NEURAL MECHANISMS OF MOTOR CORTICAL REPRESENTATION MODULATION

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Authors Declaration

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Abstract

TMS can be used to generate representational maps by delivering pulses at throughout a grid, centered over the most sensitive spot to elicit a resulting MEP called the motor hotspot. The areas of these maps are modulated by muscle contraction and have been shown to increase in area with increasing contraction intensity. Both intracortical inhibition (SICI), and intracortical facilitation (ICF) are paired pulse paradigms in which contraction causes a reduction in magnitude. The present study aimed to categorize changes in the above circuits and representational maps as well as expose a possible relationship between both metrics in the context of graded contraction. To study these questions 15 healthy, right-handed volunteers participated in a study measuring SICI, ICF and cortical maps under conditions of REST, 10, 20 and 30% of MVC of the right FDI muscle. SICI and ICF showed significant reduction between REST and no differences amongst contraction levels. However, SICI displayed a graded reduction through contraction levels when analyzed on a trial-by-trial basis sorted by actual contraction level. Cortical representational area increased from REST to all contraction states and between 10 and 30% MVC confirming the graded growth observed in previous studies. Further, analysis shows that SICI, ICF and area all exhibited the majority of their modulation within the first 10% of contraction. Both SICI and ICF were not significantly correlated to the growth in representational area. This may be in part due to participants' variability in the level of contraction sustained during measures, which also made it unfeasible to conduct a correlation of trial-by-trial data between map area and circuit magnitudes. We present evidence to corroborate previous findings for the effects of

contraction on intracortical circuits and representational area during graded contraction as well as contribute to the methodology of such investigations concerning the control of varied contraction.

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List of Abbreviations

ADM- Adductor digiti minimi
AMT- Active motor threshold
CoG- Center of gravity
CS- Conditioning stimulus
EMG- Electromyography
FDI- First dorsal interosseus
fMRI- Functional magnetic resonance imaging
LIHI- Long latency interhemispheric inhibition
TS- Test stimulus
M1- Primary motor cortex
MEG- Magnetoencephalography
MEP- Motor evoked potential
MSO- Maximum stimulator output
MVC- Maximum voluntary contraction
PET- Positron emission tomography
RMT- Resting motor threshold
SICI- Short latency intracortical inhibition
SIHI Short latency interhemispheric inhibition
TMS- Transcranial magnetic stimulation

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Chapter 1: Introduction

Overview of Thesis

This thesis investigated the effects of graded muscle contraction on both the magnitude of intracortical circuitry and the area of motor cortical representations, known as 'maps', to better understand how map modulation occurs. Previous research has proven that representational area expands with increasing contraction intensities (Van de Ruit & Grey 2016). The literature also provides evidence that would indicate intracortical circuitry, both inhibitory and facilitatory, also decrease when muscle is voluntarily activated. The primary hypothesis for the mechanism behind the expansion of maps was that a reduction of inhibitory input would allow the observed expansion.

Goals of the thesis

The aim of this thesis was to both characterize changes in intracortical circuit magnitude and representational maps as well as attempt to expose a relationship between the two metrics. This was accomplished by assessing intracortical circuits and motor cortical representations using transcranial magnetic stimulation (TMS). Intracortical circuitry was assessed by paired-pulse TMS of varying interstimulus intervals (ISI). Motor cortical representations were assessed by single pulses of TMS delivered systematically throughout a grid laid over the primary motor cortex (M1). These measures were repeated under conditions of rest, 10, 20, and 30% of the target muscle's maximum voluntary contraction (MVC).

Significance

Motor cortical representations ('maps') are vital to the understanding of the neural control of movement. Cortical representations provide a tangible representation of the muscle within the cortex and how much of the cortical territory is devoted to that specific muscle. The cortical maps also allow researchers to have a visual representation of the alterations caused by various neural processes. The study of muscle specific cortical maps has allowed researchers to examine the effects of interventions across the cortical representation of a muscle rather than at one focal point, which had made it a well-suited target to elucidate neural processes that 'modulate' the muscle response. If we can identify the intracortical circuits that contribute to modifying muscle representations, future research can aim to modify these circuits and ultimately change the motor cortical representations. Motor representations are observed to be abnormal in neurological disorders, which have known motor impairments, increasing the necessity of study in this area to discover the mechanisms for these dynamic alterations (Liepert et al. 2000; Schabrun et al. 2007). The information obtained from the proposed research will have applications in neurological disorders such as Parkinson's, dystonia and stroke, all of whom show abnormal intracortical circuits. These abnormalities may be indicative of an underlying impairment in neural function, which may be related to impairments in motor representations (Bâres et al. 2003; Stinear & Byblow 2004).

The investigation of neural circuits alongside motor cortical representations brings together two independently researched components of motor control in a novel manner.

By also characterizing intracortical circuits and motor map modulation in response to graded muscle contraction, a more informative relationship between the two could be exposed. The implied mechanisms will depend on which intracortical circuits are found to influence the cortical map area. The findings of this study will elucidate the key circuits that mediate the cortical representations of upper limb muscles and thus upper limb motor control.

Chapter 1: Review of Relevant Literature

Imaging the Dynamic Motor Cortices

Organization

The motor cortex is composed of mosaic-like somatotopic representations of the muscles it outputs to. Penfield and Boldrey (1937) gathered the original evidence of the phenomena utilizing electrical stimulation to map the motor cortex during neurosurgery. A pattern of representations was discovered, which we commonly know now as the motor homunculus, in which body areas are represented discretely in a medial to lateral fashion (Penfield and Boldrey 1937). This was the first study to look inside the motor cortex to find an organized layout of activation, and catalyzed the use of other imaging modalities to characterize both organization and stimulus driven changes in organization.

Non-Invasive Neuroimaging Techniques

Non-invasive imaging techniques were developed as an alternative to look at cortical structure and function without the need for alterations of the skull and neural membranes commonly seen in animal research. Functional magnetic resonance imaging (fMRI) has commonly been used to track the hemodynamic response within brain regions following activation. The blood oxygen level dependent (BOLD) signal fMRI detects is an indirect measure of neurotransmitter activity which indicates neuronal signaling and activation (Matthews & Jezzard 2004). Research using fMRI has revealed a medial to lateral somatotopic pattern specifically of the foot, elbow and hand (Rao et al. 1995; Schieber 2001). This technique has a high degree of spatial acuity and as such has been used to map the M1 with enough specificity to uncover contraindications to the original

organization put out by Penfield and Boldrey. One such contraindication is a much more overlapping pattern of arm muscles where the different areas of the upper and lower arm have been shown to overlap with both a dorsal and ventral region representation separate from that of the fingers (Meier et al. 2008). Positron emission tomography (PET) measures radioactive markers in the blood to provide an indirect measure of neuronal activity using the associated increases in cellular metabolism. PET imaging has yielded a similar medial to lateral representation pattern of the toes, fingers and tongue (Grafton et al. 1991). Magnetoencephalograpy (MEG), a technique which measures the magnetic fields produced by electrical neuronal activity is yet another method that has confirmed the aforementioned pattern of muscle representation (Cheyne et al. 1991). Clearly, many imaging techniques have confirmed the homuncular pattern of muscles in M1. However, it is important to note that M1 is not simply a static collection of muscle representations but a dynamic representation of our skeletal muscle system that can undergo plastic changes with various stimuli, including motor training and muscle contraction

Dynamic Modulation

It is widely known that motor cortical representations have the capacity to be dynamically altered in human and animal models. Intracortical microstimulation studies exploring animal models of behavioural plasticity have exemplified an immense capacity for M1 to undergo change (Nudo et al. 1990; Donoghue et al. 1992 & 1994; Nudo et al. 1996; Kleim et al. 1998; Remple et al. 2001; Kleim 2004). Long term motor training of fine digit control in non-human primates resulted in an increase in cortical motor

representation area of the digits while the less used forearm muscles saw a decrease in representation (Nudo et al. 1996). Even simple activation via repetitive microstimulation of M1 for ~ 10-20 minutes increased the representational area of the stimulated area of M1, which highlights the motor cortex's propensity for change (Nudo et al. 1990). Rodent models have also shown a capacity to change with stimuli including voluntary reaching and involuntary postural muscle stretch, which expanded forelimb representations (Kleim et al. 1998). Dynamic modulations outlined in animal studies have clearly shown that M1 representations change and more specifically areas corresponding to muscles trained, stimulated or more frequently used experience growth. These animal studies all involved the use of microstimulation, which necessitates the removal of a portion of the skull under anesthesia to allow microelectrode needles to be placed into the brain at a minute depth to electrically stimulate neurons. This technique is extremely focal and has the best temporal accuracy but is far too invasive for human participants and thus more complex and less invasive techniques have been developed.

Human studies rely on imaging modalities reliant on more indirect measures such as fMRI, PET and functional near-infrared spectroscopy (fNIRS), which is another method of assessing the hemodynamic response via infrared light absorption spectra. fMRI BOLD signals acquired from M1 and supplementary motor areas increased when participants increased the force at which they completed a key-pressing task (Dettmers et al, 1995 & 1996; Thickbroom et al. 1998 & 1999). PET scans taken during bilateral motor tasks of the upper limb and hand (Ingvar & Philipson 1977; Colebatch et al.

1991a), and during voluntary breathing (Colebatch et al. 1991b) have also shown increased regional blood flow in M1 and supplementary motor areas. Furthermore, PET imaging after administration of ^{15}O-labeled water found increases in blood flow to somatopically located tongue, fingers and toes (Grafton et al. 1991). fNIRS imaging has reliably shown increases in oxygenated blood flow within the motor cortices during non-fatiguing simple motor tasks such as finger movements (Maki et al. 1995). More recently, fNIRS imaging has shown significant differences in oxygenation levels corresponding to both contralateral and ipsilateral M1 during a graded pinch grip task ranging from 20-60% maximum voluntary contraction (MVC) (Shibuya et al. 2014). Together, these imaging modalities have provided consistent evidence for the modulatory capacity of motor cortical representations. However, these imaging techniques are costly and record metabolic or blood dependent response, which lag temporally behind the actual firing of neurons (Hallett 2007). Promising results have been obtained with the aforementioned imaging however, a comparably less expensive imaging modality of the cortices exists in the form of transcranial magnetic stimulation (TMS).

Transcranial Magnetic Stimulation

In 1985, a technique utilizing magnetic induction as a means of stimulating the brain was developed (Barker et al. 1985). This new technique offered more comfort and a less invasive experience for participants when compared to existing electrical stimulation techniques such as transcranial electrical stimulation (TES) discovered by Merton and Morton (1980). TMS indirectly stimulates neurons with a short, high current pulse

through a coil of wire, which produces a magnetic field perpendicular to the coil. This magnetic field induces a perpendicular electrical field relative to the magnetic field and parallel to the coil orientation, which is tangential to the skull (Hallett 2007). There are numerous coil designs aimed at stimulating different brain areas. The most commonly used coil design is the figure-of-eight coil, made up of two side-by-side circular coils with the greatest current induced at the intersection of the two circular windings of wire. Although TMS can stimulate underlying neurons, it is limited to superficial cortical grey matter as the magnetic pulse is attenuated to 30% of the strength produced at the coil, after travelling approximately 4 cm into the cortices (Siebner et al. 2009).

The delivery of a TMS pulse at a sufficient intensity depolarizes a group of neurons, which causes a resultant descending corticospinal volley. The earliest volley is thought to be a result of direct activation (D-wave) of the corticospinal axons of pyramidal tract neurons (PTN) thought to be located in the subcortical white matter because the volley is not influenced by changes in cortical excitability (Di Lazzaro et al. 2008). Indirect waves (I-waves) follow the D-wave in latency and result from trans-synaptic activation of the very same PTNs activated to produce the earlier D-wave. Subsequent I-waves appearing at increased latencies are elicited with increasing TMS stimulation and are separated by a 1.5 ms interval thought to reflect the firing rate of the PTNs (Di Lazzaro et al. 2008).

Parameters

The parameters under which the TMS stimulation is delivered can greatly impact the physiological response. M1 serves as a reference as it is the most frequently studied region. Small changes in coil orientation relative to the intact skull change the characteristics of the descending volleys, for example changing the current direction from posterior-anterior to anterior-posterior has been shown to cause slightly different latencies and longer duration (Di Lazzaro et al. 2012). Posterior-anterior stimulation has also been shown to reverse the order in which the volleys are recruited, displaying indirect I waves before the D wave (Di Lazzaro et al. 2012). Changing the stimulation intensity of the TMS pulse has been observed to alter the response, such that higher intensities induce more I-waves at increasing latencies and thus a greater response in the muscle targeted by the stimulation. The frequency of stimulation can also affect responses concerned with neuroplasticity. Repeated TMS pulses at low frequency (<1 Hz) have been shown repeatedly to suppress MEP amplitudes elicited from the motor cortex while high frequencies (>1 Hz) increased the amplitude (Chen et al. 1997, Berardelli et al. 1998).

Muscle Response

The motor evoked potential (MEP) displayed in Figure 1 displays the activity measured in the muscle following a TMS pulse delivered to its corresponding cortical representation. The muscle can be preferentially activated by locating what is known as a motor ‘hotspot’ which is defined as the site of stimulation along the scalp that elicits the largest MEP from the muscle of interest. MEPs are measured with the use of

electromyography (EMG), which involves the application of recording electrodes on the targeted muscle. TMS elicits corticospinal volleys down the tract where they eventually synapse onto alpha motor neurons in the spine, which send an efferent signal to the targeted muscle resulting in activity. Changing stimulation parameters has been shown to cause a change in the resultant MEP magnitude by activating more or less I-waves, thus changing the strength of the descending corticospinal volleys, resulting in different levels of activation of the alpha motor neuron pool.

Paired Pulse Paradigms

Paired pulse paradigms utilize two TMS pulses. The first pulse, known as the conditioning stimulus (CS), which has a modulatory effect on the response from the subsequent test stimulus (TS). The modulatory effect can either be in the form of facilitation or inhibition of the test pulse depending on stimulation intensity as well as the inter-stimulus interval (ISI) length.

Inhibitory Paradigms

Paired pulse protocols are utilized to investigate both excitatory and inhibitory circuits of the brain. Long- and short-latency intracortical inhibition (SICI and LICI, respectively) are a result of GABAergic mechanisms. Specifically, SICI is modulated by $GABA_A$ receptor activity and LICI is modulated by $GABA_B$ receptor activity (Ziemann et al. 1996; Hanajima et al. 1998; Werhahn et al. 1999; McDonnell et al. 2006). SICI is elicited by a subthreshold stimulus followed ~1-6 ms later by a suprathreshold stimulus while

LICI is elicited by two suprathreshold stimuli separated by ~50-200 ms (Valls-Solé et al. 1992; Kujirai et al. 1993). Pictorial representations of the described paradigms are shown in Figure 1.

Some indication of a functional role for these circuits comes from investigations looking at populations with neurological disorders characterized by altered cortical output and function which have been linked to abnormal inhibitory circuits (Bâres et al. 2003; Stinear & Byblow 2004). Inhibitory mechanisms have been indicated to have a role in muscle fractionation; a process that inhibits certain muscles while allowing cortical output to activate others in a specified pattern resulting in a desired movement (Zoghi & Nordstrom 2003). Inhibition has been implicated in the decision to move as shown by both increased SICI and LICI immediately prior to a simple choice reaction go/no-go task (Sohn et al. 2002). These results suggest that SICI and LICI modulate output until the desired movement for a successful result is selected.

Facilitation Paradigm

When a subthreshold TMS stimulus is followed by a suprathreshold TMS stimulus with an interval of ~10-25 ms the resulting MEP amplitude is increased, a phenomenon known as intracortical facilitation (ICF) (McGinley et al. 2010). ICF is mediated through excitatory glutamatergic interneurons and N-methyl-D-asparate (NMDA) receptors (Ziemann et al. 1995; Ziemann 2003). The role of ICF in M1 is less clear than that for inhibition. However, insights into the functional relevance of ICF have been gleaned from

studies in aging or clinical populations. Aging is associated with a decline in neuromuscular function as well as observed decreases in ICF at rest relative to young controls (McGinley et al. 2010). Individuals with Parkinson's disease also exhibited a lower level of ICF and saw an increase in both motor function and ICF when administered dopamine based pharmaceuticals such as LEVDOPA (Ridding et al. 1995; Bâres et al. 2003). However, when investigated in the context of motor training protocols ICF displayed no change indicating no involvement in the associated improvements in motor function (Perez et al. 2004).

Intracortical Circuits During Muscle Activity

Intracortical circuits are reduced when the target muscle is voluntarily contracted (Zoghi et al. 2003; Zoghi & Nordstrom 2007; Ortu et al. 2008). Other researchers took the aforementioned concept further by examining the diminished effect of intracortical inhibition during a graded muscle task. One study used thumb abductions at forces of 1, 2, 3, 5 and 10 N while recording from right APB, FDI and abductor digiti minimi (ADM) and found that the CS had less influence on the TS response amplitude at forces of 2-10 N for the APB and for ADM and FDI only at 10 N (Zoghi & Nordstrom 2007). The paired pulse paradigm in the previous study was conducted with an ISI of 3 ms and as such displayed a reduction in SICI with graded thumb abduction. Reduction of LICI has also been shown in the first dorsal interosseus (FDI) muscle during index finger abduction (Hammond & Vallence 2007). An experiment where an individual was required to tap a computer mouse at a rate of 1 Hz resulted in an increase in EMG activity and a decrease

in SICI in the agonist FDI. Interestingly, recording EMG of the uninvolved surrounding abductor pollicis brevis (APB) muscle displayed a similar pattern as the agonist in half of the subjects but exhibited an increase in SICI and decreased excitability in the other half (Stinear & Byblow 2003). The results suggest that an increase in inhibition accompanied by a decrease in excitability may thwart unwanted contractions and that the uninvolved muscle acted in an inverse manner in a substantial amount of subjects (Stinear & Byblow 2003). The effect of voluntary activation of multiple muscles on intracortical inhibition has implied that inhibition may have at least some role in shaping the movements humans produce.

ICF is seldom studied in the context of muscle activation but when it has been, a reduction is generally seen. One study examined the effect of 5 % MVC contraction of the right FDI on ICF and found that ICF decreased with the contraction (Ridding et al. 1995). A later investigation also in the right FDI also found that voluntary contraction decreased ICF (Hanajima et al. 2002). Overall, ICF has been shown to decrease in the few studies investigating it within the context of contraction however, the lack of investigation highlights the need for more studies regarding ICF and contraction, specifically graded contraction.

Motor Mapping

Most imaging tools investigating the topography of the human brain are all encompassing and map all areas participating in the neural activity at that time (Karhu et al. 2014) In

contrast, TMS can be used to selectively map the brain areas necessary for the physiological response being evoked, making it uniquely suited to map M1. TMS mapping implies a causal response to the output we measure as it is caused by the stimulation itself. The maps are made by linking the observed muscle response to the position of the stimulus over M1 (Wassermann et al. 1992). TMS mapping is accomplished with the use of neuro-navigation software, which allows researchers to track the position of an individual's head and respective brain scan to the TMS coil via markers and an infrared camera. Mapping technologies are have commonly been used to evaluate neural changes or plasticity caused by neurological disorders such as stroke (Liepert et al. 2000; Schabrun et al. 2008). This technology has enabled the evaluation of functional changes in M1 to guide rehabilitation strategies. However, the size or areas of the representations are not the only metrics obtained from the technique. Further information concerning the foci of the representation with the use of a weighted center of gravity calculation as well as the degree of overlap with adjacent representations can provide additional information about the motor output. The differences in area between muscles may be a result of the increased innervation density and thus increased corticospinal density to those muscles involved in fine motor skills that have been shown to possess larger representations (Feinstein et al. 1955). Some of the earliest work to utilize TMS as a method to map the cortices was completed by Wassermann and colleagues (1992). A critical flaw in this pioneering study was that a stimulus intensity of 100% of the stimulator capacity was used and thus saturated the cortical map. This method caused the technique to be too insensitive to pick up the discrete topographic

representations described by earlier studies utilizing electrical stimulation and highlighted the need to control mapping parameters closely. Many mapping parameters including stimulation intensity, muscle contraction and processing methods can alter map characteristics.

Stimulation Intensity

The most notable change in map characteristics is the expansion of area when higher stimulus intensities are utilized (Thordstein et al. 2013). The expansion with higher intensities is very intuitive, as it is known that higher intensities activate larger areas of the cortex (Day et al, 1989). MEP amplitudes have also been shown to increase with increasing stimulation intensities (Hess et al. 1987; Day et al. 1989; Kiers et al. 1993) however the MEP amplitude does eventually saturate, observed as a plateau in MEP recruitment curves (Devanne et al. 1997). Systematic mapping with three levels of stimulation intensity ranging from 110 to 130% of RMT resulted in larger volume and area of FDI representations, which showed that the above changes do influence map characteristics (Van de Ruit & Grey 2016). In conclusion, stimulus intensity has been shown to influence both the MEP amplitude of motor responses as well as the amount of the cortex dedicated to eliciting these responses, reflected in the growth of motor representations probed at higher intensities. These findings highlight the importance of maintaining constant stimulation intensities to avoid the aforementioned confounding effects.

Muscle State

Cortical maps are most often obtained in resting muscles (Wassermann et al. 1992; Pascual-Leone et al. 1995), but have also been obtained at during contraction most commonly at low levels (Ridding et al. 1995; Ngomo et al. 2012). The state of the muscle in regards to contraction has long been known to affect the magnitude of MEPs such that increased contraction results in elevated MEP magnitudes (Hess et al, 1987; Kiers et al. 1993). Much like the effect of stimulus intensity the increased MEP magnitudes are accompanied by an expansion of representational area (Wilson et al. 1995; Classen et al. 1998; Van de Ruit & Grey 2016). The effect of muscle contraction on CoG has been less decisive as some studies displayed a shift with contraction (Wilson et al. 1995) while others saw no change (Classen et al. 1998; Ngomo et al. 2012). It is also important to note that the expansion of area seen with contraction was abolished when stimulation intensity is adjusted relative to AMT (Ngomo et al. 2012). The disappearance of expansion could be a result of decreased active motor thresholds at higher contraction intensities resulting in lower stimulation intensities, which mask the area increase normally seen. A more recent study investigated the effects of higher contraction levels with constant stimulation intensity and showed a graded increase in map area with increasing contraction as well as shifts in CoG (Van de Ruit & Grey 2016). In conclusion, contraction does expand map area and shift CoG with constant stimulation intensity but both effects are not present with adjusted intensities. The present data suggests that the representational area of a muscle does grow when that muscle is contracted under proper stimulation parameters.

Processing Techniques

The methods used to determine which motor responses will be included in the calculation of map characteristics can heavily influence the result. Most researchers have agreed that it is necessary to filter out tonic background EMG signals that are not a result of TMS stimulation. The most common method of filtering out tonic contraction has been to set an operational definition of what constitutes a response to TMS which is used as a cutoff for inclusion in the cortical map (Wassermann et al. 1992; Wilson et al. 1993; Uy et al. 2002; Van de Ruit & Grey 2016). The MEP cutoff has also been centralized on the map such that any responses below a certain percentage of the largest response in the map were not included (Wilson et al. 1993; Uy et al. 2002). Alternatively, the cutoff for MEP inclusion has been calculated based on the maximum muscle response elicited from supramaximal peripheral nerve stimulation (Wassermann et al. 1992). Utilizing a percentage of supramaximal nerve stimulation as an inclusion cutoff is believed to be problematic, as it is well known that the maximum response from nerve stimulation is greater than that from TMS. Perhaps, the most valid method is to anchor the cutoff to the level of physiologic background noise as this method ensured the map captured all physiological data without the tonic background EMG (Devanne et al. 2006). The inclusion criteria greatly influenced the resulting area of motor representations as more conservative MEP inclusion criteria resulted in smaller representations and vice versa (Uy et al. 2002). Although, representational area calculated with the use of TMS will always be greater than the actual representation due to current spread the aforementioned processing techniques look to limit the saturation of the motor map grid (Mortifee et al.

1994) Selection of proper stimulation intensities as well as specific inclusion criteria has allowed researchers to closely approximate representational area, which remains stable over time (Wilson et al. 1993).

Chapter 3: Modulation of Intracortical Circuits and Motor Maps with Contraction

Introduction

It is widely known that motor cortical representations have the capacity to be dynamically altered in human and animal models. Intracortical microstimulation studies exploring animal models of behavioural plasticity have exemplified an immense capacity for M1 to undergo change (Nudo et al. 1990; Donoghue et al. 1992 & 1994; Nudo et al. 1996; Kleim et al. 1998; Remple et al. 2001; Kleim 2004). Many different factors induce a growth in M1 representations including motor training (Nudo et al. 1996), direct micro-stimulation (Nudo et al. 1990), voluntary and involuntary movement (Sanes et al. 1992; Kleim et al 1998).

TMS-evoked measures of SICI and ICF are assessed using a paired-pulse TMS protocol where a CS inhibits or facilitates the activity elicited from a subsequent TS (Valls-Solé et al. 1992; Kujirai et al. 1993). SICI and LICI have been implicated in muscle fractionation; a phenomenon that allows coordinated, sequential movements, of multiple muscles necessary to produce desired movements (Liepert et al. 1998; Zoghi et al. 2003; Zoghi & Nordstrom 2007; Hammond & Vallence 2007). Parkinson's patients have been observed to exhibit abnormally decreased SICI (Ridding et al. 1995a; Bareš et al. 2003) as well as increased LICI (Priori et al. 1994; Berardelli et al. 1996), while the role of ICF is unclear with some research pointing towards decreases (McGinley et al. 2010) as well as null findings (Ridding et al. 1995a). Individuals with focal hand dystonia (FHD) also demonstrated abnormal circuitry with reduced SICI (Ridding et al. 1995b; Stinear & Byblow 2004) and LICI (Chen et al. 1997; Espay et al. 2006), while ICF appeared to

show no change (Ridding et al. 1995b). Furthermore, SICI, LICI and ICF are reduced with low-level muscle contraction relative to rest as well as during increasing graded contraction intensity (Ridding et al. 1995c; Hanajima et al. 2002; Zoghi & Nordstrom 2007; Hammond & Vallence 2007). The prevalence of altered modulation of intracortical circuits in individuals with movement disorders along with their reductions from rest to contraction further implicates their importance to normal motor output and function.

TMS can be utilized to systematically map the territory of M1 dedicated to a muscle of choice. This is accomplished by delivering TMS pulses throughout a grid centered over the area of the brain which elicits the strongest response in the muscle of interest, known as the motor hotspot (Wasserman et al. 1992). Voluntary muscle activity has been shown to increase cortical territory, most commonly in intrinsic hand muscles such as the FDI muscle of the index finger (Wilson et al. 1995; Van de Ruit & Grey 2016). This increase in cortical territory has also been observed to have a dose-dependent response such that greater muscle contraction intensity results in further map growth (Van de Ruit & Grey 2016). The reason for these changes remains largely unclear, however it has been hypothesized that a reduction of inhibitory inputs during contraction allows the growth in cortical territory (Jacobs & Donoghue 1991; Ziemann et al. 1998). The effects of context dependent contraction are well characterized for intracortical and cortical territory modulation. However, the effects of graded contraction on the circuits alone and the relationship between them and cortical territory with graded contraction is poorly understood.

The goal of the present study was to explore a possible relationship between SICI, and ICF and the cortical territory representing this muscle. To test this, each measure as well as a map of the cortical territory was probed at rest and three increasing contraction intensities (REST, 10, 20 and 30% MVC). This allowed for an investigation into potential exogenous targets for proven pharmaceutical interventions to alter neural circuits and thus modulate cortical maps in pathologies exhibiting altered motor characteristics.

Methods

i. Subjects

15 healthy, right-handed subjects (18-25 years old) were recruited to participate in this study. Right hand dominance was confirmed with a handedness questionnaire (Oldfield 1971). No testing was commenced until all participants had given written informed consent and had passed a TMS safety questionnaire. All methods and protocols mentioned herein were previously approved by the McMaster Research Ethics Board in accordance with the *Declaration of Helsinki.*

ii. Transcranial Magnetic Stimulation

Single-pulse TMS was administered using a Masgstim 200^2 stimulator (Magstim, UK) attached to a 70 mm figure-of-eight coil to send pulses to the right FDI area in the left M1. The right FDI hotspot, defined as the area of M1 eliciting a maximal response in the right FDI muscle, was located for each individual using the BrainSight Neuronavigation system (Rogue Research, Canada). TMS stimulation intensities were calculated as a

percentage of the individuals resting or active motor threshold. RMT was defined as the minimum stimulus intensity required to elicit a peak-to-peak amplitude response of greater than 50 μV in 50% of trials. Active motor threshold (AMT) was defined as the minimum stimulation intensity required to elicit a 200 μV peak-to-peak amplitude response (Rossini et al. 2015). Both RMT and AMT were determined using a Bayesian adaptive estimation method contained in the TMS MTAT software (Clinical Researcher, USA). The coil was held perpendicular to the scalp and 45 degrees from the midline throughout all measures.

iii. Electromyography

EMG activity was recorded from the muscle belly of the right and left FDI muscle using two Ag/Ag-Cl adhesive electrodes. A wet ground was wrapped around the forearm to ensure low signal noise. EMG recordings were band-pass filtered between 20 Hz and 2.5 kHz, amplified 1000x (Intronix Technologies Corporation Model 2024F, Bolton, Canada), digitized using an analog-to-digital converter at 5 kHz (Power1401, Cambridge Electronics Design, Cambridge, UK) and stored on a secure computer for offline analysis (Signal v6.02, Cambridge Electronics Design, Cambridge, UK).

iv. MVC and Background EMG

Maximum voluntary contraction (MVC) was obtained by converting a 50 ms window of linear envelop EMG obtained from electrodes on the right FDI muscle during 3 maximal bouts of index finger abduction to RMS EMG (root mean square) with a custom

MATLAB script (MATLAB 2017b, MathWorks, Massachusetts, USA). The MATLAB script took the largest RMS amplitude of all 3 trials as the MVC of a particular participant. Background noise was assessed for each measure at each level of contraction sustained during the acquisition of cortical maps and intracortical circuits. A 50 ms window per trial of linear envelope EMG recorded from electrodes on the right FDI was converted to RMS (root mean square) EMG for all trials of a given condition using an adapted custom MATLAB script. This RMS data was then expressed as a percentage of the MVC EMG mentioned previously obtained during the 3 maximal bouts.

v. Intracortical Circuits

SICI and ICF were elicited in the right FDI muscle using the protocol established by Kujirai and colleagues in 1993 wherein a subthreshold conditioning TMS pulse (SI) is followed by a suprathreshold test TMS pulse (S2). SICI was probed over the previously found motor hotspot for the right FDI muscle utilizing an inter-stimulus interval (ISI) of 2 ms while ICF utilized an ISI of 10 ms (Kujirai et al. 1993). All intracortical paradigms used in this experiment are displayed in figure 1.

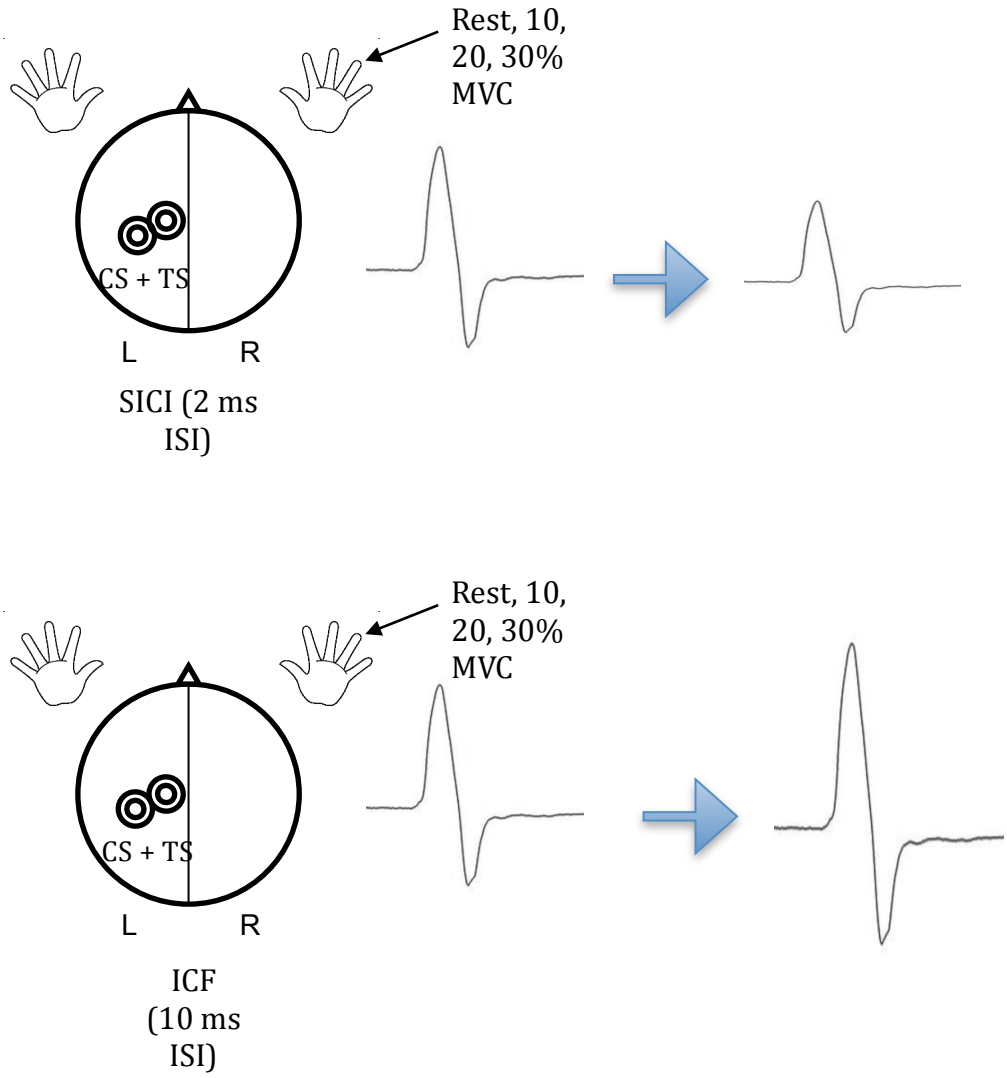

Figure 1. The coil's 45-degree orientation in the contralateral hemisphere is displayed as well as the MEP modulation that occurs with the respective paired pulse paradigms.

vi. Cortical Mapping

A cortical map is a visual representation of the size and level of activation for a specific area of the brain. In the case of this experiment, a cortical map was obtained for the right FDI muscle, centered on the muscle's hotspot in the left hemisphere. A 5 x 5 cm square

grid with 25 individual grid points as shown in figure 2, was utilized for each cortical map with five TMS pulses delivered at each point guided by neuro-navigation software. The intensity of the stimulation at each grid point was set at 110% of the respective muscles RMT.

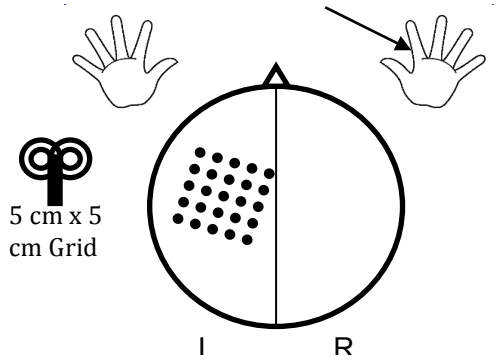

Figure 2. The 5 x 5 cm grid is overlaid on the left motor cortex with the 4 contraction conditions of the right FDI listed.

vii. Experimental Protocol

The subject was first seated into a comfortable position with both hands and forearms relaxed on a pillow in their lap. Hotspots, RMT and AMT of the right FDI muscle were found next using neuro-navigated TMS stimulation. Next, the individuals MVC was determined by having them maximally contract their right FDI by abducting their index finger against an immovable pole with EMG data displayed on an oscilloscope for participant feedback and for the experimenter to mark the participant's approximate MVC. Next, SICI and ICF were measured while the right FDI is contracted at one of the four contraction intensities of rest, 10%, 20%, and 30% MVC. The participant maintained

the mentioned contractions by matching their contraction to a predetermined line displayed on an oscilloscope. The order of the type of stimulation, as well as the contraction intensity required was randomized. However, it is important to note that only the right FDI was ever contracted as the left FDI was being observed for background activity. SICI and ICF conditioning pulse intensity was set at 90% of the individuals AMT in the stimulated muscle and the TS was set at 130% of the individuals RMT for rest trials and 130% of the AMT obtained under each respective contraction intensity for active trials. The aforementioned stimulation parameters remained constant across all SICI and ICF measurements at all levels of contraction. SICI and ICF were determined using 30 pulses; 15 TS alone pulses and CS/TS conditioned pulses.

The cortical maps were collected using an intensity of 110% RMT. The right FDI cortical map was obtained by centering the 5 x 5 cm grid over the motor hotspot for the muscle and then delivering five pulses (ISI-5 s) at each of these grid points for a total of 125 pulses per map.

Cortical maps were produced by including individual MEPS that were equal to or larger than 20% of the max MEP amplitude of that respective grid (Wilson et al. 1993). The centre of gravity (CoG) was calculated to find the amplitude weighted center of the cortical territory using the equation; $CoG_{(x)} = \Sigma\alpha_i X_i / \Sigma\alpha_i$ 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 (Wassermann et al. 1992).

viii. Statistical analysis

Each dependent measure was assessed for normality (Shapiro Wilks) and outliers. Non-normal data was assessed using a Friedman's test. A one-way RM ANOVA with within subjects factor of CONTRACTION (REST, 10, 20 and 30% MVC) was used to assess the effects of contraction on SICI and ICF while a Friedman's test with the same conditions was used to assess the effects of contraction on representational area. SICI, ICF and representational area also had the percent change between contraction levels analyzed via a Friedman's test with the factor of INTERVAL (REST-10%, 10-20%, and 20-30% MVC). Changes in TS amplitudes across contraction levels were also assessed using a Friedman's test with the factor of CONTRACTION (REST, 10, 20 and 30% MVC). Correlational analysis comparing the percent change in SICI and ICF magnitude with the percent change in representational area were completed using a two-tailed Spearman correlation. Lastly, all SICI trials were sorted by the level of contraction actually obtained in each respective trial and were sorted into groups ($0.1 \geq x \leq 1.3\%$, $1.3 \geq x < 10\%$, and $10 \geq x < 20\%$ and $20 \geq x \leq 30\%$ MVC). Spearman correlations were run correlating the magnitude of SICI during each of the aforementioned intervals. Lastly, a one-way RM ANOVA was completed with the factor of GROUP for the sorted SICI data.

Results

All 15 participants successfully completed the experiment and are included in the data analyses. Table 1 summarizes the statistical analyses.

Table 1. Results of ANOVA/Friedman's Tests

Measure	Effect Size	ANOVA/Friedman's	Post-Hoc
SICI	R^2= 0.2949	$F_{(3,42)}$=10.27, p<0.001	REST vs. 10%: 0.65 ± 0.07 vs. 0.98 ± 0.05, p < 0.05 REST vs. 20%: 0.65 ± 0.07 vs. 0.95 ± 0.05, p < 0.05 REST vs. 30%: 0.65 ± 0.07 vs. 1.00 ± 0.06, p < 0.05 10% vs. 20%: 0.98 ± 0.05 vs. 0.95 ± 0.05, p > 0.05 10% vs. 30%: 0.98 ± 0.05 vs. 1.00 ± 0.06, p > 0.05 20% vs. 30%: 0.95 ± 0.05 vs. 1.00 ± 0.06, p > 0.05
ICF	R^2=0.2163	$F_{(3,42)}$= 5.61, p=0.0025	REST vs. 10%: 1.14 ± 0.05 vs. 0.95 ± 0.06, p < 0.05 REST vs. 20%: 1.14 ± 0.05 vs. 0.86 ± 0.04, p < 0.05 REST vs. 30%: 1.14 ± 0.05 vs. 0.94 ± 0.04, p < 0.05 10% vs. 20%: 0.95 ± 0.06 vs. 0.86 ± 0.04, p > 0.05 10% vs. 30%: 0.95 ± 0.06 vs. 0.94 ± 0.04, p > 0.05 20% vs. 30%: 0.86 ± 0.04 vs. 0.94 ± 0.04, p > 0.05
Cortical Territory	Kendall's W=0.743	$X^2_{(3)}$= 33.087, p<0.001,	REST vs. 10%: 10.0 ± 0.98 vs. 19.93 ± 0.59, p < 0.001, r=-0.603 REST vs. 20%: 10.0 ± 0.98 vs. 21.33 ± 1.00, p < 0.001, r=-0.602 REST vs. 30%: 10.0 ± 0.98 vs. 22.40 ± 0.70, p < 0.001, r=-0.624 10% vs. 20%: 19.93 ± 0.59 vs. 21.33 ± 1.00, p = 0.088, r=-0.312 10% vs. 30%: 19.93 ± 0.59 vs. 22.40 ± 0.70, p = 0.004, r=-0.527 20% vs. 30%: 21.33 ± 1.00 vs. 22.40 ± 0.70, p = 0.134, r=-0.274

**Parametric data used ANOVA and Tukey HSD while non-parametric data used Friedman's and Wilcoxon signed rank tests.*

Intracortical Circuits

The actual contractions during the 10, 20 and 30% MVC of FDI were significantly different from each other ($X^2(3)$=133.8, P<0.0001) and are shown in table 2. Figure 3Ai depicts the group-averaged magnitude (with standard error of the mean) of SICI for each level of contraction. One-way ANOVA revealed a main effect of contraction ($F_{(3,42)}$=10.27, p<0.001) such that SICI was greater in REST compared to all other conditions only ($p < 0.05$) with no differences among the levels of muscle contraction. To explore this further, percent change for SICI was calculated within each bin (REST to 15.56±1.87%, 15.56±1.87 to 24.93±2.27%, 24.93±2.27 to 36.77±4.12%) as seen in Figure 3Aii. One-way ANOVA revealed a main effect of contraction on the percent change of SICI between the interval bins (REST to 10%, 10 to 20%, 20 to 30%) for SICI ($F_{(2,28)}$= 7.422, p=0.0026) such that the contraction intervals showed decreased magnitude of SICI when compared to the REST to 10% MVC interval (Table 3). A main effect of contraction was found for TS amplitude ($X^2(3)$=260.973, P<0.001) indicating that amplitudes were significantly increasing throughout all contraction levels (p<0.01) (Figure 3Aiii) A depiction of SICI can be seen by comparing the amplitude of the CS-TS dashed trace in figures 3Bi-iv to that of the solid TS alone at each contraction intensity. The CS-TS trace appears to be of lower amplitude at rest reflecting SICI while matching the TS alone amplitude during contraction reflecting an abolishment of the inhibition

(Figures 3Bi-iv). A graphical representation of the magnitude of SICI across all conditions, and for all participants can be seen in figure 4 with the 10 out of 15 participants highlighted who followed the trend outlined above.

Table 2. RMS Background Data

Measure	10% MVC	20% MVC	30% MVC
SICI/ICF	15.56 ± 1.87	24.93 ± 2.27	36.77 ± 4.12
Cortical Mapping	15.75 ± 1.67	26.32 ± 2.79	36.43 ± 3.96

**All RMS data was sampled during a 50 ms pre-stimulus window.*

Table 3. Results of Percent Change Friedman's Tests

Measure	Effect Size	Friedman's	Outcome
SICI	Kendall's W=0.253	$X^2_{(3)}$=19.55, p< 0.001	REST-10% vs. 10-20%: 82.15 ± 26.65 vs. -0.8223 ± 5.035, p=-.001, r=-0.591 REST-10% vs. 20-30%: 82.15 ± 26.65 vs. 7.342 ± 6.122, p=0.001, r=-0.602 10-20% vs. 20-30%: -0.8223 ± 5.035 vs. 7.342 ± 6.122, p=0.433, r=-0.143
ICF	Kendall's W=0.058	$X^2_{(3)}$= 1.733, p=0.420	REST-10% vs. 10-20%: -15.14 ± 6.486 vs. -2.405 ± 8.339, p=0.609, r=-0.093 REST-10% vs. 20-30%: -15.14 ± 6.486 vs. 13.24 ± 7.564, p=0.069, r=-0.332 10-20% vs. 20-30%: -2.405 ± 8.339 vs. 13.24 ± 7.564, p=0.211, r=-0.228
Cortical Territory	Kendall's W=0.652	$X^2_{(3)}$= 19.55, p<0.0001	REST-10% vs. 10-20%: 120.9 ± 18.11 vs. 6.737 ± 3.835, p=0.001, r=-0.591 REST-10% vs. 20-30%: 120.9 ± 18.11 vs. 7.339 ± 4.617, p=0.001, r=-0.602 10-20% vs. 20-30%: 6.737 ± 3.835 vs. 7.339 ± 4.617, p= 0.433, r=-0.143

Figure 3

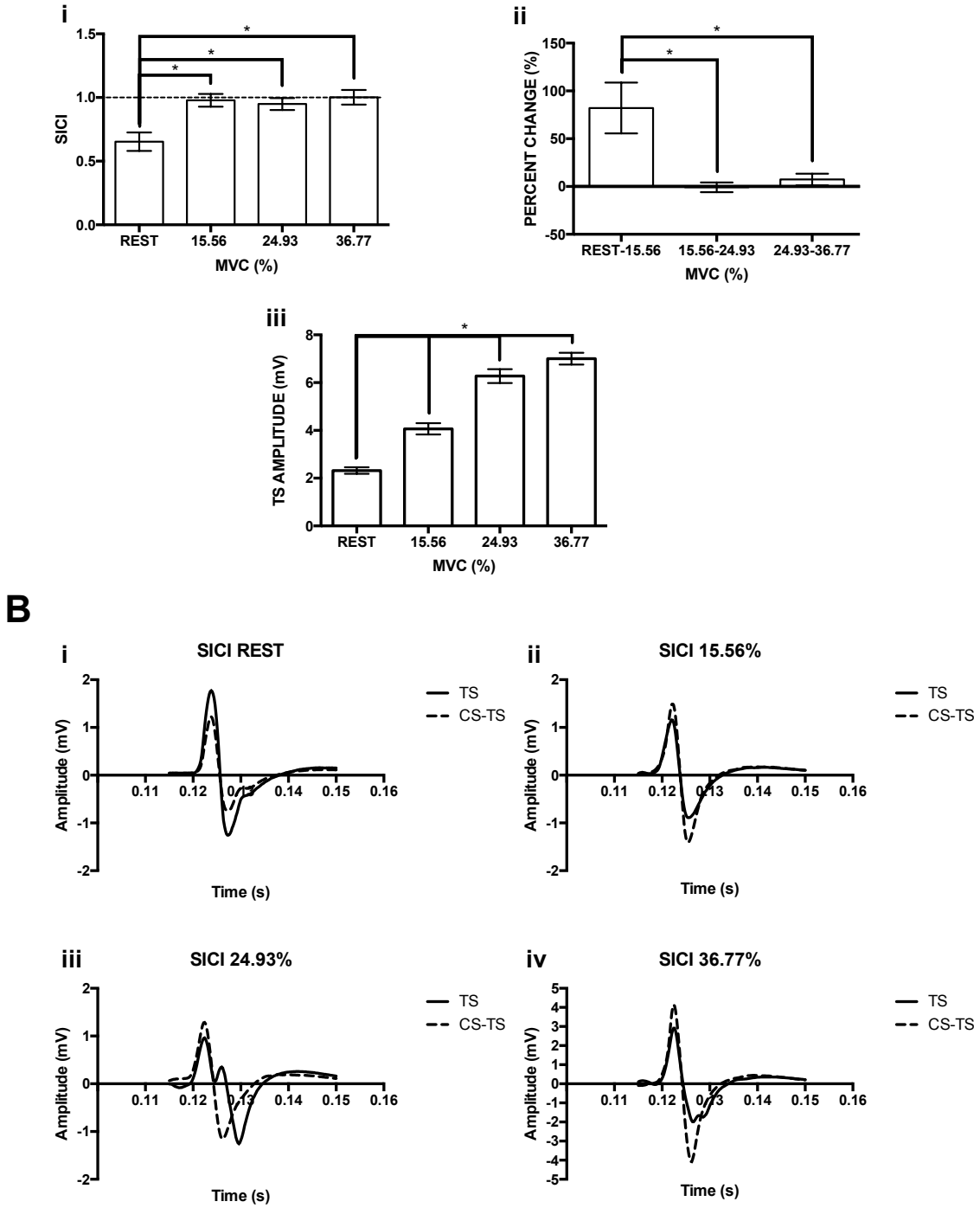
A
i
SICI
1.5
1.0
0.5
0.0
REST
15.56
24.93
36.77
MVC (%)
ii
PERCENT CHANGE (%)
150
100
50
0
-50
REST-15.56
15.56-24.93
24.93-36.77
MVC (%)
iii
TS AMPLITUDE (mV)
8
6
4
2
0
REST
15.56
24.93
36.77
MVC (%)
B
i
SICI REST
ii
SICI 15.56%
iii
SICI 24.93%
iv
SICI 36.77%
TS
CS-TS
Amplitude (mV)
Time (s)
0.11
0.12
0.13
0.14
0.15
0.16

Figure 3. Differences in SICI magnitude amongst and between the contraction conditions. Ai) The differences in SICI magnitude (mean± SEM) indicating the REST condition is different than all other contraction levels ($p<0.05$) and Aii) showing the percent change data (mean± SEM) for the magnitude of SICI, which indicates the majority of the decrease in SICI happens in the REST-15.56% MVC interval ($p<0.001$). Aiii) depicts the TS amplitudes at each contraction level with SEM error bars and shows that TS amplitude grows significantly with contraction ($p<0.001$). Bi-iv) show representations of EMG traces from the FDI muscle as a result of CS-TS paired pulses (dashed line) and single TS pulses (solid line) for REST, 15.56, 24.93, and 36.77% MVC conditions respectively. The CS-TS pulse becomes closely matched to or larger than the TS pulse at 15.56% MVC and higher indicating a loss of SICI.

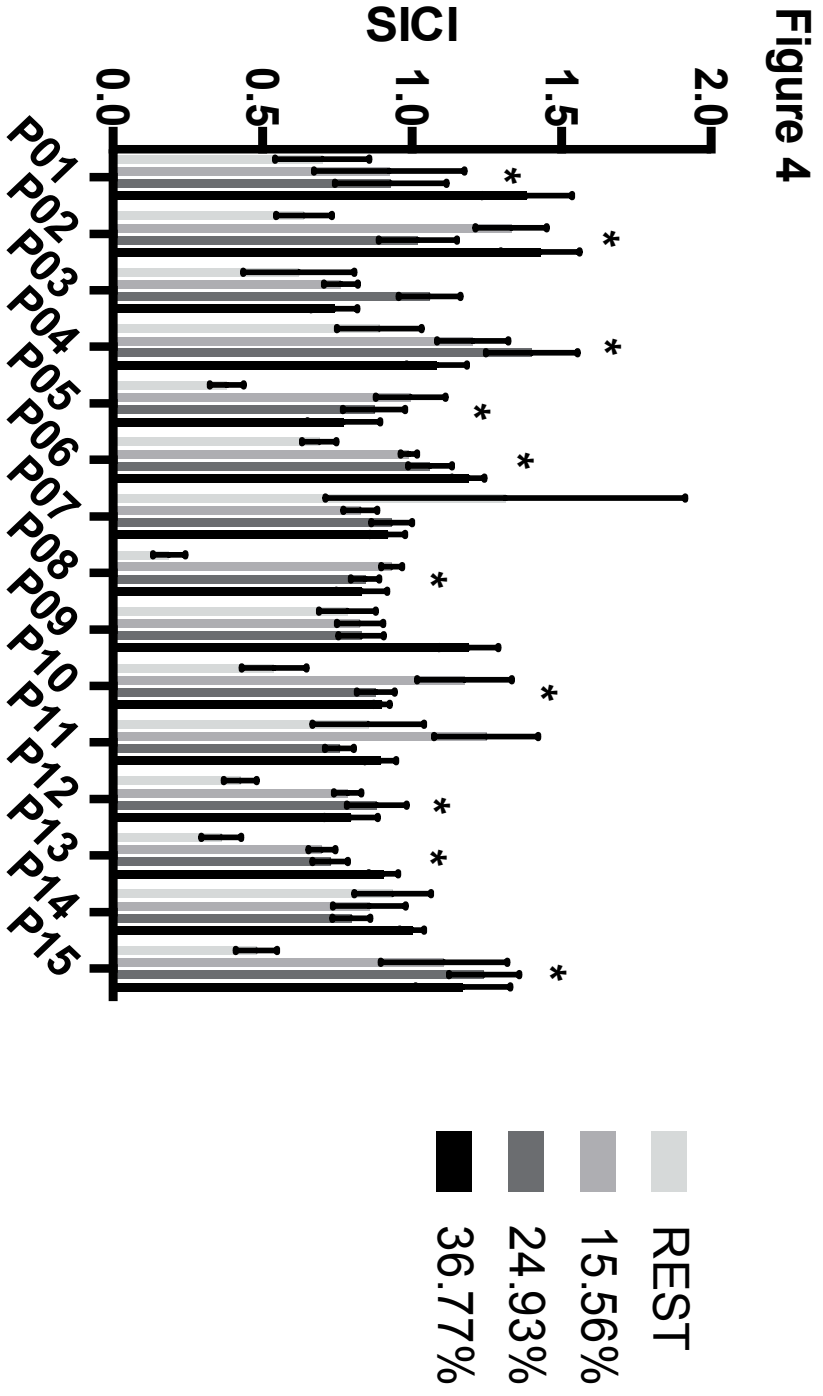
SICI
0.0
0.5
1.0
1.5
2.0
P01
P02
P03
P04
P05
P06
P07
P08
P09
P10
P11
P12
P13
P14
P15
REST
15.56%
24.93%
36.77%

Figure 4

Figure 4. Difference in SICI magnitude (mean ± SEM) for all participants across all contraction conditions tested.

For ICF, a one-way ANOVA demonstrated a main effect of condition ($F_{(3,42)}$=5.61, p=0.0025) such that ICF was greater during REST compared to all other conditions only ($p < 0.05$) (Figure 5Ai). A one-way ANOVA was also completed on the percent change data between the same bins used for SICI but showed no significant changes with the condition of contraction ($F_{(2,28)}$= 2.588, p=0.0930) (Figure 5Aii). The same pattern of increasing TS amplitudes was seen with ICF as they were acquired during the same acquisition script and thus may also be suppressing the level of ICF at higher intensities (Figure 5Aiii). A depiction of ICF is shown by comparing the amplitude of the CS-TS dashed trace in figures 5Bi-iv to that of the solid TS alone at each contraction intensity. The CS-TS trace appears to be of higher amplitude at rest reflecting ICF while matching or showing a decrease relative to the TS alone amplitude during contraction reflecting an abolishment during contraction and even a reversal of the facilitation at the highest level (36.77±4.12% MVC) (Figures 5Bi-iv).

Figure 5

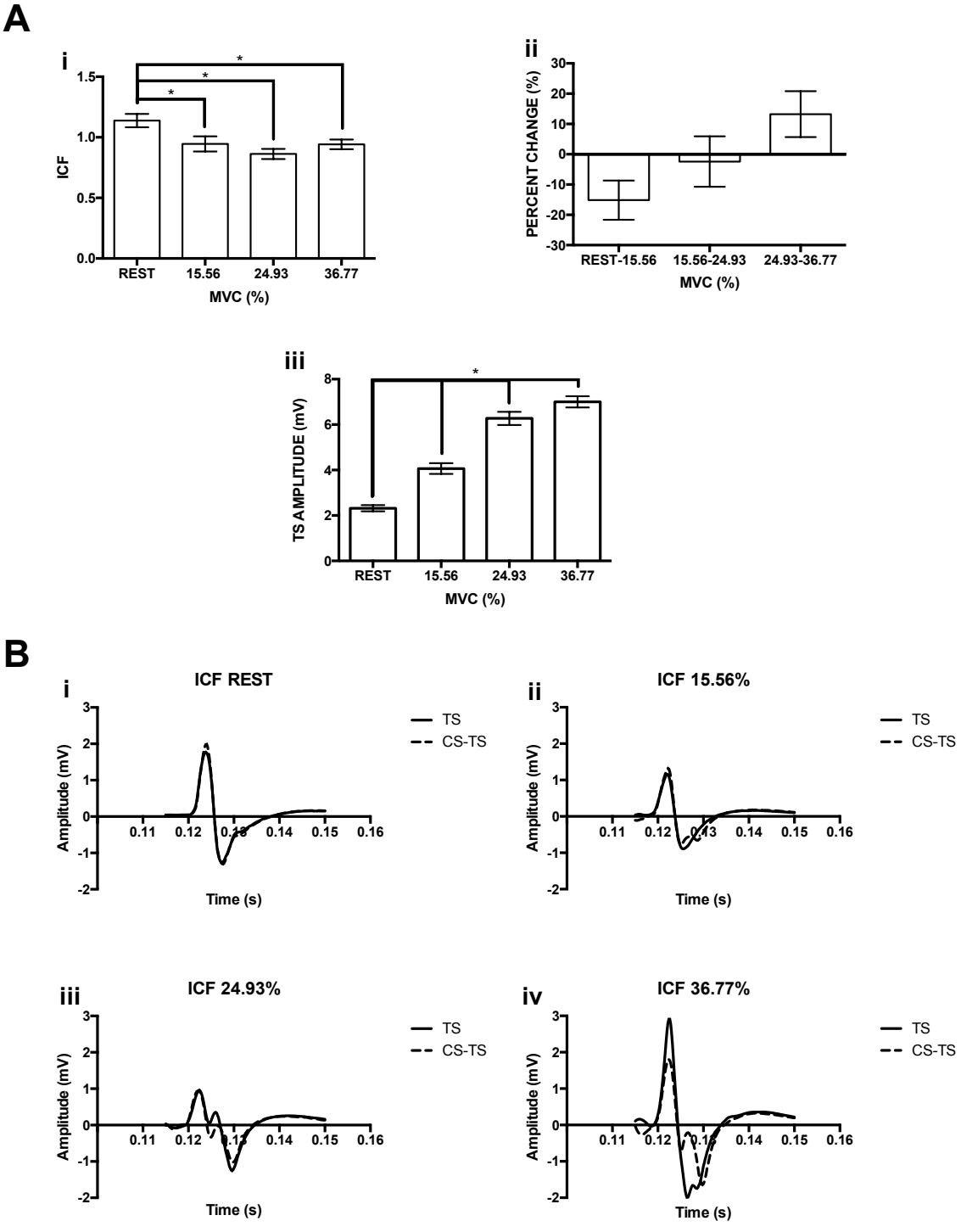
A
i
ICF
1.5
1.0
0.5
0.0
*
*
*
REST
15.56
24.93
36.77
MVC (%)
ii
PERCENT CHANGE (%)
30
20
10
0
-10
-20
-30
REST-15.56
15.56-24.93
24.93-36.77
MVC (%)
iii
TS AMPLITUDE (mV)
8
6
4
2
0
*
REST
15.56
24.93
36.77
MVC (%)
B
i
ICF REST
Amplitude (mV)
3
2
1
0
-1
-2
0.11
0.12
0.13
0.14
0.15
0.16
Time (s)
TS
CS-TS
ii
ICF 15.56%
Amplitude (mV)
Time (s)
TS
CS-TS
iii
ICF 24.93%
Amplitude (mV)
Time (s)
TS
CS-TS
iv
ICF 36.77%
Amplitude (mV)
Time (s)
TS
CS-TS

Figure 5. Differences in ICF magnitude amongst and between the contraction conditions. Ai) The differences in ICF magnitude (mean± SEM) indicating the REST condition is different than all other contraction levels ($p<0.05$) and Aii) showing the percent change data (mean± SEM) for the magnitude of ICF, which indicates the majority of the decrease in ICF appears to happen in the REST-15.56% MVC interval however no significant differences were found ($p= 0.420$). Aiii) depicts the TS amplitudes at each contraction level with SEM error bars and shows that TS amplitude grows significantly with contraction ($p<0.001$). Bi-iv) show representations of EMG traces from the FDI muscle as a result of CS-TS paired pulses (dashed line) and single TS pulses (solid line) for REST, 15.56, 24.93, and 36.77% MVC conditions respectively. The CS-TS pulse becomes closely matched to or smaller than the TS pulse at 15.56% MVC an higher indicating a loss of ICF.

Cortical Territory

A Friedman's test showed a main effect of contraction ($X^2(3)=33.087$, $P<0.001$) indicating that cortical territory during the REST condition was smaller compared to all levels of contraction (Figure 6Ai). Cortical territory was observed to also increase from the lowest level of contraction (10%) to the highest level of contraction (30%) ($Z=-2.885$, $p=0.004$), shown graphically in Figure 6Ai. A Friedman's test of the percent change of cortical territory indicates a main effect of contraction ($X^2(3)= 19.55$, $p< 0.0001$) shown in Figure 6Aii, again indicating that most change occurs within the REST to 10% MVC interval. Pictorial representations of the cortical territory dedicated to the FDI muscle can

be seen in the pixel maps in figures 6Bi-iv where a red square signifies an active cortical site with the darker square signifying higher amplitude responses in the muscle.

Figure 6

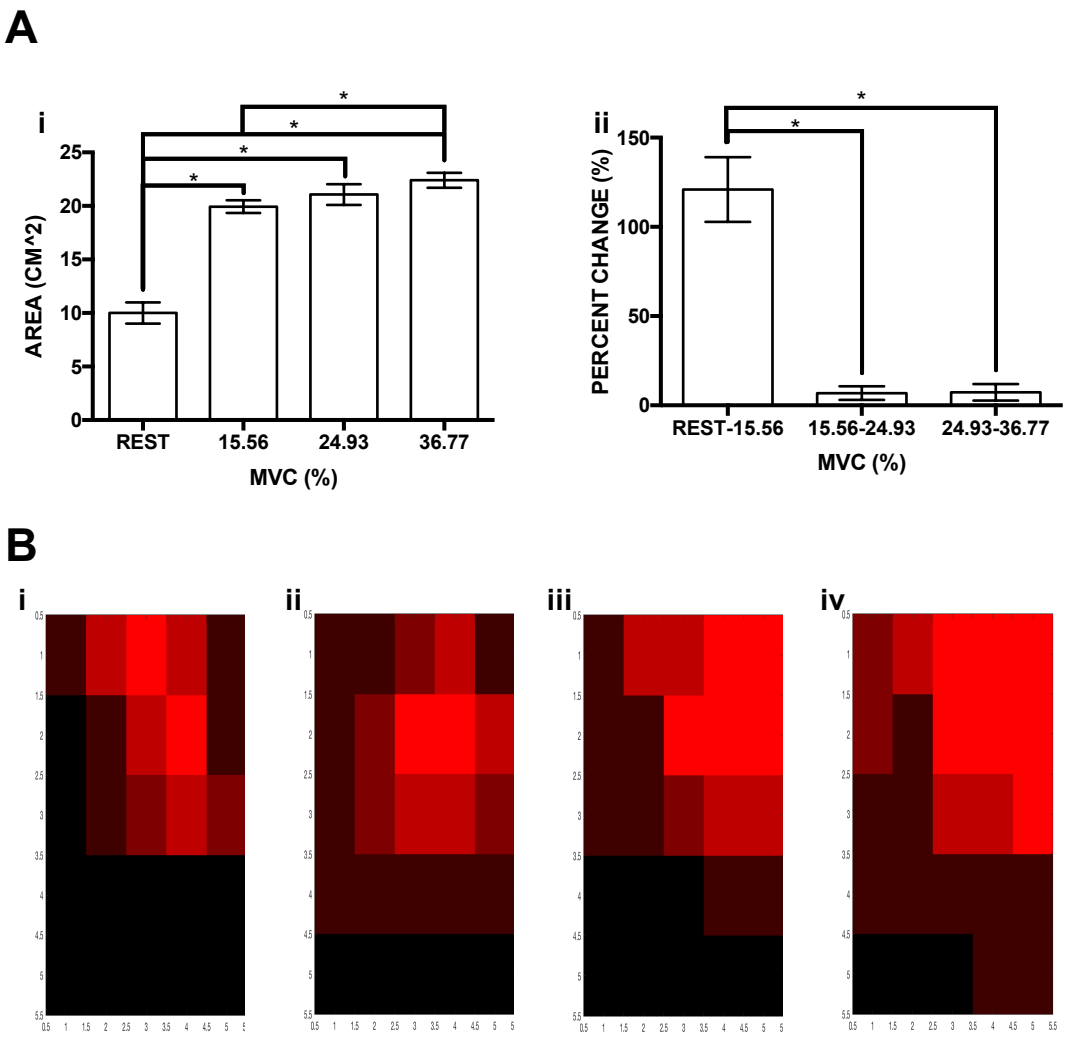

Figure 6. Differences in representational area measured amongst and between the contraction conditions. Ai) The area during REST is significantly smaller than during any

contraction level ($p<0.001$) and the area during 15.56±1.87% MVC is significantly less than that at 36.77±4.12% MVC ($p=0.004$) indicating a graded increase in area with contraction. Aii) The percent change of area indicates that the majority of the change in representational area occurs in the REST-15.56% MVC interval ($p=0.001$). Bi-iv) are pixel maps which represent the cortical territory of the FDI muscle wherein a red square signifies an active cortical site with the darker square signifying higher amplitude responses in the muscle.

Relationship Between Intracortical Circuits and Cortical Territory

Correlational analysis comparing the percent change in the REST-10% MVC interval of cortical territory and SICI and ICF magnitude are plotted in Figure 7A and B, respectively. No significant correlations were observed (SICI: $r= 0.2004$, $p=0.2355$, $r= -0.4204$, $p=0.0589$). These data indicate that neither SICI nor ICF play a significant role in the contraction interval where the majority of the growth of motor representational area exists.

Figure 7

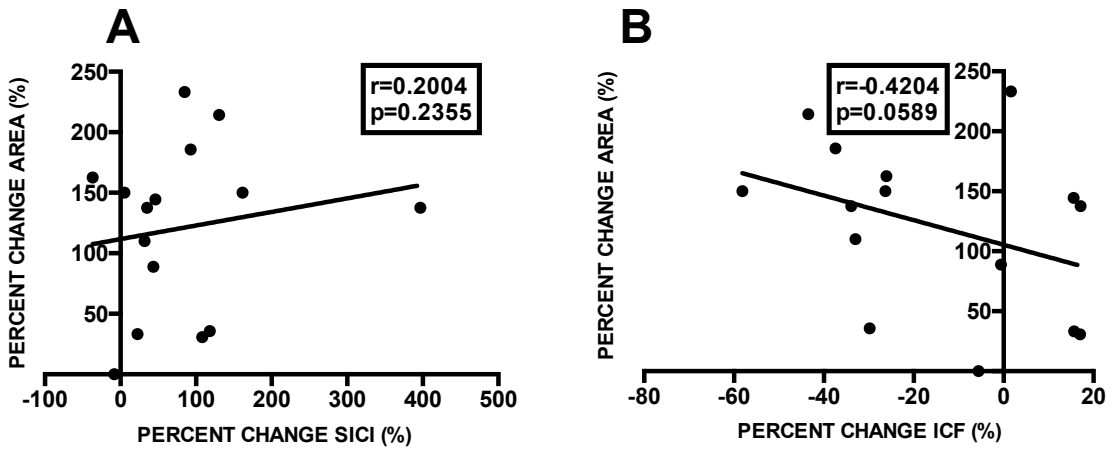

Figure 7. Correlational analyses of the percent change in area and SICI and ICF during the REST-15.56% MVC interval. A) No significant correlation was found (p=0.2355) between the changes in SICI and area and the data points have a poor fit (R^2=0.02726). B) A negative correlation trending toward significance (p=0.0589) was found between the changes in ICF and area however, ICF also had a poor fit (R^2=0.1350).

Changes in SICI with Contraction: Trial-by-Trial Analysis

The analyses presented above indicated that individuals deviated from their intended contraction level (Table 2). The subsequent analyses quantified the relationship between the actual MVC on a given trial, with the magnitude of inhibition (for SICI) or facilitation (for ICF) within that trial. To achieve this, the data were sorted on a trial-by-trial basis according to the actual level of contraction obtained during all individual trials measuring SICI (n=90). The results indicate that varying numbers of trials were present in each of

the contraction bins; 0.1≥x≤1.3% MVC (N=221), 1.3≥x<10% MVC (N=105), 10≥x<20% MVC (N=223) and 20≥x≤30% MVC (N=155). The REST group encompasses those trials that had a contraction level below 2SD above the average RMS EMG during trials where participants were instructed not to contract. By pooling all data points into the aforementioned bins a more accurate and tighter coupling of the effects of contraction on the magnitude of inhibition can take place, which can be seen in table 4 and graphically in figures 8Ai-iv. A Friedman's test revealed a main effect of contraction ($X^2(3)$=61.286, P<0.001, N=699) indicating that the resting tonic contraction group exhibits significantly more SICI than any of the contraction conditions (Figure 8Bi). The magnitude of SICI was also significantly lower in the 1.3≥x<10% MVC interval (N=105) when compared to the 10≥x<20% MVC (N=223) (Z=-3.656, p<0.001) and 20≥x≤30% MVC interval groups (N=155) (Z=-4.202, p<0.001) (Figure 8Bi). Figure 8Bii displays the same data as figure 8Bi in the form of side-by-side boxplots that illustrates the graded decrease in SICI and the similar spread of data across contraction levels. The trial-by-trial results seem to refute our previous findings that SICI was initially decreased at the onset of contraction and is rather decreased in a graded fashion with increasing contraction.

Table 4. Friedman's Test on Trial-by-Trial SICI Data

Measure	Effect Size	Friedman's	Post-Hoc
SICI	Kendall's W= 0.195	$X^2_{(3)}$=61.286, p<0.001	REST vs. 10%: 0.66 ± 0.05 vs. 0.81 ± 0.04, p = 0.004 REST vs. 20%: 0.66 ± 0.05 vs. 1.00 ± 0.03, p < 0.001 REST vs. 30%: 0.66 ± 0.05 vs. 1.01 ± 0.03, p < 0.001 10% vs. 20%: 0.81 ± 0.04 vs. 1.00 ± 0.03, p < 0.001 10% vs. 30%: 0.81 ± 0.04 vs. 1.01 ± 0.03, p < 0.001 20% vs. 30%: 1.00 ± 0.03 vs. 1.01± 0.03, p = 0.690

**Friedman's test used N=105 of smallest group as group sizes were unequal in trial-by-trial data.*

Figure 8

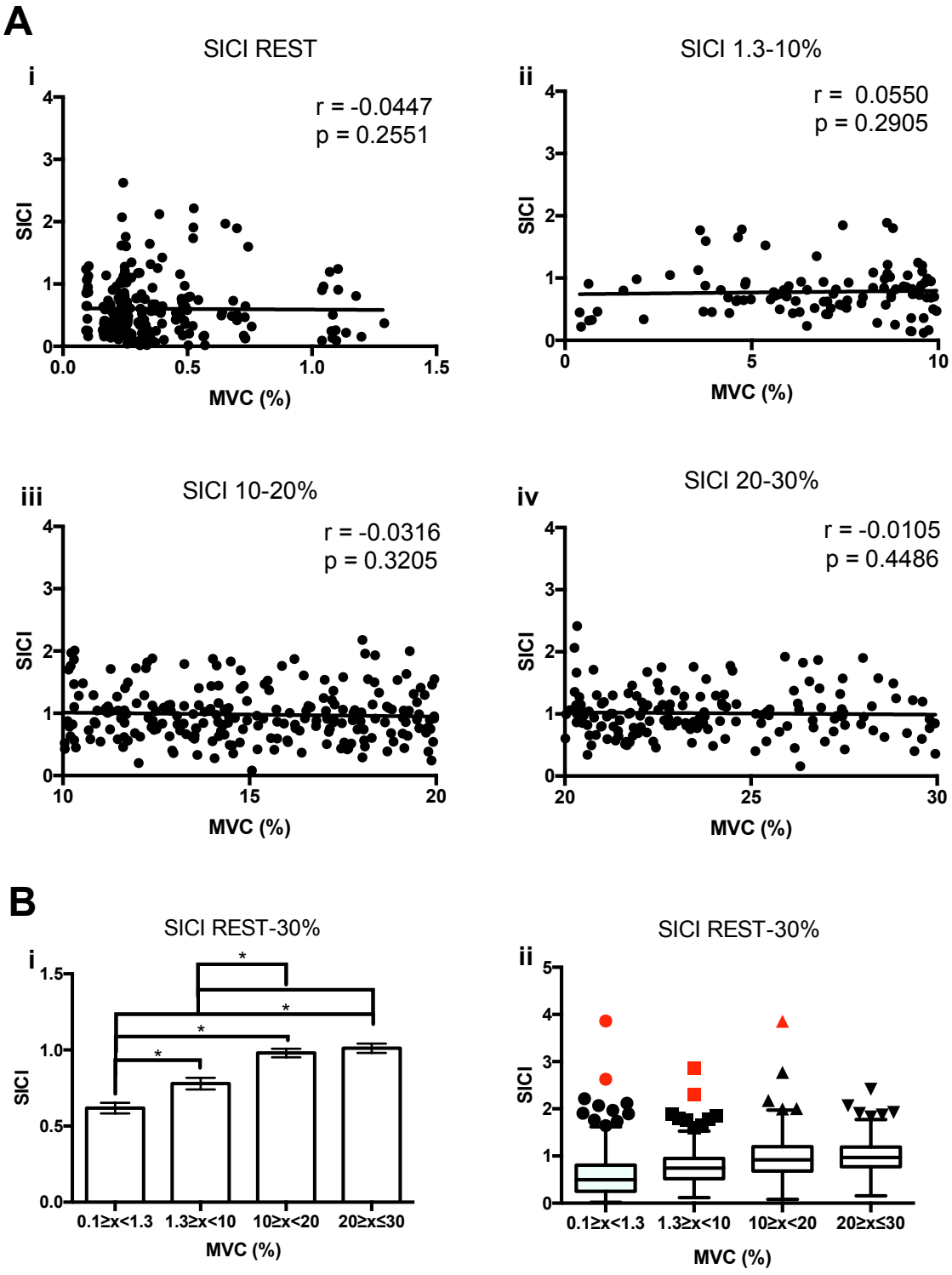
A
SICI REST
i
r = -0.0447
p = 0.2551
SICI
MVC (%)
SICI 1.3-10%
ii
r = 0.0550
p = 0.2905
SICI
MVC (%)
SICI 10-20%
iii
r = -0.0316
p = 0.3205
SICI
MVC (%)
SICI 20-30%
iv
r = -0.0105
p = 0.4486
SICI
MVC (%)
B
SICI REST-30%
i
SICI
0.1≥x<1.3
1.3≥x<10
10≥x<20
20≥x≤30
MVC (%)
SICI REST-30%
ii
SICI
0.1≥x<1.3
1.3≥x<10
10≥x<20
20≥x≤30
MVC (%)

Figure 8. Changes amongst and between the contraction levels using trial-by-trial SICI data. Ai-iv) are scatterplots showing the magnitude of SICI (mean±SEM) at contraction levels during the intervals; 0.1≥x<1.3, 1.3≥x<10, 10≥x<20, 20≥x≤30% MVC (N=219, r=-0.04473, p=0.2551). Bi) Displays that the magnitude of SICI (mean±SEM) during resting tonic contraction is significantly stronger than any other contraction level. The level of SICI magnitude during the 1.3≥x<10 was also significantly less than during the 10 ≥x<20 and 20 ≥x≤30% MVC intervals. Bii) A boxplot showing the same data as Bi) allows a better visualization of the changes in SICI magnitude as well as outlying data points. Note that one significant outlier in the 0.1≥x<1.3% MVC interval is not shown for the purposes of y-axis scaling.

Discussion

This study was the first to examine intracortical circuits and representational maps concurrently under conditions of graded contraction. The experiment examined the modulatory effects of graded muscle contractions of the right FDI muscle on SICI, ICF and the area of its cortical representation. Several lines of previous research were corroborated and although the hypothesized novel relationship of circuits and representational maps was not exposed, the possible reasons for this and their implication for future study will be discussed herein.

Methodological Considerations

Trial-by-Trial Analysis

Most TMS studies investigating SICI take the average CS-TS/TS ratio as a participant's SICI magnitude, thus this investigation began by employing the same technique (Kujirai et al. 1993, Di Lazzaro et al, 1998). The traditional technique found that SICI magnitude decreased substantially from rest to any level of contraction but was not different amongst contraction conditions (Figure 3Ai,ii). As mentioned above, the contraction levels actually achieved varied significantly from expected levels which may have altered the effectiveness of the stimulation parameter as contraction is known to increase MEP size (Hess et al. 1987; Kiers et al. 1993). To combat this variability the data was investigated in a novel manner whereby the SICI data was analyzed on a trial-by-trial basis to match the CS-TS MEP to the specific contraction level during a trial. Next, the trials and their associated contraction level and CS-TS amplitudes were sorted according to ascending contraction level allowing for a much more accurate look at the effects of increasing contraction. This analysis was successful in displaying a graded decrease in SICI with contraction up until 20% MVC of the right FDI, which highlights the importance of controlling contraction variability in active TMS experiments. The present results can inform future studies to tightly regulate contraction through increased biofeedback and more accurate EMG recording. Even where the aforementioned technology for more accurate recording may not be available the trial-by-trial analysis put forth in the current study proved to be a viable option to correct for contraction variability.

Intracortical Circuit Modulation

The Effect of Contraction

This study's findings indicated that SICI magnitude was continually reduced as contraction intensity increased from rest to 20% MVC of the right FDI muscle which indicated a graded reduction; after this point SICI appeared to be abolished (Figure 6Ai). ICF showed a reduction from rest to all contraction levels (Figure 5Ai) but did not show a significant pattern of change through intervals of increasing contraction intensity when examined in terms of percent change between those intervals. (Figure 5Aii). These findings indicated that ICF did not respond continually in one direction past the relaxed-contraction transition, which indicated a lack of graded reduction seen with SICI. This finding aligned with previous investigations showing a reduction of ICF during low level contraction relative to rest (Ridding et al. 1995; Hanajima et al. 2002). Previous work has seen a reduction of SICI up until 25% MVC with graded contraction starting at 10% MVC and ending at 50% MVC. Though both specified levels of muscle contraction, one used EMG displayed on an oscilloscope (Ortu et al. 2008), while the other used force measurement to assess contraction intensity (Zoghi & Nordstrom 2007).

In order to measure SICI, a subthreshold CS must precede a suprathreshold TS delivered 1-6 ms later (Kujirai et al. 1993). However, 10% MVC contraction has a lowering effect on motor threshold on the order of 8-10% of maximum stimulator output (MSO) and in order to maintain the paradigm to elicit SICI the change in threshold must be adjusted for. The present study controlled for the change in threshold by setting CS and TS intensities to RMT for the relaxed condition and AMT acquired at the respective contraction level

for active conditions. However, the adjustment was unsuccessful as the TS amplitudes increased significantly throughout contraction levels. The resulting increased TS amplitudes could have been the reason why SICI decreased, as a given CS intensity is less effective at inhibiting increasingly large TS amplitudes. TS intensities of 0.2, 1 and 4 mV with consistent CS intensity (80% RMT) have been investigate previously and found that SICI increased from 0.2 mV to 1 mV but showed a trend to decreases between 1 and 4 mV (Sanger et al. 2001). These findings indicate that although SICI is ideal at 1 mV it may begin to decrease at higher TS intensities like the ones obtained in the present study amongst contraction (Figure 3aiii).

Map Growth and its Contributors

Intracortical circuits were not related to map growth at any intensity suggesting these two measures are not related. The trial-by-trial analysis indicated a graded decrease in SICI an inverse of the effect seen in the original analysis of representational area. However, a comparison between the two was not feasible due to inconsistent contraction level at the map acquisition grid points. Therefore, the question of what is causing the expansion of cortical muscle representation especially at higher contraction levels remains.

Low-level Contraction Expansion of Motor Maps

The map expansion seen during the production of contraction up to 10% MVC most likely drew from a host of excitability increasing sources. It is known that MEP size increases dramatically with contraction intensity (Hess et al. 1987, Kiers et al. 1993) up

until 10% MVC where the increase plateaus (Helmers et al. 1989, Taylor et al. 1997). The expansion may also be attributed by some degree to reduced motor threshold with muscle activity. Motor thresholds have been observed to decrease by 8-10% MSO when stimulating active muscle (10% MVC) versus resting muscle (Devanne et al. 1997, Wassermann 2002), which plays a role in the expansion of maps when the change in threshold is not accounted for. Interestingly, when the 8-10% MSO decrease is accounted for, representational maps show no growth (Ngomo et al. 2012). However, just like the increasing MEP excitability, the lowering of motor threshold changes very minimally past 10% MVC thus it cannot explain the growth during stronger contractions (Devanne et al. 1997). There is also a possibility that although the relationship between SICI and representational area was not statistically significant, it still plays a minor role in the expansion of area related to the reduction in its magnitude over contraction levels up to 24.93±2.12% MVC. However, variability in the contraction level during map measurement did not allow for correlational analysis with SICI. There are many proposed reasons for map expansion with low level contraction and the mechanism is more than likely a combination of several of the aforementioned factors, but what remains to be explained is the continued growth of area at levels where many of these factors exert negligible effects.

High-level Contraction Expansion of Motor Maps

Representational maps not only increased from rest to 10% MVC but also significantly increased from 10-30% MVC (Figure 4B) which falls in line with previous work by Van

de Ruit and colleagues (2016). The current investigation was the first to investigate the decreases in intracortical inhibition as a driver of representation growth but unfortunately was unsuccessful, thus we must turn to the contribution of spinal contributions.

Spinal Contributions

TMS and nerve stimulation can be used to assess the excitability of the spinal cord and its associated motor neuron pool. H- reflexes elicited via TMS are an indirect measure of spinal excitability and have exhibited increased magnitude as well as decreased thresholds with low-level contraction (Mazzochio et al. 1994). These modulations in H-reflex indicated an increase in motorneurone pool excitability, which caused more neurons to synapse to the muscle with a given TMS stimulus (Maertens de Noordhout et al 1992, Ugawa et al. 1995). It is hypothesized that during low-level contraction spinal neurons are activated to produce the contraction but other surrounding motor neurons are 'subliminally' active and thus more easily activated by subsequent TMS stimuli. This subliminal activation may be the reason for increased H- reflex magnitude and TMS evoked MEP with contraction (Mazzochio et al. 1994; DeBruin et al. 2006). Unfortunately, active H-reflex studies have been limited to low-level contraction and thus cannot provide evidence for a spinal contribution to representational growth at upper contraction levels. However, alternative measures of spinal have exhibited continued increases past low-level contraction. When a motor fiber is maximally stimulated two responses are recorded resulting from the direct orthodromic response (M-wave) and a second response resulting from antidromic firing of anterior horn cells of the spinal cord

known as the F-wave (Suzuki et al. 1993). F- wave magnitude as measured by a F/M wave amplitude ratio increased as contraction increased up to 100% MVC which would indicate that the motoneurone pool continues to be excited at upper contraction levels (Suzuki et al. 1993).

Limitations

One important limitation of this study was the participants' inability to control FDI contraction at prescribed levels as well as certain limiting factors in our ability to account for this variability. Using traditional averaged analysis the variable contraction caused the graded decrease of SICI with contraction to be masked. It was not until the CS-TS response was sorted according to actual contraction level that the graded decrease in SICI emerged which proves the prior masking notion. Unfortunately, the trial-by trial analysis was a simple solution to the contraction variability only for SICI measurement and could not be applied to cortical map data, as the MEP responses are position dependent. By, extension the inconsistent contraction during cortical map measurement made a correlation between the graded decrease in SICI to the graded increase in map area unfeasible. This limitation made a relationship between SICI magnitude and cortical area impossible to probe using a trial-by-trial design sorted by contraction. Although, the trial-by-trial analysis was a simple solution for SICI and other intracortical circuit measures it is essential to achieve consistent contraction during map acquisition especially if comparisons are to be drawn between the two. A solution could be to employ additional biofeedback metrics to signify when participants are varying contraction beyond

prescribed levels. One study used a force transducer that had an output trace on the screen in front of the participant as well as lines surrounding the target signifying 10% deviation which would cause the bar to turn red when exceeded (Van de Ruit et al. 2016).

Another limitation of this study was the lack of assessment of spinal excitability; it is likely that the growth in area during the first 10% MVC was caused by a combination of spinal and cortical sources (Maertens de Noordhout et al. 1992; Ugawa et al. 1995; Di Lazzaro et al. 1998). Low-level contraction has been shown to bring additional motor neurons close to discharge and the subsequent TMS provides the small bit of stimulus still needed to discharge. This notion presents the idea that the expansion in cortical territory and decrease in inhibition may be influenced by these subliminally active spinal motor neurons and not solely from cortical sources. However, the present study cannot confirm or deny the contribution of spinal contributions at low and high level of contraction, which could have been rectified by adding a measure of H-reflex at the prescribed contraction levels.

The current study only accounted for EMG activity of one intrinsic hand muscle and ignored the surrounding musculature. This was a limitation of the current investigation because even humans possessing the most dexterous hand movements are unable to independently activate their fingers and as a result have a substantial amount of activation coupling between the digits. The process of involuntary movement of other muscles is known as enslaving and can be due to both biomechanical (Keen & Fuglevand 2003;

Reilly & Schieber 2003) and neural constraints (Sanes et al. 1995), which have been shown worsen at higher levels of force production (Slobounov et al. 2002). The human motor cortex attempts to combat this through the use of surround inhibition in which surrounding muscles are inhibited to focus the motor output to a target muscle. However, this phenomenon is often restricted to movement production and is inactive during contraction maintenance, which was required isometric contraction of the current experiment (Beck et al. 2008). Furthermore, muscles commonly activated together such as the abductors of the fingers are more synergistic and harder to independently activate thus multi-muscle contraction was more than likely present during the present index finger abductions (Kilbreath and Gandevia 1993). The current study did not measure the activity of these coupled muscles and their possible implications to the measures. Neighbouring flexor muscles were more than likely increasingly active during the increasing contractions of the FDI and may have been contributing to the EMG signal picked up by disk electrodes which have poor spatial acuity. The unexpected recording of neighbouring muscles may have contributed to the 'growth' of the FDI cortical territory. Future studies should ensure that EMG is recorded from neighbouring muscles as well as the target in order to minimize surround activation to obtain a more focused cortical representation of the target.

Conclusion

SICI, ICF and representational area were all modulated the most during the transition from a relaxed state to a contracted state. SICI and ICF both showed a reduction in

magnitude while representational area grew with the production of contraction. SICI trial-by-trial analysis and representational area decreased and grew, respectively, in a graded manner with increased contraction intensity. However, the modulation of the two could not be related, as the trial-by-trial sorting used for SICI could not be used on the location dependent trials during map acquisition. Therefore, future analyses need to ensure control over contraction to allow a possible relationship between SICI and representational map area modulation to be exposed.

References

Bares, M., Kanovský, P., Klajblová, H., & Rektor, I. (2003). Intracortical inhibition and facilitation are impaired in patients with early Parkinson's disease: a paired TMS study. *European Journal of Neurology : The Official Journal of the European Federation of Neurological Societies*, *10*(4), 385–9.

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex, 1106–1107.

Beck, S., Richardson, S. P., Shamim, E. A., Dang, N., Schubert, M., & Hallett, M. (2008). Short intracortical and surround inhibition are selectively reduced during movement initiation in focal hand dystonia. *Journal of Neuroscience*, *28*(41), 10363–10369. https://doi.org/10.1523/JNEUROSCI.3564-08.2008

Chen, R. (2004). Interactions between inhibitory and excitatory circuits in the human motor cortex. *Experimental Brain Research*, *154*(1), 1–10. http://doi.org/10.1007/s00221-003-1684-1

Chen, R., Wassermann, E. M., Caiios, M., & Hallett, M. (1997). Impaired inhibition in writer ' s cramp during voluntary muscle activation. *Neurology*, *49*, 1054–1059.

Cheyne, D., Kristeva, R., & Deecke, L. (1991). Homunclar organization of human motor cortex as indicated by neuromagnetic recordings. *Neuroscience Letters*, *122*, 17–20.

Classen, J., Knorr, U., Werhahn, K. J., Schlaug, G., Kunesch, E., Cohen, L. G., … & Benecke, R. (1998). Multimodal output mapping of human central motor representation on different spatial scales. *J Physiol*, *512 (Pt 1*(0022–3751 LA–eng PT–Journal Article SB–IM), 163–179. http://doi.org/10.1111/j.1469-7793.1998.163bf.x

Colebatch, J. G., Deiber, M., Passingham, R. E., Friston, & Frackowiak, R. S. (1991a). Regional cerebral blood flow during voluntary arm and hand movements in human subjects. *Journal of Neurophysiology*, *65*(6), 1392–1401.

Colebatch, J. G., Adams, L., Murphy, K., Martin, A. J., Lammertsma, A. A., Tochon-Danguy, H. J., & Guz, A. (1991b). Regional cerebral blood flow during volitional breathing in man. *Journal of Physiology*, *443*, 91–103.

Darling, W. G., Wolf, S. L., & Butler, A. J. (2006). Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. *Experimental Brain Research*, *174*(2), 376–385. http://doi.org/10.1007/s00221-006-0468-9

Daskalakis, Z. J., Christensen, B. K., Fitzgerald, P. B., Roshan, L., & Chen, R. (2002). The mechanisms of interhemispheric inhibition in the human motor cortex. *Journal of Physiology*, *543*(1), 317–326. http://doi.org/10.1113/jphysiol.2002.017673

Day, B. L., Dressler, D., Maertens de Noordhout, A., Marsden, C. D., Nakashima, K., Rothwell, J. C., & Thompson, P. D. (1989). Electric and Magnetic Stimulation of Human Motor Cortex: Surface EMG and Single Motor Unit Responses. *J Physiol*, *412*, 449–473.

De Noordhout, A. M., Pepin, J. L., Gerard, P., & Delwaide, P. J. (1992). Facilitation of responses to motor cortex stimulation: Effects of isometric voluntary contraction. *Annals of Neurology*, *32*(3), 365–370. http://doi.org/10.1002/ana.410320310

Dettmers, C., Connelly, a, Stephan, K., Turner, R., Friston, K., Frackowiak, R., & Gadian, D. (1996). Quantitative comparison of functional magnetic resonance imaging with positron emission tomography using a force-related paradigm, *209*(4), 201–209. Retrieved from http://discovery.ucl.ac.uk/112325/

Devanne, H., Lavoie, B. A., & Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Experimental Brain Research*, *114*(2), 329–338. http://doi.org/10.1007/PL00005641

Devanne, H., Cassim, F., Ethier, C., Brizzi, L., Thevenon, A., & Capaday, C. (2006). The comparable size and overlapping nature of upper limb distal and proximal muscle representations in the human motor cortex. *European Journal of Neuroscience*, *23*(9), 2467–2476. http://doi.org/10.1111/j.1460-9568.2006.04760.x

Di Lazzaro, V., Profice, P., Ranieri, F., Capone, F., Dileone, M., Oliviero, A., & Pilato, F. (2012). I-wave origin and modulation. *Brain Stimulation*, *5*(4), 512–525. http://doi.org/10.1016/j.brs.2011.07.008

Di Lazzaro, V., Ziemann, U., & Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. *Brain Stimulation*, *1*(4), 345–362. http://doi.org/10.1016/j.brs.2008.07.004

Donoghue, J. P., Leibovic, S., & Sanes, J. N. (1992). Organization of the forelimb area in squirrel monkey motor cortex: representation of digit, wrist, and elbow muscles. *Experimental Brain Research*, (89), 1–19.

Espay, A. J., Morgante, F., Purzner, J., Gunraj, C. A., Lang, A. E., & Chen, R. (2006). Cortical and spinal abnormalities in psychogenic dystonia. *Annals of Neurology*, *59*(5), 825–834. http://doi.org/10.1002/ana.20837

Feinstein, B., Lindegård, B., Nyman, E., & Wohlfart, G. (1955). Morphologic Studies Of Motor Units In Normal Human Muscles. *Cells Tissues Organs*, *23*(2), 127–142. http://doi.org/10.1159/000140989

Fink, G. R., Lemon, R. N., Stephan, K. M., Passingham, R. E., Ridding, M. C., Brooks, D. J., & Frackowiak, R. S. (1995). Relation between cerebral activity and force in the motor areas of the human brain. *Journal of Neurophysiology*, *74*(2), 802–815. http://doi.org/10.1152/jn.1995.74.2.802

Grafton, S. T., Woods, R. P., Mazziotta, J. C., & Phelps, M. E. (1991). Somatotopic mapping of the primary motor cortex in humans: activation studies with cerebral blood flow and positron emission tomography. *J.Neurophysiol.*, *66*(3), 735–743. http://doi.org/10.1152/jn.1991.66.3.735

Hallett, M. (2007). Transcranial Magnetic Stimulation: A Primer. *Neuron*, *55*(2), 187–199. http://doi.org/10.1016/j.neuron.2007.06.026

Hammond, G., & Vallence, A. M. (2007). Modulation of long-interval intracortical inhibition and the silent period by voluntary contraction. *Brain Research*, *1158*(1), 63–70. http://doi.org/10.1016/j.brainres.2007.05.014

Hanajima, R., Ugawa, Y., Terao, Y., Sakai, K., Furubayashi, T., Machii, K., & Kanazawa, I. (1998). Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. *The Journal of Physiology*, *509*, 607–18. http://doi.org/10.1111/j.1469-7793.1998.607bn.x

Hanajima, R., Ugawa, Y., Terao, Y., Enomoto, H., Shiio, Y., Mochizuki, H., … & Kanazawa, I. (2002). Mechanisms of intracortical I-wave facilitation elicited with paired-pulse magnetic stimulation in humans. *Journal of Physiology*, *538*(1), 253–261. http://doi.org/10.1113/jphysiol.2001.013094

Helmers, S. L., Chiappa, K. H., Cros, D., Gupta, N., & Santamaria, J. (1989). Magnetic stimulation of the human motor cortex: facilitation and its relationship to a visual motor task. *Journal of Clinical Neurophysiology*, *6*(4), 321–332.

Hess, C. W., Mills, K. R., & Murray, N. M. F. (1987). Responses in small hand muscles from magnetic stimulation of the human brain. *Journal of Physiology*, *388*, 397–419.

Ingvar, D. H., & Philipson, L. (1977). Distribution of cerebral blood flow in the dominant hemisphere during motor ideation and motor performance. *Annals of Neurology*, *2*(3), 230–237. http://doi.org/10.1002/ana.410020309

Jacobs, K. M., & Donoghue, J. P. (1991). Reshaping the Cortical Motor Map by Unmasking Latent Intracortical Connections. *Science*, *251*(4996), 944–947.

Karhu, J., Hannula, H., Laine, J., & Ruohonen, J. (2014). Navigated transcranial magnetic stimulation: Principles and protocol for mapping the motor cortex. *Neuromethods*, *89*, 337–359. http://doi.org/10.1007/978-1-4939-0879-0_16

Keen, D. A., & Fuglevand, A. J. (2003). Role of intertendinous connections in distribution of force in the human extensor digitorum muscle. *Muscle and Nerve*, *28*(5), 614–622. https://doi.org/10.1002/mus.10481

Kilbreath, S. L., & Gandevia, S. C. (1993). Neural and biomedical specializations of human thumb muscles revealed by matching weights and grasping objects. *Journal of Physiology*, *472*, 537–556.

Kleim, J. A. (2004). Cortical Synaptogenesis and Motor Map Reorganization Occur during Late, But Not Early, Phase of Motor Skill Learning. *Journal of Neuroscience*, *24*(3), 628–633. http://doi.org/10.1523/JNEUROSCI.3440-03.2004

Kleim, J. A., Barbay, S., & Nudo, R. (1998). Functional reorganization of the rat motor cortex following motor skill learning. *Journal of Neurophysiology*, *80*, 3321–3325. Retrieved from http://jn.physiology.org/content/80/6/3321.short

Li, X. (2014). Functional Magnetic Resonance Imaging Processing, 6–12. http://doi.org/10.1007/978-94-007-7302-8

Liepert, J., Bauder, H., Miltner, W. H. R., Taub, E., & Weiller, C. (2000). Treatment-Induced Cortical Reorganization After Stroke in Humans. *Stroke*, *31*(6), 1210–1216. http://doi.org/10.1161/01.STR.31.6.1210

Maki, A., Yamashita, Y., Ito, Y., Watanabe, E., Mayanagi, Y., & Koizumi, H. (1995). Spatial and temporal analysis of human motor activity using noninvasive NIR topography. *Medical Physics*, *22*(12), 1997–2005. http://doi.org/10.1118/1.597496

Mazzocchio, R., Rothwell, J. C., Day, B. L., & Thompson, P. D. (1994). Effect of tonic voluntary activity on the excitability of human motor cortex. *The Journal of Physiology*, *474*(2), 261–267. http://doi.org/10.1113/jphysiol.1994.sp020018

McDonnell, M. N., Hillier, S. L., Ridding, M. C., & Miles, T. S. (2006). Impairments in precision grip correlate with functional measures in adult hemiplegia. *Clinical Neurophysiology*, *117*(7), 1474–1480. http://doi.org/10.1016/j.clinph.2006.02.027

Mcginley, M., Hoffman, R. L., Russ, D. W., Ph, D., Thomas, J. S., & Clark, B. C. (2011). Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. *Experimental Gerontology*, *45*(9), 671–678. https://doi.org/10.1016/j.exger.2010.04.005.Older

Meier, J. D., Aflalo, T. N., Kastner, S., & Graziano, M. (2008). Complex organization of human primary motor cortex: a high-resolution fMRI study. *Journal of Neurophysiology*, *100*, 1800–1812. http://doi.org/10.1152/jn.90531.2008.

Merton, P. A., & Morton, H. B. (1980). Stimulation of the cerebral cortex in the intact human subject. *Nature*, *285*, 227.

Mortifee, P., Stewart, H., Schulzer, M., & Eisen, a. (1994). Reliability of transcranial magnetic stimulation for mapping the human motor cortex. *Electroencephalography and Clinical Neurophysiology*, *93*(2), 131–7. http://doi.org/doi: DOI: 10.1016/0168-5597(94)90076-0

Nakamura, H., Kitagawa, H., Kawaguchi, Y., & Tsuji, H. (1997). Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *Journal of Physiology*, *498*(3), 817–823. https://doi.org/10.1113/jphysiol.1997.sp021905

Ngomo, S., Leonard, G., Moffet, H., & Mercier, C. (2012). Comparison of transcranial magnetic stimulation measures obtained at rest and under active conditions and their reliability. *Journal of Neuroscience Methods*, *205*(1), 65–71. http://doi.org/10.1016/j.jneumeth.2011.12.012

Nudo, R. J., Jenkins, W. M., & Merzenich, M. M. (1990). Repetitive Microstimulation Alters the Cortical Representation of Movements in Adult Rats. *Somatosensory and Motor Research*, *7*(4), 463–483. http://doi.org/10.3109/08990229009144720

Nudo, R. J., Milliken, G. W., Jenkins, W. M., & Merzenich, M. M. (1996). Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(2), 785–807. http://doi.org/8551360

Oldfield, R. (1971). The assessment and analysis of handedness. *Neuropsychologia*, *9*(1), 97–113.

Ortu, E., Deriu, F., Suppa, A., Tolu, E., & Rothwell, J. C. (2008). Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. *Journal of Physiology*, *586*(21), 5147–5159. http://doi.org/10.1113/jphysiol.2008.158956

Pascual, L. A., Nguyet, D., Cohen, L. G., Brasil, N. J. P., Cammarota, A., & Hallett, M. (1995). Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol.*, *74*(3), 1037–1045.

Penfield, W., & Boldrey, E. (1937). Somatic Motor and Sensory Representation in Man. *Brain*, 389–443. http://doi.org/10.1093/brain/60.4.389

Perez, M. A., & Cohen, L. G. (2008). Mechanisms Underlying Functional Changes in the Primary Motor Cortex Ipsilateral to an Active Hand. *Journal of Neuroscience*, *28*(22), 5631–5640. http://doi.org/10.1523/JNEUROSCI.0093-08.2008

Perez, M. A., Lungholt, B. K. S., Nyborg, K., & Nielsen, J. B. (2004). Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Experimental Brain Research*, *159*(2), 197–205. http://doi.org/10.1007/s00221-004-1947-5

Priori, A., Berardelli, A., Inghilleri, M., Accornero, N., & Manfredi, M. (1994). Motor Cortical Inhibition and the Dopaminergic System - Pharmacological Changes in the Silent Period After Transcranial Brain-Stimulation in Normal Subjects, Patients With Parkinsons-Disease and Drug-Induced Parkinsonism. *Brain*, *117*(2), 317–323. http://doi.org/10.1093/brain/117.2.317

Rao, S. M., Binder, J. R., Hammeke, T. A., Bandettini, P. A., Bobholz, J. A., Frost, J. A., & Hyde, J. S. (1995). Somatotopic mapping of the human primary motor cortex with functional magnetic resonance imaging. *Neurology*, *45*, 919–924.

Reilly, K. T., & Schieber, M. H. (2003). Incomplete functional subdivision of the human multitendoned finger muscle flexor digitorum profundus: an electromyographic study. *Journal of Neurophysiology*, *90*(4), 2560–2570. https://doi.org/10.1152/jn.00287.2003

Remple, M. S., Bruneau, R. M., VandenBerg, P. M., Goertzen, C., & Kleim, J. A. (2001). Sensitivity of cortical movement representations to motor experience: evidence that skill learning but not strength training induces cortical reorganization. *Behavioural Brain Research*, *123*(2), 133–141. http://doi.org/10.1016/S0166-4328(01)00199-1

Ridding, M. C., Sheean, G., Rothwell, J. C., Inzelberg, R., & Kujirai, T. (1995a). Changes in the balance between motor cortical excitation and inhibition in focal, task specific dystonia. *Journal of Neurology, Neurosurgery and Psychiatry*, *59*(5), 493–498. http://doi.org/10.1136/jnnp.59.5.493

Ridding, M. C., Inzelberg, R., & Rothwell, J. C. (1995b). Changes in Excitabdity of Motor Cortical Circuitry in Patients with Parkinson's Disease. *Annals of Neurology*, *37*(2), 181–188.

Ridding, M. C., Taylor, J. L., & Rothwell, J. C. (1995c). The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. *Journal of Physiology*, *487 (Pt 2*, 541–548.

Sanes, J. N., Donoghue, J. P., Thangaraj, V., Edelman, R. R., & Warach, S. (1995). Shared neural substrates controlling hand movements in human motor cortex. *Science*, *268*(5218), 1775–1777.

Schabrun, S. M., Stinear, C. M., Byblow, W. D., & Ridding, M. C. (2009). Normalizing motor cortex representations in focal hand dystonia. *Cerebral Cortex*, *19*(9), 1968–1977. https://doi.org/10.1093/cercor/bhn224

Schieber, M. H. (2001). Constraints on Somatotopic Organization in the Primary Motor Cortex. *Journal of Neurophysiology*, *86*(5), 2125–2143. http://doi.org/10.1152/jn.2001.86.5.2125

Shibuya, K., Kuboyama, N., & Tanaka, J. (2014). Changes in ipsilateral motor cortex activity during a unilateral isometric finger task are dependent on the muscle contraction force. *Physiological Measurement*, *35*(3), 417–428. http://doi.org/10.1088/0967-3334/35/3/417

Siebner, H. R., Bergmann, T. O., Bestmann, S., Massimini, M., Johansen-Berg, H., Mochizuki, H., … Rossini, P. M. (2009). Consensus paper: Combining transcranial stimulation with neuroimaging. *Brain Stimulation*, *2*(2), 58–80. http://doi.org/10.1016/j.brs.2008.11.002

Slobounov, S., Johnston, J., Chiang, H., & Ray, W. (2002). The role of sub-maximal force production in the enslaving phenomenon. *Brain Research*, *954*(2), 212–219. https://doi.org/10.1016/S0006-8993(02)03288-2

Sohn, Y. H., Wiltz, K., & Hallett, M. (2002). Effect of volitional inhibition on cortical inhibitory mechanisms. *Journal of Neurophysiology*, *88*(1), 333–338. http://doi.org/10.1152/jn.00863.2001.

Stinear, C. M. (2002). Role of Intracortical Inhibition in Selective Hand Muscle Activation. *Journal of Neurophysiology*, *89*(4), 2014–2020. http://doi.org/10.1152/jn.00925.2002

Stinear, C. M., & Byblow, W. D. (2004). Impaired Modulation of Intracortical Inhibition in Focal Hand Dystonia. *Cerebral Cortex*, *14*(5), 555–561. http://doi.org/10.1093/cercor/bhh017

Suzuki, T., Fujiwara, T., & Takeda, I. (1993). Excitability of the spinal motor neuron pool and F-waves during isometric ipsilateral and contralateral contraction. *Physiotherapy Theory and Practice*, *9*(1), 19–24. http://doi.org/10.3109/09593989309036482

Taylor, J. L., Allen, G. M., & Butter, J. E. (1997). Effect of contraction strength on responses in biceps brachii and adductor pollicis to transcranial magnetic stimualtion. *Experimental Brain Research*, *117*, 472–478.

Thickbroom, G. W., Phillips, B. A., Morris, I., Byrnes, M. L., & Mastaglia, F. L. (1998). Isometric force-related activity in sensorimotor cortex measured with functional MRI. *Experimental Brain Research*, *121*(1), 59–64. http://doi.org/10.1007/s002210050437

Thickbroom, G. W., Phillips, B. A., Morris, I., Byrnes, M. L., Sacco, P., & Mastaglia, F. L. (1999). Differences in functional magnetic resonance imaging of sensorimotor cortex during static and dynamic finger flexion. *Experimental Brain Research*, *126*(3), 431–438. http://doi.org/10.1007/s002210050749

Thordstein, M., Saar, K., Pegenius, G., & Elam, M. (2013). Individual effects of varying stimulation intensity and response criteria on area of activation for different muscles in humans. A study using navigated transcranial magnetic stimulation. *Brain Stimulation*, *6*(1), 49–53. http://doi.org/10.1016/j.brs.2012.01.004

Ugawa, Y., Terao, Y., Hanajima, R., Sakai, K., & Kanazawa, I. (1995). Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. *Electroencephalography and Clinical Neurophysiology*, *6*(97), 451–454. http://doi.org/0.1016/0924-980X(95)00214-6

Uy, J., Ridding, M. C., & Miles, T. S. (2002). Stability of maps of human motor cortex made with transcranial magnetic stimulation. *Brain Topogr*, *14*(4), 293–297.

Valls-Solé, J., Pascual-Leone, A., Wassermann, E. M., & Hallett, M. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalography and Clinical Neurophysiology/ Evoked Potentials*, *85*(6), 355–364. http://doi.org/10.1016/0168-5597(92)90048-G

Van de Ruit, M., & Grey, M. J. (2016). The TMS Map Scales with Increased Stimulation Intensity and Muscle Activation. *Brain Topography*, *29*(1), 56–66. http://doi.org/10.1007/s10548-015-0447-1

Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin Neurophysiol*, *113*(7), 1165–1171. http://doi.org/S138824570200144X [pii]

Wassermann, E. M., McShane, L. M., Hallett, M., & Cohen, L. G. (1992). Noninvasive mapping of muscle representation in human motor cortex. *Electroehceph Clin Neurophysiol*, *85*, 1–8.

Werhahn, K. J., Kunesch, E., Noachtar, S., Benecke, R., & Classen, J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *Journal of Physiology*, *517*(2), 591–597. http://doi.org/10.1111/j.1469-7793.1999.0591t.x

Wilson, S. A., Thickbroom, G. W., & Mastaglia, F. L. (1995). Comparison of the magnetically mapped corticomotor representation of a muscle at rest and during low-level voluntary contraction. *Electroencephalography and Clinical Neurophysiology/ Electromyography*, *97*(5), 246–250. http://doi.org/10.1016/0924-980X(95)80001-8

Wilson, S. A., Thickbroom, G. W., & Mastaglia, F. L. (1993). Transcranial magnetic stimulation mapping of the motor cortex in normal subjects. *Journal of the Neurological Sciences*, *118*(2), 134–144. http://doi.org/10.1016/0022-510X(93)90102-5

Ziemann, U., Lonnecker, S., & Paulus, W. (1995). Inhibition of human motor cortex by ethanol. A transcranial magnetic stimulation study. *Brain : A Journal of Neurology*, *118 (Pt 6*(July), 1437–1446.

Ziemann, U., Lonnecker, S., Steinhoff, B. J., & Paulus, W. (1996). Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann Neurol*, *40*(3), 367–378. http://doi.org/10.1002/ana.410400306

Ziemann, U. (2003). Chapter 23 Pharmacology of TMS. *Supplements to Clinical Neurophysiology*, *56*(C), 226–231. http://doi.org/10.1016/S1567-424X(09)70226-0

Zoghi, M., & Nordstrom, M. A. (2007). Progressive suppression of intracortical inhibition during graded isometric contraction of a hand muscle is not influenced by hand preference. *Experimental Brain Research*, *177*(2), 266–274. http://doi.org/10.1007/s00221-006-0669-2

Zoghi, M., Pearce, S. L., & Nordstrom, M. A. (2003). Differential Modulation of Intracortical Inhibition in Human Motor Cortex during Selective Activation of an Intrinsic Hand Muscle. *The Journal of Physiology*, *550*(3), 933–946. http://doi.org/10.1113/jphysiol.2003.042606