA decreased their coupling with left M1. The relative decrease in coupling between

left PMd and left area 4p following 5Hz rTMS in Chapter 4 and between right PMd and CMA and left M1 in chapter 5 may both reflect a shift of metabolic activity during task performance towards an area of enhanced excitability (and hence responsiveness to cortical inputs) at the site of stimulation with rTMS.

Chapter 6 aimed to test if similar reorganization to the aforementioned studies occurs when 5Hz rTMS is delivered to the lateral prefrontal cortex responsible for generating a more complex pattern of motor behaviour, which might lead to a worsening of task performance. Previous evidence from a behavioural study on reorienting attention (Siebner *et al.* 2003) had shown that 5Hz rTMS over the right DLPFC might compromise healthy subjects' ability to reorient their attention in a cued reaction time task when the cue was invalid. This raised two questions dealt in Chapter 6 and 7 respectively. Firstly, the question was to find whether this behavioural finding could be replicated and investigate what the neural correlates of altered DLPFC excitability during a reorienting attention task would be using functional neuroimaging (Chapter 6). Second, to understand whether 5Hz rTMS over the DLPFC in the reorienting Posner-type task used in Chapter 6 would affect healthy subjects' ability to covertly reorient their spatial attention towards another site or whether it would affect their ability to cancel a previously prepared motor response in order to perform a movement in another direction (Chapter 7).

The Posner-type cued reaction time task in Chapter 6 activated a right-lateralised fronto-parietal network previously described in the literature (Corbetta and Shulman 2002) along with the left IPS probably involved in motor attention (Rushworth *et al.* 2001). 5Hz rTMS over the DLPFC did not lead to any significant behavioural change or any change in activity in the stimulated area but led to prominent decreases in VLPFC BOLD activity in invalidly cued trials as revealed in the simple main effects of 5Hz rTMS following left DLPFC conditioning or in the validity-by-rTMS interaction following right DLPFC conditioning. These findings shed some new light into the dynamic relationship between the DLPFC and VLPFC and their involvement in a reorienting task. In addition, given the absence of a behavioural effect and given the fact that the cognitive demands of the task were not changed, the decreases in VLPFC activity adjacent to the stimulated site might constitute some adaptation of the orienting system to altered cortical

excitability caused by 5Hz rTMS conditioning, as was the case in the movement-related activity changes observed in the PET experiments. The lack of behavioural effects in this study, particularly in invalidly cued trials following right DLPFC conditioning, as had been shown in Siebner's et al. study (2003) supports many prefrontal studies on patients and animals (D'Esposito and Postle 1999, Sahraie et al. 1997, Latto and Cowey 1971), which suggest that behavioral effects are very hard to obtain following prefrontal lesions, but is also reminiscent of the difficulty in obtaining any behavioural changes following rTMS, such as in studies looking for effects of 1Hz rTMS on M1 (Muellebacher *et al.* 2000). The subtle effects of 5Hz rTMS were, however, demonstrated in the study presented in Chapter 7, where this conditioning protocol did lead to a change in behaviour in a task that tested specifically for effects on motor and spatial attention.

Chapter 7 investigated the effect of 5Hz rTMS conditioning over DLPFC on motor versus visual attention. This study extends previous results (Siebner *et al.* 2003, Chapter 6) by showing an effect on performance in reorienting attention when the left-lateralised fronto-parietal network, known to be dominant for motor attention, was targeted in a task exclusively testing the ability of subjects to shift the direction of a prepared motor response. In this study, the ability to shift spatial attention was not impaired following 5 Hz rTMS to the right DLPFC, suggesting that the deficit found in Siebner et al's study was instead likely to have reflected the task's motor demands (although subtle differences existed in the nature of the attentional precues used in the two studies in that they were endogenous in the current research and exogenous in Siebner et al's study). The hemispheric discrepancy revealed in the two studies might be related to the nature of the stimulus-response associations used in the two tasks. In Chapter7, the motor attention task used a rather abstract S-R link requiring a complex process of translation (color to hand of response). By contrast, Siebner et al used a very direct S-R association (position to hand of response) that was very spatial in nature. These kinds of S-R associations are so automatic that they often interfere with responses when the spatial position of the response is irrelevant to the task that is being performed (Cho and Proctor 2004, the "Simon effect"). These results suggest that left and right DLPFC may be differentially involved in the selection of motor responses in

tasks with different stimulus-response demands, with the right DLPFC playing a greater role when the movement is specified in a spatially congruent manner.

Taken together, the findings in this thesis suggest that following high-frequency (5Hz) rTMS conditioning there may or may not be a change in behavioural performance depending on the ability of the brain to compensate for the change in excitability induced with rTMS by adjusting its pattern of task related activity. It seems that when performance of the task is maintained, changes in task-related activity may be observed in adjacent or remote regions, depending on the effective connectivity of the region conditioned by rTMS with other areas involved in the task. Alternatively, if performance is affected, it may be because an area's specific function could not be compensated for by other areas in the network, which depends on a few factors, such as: 1) whether the brain system being studied displays characteristics of degeneracy 2) whether the task is easy or complex, which would determine other areas' dynamic capacity to take up more function.

8.2 COUPLING OF PHYSIOLOGICAL WITH METABOLIC CHANGES

One of the main issues faced in the work presented in this thesis was about the nature of the neuronal signal picked up with current neuroimaging techniques such as PET or fMRI, particularly concerning the issue of whether no change in metabolic or BOLD activity really means a lack of neuronal signal and whether increases or decreases in metabolic activity really mean increases or decreases in neuronal activity. The nature of these signals has been introduced in the introductory chapters and discussed extensively in the relevant experimental chapters. However, it is interesting to note here a few lessons learnt from studying rTMS effects.

In Chapter 4, we observed that the main effects of rTMS, whether stimulating at 1 or 5Hz, on rCBF were increases located in the motor system. This is despite neurophysiological evidence that these frequencies of stimulation have opposite effects on cortical excitability. The latter was demonstrated by the opposite effects these frequencies of stimulation had on movement-related activity and effective connectivity. It

seems therefore, that examining the effects of rTMS on metabolic activity during movement, and more specifically during performance of particular tasks is more informative in assessing reorganisation of the motor system following these "virtual lesions".

The main effects of 5Hz rTMS on the left PMd observed in Chapter 5 showed to be completely different than 5Hz rTMS over M1 (Chapter 4). However, they were similar to the main effects described in the study by Siebner et al. (2003) on dystonia patients, in that they found decreases in rCBF over the motor system. It seems, therefore, that the site of stimulation might show characteristic main effects, independent of the physiological effects a particular rTMS protocol might have on the stimulated site. This should be tested in future studies with other protocols of rTMS such as TBS.

In all the neuroimaging studies described in the previous chapters, 5Hz rTMS did not lead to any task-related changes in activity of the stimulated site. This has been confirmed in previous studies in the literature (Bestmann 2003, 2004, Lee et al. 2003). Instead, task-related changes in activity were observed mainly in adjacent areas. Given the physical properties of TMS (Chapter 3), in that the intensity of stimulation decays exponentially with distance from the stimulated site, and given that we did not observe any measurable changes in behavioural performance, we hypothesise that these changes are compensatory rather than due to a direct conditioning of these areas.

8.3 REORGANISATION

The hypothesis is that having altered the stimulated area's sensitivity to inputs with various protocols of rTMS, task performance may be maintained by changing task-related activity in areas showing anatomical and functional connectivity to the stimulated site. There are several ways in which operational remapping of the motor networks as a compensatory mechanism to rTMS conditioning may occur.

As mentioned in Chapter 1, many studies have shown evidence for overlapping sensorimotor representations of distinct hand movements in monkeys (Graziano et al. 2001) and in humans as revealed in recent neuroimaging experiments (Sanes et al.

1995, Rao et al. 1995). The changes observed in the experiments presented in this thesis may be explained by these studies, which provide the basic framework supporting the idea of degeneracy. The concept of degeneracy, introduced in Chapter2, implies that more than one set of cortical structures can support the same function. This may explain the observation, in a study by Strens et al. (2003), that subthreshold 5Hz rTMS to either the contralateral or ipsilateral M1 had very limited effects on performance of a precision finger tapping task, whereas bilateral stimulation of the motor cortices simultaneously led to a marked and prolonged detrimental effect on performance. The authors suggested that when one primary motor cortex was disrupted during motor performance, the other motor cortex provided functionally significant compensation, which was disrupted when both were stimulated.

In addition, the fact that reorganisation following rTMS occurs so rapidly is supported by evidence that plasticity in the rat motor cortex has been shown to occur within hours of motor nerve lesion (Donoghue et al. 1990), or repetitive intracortical microstimulation (Nudo et al. 1990). In humans, such transient and reversible reorganisation within the primary sensorimotor cortex has been shown to occur in studies by Brasil-Neto et al. (1993) and Ziemann et al. (2002) following ischaemic nerve block of healthy subjects' forearm.

8.4 THE FUTURE

The work presented in this thesis attempted to investigate the nature and the extent of brain reorganisation following altered periods of cortical excitability induced by rTMS.

The first interesting finding provided by functional neuroimaging techniques has been the observation that areas known to show reorganisation following recovery from stroke (e.g. left PMd followinf left M1 lesions), or during physiological processes such as learning, revealed task-related changes in activity and coupling following rTMS conditioning. This suggests that rTMS could be used as a technique to provide new insights into compenstory plasticity of the human brain and may help understand how the brain reacts in response to more permanent lesions, such as stroke. It appears that

the patterns of reorganisation observed in the previous chapters may be task dependent. This has implications for neurorehabilitation and could be tested by directly comparing activity patterns in tasks implicating various known interconnected brain regions. The site of lesion also plays an important role so these should be examined sytematically. Additional connectivity information using new techniques such as Diffusion Tensor Imaging (DTI) combined with connectivity analyses might shed some new light in the nature of network reorganisations that occur in stroke as they would allow the identification of remaining cortico-cortical, cortico-subcortical and cortico-spinal connections.

Chapter 4 revealed bidirectional changes in the reorganisation and coupling of areas 4p and the left PMd that depended on the frequency of rTMS stimulation which in turn is known to determine the side of excitability changes induced with conditioning. This provides reassuring evidence that the changes observed electrophysiologically can be confirmed to occur also in remote areas as measured with functional neuroimaging techniques, thus allowing the experimenter to modulate a particular cortical network at will during various kinds of task performance. This bidirectional effect could be tested with the newly developed TBS method. Bidirectional induction of plasticity opens a range of new experimental protocols that could be used and combined with neurorehabilitative methods in patients in order to modulate the excitability of areas known to be crucial in the pathology of the disease.

The experiments in Chapters 5 to 7 tested how varying other parameters in an "off-line" virtual lesion rTMS paradigm, such as the site of stimulation or the task, would affect behavioural perfomance as well as the pattern of cerebral activity during the performance of these tasks. A behavioural effect was only obtained with rTMS conditioning when testing for a very specific function of an area that might have been irreplaceable in the performance of the task.

There are two reasons, which may not be mutually exclusive, why a change in the pattern of brain activity measured with functional neuroimaging methods in this thesis could have been paired with a lack of any behavioural effect following rTMS conditioning: 1) the tasks being used were so simple that subjects could still perform them even after rTMS conditioning and/or 2) the task could still be performed thanks to operational

remapping of the motor networks as a compensatory mechanism. To test the first hypothesis, new experiments with more subtle measures of behaviour or better controls need to be put in place in order to detect any subtle changes in behaviour. One such example in this thesis was the development of new tasks in Chapter 7. Alternatively, in order to study these effects on M1, tasks in which difficulty could be increased parametrically could allow the identification of even subtle alterations (improvements or worsening) in behaviour. For example, although 1Hz rTMS had not been shown to have any effects on basic motor behaviour (Muellbacher et al. 2000), motor learning was affected following 1Hz rTMS over M1 (Muellebacher et al. 2002). We need to establish whether these effects are just occurring during learning or whether they are related to task difficulty. The second hypothesis has been partly demonstrated in this thesis' chapters. Having identified the areas that may contribute to compensatory mechanisms during periods of altered cortical excitability, their functional relevance could be tested using disruptive "on-line" TMS or rTMS virtual lesion techniques as in the studies by Johansen-Berg *et al.* (2002) and Strens *et al.* (2003). In addition, the newly developed dynamic causal modelling method (Friston *et al.* 2003) combined with Bayesian model comparison (Penny *et al.* 2004) could be used to examine the changes in effective connectivity of candidate areas more accurately than with the PPI techniques used in the PET experiments in these chapters (4 and 5).

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