EXPERIMENTALLY INDUCED CORTICAL PLASTICITY: NEUROPHYSIOLOGICAL AND FUNCTIONAL CORRELATES IN HEALTH AND DISEASE

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by

Siobhan M Schabrun

B. Physio (Hons)

Research Centre for Human Movement Control

Discipline of Physiology

School of Molecular and Biomedical Science

The University of Adelaide University

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Abstract

Neuroplasticity provides the basis for many of our most fundamental processes including learning, memory and the recovery of function following injury. This thesis is concerned with the neurophysiological and functional correlates of sensorimotor neuroplasticity in the healthy and focal dystonic populations.

My initial experiments were conducted to determine the functional correlates of neuroplasticity induced in the primary motor (M1) and primary sensory (S1) cortices during a grip lift task. In healthy subjects these experiments further quantified the role of M1 in the anticipatory control of grip force scaling and demonstrated a role for S1 in triggering subsequent phases of the motor plan. My second series of experiments served to extend these findings by examining the functional correlates of neuroplasticity induced in the supplementary motor area (SMA). This study provided evidence for the role of left SMA in the control of grip force scaling and a role for left and right SMA in the synchronization of grip force and load force during the grip-lift synergy.

Afferent input is known to be a powerful driver of cortical reorganisation. In particular, the timing and pattern of afferent input is thought to be crucial to the induction of plastic change. In healthy subjects, I examined the neurophysiological effects of applying "associative" (synchronous) and "non-associative" (asynchronous) patterns of afferent input to the motor points or digits of the hand. I observed an increase in the volume and area of the cortical representation of stimulated muscles when associative stimulation was applied over the motor points of two hand muscles. This pattern of stimulation also caused the centres of gravity of the stimulated muscles to move closer together, mimicking the maladaptive changes seen in

focal hand dystonia. Non-associative stimulation and stimulation applied to the digits did not produce such an effect.

Task-specific focal dystonia is characterised by excessive representational plasticity resulting in cortical representations which are significantly larger, and demonstrate greater overlap, than those seen in healthy individuals. These changes are thought to be driven, in part, by repetitive movement patterns which promote associative patterns of afferent input over an extended time period. On the basis of this knowledge, I applied non-associative stimulation to the hand muscles of dystonic subjects. Following this intervention, I noted a contraction of representational maps and a separation in the centres of gravity of the stimulated muscles. These neurophysiological changes were accompanied by improvements on a cyclic drawing task.

This thesis demonstrates the functional correlates of neuroplasticity in M1, S1 and SMA during object manipulation using a precision grasp. These findings further extend our knowledge on the mechanisms underlying effective grasp control and assist us in the development of future rehabilitation protocols for neurological conditions involving grasp dysfunction. In addition, this thesis is the first to demonstrate an improvement in both neurophysiological and functional measures in focal dystonia following a period of non-associative afferent stimulation. These results open up exciting new avenues for the development of effective treatment protocols in those with focal hand dystonia.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Siobhan Schabrun and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Aims and general introduction

In recent years our understanding of the human central nervous system has advanced considerably. Where the structure and function of the adult central nervous system was once thought to be static, it is now clear that this system retains the ability to restructure and reorganise throughout life. This property, known as plasticity, is of significant importance in learning and memory, and is likely to play a key role in recovery of motor function following injury.

Reorganisation of the human cortex has been demonstrated using a number of experimental paradigms. However, evidence for a concomitant and related functional effect is limited. Investigation of the functional effects associated with cortical reorganisation is of critical importance if novel and effective rehabilitation strategies are to be developed for those with neurological conditions.

The neurophysiological correlates of cortical plasticity may be measured using transcranial magnetic stimulation (TMS). Using the principles of electromagnetic induction, TMS triggers neuronal depolarisation and the propagation of a descending volley of action potentials in the corticospinal tract. This volley activates motor neurons and induces a transient electromyographical (EMG) response in the target muscle known as a motor evoked potential (MEP). Changes in MEP amplitude reflect changes in the excitability of the corticospinal projection to the target muscle and are used as a marker of plasticity induction.
TMS may also be applied repetitively (rTMS) as a tool to induce cortical reorganisation. This approach can be used to induce a temporary “virtual lesion” that interrupts activity in a specific cortical region or, by altering the frequency, intensity or direction of the stimulation,

rTMS can be used to transiently alter synaptic strength. The functional correlates of rTMS-induced plasticity may then be determined.

In my first series of experiments I used the technique of rTMS to induce plasticity in the primary motor (M1) and primary sensory (S1) cortices of healthy subjects performing a grip-lift task. The grip-lift task has been shown to be a sensitive, objective measure of hand dexterity in both healthy subjects and in those with neurological conditions. As one limitation of previous studies has been the use of functional measures with insufficient sensitivity to detect subtle changes in performance, a grip-lift apparatus was considered the most appropriate tool for this study. The results of this study, detailed in Chapter 2, demonstrate functional effects which are highly correlated with the induction of plasticity in M1 and S1. Specifically, rTMS applied over M1 disrupted the ability to accurately anticipate the grip force needed to lift a small object, while rTMS applied over S1 hampered the ability to initiate subsequent phases of the motor plan.

A second series of experiments extended these findings by applying rTMS over the supplementary motor area (SMA) using a similar paradigm and the same grip-lift task. In healthy subjects, application of rTMS led to changes in the temporal and dynamic aspects of the grip-lift task and these changes demonstrated a hemispheric lateralisation. Disruption to left SMA produced a significant increase in the grip force needed to lift an object regardless of the hand used in the task. Conversely, disruption to right SMA reduced the synchronisation of the grip force to the object load force. These experiments are described in Chapter 3.

In animal models, temporally coupled afferent inputs have been shown to induce cortical reorganisation characterised by expansion and greater overlap of representational zones. In human subjects, paradigms utilising this “associative input” have also been shown to induce plastic change. However, it is unclear whether plasticity induced by associative afferent input

in human subjects is characterised by representational changes analogous to those seen in animal studies. This question was addressed in Chapter 4. I used a period of associative afferent stimulation applied to two hand muscles or two digits in healthy individuals and contrasted these findings with a period of non-associative afferent stimulation. Subjects receiving associative stimulation to the motor points demonstrated cortical representations which were larger in both area and volume and centred closer together. These changes were not present following associative stimulation applied to the digits or following non-associative stimulation.

A number of neurological conditions are thought to involve abnormal cortical plasticity. In particular, task-specific focal hand dystonia (FHD) is a debilitating neurological condition characterised by aberrant and maladaptive cortical plasticity. Previous studies have shown that cortical representations in FHD are significantly larger and demonstrate greater overlap than those in healthy individuals. While the exact mechanism is unclear, it appears that a genetic predisposition coupled with repeated exposure to associative afferent inputs may trigger maladaptive cortical reorganisation. Based on this, the experiments described in Chapter 5 tested the hypothesis that non-associative stimulation applied to the motor points of affected hand muscles would promote normalisation of cortical representations and alleviate symptoms in FHD. All subjects performed a grip-lift and a handwriting task before receiving 1 hour of non-associative stimulation. A decrease in the volume and area of cortical representations, and a separation in the centres of gravity for the stimulated muscles, was observed. These changes were correlated with a functional improvement in the variability of cyclic drawing, suggesting that the induction of plasticity using a non-associative stimulation paradigm may be an exciting avenue for the development of novel and effective treatment strategies in FHD.

1. Literature review

The intent of this review is to provide an overview of the literature concerning human movement control, transcranial magnetic stimulation and the induction of cortical plasticity in healthy individuals and in those with task specific focal dystonia. The review will begin by outlining the anatomical substrates and physiological mechanisms involved in the neural control of movement. Principles of cortical plasticity, how plastic change can be investigated and paradigms for inducing plastic change will then be discussed. Finally, the pathological basis for task-specific focal dystonia and the potential for novel treatment techniques, based on the induction of cortical plasticity, to reverse the underlying pathology and alleviate symptoms will be presented.

1.1. THE NEURAL CONTROL OF MOVEMENT

Each day humans perform a myriad of tasks with their hands. Successful completion of these tasks relies upon accurate movement control. Consider, for example, the movement complexity involved in reaching, grasping and manipulating a full teacup without spilling the contents. In this sequence the movement pattern must be executed within a fine margin of error to allow satisfactory task completion. Such precise control results from a number of distinct and interconnected cortical regions, most notably the primary motor, premotor and somatosensory cortices.

1.1.1. The human motor cortex

The human motor cortex was classically defined as the region of cerebral cortex requiring the least amount of electrical current to elicit movement (Penfield and Rasmussen 1950). In recent years a growing body of anatomical, physiological and functional evidence has expanded upon this early definition and the motor cortex is now considered to be comprised

of a number of distinct cortical regions including the primary motor cortex (Brodmann's area 4; M1), the supplementary motor area (Brodmann's area 6); the premotor cortex and the cingulate motor area (Donoghue and Sanes 1994; Wu et al. 2000).

In a seminal study, Penfield and Rasmussen (1950) used direct electrical stimulation to map the organisation of M1 in neurosurgical patients. This work revealed a topographical organisation, frequently depicted as the motor homunculus, where body parts are represented from medial (foot and leg representations) to lateral (head and face representations) along the cerebral surface. While the global division of body parts in M1 is still considered accurate, movement representations demonstrate significant overlap and are distributed at multiple, spatially distinct locations (Gould et al. 1986; Huntley and Jones 1991; Donoghue et al. 1992; Sanes and Donoghue 1992). Indeed, experiments employing spike-triggered averaging of neuronal firing suggest that each M1 neuron maps its output to a specific movement pattern rather than an individual muscle, creating a system with a large degree of flexibility (Buys et al. 1986; Fetz et al. 1989). This inherent flexibility is likely to underlie the extraordinary ability of the human brain to reorganise its structure and function (plasticity) in response to learning and injury.

The flexibility of M1 is supported by a complex cellular organisation. Cells are arranged horizontally in six layers (labelled I - VI from the superficial aspect of the cortex) and vertically in columns. Horizontal layers are segregated primarily by cell type, allowing functional organisation of cells based on individual input and output profiles (Kandel et al. 2000). By comparison, cells arranged in vertical columns form discrete processing networks. These networks are thought to be the primary computational units of the cortex and are characterised by extensive synaptic communication between local neurons, adjacent vertical columns and remote cortical regions (Mountcastle 1997; Jones 1983).

The most direct pathway for movement execution originates with large pyramidal cells located predominantly in layers III, V and VI of the cortex (Weber and Eisen 2002). The long axons of the large pyramidal cells project extrinsically, travelling via the corticospinal tract to synapse with alpha motoneurons in the spinal cord. In addition, the dendrites of pyramidal cells form extensive intrinsic networks with all cortical layers. Such extensive cortical networks serve to further enhance the plastic capabilities of this system.

In contrast to pyramidal cells, stellate cells produce axonal and dendritic projections which are almost exclusively intrinsic to the cortex. These cells make up 20-25 % of M1 neurons and are responsible for forming excitatory and inhibitory interneuronal connections (Rothwell 1994). The presence of interneurons has been identified as an integral component of precise movement control, permitting tight feedforward and feedback control of the entire system (Freund and Buzsaki 1996). The most common form of M1 stellate cell, the basket cell, uses the gamma-aminobutyric acid (GABA) neurotransmitter to produce inhibitory activity in post-synaptic pyramidal cells (Jones 1983). Through these mechanisms, interneuronal inhibition is thought to play an important role in maintaining normal cortical representations and in the production of fractionated movements (Sanes et al. 1988; Ridding et al. 1995; Liepert et al. 1998).

The primary motor cortex is also the recipient of extensive projections from premotor cortical areas (Donoghue and Sanes 1994). Traditionally, the presence of these connections gave rise to the view that M1 acted as the final common pathway for all voluntary movement commands, with the premotor areas acting only to integrate and dispatch information to M1. However, it is now clear that the premotor areas contain the anatomical substrate necessary for movement generation and thus, play a more complex role in motor behaviour.

1.1.2. The premotor cortex

The premotor cortex is a mosaic of interconnected areas situated in the frontal lobe. The region is characterised by the presence of extensive projections to M1. However, the premotor areas also project directly to the spinal cord (Murray and Coulter 1981; Martino and Strick 1987; Hutchins et al. 1988; Dum and Strick 1991). In fact, the number of corticospinal neurons present in premotor areas is similar to the number of corticospinal neurons contained in M1 (Dum and Strick 1991; He et al. 1995). In addition, the premotor areas demonstrate a high degree of topographic organisation with distinct projections to proximal and distal muscle groups as well as to individual arm and leg regions (He et al. 1993; He et al. 1995). These findings indicate that premotor areas are able to influence motor behaviour independently of M1. Each premotor area is also the recipient of an exclusive set of inputs from posterior parietal and prefrontal cortical areas (Dum and Strick 1991; 1992). The existence of a unique input and output profile for each premotor region provides the anatomical framework necessary for each region to make a distinct contribution to motor control (Dum and Strick 2002).

Functionally, the premotor areas consist of the premotor cortex (PMC), the supplementary motor area (SMA) and the cingulate motor area (CMA), although an increasing body of evidence indicates that these regions can be further subdivided (Roland and Zilles 1996). Numerous studies demonstrate activation in PMC during movement preparation, particularly when movements are triggered by external sensory events (Gemba and Sasaki 1984; Passingham 1986; Kurata and Wise 1988; di Pellegrino and Wise 1993; Kurata 1994). The PMC also plays a key role in the preparation and execution of delayed movements in response to specific cues and has been implicated in hand shaping during object manipulation (Rizzolatti et al. 1988; Murata et al. 1997; Rizzolatti and Luppino 2001; Davare et al. 2006). In contrast, SMA is active during movement preparation only when movements are initiated

through internal cues and are rehearsed from memory (Roland et al. 1980; Passingham 1986). Evidence also exists suggesting a role for SMA in bimanual coordination (Gentilucci et al. 1988; Freund 1990; Serrien et al. 1997). Finally, CMA has an important role in the motivational and cognitive aspects of voluntary movement control. In particular, evidence suggests that CMA is instrumental in selecting the motor output pattern with the highest reward potential when a multitude of options are available (Shima and Tanji 1998). Thus, the premotor areas have a clear role in the control of voluntary movement, acting both in concert with M1 and as separate entities to prepare, generate and execute motor commands.

1.1.3. The corticospinal tract

The corticospinal tract is the largest of the descending fibre systems, containing axons from primary motor, premotor, parietal and somatosensory regions (Brodal 1969; Galea and Darian-Smith 1994). Approximately 60 percent of the 1 million axons contained in the corticospinal tract originate in layer V of the primary motor cortex (Jane et al. 1967). Descending fibres pass through the internal capsule before the majority (75 %) decussate in the lower medulla (Brinkman and Kuypers 1973). Crossed fibres continue in the spinal cord as the lateral corticospinal tract. A small proportion of uncrossed fibres descend in the ventral columns of the spinal cord as the ventral corticospinal tract, while the remainder continue to project ipsilaterally, joining crossed fibres in the lateral corticospinal tract (Brodal 1969).

At the level of the spinal cord, many corticospinal tract fibres form excitatory, mono-synaptic connections with spinal motor neurons, forming the cortical motor neuronal (CM) system. Each spinal motor neuron is the centre for converging CM inputs, receiving information from a number of CM cells (Weber and Eisen 2002). Functionally, these connections are important for the production of fractionated finger movements and are thought to underpin the ability to perform a variety of dextrous movements with a single muscle (Rothwell 1994; Porter and

Lemon 1995). Indeed, CM control is responsible for precise actions such as the human precision grip and is likely to play a key role in the acquisition of new skills (Weber and Eisen 2002). In addition, corticospinal tract fibres synapse with spinal interneurons in the ventral intermediate layers of the spinal cord, creating indirect pathways important for the coordination of large, proximal muscle groups, such as those involved in reaching and lifting (Donoghue and Sanes 1994).

1.1.4. The somatosensory cortex

The primary motor cortex, premotor areas and the corticospinal tract form the key components of the motor output system. However, even with a functioning output system, dextrous movement is not possible without modulation from sensory feedback systems. In fact, the reliance upon sensory information is such that individuals with absent or impaired somatic sensation are unable to perform dextrous movements without visual guidance (Rothwell et al. 1982).

The somatosensory cortex, along with ascending spinothalamic pathways, plays a key role in the provision of sensory information to the motor output system. The somatosensory cortex is comprised of four regions, Brodmann's areas 1, 2, 3a and 3b. Neurons in areas 1 and 3b process primarily cutaneous stimuli, neurons in area 3a proprioceptive inputs and neurons in area 2 receive information from both cutaneous and proprioceptive stimuli (Jones et al. 1978; Leichnetz 1986; Jones 1987; Ghosh et al. 1987). Human mapping studies demonstrate that each of these somatosensory areas contains a complete representation of the human body (Penfield and Boldrey 1937). As in M1, cells are arranged in vertical columns with each column forming a discrete computational unit (Jones 1983; Mountcastle 1997). In the somatosensory cortex, greater numbers of computational units and hence, greater amounts of

cortical territory, are devoted to those body regions most sensitive to tactile stimuli (Penfield and Boldrey 1937).

Sensory information passes from the periphery to the somatosensory cortex via ascending pathways. Sensory information arising from peripheral receptors in skin, muscles and joints travels to the dorsal horn of the spinal cord. Here, axons either terminate locally, contributing to spinal reflex circuits, or continue ascending to reach the medulla. Axons ascending in the spinal cord are characterised by a somatotrophic organisation, with axons from distal regions entering the dorsal column in the midline and axons from proximal structures entering at progressively more lateral positions (Kandel et al. 2000). Sensory fibres maintain this topographical organisation throughout the ascending pathway.

Modulation of sensory information for motor control begins at the level of the spinal cord. Indeed, many descending inputs terminate in the spinal dorsal horn and these projections are likely to be important for the control, selection and interruption of incoming sensory information (Lemon 1999). From the spinal cord, fibres carrying cutaneous inputs decussate in the medulla before terminating in the ventral posterior lateral nucleus (sensory information arising from the body and posterior head) and the ventral posterior medial nucleus (sensory information arising from the face) of the thalamus. Cutaneous and proprioceptive information is projected from the thalamus predominantly to layer IV of the somatosensory cortex, although a number of fibres also project directly to pyramidal cells in M1 (Asanuma and Arissian 1984). The close relationship between the somatosensory cortex and M1 is further evidenced by the significant number of corticomotor neurons containing receptive fields which closely resemble those of the somatosensory cortex (Asanuma et al. 1968). Thus, motor output is modulated by sensory information at the level of the spinal cord, by direct

thalamocortical projections to M1 and indirectly via axonal connections with the somatosensory cortex.

1.1.5. Summary

In summary, it is clear that accurate movement control is reliant upon a widespread network of motor, premotor and somatosensory cortical regions, each performing specific functions in the preparation and execution of voluntary movement. While outside the scope of this thesis, it is also noteworthy that a number of other cortical and subcortical areas including the visual cortex and the cerebellum, are likely to be needed for accurate task completion. The widespread nature of this network, in conjunction with its considerable cellular and functional diversity, provides an excellent framework for the induction of cortical plasticity.

1.2. TRANSCRANIAL MAGNETIC STIMULATION

Early studies into human cortical plasticity were performed using the technique of transcranial electrical stimulation (TES). While TES is still used experimentally, its application results in significant discomfort (Weber and Eisen 2002). As a result, TES is considered inappropriate for clinical use. In 1985 TES was superseded by transcranial magnetic stimulation (TMS), a non-invasive, non-painful method of cortical stimulation (Barker et al. 1985). Since this time, TMS has emerged as an important tool in the investigation of human cortical plasticity.

Transcranial magnetic stimulation adheres to the principles of electromagnetic induction. The magnetic stimulator first discharges a capacitor, producing a brief high-current pulse in an insulated coil (Barker et al. 1985). Stimulating coils vary in shape and size. A circular coil delivers a large and diffuse field over the cortex, while a figure-of-8 coil produces a weaker but more focal field. The rapidly changing electrical current passing through the stimulating

coil induces a magnetic field perpendicular to the plane of the coil. This magnetic field passes unimpeded through the skull and elicits electrical eddy currents in the underlying neural tissue (Barker et al. 1988). If the correct stimulus waveform is used and the stimulus intensity is sufficient, corticospinal neurons are depolarised (Boniface and Ziemann 2003).

Neuronal depolarisation elicits a complex descending volley of action potentials. This volley is propagated in the corticospinal tract resulting in depolarisation of spinal motoneurons and the induction of a transient electromyographic (EMG) response in the target muscle (Burke et al. 1993). This response, measured using EMG electrodes, is termed a motor evoked potential (MEP). Direct electrical stimulation of the cortex in primates illustrates that the descending volley is made up of a number of waves occurring at approximately 1.5 ms intervals (Patton and Amassian 1954). The initial part of the descending volley results from direct depolarisation of corticospinal neurons or axons, producing a D-wave (direct). Subsequent components of the volley are triggered indirectly (I-waves) via excitatory synaptic inputs from axons stimulated by the TMS pulse. TES applied in human subjects has been shown to recruit a similar pattern of D and I waves (Day et al. 1989; di Lazzaro et al. 1998). In contrast, direct recordings of the descending volley in response to TMS demonstrate that this technique activates corticospinal neurons indirectly, preferentially evoking an I- wave (di Lazzaro et al. 1998). As the stimulus intensity increases, later I-waves are recruited (di Lazzaro et al. 1998). At very high intensities TMS may also activate corticospinal neurons directly, eliciting a D-wave (Mills 1999). This pattern of recruitment suggests that TMS predominantly activates corticospinal neurons trans-synaptically rather than directly as appears to be the case in TES (Day et al. 1989). The ability to depolarise corticospinal neurons via synaptic mechanisms makes TMS a highly sensitive measure of cortical excitability (Day et al. 1991).

1.2.1. Cortical motor threshold and MEP amplitude

A number of TMS techniques are used in the investigation of human cortical plasticity. These include measures of cortical motor threshold and MEP amplitude. Cortical motor threshold is defined as the lowest stimulator intensity needed to elicit an MEP of 50 μV peak to peak amplitude in the resting target muscle in five out of 10 trials (Rossini et al. 1994). The clinical usefulness of this parameter is well established. For example, motor threshold has been shown to be elevated in multiple sclerosis (Hess et al. 1987; Ravnborg et al. 1991), stroke (Xing et al. 1990), some forms of amyotrophic lateral sclerosis (Eisen et al. 1990) and migraine (Maertens de Noordhout et al. 1992). Conversely, a reduction in motor threshold is seen in generalised epilepsy (Reutens and Berkovic 1992), myoclonic epilepsy (Reutens et al. 1993) and other cases of amyotrophic lateral sclerosis (Mills and Nithi 1997). Cortical motor threshold is thought to reflect the excitability of the neuronal cell membrane, making it a useful tool in the assessment of membrane excitability following experimental and therapeutic interventions (Cohen et al. 1998). More recently, evidence has emerged suggesting that cortical motor threshold also reflects the maturity and integrity of the underlying white matter (Kloppel et al. 2008).

Perhaps the most common TMS measure, MEP amplitudes are widely used to study corticospinal excitability. The MEP amplitude is dependent on four physiological factors; the number of corticospinal neurons activated, the number of motor neurons recruited in the spinal cord, the number of motor neurons discharging more than once in response to the TMS stimulus, and how well these motor neuron discharges are synchronised (Rosler and Magistris 2008). As such, changes in the MEP amplitude give an indication of both the size and excitability of the corticospinal projection. The MEP amplitude exhibits considerable inter- and intra-individual variation (Kobayashi and Pascual-Leone 2003) and is further affected by stimulus intensity, coil shape and position and the level of background activity at the time of the TMS pulse (Hess et al. 1986; Anderson et al. 1999). Despite this, MEPs are frequently

used as a marker for the induction of plastic change following experimental interventions and evidence also exists for their use as a diagnostic and prognostic tool (Heald et al. 1993; Curt et al. 1998).

1.2.2. Cortical mapping

Single pulse TMS can also be used to create a scalp map of the underlying cortical representation of a specific muscle. A figure-of-8 coil is used to evoke EMG responses from a standardised grid (Wassermann et al. 1992). Stimulation of each point on the grid occurs at random until an EMG response is absent in all border sites. The MEP amplitude can then be used to create a topographical representation of the stimulated area (Wassermann et al. 1992; Wilson et al. 1993). Cortical maps provide four key pieces of information, the optimal position for obtaining the largest MEP response (hotspot), the excitability of the corticospinal projection to the target muscle (volume), the map centre of gravity and the area of the scalp where MEP responses can be obtained for a particular muscle (Rothwell 2003). These parameters are used to quantify cortical reorganisation and plastic changes in both the experimental (e.g. Brasil-Neto et al. 1992) and clinical settings (Ridding and Rothwell 1995; Liepert et al. 2000).

The centre of gravity (CoG) is the amplitude weighted centre of the cortical representation (Thickbroom et al. 1999). This point is usually tightly correlated with the optimal stimulus site (Wilson et al. 1993). Changes in the CoG are used to identify shifts in the location of the cortical representation and such changes are thought to reflect a true reorganisation of the anatomical projection to the target muscle (Rothwell 2003). Shifts in the CoG have been demonstrated in patients with Writer's Cramp (Byrnes et al. 1998) and in those with facial nerve palsy (Rijntjes et al. 1997).

The CoG has been shown to be a reliable and stable measure over time. For example, Miranda et al. (1997) demonstrated that the CoG coordinates for the left Abductor Digiti Minimi (ADM) muscle were reproducible within ± 3 mm across three separate mapping sessions one week apart. Similarly, Thickbroom et al. (1999) reported the standard deviation of the CoG coordinates to be 1.1 mm in latitude and 1.3 mm in longitude for the left Abductor Pollicis Brevis (APB) muscle. In a more recent study, the CoGs of three intrinsic hand muscles were found to be reproducible within 4 mm at intervals of 24 hours, one week and two weeks (Uy et al. 2002).

Several authors have also addressed the reliability of the map area and volume parameters. Using a coil guidance system, Mortifee et al. (1994) demonstrated that map area and volume are stable and reproducible over a period of 23-84 days. Wilson et al. (1993) reported a similar finding, with map area, volume and mean location remaining stable from 21-181 days. However, it is noteworthy that the area of the map obtained using the TMS mapping technique is a scalp surface representation and is therefore larger than the true area of the underlying cortical representation (Mortifee et al. 1994; Pascual-Leone et al. 1999). Despite this, cortical mapping has emerged as a useful and reliable tool in the investigation of cortical plasticity. This approach is used to examine changes in cortical reorganisation in response to an electrical stimulation paradigm in healthy subjects in Chapter 4 and in those with focal hand dystonia in Chapter 5.

1.2.3. Paired-pulse techniques

Paired-pulse TMS encompasses several protocols used to investigate the excitability of the local circuitry in human motor cortex. The protocol involves a single, conditioning stimulus given at varying intervals before a second test stimulus. The length of the inter-stimulus interval and the stimulus intensity determines whether effects are facilitatory or inhibitory.

Activation of local circuitry can include intracortical, interhemispheric or intrahemispheric connections (Hanajima and Ugawa 2008).

Intracortical effects are obtained by delivering two TMS stimuli through the same coil. For example, a subthreshold conditioning stimulus paired with a suprathreshold test pulse at an inter-stimulus interval of 1-4 ms produces MEP suppression. This effect, termed short-interval intracortical inhibition (SICI), is thought to reflect $GABA_A$ receptor-mediated interneuronal inhibition at the level of the primary motor cortex (Kujirai et al. 1993; Ilic et al. 2002). Using this same protocol, longer inter-stimulus intervals (5-15 ms) have been shown to produce MEP facilitation (intracortical facilitation; ICF). Pharmacological studies using N-methyl-D-Aspartic acid (NMDA) receptor antagonists demonstrate that this effect is mediated by glutamatergic NMDA receptors (Ziemann et al. 1998). Conversely, short-interval intracortical facilitation (SICF) is produced when a suprathreshold conditioning stimulus is paired with a subthreshold test pulse (Ziemann et al. 1998; Ilic et al. 2002). This protocol results in MEP facilitation at discrete inter-stimulus intervals of 1-1.5 ms, 2.5-3 ms and ~ 4.5 ms (Ziemann et al. 1998; Ilic et al. 2002). These intervals mimic the same periodicity as the I-waves elicited by TMS, suggesting that MEP facilitation results from transysnaptic activation of excitatory interneurons (Hanajima et al. 2002, Ilic et al. 2002). One final variation on this protocol is to deliver two suprathreshold stimuli at inter-stimulus intervals between 50 and 200 ms (Claus et al. 1992). In contrast to SICI, this long-interval intracortical inhibition (LICI) is thought to reflect $GABA_B$ receptor-mediated inhibition in M1 interneurons (McDonnell et al. 2006).

Interhemispheric facilitation and inhibition can also be obtained using paired pulse techniques. Two magnetic stimulating coils are used to deliver a conditioning stimulus to one hemisphere and a test stimulus to the second hemisphere. At inter-stimulus intervals between

8 – 12 ms the MEP is suppressed, while inter-stimulus intervals of 4-5 ms produce weak MEP facilitation (Ferbert et al. 1992; Hanajima et al. 2001). Interhemispheric inhibitory and facilitatory effects are reportedly mediated by transcallosal motor fibres (Ferbert et al. 1992; Hanajima et al. 2001).

Finally, paired pulse techniques can be used to produce activation of local intrahemispheric circuitry. For example, modulation of M1 excitability can be obtained through the application of a conditioning stimulus to the ipsilateral premotor or supplementary motor areas and a test stimulus over M1. Indeed, experimental studies demonstrate MEP inhibition if the conditioning stimulus is subthreshold with an inter-stimulus interval of 6 ms (Civardi et al. 2001). Alternatively, a suprathreshold conditioning stimulus delivered at the same inter-stimulus interval results in MEP facilitation (Civardi et al. 2001). This protocol allows investigation of the functional connectivity between M1 and higher order motor areas (Hanajima and Ugawa 2003).

1.3. FUNCTIONAL MEASURES OF PLASTIC CHANGE IN HUMANS

Cortical plasticity can also be investigated by measuring effects on function. For example, training healthy individuals to perform a particular keyboard sequence over a period of five days has been shown to induce plastic changes in M1 (Pascual-Leone et al. 1995). These changes were evidenced neurophysiologically, by an expansion in the cortical representation of muscles involved in the task, and functionally, by an improvement in the performance error rate (Pascual-Leone et al. 1995). The link between cortical reorganisation and motor behaviour makes functional outcomes a useful tool for the assessment of plastic change in both the healthy and patient populations. As a large number of tasks can be used to measure

the functional correlates of cortical plasticity, here I will focus only on those central to this thesis, namely the grip-lift task and the analysis of handwriting.

1.3.1. The grip-lift task

The grip-lift task was first designed by Westling and Johansson in 1984. Using a custom built manipulandum, the authors examined the coordination between the grip and load forces generated during object manipulation. Execution of the task required subjects to grasp the manipulandum between the thumb and index finger (precision grip), lift the object to the height specified, hold it stationary for 15 s and then slowly release the thumb and index finger until the object slipped. The manipulandum was comprised of two calibrated strain gauges which measured the horizontal grip force (GF) and the vertical load force (LF). In these initial experiments, the weight of the object and the texture of the gripped surfaces varied. Measurements of the slip force (minimal GF needed to prevent the object from slipping through the fingers), coordination between GF and LF profiles and the optimisation of these parameters across a series of lifts were made.

Since these early experiments a large number of studies have been completed using the grip-lift task as a measure of hand dexterity. These studies have used the grip-lift task to examine the mechanisms underlying object manipulation in healthy individuals, to quantify deficits in hand dexterity seen in a variety of pathological conditions and as a measure of cortical plasticity following experimental and clinical interventions. A summary of the key findings from studies involving healthy individuals is provided below. Information on the grip-lift task as a measure of cortical plasticity is detailed in section 1.5.4. Findings as they relate to focal hand dystonia are outlined in section 1.7.3.

- The ability to manipulate small objects requires precise scaling of the GF to the weight and frictional properties of the object (Westling and Johansson 1984). Accurate GF scaling prevents the object from slipping through the fingers as well as the generation of excessive crushing forces.

- The usual close temporal coupling of GF to LF is thought to be the product of an internal feed-forward model. This model generates an appropriate GF prior to the lifting movement and in advance of sensory feedback (Johansson and Westling 1987; Flanagan and Wing 1997; Flanagan and Johansson 2002).

- The internal model relies on a memory trace of an object's properties. This trace is obtained from prior experience and is used to estimate the LF based on predicted sensory input (Flanagan and Wing 1997; Flanagan and Johansson 2002).

- At the commencement of the lift, sensory feedback arising from cutaneous mechanoreceptors updates the internal model to optimise fingertip forces. In healthy individuals, GF is optimised after 1-3 lifts (Flanagan and Johansson 2002).

1.3.2. Handwriting analysis

The kinematic analysis of handwriting provides an objective means of quantifying impairment in some movement disorders. Over the last decade a wealth of literature has emerged utilising this functional tool, most notably in the areas of Parkinson's disease (e.g. Siebner et al. 1999; Tucha et al. 2006) and focal hand dystonia (e.g. Wissel et al. 1996; Zeuner et al. 2005).

Handwriting is a highly skilled and complex fine motor activity (Tucha et al. 2006). In healthy individuals, writing strokes are performed in rapid succession utilising movements

from the forearm, wrist and hand. These movements are an example of automated, open-loop processing and a number of studies have demonstrated that minimal attention is required for accurate task completion (Meulenbroek and Van Galen 1988; Tucha et al. 2001). In pathological conditions, deficits in handwriting affect not only stroke amplitude but also kinematic variables such as velocity, acceleration, pressure and stroke frequency and duration (e.g. Tucha et al. 2006; Zeuner et al. 2007).

Assessment of handwriting is completed using a pressure sensitive digitising tablet and an inking, digitising pen. Subjects are asked to write a simple sentence such as "Sheila collects shells" 10 times in their normal handwriting. Task complexity can be increased by asking the subject to draw superimposed circles, both with the eyes open and with the eyes closed. In those with focal hand dystonia, cyclic drawing has been shown to be a more sensitive measure of dexterity than handwriting (Zeuner et al. 2007). Abnormalities in the handwriting and cyclic drawing profiles of those with focal hand dystonia and the use of this technique to measure plastic change are further outlined in section 1.7.3.

1.4. CORTICAL PLASTICITY

Cortical plasticity was once thought to be a process possible only in the very young. However, there is now a large body of evidence which demonstrates that the human brain retains the ability to reorganise throughout life. This discovery has opened up a number of exciting new avenues in neuroscience research, most notably in the development of novel rehabilitation strategies for neurological conditions.

The term cortical plasticity refers to the reorganisation of the morphological or structural properties of the cortex (Donoghue et al. 1996). This dynamic ability is the foundation for learning, memory and the restoration of cortical function following injury (Rossini and Dal

Forno 2004). Plastic changes have been demonstrated in a wide variety of human brain regions including the sensory (Wu et al. 2005), motor (Donoghue 1995; Karni et al. 1995; Hamdy et al. 1998) and auditory (Jancke et al. 2001) cortex.

1.4.1. Mechanisms underlying plastic change

The evidence for the mechanisms underlying plastic change in the human brain is drawn primarily from analogies with animal studies. In animal models, plastic change can be observed at the molecular, synaptic and functional levels (Boniface and Ziemann 2003). Plastic change can be initiated by many phenomena, including trauma, pathological disturbance, pharmacological interventions, manipulation of sensory input and motor learning (Sanes and Donogue 2000).

The mechanisms underlying cortical reorganisation can be broadly divided into two categories, those supporting rapid plasticity, and those involved in the long-term reorganisation of cortical circuits. The mechanisms underlying rapid changes in organisation include the unmasking of latent horizontal connections and activation of silent synapses, modulation of activity-dependent synaptic plasticity and generalised changes in the excitability of postsynaptic neurons.

The unmasking of existing but silent synaptic connections is a likely mechanism for rapid plasticity in response to the manipulation of sensory input (Jacobs and Donoghue 1991; Kaas 1991; Merzenich and Shameshima 1993). Silent synapses have been identified as those displaying no alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-mediated glutamate responses (Liao et al. 1995; Isaac et al. 1995). Rapid unmasking of these connections may be due to the insertion of postsynaptic AMPA receptors (Gomperts et al.

1998; Liao et al. 1999), increased release of the excitatory neurotransmitter glutamate, or more likely, a reduction in GABAergic inhibition (Kaas 1991; Chen et al. 2002).

Considerable evidence exists demonstrating the importance of GABA-mediated inhibition in the regulation of cortical representations. In the rodent model, administration of the GABA antagonist bicuculline methobromide results in rapid reorganisation of the size and distribution of cortical representations (Jacobs and Donoghue 1991). Similarly, plasticity is enhanced in human motor cortex following cortical disinhibition induced by ischemic deafferentation/deefferentation (Ziemann et al. 2001), while plasticity is depressed following application of the $GABA_A$ receptor agonist lorazepam (Ziemann et al. 2001). These findings indicate that GABA-mediated inhibition plays an important role in the unmasking of silent connections during rapid cortical reorganisation.

The most widely studied mechanism of rapid plasticity is the modulation of activity-dependent synaptic plasticity. In a seminal study, Bliss and Lomo (1973) described the modulation of synaptic efficacy in the rabbit hippocampus. The authors noted that high frequency repetitive stimulation of excitatory afferents produced an increase in the amplitude of postsynaptic potentials, enhancing the efficacy of synaptic transmission. This enhancement, termed long term potentiation (LTP), has since been demonstrated in a number of cortical regions including the motor cortex (Baranyi et al. 1991; Aou et al. 1992; Ziemann et al. 1998b).

The induction of most forms of LTP is dependent on activation of the NMDA type of glutamate receptor (Bliss and Collingridge 1993). The reliance on NMDA receptors gives rise to several important requirements of the LTP pathway. The first, known as cooperativity, dictates that several afferent axons must be activated together (Bliss and Lomo 1973). This

produces depolarisation in the post-synaptic membrane of a magnitude sufficient to remove the magnesium channel block and allow calcium influx. Similarly, concomitant activity must occur in both the pre and post-synaptic cells for adequate depolarisation to occur (Debanne et al. 1998). This property is consistent with Hebbs postulate where strengthening of synaptic efficacy occurs "when an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it" (Hebb 1949; p. 62).

The strength of synaptic connections is also dependent on the frequency, timing and intensity of inputs (Dinse et al. 1993). While high frequency stimulation induces LTP, low frequency stimulation produces long term depression (LTD) of synaptic efficacy (Dudek and Bear 1992). In addition, Hebbian plasticity is input specific. When the temporal order of paired inputs is reversed, LTD rather than LTP is induced (Levy and Steward 1983). While the role of LTD in plastic change is less clear than that of LTP, LTD-like mechanisms are implicated in the reorganisation of cortical maps (Buonomano and Merzenich 1998).

The activity dependent nature of LTP and LTD, coupled with the restriction of plastic changes to synapses and their target neurons, makes modulation of synaptic efficacy an attractive mechanism for rapid plasticity. However, evidence also exists for more generalised excitability changes in post synaptic neurons. For example, Brons and Woody (1980) demonstrated long-lasting increases in the excitability of post-synaptic M1 neurons following pavlovian conditioning in the cat. The long-lasting nature of this change makes it a feasible mechanism for memory consolidation (Moyer et al. 1996). However, such changes alter the effectiveness of all synapses to a target neuron and are therefore less specific than those induced by LTP or LTD.

Finally, mechanisms involved in long-term reorganisation are thought to include morphological changes such as neurogenesis, synaptogenesis and synaptic remodelling. Neurogenesis is increased in the hippocampus following an associative learning task (Gould et al. 1999), while motor skill learning and exposure to an enriched environment have been shown to increase the number of synapses present per neuron in M1 (Kleim et al. 1996) and the cerebellum (Kleim et al. 1997; 1998). Increases in spine density (Moser et al. 1997) and changes in dendritic spine morphology (Comery et al. 1996) have also been demonstrated following exposure to an enriched environment. Whether LTP might also induce synaptogenesis or synaptic remodelling is unclear. Using imaging techniques several authors have shown the generation of new synapses 30-60 minutes following induction of LTP in hippocampal slice preparations (Engert and Bonhoeffer 1999; Toni et al. 1999). However, the role of synaptogenesis in LTP is yet to be proven.

1.4.2. Use-dependent plasticity in humans

LTP and LTD-like plastic changes are induced in M1 following motor learning, motor practice and use. This "use-dependent" plasticity is responsible for improvements in motor performance and is thought to underlie skill acquisition in human motor cortex (Classen and Cohen 2003). As a result, use-dependent plasticity is likely to play a key role in motor learning and in functional recovery following CNS injury or pathology.

The LTP and LTD-like changes underlying use-dependent plasticity are evidenced by pharmacological manipulations and through similarities with animal studies. Pharmacological experiments demonstrate that use-dependent plasticity is reduced by drugs which enhance $GABA_A$ receptor function (Lorazepam; Wong et al. 1988) and block NMDA receptor activity (Dextromethorphan; Wong et al. 1988). As the induction of LTP in M1 slice preparations requires NMDA receptor activation and is facilitated by a decrease in GABAergic inhibition,

these findings provide evidence that use-dependent plasticity is mediated by LTP/LTD-like mechanisms similar to those present in animal models (Hess et al. 1996). Additional similarities with animal studies, such as an effect duration of 30- 60 minutes and the presence of input specificity, provide further support for this hypothesis (Stefan et al. 2002; Wolters et al. 2003).

The induction of use-dependent plasticity has been extensively demonstrated following motor training in human subjects. Indeed, a number of studies show a strong association between long-term skill acquisition and changes in cortical organisation. One early example of this phenomenon showed that the motor representation of the "reading" finger is enlarged in blind individuals learning to read Braille (Pascual-Leone et al. 1993). Similar expansions in cortical territory have been reported for the digits of the skilled hand in string musicians (Elbert et al. 1995) and elite badminton players (Pearce et al. 2000). In both cases cortical excitability was increased in the hemisphere contralateral to the skilled hand. No changes were seen in the ipsilateral hemisphere (contralateral to the unskilled hand) or in the cortical representations of untrained individuals.

Similar reorganizational changes have been reported in response to short-term motor training. In fact, changes in M1 have been reported within 15 minutes of training on a repetitive thumb movement task (Classen et al. 1998). This rapid adjustment is consistent with the notion of M1 as a cortical region able to continuously adapt to new environmental requirements (Classen and Cohen 2003). Pascual-Leone and colleagues (1995) investigated changes in motor maps following 5 days of training on a one-handed, five finger piano exercise compared with random key presses and pure mental practice. The authors found that repetitive movement practice with a clear performance goal resulted in increased excitability of M1. Similar changes were not observed following random key presses or pure mental

practice. Synchronised thumb and foot movements also produced reorganisation of M1 (Liepert et al. 1999). Cortical reorganisation was manifest as a temporary (less than 60 minutes) shift of the thumb representation medially towards that of the foot area (Liepert et al. 1999). Asynchronous movements of the thumb and foot did not result in cortical reorganisation (Liepert et al. 1999).

The experimental examples given above demonstrate the importance of skilled practice in the induction of plasticity. However, cortical reorganisation is also present following non-use of a body part. For example, transient deafferentation of the forearm and hand results in a rapid increase in cortical excitability and expansion of the cortical territory of muscles proximal to the block (Brasil-Neto et al. 1993). In addition, ischemic deafferentation produces an increase in motor cortical excitability to muscles in the opposite hand and forearm (Werhahn et al. 2002). The mechanism for this rapid change is thought to be a reduction in GABAergic inhibition in the motor cortical representations of the contralateral upper arm and the ipsilateral hand and forearm (Levy et al. 2002). Similar findings have also been reported in amputees, where increased cortical representations have been demonstrated for muscles proximal and ipsilateral to the stump (Cohen et al. 1991).

Paradigms for inducing cortical plasticity are many and varied. The paradigms outlined above predominantly describe examples of training-induced plasticity. However, similar changes have also been observed following a number of other experimental paradigms. The experimental chapters of this thesis employ two protocols for the induction of cortical plasticity, repetitive TMS and electrical somatosensory stimulation. These two protocols are discussed in detail in the following section.

1.5. CENTRAL STIMULATION: REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION

Cortical plasticity may be induced by repetitive transcranial magnetic stimulation (rTMS) applied over the cortex. This approach is particularly beneficial in the study of structure-function relationships where rTMS can be used to disrupt activity in a specific cortical region and the effect of this disruption on function determined (Classen and Ziemann 2003). For example, rTMS has been widely used as a method to investigate cortical regions involved in object manipulation. While the exact mechanisms underlying rTMS-induced plasticity are unclear, effects have been linked to LTP and LTD-like mechanisms. In addition, rTMS has received considerable attention due to its therapeutic potential in neuropsychiatric disorders (Classen and Ziemann 2003).

rTMS protocols involve trains of TMS stimuli delivered at constant or varying interstimulus intervals. Repetitive trains of TMS stimuli have been shown to induce changes in corticomotor excitability which outlast the intervention period. The precise physiological response to rTMS is dependent on a number of experimental parameters including stimulus frequency and intensity, pulse configuration, total number of stimuli and duration of the application (Pascual-Leone et al. 1994).

1.5.1. High frequency repetitive TMS

High frequency (5 - 20 Hz) rTMS protocols generally produce an increase in cortical excitability. For example, in the first study to use such a protocol, Pascual-Leone and colleagues (1994a) demonstrated an increase in MEP amplitude lasting 3–4 minutes after the stimulation period. The experimental paradigm consisted of 10 TMS pulses delivered over M1 at a frequency of 20 Hz and a stimulus intensity of 150 % resting motor threshold. Similar

findings were reported by Berardelli et al. (1998). Using a 5 Hz, rTMS train of 20 stimuli and a stimulus intensity of 120 % the authors reported an increase in MEP amplitude outlasting the stimulation period by 600 – 900 ms.

Since these early experiments, high frequency rTMS has been investigated as a therapeutic tool in a variety of clinical populations. For example, rTMS applied to the left dorsolateral prefrontal cortex at 10-20 Hz over 5 daily sessions are reported to alleviate symptoms in those with drug resistant depression for up to 6 weeks (Pascual-Leone et al. 1996). In those with Parkinson's disease, subthreshold 5 Hz, rTMS significantly shortened choice reaction time and led to improved performance on a grooved peg board task. Positive effects of high frequency rTMS have also been shown in chronic pain syndromes (e.g. Amassian et al. 1997) and with the application of 10 Hz rTMS over the lesioned hemisphere in stroke patients (Kim et al. 2006).

Despite the obvious therapeutic potential of high frequency rTMS, relatively few studies exist examining the underlying physiological mechanisms. However, several studies have demonstrated a reduction in SICI following subthreshold (di Lazzaro et al. 2002; Quartarone et al. 2005) and suprathreshold (Wu et al. 2000b) 5 Hz rTMS. For example, Wu et al. (2000b) reported a decrease in SICI which outlasted the stimulation period by approximately 3 minutes. In addition, several pieces of evidence support the hypothesis that rTMS effects are mediated at the level of the cortex. Using epidural spinal recordings of descending cortical volleys in humans, di Lazzaro et al. (2002) illustrated that the reduction in SICI following rTMS was due to changes in cortical circuits and not due to effects originating from subcortical or spinal structures. Similarly, studies conducted using brain-stem electrical stimulation demonstrate that changes induced by 5 Hz rTMS are generated at the cortical level (Quatarone et al. 2005). These findings have led to the suggestion that high frequency

rTMS results in enhanced synaptic transmission to pyramidal neurons through the induction of LTP (Quartarone et al. 2005).

Finally, it is prudent to mention the effects of interactions between frequency, intensity and rTMS duration. While high frequency rTMS generally produces an increase in cortical excitability, studies have shown that high frequency rTMS applied at low stimulus intensities results in MEP suppression (Todd et al. 2006). Similarly, short stimulus durations (< 20 s) tend to result in decreased cortical excitability, regardless of the stimulation frequency, while longer trains tend to increase cortical excitability (Modugno et al. 2001). The explanation for these findings appears to relate to the build-up of inhibitory and facilitatory effects in the cortex, with inhibitory effects taking a shorter time to reach their maximum effect (Modugno et al. 2001).

1.5.2. Low frequency repetitive TMS

Low frequency rTMS protocols are widely used to produce a decrease in cortical excitability. This effect is illustrated in studies by Chen et al. (1997) and Muellbacher et al. (2000). Using suprathreshold, 0.9 Hz rTMS Chen and colleagues (1997) demonstrated a reduction in MEP amplitude of approximately 20 %. This effect was sustained for a period greater than 15 minutes following the intervention. In comparison, Muellbacher et al. (2000) reported suppression of the MEP input-output curve lasting up to 30 minutes after stimulation. Effects have been shown to be specific for the cortical representation targeted by stimulation (Muellbacher et al. 2000). However, it is noteworthy that effects produced by rTMS exhibit considerable inter-individual variability (Maeda et al. 2000). The reason for this remains unclear, although a number of factors such as activation history (Iyer et al. 2003), time of day (Sale et al. 2007) and genetics (Cheeran et al. 2008) may play a role.

The inhibitory effects produced by low frequency rTMS make it possible to induce temporary disruption of function in specific cortical regions and thereby examine their role in the performance of various tasks. This approach is often referred to as inducing a "virtual lesion" in the stimulated region (Walsh and Rushworth 1999). As such, low frequency rTMS is one of the most commonly used protocols in the study of structure-function relationships. For example, a 1 Hz rTMS paradigm applied over the ipsilateral motor cortex was found to influence the temporal accuracy of unilateral finger movements, evidenced by an increase in touch duration and a decrease in the inter-tapping interval (Avanzino et al. 2008). These effects were present for up to 30 minutes following stimulation (Avanzino et al. 2008). The authors used this information to conclude that M1 plays a key role in the execution of sequential finger movements. Similar investigations have been performed in the area of object manipulation, using the grip-lift task. The literature pertaining to this topic is outlined in section 1.5.4.

The literature addressing the possible mechanisms underlying low frequency rTMS is conflicting. An increase in resting motor threshold has been reported after 1 Hz rTMS (Muellbacher et al. 2000). However, this increase did not correspond well with the duration of MEP suppression and several more recent studies have failed to demonstrate effects on resting motor threshold following similar rTMS protocols (Bagnato et al. 2005; Heide et al. 2006). Thus, it is unclear whether changes in neuronal membrane excitability contribute to the decrease in cortical excitability seen with low frequency rTMS. Further research has examined the contribution of GABAergic inhibition and NMDA receptor activation using pharmacological blockade. The administration of Lorazepam (allosteric positive modulator at the $GABA_A$ receptor) and Dextromethorphan (NMDA receptor antagonist) blocked plastic changes following subthreshold, 1 Hz rTMS (Fitzgerald et al. 2005). These findings indicate that effects of low frequency rTMS are dependent on both $GABA_A$ and NMDA receptor

activation, presumably through the induction of LTD-like mechanisms (Fitzgerald et al. 2005). Finally, evaluation of H-reflexes suggests that plastic changes induced by low frequency rTMS are mediated at the level of the cortex (Modugno et al. 2001; Touge et al. 2001).

1.5.3. Theta-Burst stimulation

Theta-burst stimulation (TBS) is a recently developed rTMS paradigm employing low-intensity bursts of high-frequency stimulation. The overarching pattern of TBS involves three magnetic pulses given at a frequency of 50 Hz and repeated at an interval of 200 ms (Huang et al. 2005). This novel approach has been shown to induce long-lasting inhibitory or facilitatory effects depending on the temporal pattern of the stimuli (Huang et al. 2005). Continuous TBS (cTBS) applied for a period of 20 s at an intensity of 80 % resting motor threshold decreases cortical excitability for a period of 60 minutes, while intermittent TBS (iTBS) produces an increase in cortical excitability lasting up to15 minutes (Huang et al. 2005).

Indirect evidence suggests that the decrease in cortical excitability produced by cTBS is due to an LTD-like effect on the excitatory synaptic connections within the cortex (Huang et al. 2005; Huang et al. 2007). Huang and colleagues (2007) recently demonstrated that memantine, a NMDA receptor antagonist, blocks the inhibitory effect of TBS. Changes in H-reflexes are also absent following cTBS, indicating a cortical origin for the induced plastic changes. This finding is supported by work from di Lazzaro et al. (2005) demonstrating a decrease in the corticospinal I1-wave which is correlated with the period of MEP suppression. Conversely, iTBS is thought to produce MEP facilitation by increasing the effectiveness of synaptic transmission through LTP-like mechanisms (Huang et al. 2005).

1.5.4. Functional correlates of cortical plasticity: the grip-lift task

rTMS and TBS have been used extensively to investigate the cortical network contributing to object manipulation. In the contralateral primary motor cortex, high frequency rTMS has been shown to disrupt accurate GF scaling, resulting in a significant increase in peak GF across a series of precision lifts (Nowak et al. 2005). Similarly, rTMS induced disruption of M1 causes the object to be underestimated 10-30 s after the previous set of lifts and overestimated 30-60 s later (Berner et al. 2007). These results provide evidence for the role of M1 in the establishment of a sensorimotor memory during object manipulation. The ipsilateral primary motor cortex has been shown to make a significant contribution to the timing of muscle recruitment during object manipulation (Davare et al. 2007b). This finding was manifest as a decrease in the coordination of the grip and lift profiles during a grip-lift task following high frequency rTMS applied to ipsilateral M1. It seems likely that ipsilateral M1 exerts its influence on muscle recruitment through transcallosal inhibitory or facilitatory connections with the contralateral M1 (Davare et al. 2007b).

Comparable results have been reported following rTMS-induced virtual lesions in premotor areas. For example, Davare and colleagues (2007) demonstrated an increase in peak GF following disruption to the anterior intraparietal region, indicating that GF modulation is processed in more than one cortical region. In addition, rTMS of the dorsal premotor cortex has been shown to disrupt the ability to use cued information in the planning of subsequent lifts (Chouinard et al. 2005). Finally, evidence for a differential role of the ventral and dorsal premotor cortex in fingertip positioning and intrinsic muscle recruitment has been demonstrated during a grip-lift task (Davare et al. 2006).

Despite these findings, relatively little is known about the neural mechanisms and cortical regions involved in feed-forward anticipatory control. In particular, the contribution of the

primary sensory cortex and the supplementary motor area to anticipatory scaling of fingertip forces remains unclear. These questions formed the research hypotheses for Chapters 2 and 3 of this thesis. The protocols of high frequency rTMS and TBS were used to induce LTD-like plastic changes in S1 and SMA during object manipulation. The grip-lift task was employed as a functional correlate of cortical plasticity, allowing the effect of disruption to these particular cortical regions to be quantified. The experimental set-up and subsequent results are discussed further in Chapter 2 (S1) and Chapter 3 (SMA).

1.6. PERIPHERAL STIMULATION: ELECTRICAL SOMATOSENSORY STIMULATION

Sensory input is known to be a powerful driver of cortical reorganisation. In fact, an extensive body of research exists illustrating the critical role of sensory information in the maintenance of cortical representations. For example, a reduction in the flow of sensory input to the cortex through amputation (Cohen et al. 1991; Ridding and Rothwell 1995), temporary ischemic block (Ridding and Rothwell 1995), thalamic lesions (Miles et al. 2005) or prolonged positional adjustments (Sanes et al. 1992) results in rapid and dramatic reorganisation of M1 representational areas. These changes are supported by the presence of direct connections between S1 and M1. These connections provide the anatomical substrate necessary for changes in sensory information to induce plastic change in M1 representational areas (Wu and Kaas 2002).

Cortical plasticity may also be induced through the addition of sensory information. The most common approach involves the use of electrical somatosensory stimulation, although other sensory paradigms such as exposure to an enriched environment (e.g. Turner and Greenough 1985) have also been shown to induce cortical reorganisation. Indeed, a wealth of evidence now exists examining a range of electrical stimulation paradigms in both healthy subjects and

in those with various pathologies. For this reason, discussion in the following sections will be limited to those paradigms which are directly relevant to the studies conducted in this thesis.

In healthy individuals, electrical stimulation of the periphery results in reorganisation of M1 and S1 cortical regions. In experiments by Hamdy et al. (1998) 10 minutes of repetitive electrical stimulation applied to the pharyngeal region led to a significant increase in M1 excitability and an increase in the cortical representation for the stimulated muscles. The authors also examined brainstem-mediated reflexes and reported no change following electrical stimulation, indicating that reorganisational changes were likely of cortical origin.

Relatively short periods of electrical stimulation (2 hours) have been shown to result in reorganizational changes which are topographically specific and persist in the cortex for up to 2 hours (Kaelin-Lang et al. 2002; Charlton et al. 2003). For example, repetative peripheral nerve stimulation applied to the ulnar or radial nerve in a single, 2 hour session is characterised by an increase in cortical excitability, and a shift in the centre of gravity of the cortical representation for stimulated muscles (Ridding et al. 2000; Ridding et al. 2001; Charlton et al. 2003). Cortical reorganisation induced by electrical stimulation is blocked by lorazepam, but is unaffected by administration of dextromethorphan (Kaelin-Lang et al. 2002). Thus, a reduction in GABAergic inhibition is likely to be a key mechanism in the induction of cortical plasticity following electrical stimulation protocols.

The ability of electrical stimulation paradigms to induce cortical plasticity has opened up exciting new avenues for the development of other novel methods to produce functionally beneficial cortical reorganisation. One such method is that of associative electrical stimulation where tightly correlated afferent inputs are delivered to the digits or muscle motor points using electrical stimuli.

1.6.1. Associative electrical stimulation

The temporal characteristics of synaptic inputs to neurones are thought to be of crucial importance in the induction of plasticity (Hebb 1949). In particular, afferent input which is temporally correlated, or "associative" has been shown to exert a significant influence on the topographical organisation of the cortex. Examples of this principle can be found in both the animal and human literature. In early animal models, Clark and colleagues (1988) increased the amount of correlated associative input reaching the cortex by surgically connecting two adjacent digits (syndactyly) in adult owl monkeys. Several months post surgery, cortical mapping revealed representational enlargements, blurring between the representations of adjacent, joined, digits and greater representational overlap in S1. In the rat, associative pairing of weak electrical stimuli applied simultaneously to the digits for a period of 6 -15 hours also resulted in representational changes in S1 (Godde et al. 1996). These changes were characterised by enlargement and greater overlap of stimulated receptive fields. Electrical stimuli applied in the absence of associative pairing failed to produce analogous effects (Godde et al. 1996). Similar changes are also reported in M1. Using intracortical microstimulation in adult squirrel monkeys, Nudo and colleagues (1996) demonstrated that muscles that co-contract during motor training, and therefore produce associative patterns of afferent input, have representations which are enlarged and centred closer together following training.

Associative stimulation applied in human subjects has also been shown to induce changes in cortical excitability. These changes were characterised by an increase in the MEP amplitude for stimulated muscles and an increase in ICF and SICF at interstimulus intervals of 2.3-3.3 ms (Pyndt and Ridding 2004). Despite these findings, it is currently unclear whether associative stimulation applied in human subjects results in representational changes

analogous to those seen in animal models. This question was investigated in Chapter 4 of this thesis using the technique of TMS mapping. Measurements of the area, volume and centre of gravity of the cortical representations for muscles receiving associative stimulation were obtained.

In human subjects, associative stimulation has also been shown to produce changes in function. For example, Godde et al. (1996) applied associative tactile stimulation over two skin sites and reported improvements on a spatial discrimination task. Comparable results have also been reported in subjects with stroke. In a recent randomised controlled trial, stroke subjects who received associative stimulation combined with task-specific training exhibited significantly greater improvements on the grip-lift task than subjects receiving training alone (McDonnell et al. 2007). This property of associative stimulation makes it a novel method of inducing functionally beneficial plasticity in those with neurological conditions and it may have particular relevance to the condition of task-specific focal hand dystonia.

1.7. TASK-SPECIFIC FOCAL HAND DYSTONIA

Task-specific focal hand dystonia (FHD) is a movement disorder characterised by excessive and sustained muscle activity and an inability to produce fractionated movements. Symptoms manifest during highly-skilled, fine motor tasks and result in slow, clumsy movement patterns and diminished task performance (Zeuner et al. 2005). The two most common examples of FHD are musician's dystonia and writer's cramp (WC), in which symptoms are localised to the muscles of the hand and forearm (Byl et al. 2003, Candia et al. 2003). Despite the significant impact upon the daily lives and careers of those with FHD, there is currently a paucity of effective treatment strategies.

1.7.1. Pathophysiology: the role of maladaptive cortical plasticity

The focal dystonias were initially ascribed to psychiatric illness. However, it is now widely accepted that this condition has a neurological basis (Nadeau et al. 2004). In particular, it has been suggested that repetitive performance of highly-skilled motor tasks may produce correlated patterns of afferent input which drive maladaptive cortical reorganisation and contribute to the onset of dystonic symptoms (Tempel and Perimutter 1990; Grunewald et al. 1997; Tinazzi et al. 2000). Indeed, the importance of specific patterns of afferent input has been clearly demonstrated in several animal studies. Byl and colleagues (1996, 1997) trained primates in a repetitive motor task over 12 to 25 weeks and concluded that repetitive sensorimotor training disrupts the organisation of the primary sensory cortex, contributing to the onset of dystonia. This disruption was characterised by a loss of differentiation in the cortical representation of the involved muscles, receptive fields that were 10 to 20 times larger than normal and extended across two or more digits and receptive fields which exhibited significantly greater overlap. In human subjects, further evidence for this hypothesis arises from a number of studies demonstrating excessive representational plasticity (Quartarone et al. 2003), abnormal cortical representations (Byrnes et al. 1998; Bara-Jimenez et al. 1998) and reduced intracortical inhibition (Ridding et al. 1995; Stinear and Byblow 2004b, 2004c; Bütefisch et al. 2005) in FHD. For example, Quartarone et al. (2003) demonstrated that in subjects with FHD, paired associative stimulation produces an abnormal increase in corticospinal excitability. Studies using TMS mapping in patients with writer's cramp show distortion in the lateral borders of representational maps, the presence of discrete secondary motor areas and displacement of maps relative to healthy control subjects (Byrnes et al. 1998). In addition, the degree of map distortion and displacement has been shown to be strongly correlated with the duration of symptoms (Byrnes et al. 1998). Finally, in healthy human subjects, short interval intracortical inhibition is decreased for muscles required in the execution of a voluntary motor task (Ridding et al. 1995) and increased or maintained for

adjacent muscle groups that need to remain relaxed (Liepert et al. 1998; Stinear and Byblow 2003; Zoghi et al. 2003). This process is likely important for the fractionation of motor outputs from the primary motor cortex, creating isolated movements in the absence of muscle overflow and co-contraction. Interestingly, intracortical inhibitory networks are impaired or poorly modulated in FHD (Ridding et al. 1995; Stinear and Byblow 2004b, 2004c; Bütefisch et al. 2005). As cortical representations are thought to be maintained and adjusted by intracortical inhibitory circuits, primarily through the modulation of the inhibitory neurotransmitter GABA, impairment of this inhibition is likely to contribute to the abnormal cortical representations and excessive muscle activity present in FHD (Sanes et al. 1988; Ridding et al. 1995; Liepert et al. 1998). Indeed, reductions in GABA neurotransmission have been shown to result in inappropriate neuronal activity. For example, application of the GABA antagonist bicuculline produces an increase in the size of receptive fields in the primary sensory cortex (Matsumura et al. 1991). Similarly, administration of bicuculline produces performance deficits characterised by muscle co-contraction in the primate (Matsumura et al. 1991).

Taken together, the findings outlined above suggest that repetitive, tightly correlated afferent inputs may drive excessive representational plasticity in those with FHD. This excessive plasticity may be facilitated by a decrease in the normal levels of intracortical inhibition. While the reason for such a reduction remains unclear, the genetic contribution to the dystonias is increasingly recognised (Defazio et al. 2007), indicating that repetitive, stereotyped afferent inputs may lead to late-onset dystonia, such as FHD, more rapidly in genetically susceptible individuals.

1.7.2. Novel rehabilitation strategies

The importance of afferent input in driving both functionally beneficial and maladaptive cortical plasticity has opened up exciting new avenues for the development of novel rehabilitation strategies in FHD. One such strategy involves the provision of specific patterns of afferent input through electrical stimulation or sensorimotor retraining. Indeed, several studies have examined the effect of manipulating afferent input on sensorimotor reorganisation and symptom alleviation in FHD. For example, in healthy subjects muscle vibration produces a reduction in cortical inhibition for the M1 representation projecting to the motor neurons of the stimulated muscle (Rosenkranz and Rothwell 2003). Conversely, adjacent, non-vibrated muscles exhibit an increase in cortical inhibition (Rosenkranz and Rothwell 2003). In FHD the topographic specificity of this effect is disturbed (Rosenkranz et al. 2005). Interestingly, Rosenkranz and colleagues (2008) demonstrated that a period of vibratory proprioceptive training could normalise the response to muscle vibration in participants with musician's dystonia, but not those with writer's cramp. Tinazzi and colleagues (2006) reported that MEP amplitudes are reduced in normal subjects after a single session of transcutaneous electrical nerve stimulation (TENS) but are unchanged in patients with FHD. However, if TENS is applied repeatedly, FHD subjects demonstrate MEP suppression which is associated with an improvement in handwriting. These findings indicate that specific patterns of afferent stimulation which result in decreased cortical excitability may be important for functional improvements and symptom alleviation in those with FHD.

Several studies have also examined the effect of sensorimotor "retuning" and more complex paradigms such as sensory discrimination combined with fitness exercises on cortical reorganisation in FHD. Sensorimotor retuning, which involves splinting of the digits of the affected hand while one digit is systematically trained, has been examined in patients with both musicians dystonia and writer's cramp (Candia et al. 1999; 2003; Zeuner et al. 2005).

Using magnetoencephalography and transcranial magnetic stimulation these studies demonstrated increased distances between cortical representations of the digits of the affected hand and a more orderly representation within the cortex. Subjective improvements in performance and function and objective enhancement of movement smoothness and handwriting were also reported. Byl and McKenzie (2000) developed a more complex training program which combined sensory discrimination training with general fitness exercises. The authors reported improvements in motor control, motor accuracy, sensory discrimination and physical performance following training. These results indicate that an intervention strategy based on the principles of neuroplasticity may improve cortical structure and clinical function in FHD.

It has recently been demonstrated that it is possible to induce plastic changes in the motor cortex similar to those seen during sensorimotor training by applying specific patterns of afferent stimulation (Ridding and Uy 2003). This stimulation paradigm involves the use of synchronous and convergent stimuli from two hand muscles ("associative stimulation"). However, if stimuli are given asynchronously ("non associative stimulation"), organisational changes are not induced in healthy subjects. Associative input is also characteristic of that seen during the performance of highly-trained stereotyped motor tasks and is thought to contribute to the maladaptive plasticity seen in FHD. Based on this, one strategy to re-establish discrete representational zones in FHD, which appears vital for the skilled performance of complex tasks, may be to provide independent input from individual muscles. Such a hypothesis is supported by the finding that reducing correlated input from adjacent digits, by surgical separation of syndactyly, produces separation of digital cortical representations (Mogilner et al. 1993). Therefore, it seems feasible that asynchronous, and non-associative, stimulation of hand muscles may temporarily reverse representational changes characteristic of FHD. In experiments described in Chapter 5, I investigated the

therapeutic potential of this approach in subjects with musician's dystonia and writer's cramp and compared these results with those of a healthy control group. Cortical reorganisation was examined using the technique of TMS mapping. Improvements in function were investigated using the grip-lift task and a kinematic evaluation of handwriting. The results of these experiments are detailed in Chapter 5.

1.7.3. Investigation of function in focal hand dystonia

Deficits in movement prevention and control have been extensively investigated using both the grip-lift task and kinematic analysis of handwriting in FHD. In the grip-lift task, patients with FHD employ an increased safety margin between the weight of the object and the GF applied to lift and hold the load. In fact, the average GF used by dystonic subjects is elevated by an average of 4 N when compared with healthy controls (Odergren et al. 1996; Serrien et al. 2000; Nowak et al. 2005b). The reason for this increase was initially thought to be due to impairments in sensorimotor integration (Odergren et al. 1996; Serrien et al. 2000). However, visual feedback training is successful in reducing GF levels in writer's cramp (Schenk and Mai 2001) suggesting that excessive GF may be a learned, compensatory strategy (Nowak et al. 2005b). Interestingly, anticipatory GF scaling is preserved in FHD, indicating that the underlying pathophysiology does not interfere with the generation or execution of the feed-forward model (Odergren et al. 1996; Nowak et al. 2005b). Deficits in other parameters of the grip-lift task, such as the preload duration or the correlation of GF and LF have not been demonstrated.

The most consistent deficit in handwriting among FHD patients is a decrease in the stroke frequency (Zeuner et al. 2007). A reduction in this parameter indicates significant impairment in movement fluency and automaticity (Zeuner et al. 2007). Abnormalities in vertical pen pressure have also been reported (Siebner et al. 1999b; Zeuner et al. 2007). Siebner and

colleagues (1999b) used kinematic analysis of handwriting as a measure of function before and after 1 Hz rTMS in dystonic subjects. The authors demonstrated an improvement in mean vertical writing pressure following the intervention. This result suggests that the kinematic analysis of handwriting is a sensitive measure of functional change in FHD. Due to the wealth of evidence already accumulated in FHD using the grip-lift task and handwriting analyses, these measures were chosen to examine the induction of plasticity in Chapter 5.

1.8. SUMMARY

Dextrous movement control is an intricate and complex skill. A large number of cortical regions are required for accurate movement execution including M1, S1 and the premotor areas. The cellular and functional diversity of this system provides an excellent framework for the induction of plasticity in both healthy individuals and in those with neurological conditions. This system is susceptible to repetitive, stereotypical afferent inputs however, and in those with FHD these aberrant inputs are thought to drive maladaptive plasticity, leading to impaired movement control and diminished task performance. As cortical plasticity is known to be driven by afferent input, the addition of controlled afferent information may provide the basis for novel and effective treatment techniques in those with FHD.

Using the system for the neural control of movement and exploiting the principles of cortical plasticity, the following chapters describe a series of experiments designed to investigate the neurophysiological and functional correlates of cortical plasticity. Chapters 2 and 3 utilise rTMS and TBS paradigms to induce plasticity in M1, S1 and SMA during a grip-lift task. In Chapters 4 and 5, associative electrical stimulation is applied over M1 in healthy subjects, and then in those with FHD. In these chapters neurophysiological measures are made using the technique of TMS mapping and the functional correlates of plasticity are identified using the grip-lift task and, in those with FHD, a kinematic analysis of handwriting.

2. Role of the primary motor and sensory cortex in precision grasping

2.1. Abstract

Human precision grip requires precise scaling of the grip force to match the weight and frictional conditions of the object. The ability to produce an accurately scaled grip-force prior to lifting an object is thought to be the result of an internal feed-forward model. However, relatively little is known about the roles of various brain regions in the control of such precision grip-lift synergies. Here I investigate the role of the primary motor (M1) and sensory cortices (S1) during a grip-lift task using inhibitory transcranial magnetic theta burst stimulation (TBS). Fifteen healthy individuals received 40 s of either i) M1 TBS, ii) S1 TBS, or iii) sham stimulation. Following a 5-minute rest subjects lifted a manipulandum 5 times using a precision grip, or completed a simple reaction time task. Following S1 stimulation, the duration of the preload phase was significantly longer than following sham stimulation. Following M1 stimulation the temporal relationship between changes in grip and load force was altered, with changes in grip force coming to lag changes in load force. This result contrasts with that seen in the sham condition where changes in grip force preceded changes in load force. No significant difference was observed in the simple reaction task following either M1 or S1 stimulation. These results further quantify the contribution of the M1 to anticipatory GF scaling. In addition they provide the first evidence for the contribution of S1 to object manipulation, suggesting that sensory information is not necessary for optimal functioning of anticipatory control.

2.2. Introduction

The ability to lift and manipulate small objects using a precision grip requires precise scaling of the grip force (GF) to the weight and frictional conditions (load force, LF) of the object

(Johansson and Westling 1984; Flanagan and Johansson 2002). Accurate GF scaling prevents both slipping of the object through the fingers and excessive crushing forces (Westling and Johansson 1984). The close temporal coupling of GF to the LF is thought to be the product of an internal feed-forward model, which generates an appropriate GF in advance of both the lifting motion, and the availability of sensory feedback during the movement (Johansson and Westling 1987; Flanagan and Wing 1997; Flanagan and Johansson 2002). This model requires a memory of object properties obtained from previous experience, which is then used to estimate the load force required and to specify the motor commands using predicted sensory input (Flanagan and Wing 1997; Flanagan and Johansson 2002). When the lift begins, sensory feedback arising from the frictional conditions, object weight and speed of movement updates the internal model to optimise the forces exerted by the finger muscles. When an unfamiliar object is lifted repeatedly, the scaling of GF reaches its optimum after 1-3 lifts (Flanagan and Johansson 2002).

Repetitive transcranial magnetic stimulation (rTMS) can induce short-term changes in the excitability of circuits in a number of cortical regions, including the motor cortex (e.g. Muellbacher et al. 2000; Tsuji and Rothwell 2002; Siebner and Rothwell 2003; Quartarone et al. 2005) and the sensory cortex (Ogawa et al. 2004; Karim et al. 2006; Pleger et al. 2006). A recent rTMS paradigm employing low-intensity bursts of high-frequency stimulation, so called "theta burst stimulation" (TBS), can induce a lasting reduction in cortical excitability (Huang et al. 2005). Such inhibitory rTMS paradigms make it possible to induce temporary disruption of function in specific cortical regions and thereby to examine their role in the performance of various tasks (Chen et al. 1997; Muellbacher et al. 2000; 2002; Todd et al. 2006). This is evidenced by a number of studies which demonstrate deficits in behavioral tasks following inhibitory rTMS applied to a specific brain region (Chouinard et al. 2005; Nowak et al. 2005; Hutton and Weekes 2007).

The role of various cortical regions in the grasping and lifting of objects has been investigated with rTMS. For example, rTMS applied to the motor cortex after subjects had performed a number of precision lifts resulted in a significant increase in peak GFs in subsequent lifts, which suggests an important role for this cortical region in GF modulation (Nowak et al. 2005). Davare et al. (2007) have demonstrated similar findings in the anterior intraparietal region suggesting that GF modulation is processed in more than one cortical region. In addition, Chouinard et al. (2005) have recently reported that rTMS of the dorsal premotor cortex area disrupts the ability of subjects to use cued information in the planning of subsequent lifts. Evidence for a differential role of the ventral and dorsal premotor cortex in fingertip positioning and intrinsic muscle recruitment during object manipulation has also been demonstrated (Davare et al. 2006). Despite these findings, relatively little is known about the neural mechanisms underlying the internal model and the cortical regions involved in the control of the anticipatory command. Furthermore, the contribution of the sensory cortex and the way in which sensory information contributes to anticipatory control remains unclear. It seems likely that different aspects of the grip-lift task are reliant upon different cortical regions for their accurate execution.

Inhibitory TBS applied to the motor cortex has also been shown to delay simple reaction times in normal subjects (Huang et al. 2005). The grip-and-lift task has some of the characteristics of a reaction time task, where contact of the fingers with the object could be considered as the stimulus, time from first contact to lift initiation (preload duration) as the reaction time, and the lift as the reaction-time response which begins about 150 ms later. Some support for this hypothesis is provided by corresponding changes in preload duration and reaction time in several clinical situations. For example, both preload duration and reaction time increase in stroke patients (Nowak et al. 2003; Hermsdorfer et al. 2003; McDonnell et al. 2006; Miscio et al. 2006) and in Parkinsonian patients (Müller and Abbs,

1990; Gauntlett-Gilbert and Brown 1998). Longer preload durations are also found in very young children (Flanagan and Johansson 2002) who are known to have long (and variable) reaction times (Surwillo 1972; 1974). An understanding of the mechanism underlying the preload duration would further assist us in determining the contribution of sensory information to anticipatory GF control.

Thus, in the present study I sought firstly, to examine the temporal parameters relating to anticipatory control following TBS applied to the primary motor (M1) and sensory cortices (S1) and secondly to determine whether the preload duration in the grip-lift task is analogous to a simple reaction time task.

2.3. Methods

2.3.1. Subjects

Fifteen healthy subjects (8 males, 7 females, age 28 ± 12 years mean ± SD, range 18-59 years) participated in the study. All subjects were right-hand dominant as assessed by the Edinburgh Oldfield Handedness Inventory (Oldfield 1971). The University of Adelaide Human Research Ethics Committee approved the protocols and all subjects gave written, informed consent in line with the declaration of Helsinki. In addition, all subjects successfully completed a TMS safety screen before participating (Keel et al. 2001).

2.3.2. Grip-lift manipulandum

GF, LF and vertical acceleration were measured using a custom-built manipulandum based upon the design originally described by Westling and Johansson (1984). This device, weighing 350 g, was comprised of two lightweight load cells that measured the horizontal

grip force (GF) exerted by the index finger and thumb on two polished brass surfaces 35 mm apart, and the vertical load force (LF). In addition, an accelerometer attached to the manipulandum signalled the onset of the lift. All GF, LF and acceleration signals were low-pass filtered at 100 Hz, sampled at 400 Hz and stored on a computer for off-line analysis.

2.3.3. Electromyographic (EMG) recording

The surface EMG of the first dorsal interosseous muscle (FDI) of the right hand was recorded with surface silver/silver chloride electrodes. FDI was chosen as this muscle is directly involved in generating the precision grip. One electrode was placed over the muscle belly and the other electrode over the metacarpophalangeal joint of the index finger. Responses were amplified 1000x and sampled at 5 kHz in the bandwidth 20-1000 Hz.

2.3.4. Transcranial magnetic stimulation (TMS)

Focal TMS was applied to the motor cortex using a Magstim Rapid Stimulator and a figure-of-eight coil (Magstim Co. Dyfed, UK, external wing diameter 9 cm). The coil was orientated over the left hemisphere with the handle of the coil pointing posteriorly and angled at approximately 45° to the sagittal plane. The optimal scalp site for evoking motor evoked potentials (MEPs) in the relaxed FDI was determined for each subject with the stimulator in single pulse mode, and this point was marked on the scalp. In addition, active motor threshold was determined during a voluntary contraction of FDI (approximately 20% of maximal voluntary force). Active motor threshold was defined as the stimulator intensity at which 5 of 10 single-pulse TMS applied at the optimal scalp site evoked a MEP in active FDI of approximately 100 μV peak-to-peak in amplitude.

2.3.5. Theta burst stimulation (TBS)

Each subject received TBS on three separate occasions at least three days apart. Stimulation consisted of either M1 stimulation, S1 stimulation or sham stimulation. The order of the three stimulus conditions was randomised between subjects. For S1 stimulation, the coil was positioned 2 cm posterior to the optimal motor site for evoking MEPs in the FDI with the coil oriented as for M1 stimulation (Maldjian et al. 1999; Ragert et al. 2003). Sham stimulation was performed using a sham rTMS coil (placebo coil PN 3285-00, The Magstim Co. Ltd, Dyfed, UK) positioned and oriented as for M1 stimulation. The sham coil produces an auditory stimulus similar to that produced by real stimulation but does not result in stimulation of the brain.

The TBS protocol consisted of bursts of 3 stimuli at 50 Hz repeated every 200 ms for a period of 40 seconds (Huang et al. 2005; Nowak et al. 2005) at an intensity of 80% of active motor threshold. In Huang et al.'s (2005) nomenclature, this is described as continuous theta burst stimulation (cTBS600). At this intensity, TBS did not evoke MEPs in either the M1 or S1 conditions.

2.3.6. Experimental procedure

Subjects washed their hands thoroughly with soap and water and sat at a low table. Prior to lifting the manipulandum, each subject was asked to position their forearm and hand in a comfortable position to lift the device. The manipulandum was then positioned for each subject to ensure shoulder and wrist joints remained in a neutral position throughout the lift so that the lift was performed primarily by elbow flexion. The manipulandum was not lifted prior to TBS. Each subject received 40 s of motor, sensory or sham TBS, after which they were instructed to rest, keeping the right hand still for the following 5 minutes.

Following this rest period, subjects were instructed to lift the manipulandum to the height indicated (10 cm), hold it steady for 3 s and then lower it to its original position on the table. Each subject performed 5 consecutive lifts, 5 s apart. It has been shown previously that a single trial is sufficient to update the internal model and achieve an optimal GF/LF ratio when lifting the same load repeatedly (e.g., Johansson and Westling 1988 ; Flanagan and Johansson 2002; Huang et al. 2005), thus 5 lifts is sufficient to examine the effect of TBS stimulation on the parameters involved in the grip-lift task. This protocol was followed on three separate occasions, with one of the three stimulation conditions being used on each experimental day.

2.3.7. Simple reaction time studies

Eight of the same 15 subjects (4 males, 4 females, age 29 ± 12 years mean ± SD, range 19-49 years) participated in a simple reaction time task on a further three separate occasions. The experimental protocol including the 3 different TBS protocols (M1 TBS, S1 TBS or sham stimulation) was essentially the same, except that the task was to grasp the manipulandum with as much force as they thought necessary to lift it, and then to lift it as soon as possible after receiving the "Go" signal. An auditory warning signal was delivered at random intervals (between 4-6 seconds) through headphones. The "Go" signal was an innocuous electrical stimulus delivered to the ipsilateral index finger that was given at varying intervals of between 500 ms and 1 s following the warning signal. Fifty trials, 10 blocks of 10 lifts with a 1 minute rest in between each block were collected for each subject. Simple reaction time was defined as the time from the "go" signal to when the load force first became positive for each trial.

2.3.8 Data analysis - main experiments

The following parameters of the grip-lift task were measured in each trial: preload duration which is the time between the onset of GF and the onset of positive LF as the manipulandum was lifted from the table (T1-T0, Figure 2.1) and GF_{max} (T2, Figure 2.1).

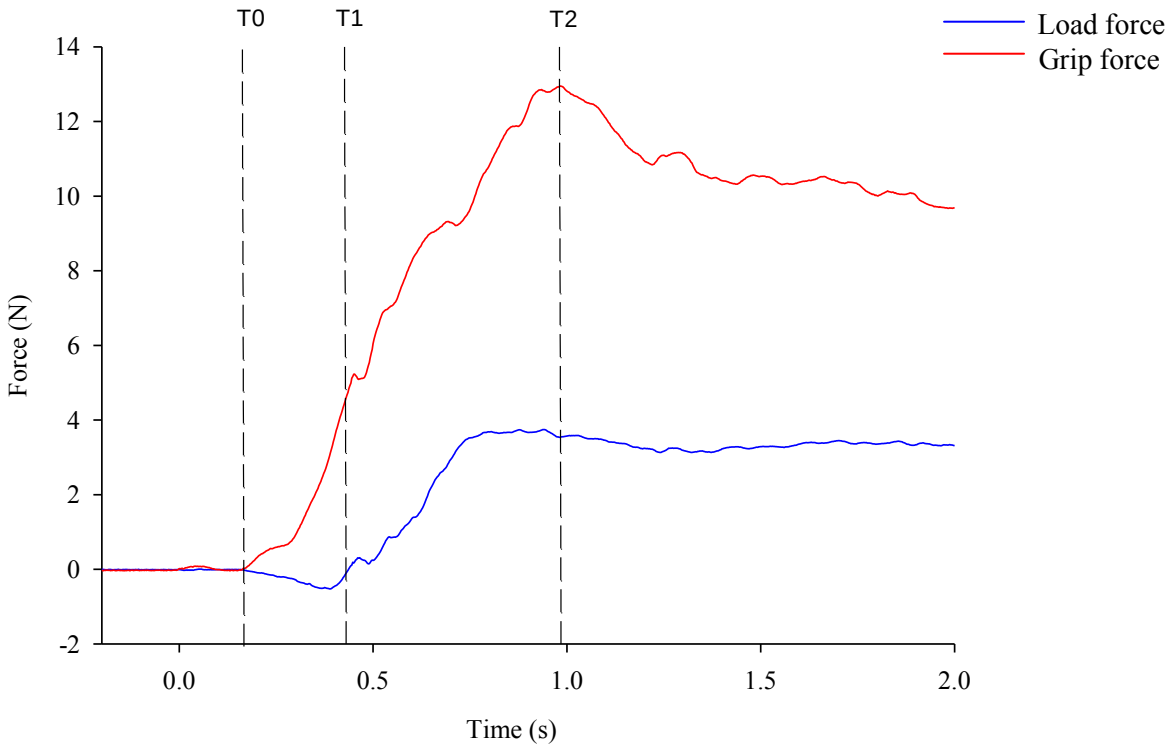


Figure 2.1 *Example of grip force and load force traces obtained during a single lift under sham stimulation. The lines show the different parameters of the grip-lift task that were analysed, namely preload phase (T1-T0) and GF_{max} (T2). The GO signal equates with time zero.*

In addition, the rate of change of GF (dGF/dt) in the lift phase (which spans a period of approximately 0.3 s before and after lift-off) was correlated with the rate of change of LF (dLF/dt) at each of a series of time points when one signal was shifted in increments of 2 ms with respect to the other (Duque et al. 2003; Mcdonnell et al. 2005). The time-shift at which

the correlation was maximal ($Timeshift_{max}$) and the value of the maximal correlation were then noted for each individual trial. This process determines the delay between the rate of change of GF and LF at the point where the correlation is maximal during the lift phase. A positive value indicates that changes in GF lead changes in LF, whereas a negative value indicates that changes in GF trail changes in LF.

Statistical analysis was performed using a two-way repeated-measures analysis of variance (ANOVA) on each of the above parameters. Data which did not follow a normal distribution was transformed using a logarithmic transformation. The between-subjects factor was stimulation condition (M1, S1 or sham) and the within-subjects factor was the lift number (1–5). *Post-hoc* analyses were performed using Bonferroni correction.

2.3.9. Data analysis – simple reaction time studies

Simple reaction time data were averaged for each block of 10 trials. Individual trials were excluded if they fell more than 2 standard deviations outside the mean (Seitz and Rakerd 1997). A two-way ANOVA with the between-subjects factor stimulation condition (M1, S1 or sham) and the within-subjects factor trial block number (1-5) was performed. A $p < 0.05$ was considered statistically significant.

2.4. Results

All group data are expressed as mean ± standard deviation. Mean values obtained for each of the grip-lift parameters under each of the stimulation conditions are presented in Table 2.1. No significant difference was observed in the maximal correlation parameter with respect to TBS condition or lift number. There was no significant condition/lift interaction present.

Table 2.1 *Mean ± SD values for each of the grip-lift parameters analysed under each stimulation condition.*

	Sham stimulation	Motor cortex TBS	Sensory cortex TBS
Preload duration (ms)	150 ± 14	169 ± 14	226 ± 18*
GF_{max} (N)	6.5 ± 3.7	6.8 ± 4.1	7.6 ± 5.2
Maximal correlation (r)	0.78 ± 0.12	0.76 ± 0.12	0.78 ± 0.1
$Timeshift_{max}$ (ms)	20 ± 7	-20 ± 7*	8 ± 6

2.4.1 Optimisation of GF_{max} following repeated lifts

There was a significant main effect of lift number on GF_{max} (ANOVA, $F_{4,112} = 25.6$; $p < 0.001$), across all conditions. Significant differences were observed between the first and second lifts and all subsequent lifts in a given run of 5 trials (lifts 1 vs 3; t = 6.8, $p < 0.001$, lifts 1 vs 4; t = 7.9, $p < 0.001$, lifts 1 vs 5; t = 9.2, $p < 0.001$).

2.4.2 Effect of TBS on preload duration

There was no significant condition/lift interaction (ANOVA, $F_{8,112} = 0.9$; $p = 0.5$) and no significant effect of lift number on preload duration (ANOVA, $F_{4,112} = 2.3$; $p = 0.07$). Therefore, data from each of the 5 lifts were combined for further analysis. There was a significant effect of stimulation condition on preload duration (ANOVA, $F_{2,112} = 6.5$; $p = 0.005$). *Post-hoc* testing revealed a significant increase in preload duration following S1 stimulation (226 ± 18 ms) when compared with both M1 stimulation (t = 2.4, $p = 0.023$; 169 ± 14 ms) and sham stimulation (t = 3.5, $p = 0.001$; 150 ± 14 ms, Figure 2.2). There was no significant difference between sham stimulation and M1 stimulation (t = 1.1, $p = 0.28$).

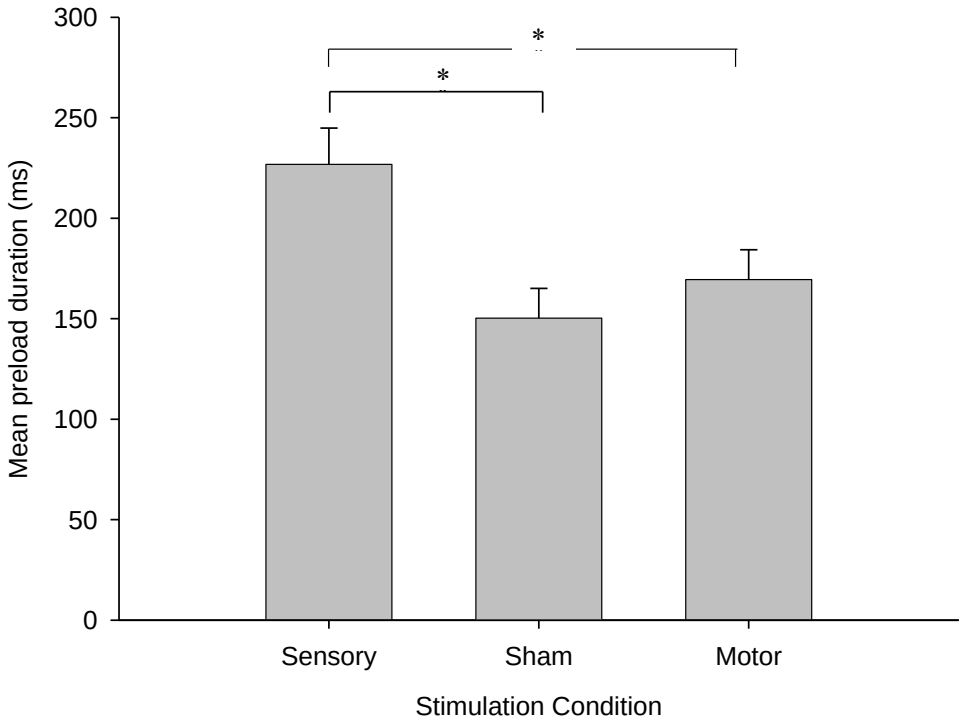


Figure 2.2 *The mean preload duration (+ SD) recorded following TBS applied to the motor cortex, sensory cortex or following sham stimulation. Mean preload duration following stimulation of the sensory cortex was significantly greater than following sham stimulation (* $p < 0.05$). There was no significant difference in the preload duration between motor cortex stimulation and sham stimulation conditions.*

2.4.3. Effect of TBS on $Timeshift_{max}$

There was no significant condition/lift interaction (ANOVA, $F_{8,112} = 1.2$; $p = 0.3$) and no significant main effect of lift number on $Timeshift_{max}$ (ANOVA, $F_{4,112} = 0.3$; $p = 0.8$). Therefore, data from each of the five lifts were combined for further analysis. There was a significant effect of stimulation condition on $Timeshift_{max}$ (ANOVA, $F_{2,112} = 4.1$; $p = 0.027$). Figure 2.3 shows the average time-shift under each stimulation condition when the GF and LF signals were maximally correlated.

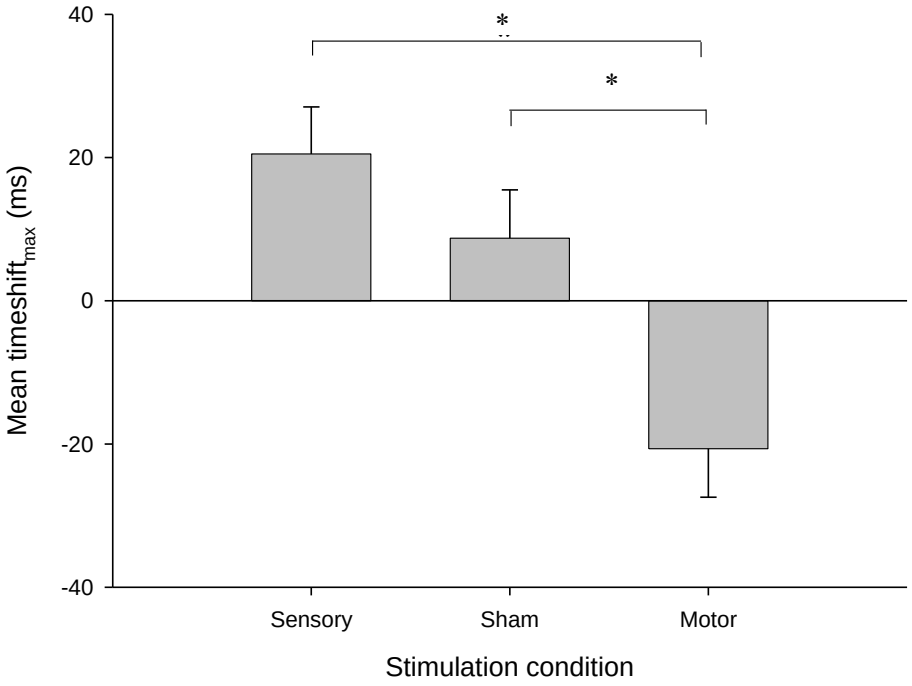


Figure 2.3 *Mean Timeshift$_{max}$ data (+ SD) following TBS of the motor cortex, sensory cortex or sham stimulation. The Timeshift$_{max}$ data following motor cortex stimulation was significantly different to that seen following sham stimulation (*p < 0.05). Timeshift$_{max}$ values were not significantly different between sensory cortex stimulation and sham stimulation conditions.*

Following M1 stimulation, changes in GF lagged changes in LF by -20 ± 7 ms. This is in contrast to results seen in the S1 condition where changes in GF preceded changes in LF by 20 ± 7 ms ($t = 2.8$, $p = 0.008$) and results seen in the sham condition where changes in GF preceded changes in LF by 8 ± 6 ms ($t = 1.9$, $p = 0.045$). Data from a representative subject is shown in figure 2.4. There was no significant difference between the values of Timeshift$_{max}$ following S1 stimulation and sham stimulation ($t = 0.9$, $p = 0.3$).

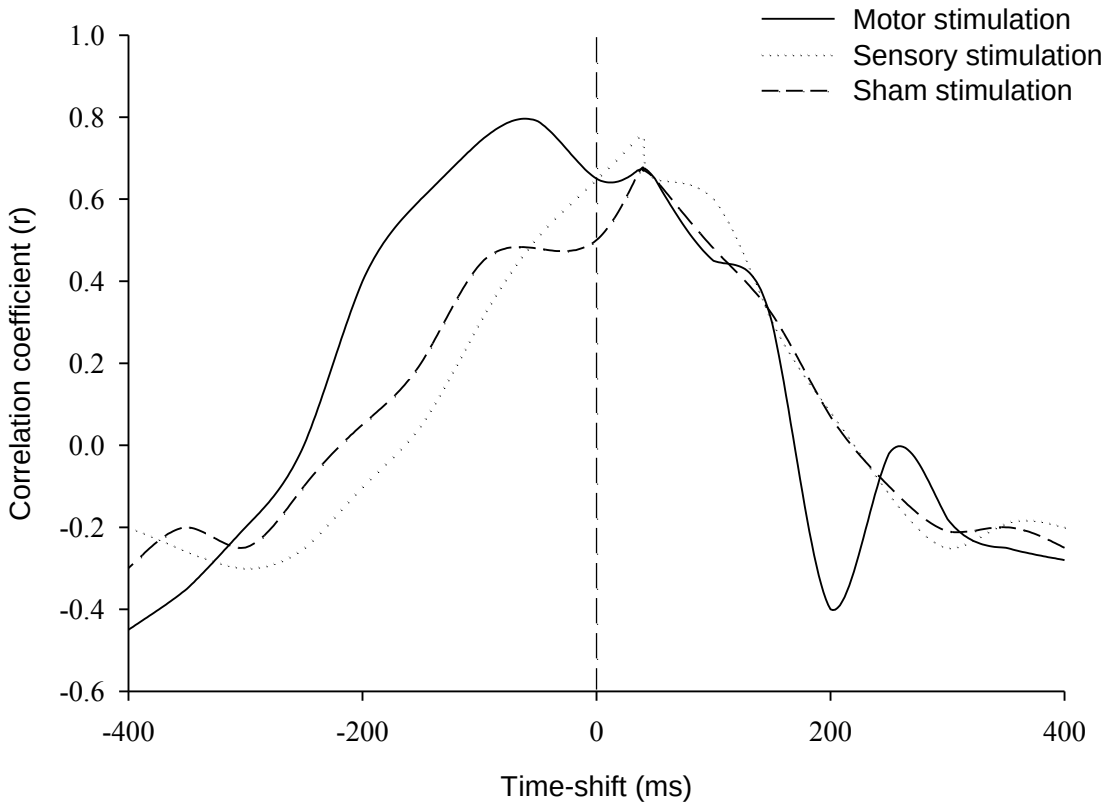


Figure 2.4 *Representative data from one subject demonstrating the time-shift at the point of maximal correlation following TBS of the motor cortex, sensory cortex or sham stimulation. Data is for the mean of 5 trials. There was a negative time-shift following motor cortex stimulation which is indicative of a more reactive grip strategy. Sensory stimulation and sham stimulation produced positive time-shifts indicative of anticipatory grip strategies.*

2.4.4. Effect of TBS on a simple reaction time task

There was no significant effect of stimulation condition or trial block number on simple reaction time (sham stimulation 193 ± 52 ms, motor cortex 235 ± 100 ms; sensory cortex 214 ± 68 ms, ANOVA, $F_{2,105} = 2.3$; $p = 0.1$).

2.5. Discussion

The present study provides evidence for the differential contribution of the motor and sensory cortices to parameters of the grip-lift task and in particular, to control of the anticipatory command. Disruption to the motor cortex produced a clear deficit in normal anticipatory control of GF scaling. Conversely anticipatory control was maintained following disruption to the sensory cortex, confirming the hypothesis that anticipatory scaling is the product of an internal model located elsewhere in the cortex. Interestingly, the preservation of anticipatory control following S1 stimulation suggests that sensory information is not required for the internal model to function accurately.

2.5.1. Mechanisms of action of TBS

Previous studies have shown that TBS, as used in the present study, depresses motor cortical excitability for a period of approximately 30 minutes (Huang et al. 2005). Huang et al. (2007) recently demonstrated that Memantine, a *N*-methyl-d-aspartate (NMDA) receptor antagonist, blocks the inhibitory effect of TBS, suggesting that the effects of TBS may be due to long-term depression (LTD) like effects within the motor cortex. A number of rTMS protocols have been shown to have qualitatively similar effects in a number of non-motor cortical regions as they do in motor areas (Boroojerdi et al. 2000; Knecht et al. 2003). It is therefore likely that TBS resulted in a similar inhibitory effect in the M1 and S1 cortical regions in the present study. The finding of clear and differential effects of M1 and S1 stimulation on various components of the grip-lift task (described below) supports this conclusion.

2.5.2. Optimisation of GF_{max}

The close temporal coupling of GF to LF is responsible for the idea that the grip-lift movement is the output of a feedforward motor plan that is based on earlier experiences. It is proposed that the sensory input produced by the contact of the fingertips with the manipulandum triggers the motor plan which then generates an appropriate GF before the lifting motion begins and hence before sensory feedback arising from the movement can begin (Flanagan and Wing 1997; Flanagan and Johansson 2002). Once the lift begins, signals are generated from sensory receptors because of the weight and frictional conditions of the object and the speed of the lift: these then modulate the output of the movement plan in a manner that optimises GF. This results in rapid optimisation of grip force across a series of lifts. In fact a single trial is sufficient to update the internal model and achieve optimal GF scaling when the same object is repeatedly lifted (e.g., Flanagan and Johansson 2002). In the present study the maximal GF (GF_{max}) was significantly higher in the first lift than in all subsequent lifts under all conditions; a finding consistent with this previous report of rapid grip optimisation.

2.5.3. Effect of TBS on preload duration

Preload duration is the delay from when the fingers first touch the manipulandum until the time that the lift begins, and reflects the time needed to establish the grip and initiate the lift. In the present study the pre-load duration was 150 ± 14 ms in the sham stimulation condition. This is similar to the delays reported in most other studies in the normal hand (Duque et al. 2003; Nowak et al. 2003; Raghavan et al. 2006), although a delay as short as 80 ms was reported by Johansson and Westling (1984). When TBS was applied to the sensory cortex 5 minutes before the lift trials began, the preload duration was significantly increased when

compared to the sham stimulation condition. In contrast to this, the preload duration was not significantly different to the sham value following M1 TBS.

While reflecting on possible mechanisms for this change in preload duration following S1 stimulation, we considered the possibility that the sequence of events in the grip-lift task has elements that are analogous to a reaction time task. Perhaps, then, the TBS-induced increase in the preload duration was due to an increase in a reaction time response. To test this hypothesis we re-tested 8 of our original 15 subjects to determine whether the same TBS protocols induced an increase in simple reaction time when a weak electrocutaneous stimulus to the finger signalled that the subject should lift the manipulandum as quickly as possible. If the central process underlying the delay between finger contact and lift onset in the manipulandum task was similar to the central processing in a reaction time scenario, I would have expected to see the same pattern of change induced by TBS in the reaction time experiments. However, the observation that simple reaction time does not react in the same way to TBS as preload duration leads to the conclusion that preload duration in the grip-lift task is a more complex variable that is not simply the result of a simple reaction time delay. This suggests that preload duration is, instead, dictated by other factors such as engagement of the movement plan.

Thus, the increase in preload duration induced by S1 TBS is likely to be the result of a more general interference with the processing of the sensory signals from the fingertips during the grip-lift task (Westling and Johansson 1987). Fast adapting type II afferents are thought to play an important role in triggering the descending motor commands required for subsequent movement phases during the grip-lift task. Westling and Johansson (1987) demonstrated that type II afferents respond with distinctive firing rates to signal initial object contact, the moment of lift off and terminal contact as the object is replaced on the table. The absence of

these signals following digital anaesthesia has been shown to delay the development of accurate lifting forces (Johansson and Westling 1984). Furthermore, Collins et al. (1999) observed that successive phases of the movement plan failed to be executed when contact signals from the fingertips were absent. Thus, one explanation for the significant increase in preload duration induced by S1 TBS is that this intervention delays the integration of the sensory signal arising from first contact with the object into the motor plan. Disruption to the integration of this signal would feasibly result in a delay in the readout of the motor plan for the next phase of the task, namely the lifting phase, resulting in an increase in the time between first contact with the object and the onset of lift.

2.5.4. Effect of TBS on $Timeshift_{max}$

The temporal relationship between changes in GF and LF indicated that with sham stimulation changes in GF precede changes in LF. Similar observations have been made in earlier studies in which this relationship was interpreted to mean that the strategy for the lift is normally based on a movement plan that anticipates the weight and frictional properties of the object (e.g., Johansson and Westling 1987; Johansson and Westling 1988; Flanagan and Wing 1997). It has been proposed that such feed-forward control utilises an internal model containing a memory of the properties of familiar objects (Johansson and Westling 1988).

Once the appropriate internal model is selected, predicted sensory inputs are used to establish motor commands prior to the commencement of the task, thereby giving anticipatory control of GF (Flanagan and Wing 1997; Flanagan and Johansson 2002). When the movement begins, real sensory input from cutaneous mechanoreceptors is compared with predicted sensory information and the internal model is updated and executed accordingly (Johansson and Westling 1987). Anticipatory GF scaling is maintained in healthy subjects during digital

anaesthesia and in patients with peripheral nerve damage, which indicates that sensory signals from feedback circuits are not directly involved in anticipatory control (Nowak et al. 2001; 2003).

However, in the present study TBS applied to M1 resulted in a change in the overall timing of changes in GF relative to changes in LF. While GF obviously still begins before LF increases, the net relationship of these two variables across the whole lift phase changed so that instead of leading changes in LF, changes in GF now lagged it overall (Figure 2.3, Figure 2.4). The implication is that M1 TBS subtly changed the grip strategy during the lift phase. Direct disruption to the internal model with M1 TBS is one possible mechanism behind the shift towards a more reactive grip strategy. This would indicate that the internal model is located at the level of the cortex. However, a number of studies demonstrating pathological deficits in GF scaling conclude that the position of the internal model lies below the level of the cortex (Flanagan and Wing 1997). For example, subjects with cortical lesions such as stroke, show only minor deficits in anticipatory GF scaling, suggesting that feed-forward control mechanisms remain intact with direct cortical lesions (Nowak et al. 2003; Hermsdorfer et al. 2003). Data from patients with Parkinson's disease demonstrates a similar pattern, with subjects consistently able to accurately scale their GF in response to fluctuating LFs (Muller and Abbs 1990). Furthermore, anticipatory GF scaling has been shown to be disrupted in those with cerebellar lesions suggesting that the internal model may be localised in the cerebellum (Nowak et al. 2002). This hypothesis is further supported by data from the present study which demonstrate that S1 TBS does not affect those parameters of the grip-lift task involved in anticipatory GF control. Thus, it seems unlikely that the shift towards a reactive grip strategy is due to a disruption of the internal model itself.

One alternative explanation for the shift towards a reactive grip strategy is as follows. Clearly, there was still an anticipatory (feedforward) motor plan that directed the initiation of the GF and the initial phase of the lift with the normal preload delay after the TBS. However, during the course of the lift phase, the strategy for the lift changed so that it became more reactive; that is, as the lift phase progressed, changes in the GF began to lag changes in the LF. This could arise from an increased dependence of GF modulation on sensory feedback signalling object weight and frictional conditions. The utilisation of such sensory signals by supraspinal structures involves finite delays. For example, the minimal reflex loop time for the long-latency stretch reflex in the long thumb flexor is about 50 ms: this delay includes the conduction time from muscle spindles in the hand to the motor cortex and back to modulate the EMG in this muscle (Matthews et al. 1990; Wallace and Miles 1998). There is a further delay arising from electromechanical coupling before the GF can change. Thus a net change in the overall timing of dGF/dt to dLF/dt to a more reactive strategy following motor cortex stimulation could arise from a disturbance in the readout of the motor plan that led to an increased reliance on sensory signals.

Several studies have demonstrated disruption to predictive grip-force processing for a period of several minutes following a similar train of M1 rTMS (Nowak et al. 2005; Berner et al. 2007). These authors suggest that this is evidence for a role for the primary motor cortex in establishing sensorimotor memory related to the weight and frictional properties of the lifted object and in particular that the motor cortex is involved in the integration of these sensory signals with the descending motor command (Berner et al. 2007). However, as accurate grip-force scaling is not impaired in people with sensory deficits, it seems unlikely that the switch to a reactive grip force strategy following M1 TBS is purely the result of disruption to the integration of sensory signals (Nowak et al. 2001; 2003). Furthermore, the changes seen in motor output in this study were induced by M1 but not S1 TBS, which suggests that the

switch to a more reactive GF strategy may be the result of disruption to the normal descending motor command rather than to the integration of sensory signals arising from cutaneous mechanoreceptors. This disruption could feasibly occur at the level of the motor cortex where TBS is known to induce a decrease in cortical excitability or in the cortico-cerebellar circuitry between the internal model and the motor cortex. This hypothesis receives some support from data which suggests that exporting sensorimotor knowledge from the cerebellum to the motor cortex allows the more efficient execution of learned motor responses (Hua and Houk 1997).

Finally, it is interesting to compare these results to those obtained in subjects with congenital hemiplegia. Duque et al. (2003) demonstrated disruption in the temporal parameters of the grip-lift task (namely $timeshift_{max}$) in those with congenital hemiplegia. However, in contrast to the present study, subjects with congenital hemiplegia appeared to use significantly larger anticipatory strategies, demonstrated by an increase in $timeshift_{max}$ values (mean ± SD, 72 ± 39.1 ms), when compared with control subjects (24 ± 16.3 ms). While the reason for this discrepancy remains unclear it may be due to the presence of compensatory mechanisms in those with long standing congenital disorders when compared with the acute, temporary lesions induced in the present study.

2.6. Conclusion

Disruption of sensory cortex function by TBS increases the delay between initial contact with the object and the initiation of the lift (ie preload duration) in a simple grip lift task. One possible mechanism for this is that disrupting sensory cortex function delays the integration of sensory signals arising from contact with the object into the motor plan. In contrast, TBS of the M1 changes the grip strategy after grip onset in a manner that suggests that the role of

sensory signals in matching GF to LF in this task becomes more dominant. These novel findings provide further evidence of the specific contribution of M1 to anticipatory GF scaling. In addition I provide evidence for the contribution of the sensory cortex to object manipulation, suggesting that sensory information is not necessary for optimal functioning of anticipatory control but plays a key role in triggering subsequent phases of the motor plan.

3. The role of the supplementary motor area in grip force scaling during precision grasping

3.1. Abstract

Anticipatory scaling of grip force (GF) is critical for effective object manipulation. Recent imaging studies demonstrated activation in a large network of cortical areas, including the supplementary motor area (SMA), during precise GF scaling. However, the exact contribution of this particular area remains unclear. Here I examined the role of SMA in GF scaling during a precision grip and sought to determine the hemispheric lateralization and timing of the SMA contribution to the grip-lift task. To address these issues I induced virtual lesions of the left or right SMA in healthy subjects performing a standard grip-lift task. I found that a virtual lesion of the left SMA yielded an increase in peak GF and in the rate of GF generation, irrespective of the hand used to perform the task; left SMA lesions also decreased the preloading phase duration but only when the task was performed with the contralateral hand. Transcranial magnetic stimulation (TMS) applied over the right SMA did not affect peak GF and only led to a decrease in the preloading phase duration for contralateral hand movements. The present study suggests that left SMA makes a significant contribution to the control of GF scaling during object manipulation. In addition, I demonstrate that both left and right SMA contribute to the synchronization between the grip and loading phase of grasping movements. Thus, I provide evidence for the hemispheric lateralization of the dynamic and temporal aspects of the grip-lift task and provide further insight into the neural correlates of grasp control.

3.2. Introduction

Successful object manipulation is a skill integral to numerous human activities. In pathologies where precision grasping is impaired, individuals may apply excessive crushing forces or under grip the object, causing object damage or allowing the object to slip and fall through the

fingers (Nowak et al. 2003). In healthy individuals, the grip force (GF) applied to lift an object is accurately scaled to meet the weight and frictional properties of the object, even on the first lift (Flanagan and Wing 1997; Flanagan and Johansson 2002). The ability to anticipate accurately an object's weight and frictional properties prior to lifting is thought to be the product of an internal model. Such a model contains a representation of object properties built from prior experience, allowing the correct GF to be determined in advance of the lifting movement (Forssberg et al. 1992; Flanagan and Wing 1997; Flanagan and Johansson 2002).

While precise GF scaling is central to effective object manipulation, relatively little is known about the cortical regions contributing to its control. A recent study by Bursztyn and colleagues (2006) identified a number of cortical regions thought to be involved in anticipatory GF control. Using functional magnetic resonance imaging (fMRI), significant activity was demonstrated in the primary motor cortex (M1), primary sensory cortex (S1) and the supplementary motor area (SMA) during a grip-lift task. While the role of M1 and S1 have been further investigated using repetitive transcranial magnetic stimulation (rTMS) induced virtual lesions (Huang et al. 2005; Jenmalm et al. 2006; Davare et al. 2007; Schabrun et al. 2008), relatively little is known about the role of SMA in precision grasping. In non-human primates unilateral SMA ablation leads to an excessive increase in GF during object manipulation with the contralateral hand (Smith et al. 1981). In human subjects, functional imaging studies suggest that SMA may be important for grasp control (Bursztyn et al. 2006), particularly when fine fingertip forces are used to gently grip and manipulate an object (Kuhtz-Buschbeck et al. 2001). These findings suggest that SMA may play a key role in the control of fingertip scaling although its exact contribution remains unclear.

Thus, the aim of the present study was to investigate the role of SMA in GF scaling during a precision grip task. In addition, I aimed to determine the hemispheric lateralization of this processes. To address these issues I used rTMS to induce virtual lesions in right or left SMA in order to interfere transiently and reversibly with their functions. Such an approach allows the determination of a causal relationship between the activation of SMA shown by neuroimaging studies and its possible contribution to the different parameters of the precision grip task. Furthermore, I sought to determine the timing of the SMA contribution to grip force scaling using a paired pulse TMS technique.

3.3. Materials and methods

3.3.1. Subjects

Twelve healthy subjects (8 males, aged 27 ± 2.7 years, mean ± SD) participated in the study. Subjects had no history of neurological impairment and were assessed as right hand dominant using the Edinburgh Handedness Inventory (Oldfield 1971). Written, informed consent and the successful completion of a TMS safety screen (Keel et al. 2001) were obtained from each subject prior to participating. All experimental protocols were approved by the Ethics Committee of the Université catholique de Louvain.

3.3.2. Grip-lift manipulandum

Subjects grasped a 275 g manipulandum. This device comprised two 3D force-torque sensors (Mini 40 F/T transducers; ATI Industrial Automation, Garner, NC) covered by two vertical parallel grip surfaces (40 mm diameter, 30 mm apart). Three orthogonal forces (F_x, F_y and F_z) and three torques (T_x, T_y and T_z) were measured by each sensor. The vertical LF was then computed using the vectorial sum of F_x and F_y and the horizontal GF computed using F_z. Force signals were low-pass filtered at 15 Hz (fourth order, zero phase lag Butterworth filter)

and digitized on-line at 1 kHz (12-bit 6071E analogue to digital converted in a PXI chassis, National Instruments, Austin, TX, see Davare et al. 2006).

3.3.3. Transcranial magnetic stimulation (TMS)

TMS was delivered through a figure-of-eight coil connected to a rapid model 200 stimulator (Magstim, Whitland, UK). Single pulse TMS was initially delivered over M1 to determine the optimal scalp site for evoking motor evoked potentials (MEPs) in the First Dorsal Interoseous (FDI). The coil was held over the contralateral hemisphere, tangentially to the skull with the handle pointing laterally and backwards at an angle of about 45°. Resting motor threshold (rMT), defined as the minimum intensity necessary to evoke MEPs of 50 μV peak-to-peak amplitude in 5 out of 10 trials, was measured separately for right and left FDI. No statistically significant difference existed between the rMT determined for these two muscles ($t = 0.034$; $p = 0.97$; paired t-test).

3.3.4. Stimulation sites

Anatomically, SMA is located directly anterior to the M1 leg representation (He et al. 1995; Geyer et al. 2000). Thus, to target SMA the following procedure was used. First, the optimal scalp site for evoking MEPs in the Tibialis Anterior muscle (TA) was determined and a point 2 cm anterior measured and marked on the scalp (Steyvers et al. 2003). Second, the marked point was co-registered on individual anatomical magnetic resonance images for each subject. This technique has been described in detail previously (Noirhomme et al. 2004; Zosso et al. 2006; Davare et al. 2006). If necessary, the coil position was then adjusted slightly according to the MRI anatomical location of the SMA. This location was determined using the most medial part of the superior frontal gyrus, dorsal and anterior to the precentral gyrus (Picard and Strick 1996). The co-registration procedure was repeated for each subject prior to each experimental session and performed separately for the left and right SMA. At the end of the

experiments, the coordinates of stimulation sites were normalized a posteriori into the Montreal Neurological Institute (MNI) system and averaged across subjects. Here, the mean normalized MNI coordinates for left SMA stimulation were -6.6 ± 2.6, -6.5 ± 5.7, 74.1 ± 6.3 mm (x, y, z, mean ± SD) and for right SMA stimulation, 8.9 ± 3.6, -6.4 ± 4.8, 74.4 ± 6.8 mm (x, y, z, mean ± SD, Figure 3.1). These values correspond with those of activation foci reported in previous functional imaging studies investigating grasp (Ehrsson et al. 2000; Kuhtz-Buschbeck et al. 2001; Imamizu et al. 2004; Bursztyn et al. 2006).

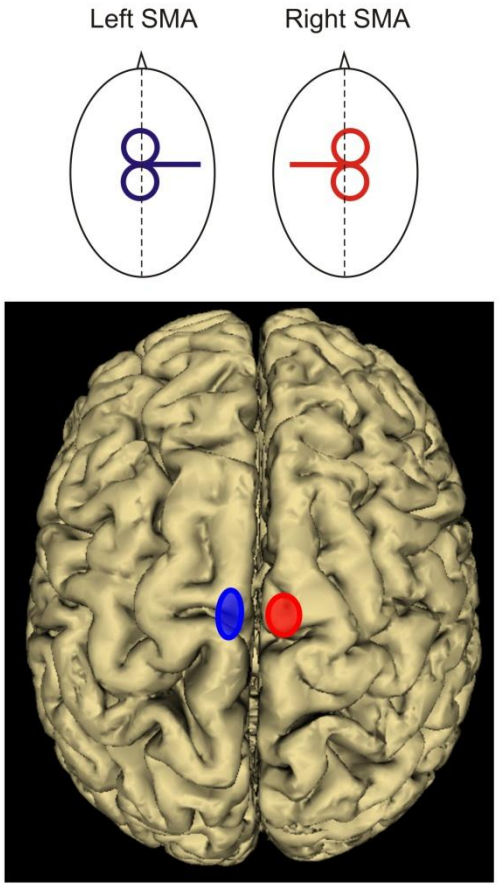


Figure 3.1 *Coil position for optimal stimulation of left and right SMA (top) and mean location of the stimulation points over left (blue; -6.6, -6.5, 74.1; x, y, z) and right (red; 8.9, -6.4, 74.4; x, y, z) SMA following normalization into the MNI coordinate system (bottom). The centre of each ellipse is located over the mean MNI coordinates of each stimulation site; the area of the ellipse indicates the 95 % confidence interval.*

3.3.5. Experiment 1 - rTMS experiment

Experiment 1 aimed to examine the role of SMA in GF scaling. Six subjects volunteered for this experiment (5 males, aged 27.8 ± 1.7 years, mean ± SD). Repetitive magnetic stimulation

(rTMS, 10 Hz, 5 pulses) was applied over either the right SMA, left SMA or with the coil held in a sham position. In order to target the SMA, the coil was orientated medio-laterally with the handle pointing towards the unstimulated hemisphere (e.g. handle pointing towards the right hemisphere for left SMA stimulation, See Figure 3.1). This orientation induced a current directed medio-laterally and has been shown to be optimal for stimulating the primary motor leg area (Priori et al. 1993). rTMS trains were separated by at least 12 s. Stimulation intensity was set at 120 % of FDI rMT. Before each experiment I checked that, at this intensity and with the coil in this position, rTMS applied over the SMA did not induce an electromyographic (EMG) response in either the ipsilateral or contralateral TA muscle, suggesting that the induced current did not spread to M1 leg area. All EMG recordings were made using silver/silver chloride surface electrodes positioned in a belly-tendon montage. Signals were amplified (CED 1902, Cambridge UK) and sampled at 5 kHz in the bandwidth 20-1000 Hz (CED, Micro 1401, Cambridge UK). In the sham condition the coil was orientated perpendicular to the scalp and positioned over either the right or left SMA.

Before each experiment, subjects washed their hands with soap and water and dried them thoroughly. An auditory GO signal indicated the beginning of the trial and was followed approximately 3 s later by a second auditory signal indicating the end of the trial. rTMS was delivered concurrently with the GO signal. Subjects were instructed to grip and lift the manipulandum using the minimum force necessary to prevent slips. The experiment consisted of 12 blocks of 12 trials. Six blocks were performed using the right hand and six using the left hand. For each hand, stimulation blocks consisted of (i) 2 blocks with rTMS delivered over left SMA, (ii) 2 blocks with rTMS delivered over right SMA and (iii) 2 blocks, 1 over each SMA, performed with the coil in a sham position. Block order was randomly determined. Between each trial subjects were asked to adopt a rest position with the hand resting on its

ulnar edge in a mid pro-supinated position and the index finger and thumb positioned approximately 4 cm apart from the manipulandum grip surfaces.

3.3.6. Experiment 2 - paired pulse TMS

In Experiment 2, I sought to determine the time course of the left SMA contribution to the GF scaling. Six subjects (3 males, aged 25.8 ± 3.3 years, mean ± SD) participated in this experiment (two having participated in Experiment 1). Paired pulse TMS was delivered using a bistim module (Magstim Company, Dyfed, UK) through one figure of eight coil. The coil position was determined using the co-registration technique described above. Intensity was set at 120 % of rMT for the right FDI. Paired pulse stimuli, separated by 5 ms, were delivered at seven different timings after the GO signal, namely 0, 50, 100, 150, 200, 250 and 300 ms. An eighth condition was a "no TMS" control condition.

The grip-lift task outlined above was performed in 8 blocks of 24 trials. Four blocks were performed using the right hand while paired pulse TMS was delivered over the left SMA at the eight different conditions (7 timings + 1 no-TMS). The remaining four blocks were performed using the left hand with paired pulse TMS delivered over right SMA. Three trials were performed at each TMS condition within each block. Thus, 12 trials per condition were available for analysis. TMS condition and block order were randomly determined.

3.3.7. Data analysis

Force signals were acquired from the ATI sensors and time locked with the GO signal. GF and LF onsets were defined as the time when the force value exceeded the mean ± 2 SD of the pre-movement value (Davare et al. 2006, 2007b). Reaction time was defined as the time between the GO signal and the onset of positive GF. The following temporal parameters of

the grip-lift task were measured for each trial (1) preloading phase duration, i.e. the time interval between the onset of GF and the onset of LF and (2) the maximum (peak) GF.

In addition, the maximum coefficient of correlation between dGF/dt and dLF/dt and the time shift that gave the maximum cross-correlation were calculated. These two values were gathered for each individual trial by computing a cross-correlation function between dGF/dt and dLF/dt and were used to provide an estimate of the overall synergy of the grip-lift movement (Duque et al. 2003). The maximum correlation coefficient permits us to quantify the similitude between the GF and LF traces, giving an indication of how accurately fingertip forces are scaled to the properties of the object. The time-shift provides a measure of the asynchrony between the two signals. A positive time-shift value indicates that GF leads LF.

3.3.8. Statistical analysis

In Experiment 1, statistical analysis was performed on all the above grip-lift parameters using a two-way analysis of variance (ANOVA) with factors CONDITION (left SMA, right SMA or sham) and HAND (left or right).

In Experiment 2 data were analysed using a two-way ANOVA with factors CONDITION (left SMA, right SMA) and TIMING (0, 50, 100, 150, 200, 250, 300, no TMS). Where appropriate, *post-hoc* analyses were performed using Bonferroni correction.

3.4. Results

3.4.1. Experiment 1 – role of left SMA in grip force scaling

A significant main effect of CONDITION on peak GF (ANOVA, $F_{2, 858} = 54.7$; $p < 0.001$) was present. *Post-hoc* analysis demonstrated that a virtual lesion of left SMA, regardless of the hand used to perform the task, led to a significant increase in peak GF of approximately 2N (21 %) when compared with both right SMA virtual lesions (Bonferroni, t = 7.8, $p < 0.001$) and the sham condition (Bonferroni, t = 9.9, $p < 0.001$; Figure 3.2).

A main effect of CONDITION was also present for the peak derivative of GF (ANOVA, $F_{2, 858} = 14.8$; $p < 0.001$). The peak derivative of GF was significantly greater following left SMA stimulation when compared with right SMA stimulation (Bonferroni, $t = 3.1$, $p = 0.007$) and the sham condition (Bonferroni, $t = 5.3$, $p < 0.001$).

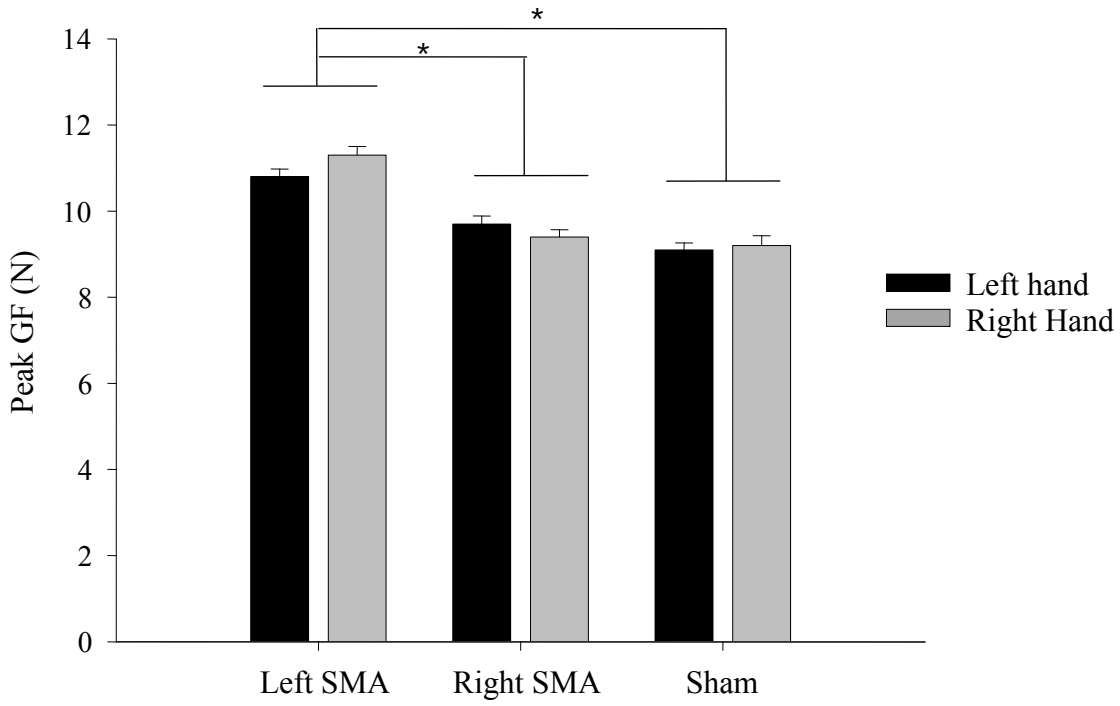


Figure 3.2 *Effect of a virtual lesion induced in left SMA, right SMA and under the sham condition on peak grip force (GF). A significant increase in peak GF was evident following left SMA stimulation, regardless of the hand used. There was no significant increase in peak GF following right SMA stimulation. * $p < 0.05$.*

In addition, a main effect of CONDITION was found for the maximal coefficient of correlation between dGF/dt and dLF/dt (ANOVA, $F_{2, 858} = 4.8$; $p = 0.008$). The maximal correlation was significantly decreased following left SMA stimulation when compared with both right SMA (Bonferroni, $t = 3.0$, $p = 0.007$) and sham (Bonferroni, $t = 2.9$, $p = 0.02$) stimulation. This effect was present regardless of the hand used. Values obtained for each movement parameter under each stimulation condition are provided in Table 3.1.

Table 3.1 *Mean ± SD values of movement parameters gathered under each stimulation condition in Experiment 1 during the grip-lift task. The task was performed with either the left (LH) or right hand (RH).*

	Sham		Left SMA		Right SMA	
	LH	RH	LH	RH	LH	RH
Peak GF (N)	9.1(1.9)	9.2(2.7)	10.8(2.2)*	11.3(2.4)*	9.7(2.3)	9.4(2.0)
dGF/dt peak (N/s)	69.5(13.3)	63.7(15.8)	80.1(13.2)*	83.9(15.5)*	76.9(18.9)	75.4(18.4)
Correlation coefficient	.85(.07)	.86(.05)	.83(.08)*	.83(.07)*	.84(.07)	.83(.08)
Time-shift (ms)	13.0(15.9)	15.9(25.6)	11.6(23.2)	16.6(19.9)	12.3(22.7)	14.6(26.1)
Preload duration (ms)	37.3(23.2)	37.2(32.0)	35.7(24.4)	27.1(18.6)*	26.9(26.6)*	34.2(24.7)

* $p < 0.05$ indicates statistical significance from control values.

3.4.2. Experiment 1 - Effect of a SMA virtual lesion on preload duration

A main effect of CONDITION (ANOVA, $F_{2, 858} = 5.9$; $p = 0.003$) and a CONDITION x HAND interaction (ANOVA, $F_{2, 858} = 7.1$; $p < 0.001$) were observed for the preload duration parameter. A significant difference was found between left SMA stimulation and sham stimulation (Bonferroni, $t = 3.1$, $p = 0.005$) and also between right SMA stimulation and sham stimulation (Bonferroni, $t = 2.7$, $p = 0.018$, Figure 3.3). Preload duration was significantly decreased following both stimulation conditions. However, this effect was only present when the contralateral hand was used to perform the task (left SMA, right hand > left hand, Bonferroni, $t = 2.4$, $p < 0.015$; right SMA, left hand > right hand, Bonferroni, $t = 2.8$, $p = 0.004$).

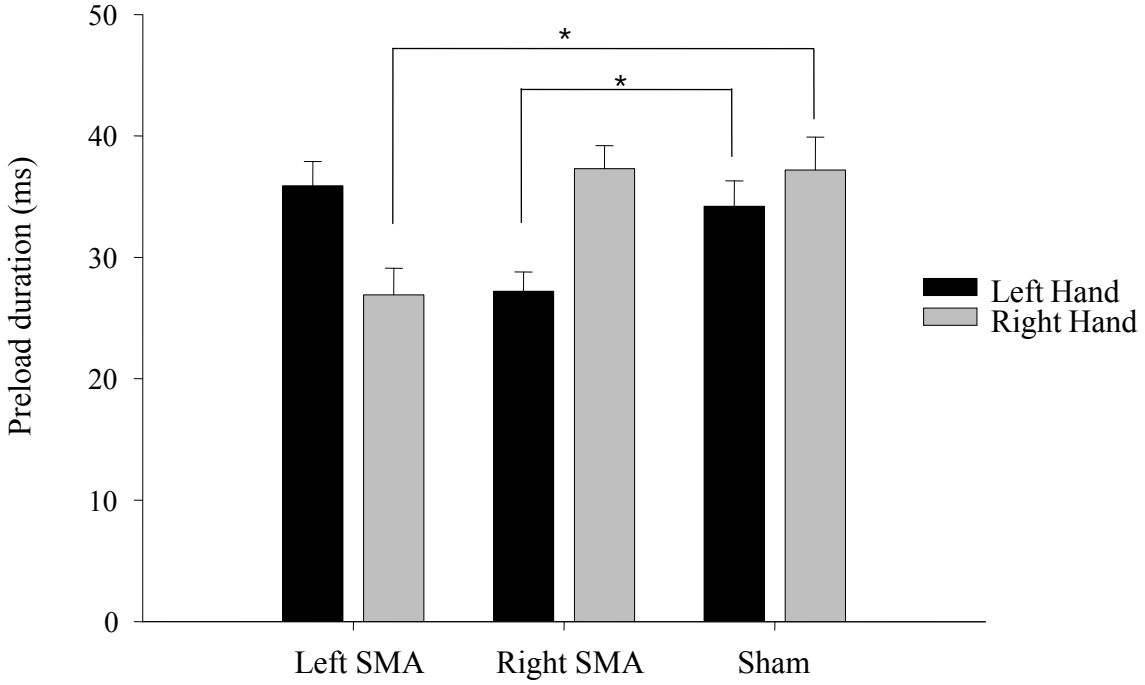


Figure 3.3 *Effect of a virtual lesion induced in left SMA, right SMA and under the sham condition on preload duration. Both left SMA and right SMA stimulation produced a significant decrease in preload duration, but only when the contralateral hand was used to complete the task. *p < 0.05.*

3.4.3. Experiment 2

In this experiment, I sought to determine the time course of the left SMA contribution to GF scaling. A significant CONDITION X TIMING interaction (ANOVA, $F_{7, 752} = 11.7$; $p < 0.001$) was present for peak GF. *Post-hoc* testing revealed that peak GF increased only when paired pulse TMS was delivered over the left SMA 200 - 250 ms after the GO signal (Bonferroni, $t = 6.1$, $p < 0.001$; Figure 3.4). Taking into account the average reaction time (385 ± 58.5 ms, mean ± SD), this indicates that left SMA makes a significant contribution to grip force scaling approximately 180-130 ms before initial contact is made with the object.

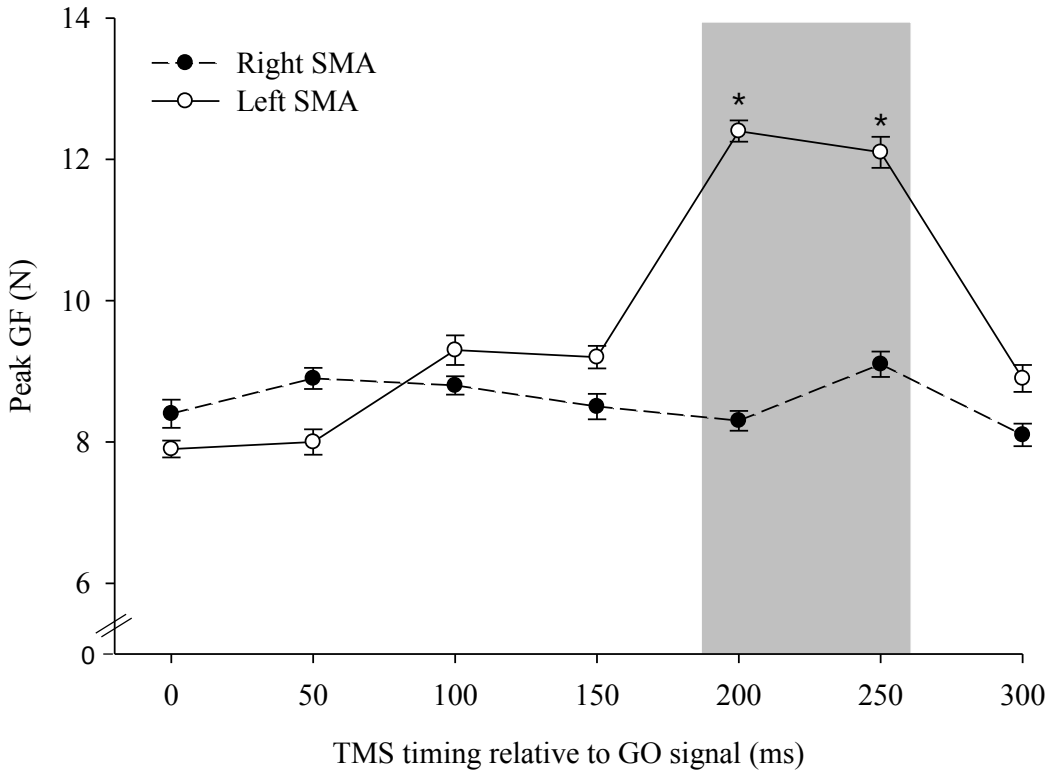


Figure 3.4 *Time course of the SMA contribution to GF scaling. Circles represent the mean (± SE) of the peak GF obtained at each timing. Peak GF increased significantly when rTMS was applied over left SMA between 200 and 250 ms after the GO signal. No increase was seen in peak GF following rTMS applied over right SMA (*p < 0.05).*

3.5. Discussion

The present study demonstrates, for the first time, a distinct role for left SMA in anticipatory control of GF scaling. Following a virtual lesion of left SMA I noted a significant increase in peak GF and in the rate of GF generation, leading to a significant decrease in the maximal correlation between the GF and LF signals, regardless of the hand used to perform the task. Furthermore, I demonstrate a role for both left and right SMA in the coordination of GF and LF during the preloading phase, but only when the movement was performed with the contralateral hand. These findings suggest that left SMA is dominant in controlling the

dynamic components of the grip-lift task, while both left and right SMA play a role in controlling the tight sequencing of the grip and lift phases in the contralateral hand.

The increased GF consequent to virtual lesions of left SMA could result either from an overestimate of the weight, or from an underestimate of the coefficient of friction of the object, leading to the application of GFs in excess of those required for efficient completion of the task. This effect was present regardless of the hand used to complete the task. Interestingly, the errors produced in GF scaling following left SMA disruption mimic those seen following stroke, where patients have been shown to apply GFs which are significantly greater than those applied by healthy controls (Hermsdorfer et al. 2003; Nowak et al. 2003). Similar deficits in GF scaling have also been demonstrated following rTMS induced virtual lesions of the left anterior intraparietal area (AIP; Davare et al. 2007b) and M1 (Nowak et al. 2005). In fact, virtual lesions of left AIP and M1 produced an increase in GF of a magnitude almost identical to that obtained for left SMA (Nowak et al. 2005; Davare et al. 2007b). One plausible hypothesis for these findings is that left AIP, M1 and left SMA are part of a cortical circuit involved in the accurate planning of predictive GF. Hence, if access to the object representation is disturbed, or if this representation is inaccurate, for example, due to a virtual lesion in left SMA, it is sensible to assume that subjects would use a greater safety margin, i.e. a greater GF, to prevent any object slips. Finally, it is interesting to note that left AIP, left SMA and M1 appear to play a complementary role in GF scaling which is not exchangeable between cortical regions. Evidence for this hypothesis arises from the observation that a virtual lesion in any one of these areas produces a deficit in GF scaling, suggesting that the intact areas are unable to compensate for the loss of function in any one area of the network. However, further investigations are required to determine the potential differences between the respective contributions of each of these three cortical areas. Particularly, as the present findings arise only from short term virtual lesion studies.

Evidence from the literature suggests that SMA is not directly involved in the *storage* of object representations but rather, is involved in the *recruitment* of these representations during movement preparation. Indeed, the cerebellum has been regarded as a good candidate for the storage of object representations due to its known role in error feedback learning (Wolpert et al. 1998; Imamizu et al. 2000). This is supported by clinical studies demonstrating significant impairment in the anticipatory control of GF scaling in individuals with cerebellar lesions (Nowak et al. 2002) and by data from imaging and neurophysiological studies (Wolpert et al. 1998; Kawato 1999; Imamizu et al. 2000). Furthermore, a recent imaging study suggests that SMA, together with M1, may be involved in the recruitment of object representations (Bursztyn et al. 2006). These authors proposed that the storage and subsequent loading of the correct object representation occurs in the cerebellum, but is accompanied by implementation of grasp control in SMA and sensory-motor preparation and task execution by M1. This conclusion is consistent with the results of the present study which demonstrate that disruption to left SMA interferes with the process of accurate grasp control.

Interestingly, the present results are strikingly similar to those previously reported by Davare et al. (2007b) following AIP virtual lesions. In the present study, left SMA was found to make its contribution to GF scaling 180-130 ms before the fingers made initial contact with the object. By comparison, Davare et al. (2007b) reported the contribution of left AIP to GF scaling 170-120 ms before initial object contact. These findings indicate that retrieval of information related to object representation and accurate GF scaling occur almost simultaneously in left AIP and left SMA and it is possible that both left AIP and left SMA transmit object related information to M1 in a parallel fashion. It is therefore sensible to assume that M1 integrates both these inputs, and sends feed-back to both areas in order to update the object representation if needed. Evidence of connections between AIP and visual areas suggest that AIP might process object weight information as estimated through visual

input (Borra et al. 2008) while SMA may rely on the internal model built from previous experience (Bursztyn et al. 2006). This indicates the possible existence of 2 parallel systems to gather object weight: the first, inclusive of AIP, processes object weight through the analysis of object visual features, while the second, inclusive of SMA, is involved in the recall of object weight information from the internal model.

Interestingly, the cortical network involved in precise GF scaling appears to be strongly lateralized in the left hemisphere. Indeed, GF scaling was significantly less accurate following both AIP and SMA virtual lesions, but only when TMS was applied over the left hemisphere (Davare et al. 2007b) and virtual lesions of the right AIP and right SMA failed to reproduce these results. By comparison, the hemispheric dominance of the temporal aspects of the grip-lift task appears to be more conventional, with each SMA exerting control over the opposite hand. The present study corroborated the conclusion of Davare and colleagues (2007b) that object weight representations are stored predominantly in the left hemisphere. Indeed, evidence exists in support of a less prominent role for motor control in the right hemisphere during hand motor skills (Amunts et al. 1997, see Serrien et al. 2006 for review). Furthermore, it has been proposed that the left hemisphere preferentially processes information related to internal representations and contains a specialization for the control of open-loop, feed-forward control (Goldberg et al. 1994; Serrien et al. 2006) In contrast, the right hemisphere specialization is thought to focus on closed-loop processing related to sensory-mediated mechanisms and processing of environmental cues (Goldberg et al. 1994; Bagesteiro and Sainburg 2003; Serrien et al. 2006).

However, since the SMA is very close to dorsal premotor areas (PMd) and M1, the question arises as to whether the functional deficit in GF scaling observed in the present study could result from current spread to neighbouring motor related areas such as M1 or PMd. I think

this unlikely for several reasons. First, rTMS applied over one SMA never produced an EMG response in either TA muscle, indicating an absence of significant current spread to ipsilateral M1 and also most likely to ipsilateral PMd. However, this finding does not allow us to rule out the possibility that rTMS applied over SMA may have influenced the excitability of these neighboring areas infraliminarly. Evidence against this hypothesis can be drawn from the distinct functional consequences of virtual lesions induced in PMd and M1 during a comparable grip-lift task. If current spread to PMd was a likely possibility to explain these results I would expect similar functional deficits to be obtained following both SMA and PMd virtual lesions. However, using the same rTMS protocol used in the present study Davare et al. (2006) reported significantly different results for PMd. Indeed, virtual lesioning of PMd produced a delay in the recruitment of the proximal muscles responsible for the lifting phase but had no effect on GF. Similarly, Schabrun et al. (2008; thesis Chapter 2) induced a virtual lesion in M1 while subjects performed a grip-lift task and demonstrated an alteration in the time-shift variable, reflecting an impaired anticipatory movement strategy. In the present study rTMS applied to either left or right SMA failed to produce a change in the time-shift variable (Table 3.1). Altogether these observations suggest that the effects reported in the present study cannot be attributed to rTMS spreading to neighboring motor-related areas. I therefore contend that the results of the current study assist in unveiling the actual function of SMA in grasping movements.

Another concern in interpreting the present results is the specificity of the effect found following left and right SMA stimulation. Indeed, if a current spread occurs to the opposite SMA, this could hamper our conclusions about the hemispheric dominance of object weight representation. However, a strong argument against current spread to the opposite hemisphere is the strikingly different functional effects found for left and right SMA in the parameters of peak GF, the derivative of peak GF and the coefficient of correlation, since only the virtual

lesion of left SMA affected these movement parameters. Significant current spread to the opposite SMA, impeding the selectivity of SMA stimulation should have led to identical behavioural deficits, irrespective of the stimulation side. Thus, I contend that the differential effects observed in the present study demonstrate a left hemispheric dominance for the dynamic aspects of the grip-lift task.

The only parameter in which right SMA stimulation induced a disruption was the preloading phase duration. Interestingly, preloading phase duration has been shown to rely heavily on sensory mechanisms. Initial object contact induces a burst of activity in cutaneous mechanoreceptors triggering the automatic readout of the next phase of the motor plan, namely the lift phase (Collins et al. 1999; Schabrun et al. 2008). In the present study both left and right SMA stimulation caused a reduction in the preloading phase duration when the task was performed with the contralateral hand. This bilateral hemispheric effect is in contrast to the left dominance noted for GF scaling. While the exact reason for this finding is unclear, it appears that left SMA may have a specialized role in the processing of the dynamic parameters of the task while bilateral processing is required for generating precisely timed sequences. In particular, bilateral SMA may play a role in the coordination of GF and LF signals through the sequencing of subsequent phases of the motor plan. Indeed, Gerloff et al. (1997) demonstrate a role for SMA as an area that combines elements of pre-planned movement and transfers them to M1 for execution. If this is the case then SMA may combine information on object load arising from the object load representation and cutaneous feedback from the fingertips to trigger the lift phase of the motor plan. This hypothesis receives further support from animal studies which demonstrate that SMA inactivation leads to deficits in the initiation of movement sequences (Kermadi et al. 1997).

3.6. Conclusions

The results of the present study reveal that left SMA makes a key contribution to GF scaling during object manipulation. This conclusion holds true irrespective of the hand used to perform the precision grip, indicating a dominant contribution of the left hemisphere in GF scaling. In addition, I demonstrate a key role for left and right SMA in the coordination between GF and LF signals in phase sequencing during a grip-lift task performed with the contralateral hand. Thus, I provide further evidence for the neural mechanisms underlying grasp control and provide initial evidence for the hemispheric lateralization of these processes in SMA.

4. The influence of correlated afferent input on motor cortical representations in humans

4.1. Abstract

Animal models reveal that correlated afferent inputs are a powerful driver of sensorimotor cortex reorganisation. Recently we developed a stimulation paradigm which evokes convergent afferent input from two hand muscles and induces reorganisation within human motor cortex. Here I investigated whether this reorganisation is characterised by expansion and greater overlap of muscle representation zones, as reported in animal models. Using transcranial magnetic stimulation, I mapped the motor representation of the right first dorsal interosseous (FDI), abductor digiti minimi (ADM) and abductor pollicis brevis (APB) in 24 healthy subjects before and after one hour of (i) associative stimulation to FDI and ADM motor points, (ii) associative stimulation to digits II and V (iii) a control condition employing non-correlated stimulation of FDI and ADM motor points. Motor point associative stimulation induced a significant increase in the number of active sites in all three muscles and volume in FDI and ADM. Additionally, the centre of gravity of the FDI and ADM maps shifted closer together. Similar changes were not observed following digital associative stimulation or motor point non-associative stimulation. These novel findings provide evidence that convergent input induces reorganisation of the human motor cortex characterised by expansion and greater overlap of representational zones.

4.2. Introduction

The synaptic organisation of the sensorimotor cortex is not fixed and can be changed by many experiences. In the primary motor cortex, behaviourally or experimentally induced reorganisations are characterised by shifts in muscle representational zones. For example,

removal of sensory input by peripheral nerve transection rapidly induces topographical reorganisation of muscle maps in the rat motor cortex (Sanes et al. 1988). Motor skill training also induces reorganisation in the rat motor cortex (Nudo et al. 1996).

Similar changes have been described in human subjects following amputation (Cohen et al. 1991; Ridding and Rothwell 1995), temporary ischemic block (Ridding and Rothwell 1995), thalamic lesions (Miles et al. 2005) and acquisition of a novel motor skill (Muellbacher et al. 2001). Likewise, a number of studies have demonstrated that periods of peripheral nerve stimulation (Hamdy et al. 1998; Ridding et al. 2000) induce reorganisations of the motor cortex.

The temporal characteristics of synaptic inputs to neurones are thought to be a crucial factor for the induction of plasticity (Hebb 1949) and, indeed, evidence exists to support this hypothesis. Clark and colleagues (Clark et al. 1988) demonstrated that increasing the amount of correlated sensory input from two adjacent digits, by surgically connecting the skin, led to both an expansion and greater overlap of the sensory representations of the fused digits. In another study, correlated input evoked by synchronous tactile stimulation of two digits resulted in similar sensory map expansions and greater overlaps (Godde et al. 1996).

We have recently developed a paradigm that involves synchronous stimulation of two hand muscles, and have demonstrated that the correlated sensory input produced by this stimulation induces significant reorganisation within the motor cortex (Ridding and Uy 2003). In transcranial magnetic stimulation (TMS) studies, such reorganisation is manifest as an increase in the excitability of the cortical projection to the stimulated muscles. Using conventional TMS approaches it is difficult to differentiate between a simple change in the excitability of an existing projection and a true representational expansion in the underlying cortex (Ridding and Rothwell 1997). In the present study, using TMS mapping techniques, I

sought to provide evidence that the change in excitability induced by associative stimulation was a reflection of the characteristic map changes reported in animal models, namely representational expansion and greater overlap of the stimulated muscles representations. In addition, I aimed to demonstrate that such changes were maximal in those muscles receiving associative stimulation.

4.3. Methods

A total of 24 healthy subjects participated in the study. All subjects were right-handed as tested using the Edinburgh Handedness Inventory (Oldfield 1971). Ethical approval was granted from the local Human Research Ethics Committee and all participants gave written, informed, consent.

4.3.1. Electromyographic recording techniques

Motor evoked potentials (MEPs) were recorded from the first dorsal interosseous muscle (FDI), abductor digiti minimi (ADM) and abductor pollicis brevis (APB) of the subject's right hand. Recordings were made using silver/silver chloride surface electrodes with the active electrode positioned over the muscle belly and the reference electrode over the adjacent metacarpophalangeal joint. Electromyographic (EMG) signals were amplified (x1000), filtered (bandwidth 50 –1000 Hz) and sampled at 5 KHz, and then stored on a computer for off-line analysis.

4.3.2. Transcranial magnetic stimulation (TMS)

Transcranial magnetic stimuli were delivered using a Magstim 200 stimulator (Magstim Co. Ltd, Dyfed, UK) and a figure of eight coil (external wing diameter 9 cm). For stimulation at all scalp sites the coil was orientated at a 45° angle to the midline with the handle of the coil pointing posteriorly. The optimal scalp site for evoking responses in FDI and ADM was

established and resting motor threshold (RMT) determined. RMT was defined as the minimum stimulator intensity at which 5 out of 10 stimuli applied at the optimal scalp site evoked a response in the target muscle (FDI or ADM) of at least 50 μV.

4.3.3. Mapping procedure

The procedure for mapping was similar to that previously described (Uy et al. 2002). Subjects wore a tightly fitting silicon cap marked with a 1 cm by 1 cm grid orientated to the vertex (point 0,0). The cap was positioned using cranial landmarks (nasion - inion) and pre-auricular creases as references. Five stimuli were applied at each scalp site, approximately once every 6 s. The stimulus intensity for mapping both before and after the intervention was set at 120% RMT. If the RMT of FDI and ADM differed by more than 1% each muscle was mapped separately using 120% of each muscles RMT. The responses for the map of APB were recorded at the intensity employed for the FDI and ADM maps. EMG activity in all three muscles was visually monitored at high gain to ensure no background muscle activity was present during the mapping procedure. Trials in which background activity was present were discarded. The order in which sites were stimulated was randomised and the number of sites stimulated was expanded until no response was evoked in all border sites. The mean amplitude of the responses evoked by the 5 stimuli at each point was then calculated.

4.3.4. Associative stimulation

The associative stimulation protocol has been described previously (Ridding and Uy 2003). In brief, stimulation consisted of paired electrical stimuli either to digits 2 and 5 using ring electrodes (referred to as digital associative stimulation; 8 subjects mean age 24 ± 8 years), or to the motor point of FDI and ADM using the EMG recording electrodes (referred to as motor point associative stimulation; 8 subjects 20 ± 1 years). In both cases, a square-wave electrical

stimulus (1 ms duration) was delivered synchronously to the stimulation sites (digits or muscle motor points) using a DS7A constant-current stimulator (Digitimer DS7A, Digitimer Ltd. Welwyn Garden City, UK). The intensity of the electrical stimuli was set to three times perceptual threshold (range 10-30mA) for digital stimulation, or at a level sufficient to evoke a just visible contraction in each stimulated muscle for motor point stimulation. The intensity of electrical stimulation was kept constant throughout the experiment. The time between each pair of stimuli (interstimulus interval) was randomised in the range 0.15 to 2.85 s (i.e. mean frequency of 1.5 Hz). This level of stimulation was not considered painful by any of the subjects.

4.3.5. Motor point non-associative stimulation

Eight subjects participated in this series of experiments (21 ± 2 years). The non-associative stimulation protocol has been described previously (Ridding and Uy 2003). It was similar to that of the associative motor point paradigm except that stimuli were never applied to the target muscles (FDI and ADM) synchronously. The mean stimulation frequency for each muscle (i.e. mean frequency of 1.5 Hz) was the same as that in the associative stimulation condition. Therefore, in this paradigm the two stimulated muscles (FDI and ADM) received the same number of stimuli as in the associative stimulation paradigm but the stimuli were never applied to the two muscles at the same time.

4.3.6. Experimental procedure

Subjects were seated in a comfortable chair with the silicon cap placed on the scalp. Baseline TMS maps were made for the three muscles. Following this a 1-hour period of stimulation (either associative digital stimulation, associative motor point stimulation, or non-associative motor point stimulation) was applied. To control for attention, subjects were given verbal

reminders to focus on their stimulated hand every five-minutes (Ridding and Uy 2003). The cap was left on during the intervention and its position checked at the end of the intervention prior to subsequent remapping. It was found that the cap remained accurately placed for all subjects and its position did not need to be adjusted. Following the intervention subjects were instructed to sit quietly for a further five minutes before the cortical mapping was repeated for each of the three muscles.

4.3.7. Data analysis

The effect of the interventions on the RMT of FDI and ADM were examined using a three-way ANOVA with between subject factors of INTERVENTION (3 levels: motor point associative stimulation, digital associative stimulation and non-associative stimulation) and with-in subject factors of TIME (2 levels: pre, post) and MUSCLE (2 levels: FDI, ADM).

Three variables were calculated for each TMS representational map before and following each intervention. These variables were:

i) The number of active scalp sites for each of the three muscles (FDI, ADM and APB). A site was considered active when the mean amplitude of the MEPs evoked at that site was greater than 0.05 mV.

ii) The "volume" of each map. The volume of each map was calculated by summing the average MEP amplitude of all active sites for each muscle.

iii) The distance between the centres of gravity (CoG) of the three muscles. The CoG is an amplitude-weighted centre of the map (Wassermann et al. 1992; Wilson et al. 1993; Uy et al. 2002) and is calculated as follows (Wassermann et al. 1992):

$$CoG = \sum V_i X_i / \sum V_i, \sum V_i Y_i / \sum V_i$$

(where V_i is the amplitude at scalp sites X_i and Y_i)

Using the data from the CoG calculation, the distance between the centres of the representational maps for the three muscles was calculated.

Data for each of the mapping variables were examined using a three-way analysis of variance (ANOVA) with the between-subject factor "INTERVENTION" (motor point associative stimulation, digital associative stimulation, non-associative stimulation) and the within-subject factors of "TIME" (pre, post) and "MUSCLE" (FDI, ADM, APB). A logarithmic transformation was performed on the non-normally distributed mapping data (active sites, map volume and CoG data) prior to statistical analysis. Where appropriate, *post-hoc* analyses were performed using 2 way ANOVAs split by intervention and Bonferroni corrected t-tests for pair wise comparisons. A $p < 0.05$ was considered statistically significant.

4.4. Results

There was no significant difference in the number of active sites, volume or distance between the CoG for all muscle pairs across the three intervention groups prior to stimulation (Table 4.1.).

4.4.1. Resting motor threshold (RMT)

There was no significant difference in RMT between individual target muscles (FDI, ADM) or across time (pre/post intervention). Additionally, there was no significant MUSCLE*TIME interaction. The average MEP at the hotspot was similar for each of the three muscles across all subjects; FDI 1.8 ±0.5 mV, ADM 0.8 ± 0.2 mV and APB 1.4 ± 0.2 mV. A one-way ANOVA with factor MUSCLE revealed no significant difference between the three muscles ($F_{2, 21} = 2.4$; $p = 0.15$).

Table 4.1 *This table summarises the motor map variables (mean ± SD) for FDI, ADM and APB under each stimulation condition.*

	MPAS[a] pre	**MPAS[a] post**	**DAS[b] pre**	**DAS[b] post**	**MPNS[c] pre**	**MPNS[c] post**
RMT (mV)						
FDI	46.2	46.6	48.1	48.1	41.4	41.3
	(2.0)	(2.1)	(3.8)	(3.9)	(1.5)	(1.5)
ADM	46.3	46.5	48.8	48.8	41.5	41.6
	(2.1)	(2.0)	(3.7)	(3.8)	(1.5)	(1.4)
Active sites						
FDI	22.4	32.4 *	23	19.4	22.6	21.1
	(2.8)	(3.3)	(2.0)	(2.1)	(1.5)	(1.1)
ADM	20	30.4 *	20	17	21.9	17.6
	(2.7)	(4.3)	(1.7)	(1.8)	(2.2)	(3.0)
APB	24	35.7 *	20.6	18.6	21.1	18.8
	(3.1)	(3.2)	(2.1)	(2.3)	(1.2)	(0.9)
Volume (mV)						
FDI	16.8	24.5 *	18.9	13.6	18.6	16.2
	(3.8)	(3.9)	(3.8)	(3.2)	(4.2)	(4.9)
ADM	6.7	14.7 *	7.8	5.9	7.9	5.7
	(0.8)	(5.1)	(0.9)	(1.2)	(1.5)	(1.8)
APB	15.1	24.4	21.8	15.8	25.6	19.3
	(2.7)	(4.9)	(5.0)	(3.6)	(5.5)	(6.9)
CoG distance (cm)						
FDI/ADM	0.45	0.23 *	0.18	0.20	0.39	0.35
	(0.02)	(0.02)	(0.04)	(0.05)	(0.05)	(0.06)
ADM/APB	0.39	0.40	0.21	0.22	0.35	0.33
	(0.06)	(0.05)	(0.05)	(0.02)	(0.02)	(0.07)
FDI/APB	0.19	0.16	0.20	0.23	0.26	0.23
	(0.005)	(0.03)	(0.04)	(0.03)	(0.07)	(0.05)

[a] MPAS - motor point associative stimulation, [b] DAS - digital associative stimulation, [c] MPNS - motor point non-associative stimulation, * Significant ($p < 0.05$) difference pre/post intervention

4.4.2. Number of active sites

A three-way ANOVA revealed a significant INTERVENTION*TIME interaction ($F_{2, 126}$ = 10.0; $p < 0.001$). Given this significant interaction, two-way ANOVAs were performed for each intervention with factors MUSCLE and TIME. A significant TIME effect was observed for motor point associative stimulation ($F_{1, 42}$ = 15.7; $p < 0.001$). Further investigation using *post-hoc* paired t-tests demonstrated a significant increase in the number of active sites in FDI (t=-3.8; $p = 0.007$), ADM (t=-3.7; $p = 0.007$) and APB (t=-4.2; $p = 0.004$) following motor point associative stimulation. No significant MUSCLE or TIME effects were observed following either digital associative stimulation or non-associative motor point stimulation.

4.4.3. Map Volume

A three-way ANOVA revealed a significant INTERVENTION*TIME interaction ($F_{2, 126}$ = 7.9; $p < 0.001$) and a significant effect of MUSCLE ($F_{2, 126}$ = 32.0; $p < 0.001$). Two-way ANOVAs performed on data for each intervention revealed significant TIME ($F_{1, 42}$ = 8.9; $p = 0.005$) and MUSCLE ($F_{2, 42}$ = 7.5; $p = 0.002$) effects following motor point associative stimulation. Bonferroni corrected t-tests demonstrated a significant increase in the map volume for FDI (t = -3.5; $p = 0.010$) and ADM (t = -2.9; $p = 0.020$) but not for APB (t = -2.1; $p = 0.075$) following motor point associative stimulation. A representative example of an individual subject's representational maps of FDI and ADM before and following motor point associative stimulation is shown in Figure 4.1a. There was no significant change in the map volumes for any of the three muscles following either digital associative or motor point non-associative stimulation (Figure 4.1b).

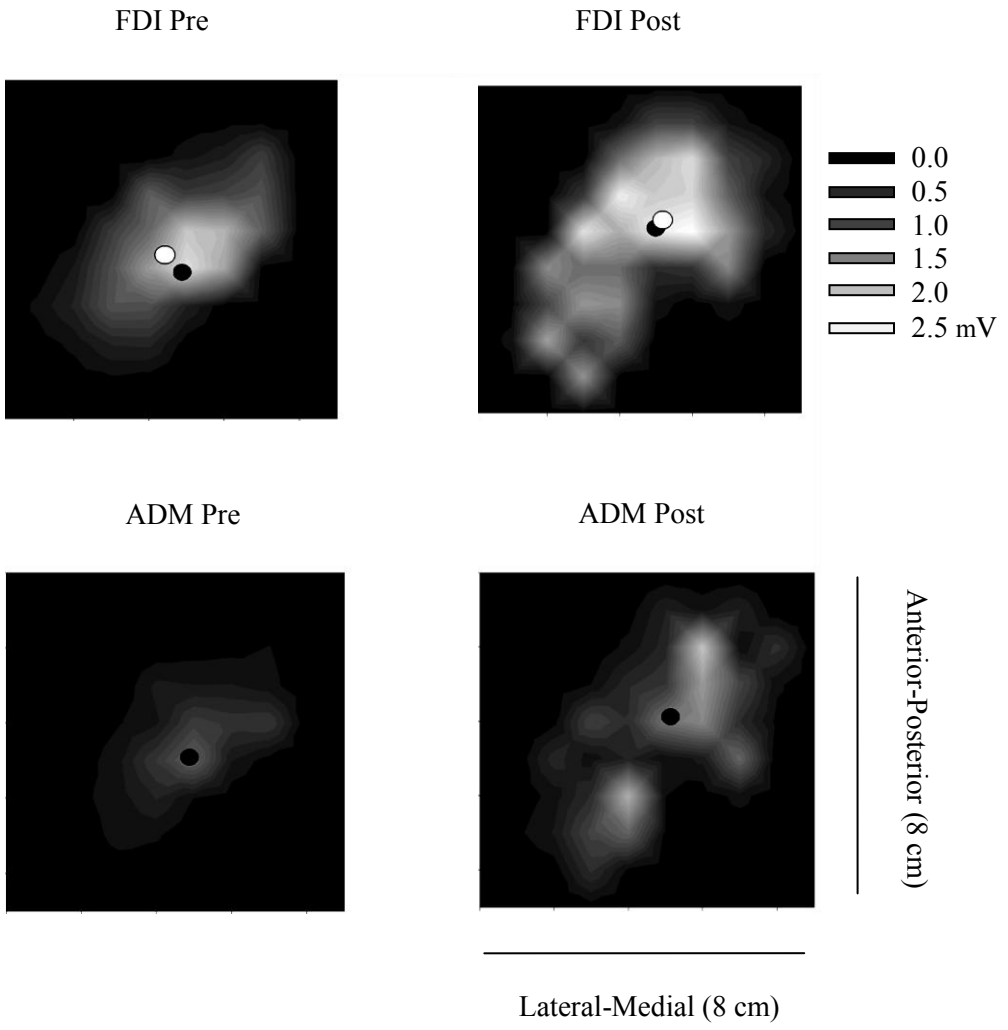


Figure 4.1a (left) *Graphical representation of the motor maps of FDI (top) and ADM (bottom) before and after motor point associative stimulation in one representative subject. Maps are referenced to the vertex in the posterior-medial corner. The average amplitude of the MEP evoked at each site is indicated by the colour scale (mV). The CoG of each FDI map is indicated by the large white dot. The CoG of each ADM map is indicated by the large black dot. The CoG of each ADM map has also been superimposed on each FDI map to demonstrate the shift in the CoG following stimulation. There is an increase in the number of active sites and map volume observed for FDI and ADM following motor point associative stimulation. Additionally, the CoG of the two muscles were significantly closer together following motor point associative stimulation (0.23 cm) than before stimulation (0.45cm).*

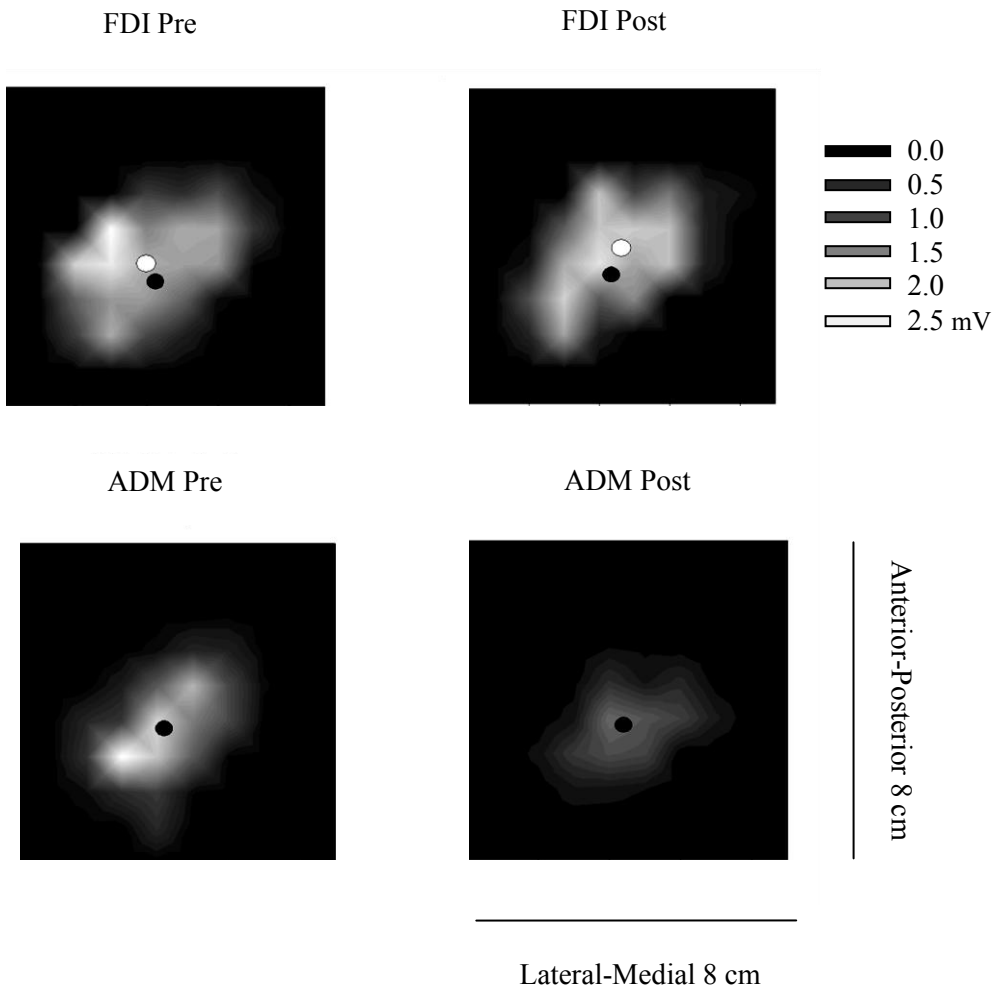


Figure *4.1b* *Graphical representation of motor maps obtained for FDI (top) and ADM (bottom) before and after motor point non-associative stimulation in the same subject. The CoG of each FDI map is indicated by the large white dot. The CoG of each ADM map is indicated by the large black dot. The CoG of each ADM map has also been superimposed on each FDI map to demonstrate the shift in the CoG following stimulation. Maps are referenced to the vertex in the posterior-medial corner. There was no significant increase in either map area or volume, and no significant change in the distance apart of the two CoG locations.*

4.4.4. Centre of gravity (CoG) measurements

The change in the distance between the CoG of the three muscles following each intervention was analysed with a three-way ANOVA which demonstrated a significant INTERVENTION*TIME interaction ($F_{2, 126} = 6.5$; $p = 0.002$) and a significant effect of

MUSCLE ($F_{2, 126} = 3.6$; $p = 0.029$). To further investigate this effect a 2-way ANOVA was performed on the data for each intervention. Significant TIME ($F_{1, 90} = 4.4$; $p = 0.038$) and MUSCLE ($F_{2, 90} = 7.3$; $p = 0.001$) effects were observed following motor point associative stimulation. *Post-hoc* paired t-tests demonstrated that the CoG of the FDI and ADM motor maps moved significantly closer together following motor point associative stimulation ($t = 2.3$; $p = 0.033$). Motor maps from a representative subject indicating changes in the CoG are shown in Figure 4.1a. The CoG of FDI motor maps demonstrated a strong trend towards a consistent anteromedial directional change with 6 out of 8 motor maps shifting anteriorly and 7 out of 8 maps shifting medially following motor point associative stimulation (Figure 4.2).

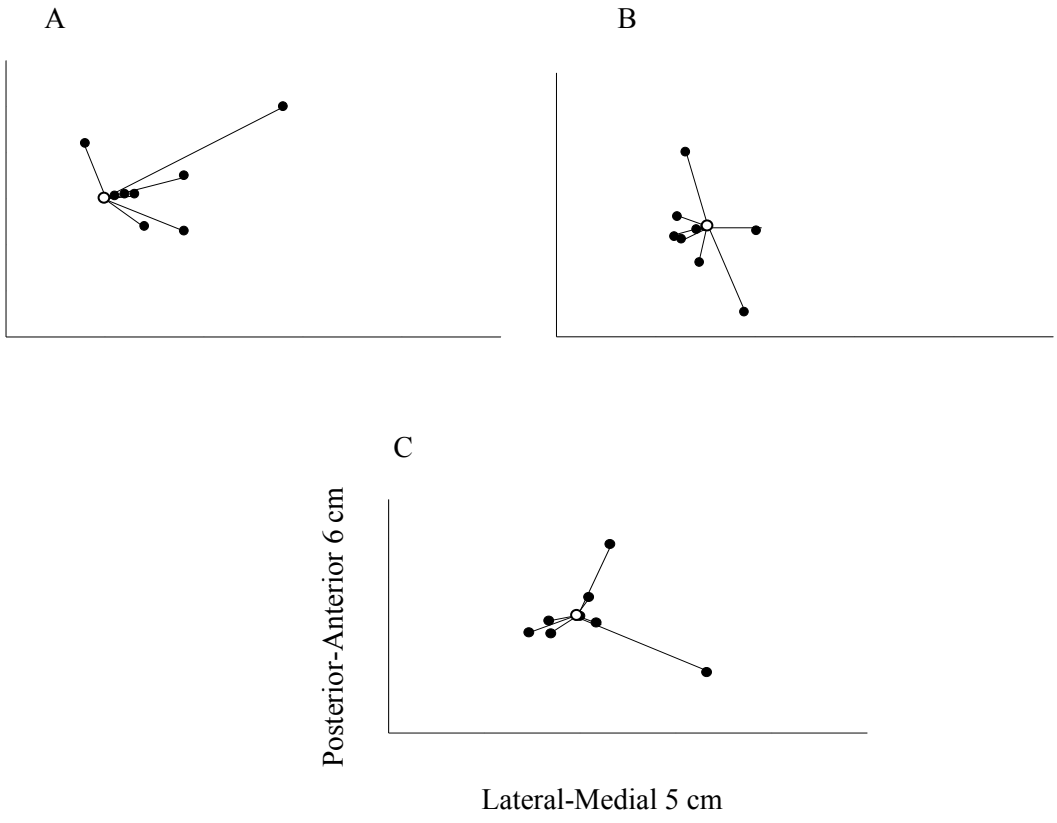


Figure 4.2 *Directional shifts in the motor maps of FDI (A), ADM (B) and APB (C) following motor point associative stimulation. Data for all 8 subjects are shown. The white circles represent the average (all subjects) pre-stimulation CoG coordinate for each muscle, FDI (4,3), ADM (3.5,2.5), APB (3,3). Black circles represent the final CoG for each subject following the period of motor point associative stimulation.*

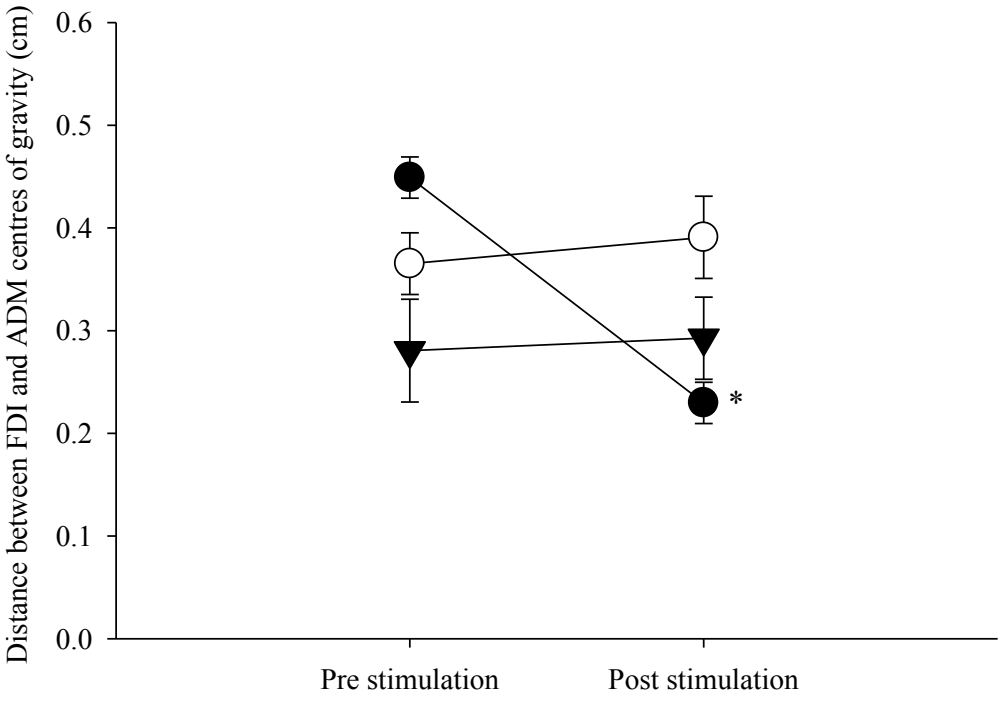


Figure 4.3 *The mean (standard error) distance (cm) between the CoG of FDI and ADM before and after motor point associative stimulation (filled circles), motor point non-associative stimulation (clear circles), and digital associative stimulation (triangles). The CoGs were significantly closer following motor point associative stimulation (*p < 0.05).*

4.5. Discussion

The main findings of the present study are, firstly, that correlated sensory input evoked by stimulating the two hand muscles, induces an increase in excitability of the cortical projections of those stimulated muscles. This confirms the results of previous studies (Ridding and Uy 2003). The second, and novel, finding is that the CoG of the scalp representational maps of the stimulated muscles moved closer together following associative stimulation. This finding suggests greater overlap of motor cortical representations of the two stimulated muscles following motor point associative stimulation. No such change was seen between stimulated and non-stimulated muscle pairs. Additionally, the associative pattern of stimulation was critical given that non-associative stimulation of the same muscles did not induce significant representational shifts. Finally, input from large diameter muscle afferent fibres appears to be an important factor in driving such reorganisation, as convergent input evoked from digital nerves failed to induce similar changes.

The organisation of the adult brain is constantly being modified and many experiences can lead to reorganisation. For example, modification of afferent input by peripheral nerve stimulation results in lasting reorganisation of the cat somatosensory cortex (Recanzone et al. 1990). This reorganisation is characterised by enlargement of the receptive fields of neurons that receive input from the stimulated peripheral region. Such expansions are seen with periods of stimulation as short as 1-2 hours. In particular, temporally correlated afferent input has been shown to exert a powerful effect on topographic organisation in a number of animal models. Clark and colleagues (Clark et al. 1988) increased the amount of correlated input from adjacent digits in adult owl monkeys by surgically connecting two adjacent digits (syndactyly). The representation of the digits in the somatosensory cortex (area 3b) was then mapped several months later, using microelectrode techniques. The maps evoked following syndactyly were markedly different from those seen in normal animals and were characterised

by a loss of the normal discontinuity between the representations of adjacent digits and an increase in the number of dual digit representational sites, indicating greater representational overlap. Godde and colleagues (Godde et al. 1996) demonstrated in the rat that paired synchronous tactile stimuli applied to the digits for 6-15 hours resulted in representational changes in the somatosensory cortex. Again, these changes were characterised by enlargement, and greater overlap, of sensory receptive fields. Similar changes are also seen in the motor cortex. Transection of the facial nerve in rats results in rapid and lasting changes in the topography of motor cortex (Sanes et al. 1990; Sanes and Donoghue 2000). These changes consisted of invasion of the previous vibrissae territory by the adjacent forelimb representation. Nudo and colleagues demonstrated in adult squirrel monkeys that training on skilled motor tasks resulted in significant reorganisations of the motor cortex (Nudo et al. 1996). Using intracortical microstimulation, they showed that these reorganisations consisted of an enlargement of the cortical area from which trained movements could be evoked. Additionally, muscles that co-contracted in the training task, and presumably generated convergent patterns of afferent input, came to be represented together in the cortex.

Transcranial magnetic stimulation has been used to investigate whether such organisational changes are seen in human subjects. Amputation (Cohen et al. 1991; Ridding and Rothwell 1995) or temporary ischaemic block (Ridding and Rothwell 1995) both induce rapid reorganisations of the motor cortex. Periods of peripheral nerve stimulation also increase the excitability of the cortical projection to stimulated muscles (Handy et al. 1998; Ridding et al. 2000). Likewise, paired stimulation of the motor points of two hand muscles in a synchronous manner increases the excitability of the corticospinal projections to the stimulated muscles (Ridding and Uy 2003). These studies have generally provided evidence of reorganisation within the motor cortex by reporting changes in cortical excitability, indicated as a change in the amplitude of MEPs.

In the current study, associative stimulation applied to the motor points of FDI and ADM resulted in an increase both in the number of active sites and the volume of the maps for the stimulated muscles, and an increase in the number of active sites but not volume in the non-stimulated APB. These findings are very similar to those reported previously where it has been shown that motor point associative stimulation preferentially increases the excitability of the cortical projection to stimulated muscles (Ridding and Uy 2003). The reason for the increase in the number of active sites for the non-stimulated APB muscle is unclear. However, other studies using peripheral stimulation have also reported some minor "overflow" of excitability changes to adjacent non-stimulated muscles (Ridding et al. 2001). Several possibilities exist to explain this relative lack of focality. Firstly, the intensities employed for mapping in the present study were determined on the basis of the RMT for FDI and ADM. Therefore, it is possible that non-optimised mapping parameters for APB may have lead to greater variability in the mapping data which would complicate interpretation. However, given that the MEPs evoked at the optimal scalp site were of similar amplitude for all three muscles I consider this an unlikely explanation. Secondly, it is known that individual corticomotoneuronal cells innervate multiple hand muscles (Cheney and Fetz 1980; Lemon et al. 1991). Therefore, I suggest a more likely explanation for the excitability change in APB is that changes in excitability of the corticomotoneuronal cells projecting to FDI and ADM may also lead to a small increase in the excitability of the projection to a wider set of upper limb muscles, including APB.

It has been generally assumed that these excitability changes reflect underlying cortical representational expansions. However, this interpretation is complicated by difficulties in differentiating between an increase in excitability of the existing representation and a true expansion of the underlying cortical representation (Ridding and Rothwell 1997). I suggest that this issue may be addressed by examining changes in the distance between the CoGs of

muscle maps. If the cortical representation of a muscle just became more excitable, without representational expansion, the CoG of the map would not be expected to shift. In contrast, expansion of the cortical representation in a non-uniform direction (which would seem likely) would cause the CoG to shift. Extending this argument, true representational expansion and overlap as well as increased co-localisation would be expected to result in a shift of the CoG of representational maps towards each other.

In the present study, I have demonstrated that the CoG for FDI and ADM moved significantly closer together following associative motor point stimulation. In addition, the CoG of the FDI and ADM motor maps tended to shift in more consistent directions (towards each other) than the CoG of the non-stimulated APB motor map. No such changes were seen following either motor point non-associative stimulation or digital associative stimulation. Additionally, the distance between the CoGs of the APB and ADM, and APB and FDI maps was not significantly different to baseline following any of the interventions. It has been reported previously that the CoG is a reliable and stable measure of the centre of motor maps (Miranda et al. 1997; Thickbroom et al.1999; Uy et al. 2002). This suggests that in the present study, the movement in the CoG in the associative condition was unlikely to be due to spontaneous spatial variability in the maps.

One factor that may complicate interpretation of the CoG data is that the MEPs seen following motor point associative stimulation were larger than in the baseline maps. This in itself may have lead to some systematic shift in the maps. However, we have previously demonstrated that larger MEPs *per se* do not significantly influence CoG calculations (Ridding et al. 2001). The results of the present study therefore suggest that the maps of FDI and ADM expanded in a non-uniform manner and towards each other, resulting in greater representational overlap. This finding is remarkably similar to that described above for the

animal models, where convergent sensory input induced expansion and greater overlap of receptive fields in the somatosensory cortex (Clark et al. 1988; Godde et al. 1996) and representation expansion and increased co-localisation of representations in the motor cortex (Nudo et al. 1996). Additionally, the present findings are similar in nature to those reported by Liepert and colleagues (Liepert et al. 1999) who performed TMS mapping of a hand muscle before and following a period of synchronized thumb and foot movements. These authors found that following the synchronised training the CoG of the hand muscle involved in the task shifted medially towards the presumed leg muscle representation. In this situation it is likely that correlated sensory input driven by synchronised muscle contraction induced qualitatively similar changes to those described here following associative stimulation.

In contrast to the results seen following motor point associative stimulation, digital associative stimulation did not induce map expansion and representational shifts. We have previously shown that prolonged digital nerve simulation does not, in contrast to mixed nerve stimulation, increase cortical excitability (Ridding et al. 2000). Digital nerves consist primarily of cutaneous afferents, so it is possible that input from large-diameter muscle afferents might be important for inducing representational expansion within the motor cortex. Non-associative motor point stimulation also had little effect on the motor maps. This is consistent with previous reports (Ridding and Uy 2003), and demonstrates the importance of correlated inputs for induction of reorganisation.

With current techniques it is impossible to provide direct evidence of the mechanisms responsible for such representational change in human subjects. However, a number of pieces of evidence point towards the possible mechanisms. Firstly, the increase in excitability seen following a period of peripheral nerve stimulation is not associated with change in spinal motoneuron excitability as assessed with F-waves (Ridding et al. 2000). Secondly, we have

previously demonstrated that MEPs evoked following transcranial electrical stimulation (TES) are, in contrast to those evoked by TMS, not facilitated following motor point associative stimulation (Ridding and Uy 2003). It is known that TES predominantly actives pyramidal cells directly while TMS involves transynaptic activation (Day et al. 1989). Therefore, the differential effect of associative stimulation on responses to TES and TMS suggests that the site of excitability modulation is within the cortex. Thirdly, studies examining the excitability of intrinsic intracortical excitatory elements using paired pulse TMS techniques (Ziemann et al. 1998) have shown that there is a significant increase in intracortical facilitation following motor point associative stimulation (Pyndt and Ridding 2004). This suggests that associative input from large diameter muscle afferents modifies the excitability of intracortical excitatory elements that in turn may be responsible for the representational map changes.

The present findings may also have relevance for our understanding of a condition associated with excessive representational plasticity (Quartarone et al. 2003; Weise et al. 2006) and abnormal cortical representations (Byrnes et al. 1998), namely, focal hand dystonia. This condition is characterised by excessive and inappropriate muscle activation. Primate models of hand dystonia have shown that this condition is likely to be associated with abnormal sensory cortical organisation characterised by increased representational overlap and co-localisation (Byl et al. 1996). These abnormalities of organisation are initiated by repetitive stereotyped patterns of hand use which, presumably, produce intense convergent afferent inputs. The findings of the present study suggest that convergent afferent input can drive short-term reorganisations within the motor cortex which are similar in nature to those described in the sensory cortex in the primate models of focal dystonia. Therefore, in predisposed individuals it is conceivable that long-term practise of tasks which produce strong

afferent input of a convergent nature may lead to motor cortical reorganisations that contribute to focal hand dystonia.

In conclusion, I have demonstrated that a period of correlated input from large-diameter muscle afferents induces motor cortical reorganisation. This reorganisation is characterised by both enlargement, and shifts, of the scalp representational TMS maps, with the stimulated muscles representations being closer together following stimulation. I propose that these results provide the first neurophysiological evidence that correlated sensory input plays an important role in driving cortical representational expansions and shifts in human subjects. Additionally, I suggest that the nature of these induced changes is very similar to those demonstrated in animal models using invasive microstimulation techniques.

5. Normalising motor cortical representations in focal hand dystonia

5.1. Abstract

Task-specific focal dystonia is thought to have a neurological basis where stereotypical synchronous inputs and maladaptive plasticity play a role. As afferent input is a powerful driver of cortical reorganisation I propose that a period of asynchronous afferent stimulation may reverse maladaptive cortical changes and alleviate symptoms. Using TMS, three hand muscles were mapped in 10 dystonics and 10 healthy controls. Mapping occurred before and after 1 hour of non-associative stimulation (NAS) to FDI and APB. Participants performed grip-lift, handwriting and cyclic drawing before and after NAS. Prior to NAS dystonics had larger maps, and the centres of gravity (CoGs) of the FDI and APB maps were closer together, when compared with those of control subjects. Dystonics demonstrated impairments in grip-lift, handwriting and cyclic drawing tasks. Following NAS, map size was reduced in all muscles in dystonic participants and FDI and APB CoGs moved further apart. Among dystonics NAS produced a reduction in movement variability during cyclic drawing. Thus, 1 hour of NAS can reduce the magnitude, and increase the separation, of TMS representational maps. I suggest these changes reflect some normalisation of the representational abnormalities seen in focal dystonia and provide initial, limited evidence that such changes are associated with improvements in circle drawing.

5.2. Introduction

Task specific focal dystonia is characterised by excessive and inappropriate muscle activation during highly skilled fine motor tasks, resulting in slow, clumsy movements and impaired task performance. The condition affects highly trained, stereotypical movement patterns, such as writing (Writer's cramp) or playing a musical instrument (Musicians dystonia; Byl et al. 2003; Candia et al. 2003). While excessive, repetitive movement patterns may play a role in

Musician's dystonia, those with Writer's cramp do not typically have a history of excessive hand use.

The focal dystonias were initially ascribed to psychiatric illness, however, their neurological basis is now widely accepted (Nadeau et al. 2004). In particular, excessive representational plasticity (Quartarone et al. 2003), abnormal cortical representations (Byrnes et al. 1998; Bara-Jimenez et al. 1998) and reduced intracortical inhibition (Ridding et al. 1995; Stinear and Byblow 2004b; 2004c; Bütefisch et al. 2005) may manifest in dystonic symptoms and deficits in movement prevention and control (Stinear and Byblow 2004a).

It has been proposed that synchronous and convergent afferent input arising from repetitive motor tasks may play an important role in driving the maladaptive cortical plasticity seen in focal hand dystonia (FHD). Such a hypothesis arises from work conducted in both animal and human subjects. For example, Byl and colleagues (1996; 1997) trained primates in a repetitive motor task over 12 to 25 weeks and noted significant disruption in the organisation of the sensory cortex, and motor symptoms similar to those seen in FHD. This disruption was characterised by enlargement, overlap, and a loss of differentiation in the cortical hand skin representation. It has also been shown that surgical joining of the skin of adjacent digits, which increases correlated sensory inputs, produces similar organisational changes (Clark et al. 1988). Likewise, in healthy human participants, Hebbian-like pairing of tactile stimuli to the digits induces similar changes in the sensory cortex (Godde et al. 1996). Also, synchronous stimulation of peripheral muscles induces organisational changes in motor representations, characterised by an increase in map size of stimulated muscles and a reduction in map separation, as assessed using transcranial magnetic stimulation (TMS) (Schabrun and Ridding 2007). Similar abnormalities of cortical organisation are seen in both motor (Byrnes et al. 1998) and sensory (Butterworth et al. 2003) representations in FHD.

Given that afferent input is known to be a powerful driver of cortical reorganisation I suggest that one strategy to re-establish discrete cortical representations and alleviate dystonic symptoms may be to provide independent input from involved muscles through asynchronous afferent stimulation in which there is no consistent temporal coupling of the evoked afferent inputs. Such a hypothesis is supported by the finding that reducing correlated input from adjacent digits, by surgical separation of syndactyly, produces separation of digital cortical representations (Mogilner et al. 1993).

Therefore, it seems feasible that asynchronous, and non-associative, stimulation of hand muscles may temporarily reverse representational changes characteristic of FHD. Therefore, the aim of this study was to examine the effect of an asynchronous ("non-associative") afferent stimulation paradigm on motor cortex representations and symptoms in task-specific FHD.

5.3. Methods

Ten participants with FHD (5 males; 56 ± 12.6 years; mean ± SD; Table 5.1.) and 10 age, handedness and sex-matched healthy participants (5 males; 55.9 ± 11.7 years) took part in the study. Dystonic participants were included if they a) had a confirmed diagnosis of FHD, b) did not suffer from dystonic symptoms at rest and c) had not received Botulinum toxin therapy in the last 6 months. All participants gave written, informed consent in line with the Declaration of Helsinki. All procedures were approved by the local Human Research Ethics Committee.

Table 5.1 *Characteristics of FHD participants*

Subject	Age (yr)	Gender	Handedness	Diagnosis	Duration (yr)	ADDS	WCRS pre	WCRS post
1	63	M	RH	WC	8	58.3	5	8
2	75	M	RH	WC	15	74.2	7	7
3	51	M	RH	MD	1	86.1	0	0
4	41	F	LH	WC	11	76.5	5	4
5	67	F	RH	WC	22	66.5	3	5
6	34	F	RH	MD	5	70.5	0	0
7	50	M	LH	WC	31	58.5	20	16
8	64	F	RH	WC	29	82.2	10	9
9	52	F	RH	WC	8	76.5	15	8
10	63	M	RH	MD	40	59	3	3

WC – Writer's Cramp; MD – Musicians Dystonia; RH – Right Handed; LH – Left Handed; ADDS – Arm Dystonia Disability Scale, max score 100%; WCRS – Writer's Cramp Rating Scale, max score 30.

5.3.1. Clinical rating of focal hand dystonia

Dystonic symptoms were assessed using the Arm Dystonia Disability Scale (ADDS; Fahn 1989) and the Writer's Cramp Rating Scale (WCRS; Wissel et al. 1996). The ADDS provides a measure of functional hand impairment on a range of everyday tasks. An ADDS score of 100 % indicates the absence of any motor impairment, while a score of 90 % denotes the presence of social restrictions but no limitation in activities. Scores below 90 % indicate significant impairment in daily activities, including writing, personal hygiene and feeding. The WCRS measures the degree of dystonic posturing and speed during handwriting. A score of 0 indicates normal posture and a score of 30 indicates severe impairment. Participants were

videotaped as they wrote the sentence "Sheila collects shells" 10 times (Zeuner et al. 2005). The WCRS was scored by an experienced assessor blinded to subject group.

5.3.2. Electromyographic (EMG) recording

Surface EMG was recorded from the FDI and APB muscle of each participant's symptomatic hand. As a control muscle not directly involved in the intervention, recordings were also made from the abductor digiti minimi (ADM) in the same hand. Recordings were made using silver/silver chloride surface electrodes positioned with the active electrode over the muscle belly and the reference electrode over the adjacent metacarpophalangeal joint. Signals were amplified (CED 1902, Cambridge UK) sampled at 5 kHz in the bandwidth 20-1000 Hz (CED, Micro 1401, Cambridge UK).

5.3.3. Transcranial magnetic stimulation (TMS)

TMS was delivered using a Magstim 200 stimulator (Magstim Co. Ltd, Dyfed, UK) and a figure of eight coil (external wing diameter 9 cm). The coil was positioned at a 45° angle to preferentially induce current in posterior-to-anterior direction in the cortex. A tightly fitting silicon cap marked with a 1 cm by 1 cm grid was positioned and orientated to the vertex (point 0,0) in each participant. The optimal scalp site for evoking responses in FDI, APB and ADM was established and the co-ordinates noted. Resting motor threshold (rMT) was determined for FDI, APB and ADM, and defined as the minimum stimulator intensity at which 5 out of 10 stimuli applied at the optimal scalp site evoked a response of at least 50 μV in the target muscle.

5.3.4. Mapping procedure

The procedure for mapping has been described in detail previously (Uy et al. 2002; Schabrun and Ridding 2007). Subjects were fitted with a silicon cap marked with a 1 cm by 1 cm grid orientated to the vertex (point 0,0). The cap was positioned using cranial landmarks and the periauricular creases. The cap was not removed during NAS and its position was remeasured to ensure accurate placement following the intervention. The stimulus intensity for mapping both before and after the intervention was set at 120% of baseline rMT. FDI and APB were mapped separately if the rMT for each muscle differed by more than 1% of mean stimulator output. EMG responses evoked in the control muscle (ADM) were recorded at the intensity employed for the FDI and APB maps. TMS was applied every 6 s to a total of 5 stimuli at each scalp site. Trials containing background EMG activity greater than 50 μV were discarded. The number of scalp sites was increased until no MEP was evoked in all border sites. The order in which sites were stimulated was randomised. The mean amplitude of the responses evoked by the 5 stimuli at each scalp site was then calculated. We have previously demonstrated that this approach yields reliable and stable area, volume and CoG measures over significant periods of time, and that such an approach is appropriate for examining the effects on an intervention on TMS representational maps (Uy et al. 2002).

5.3.5. Handwriting task

Participants were seated at a standard writing table where they completed two tasks. First, they were asked to write the sentence “Sheila collects shells” 10 times in their normal handwriting across a sheet of blank paper. Secondly, following the procedure of Zeuner and colleagues (2007) participants were asked to draw superimposed circles approximately 2 cm in diameter for a period of 10 s. On the first attempt participants were instructed to draw as quickly as possible and on the second attempt to use minimal pen pressure. Both tasks were

repeated following the intervention. The three subjects with musician's dystonia all reported mild (2) to moderate (1) difficulties with handwriting on the ADDS scale. Handwriting and cyclic drawing tasks were recorded using a pressure sensitive digitising tablet and an inking digitising pen connected to a personal computer (WACOM Intuos A4 oversize; Wacom Europe). Data were sampled at 166 Hz with a spatial resolution of 0.05 mm.

5.3.6. Grip-lift task

Participants washed their hands thoroughly with soap and water and were comfortably seated at a table. Participants lifted a 350 g manipulandum based on that originally described by Westling and Johansson (1984). The device was comprised of two lightweight load cells situated 35 mm apart and an accelerometer. Participants were instructed to lift the manipulandum to the height indicated (10 cm), hold it steady for 3 s and then replace it on the table. The lift was performed primarily by flexing the elbow. Each participant performed 5 consecutive lifts, 5 s apart. Measures of the horizontal grip force (GF) exerted by the index finger and thumb, the vertical load force (LF), and the onset of the lift were obtained. All GF, LF and acceleration signals were low-pass filtered at 100 Hz, sampled at 400 Hz and stored on a computer for off-line analysis.

5.3.7. Non-associative stimulation

The non-associative stimulation (NAS) protocol has been described previously (Ridding and Uy 2003; Schabrun and Ridding 2007), and consisted of asynchronous electrical stimuli (square-wave stimuli of 1 ms duration) applied to the motor points of FDI and APB for a period of 1 hour, using a constant-current stimulator (Digitimer DS7A, Digitimer Ltd. Welwyn Garden City, UK). There was no consistent temporal coupling between the two stimulated muscles with the time between each pair of stimuli randomised in the range 0.15 to

2.85 s (i.e. mean frequency of 1.5 Hz). Stimuli were never applied to the two muscles at the same time. Stimulus intensity was set to evoke a just visible contraction in each stimulated muscle. Stimulation was not considered painful by any of the participants.

5.3.8. Experimental procedure

Participants first completed the handwriting tasks and then the grip-lift task. TMS was then used to map the corticomotor representations of the three muscles, before a 1-hour period of NAS was applied. Participants were verbally reminded to focus on their stimulated hand every 5 minutes (Ridding and Uy 2003; Schabrun and Ridding 2007). Following the period of NAS, participants sat quietly for a further five minutes, before cortical mapping was repeated for each of the three muscles. Finally, the grip-lift and handwriting tasks were repeated. Resting motor threshold was assessed for FDI and APB prior to TMS mapping and again following the NAS intervention.

5.3.9. Data and statistical analysis

5.3.9.1. Neurophysiological data

Background EMG data was rectified and averaged in each subject for the 100 ms preceding the TMS pulse before and after NAS. A three-way analysis of variance (ANOVA) with the between-subject factor GROUP (Dystonic, Control) and the within-subject factors MUSCLE (FDI, APB, ADM) and TIME (Pre, Post) was performed. This analysis ensured that changes in map volume were not due to voluntary activity during the mapping procedure.

The number of active sites and map volume were calculated for each muscle (FDI, APB, ADM) before and after NAS. A scalp site was considered active if the mean peak-to-peak amplitude of the MEPs evoked at that site was greater than 0.05 mV. Map volume was calculated by summing the mean MEP amplitude at all the active sites for each muscle. In addition, I calculated the distance between the centres of gravity (CoG) of the three muscle maps. The CoG is the amplitude-weighted centre of the map (Wassermann et al. 1992; Wilson et al. 1993; Uy et al. 2002). Map CoGs were calculated using the formula (Wassermann et al. 1992):

$$CoG = \sum V_i X_i / \sum V_i, \sum V_i Y_i / \sum V_i$$

(V_i is the mean MEP amplitude at the scalp site with coordinates X_i and Y_i)

Using the data from the CoG calculation, the distance between the centres of each representational map was calculated in order to quantify CoG displacements.

Data for the number of active sites and map volume were examined using a three-way ANOVA with the between-subject factor GROUP (Dystonic, Control) and the within-subject factors of TIME (Pre, Post) and MUSCLE (FDI, APB, ADM). A three-way ANOVA was performed on CoG data, with the between-subject factor GROUP (Dystonic, Control) and the within-subject factors of TIME (Pre, Post) and MUSCLE PAIR (FDI/APB, APB/ADM, FDI/ADM). Correlations between the CoG and functional measures were performed using a Pearson's Product Moment Correlation with significance $p < 0.05$.

Where appropriate, all *post-hoc* analyses were performed using Bonferroni corrected t-tests for pair-wise comparisons. A $p < 0.05$ was considered statistically significant.

5.3.9.2. Handwriting and Cyclic drawing data

Kinematic variables were analysed using the MovAlyzeR 4.1 software package (NeuroScript, Arizona, USA). The mean stroke frequency (MSF; mean number of up and down strokes per second) provides a measure of the automaticity and fluency of the movement (Zeuner et al. 2007). Mean pen pressure and the coefficient of variation (CV) of the positive velocity peaks were also calculated according to the procedure described by Zeuner and colleagues (2007). The CV of positive velocity peaks provides a measure of the variability of consecutive movements (Zeuner et al. 2007). A two-way mixed ANOVA was performed for each of the above measures, the between-subjects factor was GROUP (Dystonic, Control) and the within-subjects factor TIME (Pre, Post). All *post-hoc* analyses were performed using Bonferroni corrected t-tests for pair-wise comparisons. A $p < 0.05$ was considered statistically significant.

5.3.9.3. Grip-lift data

Four variables were extracted from each trial of the grip-lift task: (1) preload duration (time between the onset of GF and the onset of positive LF), (2) the maximum grip force (GF_{max}), (3) maximum correlation coefficient and (4) $Timeshift_{max.}$ To determine the maximum correlation and the $Timeshift_{max}$ the rate of change of GF (dGF/dt) in the lift phase was correlated with the rate of change of LF (dLF/dt), by shifting one signal with respect to the other in 2 ms increments (Duque et al. 2003; Schabrun et al. 2008). This procedure determines the difference delay ($Timeshift_{max}$) between the rate of change of GF and the rate of change of LF at the point where the correlation is maximal, and is thought to reflect anticipatory or reactive scaling of GF (Duque et al. 2003; Schabrun et al. 2008). *Post-hoc* analyses were performed using Bonferroni corrected t-tests ($p < 0.05$).

Statistical analysis was performed using a three-way ANOVA for each of the above variables with between-subjects factor GROUP (Dystonic, Control) and within-subjects factors TIME (Pre, Post) and LIFT NUMBER (1–5).

5.4. Results

Mean ± Standard error values for all EMG and mapping data are given in Table 5.2.

Table 5.2 *Mean ± Standard error values for all EMG and mapping data*

Variable	Muscle	Dystonic group		Control group	
		Pre NAS	Post NAS	Pre NAS	Post NAS
Background EMG (mV)	FDI	.02 (.003)	.02 (.004)	.01 (.007)	.02 (.003)
	APB	.01 (.005)	.01 (.001)	.02 (.006)	.02 (.006)
	ADM	.02 (.005)	.02 (.006)	.02 (.006)	.03 (.001)
Resting motor threshold (% stim output)	FDI	49.5 (4.3)	49.4 (4.3)	47.1 (2.9)	47.0 (2.7)
	APB	49.5 (4.3)	49.6 (4.3)	47.3 (3.2)	47.4 (3.0)
	ADM	49.1 (4.2)	49.1 (4.3)	47.3 (3.2)	47.1 (2.6)
Mean MEP amplitude (hotspot)	FDI	1.3 (.44)	1.1 (.28)	.98 (.2)	.76 (.22)
	APB	1.2 (.43)	.91 (.31)	.65 (.31)	.82 (.22)
	ADM	.54 (.08)	.46 (.06)	1.0 (.34)	1.3 (.41)
Active sites	FDI	29.9 (2.6)	21 (1.5)*	20 (3.2)	21.5 (3.5)
	APB	27.6 (3.2)	21.3 (2.9)*	19.6 (3.2)	19.9 (2.9)
	ADM	25.8 (3.6)	21.4 (2.6)*	18.2 (3.2)	17.7 (1.8)
Volume (mV)	FDI	15.7 (4.7)	10.6 (3.5)*	6.7 (1.6)	6.3 (1.5)
	APB	15.7 (3.6)	7.9 (1.5)*	7.0 (2.2)	10.5 (3.2)
	ADM	8.6 (3.2)	6.6 (2.4)*	3.4 (1.2)	6.1 (1.6)
Distance between Centres of Gravity (cm)	FDI/APB	0.24 (0.022)	0.40 (0.02)*	0.29 (0.02)	0.33 (0.02)
	APB/ADM	0.38 (0.016)	0.44 (0.029)	0.54 (0.05)	0.42 (0.04)
	ADM/FDI	0.31 (0.024)	0.34 (0.025)	0.39 (0.03)	0.35 (0.04)

NAS – Non-associative stimulation; * $p < 0.005$

5.4.1. EMG and resting motor threshold data

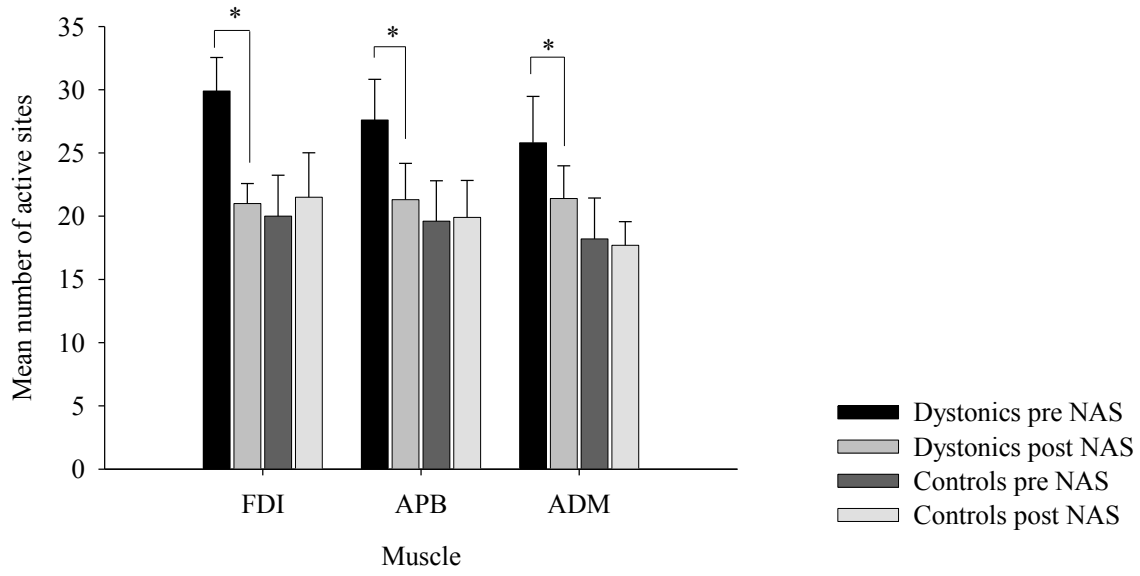
There was no main effect of GROUP ($F_{1, 119} = 0.79$; $p = 0.37$), MUSCLE ($F_{2, 119} = 2.2$; $p = 0.11$) or TIME ($F_{1, 119} = 0.54$; $p = 0.46$), on background EMG data. In addition, there was no GROUP x MUSCLE interaction ($F_{2, 119} = 1.2$; $p = 0.31$).

There were no effects of GROUP ($F_{1, 108} = 1.2$; $p = 0.26$), MUSCLE ($F_{2, 108} = 0.003$; $p = 0.99$) or TIME ($F_{1, 108} < 0.001$; $p = 0.99$) on rMT, and there were no interactions between these factors (both $F_{2, 108} < 0.002$; $p > 0.99$; see Table 5.2). The average MEP amplitude at the hotspot was similar for each of the three muscles prior to NAS (Table 5.1). A three-way ANOVA with factors GROUP (Dystonic, Control), TIME (Pre, Post) and MUSCLE (FDI, ADM, APB) revealed no significant difference between groups ($F_{1, 119} = 0.1$; $p = 0.75$), muscles ($F_{2, 119} = 1.1$; $p = 0.34$) or across time ($F_{1, 119} = 0.4$; $p = 0.5$). In addition, there was no GROUP x TIME interaction ($F_{1, 119} = 0.1$; $p = 0.7$).

5.4.2 Neurophysiological data

There was a main effect of GROUP on the number of active sites ($F_{1, 108} = 8.7$; $p = 0.004$) and map volume ($F_{1, 108} = 6.6$; $p = 0.01$). There was also an interaction between GROUP and TIME for the number of active sites ($F_{1, 108} = 4.2$; $p = 0.043$) and map volume ($F_{1, 108} = 4.5$; $p = 0.037$). The number of active sites ($t = 2.9$; $p = 0.004$) and map volume ($t = 2.6$; $p = 0.01$) were greater in the dystonic group at baseline than in the control group in all three muscles. Following NAS there was a reduction in both the number of active sites ($t = 2.7$; $p = 0.008$; Figure 5.1a) and map volume ($t = 2.2$; $p = 0.029$; Figure 5.1b) among dystonic participants. In contrast, no change was observed in either the number of active sites ($t = 0.18$; $p = 0.86$) or map volume ($t = 0.78$; $p = 0.43$) following NAS in the control group. There was no effect of MUSCLE and no MUSCLE x TIME interaction for either variable (active sites, both $F_{2, 108} < 0.6$; $p > 0.54$; map volume, both $F_{2, 108} < 2.4$; $p > 0.091$).

1a



1b

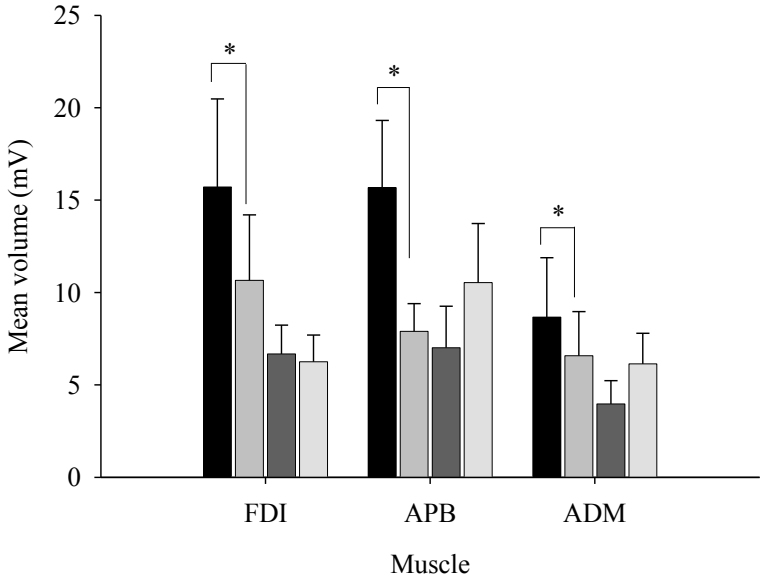


Figure 5.1 *Mean (+ SE) number of active sites (1a) and map volume (1b) for each muscle before and after NAS in dystonic and control groups. There was a decrease in the number of active sites and volume in all three muscles following NAS in the dystonic group * $p < 0.05$.*

In control participants, no changes were noted in the degree of separation of CoGs for any muscle pair following NAS (all; $t < 0.44$; $p > 0.1$). Examination of raw data suggested the increase in CoG separation was not associated with any consistent directional shift across the dystonic participants. Data from representative subjects is shown in Figure 5.2.

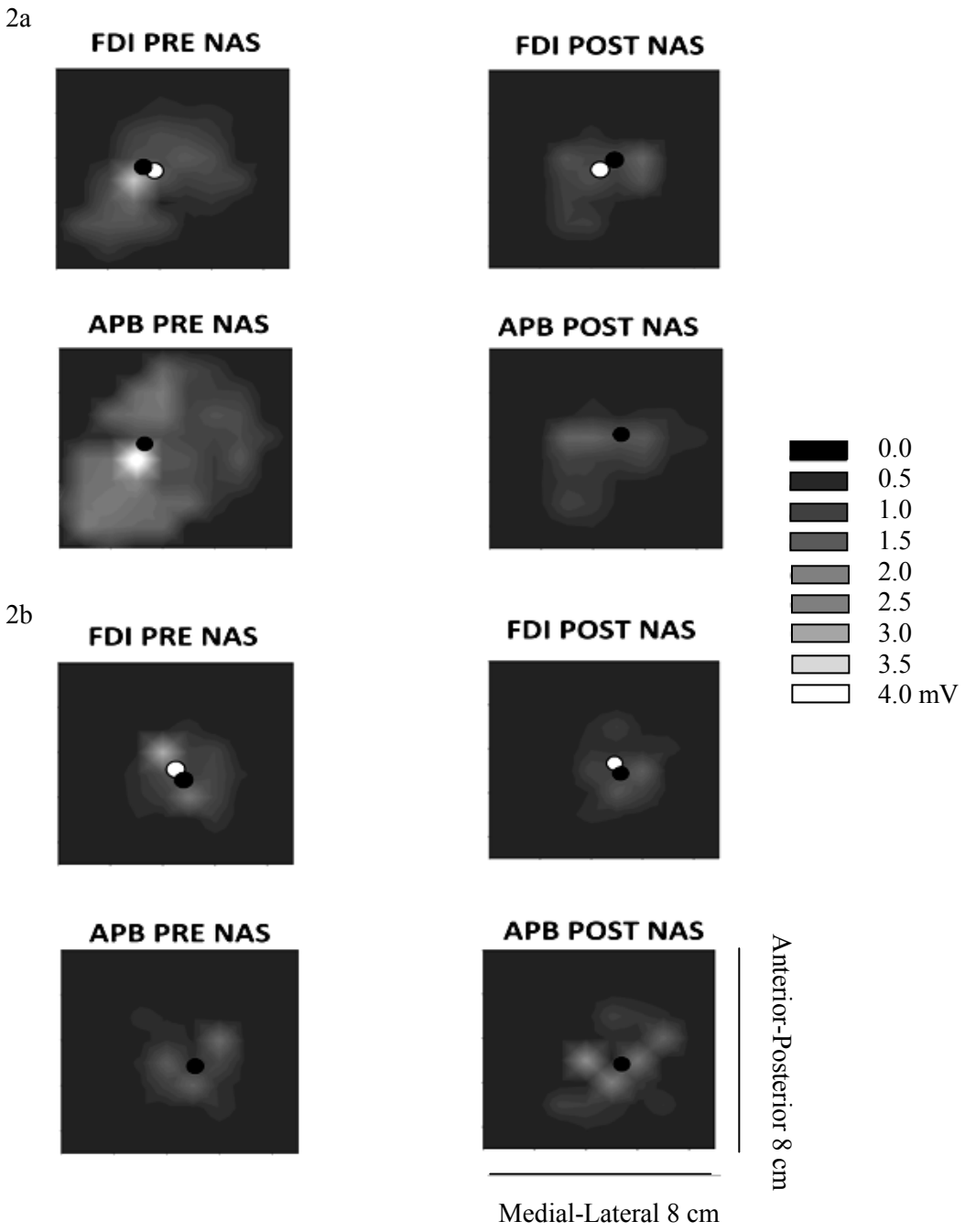


Figure 5.2a *Cortical representation obtained for FDI and APB before and after NAS in one dystonic (2a) and one control (2b) subject. MEP amplitude is given by the grey scale (mV). The CoG of each map is indicated by the circle (FDI–white; APB–black). The CoG of each APB map has been superimposed over each FDI map to demonstrate the CoG shift following NAS. A decrease in the number of active sites and map volume was observed for FDI and APB following NAS in dystonics ($p < 0.05$). Additionally, the CoGs of the two muscles were further apart after NAS (pre 0.26 cm; post 0.37 cm).* ***Figure 5.2b*** *There was no change in either map area or volume, and no change in the distance between the two CoG locations after NAS in control subjects (pre 0.23 cm; post 0.20 cm).*

There was a C

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separation for

muscle pairs.

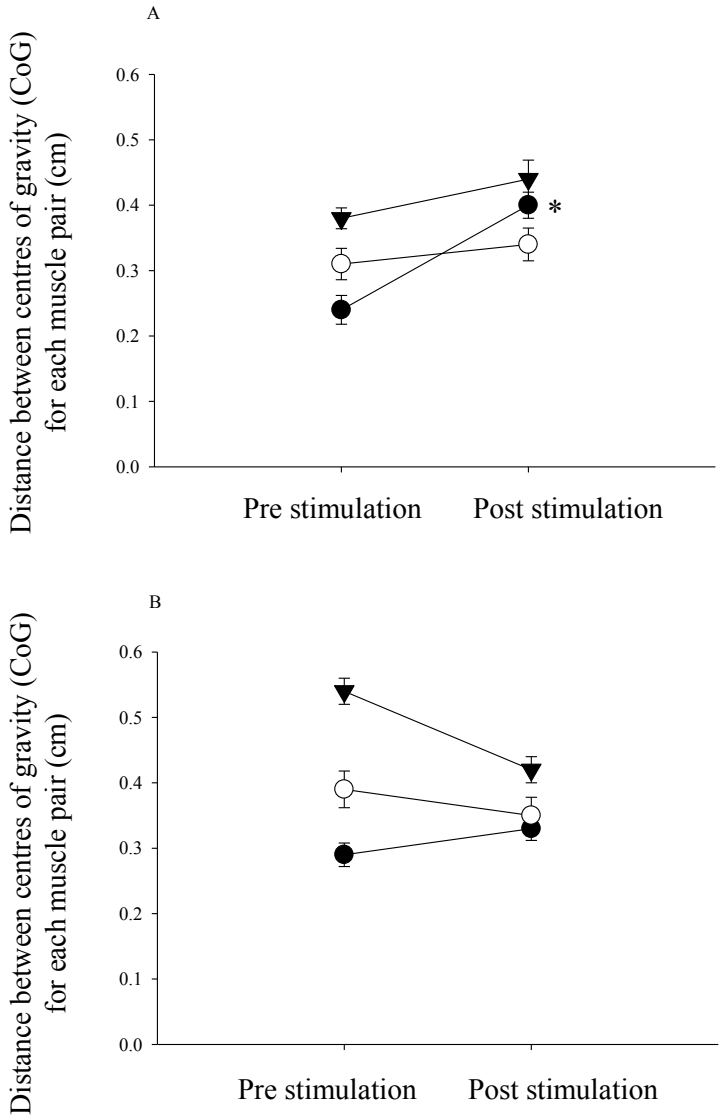


Figure 5.3 *Mean (standard error) distance (cm) between the CoG of each muscle pair before and after NAS (FDI/APB, filled circles; FDI/ADM, open circles; ADM/APB, triangles) in A) dystonic group and B) control group. The CoGs of FDI and APB were further apart in the dystonic group following NAS (* $p < 0.05$).*

5.4.2. Behavioural data

Mean ± SE values for all handwriting and grip-lift data are given in Table 5.3.

Table 5.3 *Mean ± Standard error values for all handwriting and grip-lift data.*

Task	Variable	Dystonic group		Control group	
		Pre NAS	Post NAS	Pre NAS	Post NAS
Handwriting	MSF (Hz)	2.8 ± 0.4*	3.6 ± 0.5	4.1 ± 0.6	4.7 ± 0.5
	CV	0.51 ± 0.06*	0.5 ± 0.06	0.37 ± 0.03	0.42 ± 0.04
	Pen pressure (N)	1.9 ± 0.4	1.8 ± 0.3	2.2 ± 0.3	2.0 ± 0.2
Cyclic drawing (condition 1)	MSF (Hz)	2.1 ± 0.3*	2.5 ± 0.4	3.2 ± 0.3	2.9 ± 0.3
	CV	0.52 ± 0.06**	0.26 ± 0.05	0.21 ± 0.027	0.22 ± 0.026
	Pen pressure (N)	2.5 ± 0.4*	2.0 ± 0.4	1.6 ± 0.4	1.2 ± 0.2
Cyclic drawing (condition 2)	MSF (Hz)	1.8 ± 0.2*	2.1 ± 0.3	2.9 ± 0.2	2.8 ± 0.2
	CV	0.33 ± 0.03**	0.13 ± 0.04	0.15 ± 0.068	0.16 ± 0.074
	Pen pressure (N)	1.7 ±0.4 *	1.2 ± 0.3	0.5 ± 0.1	0.4 ± 0.1
Grip-lift	Preload duration (ms)	317.5 ± 42.0*	233.7 ± 39.5	169.5 ± 24.6	180.7 ± 26.1
	Peak GF (N)	13.1 ± 0.7*	12.8 ± 0.5	9.2 ± 0.8	7.2 ± 0.6
	Correlation (r)	0.76 ± 0.01	0.76 ± 0.01	0.78 ± 0.01	0.76 ± 0.01
	$Timeshift_{max}$ (ms)	-15.5 ± 8.0	-5.8 ± 6.9	-26.8 ± 9.5	-21.2 ± 9.7

* $p < 0.005$ significant GROUP effect at baseline; ** $p < 0.005$ significant GROUP x TIME interaction; Condition 1 – quick drawing; Condition 2 – light pen pressure; NAS – Non-associative stimulation; MSF – mean stroke frequency; CV – Coefficient of variation of velocity;

Under both cyclic drawing conditions (quick drawing and light pen pressure) there was a main effect of TIME (both $F_{1, 36} > 8.4$; $p < 0.006$) and a GROUP x TIME interaction (both $F_{1, 36} > 8.5$; $p < 0.006$) for the CV of positive velocity peaks. Following NAS the CV of positive

velocity peaks was lower in dystonic participants (both $t > 4.1$; $p < 0.001$), while there was no change among control participants (both $t < 0.01$; $p > 0.98$). The degree of improvement in the CV of positive velocity peaks among dystonic participants was positively correlated with the increase in the separation of the FDI and APB CoGs ($r = 0.72$, $p = 0.018$; Figure 5.4).

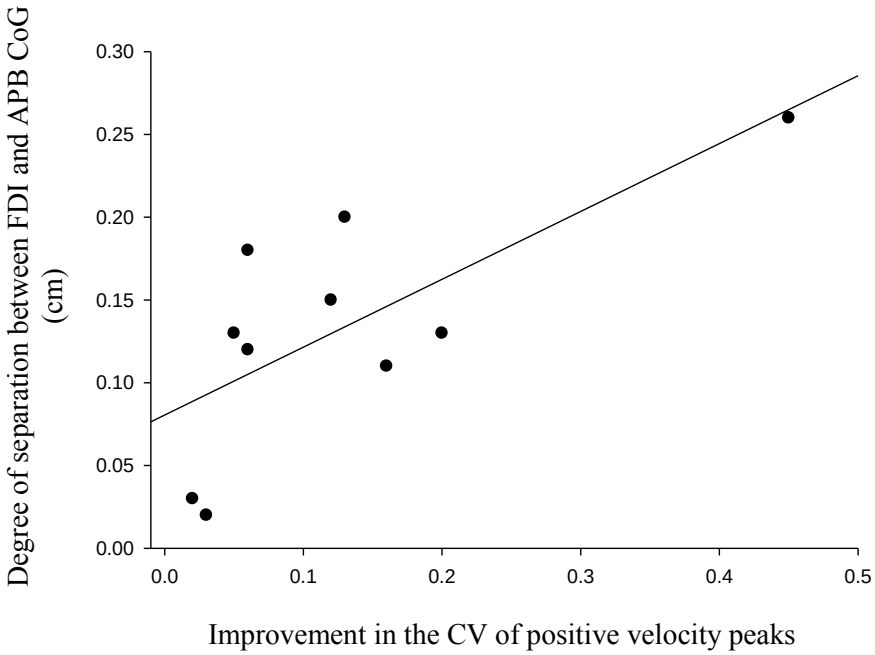


Figure 5.4 *Correlation between the degree of separation between the FDI and APB CoGs and improvement in the variability of movement profiles (CV of positive velocity peaks) during cyclic drawing with light pen pressure following NAS among dystonic participants. Greater separation of the cortical representations for FDI and APB was associated with larger decreases in the variability of movement profiles during cyclic drawing ($r = 0.72$, $p = 0.018$).*

For both cyclic drawing conditions there was an effect of Group on MSF (both $F_{1, 108} > 7.5$; $p < 0.01$), CV of positive velocity peaks (both $F_{1, 108} > 15.7$; $p < 0.001$) and pen pressure (both $F_{1, 108} > 4.3$; $p < 0.044$). Dystonic participants demonstrated lower stroke frequency (both $t > 7.5$; $p < 0.01$), higher CV of positive velocity peaks (both $t > 3.2$; $p < 0.008$), and greater pen

pressure (both t > 4.3; $p < 0.044$) under both drawing conditions when compared with control participants.

There was an effect of GROUP on MSF ($F_{1, 36} = 4.6$; $p = 0.039$) and the CV of positive velocity peaks ($F_{1, 36} = 4.3$; $p = 0.045$) during the handwriting task. Stroke frequency at baseline was lower in dystonic participants (2.8 ± 1.2 Hz; mean ± SD) than control participants (4.1 ± 2.1 Hz; $t = 2.1$; $p = 0.039$). The CV of positive velocity peaks was greater in dystonic participants (0.51 ± 0.2; mean ± SD) than control participants (0.37 ± 0.09; t = 2.1; $p = 0.045$). There was no GROUP effect on pen pressure ($F_{1, 36} = 0.42$; $p = 0.52$). There was no effect of TIME (all $F_{1, 36} < 1.69$; $p > 0.2$) and no GROUP x TIME interactions for MSF, pen pressure, or the CV of positive velocity peaks (all $F_{1, 36} < 0.24$; $p > 0.6$), indicating that the NAS protocol did not affect handwriting in dystonic or control participants.
There were significant effects of GROUP on preload duration ($F_{1, 180} = 8.5$; $p = 0.004$) and GF_{max} ($F_{1, 180} = 16.0$; $p < 0.001$) during the grip-lift task. Preload duration was longer in dystonic participants (317.5 ± 297.1 ms; mean ± SD) than control participants (169.5 ± 174.3 ms; $t = 2.9$; $p = 0.004$). Dystonic participants also applied a higher grip force during object lifting (13.1 ± 3.9 N) when compared with control participants (9.2 ± 10.7 N; t = 4.0; $p <$ 0.001). There was no difference between the dystonic and control groups with respect to the correlation coefficient or $Timeshift_{max}$ variables, indicating that anticipatory scaling of grip force is preserved in this group of patients. There was no effect of the NAS intervention on any of the grip-lift variables (all $F_{1, 180} < 1.8$; $p > 0.17$).

5.5. Discussion

The main findings of this study can be summarised as follows. First, representational motor maps in the dystonic group were larger at baseline than those obtained from the control group. Second, representational maps in the dystonic group contracted following a 1 hour period of

NAS, becoming smaller in both area and volume. In addition, the CoGs for the two stimulated muscles (FDI, APB) were further separated following NAS. Finally, I observed improvements in cyclic drawing, providing initial evidence for the amelioration of dystonic symptoms following NAS.

At baseline, maps obtained for all three muscles were considerably larger in both area and volume, and the FDI and APB CoGs were significantly closer together, in dystonic participants than in control subjects. As trials containing background EMG activity were discarded, the increase in map area and volume in dystonic participants is unlikely to be due difficulties with muscle relaxation. Furthermore, these findings are consistent with previous reports in both the animal (Byl et al. 1996; 1997) and human literature (Byrnes et al. 1998; Elbert et al. 1998). While the exact mechanism of altered cortical representations in focal dystonia remains unclear, it appears that repetitive, simultaneous afferent inputs arising from prolonged motor practice plays a key role in the development of the maladaptive reorganisation of the sensorimotor cortex. Indeed, it is well known that use-dependent plasticity of the sensorimotor cortex is heavily influenced by the pattern of afferent input (Brons and Woody 1980; Sanes et al. 1990). In particular, synchronous or tightly temporally coupled afferent inputs have been shown to be a particularly powerful driver of cortical reorganisation. For example, Godde and colleagues (1996) applied synchronous afferent stimulation to digits in the rat and reported enlargement and overlap of cortical representations. Furthermore, muscles which co-contract during a motor task, and thus produce convergent patterns of afferent input, have greater overlap of their cortical representations (Nudo et al. 1996). Conversely, surgical separation of syndactyly in humans, which reduces convergent and associative afferent input, results in separation of digital cortical representations (Mogilner et al. 1993). Therefore, it may be that the abnormal sensorimotor representations seen in FHD are due to an abnormally increased response to

repeated patterns of stereotypical and convergent afferent inputs. This hypothesis receives some support by the finding of abnormally increased representational plasticity in FHD (Quartarone et al. 2003).

A novel finding in the present study is that 1 hour of asynchronous electrical stimulation delivered to the motor points of FDI and APB is sufficient to produce a normalisation of TMS representational maps in those with focal dystonia. Following non-associative stimulation there was a decrease in MEP amplitudes in all three muscles and a separation of the CoGs for stimulated muscles (FDI and APB). No significant shift in CoGs occurred in muscle pairs containing the unstimulated ADM muscle. The decrease in the number of active sites and map volume for the non-stimulated ADM muscle is likely due to the innervation of multiple hand muscles by single corticomotoneuronal cells (Cheney and Fetz 1980; Lemon et al. 1991). Thus, changes in the excitability of individual corticomotoneuronal cells projecting to FDI and APB muscles may also induce a change in the excitability of the unstimulated ADM muscle. Indeed, other studies using peripheral stimulation have reported similar "overflow" of excitability changes to adjacent non-stimulated muscles (Ridding et al. 2001, Schabrun and Ridding 2007).

A question to arise is whether the observed separation in the CoGs of the stimulated muscles is due to the period of NAS or other technical or non-specific time effects. As the MEP amplitude evoked following NAS was smaller than baseline MEPs in the maps of the FHD patients it could be argued that this would produce changes in CoG measurements. However, I think this unlikely as we have previously demonstrated that simply changing the amplitude of MEPs in representational TMS maps does not produce significant CoG shifts (Ridding et al. 2001). The CoG is a reliable and stable measure of the point of greatest excitability within representational maps (Miranda et al. 1997; Thickbroom et al. 1999; Uy et al. 2002).

Furthermore, the distance between the CoGs of various muscles appears to be a consistent and stable measure over time (Schabrun and Ridding 2007). Therefore, I consider it unlikely that the increase in the separation of the CoG of the stimulated muscles is due to non-specific time effects. Additionally, the fact that such a shift was not seen in the control group argues against this possibility.

Motor cortex maps produced with TMS provide information on the surface topography of the corticomotor projection for each stimulated muscle (Byrnes et al. 1998). Changes induced in the spatial territory of the corticomotor projection are therefore reflected as a shift in the CoG. Thus, in the present study an increase in the distance between the CoGs of stimulated muscles likely reflects a separation in the spatial territory of the corticomotor projection to each of the stimulated muscles.

Several studies have examined the effect of afferent stimulation in FHD. Tinazzi and colleagues (2006) reported that MEP amplitudes are reduced in normal subjects after a single session of transcutaneous electrical nerve stimulation (TENS) but were unchanged in patients with FHD. However, if repeated sessions of TENS were applied, FHD subjects showed MEP suppression which was associated with an improvement in handwriting. These findings are similar to the current findings and indicate that a reduction in cortical excitability in FHD may be important for functional improvements and symptom alleviation. Several studies have also examined the effect of afferent stimulation on sensorimotor reorganisation in FHD. In normal subjects muscle vibration has a differential effect on cortical excitability with short interval intracortical inhibition being reduced in the projection to the vibrated muscles but increased for adjacent non-vibrated (Rosenkranz and Rothwell 2003). In FHD the topographic specificity of this effect is disturbed (Rosenkranz et al. 2005). Rosenkranz and colleagues (2008) demonstrated that a period of vibratory proprioceptive training could normalise the

response to muscle vibration in participants with musician's dystonia, but not those with writer's cramp. This finding suggests that there may be some differences in the pathophysiology of writer's cramp and musician's dystonia. In the present study, the small number of subjects with musician's dystonia studied makes it difficult to comment with any certainty that afferent input arising from the hand may be less important for the development of pathological changes in writer's cramp subjects than in those with musician's dystonia. However, inspection of the data revealed no obvious differences in either baseline maps, or response to NAS, between participants with writer's cramp and musician's dystonia. Finally, sensorimotor reorganisation has also been demonstrated following a period of motor training known as sensorimotor retuning (SMR). SMR involves splinting of the fingers to produce isolated movement patterns in the dystonic hand and, using the technique of magnetoencephalography, has been shown to promote more ordered and discrete cortical representations in those with FHD (Candia et al. 2003; 2005). These studies, coupled with the results reported here, indicate that modulation of afferent input is likely to be of significant importance in the successful treatment of FHD.

A question arises as to whether there is a particular importance associated with the application of asynchronous afferent input to two muscles or whether providing a similar amount of stimulation to only one muscle would produce comparable neurophysiological changes. While not examined here, previous studies have demonstrated that changes in cortical organisation can be seen following application of afferent stimulation to only one muscle (Ridding et al. 2000; Ridding et al. 2001). However, such changes occurred only after a prolonged period of stimulation. These findings suggest that stimulation applied to only one muscle is unlikely to produce effects similar to those reported here. In addition, at least in normal subjects such stimulation produces representational map enlargement, in contrast to the map reduction seen in the FHD subjects in the present study. Therefore, it seems unlikely

that isolated peripheral stimulation would be effective in producing the organisational changes reported here.

Investigation of the mechanisms by which NAS normalises cortical representations in FHD was not an aim of the present study. However, it is known that cortical representations are thought to be maintained and adjusted by intracortical inhibitory circuits, primarily through the modulation of the inhibitory neurotransmitter γ-amino-butyric acid (GABA) (Sanes et al. 1988; Ridding et al. 1995; Liepert et al. 1998). In healthy humans, GABAergic intracortical inhibition is decreased in muscles required for the execution of a voluntary motor task (Ridding et al. 1995) and increased in adjacent muscle groups that need to be maintained relaxed (Liepert et al. 1998; Stinear and Byblow 2003; Zoghi et al. 2003). This process is likely important for the fractionation of motor outputs from the primary motor cortex, creating isolated movements in the absence of muscle overflow and co-contraction. Interestingly, intracortical inhibitory networks are impaired or poorly modulated in FHD (Ridding et al. 1995; Stinear and Byblow 2004b, 2004c; Bütefisch et al. 2005) Therefore, it may be that a period of NAS exerts its effect on cortical representation by increasing GABAergic inhibition and "decoupling" overlapping muscle representations as was evident in the CoG separation observed.

NAS did not produce analogous changes in healthy subjects. This is consistent with previous reports (Schabrun and Ridding 2007). There was no change in map area, volume or the CoG in these subjects, suggesting that in healthy individuals asynchronous afferent input does not lead to rapid change in cortical representations. Therefore, it is possible that the rapid, aberrant plasticity seen in dystonic subjects is in part driven by excessive sensitivity in the sensorimotor cortex to afferent inputs. Such a mechanism receives support from several sources. Firstly, Quartarone et al. (2003) demonstrated that in subjects with FHD, paired

associative stimulation (PAS) produces an abnormal increase in corticospinal excitability, which was not confined to stimulated muscles. These findings provide support for the role of excessive plasticity in FHD. Secondly, the genetic contribution to the dystonias is increasingly recognised (Defazio et al. 2007), indicating that repetitive, stereotyped afferent inputs may lead to late-onset dystonia, such as FHD, more rapidly in genetically susceptible individuals.

In the present study I examined several behavioural tasks that have been shown to be abnormal in writer's cramp and musicians dystonia. Abnormalities in the grip-lift task have been demonstrated in both writer's cramp and musicians dystonia patients (Nowak et al. 2005). In addition, all three subjects with musician's dystonia reported mild (n = 2) to moderate (n = 1) difficulties with writing on the ADDS scale, making the inclusion of a handwriting task relevant for all subjects. Dystonic participants displayed significant impairments in grip-lift, handwriting and cyclic drawing tasks when compared with the healthy control group. In the grip-lift task they demonstrated preload durations that were double those seen in healthy controls, and peak GF levels that were elevated by 4 N on average, signifying an increased safety margin during the lift. Several previous studies have demonstrated similar increases in peak GF in those with focal dystonia (Odergren et al. 1996; Serrien et al. 2000; Nowak et al. 2005b). However, it is noteworthy that in both the present study and the previous literature, anticipatory scaling of fingertip forces is maintained in dystonic subjects. This suggests that the underlying pathophysiology does not interfere with the generation or execution of the internal, feed-forward model (Odergren et al. 1996; Nowak et al. 2005b).

Previous studies have hypothesised that increased GF levels in those with dystonia are the result of impaired sensorimotor integration (Odergren et al. 1996; Serrien et al. 2000).

However, it has also been shown that visual feedback training is successful in reducing GF levels in subjects with writer's cramp (Schenk and Mai 2001). This suggests that excessive GF may be a learned, compensatory strategy rather than a pathophysiological deficit in sensorimotor integration (Nowak et al. 2005b). If the impairments noted during the grip-lift task are the result of a prelearned strategy it is perhaps not surprising that single, short period of NAS produced no significant change in these variables.

Dystonic participants performed both the handwriting and cyclic drawing tasks with significantly lower mean stroke frequencies than control participants. In fact, mean stroke frequency consistently fell below 3 Hz, indicating significant impairment in movement fluency and automaticity (Zeuner et al. 2007). Pen pressure was also significantly increased in dystonic participants. However, this increase was only present during cyclic drawing and not during the handwriting task. These results are similar to those obtained by Zeuner and colleagues (2007) and confirm that cyclic drawing is a more sensitive measure of motor dysfunction than handwriting in focal dystonia. Dystonic participants showed greater variability in movement profiles during both the handwriting and cyclic drawing tasks than their healthy counterparts. Interestingly, the only behavioural variable that significantly improved in the FHD patients following NAS was the coefficient of variability in the movement velocity. As there was no sham condition in the present study it is difficult to completely rule out placebo effects. However, I suggest that the reported changes are unlikely due to placebo or non-specific time effects for several reasons. Firstly, although the change was only seen in one variable (coefficient of variation of positive velocity peaks) the magnitude of the change was large and highly significant ($p < 0.001$). Secondly, as there were no significant changes in the control group this suggests that these measures are reliable across time. Finally, there was a significant correlation between the separation of motor cortical representations of the stimulated muscles and improvements in the CV of positive

velocity peaks. This suggests that the improvements in performance were related to normalisation in representational maps.

The baseline CV value for FHD subjects was slightly higher than that reported previously by Zeuner et al. (2007) for circle drawing. As my testing protocol was identical, as far as I can tell, to that employed by Zeuner and colleagues (2007) I suggest that this difference in velocity variability likely reflects differences in subject characteristics between the two studies. Following the intervention, the CV of positive velocity peaks for FHD subjects were remarkably similar to those obtained for controls.

The question arises as to why changes in other functional measures were not seen. I suggest that improvements in the CV of positive velocity peaks were seen because this is a highly sensitive measure, especially when investigated during cyclic drawing tasks performed with minimal pen pressure (Zeuner et al. 2007). In contrast to the improvement in this variable there was no significant improvement in the WCRS scores following NAS. I suggest that this may reflect the relatively low sensitivity of this functional scale to small differences in hand function due to its relatively broad, and non-specific, nature. To produce additional clinical and functional gains it is likely that repeated or extended sessions of NAS would be necessary. While not examined here, previous research has demonstrated that effects on cortical organisation seen following a single session of afferent stimulation are maintained for 30-60 minutes (McKay et al. 2002). However, repeated sessions of afferent stimulation have been shown to produce longer lasting effects (McKay et al. 2002). This finding opens up the possibility that repeated sessions of NAS may be effective in producing longer lasting reversal of the cortical abnormalities seen in FHD, which may be necessary for more robust functional effects to develop. However, if the primary cause of abnormal cortical organisation

in FHD is excessive plasticity it is unlikely that the approach described here will lead to any permanent normalisation of cortical representations.

5.6. Conclusion

In conclusion, NAS resulted in a decrease in the volume and area of representational motor maps as well as a separation in the CoG of the cortical representations for stimulated muscles. NAS also produced a decrease in the variability of movement profiles during a sensitive cyclic drawing task. These results provide initial evidence that specific patterns of non-associative afferent input are capable of, at least temporarily, reversing the characteristic representational changes seen in FHD. Importantly, the present findings also indicate that such representational changes may be associated with improvements in hand function. Therefore, these findings may be important for the development of novel therapeutic strategies for the treatment of task-specific focal dystonia.

6. General Discussion

This thesis has described the neurophysiological and functional correlates of cortical plasticity in health and disease. TBS and high-frequency rTMS protocols were used to induce plasticity in the M1, S1 and SMA regions of healthy individuals during the manipulation of a small object. The precision grip-lift task was chosen to detect and quantify any resultant functional changes. Following this series of experiments, afferent electrical stimulation was used to induce plastic change in healthy individuals and in those with task-specific focal hand dystonia. Neurophysiological measures were made using the technique of TMS mapping. Functional changes correlating with the induced cortical plasticity were investigated using the precision grip-lift task and a kinematic analysis of handwriting.

The following general discussion presents a synthesis of the major findings arising from this thesis and outlines how these findings contribute to the current knowledge available in this area.

6.1. The induction of cortical plasticity in the human brain: paradigms and potential mechanisms

The human cortex is capable of rapid and significant plastic change. With the advent of new technologies it has become possible to examine the mechanisms underlying a diverse range of plasticity inducing paradigms. In this thesis, three major paradigms were utilised to induce cortical plasticity; high-frequency TBS, rTMS and afferent electrical stimulation. While not tested directly in the experiments outlined in this thesis, a large number of previous studies provide evidence of this form of plasticity being rapidly expressed, temporary and reversible. This observation suggests that reorganisation induced by rTMS, TBS and afferent stimulation results from modifications of pre-existing neural circuitry and does not involve morphological changes such as neurogenesis, synaptogenesis and synaptic remodelling.

Evidence suggests that the reorganisational changes induced by these methods are mediated at the synaptic level through LTP and LTD-like mechanisms. Indeed, the most likely explanation for the increase in cortical excitability seen with associative afferent stimulation, and the decrease seen with TBS, rTMS and non-associative stimulation, is a shift in the balance of excitation and GABA mediated inhibition in the cortex. In the case of associative afferent stimulation (Chapter 4) it is likely that a reduction in tonic inhibition led to an increase in the size and overlap of M1 representational zones through the unmasking of latent horizontal connections (Jacobs and Donoghue 1991; Kaas 1991; Merzenich and Shameshima 1993) and an increase in synaptic efficacy (Baranyi et al. 1991; Aou et al. 1992; Ziemann et al. 1998b). Conversely, non-associative stimulation (Chapter 5) is likely to have increased levels of GABA-mediated inhibition in the cortex, presumably leading to down-regulation of synaptic efficacy and LTD-like changes (Fitzgerald et al. 2005). This hypothesis receives some support from data demonstrating that intra-cortical inhibitory networks are impaired in those with FHD (Ridding et al. 1995; Stinear and Byblow 2004b, 2004c; Bütefisch et al. 2005), a factor which is likely to contribute to the excessive expansion and overlap of cortical representational zones (Sanes et al. 1988; Ridding et al. 1995; Liepert et al. 1998). While not tested in the current thesis, it is feasible that non-associative stimulation may increase levels of inhibition in the affected sensorimotor cortex leading to a normalisation of cortical representations and alleviation of symptoms in those with FHD.

6.2. The functional correlates of rTMS and TBS induced plasticity in the neural control of grasp

The accurate and effective manipulation of objects in our environment is important for learning, completion of everyday tasks, social participation and our ability to function independently. Central to this skill is the ability to select and apply the accurate amount of force needed to grip and lift an object. Selection of the appropriate GF is thought to rely on an

internal feed-forward model (Johansson and Westling 1987; Flanagan and Wing 1997; Flanagan and Johansson 2002). This model, built from prior experience, contains information relating to an object's properties (weight, friction etc), allowing the correct GF to be executed well in advance of sensory feedback and the onset of the lifting movement (Flanagan and Wing 1997; Flanagan and Johansson 2002).

The neural control of grasp relies on a complex network of cortical regions. The development of the precision grip-lift task, in conjunction with the ability to localise cortical disruption using rTMS protocols, has allowed us to examine the role of individual cortical regions in grasp control in healthy and patient population groups. A thorough understanding of the regions involved in the neural control of movement, and the contribution each makes to accurate task completion, may be important for the future development of treatment strategies in those with grasp deficits, including those with stroke, Parkinson's disease and FHD.
In Chapters 2 and 3, TBS and high-frequency rTMS protocols were used to induce temporary disruption of activity in M1, S1 and SMA during a grip-lift task. The grip-lift task is a highly sensitive measure of hand function and allows quantification of the grip force, load force and acceleration present during object manipulation. Based on this, the grip-lift task was chosen to evaluate the functional effects of TBS and rTMS induced plasticity. These experiments are the first to quantify the contribution of S1 and SMA to object manipulation using rTMS and TBS techniques.

In Chapter 2, TBS applied over M1 affected the temporal relationship of the GF and LF profiles. The findings appear to provide evidence for the role of M1 in the anticipatory control of GF scaling. While the exact mechanism underlying such a change is unclear, a logical hypothesis is that disruption to M1 leads to a greater reliance on sensory feedback as the lift progresses. These findings are the first to demonstrate a role for M1 in the anticipatory control

of GF. Interestingly, disruption of S1 did not affect anticipatory GF control. This finding provides the first evidence that sensory information is not required for accurate execution of the anticipatory command. In contrast, a virtual S1 lesion produced an elongation of the preloading phase. While the mechanism underlying this change is again unclear, the results suggest that disruption to S1 may hamper the integration of sensory signals into the motor plan, prolonging the time between initial object contact and the onset of the lift. However, further research into the exact mechanisms underlying these changes is needed before firm conclusions can be drawn.

The results obtained in Chapter 3 demonstrate a role for left SMA in the accurate scaling of peak GF, a role for right SMA in the preloading phase duration and a role for both left and right SMA in the synchronisation of the GF and LF profiles during object manipulation. Of particular interest, is initial evidence demonstrating the hemispheric lateralisation of the dynamic and temporal aspects of the grip-lift task. Based on the current findings it appears that the cortical network involved in GF control and storage of object weight representations is strongly lateralised in the left hemisphere. Conversely, the right hemisphere may play a key role in aspects of the grip-lift task relating to sensory mediated, open loop processing.

While this study is the first to examine the contribution of SMA to object manipulation, comparable findings have been demonstrated in the left anterior intraparietal (AIP; Davare et al. 2007b) and M1 (Nowak et al. 2005) regions. Indeed, these authors reported strikingly similar deficits in GF scaling, and a pattern of hemispheric lateralisation akin to that seen in Chapter 3, following the application of rTMS over left AIP and M1. Taken together with the current findings, this suggests that left SMA, left AIP and M1 may be part of a cortical network involved in the recruitment of object representations required for accurate GF scaling. Within this network, these cortical regions appear to play a complimentary role which

cannot be interchanged. Such a hypothesis is evidenced by the observation that disruption to any of these regions causes a deficit in GF scaling, indicating that the intact regions are unable to compensate for impairments produced elsewhere in the network. Additional research is needed however, before the precise contribution of each of these regions can be elucidated.

As in all research, the studies included in this thesis were not without limitations. In particular, the appropriateness of the sham stimulation is worthy of further discussion. A number of different methods of sham stimulation are used in TMS research and all have limitations. For example, one approach is to stimulate a remote cortical site using a conventional TMS coil. While this approach has the benefit of producing auditory and cutaneous sensations well matched to the active stimulation condition, it will also activate the underlying cortical region. For tasks such as object manipulation which rely on a large, distributed cortical network this method poses obvious problems. In Chapter 2, a sham coil producing an auditory stimulus but which did not induce cutaneous scalp sensations was used. However, given the low intensity of stimulation used in this study (80 % active motor threshold) scalp sensations produced by the active TMS condition are likely to have been minimal, making this form of sham stimulation relatively well matched to the active condition. In Chapter 3 a different sham technique was employed, where the coil is tilted at a 90 degree angle from the scalp. This approach again produces an auditory stimulus but does not result in cutaneous scalp sensations. Thus, none of the currently available methods is perfectly matched to the active TMS condition. Future research into the development of appropriate sham techniques is therefore necessary to improve the methodological rigour of TMS experiments.

Despite these limitations, the novel results illustrated in Chapters 2 and 3 suggest a distributed cortical network for object manipulation with contributions from M1 for anticipatory GF scaling, left SMA for grasp control, left and right SMA for GF and LF synchronisation and S1 for sensorimotor integration and phase triggering. These findings have relevance for our understanding of the mechanisms underlying grasp control in healthy individuals and may also assist in pinpointing problem areas in those with grasp deficits. Further clarity in this area may also provide the basis for novel, therapeutic approaches to improve grasp in those with neurological conditions. For example, in those patients who use significantly greater GFs than necessary to manipulate small objects, excitatory rTMS applied over left SMA may be successful in up-regulating this area and reducing the GF overshoot. Similarly, in patients with an increased preloading phase, up-regulation of S1 may provide an effective therapeutic option.

6.3. The neurophysiological and functional correlates of afferent stimulation in health and disease

Afferent input is known to be a powerful driver of plastic change. In particular, temporally coupled afferent inputs have been shown to increase the excitability of corticospinal projections to stimulated hand muscles, a phenomenon characterised by enlargement and greater overlap of cortical representational zones in animal models (Byl et al. 1996; 1997). The experiments described in Chapter 4 aimed to resolve whether a period of temporally coupled afferent inputs (associative stimulation) applied to either the motor points of two hand muscles or the digits of the hand, produced changes in cortical representations analogous to those seen in animal studies.

Associative stimulation applied to the motor points of healthy subjects led to an increase in corticospinal excitability. TMS mapping revealed that the increase in excitability was

discernible in M1 as an increase in the area and volume of the cortical representation. In addition, the CoG of the cortical representations for stimulated muscles shifted closer together following the period of stimulation. Such changes were not observed following associative stimulation applied to the digits or following a control condition employing non-associative stimulation. These findings are remarkably similar to those obtained using intracortical microstimulation techniques in animal models. Indeed, several studies have shown that increasing the amount of correlated afferent input to the sensory cortex results in expansion and greater overlap of sensory receptive fields (Clark et al. 1988; Godde et al. 1996). Likewise, muscles which co-contract during motor training, and therefore produce convergent patterns of afferent input, are larger and exhibit greater overlap in the motor cortex (Nudo et al. 1996). The novel findings outlined in Chapter 4 expand on these results by providing evidence for the role of correlated afferent inputs in driving representational expansion and CoG shifts in human subjects.

Paradigms which increase cortical excitability have received considerable interest in recent years based on their potential to improve function in those with neurological conditions. For example, previous studies have demonstrated functional improvements in stroke patients following associative afferent stimulation with and without combined rehabilitation therapy (Uy et al. 2003; McDonnell et al. 2007). The experiments outlined in Chapter 4 make an important contribution to this area by illustrating the neurophysiological correlates of plasticity induced by associative afferent stimulation. A clearer understanding of the reorganizational changes accompanying cortical plasticity in humans may be important for the development of future rehabilitation protocols utilising associative afferent stimulation. The results of these experiments also make a significant contribution to our understanding of conditions associated with excessive representational plasticity. Conditions such as FHD are characterised by enlarged and overlapping representational zones, factors which are likely to

contribute to the loss of isolated, dextrous movements common in those with FHD (Byrnes et al. 1998; Bara-Jimenez et al. 1998). These maladaptive plastic changes are thought to be driven in part, by repetitive, tightly correlated afferent inputs (Tempel and Perimutter 1990; Grunewald et al. 1997; Tinazzi et al. 2000). This hypothesis receives some support from the current study. The neurophysiological findings obtained following a period of associative afferent stimulation in Chapter 4 closely mimic the maladaptive representational changes seen in FHD. For example, in both healthy subjects receiving associative stimulation and in FHD, representational zones were larger and exhibited greater overlap when compared with those of healthy controls. These findings provided the basis for the experiments conducted in Chapter 5.

The rationale in Chapter 5 centred around the idea that if associative, synchronous inputs drive representational expansion and greater overlap then non-associative, asynchronous stimulation may drive contraction and separation of these same representational zones. Based on this, I further hypothesised that normalisation of representational zones in those with FHD may be accompanied by alleviation of symptoms and improvements in task performance. This hypothesis is compatible with previous research targeting cortical reorganisation through a motor training technique known as sensorimotor retuning (SMR; Candia et al. 2003; 2005). SMR modulates afferent input entering the cortex using a combination of splinting and sensorimotor retraining techniques. This form of training produces isolated movement patterns in the dystonic hand and has been shown to promote discrete and ordered cortical representations in FHD (Candia et al. 2003; 2005). The studies by Candia and collegues (2003; 2005) support the hypothesis that modulation of afferent input may be of significant importance in the treatment of FHD.

To test the hypothesis that normalisation of cortical representational zones alleviates symptoms and improves performance, subjects with writer's cramp and musician's dystonia received a period of non-associative stimulation delivered to the motor points of two hand muscles. TMS mapping was used to examine the neurophysiological changes arising from the induction of plasticity. Functional improvements were assessed using the grip-lift task and a kinematic analysis of handwriting. The same experimental protocol was repeated in a group of healthy control subjects.

The results of these experiments were two-fold. First, they confirmed that cortical scalp representations obtained with TMS mapping are larger and demonstrate significantly greater overlap in those with FHD when compared with healthy controls. These findings are similar to those obtained from both animal (Byl et al. 1996; 1997) and human studies (Byrnes et al. 1998; Bara-Jimenez et al. 1998) and provide further support for the role of maladaptive plasticity and abnormal representational zones in the pathophysiology of FHD. Importantly, a greater understanding of the pathophysiology underlying dystonic symptoms will assist in directing future research concerned with the development of suitable treatment strategies. Second, the results showed that a period of non-associative afferent stimulation produces contraction and separation of cortical representations in those with FHD. These neurophysiological changes were accompanied by improvements in the variability of movement profiles during cyclic drawing. These findings suggest that non-associative stimulation is successful in normalising representational abnormalities in FHD. In addition, these findings provide initial, limited evidence that normalisation of cortical representations is associated with improvements on a cyclic drawing task. While it must be noted that an association between map changes and improvements in performance does not provide direct evidence of cause and effect, these novel and exciting findings may be important for the development of new therapeutic strategies for the treatment of FHD.

It must be noted however, that the functional improvements seen following non-associative stimulation were small with only one parameter, the coefficient of variation (CV) of the positive velocity peaks, demonstrating a functionally-beneficial change. In addition, the change in the CV of positive velocity peaks was observed only during a cyclic drawing task performed with minimal pen pressure. There are several possible explanations for this finding. Firstly, the CV of positive velocity peaks may be a highly sensitive measure, particularly when examined using a cyclic drawing task. Indeed, previous research demonstrates that cyclic drawing is a more sensitive measure of impairment in those with FHD than handwriting (Zeuner et al. 2007). Secondly, this study employed only a single session of non-associative stimulation. This short application may be responsible for the limited functional improvement observed. However, it seems feasible that repeated sessions of non-associative stimulation may induce longer-lasting reversal of representational abnormalities, producing more robust functional improvements. Thus, future research utilising repetitive periods of non-associative stimulation is certainly warranted.

Finally, as the abnormal representations present in FHD are thought to be driven by excessive representational plasticity coupled with a genetic predisposition, it must be acknowledged that non-associative stimulation is unlikely to provide a cure for FHD. Rather, non-associative stimulation may provide an effective means of alleviating symptoms and improving function and performance in those who suffer from this debilitating condition. Given the paucity of effective treatment strategies available for FHD, symptom alleviation will undoubtedly be a positive step for the successful management of this condition.

6.4. Concluding remarks

The discovery that the brain is plastic is one of the most exciting scientific breakthroughs to occur in recent years. This new understanding has revolutionised the way neuroscientists think and has given rise to a wealth of literature examining the basic mechanisms underlying this remarkable ability. The vast majority of this work is carried out in the hope that we may one day be able to harness brain plasticity to improve the functional outcomes of those suffering from a variety of neurological conditions. Indeed, it is already possible to induce plasticity using specific experimental interventions and the application of these interventions in clinical populations is emerging as a promising research nexus.

This thesis makes an important contribution to this exciting and dynamic area. Using the precision grip-lift task I have demonstrated the functional correlates of rTMS and TBS induced plasticity in M1, S1 and SMA. These results illustrate the differential contributions of each area to successful object manipulation. With further research, these findings may one day contribute to the development of therapeutic interventions for those living with grasp deficits. In addition, I have shown that a period of non-associative afferent stimulation can normalise cortical representations and lead to initial, small improvements in function in those with task-specific focal hand dystonia. As the effectiveness of this paradigm is further elucidated, longer lasting changes in cortical representations and more robust functional improvements may be induced. In the longer term, research investigating the efficacy of this, and other paradigms may lead us to uncover the optimal rehabilitation strategy for those with FHD.

7. Appendices

7.1. Appendix I: Contribution statement Chapter 2

Schabrun SM, Ridding MC, Miles TS. 2008. Role of the primary motor and sensory cortex in precision grasping: a transcranial magnetic stimulation study. Eur. J. Neurosci. 27(3): 750-56.

Contributor	Statement of contribution
Siobhan Schabrun	Experimental design, subject recruitment, data collection, data analysis, interpretation of results, writing of manuscript.
Dr. Michael Ridding	Aided experimental design and interpretation of results, editing of manuscript.
Professor Tim Miles	Aided experimental design and interpretation of results, editing of manuscript.

The authors listed here certify that they agree to the use of the publication in this thesis.

Dr. Michael Ridding

Signature

12/1/09

Date

Professor Tim Miles

Signature

05/01/09

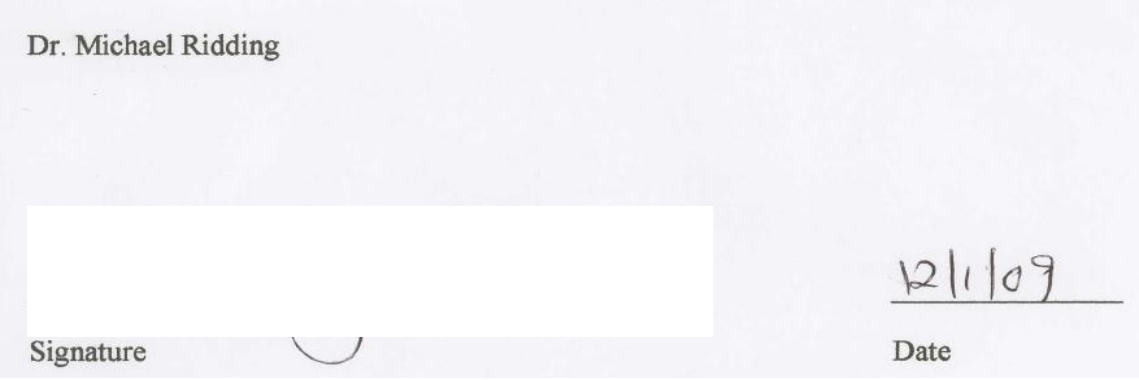
Date

7.2. Appendix II: Contribution statement Chapter 4

Schabrun SM, Ridding MC. 2007. The influence of correlated afferent input on motor cortical representations in humans. Exp. Brain Res. 183: 41- 9.

Contributor	Statement of contribution
Siobhan Schabrun	Experimental design, subject recruitment, data collection, data analysis, writing of manuscript.
Dr. Michael Ridding	Aided experimental design and interpretation of results, editing of manuscript.

The authors listed here certify that they agree to the use of the publication in this thesis.



7.3. Appendix III: Contribution statement Chapter 5

Schabrun SM, Stinear CM, Byblow WD, Ridding MC. 2008. Normalising motor cortex representations in focal hand dystonia. Cereb. Cortex (in press, accepted 28th October 2008).

Contributor	Statement of contribution
Siobhan Schabrun	Experimental design, subject recruitment, data collection, data analysis, interpretation of results, writing of manuscript.
Dr. Cathy Stinear	Aided experimental design, subject recruitment and interpretation of results, editing of manuscript.
Professor Winston Byblow	Aided experimental design, subject recruitment and interpretation of results, editing of manuscript.
Dr. Michael Ridding	Aided experimental design and interpretation of results, editing of manuscript.

The authors listed below certify that they agree to the use of the publication in this thesis.

Dr. Cathy Stinear

08/01/09

Signature Date

Professor Winston Byblow

5/1/9

Signature Date

Dr. Michael Ridding

12/1/09

Signature Date

7.4. Appendix IV: Publications arising from this thesis

Schabrun SM, Stinear CM, Byblow WD and Ridding MC (2008): Normalising motor cortex representations in focal hand dystonia. Cerebral Cortex (*in press, accepted 28th October 2008)*.

Schabrun SM, Ridding MC and Miles TS. Role of the primary motor and sensory cortex in precision grasping: a transcranial magnetic stimulation study. *Eur J Neurosci* 2008 27 (3): 750-756.

Schabrun SM and Ridding MC. The influence of correlated afferent input on motor cortical representations in humans. *Exp Brain Res* 2007 183: 41- 49.

7.5. Appendix V: Other related publications

Schabrun SM, Hillier S. Evidence for the retraining of sensation after stroke: a systematic review. *Clin Rehab* 2009 23: 27-39.

7.6. Appendix VI: Presentations and abstracts arising from this thesis

7.6.1. Presentations

Schabrun S, Stinear CM, Byblow WD, Ridding MC (2008): Separation of motor cortex representational maps is positively correlated with improvements in function in Writer's Cramp patients. Proceedings of the 3rd International Conference on Transcranial Magnetic stimulation and Direct Current Stimulation: Göttingen, Germany 3rd October.

Schabrun S and Hillier S (2008): Evidence for sensory retraining post stroke. Proceedings of the 2008 Stroke Conference: Sydney, Australia 14th August.

Schabrun S (2007): Sensory retraining following stroke. Proceedings of the 7th National Conference of Emerging Researchers in Ageing: Adelaide, Australia 20th November, p.16.

Schabrun S (2007): Disruption to the Precision Grip synergy with Theta burst stimulation. Laboratory of Neurophysiology, School of Medicine, Universite Catholique de Louvain, Brussels, Belgium. September 28th (invited seminar).

Schabrun S (2007): The role of SMA in recruitment of the internal model. Laboratory of Neurophysiology, School of Medicine, Universite Catholique de Louvain, Brussels, Belgium. September 7th (invited seminar).

Schabrun S (2007): Retraining the brain in focal dystonia. Movement Neuroscience Laboratory, Department of Sport and exercise Science, The University of Auckland, Auckland, New Zealand. May 1st (invited seminar).

7.6.2. Published abstracts

Schabrun SM, Stinear CM, Byblow WD, Ridding MC (2008): Separation of motor cortex representational maps is positively correlated with improvements in function in Writer's Cramp patients. Brain Stimulation: 1 (3).

Schabrun SM, Hillier S (2008): Evidence for sensory training post stroke. Internal Medicine Journal: 38 (Supplement 4): 87.

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