HUMAN MOTOR CORTEX ORGANIZATION: HOMUNCULAR PLASTICITY AND ITS MECHANISM

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Abstract

The primary motor cortex (M1) contains a somatotopic progression with highly overlapping areas outputting to muscles of the upper limb. This organization is modified by muscle activity and neurological injury such as spinal cord injury (SCI). To date, bilateral M1 organization in controls and SCI has been minimally explored, and no study has examined the cortical territory that directs output to multiple muscles thought to be involved in movement synergies. An initial study was conducted to characterize the bilateral organization and representational overlap for muscles of the upper limb in incomplete spinal cord injury relative to uninjured individuals. Differences in symmetry and amount of overlapping territory were observed between groups, possibly reflecting differences in synergistic muscle use. The second study examined transcallosal communication between the two motor cortices and its role in dynamically modulating motor representations during unilateral contraction. The depth of interhemispheric inhibition (IHI) was examined in a muscle of the right hand by delivering a conditioning stimulus to ipsilateral M1 followed by a test stimulus to contralateral M1. Reduced IHI corresponded to larger cortical territory, a relationship that existed for both contralateral and ipsilateral contraction. These data demonstrate that the magnitude of IHI in a hand muscle predicts the size of the cortical territory occupied by that muscle. We present a mechanistic model to explain these findings that further elucidate the role of interhemispheric communication in shaping motor output. This interaction between

transcallosal inhibition and motor output may act as a component to experience-dependent plasticity within M1. By targeting this interaction, it may be possible to facilitate motor learning and performance or promote recovery of function following neurological injury. Further study examining the role of various intracortical circuits on representational plasticity and modulation of these interactions may yield advances in both basic and clinical neuroscience.

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List of Abbreviations

ADM – Adductor digiti minimi

AMT – Active motor threshold

ASIA – American spinal injury association

BB – Biceps brachii

CoG – Center of gravity

CS – Conditioning stimuli

EMG – Electromyography

FCR – Flexor carpi radialis

FDI – First dorsal interosseous

fMRI – Functional magnetic resonance imaging

IHI – Interhemispheric inhibition

IHI_{INT} – Interhemispheric inhibition interneuron

LIHI – Long latency interhemispheric inhibition

LTP – Long-term potentiation

TS – Test stimuli

M1 – Primary motor cortex

MEG – Magnetoencephalography

MEP – Motor evoked potential

MSO – Maximum stimulator output

MVC -Maximum voluntary contraction

PET – Positron emission tractography

RMT – Resting motor threshold

rTMS – Repetitive transcranial magnetic stimulation

S1 – Primary somatosensory cortex

SCI – Spinal cord injury

SICI – Short latency intracortical inhibition

SIHI – Short latency interhemispheric inhibition

TMS – Transcranial magnetic stimulation

Chapter 1 Review of the Literature

Organization of the Motor Cortex

The primary motor cortex (M1) is situated just anterior to the central sulcus with a finger-like appearance stretching laterally from the vertex within the precentral gyrus. This region of the cortex is characterized by the presence of large pyramidal neurons in layer V with long axons that descend through the spinal cord to synapse with lower motor neurons (Dum and Strick 2002; Maier et al. 2002). These descending projections from M1 are the largest contributor to motor output as evidenced by the greater density of corticomotor synapses with alpha motor neurons in the spinal cord from M1 projections compared to those originating in other motor areas (Dum and Strick 2002; Maier et al. 2002). By probing the intact brain, the organization of M1 has been shown to be somatotopic with discrete regions of cortex devoted to separate body parts. Early electrical mapping of the human brain was conducted in the late 1930's when Penfield and Boldrey mapped the motor cortex of patients using electrical stimulation during neurosurgery (Penfield and Boldrey 1937). Their findings indicated a predictable pattern such that the face, hand, arm, torso, and legs were represented in a medial to lateral orientation along the precentral gyrus (M1), respectively (Penfield and Boldrey 1937). However, the size of the individual muscle representations was unrelated to the physical size of the body parts. For example the representation of the lips and hands had unusually large representations while proximal muscles of the limbs and torso had small representations (Penfield and Boldrey 1937). The disproportionate territory for certain body parts such as the hands and face within this homuncular organization is thought to be due to the fine motor control required by those body regions (Penfield and Boldrey 1937; Schieber 2001). This idea is further supported by examination of the corticospinal projection density to these muscles where muscles of the hand are more densely innervated than those of the upper arm (Feinstein et

al. 1955). Thus, a relationship exists between cortical topography within M1 and motor output to the body.

Neuroimaging the Human Motor Cortex

Since the time of Penfield's neurosurgeries, many technologies have been developed for non-invasively probing the human brain. These include techniques such as magnetic resonance imaging (MRI), magnetoencephalography (MEG), positron electron topography (PET), and transcranial magnetic stimulation (TMS). These advances have expanded the capacity for probing the organization and function of the human motor cortex as researchers can more readily study the living brain in controlled settings. Extensive work looking at the activation within M1 during various voluntary motor tasks have been conducted (Grafton et al. 1991; Guye et al. 2003; Kleinschmidt et al. 1997; Rao et al. 1995). In attempts to characterize the somatotopy of the human M1, PET has been used to record blood flow through radioactively labelled oxygen molecules in the brain during motor tasks using the toes, fingers, or tongue (Bruehlmeier et al. 1998; Grafton et al. 1991). They found that gross organization demonstrated a predictable organization within M1 with medial to lateral locations of toe, finger, and tongue activation, respectively (Grafton et al. 1991). PET is a logical means to examine cortical activation as neuronal activity increases metabolic activity. However, the metabolic changes that occur lag behind the actual firing of neural components, resulting in poorer temporal resolution (Hallett 2007). The use of MEG to image the motor cortex is an attractive tool as it records the small magnetic fields produced by neuronal activity of the brain. This technique has also confirmed the predictable homuncular pattern during voluntary activity of various muscles (Cheyne et al. 1991). However, this method has low spatial resolution due to the inability to pick up radial sources of electrical activity (Hallett 2007). Studies utilizing fMRI, which measures blood flow within the brain via strong magnetic fields have also

demonstrated somatotopic organization with the foot, elbow, and hand being located from medial to lateral along the precentral gyrus (Rao et al. 1995; Schieber 2001). High definition fMRI was the first to statistically quantify properties of single muscle representations by calculating the center of mass of the representations of finger muscles (Beisteiner et al. 2001; Kleinschmidt et al. 1997). Notably, the individual finger representations followed patterned somatotopy as indicated by the original motor homunculus. Yet, significant overlap between the representations of finger muscles has been observed with fMRI (Beisteiner et al. 2001; Donoghue and Sanes 1994; Kleinschmidt et al. 1997; Pascual-Leone et al. 1995a). However, the blood oxygenation that is tracked with MRI is secondary to neuronal metabolism, making it a more indirect method of tracking neural activity. Thus, despite the strengths and weaknesses of each technique, multiple neuroimaging tools have been used to confirm the overall pattern observed by Penfield's electrical stimulation studies; suggesting that the presence of a gross motor homunculus is reliably present.

Implementation of PET, MEG, and MRI measures make accurate resolution and quantification of discrete muscle representations difficult due in part to the temporal or spatial resolution afforded to each or the reliance on indirect metabolic measures to infer neural activity (Walsh & Cowley, 2000; Hallet, 2007). It is also important to note the cost of utilizing these techniques to collect and analyze brain scans. However, the use of TMS to non-invasively probe the human brain with high temporal and spatial resolution is relatively cost-effective (Hallett 2007; Walsh and Cowey 2000). This tool was introduced 1985 and provides a means to non-invasively and painlessly stimulate areas of the brain, producing evoked potentials by eliciting neuronal discharges down the corticospinal tract (Barker et al. 1985). This contrasts with many neuroimaging techniques such as MRI or PET that measure metabolic changes that are secondary to neuronal firing (Cheyne et al.

1991; Grafton et al. 1991). TMS has allowed researchers to examine the organization of various cortical regions as well as probe mechanisms of plasticity (Siebner and Rothwell 2003a), making it a viable candidate for extensive characterization of M1 topography. In fact, the accuracy of TMS to explore organization of M1 in humans has been demonstrated to be similar to locating representations with PET (Wassermann et al. 1996)and fMRI (Boroojerdi et al. 1999; Lotze et al. 2003). Thus, TMS is a cost-effective tool to reliably assess M1 circuitry outputting to various corresponding body parts.

Transcranial Magnetic Stimulation

The introduction of non-invasive magnetic cortical stimulation techniques has allowed researchers to gain new insight into the organization of the human motor cortex. The method by which TMS activates cortical tissue involves a coil housing that contains coiled wires. When a large electrical current is supplied to these circular wires, a magnetic field is induced perpendicular to the plane of the coil. This magnetic field will subsequently produce a perpendicular electrical field in a direction opposite to the original field created by the stimulating coil. Typically, the TMS coil housing such coiled wires is held tangentially to the scalp. Thus, the induced magnetic field will pass through the scalp and cortical tissue with up to approximately 2 Tesla (Hallett 2007). Subsequent electrical currents can be induced in excitable material or mediums such as cortical neurons within the magnetic field. These currents flow in loops parallel to the stimulating coil that may excite neural tissue to elicit neuronal depolarization (Barker et al. 1985; Hallett 2000; Hallett 2007; Siebner and Rothwell 2003a). Thus, TMS can be used to non-invasively produce cortical activity as the magnetic field is capable of passing through the scalp with little interruption (Hallett 2000). The shape of the coil is an important factor in the use of TMS since the field strength of

induced currents is strongest along the circumference of the coil, directly below the coiled wires. Below the center of the coil, where there is no wire, there is almost no induced current (Hallett 2007). Consequently, a round coil may produce distributed currents in underlying tissue while figure of eight coils may evoke a focal current at the intersection of two coiled wire components (Hallett 2007). With regards to M1, a TMS pulse may excite enough cortical neurons to generate an action potential in a descending corticomotor neuron that will activate the corticospinal tract and ultimately produce activity within a muscle of the body. This brief muscle contraction is known as a motor evoked potential (MEP) and can be observed via surface electromyography (EMG) (Hallett 2000; Siebner and Rothwell 2003a). It is through this phenomenon that the organization of the body within M1 can be examined using TMS.

Mapping the Human Motor Cortex with Transcranial Magnetic Stimulation

By applying multiple TMS pulses, typically with a figure of eight coil, to the motor cortex across a grid system or within a predefined area, the cortical tissue corresponding to skeletal muscles can be identified by analyzing the presence and amplitudes of MEPs (Rossini et al. 2015). These motor representations correspond to the cortical territory devoted to each muscle of the body and can be characterized by a number of features. Properties include the area of representation (typically given in cm^2), center of gravity, and overlap with other muscle representations (van de Ruit and Grey 2016). Each of these characteristics affords information regarding the output to the muscle being mapped. A muscle with a smaller area of representation may have a lesser corticospinal output to that muscle (Freund et al. 2011), this phenomenon may be explained by the differences in density of corticospinal projections as evidenced by greater innervation ratios to muscles typically involved in fine motor control (Feinstein et al. 1955). The center of gravity of a motor

representation corresponds to the amplitude weighted center of the map and is commonly used to define the location of a motor representation (Thickbroom et al. 1999). Lastly, the overlap between two motor representations can be assessed by finding the proportion of each motor representation's total area that is shared with the motor representation for another muscle (Jono et al. 2015; Marconi et al. 2007; Melgari et al. 2008). Together, these properties have given researchers insight into the organization of cortical territory within M1. However, the characteristics of motor representation's may be influenced by aspects of stimulation and analysis rather than the true organization of M1. Modulation of area, CoG, and overlap can occur as a result of inconsistent or inappropriate map generation parameters. Namely, stimulation intensity (Day et al. 1989; Hess et al. 1987; Kiers et al. 1993; Rothwell 1991; Thordstein et al. 2013), state of the muscles being tested (Hess et al. 1987; Ngomo et al. 2012; van de Ruit and Grey 2016), and data analysis methods (Uy et al. 2002; Wassermann et al. 1992).

Effect of Stimulus Intensity

The stimulus intensity used to create motor maps will impact properties such as the area as increasing stimulator output will presumably recruit greater numbers of motor neurons (Devanne et al. 1997). This may expand the motor representation area as corticomotor neurons with lower thresholds will have a higher probability of contributing to an motor representation with greater TMS intensities (van de Ruit and Grey 2016). Stimulus-response relationships for various muscles have demonstrated a general sigmoidal shape often known as a recruitment curve (Carroll et al. 2002; Devanne et al. 1997; Rossini et al. 2015). This sigmoid curve begins where no response is observed and begins to increase its slope at the intensity corresponding to motor threshold. The MEP amplitude increases rather linearly through a range of approximately 120% RMT to 140%

RMT before plateauing (Rossini et al. 2015). Recently, the effects of increasing stimulus intensity on the motor representation for an intrinsic hand muscles has been reported (van de Ruit and Grey 2016). These researchers systemically mapped out the cortical representation of the FDI muscle of the hand at 110%, 130%, and 130% and observed that the area of the motor representation increased linearly without altering the CoG or shape (van de Ruit and Grey 2016). However, previous work has demonstrated that the motor representation area may respond uniquely in separate motor representations when increasing stimulus intensity (Thordstein et al. 2013). Therefore, it is important to select consistent stimulation intensities if group comparisons of motor representation area will be performed.

Effect of Resting Versus Active Contraction of the Muscle

The state of the muscle is an important consideration when mapping the motor cortex because it will determine the latent activity within spinal motor neurons during stimulation, contributing to the variability of MEP responses (Kiers et al. 1993; Siebner and Rothwell 2003a). Thus, maps generated in the active condition are sometimes utilized as they normalize spinal motor neuron synaptic activity (Siebner and Rothwell 2003a). When using this method, it is typical for slight muscle activation, around 5-10% of maximum voluntary contraction to be sustained (Byrnes et al. 1999; Wilson et al. 1993). In comparison to maps generated at rest, it has been shown that map area is larger and the CoG shifts when the target muscle maintains low level contraction (Wilson et al. 1995). Yet, when the intensity of stimulation is relative to active motor threshold rather than resting motor threshold reports have shown the map area and CoG location is unchanged (Ngomo et al. 2012). A recent study evaluated the methodological differences in cortical motor mapping by examining motor representation properties of an intrinsic hand muscle in various levels of

contraction (van de Ruit and Grey 2016). The effects of such modulations showed that the area and volume of the motor representation increased linearly with greater muscle contraction with no change in CoG or map shape (van de Ruit and Grey 2016). However, it is unclear whether observed differences with muscle contraction is due to the increased excitability of the corticospinal system or due to reductions in motor threshold in the contracted state (van de Ruit and Grey 2016). Therefore, active contraction may yield a more accurate depiction of cortical representations of the muscle but care should be taken in establishing the level of contraction and stimulus intensity of active mapping if comparisons to resting maps are to be made.

Effect of Data Analysis Methods

Lastly, the criteria used to determine the inclusion of MEPs and map characteristics impact the resultant motor representations. It is typical for an amplitude cutoff to be used to define the presence or absence of an MEP (Uy et al. 2002; van de Ruit and Grey 2016; Wassermann et al. 1992; Wilson et al. 1993). Others have examined MEPs on the basis of their amplitude relative to the baseline EMG activity during a pre-stimulus time window (Devanne et al. 2006; Freund et al. 2011). By rooting the MEP inclusion criteria to the level of background muscle activation, it is likely a more accurate depiction of physiological responses. Additionally, the calculation methods of map parameters, particularly area are influential to final motor representation descriptors. Many researchers use a threshold value relative to the maximum MEP elicited (Uy et al. 2002; Wilson et al. 1993) or relative to the maximum compound muscle action potential evoked with supramaximal nerve stimulation (Levy et al. 1990; Streletz et al. 1995; Wassermann et al. 1992). One such threshold may allow for any scalp sites with an average MEP area exceeding 12.5% of the maximum average MEP in that map to be counted in area calculation (Thickbroom et al. 1999).

However, it has been shown that varying thresholds based on maximum response do show area changes with stricter criteria (closer to 100% maximum response) producing smaller motor representations yet criteria do not change the variability of motor representation area across participants (Uy et al. 2002). In summary, it is critical to set clearly defined MEP inclusion criteria and methods of motor representation attribute calculations.

Motor Representations in Healthy Individuals

Early human mapping studies using TMS highlighted the organization of the motor cortex with regards to various muscles of the upper limb as a function of MEPs from muscles of the upper arm (Metman et al. 1993; Wassermann et al. 1992; Wilson et al. 1993). Similar to the findings of Penfield, the layout of M1 when probed with TMS has shown a rough overall somatotopic organization in the medio-lateral plane (Metman et al. 1993; Penfield and Boldrey 1937; Wassermann et al. 1992; Wassermann et al. 1994). For example, the FDI was found to be lateral to biceps, which was also lateral to the deltoid representation (Wassermann et al. 1994). This supports the notion of a motor homunculus in that the face laterally neighbors the hand region of M1, which is subsequently lateral to the proximal arm representations. Furthermore, the disconnect between muscle size and motor representation area was also replicated using TMS; the representation of the hand and face was large compared to proximal upper arm muscles (Metman et al. 1993; Wassermann et al. 1992; Wassermann et al. 1994; Wilson et al. 1993). Thus, similar to other imaging tools (Cheyne et al. 1991; Grafton et al. 1991; Kleinschmidt et al. 1997) it appears that the original motor homunculus is reproducible when assessed via TMS. However, rather than being discrete representations within M1, TMS cortical mapping studies have shown considerable overlap between motor representations of various muscles (Metman et al. 1993; Wassermann et

al. 1992; Wassermann et al. 1994; Wilson et al. 1993). The maps of two intrinsic hand muscles were shown to be highly overlapped with the thumb motor representation being shared by the adductor digiti minimi muscle (Wilson et al. 1993). These two muscles are situated in close proximity to each other and may have some collaborative functions in hand posture and movement. However, other studies have revealed considerable overlap between muscles of the hand and of the upper arm such as biceps brachii or deltoid that do not share a straightforward relationship in location nor function (Devanne et al. 2006). Therefore, the organization of the motor cortex does follow a predictable, somatotopic pattern overall with complex connectivity within and between motor representations of the cortex. By studying the effects of various stimuli on motor representation plasticity, we may gain insight into the functional relevance of motor cortical representations. Perhaps allowing them to be utilized as a diagnostic tool in motor learning and rehabilitation.

Motor Representation's Following Neurological Injury

Spinal Cord Injury

Spinal cord injury involves damage to the central nervous system (CNS) at a spinal level involving either incomplete or complete transection of the spinal cord. This mechanism of injury may yield considerable reorganization in M1 as the corticospinal tract, and thus communication between the cortex and the body, has been impaired. Researchers have been interested in identifying organizational changes within the cortical tissue in efforts to predict or rehabilitate functional recovery. The CNS has a restricted ability to regenerate and it is common to see cortical changes in response to spinal cord injury as the lesioned areas of the spinal cord do not completely

regenerate. Following transection of the spinal cord, cortical representations within the motor cortex become denervated and the ability to communicate to skeletal muscles via efferent outputs becomes impaired. Plastic changes within the cortex may help those with SCI maintain and strengthen preserved connections or regain some function in impaired muscles.

TMS Evoked Motor Representations Following Spinal Cord Injury

Studies examining TMS evoked motor representations have shown reorganization following spinal cord injury in humans (Brouwer and Hopkins-Rosseel 1997; Cohen et al. 1991; Cortes et al. 2016; Levy et al. 1990; Streletz et al. 1995). For example, early case reports of motor representation plasticity following SCI used TMS to noninvasively map the motor cortex of two individuals with traumatic quadriplegia during intensive physiotherapy (Levy et al. 1990). In both cases, the representations of the biceps and triceps of the arm, which were the most caudally spared muscles, were significantly larger than the maps of the same muscles in normal controls. The authors propose that this cortical takeover is linked to residual function and that the enlargement of upper arm muscle motor representations was likely facilitated by the extensive therapy they were performing (Levy et al. 1990). These findings were later replicated with muscles innervated by spinal levels immediately proximal to the injury site demonstrating enlarged cortical territories (Cohen et al. 1991). Further study revealed the difficulty in cortical mapping in SCI patients due to the discovery that it may not be possible to elicit MEPs at all in severely impaired muscles when TMS was delivered in the resting state (Brouwer and Hopkins-Rosseel 1997; Streletz et al. 1995). Thus, active contraction during motor representation generation has allowed for improved mapping in this population as the threshold for an MEP response is reduced with low-level sustained contraction (Freund et al. 2011; van de Ruit and Grey 2016). Although the addition of

active contraction may help in eliciting responses from impaired muscles, the enlargement of motor representation areas in preserved muscles and shrinking of motor representation areas in impaired muscles is maintained (Brouwer and Hopkins-Rosseel 1997; Freund et al. 2011). Thus, the typical result of reorganization in chronic SCI seems to be that the cortical territory that corresponded to the denervated muscles get smaller and begin to reorganize to innervate neighboring muscles to which the descending tracts are still functionally intact (Cohen et al. 1991; Freund et al. 2011; Streletz et al. 1995).

Insights into the Timeline of Motor Representational Map Reorganization

The outlined studies in chronic SCI populations have succeeded in identifying the presence of reorganization within human sensorimotor cortices. However, the timeline or rate of this reorganization remains unclear. A series of cases reporting motor representation changes in the acute stages of cervical incomplete SCI have shed some light on the timeframe of reorganization (Streletz et al. 1995). The patients were tested 6-17 days following cervical injury and reorganization had already occurred. Similar to previous work (Cohen et al. 1991; Levy et al. 1990), the biceps representations were significantly larger in SCI patients compared to controls (Streletz et al. 1995). Thus, in the acute phase of injury, cortical changes have already occurred. This rapid reorganization of M1 is supported by work in able-bodied subjects that were given anesthetic nerve block of the median nerve (Rossini et al. 1996). This group used TMS to generate motor representations of the adductor digiti minimi (ADM) and FDI muscles of the hand before and after application of an anesthetic block of the median and radial nerves at the wrist. When the anesthetic prevented peripheral communication between the hand and cortical tissue the motor representation of the first digit interosseous (FDI) muscle had a reduced cortical area (Rossini et

al. 1996). This observation supports the theory that cortical maps are maintained by tonic sensory input and integration within M1 since FDI is innervated by the ulnar nerve which was not blocked. Therefore, the authors conclude that the lack of sensory input to the cortex from the dorsum of the hand drives changes in M1 motor maps as the sensory, proprioceptive information from the FDI muscle was still intact (Rossini et al. 1996).

Therefore, although the exact timeframe requires further study, it has consistently been seen that cortical reorganization occurs following SCI. However, the reorganization within M1 seems to be loosely constrained as the degree of overlap in motor maps is still large and retains the overall somatotopic organization post-injury (Cohen et al. 1991; Freund et al. 2011; Levy et al. 1990; Streletz et al. 1995). Despite shrinking of motor maps for distal muscles that have lost function following SCI, the encroachment of the cortical territories of proximal muscles that are spared into the deprived cortical regions does not disturb this overall organization of M1 (i.e. proximal muscles of the upper arm located medially followed by the hand and face areas more laterally). Future works should attempt to identify a relationship between motor representation properties and muscle function. Any such relationship may support the opportunity to quantify motor output to specific muscles as a means of identifying and guiding potential strategies.

Neural Mechanisms of M1 Reorganization Following Neurological Injury

Hebbian Learning

It is clear from examination of cortical motor maps that reorganization of M1 occurs following SCI. However, the exact physiological mechanism behind this cortical reorganization after SCI

has not been established. Levy and colleagues speculated that reorganization is due to sensory loss and may occur due to a number of neurophysiological responses (Levy et al. 1990). First, they proposed that reorganization may be due to strengthening of preexisting synapses within M1 due to disinhibition of connections between sustained and denervated cortical regions. A second hypothesis is collateral sprouting of corticospinal axons rostral to the lesion level to innervate more proximal, spared muscles (Levy et al. 1990). However, their work with TMS mapping could not differentiate between the two. More recently, considerable work to establish the mechanism of cortical plasticity, and more specifically synaptic plasticity has been performed (Buonomano and Merzenich 1998). The framework under which current hypotheses on synaptic plasticity operates is that of Hebbian learning. That is, synaptic strength between neurons that fire together are strengthened while those that do not fire together are not reinforced (1950). It is thought that this mechanism of synaptic strengthening occurs for neurons that fire together temporally, potentially explaining the somatotopic organization that is seen in many primary sensorimotor cortices as they receive input from neighboring parts of the body and often recruit synergistic or neighboring muscles to respond to such stimuli (Buonomano and Merzenich 1998; Clark et al. 1988). Additionally, this theory can be expanded to encompass changes that occur following SCI as denervated cortical regions lose synaptic strength and spared cortical inputs strengthen surrounding connections to expand their cortical representations. The exact mechanism of this associative strengthening is known as long-term potentiation (LTP) which involves strengthening of neuronal connections that fire together. It is thought that this strengthening is due to the detection of coincident firing by the NMDA glutamate receptor which mediates Ca^{2+} permeability to induce LTP (Crair and Malenka 1995). This phenomenon has allowed several specific mechanisms of cortical reorganization following SCI to be proposed.

Plasticity of Cortical Regions

This associative plasticity is largely thought to cause reorganization by altering the activation of horizontal inhibitory pathways within M1 (Hess and Donoghue 1994). Predating human mapping studies of M1, a group of researchers examined changes in the somatotopic map of S1 in primates following median nerve transection (Merzenich et al. 1983). They found that S1 reorganized as the 3rd and 4th digits of the hand, that were still supplying sensory input, expanded into the denervated region of the cortex that previously corresponded to digits 1-3. Over time, this expansion continued until the entire denervated area was supplied by spared sensory inputs from the 3rd and 4th digits. The authors interpreted their findings as the unmasking of pre-existing horizontal synapses within S1 that were disinhibited as a result of median nerve transection (Merzenich et al. 1983). Similar circuits are likely present in M1 as it has extensive connections with S1. Studies such as these have not been performed in humans due to the invasive nature of the protocols. However, one area that provides compelling evidence for horizontal connectivity as the driving factor for cortical organization has been demonstrated in humans undergoing surgical reversal of congenital digital syndactyly (Mogilner et al. 1993). When mapping S1 using magnetoencephalography (MEG), the cortical representations of the fused fingers (syndactyly) had very extensive overlap prior to surgery. After the surgery, mapping revealed that the cortical territories of the two previously fused fingers reorganized to move apart from each other (Mogilner et al. 1993). It may be that similar mechanisms of reorganization in the human motor cortices occurs with SCI, but in the opposite direction as gross motor output tends to be left intact with fine motor control being lost. Thus, functional relevance of such horizontal disinhibition and

encroachment on deprived regions of the cortex following SCI is thought to be adaptive as it allows for better cortical control of spared muscles.

Plasticity of Subcortical Regions

Another possible mechanism of reorganization involves subcortical changes within circuitry of the spinal cord. This type of adaptation is likely more prevalent in incomplete rather than complete SCI as parts of the ascending and descending tracts remain intact. The view of the motor system as a highly distributed system comprised of both serial and parallel pathways throughout descending tracts may explain reorganization observed in motor representations (Raineteau and Schwab 2001). Under this construct, reorganization within subcortical pathways may occur following transection of corticospinal tracts. In particular, the red nucleus has been shown to undergo plastic changes following lesions to the corticospinal tract in animal models (Belhaj-Saïf and Cheney 2000; Z'Graggen et al. 2000). These changes have been demonstrated to be due to axonal sprouting and may provide a means of compensation through the rubrospinal tracts for transmission of motor commands through alternate pathways to partially recover motor function (Raineteau and Schwab 2001). Other locations of subcortical plasticity include the corticospinal tract itself. When incomplete spinal cord lesion is induced in animal models, collateral sprouting of preserved descending fibers has been demonstrated, although this reorganization appears to be more prevalent in young, developing animals (Bregman and Goldberger 1983; Kuang and Kalil 1990). This collateral sprouting may contribute to functional recovery or maintenance of motor ability following incomplete spinal cord injury. The mechanisms of collateral sprouting and axonal growth in adults is relatively unclear although developmental models in animals suggest that neurotrophic factors facilitate axonogenesis and that attracting factors direct new axon collaterals

to the denervated tissues (Gallo and Letourneau 1998; Schnell et al. 1994). Thus, subcortical anatomic plasticity may play a role in establishing extensive connections to spared muscles. This increased innervation to proximal muscles after SCI may provide part of the explanation for cortical reorganization of M1 that has been demonstrated in this population.

Therefore, there are a number of available mechanisms and pathways that contribute to cortical reorganization of the cortex. By identifying the extent and location of cortical reorganization in certain clinical populations, such as SCI, we may be able to target those areas using various rehabilitative technologies. This may provide a means to promote reversals of the observed changes in the sensorimotor cortices to facilitate increased cortical output and/ or functional capacity and performance of impaired movements.

Functional Relevance of TMS Evoked Motor Representational Maps

The differences in M1 topography has been confirmed in humans with spinal cord injury (Cohen et al. 1991; Freund et al. 2011; Levy et al. 1990; Streletz et al. 1995). Yet, any relationship between representational organization within M1 and muscle function or capacity is an area of little study in human research (Freund et al. 2011; Melgari et al. 2008; Pearce et al. 2000; Tyč et al. 2005). However, animal literature has more definitively exposed a number of factors that may alter the properties of motor representation's in conjunction with skilled motor performance on a task (Kleim 2004; Kleim et al. 1998; Nudo et al. 1996a; Remple et al. 2001). Identification of specific motor representation properties such as area, CoG, and overlap changes following improvements

in skilled motor capacity will impact the use of representational plasticity in human rehabilitation following injury or disease.

Animal Studies Relating Motor Representations and Motor Function

The effects of behavioral modification on representational plasticity within M1 has been examined in more detail using animal models (Barbay et al. 2013; Kleim 2004; Kleim et al. 1998; Nudo et al. 1996a; Remple et al. 2001). Motor training in non-human primates involving long-term use of fine digit manipulation versus gross grasping to obtain food from differentially sized wells has shown to increase motor representation areas for the digits (Nudo et al. 1996a). Similar work utilizing skilled or unskilled reaching in rodents has demonstrated expansion of forelimb and wrist motor representation areas (Kleim et al. 1998). Thus, it seems that representational plasticity can be manipulated through acquisition and practice of complex motor tasks. The specificity of motor experience that evokes changes in motor representations has also been considered. When rats were strength trained, control trained, or untrained for a reach and grasp task, both the strength and control trained rats experienced expansion of the forelimb motor representation areas (Remple et al. 2001). Thus, it appears that skilled performance, and not strength training is influential to motor representation characteristics (Adkins et al. 2006; Remple et al. 2001). When looking at a model of cortical injury in rodents, skilled training reduces the motor representation area of the more impaired limbs (Barbay et al. 2013). This may be due to improved motor coordination following training such that rodents are no longer as reliant on compensatory mechanisms that may have caused expansion of motor representation area and overlap involved with co-contractions. Therefore, animal models offer the opportunity to speculate at the types of motor experience that modulate motor representation plasticity in humans. Further study is required to identify the impact

and application of skilled behavioral modification and cortical plasticity in humans. This may provide an avenue for motor representation targeted rehabilitation following spinal cord injury or other movement disorders.

Human Studies Relating motor representations and Motor Function

A handful of human studies have used motor tasks to assess changes in TMS-evoked motor representations (Melgari et al. 2008; Pearce et al. 2000; Tyč et al. 2005). First, by mapping multiple muscles of the upper limbs in right-handed healthy individuals, motor representation differences were observed in the overlapping territory (Melgari et al. 2008). Specifically, they reported higher overlap for motor representations of wrist and forearm muscles and relatively smaller overlap for upper arm and hand muscles. Demonstrating that the overlap of motor representations may play a role in synergistic muscle activations (Melgari et al. 2008). Furthermore, hemispheric differences illustrated significantly larger overall overlap in the dominant hemisphere (left) which the authors attributed to long-term differences in skilled practice of the dominant and non-dominant arms (Melgari et al. 2008). Thus, the overlapping nature of motor representation's is likely involved with synergistic motor performance, although muscle function was not assessed in this study. The impact of motor behavior on motor representations in humans is further supported by comparing motor representations of elite athletes and control subjects (Pearce et al. 2000; Tyč et al. 2005). High-level badminton players were seen to have larger motor representation areas for the hand muscles with a lateral shift in the CoG in the dominant playing hand (Pearce et al. 2000). Comparisons of the arm muscle motor representations of elite volleyball players to runners has highlighted expansion of the motor representation area for medial deltoids and a greater overlap between the deltoids and forearm muscles in the highly trained volleyball players (Tyč et al. 2005).

These results suggest that the changes observed in motor representations of highly skilled individuals are due to chronic, highly skilled, motor practice of the muscles being tested. Lastly, examination of cortical changes following injury may work through similar mechanisms. Lack of skilled training may reduce motor representation areas or shift the CoG within M1. This phenomenon may be supported by changes in residual muscle function following SCI and cortical output as assessed by motor representations. Although this functional relationship has not been extensively studied to date, it has been shown that the AMT and cortical silent period observed in a muscle is inversely related to the amount of spinal cord atrophy at the injury site (Freund et al. 2011). Therefore, there seems to be a link between muscle function via skilled motor performance and motor representation properties. Although these studies did not ascribe long term motor training to individuals, they highlight reorganizational changes in motor representation properties due to long-term motor behavior or injury. The use of motor training to induce motor representation changes has been much more prevalent in non-human research.

Unanswered Questions

The innovation of non-invasive methods of brain imaging have allowed human brain somatotopy to be extensively examined. Through the use of TMS, an efficient, cost-effective analysis of representational organization and plasticity within M1 can be explored. To date, however, many questions remain to be answered regarding the use of specific representational properties to assess the functional capacity of the muscles. For example, how do changes in specific motor representation characteristics influence motor output to the muscles and their subsequent functional capacity? What factors are influential in shaping and defining motor representation properties? This thesis aims to address these questions as the answers could implicate the use of

strategic rehabilitative interventions to promote plastic changes in M1 at the representational level to benefit motor control.

Chapter 2 Experiment 1: Altered overlapping organization within bilateral motor cortex following spinal cord injury

Abstract

The primary motor cortex (M1) contains a somatotopic progression with highly overlapping areas outputting to muscles of the upper limb. This organization is modified following spinal cord injury (SCI) and can be characterized via transcranial magnetic stimulation (TMS). To date, bilateral M1 organization in controls and SCI has been minimally explored, and no study has examined the cortical territory that directs output to multiple muscles thought to be involved in movement synergies. This study examines the bilateral organization and representational overlap for muscles of the upper limb in incomplete spinal cord injury relative to uninjured individuals. Nine individuals with chronic cervical SCI (46.1 ± 12.5 yrs; two female) and nine age, sex and handed-matched controls (40.3 ± 13.5 yrs; two female) were studied. TMS was delivered over a 6x5 point grid anchored to the C3 electrode position using a TMS intensity specific to the resting motor threshold for biceps, flexor carpi radialis, and abductor pollicus brevis muscles obtained in each participant. For each muscle and hemisphere, the cortical territory (cm^2), overlapping territory (cm^2) of the target muscles, and center of gravity ($CoG_{(AP,ML)}$) were computed. Hemispheric asymmetries were observed in controls such that muscle representations were more laterally located in the non-dominant versus dominant hemisphere. In contrast, these locations were symmetrical in SCI. Second, overlapping cortical territory was greatest for all three muscles in controls and greatest for only two muscles following SCI. Our findings support a highly-overlapped organization of M1 that is preserved following SCI. However, the extent of representation overlap varies between groups, possibly reflecting differences in synergistic muscle use. M1 organization appears to reflect use-dependent preference for the dominant limb in controls (i.e. hemispheric asymmetry) and increased reliance on bimanual movement following SCI (i.e. hemispheric symmetry).

Introduction

Non-human primates demonstrate a complex organization of muscle representations within the primary motor cortex (M1) due to cortical territory that simultaneously innervate multiple muscles (Donoghue et al. 1992; Park et al. 2001; Schieber and Hibbard 1993). Such overlapping muscle representations are thought to allow for coordinated upper limb movement (Graziano and Aflalo 2007). Cortical territory allocated to the hand is represented centrally and surrounded by territory dedicated to the forearm (Park et al. 2001). In human M1, a medial to lateral progression of proximal to distal muscles of the upper limb is reported via functional magnetic resonance imaging (fMRI) (Beisteiner et al. 2001; Boroojerdi et al. 1999; Hlustik et al. 2001; Lotze et al. 2003) and transcranial magnetic stimulation (TMS) (Wassermann et al. 1992; Wilson et al. 1993). However, deviations from somatotopy are also reported, as forearm territory is found both medial and lateral to the hand region (Meier et al. 2008). Further, TMS data reveal extensive overlap between upper limb muscle representations (Devanne et al. 2006) thought to reflect regions of cortex that contribute to synergistic movements involving multiple muscles (Jono et al. 2015; Schieber 2001). Using fMRI, the representational overlap of hand muscles may be predicted by the usage of the hand (Ejaz et al. 2015). Thus, the organization of primate M1 appears to include largely intertwined cortical territories for muscles of the upper limb (Devanne et al. 1997; Holdefer and Miller 2002; Meier et al. 2008; Nudo et al. 1996a).

Learning and task-experience modifies M1 organization in humans (Pascual-Leone et al. 1993; Pascual-Leone et al. 1995a; Pearce et al. 2000; Tyč et al. 2005), monkeys (Nudo et al. 1996a), and rats (Adkins et al. 2006; Kleim et al. 1998). It is therefore likely that muscle representations differ between hemispheres as a reflection of the use of the dominant versus non-dominant limb. In

humans, the dominant hemisphere (i.e. contralateral to the dominant upper limb) has a longer central sulcus (Amunts et al. 2000) and demonstrates greater movement-related activation (Dassonville et al. 1997) relative to the non-dominant hemisphere. Cortical territory is larger for proximal arm muscles in the non-dominant hemisphere (Wassermann et al. 1992) but is similar across hemispheres for intrinsic hand muscles (Wilson et al. 1993). Whether the two hemispheres differ in somatotopy and/or muscle representation overlap remains to be explored. However, given the lifelong preference of one hand over the other it is likely that experience modifies and creates asymmetries in the homologous M1 representations according to usage (Adkins et al. 2006; Buonomano and Merzenich 1998; Nudo et al. 1996b; Pascual-Leone et al. 1995a).

Neurological injury also modifies muscle representations in human M1 (Brouwer and Hopkins-Rosseel 1997; Cohen et al. 1991; Cortes et al. 2016; Freund et al. 2011; Levy et al. 1990; Streletz et al. 1995). Following incomplete spinal cord injury (SCI), damage to the corticospinal tract may lead to reorganization within M1. For example, cortical territory, as assessed by TMS, increases following complete transection of the spinal cord (Cohen et al. 1991; Levy et al. 1990; Streletz et al. 1995) and does not change following incomplete SCI (Brouwer and Hopkins-Rosseel 1997; Cortes et al. 2016). Further, muscle representations within M1 may shift (Freund et al. 2011) or not change (Brouwer and Hopkins-Rosseel 1997; Cohen et al. 1991; Cortes et al. 2016; Levy et al. 1990; Streletz et al. 1995) after SCI. To date, no TMS study has quantified the cortical territory dedicated to overlapping representations in clinical populations, yet this is thought to be a dominant feature of M1 organization (Meier et al. 2008; Schieber 2001) that is considered to underpin muscle synergies (Graziano and Aflalo 2007). Further, research has yet to examine hemispheric asymmetries in SCI that are likely to reflect experience-dependent plasticity

associated with different movement dynamics in this population compared to uninjured controls. For example, it has been demonstrated that greater reliance on bimanual movement during daily activities often accompanies neurological injury such as SCI (Brogioli et al. 2016) (i.e. manual wheelchair operation).

The goal of the present study was to explore and compare the organization of bilateral M1 muscle representations of the upper limb in chronic incomplete cervical SCI and uninjured controls. TMS was used to quantify the size of cortical territories, cortical territory overlap, and somatotopy.

Methods

Participants

Eighteen individuals, nine SCI (46.1 ± 12.4 years) and nine age and gender matched uninjured controls (40.3 ± 13.5 years) participated ($T_{(16)} = 0.941$, $p = 0.360$) (Table 1.). SCI participants presented with chronic (defined as a minimum of 2 years post-injury) incomplete injury at the level of C3 – T1 with a classification on the American Spinal Injury Association (ASIA) scale of C (Kirshblum et al. 2011) (Table 1). The study conformed to the declaration of Helsinki and was approved by the Hamilton Integrated Research Ethics Board. All individuals provided written consent prior to participation.

Electromyography (EMG)

EMG was recorded bilaterally from biceps brachii (BB), flexor carpi radialis (FCR), and abductor pollicus brevis (APB) muscles using surface electrodes (9 mm diameter Ag-AgCl). Recordings from BB and FCR used a bipolar montage over the muscle belly. EMG from APB was measured

via a monopolar configuration with one electrode placed over the muscle belly and the other over the metacarpal-phalangeal joint. All EMG recordings were band-pass filtered between 20 Hz and 2.5 kHz, amplified 1000x (Intronix Technologies Corporation Model 2024F, Bolton, Canada), and an analog-to-digital interface was used to digitize recordings at 5 kHz (Power1401, Cambridge Electronics Design, Cambridge, UK).

Maximum voluntary contraction

MVC was obtained to identify the dominant arm and to assess the volitional output to the specific muscle. Maximum voluntary contraction (MVC) was obtained for APB, FCR, and BB bilaterally via maximal isometric contraction of the target muscle against an immoveable apparatus. Trials were 5 s in duration with a 1 min break between each trial. Three trials were performed for each muscle. The largest peak-to-peak EMG amplitude was identified from these trials and determined to be the MVC for that muscle. In addition, a modified handedness questionnaire was performed (Oldfield 1971). Seven SCI participants identified as right-hand dominant (before and following injury), while the remaining were left-handed (one of whom was right-handed prior to the injury). Control participants were matched to SCI individuals for age, gender and handedness. In all cases, the largest MVC for each muscle was obtained from the limb identified as the dominant arm as per the results of the handedness questionnaire.

M1 Mapping Protocol

All TMS delivery was done with a customized 50-mm diameter figure-of-eight branding coil connected to a Magstim Plus stimulator (Magstim, Whitland, UK). When delivering TMS to M1, motor responses can be obtained from the muscle via EMG that are known as motor evoked

potentials (MEPs). Figure 1A displays a sample 6x5 point grid space (5 cm from medial to lateral and 4 cm from anterior to posterior) with 1 cm spacing. Using Brainsight Neuronavigation (Rogue Research, Montreal, Canada), the grid was centered on a location 1.5 cm medial to C3 (International 10-20 system). The grid placement allowed for cortical territory extending in the anterior-posterior direction to center on the precentral gyrus while encroaching minimally on the premotor (anterior) and somatosensory (posterior) cortices. The grid was rotated 45 degrees from the sagittal plane to approximate the orientation of the central sulcus. During stimulation, the coil was always oriented 45 degrees from the sagittal plane. Following grid placement, the motor hotspot was determined for each muscle by delivering a single pulse per grid point at an intensity corresponding to 80% of maximum stimulator output (MSO). The location in the grid whereby the largest peak-to-peak MEP was measured for the target muscle was identified as the motor hotspot (i.e. location for APB, location for FCR, etc.). Next, resting motor threshold (RMT) was obtained at the motor hotspot for each muscle and was defined as the percentage of the MSO that produced a MEP of ≥ 50 μV peak-to-peak amplitude in 5 out of 10 consecutive trials (Rossini et al. 2015). This procedure was performed for each muscle in each hemisphere. The subsequent TMS intensity for mapping was set to 120% RMT specific to the muscle tested (Figure 1A, right). Maps were generated by delivering three TMS pulses to each grid point with an interstimulus interval of 5 s (i.e. a total of 9 stimuli were delivered to each grid point; 3 at 120% RMT for BB, 3 at 120% RMT for FCR, and 3 at 120% RMT for APB). In all cases, 120% RMT never exceeded 100% MSO. This procedure was performed over both hemispheres simultaneously (i.e. alternating hemispheres for each grid point stimulated) to obtain maps moving lateral to medial over the left hemisphere and medial to lateral over the right hemisphere.

Analysis of M1 maps

Individual MEPs were included in analysis if the peak-to-peak amplitude was ≥ 150% of the peak-to-peak EMG signal obtained during a 30 ms interval prior to the TMS pulse. This was done to eliminate trials that either failed to elicit an MEP or contained background EMG activity. All MEPs that did not pass this criterion were assigned a value of zero, indicating no MEP response for the given pulse (Wassermann et al. 1992). Subsequently, the peak-to-peak MEP amplitudes were averaged for the three TMS stimuli at each grid point (including the zero value for lack of MEP) (Wassermann et al. 1992). The cortical territory dedicated to a given muscle (in cm^2) included only grid points that elicited MEPs exceeding 10% of the maximum MEP for that muscle (Figure 1B) (Uy et al. 2002; van de Ruit and Grey 2016; Wilson et al. 1993). Centre of gravity (CoG) for each map was calculated to obtain the amplitude weighted center in the anterior-to-posterior (CoG_{AP}) and medial-to-lateral (CoG_{ML}) orientations (Figure 1B) (Equation 1: $CoG_{(AP)} = \Sigma\alpha_i X_i / \Sigma\alpha_i$ (Wassermann et al. 1992) where α_i is the average amplitude at a given grid point and X_i is the position (row or column number) of the point). The Euclidean distance was calculated between the CoG and the motor hotspot for each muscle representation (Equation 2: $Distance_{(x,y)} = \sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2}$, where CoG is ($X_1$, Y_1) and motor hotspot is (X_2, Y_2)). The overlapping cortical territory (cm^2) was calculated as the sum of the number of grid points that elicited responses from multiple muscles (Figure 1C). For example, in Figure 1C, 'No overlap' refers to the 8 cm^2 territory dedicated to one muscle representation only (i.e. APB, FCR, BB), 'Two overlap' is the 8 cm^2 dedicated to the representation shared by any two muscles, and 'Three overlap' is the 5 cm^2 that is shared by three muscles.

Statistical Analysis

Each dependent measure was assessed for normality (Shapiro-Wilks) and outliers. Non-normal data was ranked and assessed using a Conover's ANOVA (Conover and Iman 1982). RMT, cortical territory (cm^2), CoG, MVC, and motor hotspot location were compared using three-way ANOVA with between-subject factor GROUP (2 levels: SCI, Control) and within-subject factors MUSCLE (3 levels: APB, FCR, BB) and HEMISPHERE (2 levels: Dominant, Non-Dominant). Cortical territory overlap was assessed using a three-way ANOVA with between-subject factor GROUP (2 levels: SCI, Control) and within-subject factors COMBINATION (3 levels: No overlap, Two overlap, Three overlap) and HEMISPHERE (2 levels: Dominant, Non-Dominant). Follow-up analyses investigated 'Two overlap' via two-way ANOVA with between-subject factor GROUP (2 levels: SCI, Control) and within-subject factor DUAL COMBINATION (3 levels: APB-FCR, FCR-BB, APB-BB). Significant main effects and interactions were assessed using Tukey's HSD for normally distributed data, while Mann Whitney-U and Wilcoxon Signed Rank were used for non-normally distributed data. Lastly, to assess whether cortical territory predicts muscle function, correlational analyses were performed on the cortical territory and the MVC for each muscle for the SCI and control groups. Effect sizes for all significant findings were calculated using Cohen's d. Results exceeding a level of significance of $\alpha < 0.05$ are reported.

Results

All participants successfully completed the experiment. In the SCI group, data was not obtained from the APB muscle in two participants, from the FCR muscle in one individual and the BB muscles from one individual. In these instances, TMS set to 100% MSO did not evoke MEPs in resting muscle.

For RMT, three-way ANOVA revealed a main effect of GROUP ($F_{(1, 16)} = 7.42$; $p = 0.015$) plotted in Figure 2A. The RMT was greater in SCI participants compared to controls ($p < 0.001$, $d = 0.71$) in support of elevated RMT reported elsewhere (Brouwer and Hopkins-Rosseel 1997; Cortes et al. 2016). These data indicate a reduction in corticospinal excitability in upper limb muscle representations in the SCI group. Assessment of MVC revealed a main effect of GROUP ($F_{(1,12)} = 6.07$, $p = 0.029$) plotted in Figure 2B such that the MVC was smaller in SCI compared to controls.

The group-averaged cortical territory occupied by each muscle is shown in Figure 2C (cm^2 with standard error). Corresponding Conover's ANOVA revealed a GROUP*MUSCLE interaction ($F_{(2, 29)} = 6.25$; $p = 0.005$) such that cortical territory was larger for the FCR muscle in SCI compared to controls ($p = 0.005$, $d = 1.11$). Statistical analyses indicated no differences in cortical territory between hemispheres or between muscles. This can be seen in the individual data such that cortical territory does not differ greatly across hemispheres or muscles within controls (Figure 3) or SCI (Figure 4).

Cortical territory overlap is shown in Figure 2D and corresponding Conover's ANOVA revealed a GROUP*COMBINATION interaction ($F_{(2, 32)} = 10.796$; $p < 0.001$). Post-hoc analyses revealed that, in controls, a similar amount of cortex was devoted to No, Two and Three muscle overlap (Figure 2C). In SCI, No overlap and Two overlap were greater than Three overlap ($p = 0.014$, and 0.003 and $d = 0.62$ and 0.99, respectively) (shown as # on plot). This pattern held true for 6 of the 9 SCI participants (Figure 4). Between groups, SCI had greater cortical territory representing No overlap ($p = 0.016$, $d = 0.80$) and Two overlap ($p < 0.001$, $d = 0.99$), but demonstrated reduced Three overlap ($p = 0.024$, $d = 0.60$; Figure 2C) (shown as * on plot) compared to controls.

Statistical analyses indicated no differences in overlap between hemispheres. Additional analyses were performed to explore whether the cortical territory overlap differed for any of the Two overlap combinations tested (i.e. APB-FCR, APB-BB, FCR-BB overlap). A main effect of GROUP ($F_{(1,16)} = 21.04$, $p < 0.001$) was observed indicating that all dual muscles combinations were greater in SCI compared to controls ($p < 0.001$, $d = 0.47$; Figure 2E).

The group-averaged CoG (with standard error) for each hemisphere and group is plotted in Figure 5A. The general location of the CoG coordinates was similar between hemispheres for the SCI group (right) while different patterns emerge for controls (left). For $CoG_{(ML)}$ a GROUP *HEMISPHERE ($F_{(1, 16)} = 10.94$; $p = 0.004$) interaction was revealed such that the control group exhibited laterally shifted CoG in the non-dominant versus dominant hemisphere ($p < 0.001$, $d = 1.51$) as shown in Figure $5B_i$. This trend can be seen in the individual data in Figure 3 in each uninjured participant. To further examine the lateral shift, the location of the motor hotspot was examined. Three-way ANOVA on the location of the motor hotspot revealed a GROUP*HEMISPHERE ($F_{(1,15)} = 6.29$, $p = 0.024$) interaction similar to CoGy such that the control group demonstrated motor hotspots that were laterally shifted in the non-dominant vs. dominant hemisphere as shown in Figure $5B_{ii}$ ($p = 0.002$, $d = 0.94$). In contrast, no hemispheric differences in $CoG_{(ML)}$ or motor hotspot were observed for the SCI group (Figure $5B_{iii}$ & $5B_{iv}$). To determine whether the lateral shift in non-dominant CoG in controls was observed because of a greater distance between the $CoG_{(ML)}$ and the motor hotspot, we computed the Euclidean distance between these two locations for each muscle in both hemispheres. Results of the three-way ANOVA (Factors: GROUP, MUSCLE, HEMISPHERE) were non-significant (Figure 5C). Collectively, these data indicate that the distance between the CoG and motor hotspot is preserved

in both hemispheres for both groups. For $CoG_{(AP)}$ no statistical differences were revealed in the ANOVA (Figure 5D). Differences in the $CoG_{(ML)}$ between groups are plotted in Figure 5E and F. For the non-dominant hemisphere (Figure 5E (left), the CoG in the SCI group was medial relative to controls ($p < 0.001$, $d = 1.71$). This was not the case for the dominant hemisphere as shown in Figure 5E (right).

Correlational analyses between cortical territory and MVC were performed to assess whether the size of a representation within M1 is associated with the capacity for motor output to that muscle. Bivariate correlations were performed on all six muscles pooled for each group (SCI and controls) since MVC differed between groups and not between muscles. There was no significant association between cortical territory and maximal output in the control group (Figure 6A). In contrast, a significant negative correlation was observed for the SCI group (Figure 6B) such that smaller cortical territory was related to greater MVC ($r = -0.390$, $p = 0.013$). This relation indicates that regardless of muscle, greater denervation following injury (i.e. smaller MVC) is associated with greater cortical territory allocated to that muscle (Figure 6B).

Discussion

The present study examined bilateral cortical organization in uninjured participants and individuals with chronic incomplete cervical SCI. Several novel findings were revealed that provide new information regarding hemispheric asymmetries in the uninjured human M1 and the reorganizational changes that manifest following SCI. We discuss these findings, their potential mechanisms, and their implications for understanding and promoting reorganization following SCI.

Cortical Territory Overlap within M1

To our knowledge this is the first study to quantify cortical territory overlap in human M1. Our data yielded interesting results. In controls, greater cortical territory there was dedicated to three muscle overlap compared to that seen in SCI. It is thought that synergistic movements are associated with cortical territory that outputs to all muscles involved in that action (Holdefer and Miller 2002; Jono et al. 2015). For example, in monkey M1, single neurons direct output to multiple muscles that are involved in synergistic movements (Holdefer and Miller 2002). In humans, MEPs in forearm muscle are modulated by contraction of shoulder muscles (Devanne et al. 1997) in support of synergistic overlapping representations in M1 for control of the upper limb (Devanne et al. 2006; Devanne et al. 1997). Therefore, the extensive overlap of all three muscles seen in uninjured controls likely reflects the interconnectedness of M1 representations for upper limb muscles that allow coordinated, synergistic movement. In contrast, the SCI data indicate that M1 territory is largely dedicated to dual muscle overlap compared to single or three muscle overlap. In line with the notion that cortical territory overlap promotes muscle synergies, the SCI data suggest that reductions in three muscle overlap may contribute to motor impairments (Barroso et al. 2015). Reduced synergistic muscle activity in SCI relative to controls is observed during grasping (Zariffa et al. 2012) and locomotion (Barroso et al. 2015; Zariffa et al. 2012). However, we note that overlapping representations remain dominant in SCI, albeit they are dissimilar from the three muscle overlap seen in controls. These data support previous research indicating that human M1 organization is highly overlapped for muscles of the upper limb (Graziano and Aflalo 2007; Holdefer and Miller 2002; Tyč et al. 2005) and indicate that the nature of the overlap is altered following SCI.

Differences in Single Muscle Territory

A second novel observation was found in the uninjured control group; muscle representations in the non-dominant hemisphere were lateral compared to those in the dominant hemisphere regardless of whether the CoG or motor hotspot was quantified (Figures 5C & D). To our knowledge this is the first observation of such asymmetry, and this was observed in all uninjured participants (Figure 3). Previous studies using TMS have not reported such asymmetries although maximum TMS intensity was used (Tyč et al. 2005; Wassermann et al. 1992; Wilson et al. 1993) that may obscure differences in CoG (Devanne et al. 2006). In the present study, the TMS intensity was tailored to the specific threshold for a given muscle, allowing subtle differences to be revealed. Further, and critical to our observation of asymmetry, previous studies used a 'free form' grid-centered approach (Uy et al. 2002; Wassermann et al. 1992; Wilson et al. 1993) not designed to expose hemispheric asymmetries. By anchoring the grid to C3, we examined comparable anatomical space from each of the two hemispheres. Hemispheric asymmetry in controls is reported elsewhere such that the central sulcus is longer (Amunts et al. 2000) and fMRI activity is greater (Dassonville et al. 1997) in the dominant versus non-dominant hemisphere. In contrast, hemispheric asymmetries were not observed within the SCI group (Figures 5D & E) and we speculate that relative symmetry may be due to plasticity associated with increased long-term use of the non-dominant limb after SCI. Increased reliance on the non-dominant limb for the purpose of bimanual arm movements during activities of daily living have been demonstrated following SCI (Brogioli et al. 2016).

In SCI, muscles demonstrating expansion relative to controls are those involved in fine control and stability of the hand and wrist (i.e. FCR). Further, we observed an association such that the larger the cortical territory allocated to a muscle, the lower the MVC of that muscle (Figure 6B). We suggest that, in SCI, this organizational relationship between cortical territory and MVC may reflect adaptations within M1 to compensate for muscle function. Thus, we speculate that the expansion in SCI may reflect a heightened opportunity for plasticity within this specialized hand/wrist zone. In M1, cortical territory expansion occurs for muscles involved in the acquisition of motor skills (Nudo et al. 1996a; Pascual-Leone et al. 1993; Pascual-Leone et al. 1995a), and also for muscles that are essential for skilled sport performance (Tyč et al. 2005). Therefore, we suggest that the expansion observed in the SCI group, relative to controls, may serve to maximize neural output to these muscles that, despite being functionally impaired, are integral to tasks of daily living (i.e. wheelchair operation). One alternative explanation may be that expansion is compensatory and reflects reductions in spinal plasticity. Impaired spinal plasticity is suggested to explain impaired motor learning capacities observed in SCI (Bloch et al. 2016; Lungu et al. 2010; Vahdat et al. 2015). Further, trans-synaptic upper motor neuron recruitment is impaired due to altered intracortical activation within M1 (Cirillo et al. 2016) and this may contribute to the expansion of cortical territory seen in those with SCI.

Implications for Rehabilitation

In SCI, synergistic muscle activity that closely resembles that of uninjured controls is a strong predictor of functional recovery (Barroso et al. 2015; Zariffa et al. 2012). Here, we show that the territory dedicated to three muscle overlap is reduced in chronic SCI and, although we do not specifically test an intervention, the information obtained from this study may guide future

rehabilitative approaches. Based on the finding of reduced three muscle overlap, retraining synergistic multi-joint movements of the upper limb may serve to increase the interconnectedness and overlapping nature of cortical representations within M1. Specifically, we show reduced three muscle overlap for the APB, FCR, and BB muscles following SCI and training using tasks involving elbow and wrist movement such as drinking, teeth brushing and racquet sports may simultaneously engage these muscles. The ultimate goal of this approach would be to improve function by, potentially, increasing the cortical territory overlap dedicated to these three muscles. One rehabilitation method approach known as activity based therapy focuses on facilitating weight-bearing, functional synergistic movements (Harness et al. 2008; Jones et al. 2014; Quel de Oliveira et al. 2016) and has greater functional outcome compared to standard physiotherapy for the upper limb in SCI (Quel de Oliveira et al. 2016). However, such techniques may not be appropriate when dealing with severe levels of impairment. With greater impairment, functional electrical stimulation of the denervated muscles have demonstrated beneficial outcomes whereby stimulation is delivered to highly impaired muscles alongside activation of intact muscles to produce synergistic activation of the upper limb during task performance such as reaching and grasping (Popovic et al. 2006). However, cortical reorganization resulting from these therapies has not been explored. Therefore, similar to synergistic EMG profiles (Barroso et al. 2015; Zariffa et al. 2012), the differences in cortical territory overlap between those with SCI and uninjured controls may act as a biomarker for recovery or allow us to tailor rehabilitation to optimize outcomes in individuals with SCI.

Practical Implications

This is the first study to quantify differences in the cortical territory overlap between those with SCI and uninjured controls and using this approach, we can advance our understanding of the interplay between muscle representations and its value in treatment and recovery from neurological injury. Second, our data indicate that mapping the location of representations using motor hotspot (i.e. 1 maximal pulse at each grid point) does not differ significantly from the CoG data and may yield a more time efficient, simpler approach to mapping if the location of muscle representations are of interest.

Limitations

In general, our data demonstrates very little evidence of somatotopic delineations that may be attributed to the small distances between the CoG for each muscle representation (i.e. ~ 5 mm between APB and BB in Figure 5E). These small differences cannot be resolved easily with TMS (Brasil-Neto et al. 1992), as somatotopy has only been observed previously for FDI and anterior deltoid muscles when the CoG differences exceeded 1.2 cm (Devanne et al. 2006). The present study mapped flexor muscles and it is unclear whether different muscle combinations would yield the same results. For example, it is possible that muscles controlling the shoulder will also share overlapping territory with the other muscles tested, and if reduced overlap in SCI includes the proximal shoulder muscles, this may suggest that therapies aim to synergistically engage all upper limb joints (i.e. movements such as in swimming) should be pursued. Further, we include a sample size of 9 participants, that, while exceeding the sample sizes of previous reports (Brouwer and Hopkins-Rosseel 1997; Cohen et al. 1991; Cortes et al. 2016; Freund et al. 2011; Levy et al. 1990; Streletz et al. 1995), is relatively small, limited mainly by the availability of participants. Last, we mapped individuals with SCI who were taking their regular medications to control spasticity and

pain. It remains unclear how these conditions and their treatment may contribute to cortical reorganization following injury.

Conclusions

Uninjured controls demonstrate asymmetry in the anatomical location of upper limb muscle representations that is not observed in SCI. Further, the cortical territory allocated to muscle representations and their overlap differs between SCI and controls, and may reflect experience associated with synergistic movements of the upper limb. However, despite these differences the data from both populations indicate that human M1 is largely comprised of overlapping muscle representations. Importantly, the data provide evidence to suggest that rehabilitation via multi-joint synergistic movement may induce cortical gains in overlapping territory that predicts or improves upper limb function in those with SCI.

Tables and Figures

Table 1. Participant Demographics

Sub	*Age*	*Sex*	*Years Since Injury*	*Injury Level*	*ASIA Score*	*Handedness*	*Medications*	*Control Sub*	*Age*	*Sex*	*Handedness*
1	26	M	4.5	C5-C6	C	L*	fesoterodine	1	25	M	R
2	41	M	2	C6-C7	C	L	diazepam, pregabalin, cyclobenzaprine	9	45	M	L
3	39	M	39	C5	C	R	none	3	38	M	R
4	39	M	14	C6-C7	C	R	baclofen	4	27	M	R
5	47	F	17	C4-C8	C	R	botulinum toxin, percocet	7	42	F	R
6	68	M	3	C4	C	R	baclofen, pregabalin	6	68	M	R
7	55	F	2	C3-C4	C	R	gabapentin, citalopram	8	48	F	R
8	58	M	33	C6-C7	C	R	baclofen, clonazepam	4	27	M	R
9	45	M	7	C5	C	R	none	5	43	M	R

Note: ASIA scale = American Spinal Injury Association Impairment Scale; A = No sensory or motor function preserved in sacral segments; B = Sensory function is preserved with no motor function; C = Sensory function is preserved below the level of injury, most muscles below injury gave a grade less than 3; D = Motor function is preserved below the level of injury, most muscles below injury have a grade of 3 or more; E = Normal sensory and motor function. * indicates that this participant switched handedness following SCI.

Figure 1.

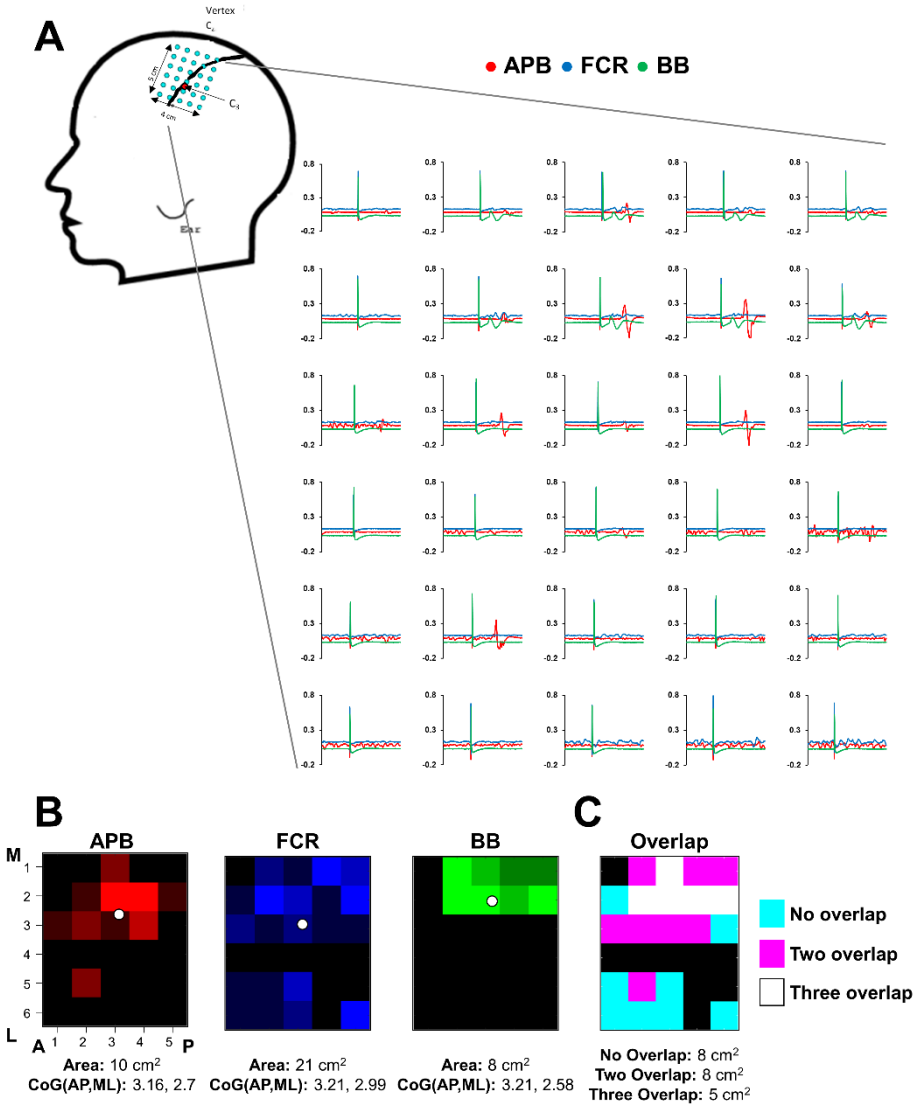

Figure 1. Sample data from a SCI participant for generation of cortical territories and their overlap. A) Raw traces are shown for each muscle at each grid point and B) the resulting cortical territories are depicted with values of area and CoG coordinates. C) The corresponding overlap and associated values for No overlap, Two overlap, and Three overlap are displayed. White circles represent the CoG for each cortical territory.

Figure 2

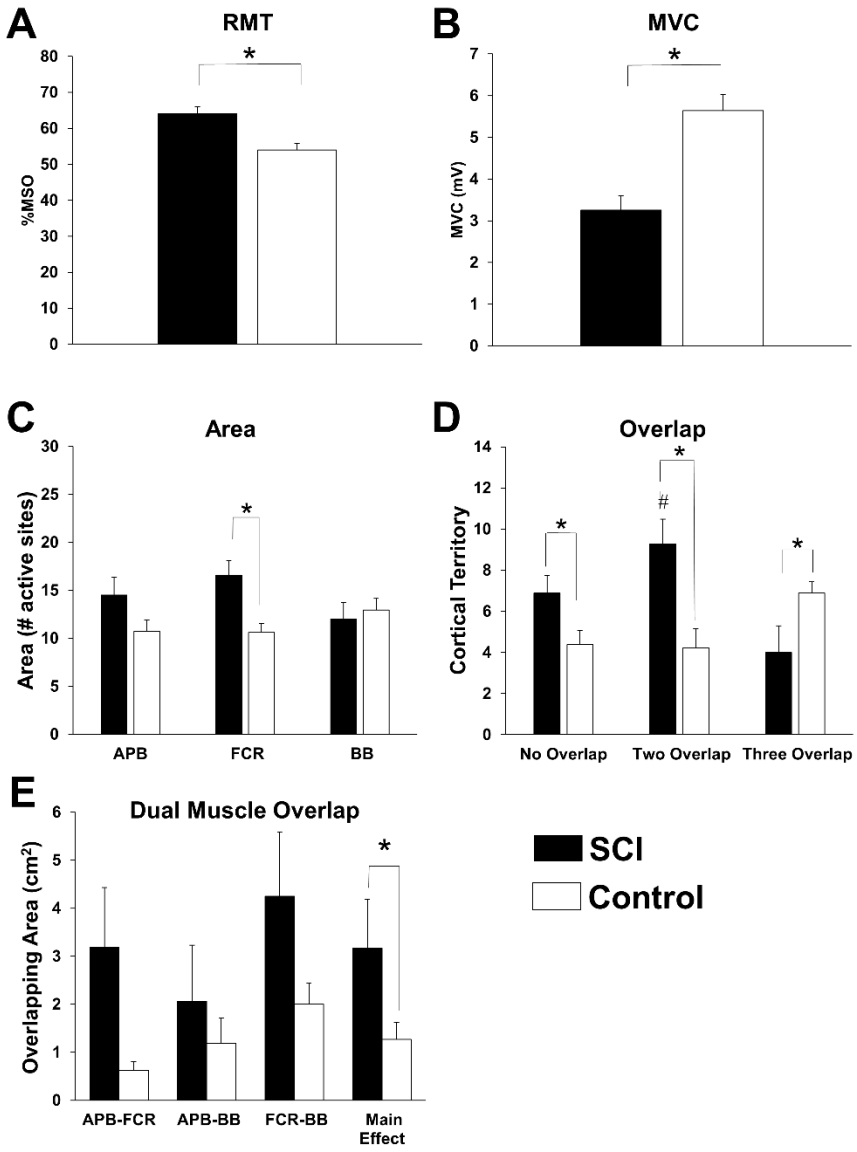

Figure 2. A) The main effect of GROUP revealed that the SCI group had larger RMTs in the relative to controls ($p < 0.001$). B) For all muscles, the SCI group had a smaller MVC than controls ($p = 0.029$). C) The FCR muscles demonstrate larger map areas (defined as the number of active grid points) in the SCI group relative to controls ($p = 0.005$). D) Overlapping area differed in the SCI group such that Three overlap was smaller than either No overlap or Two overlap ($p = 0.014$

and 0.003). Differences were seen between groups as No overlap and Two overlap was larger in SCI ($p = 0.016$ and $p < 0.001$, respectively) while Three overlap was smaller in SCI ($p = 0.024$). E) The dual muscle overlap for specific combinations is displayed. A main effect of GROUP revealed greater dual muscle overlap in the SCI group ($p < 0.001$). * indicates a significant difference between groups. # indicates a significant difference within the SCI group.

Figure 3

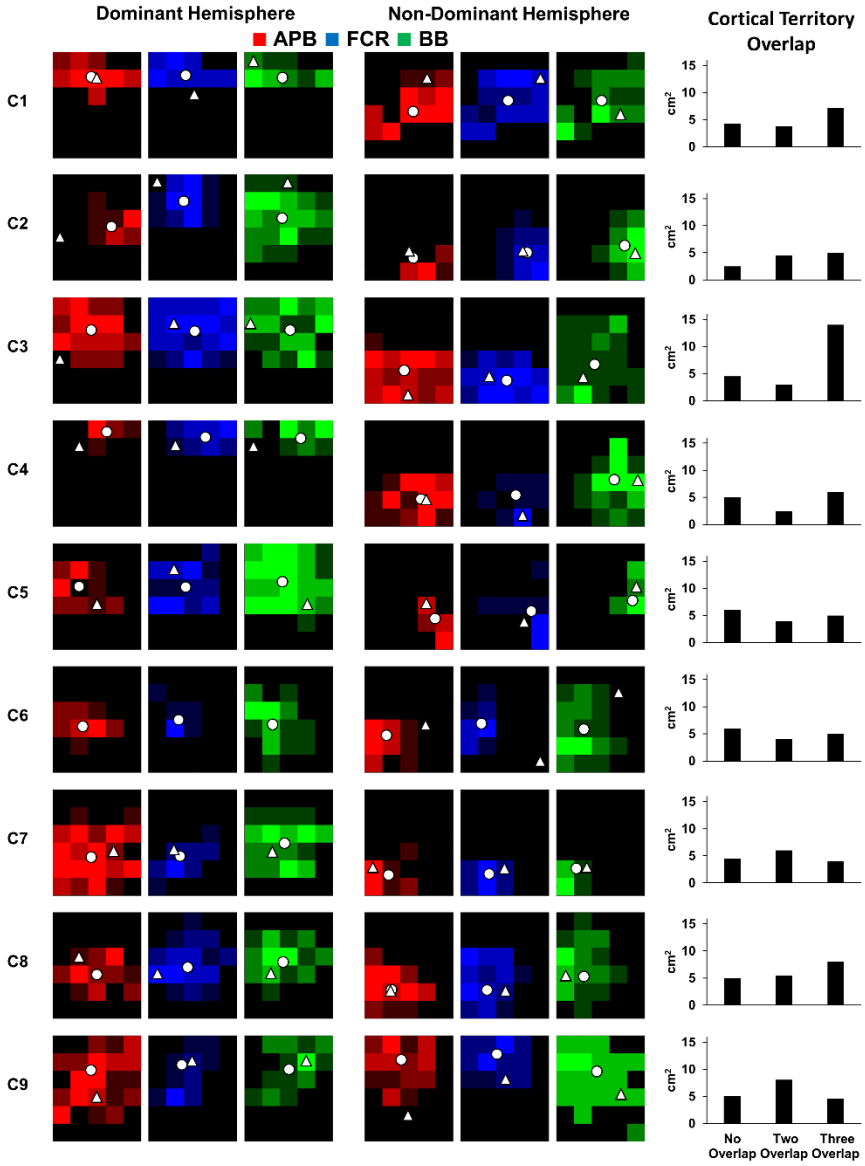

Figure 3. Individual cortical territory plots for each hemisphere and muscle for the control group. Pixel maps represent the size of the cortical territories and the white circle and triangles indicate the location of the CoG and motor hotspot, respectively. Histograms represent the magnitude of each pattern of overlap for every individual.

Figure 4

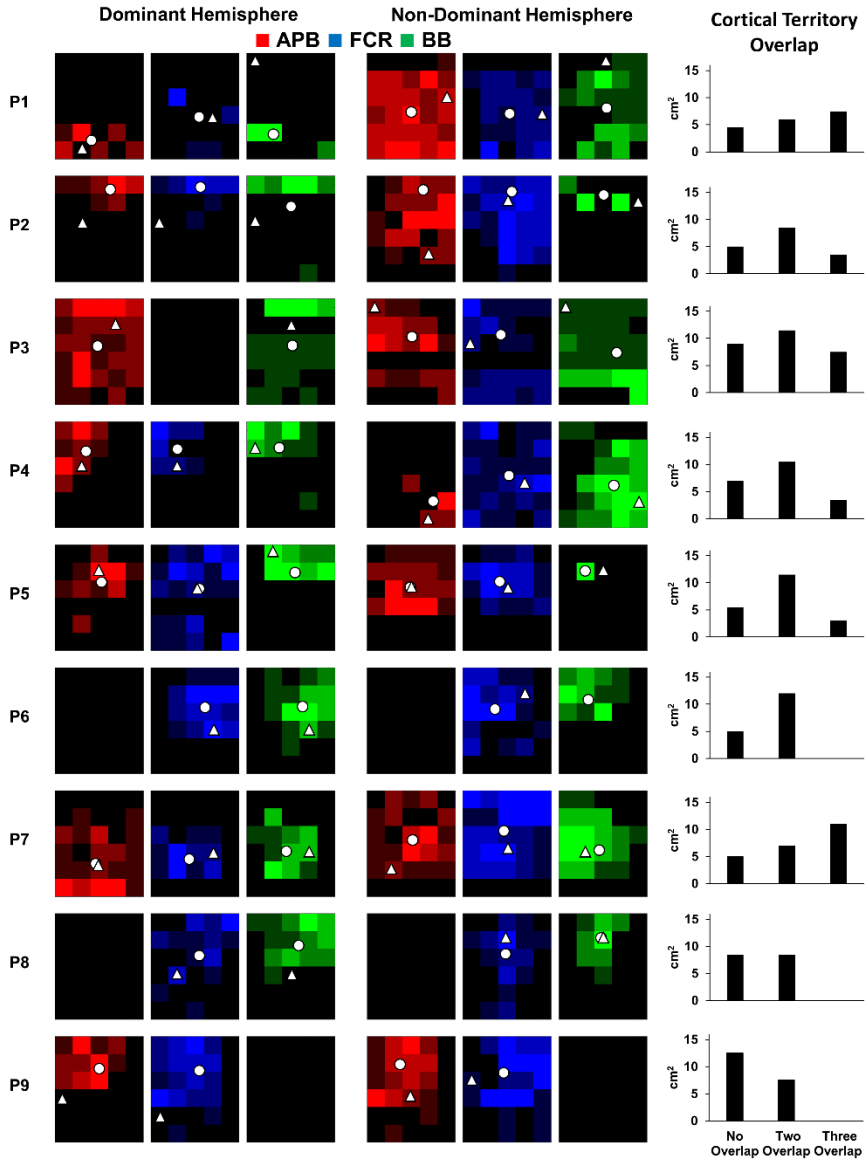

Figure 4. Individual cortical territory plots for each hemisphere and muscle for the SCI group. Pixel maps represent the size of the cortical territories and the white circle and triangles indicate the location of the CoG and motor hotspot, respectively. Histograms represent the magnitude of each pattern of overlap for every individual.

Figure 5

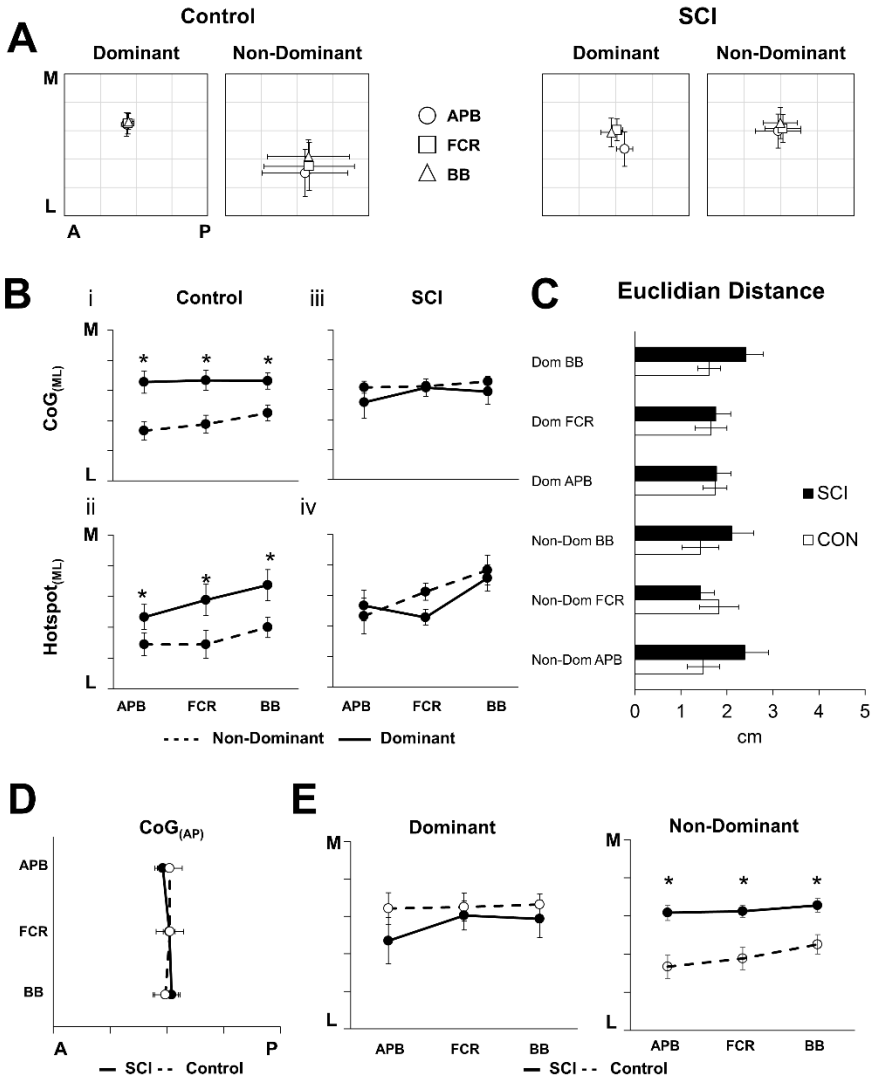

Figure 5. Differences in cortical territory somatotopy between hemispheres and groups. A) The pooled CoG across muscles for each group for each hemisphere. B) i) The medio-lateral CoG positions across hemispheres of the control group. The non-dominant hemisphere shows laterally shifted muscle representations compared to the dominant hemisphere ($p < 0.001$). ii) The medio-lateral locations of motor hotspots in the non-dominant hemisphere are significantly more lateral than the dominant hemisphere in the control group ($p = 0.002$). iii) Hemispheric differences were

not observed for the CoG of muscle representations in the SCI group. iv) The motor hotspots observed for the SCI group did not differ across hemispheres. C) The Euclidean distances between the CoG and motor hotspot for each muscle representation in each group revealed no statistical differences. D) The anterior-posterior position of muscle representations did not differ between groups, muscles, or hemispheres. E) The CoG did not differ between groups in the dominant hemisphere (left). The CoG in the non-dominant hemisphere of control participants was significantly more lateral than non-dominant SCI representations ($p < 0.001$) (right). * indicates a significant difference between groups or hemispheres.

Figure 6

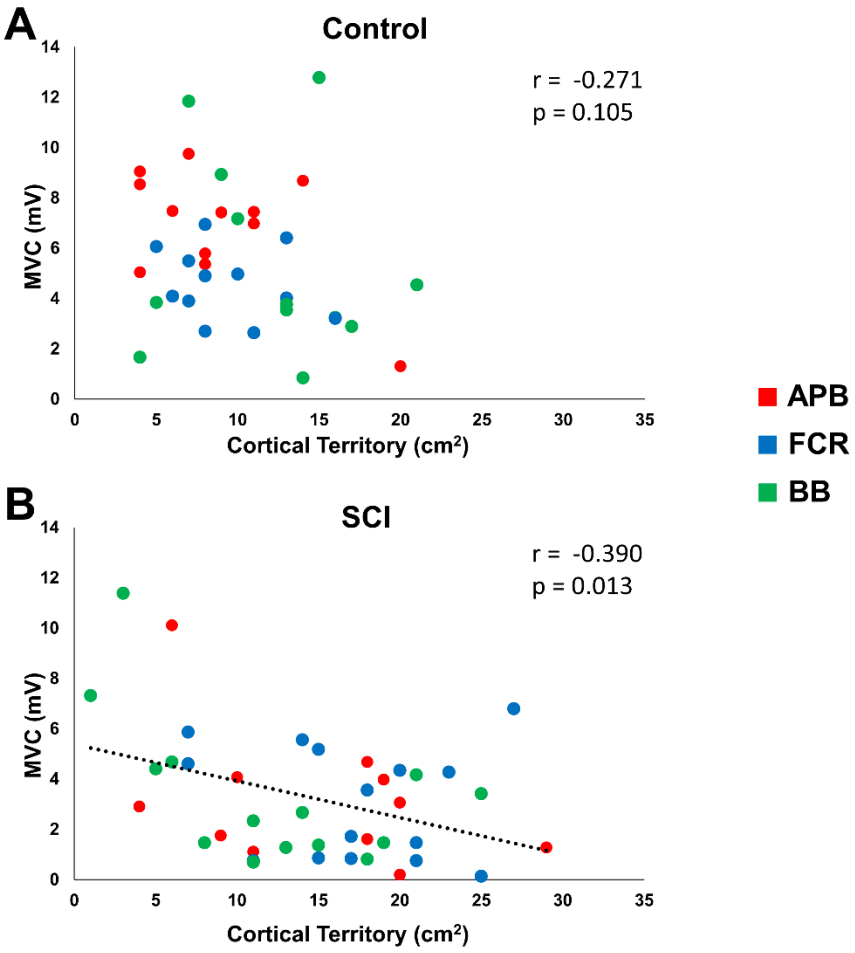

Figure 6. A) The control group did not show any correlation between the cortical territory devoted to a muscle and its MVC. B) The SCI group demonstrated a significant correlation between cortical territory and MVC such that muscles with larger cortical representations had smaller MVC.

Chapter 3 Experiment 2: Interhemispheric inhibition modulates motor representations in the healthy human motor cortex

Abstract

Interhemispheric inhibition (IHI) between motor cortices (M1) is thought to suppress unwanted mirror movements during voluntary behaviours and can be assessed using paired-pulse Transcranial magnetic stimulation (TMS). IHI may modulate the size of the cortical territory for a given muscle as a mechanism for facilitating unimanual control. To date, the relationship between IHI and cortical territory remains unknown. In the present study, IHI was obtained in the first dorsal interosseous (FDI) of the right hand in fifteen healthy, right-handed individuals at rest, during contralateral and ipsilateral contraction. The depth of IHI was examined in FDI of the right hand by delivering a conditioning stimulus to ipsilateral M1 followed by a test stimulus to contralateral M1. Cortical territory of FDI was also assessed at rest and active contraction. Cortical territory was determined by delivering suprathreshold TMS over a 5 x 5 cm grid centered on the motor hotspot for FDI of the right hand. Results indicate that IHI was positively associated with the size of the cortical territory only in the context of contraction and not when the FDI muscle was relaxed. Specifically, reduced IHI corresponded to larger cortical territory, a relationship that existed for both contralateral and ipsilateral contraction. These data demonstrate that the magnitude of IHI in a hand muscle predicts the size of the cortical territory occupied by that muscle. We present a mechanistic model to explain these findings that further elucidate the role of interhemispheric communication in shaping motor output.

Introduction

Transcallosal communication between human motor cortices (M1) can be assessed using transcranial magnetic stimulation (TMS) with activity in one cortex producing an inhibitory effect in the opposite hemisphere (Ferbert et al. 1992; Ni et al. 2009). This circuit is known as interhemispheric inhibition (IHI) and can be probed by delivering a suprathreshold stimulus to M1 in one hemisphere followed by a suprathreshold stimulus to the opposite M1 hemisphere with an interstimulus interval of 8-50ms (Ferbert et al. 1992; Ni et al. 2009). IHI consists of two phases (Ni et al. 2009) related to the short (8-10 ms, SIHI) or long (~40 ms, LIHI) interstimulus interval between the sequential TMS pulses. Voluntary contraction of a contralateral muscle reduces SIHI (Nelson et al. 2009; Perez and Cohen 2008). However, ipsilateral contraction reduces (Nelson et al. 2009; Perez and Cohen 2008) or increases (Ferbert et al. 1992; Hinder et al. 2010; Morishita et al. 2012; Uehara et al. 2014; Vercauteren et al. 2008) SIHI and/or LIHI. The functional significance of IHI remains unclear, however, IHI is reduced in populations demonstrating unwanted mirror movements that accompany stroke (McDonnell and Stinear 2017; Murase et al. 2004) and focal hand dystonia (Beck et al. 2009; Nelson et al. 2010). IHI may also act to influence the opposite M1 to prepare for future rapid engagement of the non-active homologous muscle (Nelson et al. 2009).

Motor representations within M1 can be obtained by delivering TMS systematically over the scalp to create a map of the area that elicits a muscle response (Wassermann et al. 1992; Wilson et al. 1993). During contralateral activity, the area of the TMS-evoked cortical territory increases (Wilson et al. 1995). No TMS studies have investigated motor maps during ipsilateral muscle contraction although functional magnetic resonance imaging data indicates increased activity

during such movement (Buetefisch et al. 2014; Dassonville et al. 1997). The mechanisms by which the motor map alters during contraction remains unclear, and one possibility is that the upper motor neurons are released from inhibitory inputs (Jacobs and Donoghue, 1991; Ziemann et al., 1998). SIHI and LIHI may be candidates that modulate cortical territory, leading to map expansion. Given that SIHI and LHI in a muscle are modulated by contraction of that muscle, we hypothesized that IHI circuits may be involved in altering the size of the motor representation for that muscle.

The goal of the present study was to determine whether a relationship exists between SIHI/LIHI measured in the right first dorsal interosseous (FDI) muscle and the cortical territory representing this muscle. To determine if such an association is context-dependent, we explored this relationship during muscle relaxation and contra- and ipsilateral contraction of the right index finger. Identifying a relationship between SIHI/LIHI and cortical territory advances our understanding of representational organization within M1 and provides insight into possible mechanisms that may participate in neural plasticity. Such a relation may have implications for understanding uni- vs. bimanual movements and clinical significance in populations experiencing an imbalance of transcallosal inhibition.

Methods

Fifteen healthy, right-handed individuals (22.46 ± 2.38 years, 10 female) participated in this study. To assess the changes seen following neurological injury, three individuals with spinal cord injury also participated in this experiment. All participants were screened for contraindications for TMS and provided written consent prior to participation. Handedness was confirmed using a modified

version of the Edinburgh Handedness Inventory (Oldfield 1971). This work was approved by the McMaster Research Ethics Board and conformed to the declaration of Helsinki.

Electromyography

Muscle activity was recorded using surface electrodes (9 mm diameter Ag-AgCl) placed over the left and right FDI muscles. Recordings were filtered using a band-pass of 20 Hz to 2.5 kHz and amplified 1000x (Intronix Technologies Corporation Model 2024F, Bolton, Canada) before being digitized at 5 kHz (Power 1401, Cambridge Electronics Design, Cambridge, UK). All data was stored on a secure computer for offline analysis. Maximum voluntary contraction (MVC) was obtained for the left and right FDI muscles via maximal isometric contraction of the target muscle against an immoveable apparatus. Three 5 s trials were performed in each muscle with a 1 min break between trials. The MVC obtained for the right and left FDI muscles did not differ (9.47 ± 4.381 mV vs. 9.61 ± 3.25 mV).

TMS Stimulation

TMS was delivered using a customized 50 mm diameter figure-of-eight branding coil connected to a Magstim 200 stimulator (Magstim, Whitland, UK) for all measures. The motor hotspot was found by delivering single TMS pulses to M1 to find the location that elicited the largest motor evoked potential (MEP) in the corresponding FDI muscle. The resting motor threshold (RMT) was obtained for both the left and right FDI muscles over the motor hotspot and was defined as the minimum TMS intensity required to elicit an MEP with an amplitude > 50 μV in 5 out of 10 trials (Rossini et al. 2015).

Interhemispheric Inhibition

Assessment of IHI was performed using paired-pulse TMS stimulation through two separate 50 mm diameter figure-of-eight branding coils, each connected to a Magstim 200 stimulator. IHI was recorded by delivering a conditioning stimulus (CS) to the right hemisphere followed by a test stimulus (TS) to the left hemisphere (Figure 1A). SIHI and LIHI were measured in the FDI of the right hand. The intensity of both the CS and TS corresponded to 130% RMT obtained in the left and right FDI, respectively. The two coils were oriented 45 degrees to the sagittal plane to induce a posterior to anterior current in the motor cortex. The ISI between the CS and TS was set to 10ms to assess SIHI and 40ms to assess LIHI. A block of trials consisted of 10 CS-TS for each SIHI and LIHI and 10 TS alone trials for a total of 30 trials presented randomly. The magnitude of IHI was determined as the ratio of the mean MEP amplitudes obtained during the 10 CS-TS trials to those obtained from the 10 TS alone trials for both SIHI and LIHI (i.e. IHI = [CS-TS]/TS). Thus, a value less than 1 represents inhibition of FDI output.

Cortical Motor Maps

The cortical territory dedicated to the right FDI muscle within M1 was mapped by overlaying a 5 x 5 grid with 1 cm spacing over the left hemisphere motor cortex using Brainsight Neuronavigation (Rogue Research, Montreal, Canada). The grid was centered over the motor hotspot for the right FDI and rotated 45 degrees to align with the central sulcus (Figure 1B). Four TMS stimuli were delivered to each grid point using at an intensity of 130% RMT and corresponding MEPs were recorded from the right FDI.

Cortical maps were generated by including individual MEPs whereby the peak-to-peak amplitude was ≥ 150% of the peak-to-peak EMG signal obtained during a 50ms interval prior to the TMS artefact. Responses that were below this criterion were classified as no response to TMS and were assigned a value of zero (Wassermann et al. 1992). Subsequently, the four MEP responses at each grid point were averaged (including the zero value for lack of MEP) (Wassermann et al. 1992). The area of cortical territory (in cm^2) that output to the right FDI included only grid points that elicited MEPs exceeding 10% of the maximum MEP obtained in that map (Uy et al. 2002; van de Ruit and Grey 2016; Wilson et al. 1993). The centre of gravity (CoG) was calculated to find the amplitude weighted center of the cortical territory (Equation 1: $CoG_{(x)} = \Sigma\alpha_i X_i / \Sigma\alpha_i$ (Wassermann et al. 1992) where α_i is the average amplitude at a given grid point and X_i is the position (row or column number) of the point).

Experimental Design

Cortical territory, SIHI, and LIHI were obtained with both FDI muscles at rest (REST), during low-level contraction (~10% MVC) of the right FDI (CONTRA), and during low-level contraction (~10% MVC) of the left FDI (IPSI) as depicted in Figure 1. The order of conditions as well as the order in which dependent measures were obtained was randomized across participants using a William's square design.

Statistical Analysis

Prior to statistical analyses, all measures were assessed for outliers and normality using Kolmogorov-Smirnov tests. To ensure muscle activity was similar between CONTRA and IPSI during acquisition of SIHI/LIHI and cortical territory, rectified EMG data was examined during a

50ms pre-stimulus window. The level of background EMG was compared across conditions for each dependent variable using one-way ANOVAs with a within-subject factor of CONDITION (3 levels: REST, CONTRA, IPSI) to ensure similar muscle activation between the two active conditions. One-way ANOVA was performed for right FDI cortical territory and IHI using a within-subject factor of CONDITION (3 levels: REST, CONTRA, IPSI). Conover's ANOVA was used for non-normally distributed data (Conover and Iman 1982). All significant main effects were assessed using paired t-tests for normally distributed data or Wilcoxon signed Rank for non-normal data. Bivariate correlations were computed across conditions for each dependent variable and between the depth of IHI and size of the cortical territory. Spearman's correlations were performed for non-normal data. Results exceeding the $\alpha < 0.05$ level of significance are reported.

Results

Effects of contraction on cortical territory

All fifteen participants successfully completed the experiment. The results of all statistical analyses are presented in Table 1. With the exception of cortical territory, all data were normally distributed. Pre-stimulus EMG during motor cortex mapping did not differ between IPSI and CONTRA indicating that muscle contraction was similar between conditions. CoG_{A-P} and CoG_{M-L} were unaltered by the conditions (Table 1, data not plotted). Figure 2A plots the group-averaged cortical territory (with standard error) for each condition. Cortical territory was greater in CONTRA (Figure 2A, left) compared to other conditions ($F_{(2,22)} = 36.33$, $p < 0.001$), a pattern that was observed in each participant, as shown in Figure 3. There was no difference between IPSI and REST ($p = 0.44$). Cortical territory during REST and active conditions were not correlated (Figure 2A, right), indicating that extent of activated territory during contraction was unrelated to the size of the motor representation measured when the FDI muscle was relaxed. Cortical territory was

positively correlated for CONTRA and IPSI conditions ($r = 0.751$, $p = 0.001$) such that individuals evoking small territory for CONTRA also evoked small territory for IPSI contraction (Figure 2A, right). These data indicate that IPSI creates small systematic changes in cortical territory that, while not detected with group-level statistics (Figure 2A, left), are related to the extent of CONTRA evoked activation. Therefore, the cortical territory evoked by IPSI contraction can be predicted from the CONTRA contraction and vice-versa.

Effects of contraction on interhemispheric inhibition

Pre-stimulus EMG was similar for the two active contraction tasks during acquisition of SIHI and LIHI (Table 1). SIHI was reduced during CONTRA and IPSI contraction (Figure 2B, left; $p < 0.001$ and $p = 0.002$, respectively), an effect seen in all but one participant (Figure 3). Further, SIHI in CONTRA was reduced compared to IPSI ($p = 0.002$). Similar results were observed for LIHI such that LIHI was reduced during CONTRA (Figure 2C, left; $p = 0.006$), an effect observed in 11 of 15 participants (Figure 3). In contrast to cortical territory, SIHI and LIHI were positively correlated across all conditions indicating a relationship between the depth of IHI among the resting and active states tested. Greater IHI in REST was associated with greater IHI during both CONTRA and IPSI (Figure 2B and C, right). Further, greater IHI during CONTRA was associated with greater IHI during IPSI. Therefore, the IHI depth is modulated systematically and can be predicted with or without contraction of either hand.

Relationship between interhemispheric inhibition and cortical territory

To address the main question, correlations were computed between IHI and cortical territory as shown in Figure 4A. With the muscle at REST, SIHI and LIHI were unrelated to cortical territory

(Figure 4A, top) indicating that, in relaxed muscle, the depth of IHI in the right FDI was not associated with the size of its muscle representation. In contrast, contraction of the CONTRA and IPSI muscle changed the relationship between IHI and cortical territory. For CONTRA, SIHI and LIHI were each positively correlated with cortical territory such that the cortical territory of right FDI increased with reductions in the magnitude of SIHI or LIHI measured in right FDI (Figure 4A, middle). Similarly, for IPSI, both SIHI and LIHI were negatively correlated with cortical territory (Figure 4A, bottom). Thus, decreases in the magnitude of IHI were related to increases in cortical territory in the context of muscle contraction only.

Findings from Individuals with Spinal Cord Injury

The first individual with a SCI was a 39-year-old male with a C5 injury that occurred during birth (39 years post-injury). This participant presented as ASIA C and was not taking any medications. The cortical territory of the right FDI muscle was expanded during both CONTRA and IPSI in this participant (Figure 5A, left). This demonstrates that the mechanisms governing contraction based expansion of the territory is intact, yet this modulation occurs regardless of which hand was active. Looking to the SIHI/ LIHI data, CONTRA appears to only slightly increase SIHI while IPSI reduced SIHI relative to rest (Figure 5A, right). This participant did not show any LIHI at rest or with contraction of either hand relative to rest as inhibition was never seen (Figure 5A, right). Therefore, cortical territory is increased in this individual when either hand is contracted while the ability to modulate transcallosal inhibition is reduced or absent.

The second participant was a left-handed, 41-year-old male that suffered a traumatic SCI at the level of C6-C7, 2 years prior to testing. This participant had an ASIA score of C and was regularly

using diazepam, pregabalin, and cyclobenzaprine. Similar to that seen in the group-level analysis of the control group, cortical territory of FDI during unilateral contraction was increased during CONTRA and showed a slight, if any, increase during IPSI in this individual (Figure 5B, left). The presence of contraction produced small reductions in SIHI and large reductions in LIHI compared to rest, regardless of which hand was active (Figure 5B, right). Thus, this individual demonstrated contraction related modulation of both cortical territory and SIHI/ LIHI.

The third participant was a 39-year-old, right-handed male who had experienced a traumatic SCI 14 years prior to testing. The participant was classified as ASIA C and the injury level was located at C6-C7. This participant was regularly taking baclofen. Motor responses from the right FDI muscle could not be elicited from this individual as TMS pulses at 100% MSO did not elicit any discernable MEPs. Therefore, the abductor pollicus brevis (APB) muscle, which did elicit responses to TMS, was used for EMG recordings throughout the experimental protocol. This participant showed decreases in cortical territory of the right APB muscle during unilateral contraction of either hand compared to rest (Figure 5C, left). This participant showed a decrease in SIHI during CONTRA and an increase during IPSI, an effect that was also seen for LIHI (Figure 5C, right). Therefore, this participant shows reductions in cortical territory during contraction of either hand and modulation of SIHI/ LIHI is similar to that seen in controls.

Discussion

The primary finding of the present study was the relationship between the size of the cortical territory occupied by FDI and the depth of IHI in FDI. A larger cortical map was associated with reduced transcallosal inhibition. This relationship held true irrespective of which hand was contracted, and this association was not observed in the absence of muscle contraction (i.e. REST).

To our knowledge, this is the first study to investigate the relationship between TMS evoked cortical territory and transcallosal inhibition and describe potential neural mechanisms using our working model (Figure 4B). Further, we note that the depth of IHI can be predicted from contraction of either hand or at rest, while cortical territory as measured via TMS cannot be predicted between resting and active states. We begin our discussion with the proposed neural mechanisms that mediate these findings, and highlight implications for basic and clinical neuroscience.

IHI is proposed to be mediated by an excitatory transcallosal projection that synapses on inhibitory interneurons (IHI_{INT}) within the contralateral M1 (Daskalakis et al. 2002) (Figure 4B, top). The CS excites the transcallosal projection that then inhibits the upper motor neurons in the contralateral hemisphere through IHI_{INT} producing net inhibition of the upper motor neurons when CS-TS paired pulses are delivered (Chen 2004; Daskalakis et al. 2002). It is also known that IHI_{INT} (Figure 4B, top) reduces short interval intracortical inhibition (SICI) in the target hemisphere (Daskalakis et al. 2002; Perez and Cohen 2008), and therefore both SICI and IHI_{INT} can modulate the excitability of upper motor neurons.

How do reductions in IHI increase the size of motor maps? During contralateral contraction the reduction in IHI likely reflects the weaker inhibition from IHI_{INT} (Figure 4B, middle) relative to the excited upper motor neuronal pool, similar to the decrease in IHI observed with increasing TS intensity (Ferbert et al. 1992). The present findings reveal that individuals that demonstrate larger right FDI representations exhibit the lowest levels of SIHI and LIHI in the right FDI. The increase in upper motor neuron excitability in the TS hemisphere may also be contributed by reductions in

SICI that occur during contraction of the contralateral FDI (Hammond and Vallence 2007; Ridding et al. 1995; Roshan et al. 2003). Therefore, the size of the motor representation is governed by the excitability of the motor neuronal pool that is, in turn, governed by inhibitory inputs including the IHI_{INT}. This relationship is supported by work probing neuroplastic changes whereby 1 Hz repetitive TMS delivered to M1 in one hemisphere reduces IHI (Gilio et al. 2003; Morgante et al. 2017) and increases MEP amplitudes evoked from M1 in the opposite hemisphere (Gilio et al. 2003). Therefore, the present findings that indicate a positive relationship between IHI and cortical territory support previous findings of context-dependent relationships between IHI and motor output.

During ipsilateral contraction (Figure 4B, bottom), the relationship between map size of the right FDI and IHI persists. One possibility is that the increased excitation of the CS hemisphere may increase activity in the transcallosal projection from the CS to TS hemisphere (thick line in Figure 4B). It has been shown that IHI is reduced in the presence SICI in the contralateral conditioned (i.e. CS) hemisphere (Lee et al. 2007). During ipsilateral contraction SICI would be reduced in the CS hemisphere (Hammond and Vallence 2007; Ridding et al. 1995; Roshan et al. 2003), allowing for modulation of transcallosal output that is assessed via IHI. The presence of such interactions may drive the relation between IHI and cortical territory of the right FDI during IPSI (Figure 4B). However, it is also possible that reductions in cortical territory during IPSI are contributed by changes in the interactions between IHI_{INT} and local neuronal populations (i.e. SICI, LICI) in the outputting hemisphere. It has been hypothesized that ipsilateral contraction is, in part, mediated by ipsilateral descending pathways from the motor cortex (Carr et al. 1993; Hubers et al. 2008; Mayston et al. 1999). Increased activity in the ipsilateral M1 during contraction may produce

interactions within the TS hemisphere similar to that seen in CONTRA, albeit to a lesser degree due to relatively small numbers of upper motor neurons projecting through ipsilateral paths. This may explain why cortical territory is not different from REST during IPSI (Figure 2A) yet the relationship between IHI and representation size exists.

Functional Significance of Interhemispheric Inhibition

One role for IHI may include the suppression of unwanted bimanual or mirror movements (Duque et al. 2005; Duque et al. 2007) such that the presence of mirror EMG activity is associated with decreased IHI during unilateral movements in healthy individuals (Fling and Seidler 2012; Hubers et al. 2008). Similarly, IHI is reduced in those with motor dysfunction such as focal hand dystonia (Beck et al. 2009; Nelson et al. 2010) and stroke (Murase et al. 2004). Larger cortical territory for a muscle may increase the potential for neural output to that muscle. Thus, our data corroborate these findings as reduced IHI corresponded to larger cortical territories in the target hemisphere during unilateral contraction (Figure 4B).

One functional role for modulating the depth of IHI may relate to the opportunity to engage the non-active limb during tasks that require bimanual control. For example, IHI has been shown to be reduced during contralateral and ipsilateral contraction (Nelson et al. 2009; Perez and Cohen 2008), potentially for the functional purpose of rapidly engaging the relaxed limb if required by the task. In support of this suggestion, individuals with a greater magnitude of IHI perform the poorest on skilled bimanual tasks (Fling and Seidler 2012). Further, acquisition of bimanual motor skills modulate the balance of IHI between M1 of the two hemispheres (Chieffo et al. 2016; Houdayer et al. 2016) and it is known that skill acquisition increases the cortical territory for

muscles involved in learning (Pascual-Leone et al. 1993; Pascual-Leone et al. 1995b; Tyc and Boyadjian 2011). Therefore, based on the present findings, we suggest that the relationship between cortical territory and IHI within M1 facilitates both unilateral and bimanual motor skill acquisition and performance.

An important observation in this study, was the context dependent nature of IHI and cortical territory relationship. We only observed significant associations between our dependent measures when the muscle was actively contracted and not at rest. A recent meta-analysis in post-stroke research indicates no clear association between changes in cortical territory and concomitant changes in IHI following rehabilitation (McDonnell and Stinear 2017). Based on the present findings, such discrepancies in the literature may relate to whether the context of muscle contraction was used during data acquisition. Our findings suggest that some of this discrepancy may be due to neurophysiological assessment in the resting state, where these circuits are not shown to be interacting in the uninjured cortex. The use of testing in the active rather than resting state has been proposed previously, as the active state likely allows for assessment of cortical territory in a more consistent state of excitability (Siebner and Rothwell 2003b). Future research in this area should consider assessing neural circuitry in the active state when attempting to induce plasticity promoting bimanual interactions between the limbs (Ludemann-Podubecka and Nowak 2016).

The Effects of Unilateral Contraction in Individuals with Spinal Cord Injury

The three cases of SCI that were assessed in this study do not provide consistent results regarding the modulation of cortical territory, SIHI, and LIHI during contraction. Participant 1 showed the

expected increase in cortical territory during CONTRA and this increase was not specific to which hand was active. This individual may demonstrate crossed facilitation during contraction of the hand whereby contraction of one hand increases cortical output to the non-moving hand (Muellbacher et al. 2000; Perez and Cohen 2008; 2009). The similar modulation of cortical territory during both CONTRA and IPSI may be due to impaired IHI. This participant showed alterations in SIHI that are opposite to that seen in healthy controls. SIHI was reduced during CONTRA and increased during IPSI compared to REST (Figure 5A, right). Also, LIHI was not present in any condition in this individual. Therefore, impairments in SIHI/ LIHI seen in this participant may increase mirror activation across the two hemispheres during contraction.

Participant 2 demonstrated increased cortical territory to the right FDI in CONTRA and a small change in IPSI compared to REST. This reflects the findings seen in healthy controls. However, this individual showed very small reductions in SIHI with unilateral contractions and large reductions in LIHI (Figure 5B, right). While the changes in LIHI are expected, the inability to modulate SIHI may be because this participant was medicated with diazepam. Diazepam acts on GABA-A receptors (Ziemann et al. 1996) and may reduce SIHI (Irlbacher et al. 2007). Therefore, Participant 2 appears to show contraction based modulation of cortical territory and LIHI that is representative to that seen in controls while the absence of SIHI alterations is likely mediated by diazepam.

Participant 3 showed an unexpected decrease in cortical territory during contraction of either hand. While this result has not been previously reported, several possibilities exist. These findings may be due to the effects of medications as this individual was taking baclofen daily, which impacts

GABA-B receptor activity (Ziemann et al. 1996). This participant showed that LIHI, which is thought to be modulated by GABA-B activity (Irlbacher et al. 2007), turned into facilitation during CONTRA and disappeared during IPSI, suggesting that activity based alterations of GABA-B activity is attenuated. Thus, the results in this participant may be impacted by baclofen use. Although not quantitatively assessed in this study, chronic pain disrupts M1 excitability, likely through GABAergic interactions (Parker et al. 2016) which reduces excitability within the cortical territory of a muscle at rest (Krause et al. 2006) and during contraction (Falla et al. 2017). While it is not clear why the cortical territory in this participant is reduced, it may be due to medications, pain, or their interactions.

Limitations

The present findings elucidate a relation between IHI and cortical territory targeting the dominant FDI of the right hand. It is unclear whether the same associations exist when IHI is targeting the non-dominant FDI of the left hand. Further, interactions between SICI and/or other motor circuits that may operate in conjunction with IHI should be examined for their modulatory role in shaping the cortical territory. The changes that occur with unilateral contraction may differ from that observed in those with motor impairments. The SCI cases presented herein provide inconclusive evidence regarding the interaction

Conclusions

The link between the size of a muscle representation during unilateral contraction has important implications for healthy and clinical populations. First, the impact of transcallosal inhibition on motor output furthers our understanding of the neural mechanisms guiding unilateral behaviour.

This interhemispheric modulation of cortical territory may highlight the opportunity for motor recovery following cortical injury to a single hemisphere. Last, our findings suggest that motor excitability should be assessed during active contraction, when interactions between motor circuitry are present. These findings may inform future studies examining basic and clinical neuroscience regarding the neural basis of motor control.

Tables and Figures

Table 1. Statistical Analyses

Measure	ANOVA		Outcome
EMG Activity (%MVC)	Cortical Territory	Condition $_{(2,28)}$ = 64.930, p < 0.001	Rest vs. Contra Active: 0.43 ± 0.29 vs. 11.27 ± 4.58, p < 0.001, *d* = 3.34 Rest vs. Ipsi Active: 0.43 ± 0.29 vs. 9.44 ± 2.11, p < 0.001. *d* = 5.98 Contra Active vs. Ipsi Active: 11.27 ± 4.58 vs. 9.44 ± 2.11, p = 0.161, *d* = 0.51
	IHI	Condition $_{(2,28)}$ = 52.084, p < 0.001	Rest vs. Contra Active: 0.38 ± 0.16 vs. 11.15 ± 4.75, p < 0.001, *d* = 3.20 Rest vs. Ipsi Active: 0.38 ± 0.16 vs. 8.46 ± 2.21, p < 0.001. *d* = 5.16 Contra Active vs. Ipsi Active: 11.15 ± 4.75 vs. 8.46 ± 2.21, p = 0.07, *d* = 0.73
Cortical Territory*	Condition $_{(2,28)}$ = 36.330, p < 0.001		Rest vs. Contra Active: 13.20 ± 3.45 vs. 20.93 ± 3.70, p < 0.001, *d* = 2.16 Rest vs. Ipsi Active: 13.20 ± 3.45 vs. 13.93 ± 3.51, p = 0.440. *d* = 0.21 Contra Active vs. Ipsi Active: 20.93 ± 3.70 vs. 13.93 ± 3.51, p <0.001, *d* = 1.95
CoGx	Condition $_{(2,28)}$ = 0.786, p = 0.465		
CoGy	Condition $_{(2,28)}$ = 1.190, p = 0.319		
SIHI	Condition $_{(2,28)}$ = 22.099, p < 0.001		Rest vs. Contra Active: 0.52 ± 0.28 vs. 0.89 ± 0.17, p < 0.001, *d* = 1.60 Rest vs. Ipsi Active: 0.52 ± 0.28 vs. 0.63 ± 0.31, p = 0.042. *d* = 0.37 Contra Active vs. Ipsi Active: 0.89 ± 0.17 vs. 0.63 ± 0.31, p = 0.002, *d* = 1.04
LIHI	Condition $_{(2,28)}$ = 5.650, p = 0.009		Rest vs. Contra Active: 0.64 ± 0.27 vs. 0.85 ± 0.21, p = 0.006, *d* = 0.87 Rest vs. Ipsi Active: 0.64 ± 0.27 vs. 0.72 ± 0.31, p = 0.212. *d* = 0.28 Contra Active vs. Ipsi Active: 0.85 ± 0.21 vs. 0.72 ± 0.31, p = 0.067, *d* = 0.49

Note: Results of all statistical tests are shown. * indicates data was non-normal and non-parametric Conover's ANOVA was used. p-values and Cohen's *d* are reported for all statistical tests.

Figure 1

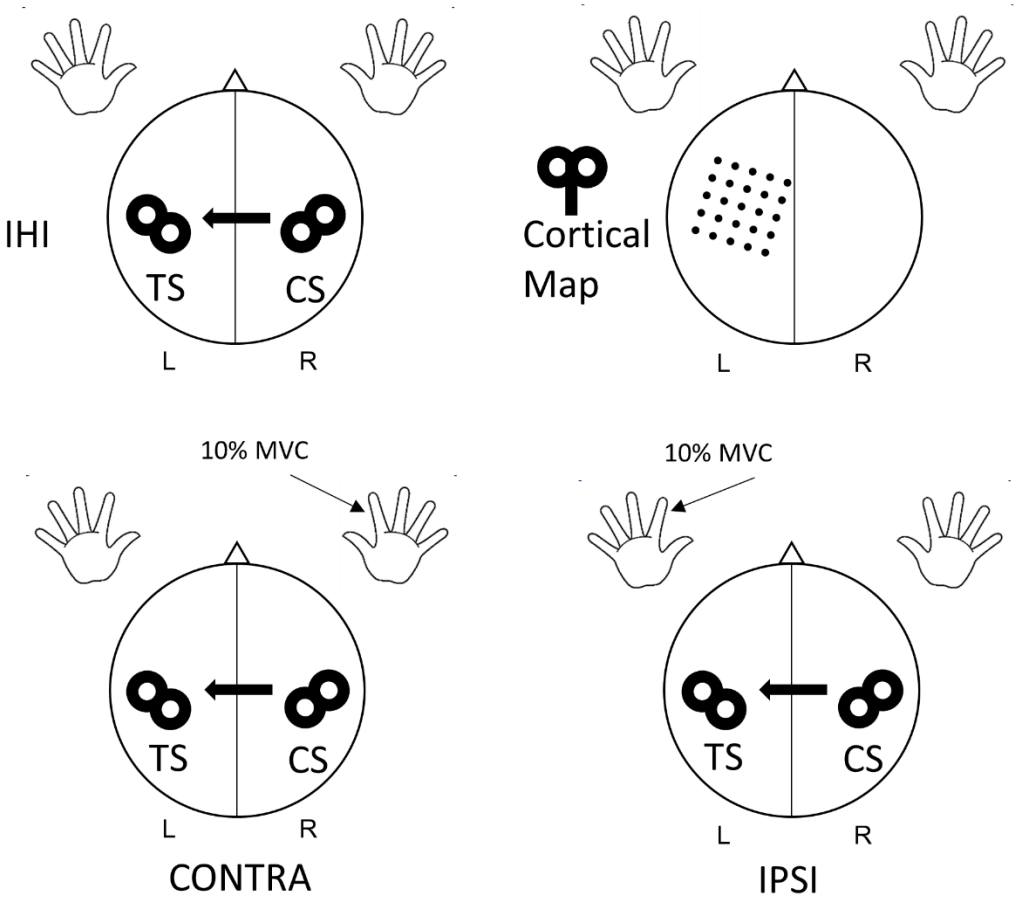

Figure 1: Depictions of the dependent variables and experimental conditions. A) Interhemispheric inhibition (IHI) was probed by delivering a conditioning stimulus over the right hemisphere at either 10 (SIHI) or 40ms (LIHI) prior to a test stimulus delivered over the left hemisphere. B) Cortical territory was obtained by delivering 4 pulses to each grid point over a 5 x 5 cm grid space that was centered on the motor hotspot for the target FDI muscle. C, D) In the experimental conditions SIHI, LIHI, and cortical territory were obtained while the right (CONTRA) or left (IPSI) FDI was isometrically contracted to 10% MVC.

Figure 2

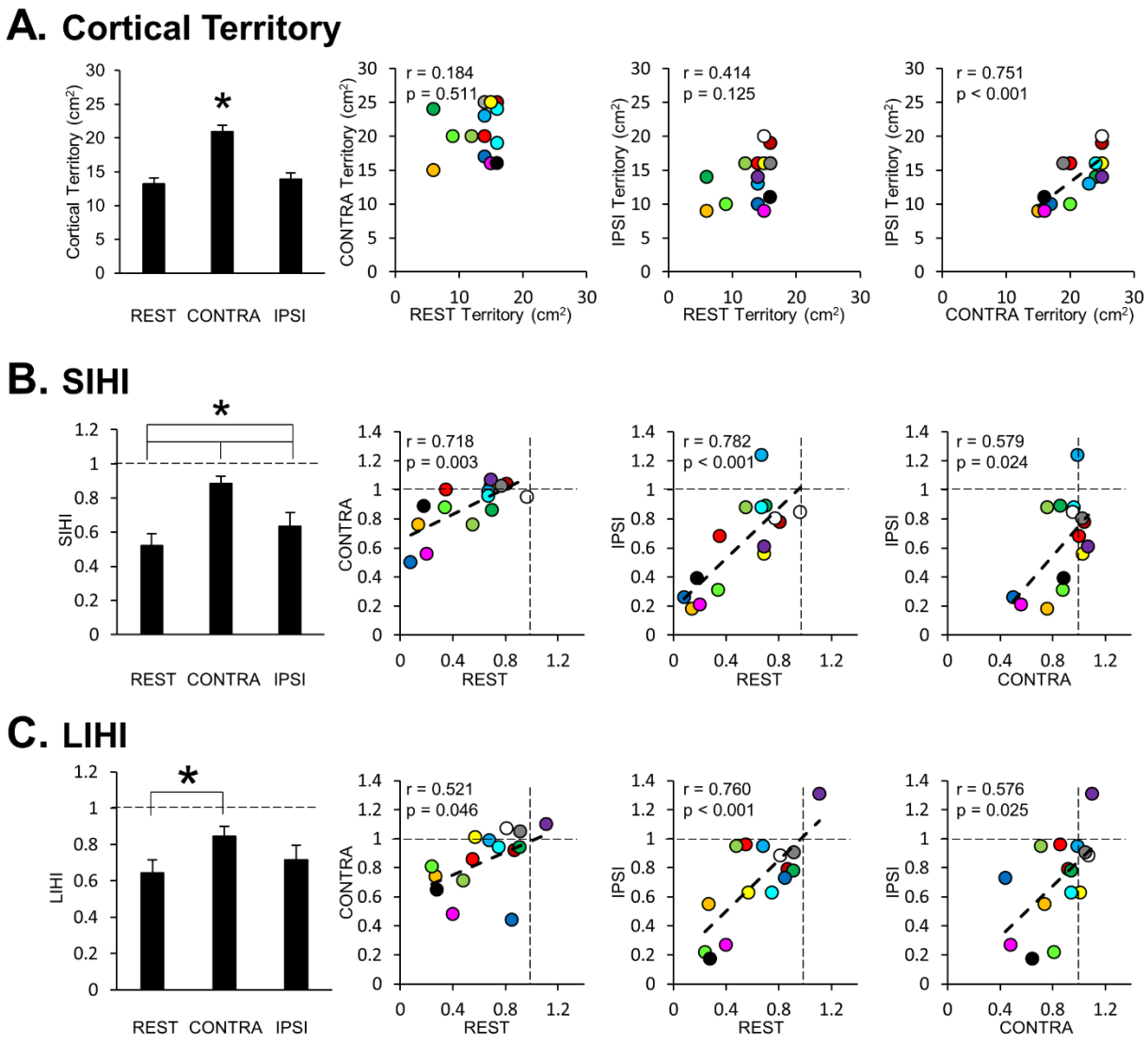

Figure 2: Effects of muscle contraction on RFDI cortical territory. A) The size of the cortical territory was greater during CONTRA relative to both REST and IPSI conditions ($p < 0.001$ for both). No significant correlations were seen between the cortical territory obtained in REST and either CONTRA or IPSI. The cortical territory for the right FDI was significantly correlated between CONTRA and IPSI conditions. B) Compared to rest, SIHI was significantly reduced from REST in both CONTRA ($p < 0.001$) and IPSI ($p = 0.042$) conditions. SIHI was reduced more so during CONTRA compared to IPSI ($p = 0.002$). SIHI observed in the REST, CONTRA, and IPSI

conditions were significantly correlated with each other. C) Compared to rest, LIHI was significantly reduced from REST in CONTRA ($p = 0.006$). LIHI observed in the REST, CONTRA, and IPSI conditions were significantly correlated with each other. * indicates a significant difference. Each point is color coded for individual participant data. Correlation coefficients and p=values are provided on the plots.

Figure 3

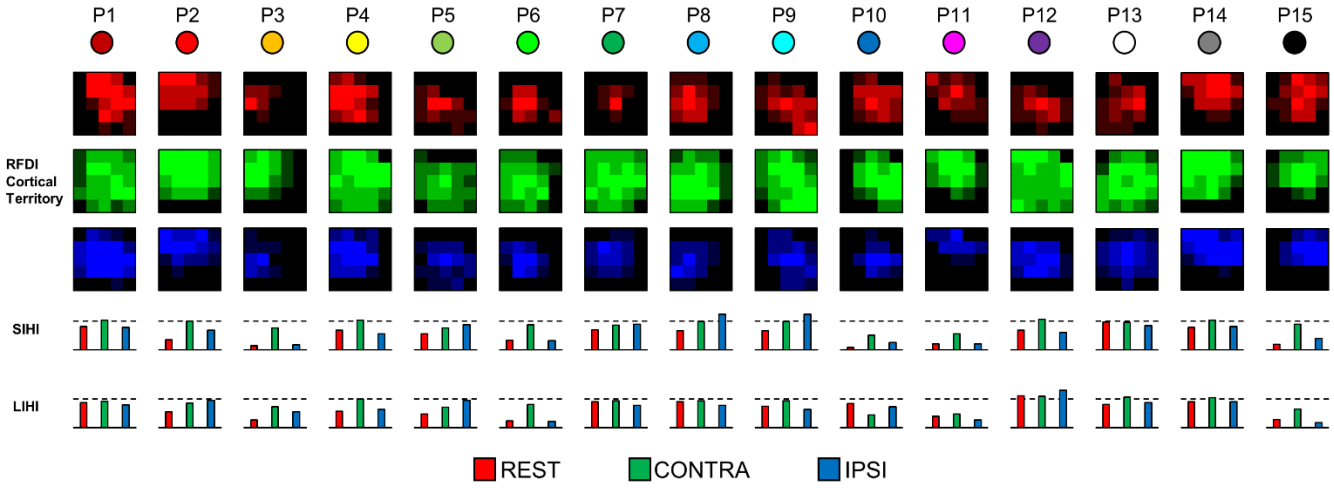

Figure 3: Individual data representing the cortical territory, SIHI, and LIHI for each participant. The color labels on the top represent the coding of individual data seen in other figures.

Figure 4

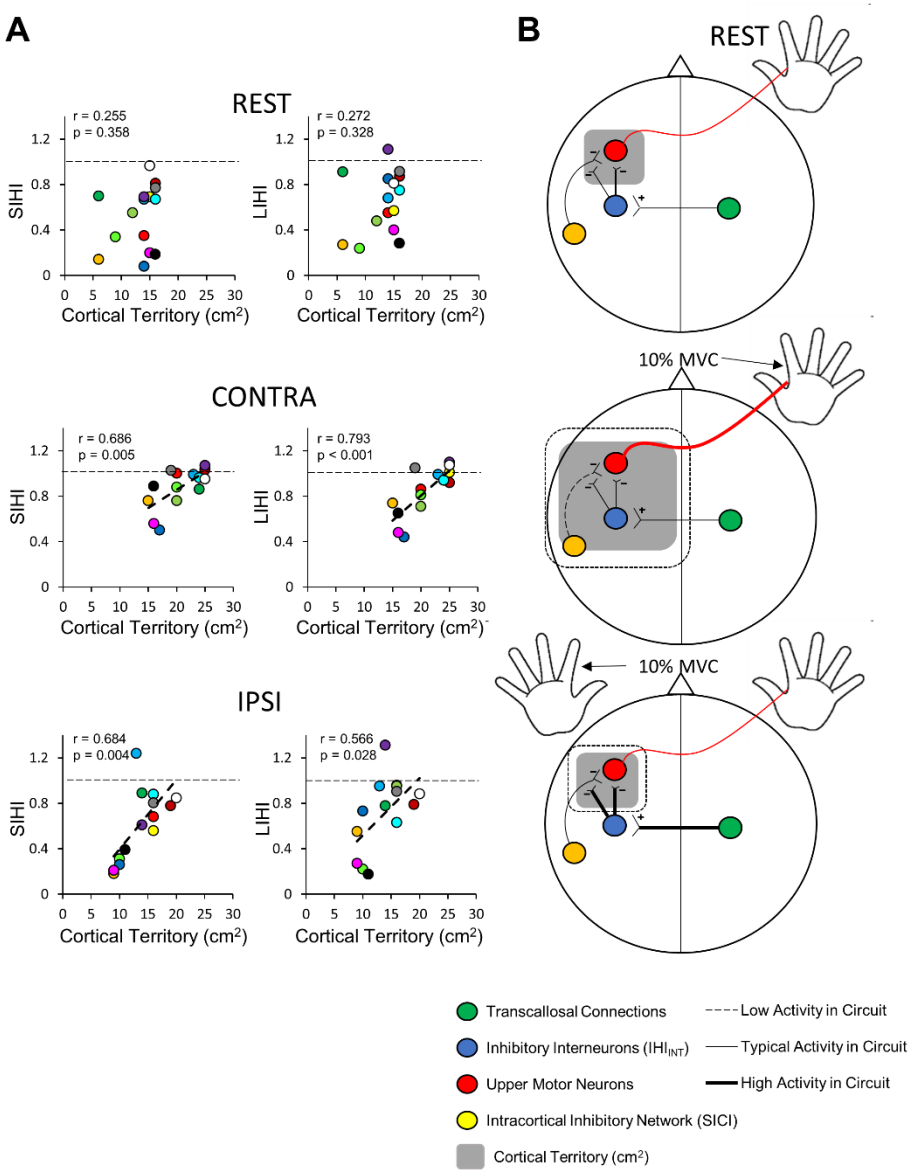

Figure 4: The relationship between SIHI and cortical territory and LIHI and cortical territory. A) No significant correlation was observed between SIHI or LIHI and cortical territory in REST (top). A significant association between the depth of both SIHI and LIHI and cortical territory was observed during CONTRA (middle). A significant association between the depth of both SIHI and LIHI and cortical territory was observed during IPSI (bottom). B) The current model of the mechanism of IHI is depicted to illustrate the relation observed in the current study. Each point is

color coded for individual participant data. Correlation coefficients and p values are provided on the plots.

Figure 5

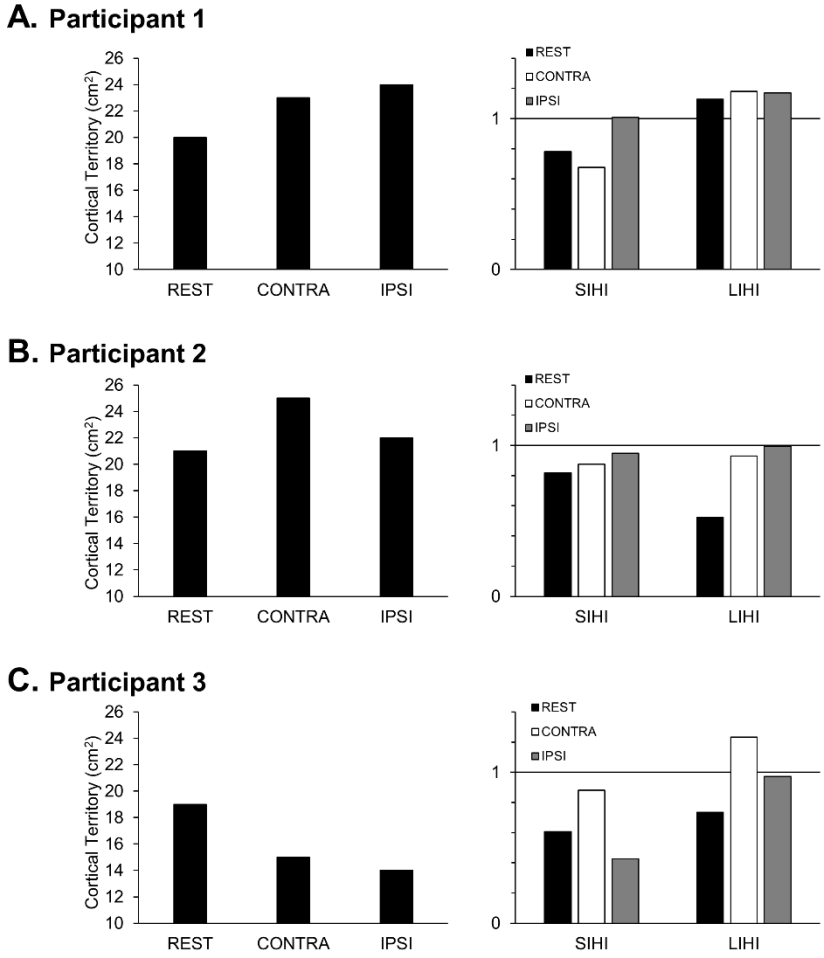

Figure 5. Cortical territory, SIHI, and LIHI for each of the three participants with spinal cord injury. A) Participant 1 shows an increase in cortical territory regardless of which hand was active and demonstrates increased SIHI during CONTRA and decreased SIHI during IPSI relative to rest. This participant did not show LIHI in any of the three conditions. B) Participant 2 shows cortical territory increases with CONTRA and no change in IPSI, similar to controls. This participant also shows a limited capacity to modulate SIHI regardless of which hand is active. LIHI is reduced compared to rest during contraction of either hand. C) Participant 3 does not show the expected modulation of cortical territory as contraction of either hand caused reductions in the size of the territory. During CONTRA SIHI is reduced and LIHI becomes facilitation while reductions in

SIHI and abolishment of LIHI are seen during IPSI. * indicates that recordings were made from the APB muscle rather than FDI for that participant.

Chapter 4 General Discussion and Conclusions

This Master's thesis explored the organization and modulation of upper limb muscle representations within the primary motor cortex (M1). Two studies were conducted to characterize the organization of upper limb motor output in healthy individuals and in individuals with spinal cord injury (SCI) to assess possible neural mechanisms that lead to alterations in muscle representations. The findings of these experiments demonstrated differences in the organization of M1 between able-bodied individuals and persons with SCI. Hemispheric asymmetry was seen in the healthy cohort that was not observed in those with SCI. Further, the pattern of overlap within M1 differed such that the amount of cortex outputting to all three tested muscles was reduced in those with SCI relative to controls. These differences may provide insight into the neural correlates of motor impairment seen following SCI. To provide clinical utility the mechanisms of cortical territory modulation were examined in able-bodied participants. The second experiment found a relation between the size of a muscle representation and the amount of transcallosal inhibition during active contraction. Thus, this thesis exposed important findings that further our understanding of how the hand and arm are represented within the motor cortex and some of the mechanisms that govern this patterned layout. The following discussion utilizes the model provided in Experiment 2 as a component of experience-dependent plasticity that govern the organization seen in healthy and spinal cord injured individuals.

Experience-Dependent Plasticity to Induce M1 Reorganization

The induction of reorganization within M1 appears to be mediated by motor experience. Skilled motor acquisition has been shown to create changes in cortical territories (Pascual-Leone et al. 1993; Pascual-Leone et al. 1995a; Pascual-Leone et al. 1995b; Tyc and Boyadjian 2006; 2011; Tyč et al. 2005) as well as IHI (Chieffo et al. 2016; Houdayer et al. 2016). Unilateral training

increases the cortical territory of muscles involved in the task (Pascual-Leone et al. 1993; Pascual-Leone et al. 1995b; Tyc and Boyadjian 2011; Tyč et al. 2005). For example, elite athletes exhibit lager deltoid representations in the dominant arm of volleyball players (Tyč et al. 2005) , and medio-laterally shifted forearm muscle representations of the playing arms in badminton players (Pearce et al. 2000). Motor training has also been shown to influence the amount of IHI between cortical areas controlling muscles engaged during the task and those of the non-moving homologs (Camus et al. 2009; Tinazzi and Zanette 1998). The plasticity of M1 that is driven by experience is proposed to be driven by GABAergic inhibition (Ljubisavljevic 2006) and appears to operate, at least in part, through this interaction between IHI and muscle representations. Therefore, this thesis exposes an interaction that is present in the active state that likely plays a role in the process of experience-dependent plasticity.

Asymmetries in Motor Output and Transcallosal Inhibition

The observation of hemispheric asymmetry in the control group during Experiment 1 lead to speculation that handedness plays a role in shaping the layout of motor representations within M1. Previous literature has suggested similar hypotheses (Hammond 2002) as structural imaging reveals a longer central sulcus in the dominant hemisphere (Amunts et al. 2000) and greater cortical activation is observed in the dominant hemisphere during contralateral hand contraction (Dassonville et al. 1997). Interestingly, similar asymmetries between the motor cortices are reported for the depth of interhemispheric inhibition (IHI) with greater inhibition from the dominant to non-dominant hemisphere in healthy individuals (Baumer et al. 2007; Duque et al. 2007). In the present work, experiment 2 revealed that IHI is capable of transiently modulating cortical territory size in the active state. Therefore, it is possible that IHI provides an influential

role in maintaining the organizational asymmetry between dominant and non-dominant hemispheres on the basis of long-term asymmetries in motor experience.

When the association between IHI and cortical territory is considered a mechanism for experience-dependent plasticity, it may also explain the relative symmetry observed in those with chronic incomplete SCI (Experiment 1). Following SCI, individuals often become much more reliant on bimanual behaviours (Brogioli et al. 2016), likely due to impairments in hand dexterity. Increased utilization of bimanual behaviours, over a long period of time, is likely to induce changes in patterns of motor output. This has been shown in able-bodied individuals whereby bimanual training, such as learning to play the piano, increases the cortical territory devoted to hand muscle bilaterally (Pascual-Leone et al. 1995a). Similarly, bimanual skill acquisition alters transcallosal communication such that the depth of IHI shifts from being deeper in the non-dominant hemisphere to a balanced amount of inhibition bidirectionally following substantial piano practice (Chieffo et al. 2016; Houdayer et al. 2016). This supports the notion that transcallosal inhibition acts to modulate the size of muscle representations and that chronic engagement of this interaction through repeated motor behaviours shapes M1 organization. Therefore, the increased use of both limbs for daily living that occurs following SCI may balance the depth of IHI and thus, the properties (i.e. size and location) of muscle representations across hemispheres. Further understanding of the mechanisms governing experience-dependent plasticity may provide value to understanding motor learning and control as well as rehabilitative strategies following neurological injury.

Neuromodulation of M1 to Promote Motor Behaviour

While the experiments comprising the thesis do not assess muscle representations or IHI over time or following any intervention, some predictions can be made when applying the results to existing literature. As noted above, motor training and skill acquisition are capable of modulating both motor representations (Pascual-Leone et al. 1993; Pascual-Leone et al. 1995a; Pascual-Leone et al. 1995b) and transcallosal inhibition (Chieffo et al. 2016; Houdayer et al. 2016). While this thesis provides the first account of a relation between muscle representation size and the depth of IHI it can be predicted that motor training acts through this interaction to produce neural markers of skill acquisition. Another possible method to induce changes in both cortical organization and motor circuitry is through non-invasive brain stimulation. Techniques such as repetitive TMS (rTMS) can induce short-term plasticity within M1. For example, the application of ischemic nerve block paired with rTMS has shown to increase the MEP size to a muscle while increasing the peak acceleration during elbow flexion tasks (Ziemann et al. 2001). Further, the interaction between IHI and motor output acts during neuromodulation as 1 Hz rTMS to one hemisphere reduces the IHI while increasing motor excitability observed in the opposite hemisphere (Gilio et al. 2003). Therefore, using these tools, it may be possible to induce cortical changes to facilitate motor learning and performance.

Targeting these interactions with neuroplasticity inducing protocols may provide benefits to motor control and learning. Motor training of one hand has been shown to increase performance of the opposite hand when performing the same task, a phenomenon named intermanual transfer of skill (Koeneke et al. 2009; Lee et al. 2010; Stockel and Wang 2011). Neurophysiological study of intermanual transfer of learning has shown that M1 excitability is greater and inhibition (including

IHI) is reduced in the hemisphere outputting to the untrained limb (Camus et al. 2009). This transfer of information from the trained to untrained cortex must operate through transcallosal connections, and the direction of change reported is consistent with the interactions between IHI and motor output seen in Experiment 2. The use of neuromodulation may, therefore, be used to target these interactions and promote or facilitate motor learning. Notably, a small number of studies have probed this possibility and showed that slow rTMS (i.e. inhibitory) delivered to M1 increase motor skill learning and performance in the ipsilateral limb (Kobayashi 2010; Kobayashi et al. 2004) while reducing motor output and skill performance in the contralateral limb (Kobayashi 2010). Thus, although more studies are needed, it appears to be plausible to target the interaction between IHI and excitability of the opposite motor representation using rTMS. This furthers our understanding of the neural correlates that underpin unilateral control and learning.

Clinical Implications

The experiments reported herein provide a characterization of M1 organization in able-bodied and motor impaired populations that may be explained by a novel relationship between transcallosal inhibition of muscle representation size. These findings demonstrate reorganization following injury that is likely driven by differences in motor experience following impairment. Further, a neural mechanism by which interhemispheric communication modulates M1 representations is exposed which may can explain some of the changes seen following injury. This interaction provides insight into the neural basis of motor control and may have clinical applications for those with movement disorders.

How do these findings impact and inform future work with clinical populations? The finding that interhemispheric interactions can shape motor output has strong implications for neurological

conditions where cortical damage or impairment is limited to a single hemisphere (i.e. stroke, TBI). In fact, previous work has shown that the size of a muscle representation predicts the capacity for recovery following stroke (Ludemann-Podubecka and Nowak 2016). However, recent reviews have determined that, while promising to date, further study is required examining the contribution of residual motor output (McDonnell and Stinear 2017) and the use of rTMS (Corti et al. 2012) to impact recovery of function following stroke. The interaction between representation size and depth of IHI observed in this thesis (Experiment 2) supports this hypothesis. Nevertheless, future study using interventions targeting increased excitability and decreased inhibition of the affected hemisphere are needed. Therefore, according to the relationship elucidated in Experiment 2, it is possible to utilize the healthy hemisphere to shape and/or rehabilitate the impaired hemisphere.

Limitations

While this work demonstrates novel advances in our understanding of cortical organization within M1, the interpretation is not without limitations. The differences observed between able-bodied and SCI individuals is hereby proposed to be due to differences in motor experience on the basis of hand dominance. However, a comprehensive metric of handedness was not utilized and the determination of a laterality quotient regarding hand dominance may provide insight into the differences in hemispheric symmetry in these groups. Further, the cohort of participants with SCI varied considerably in their injury characteristics and medications (Experiment 1, Table 1). This heterogeneity in injury demographics is common when studying those with SCI and call to question the use of group statistics. Perhaps this work, and others regarding larger samples of patients with SCI should provide findings on a case-by-case, or sub-group basis to account for this heterogeneity. Finally, the quantification of maps throughout this thesis was based on the absolute

MEP amplitudes recorded for each muscle within a participant. While this method is commonly used in the literature (van de Ruit and Grey 2016; Wassermann et al. 1992; Wilson et al. 1993), it may more informative to normalize MEP amplitudes observed to a maximum MEP or maximum M-wave response. Although neither of these measures were obtained in the current thesis work, analysis using such a normalization method may have altered the group-level findings as those with SCI did demonstrate smaller MVC and generally, lower MEP amplitudes. Despite the limitations, the organizational differences between those with and without spinal injury as well as the possible neural mechanism for these changes that this work has exposed provide significant contributions to neuroscience and motor control.

Significant Impact of the Master's Thesis Research

Experiment 1 demonstrated that differences exist between the organization of M1 in able-bodied persons and those with chronic incomplete SCI. Namely, differences in the degree of overlapping territory devoted to arm muscle and the symmetry between cortical territory location were seen. These differences may be attributable to experience dependent plasticity of the cortex as those with motor impairment perform motor tasks differently. Further, this work has demonstrated that the size of a muscle representation is inversely related to the capacity for voluntary activity for upper limb muscles following SCI. The relationship between the size of a muscle's representation and its capacity for contraction suggests that motor mapping may have clinical and rehabilitative applications following motor impairment. For example, muscles with the largest representations may benefit more from targeted rehabilitation as the cortex is attempting to maximize neural output through the residual corticospinal projections. Detailed quantifications of motor function and their link to representational organization within M1 should be pursued, particularly in clinical

populations. Therefore, the first experiment provided a characterization of how the cortical motor representations are organized in healthy and motor impaired individuals and how this organization relates to function.

The second experiment was conducted to address the question of what factors or mechanisms drive representational plasticity. In healthy participants, a relationship between the depth of transcallosal inhibition and the size of a muscle representation was observed in the active state only. Those that had less inhibition had larger cortical territories outputting to the active hand. This finding has important implications for motor control as it demonstrates that transcallosal inhibition does impact, and perhaps govern, the excitability in the opposite hemisphere during unilateral contraction. This finding offers a potential neural mechanism by which unilateral motor control and learning is achieved and shows that motor reorganization can be induced indirectly by targeting the opposite hemisphere. Future studies should consider using tools to induce plasticity such as rTMS to examine whether alterations in motor representations correspond to changes in motor learning and performance. Further, this has clinical implications for populations such as incomplete spinal cord injury and stroke where one hemisphere is intact and the other is impaired or cannot communicate through descending projections. Thus, we expose interhemispheric inhibition as a factor contributing to changes in M1 organization and this interaction likely plays a mechanistic role in experience-dependent plasticity.

This thesis aimed to address how changes in motor representation characteristics, such as those seen following neurological injury, influence output to the muscles and their function.

Furthermore, it addressed a possible factor that acts to shape and the properties of muscle representations within the cortex. This work has elucidated a number of novel findings that contribute to our understanding of motor cortex organization and provide insight for future study in both basic and clinical neuroscience.

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