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The study of Hebbian-type repetitive transcranial magnetic stimulation on impaired hand motor function in chronic stroke

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The study of Hebbian-type repetitive transcranial magnetic stimulation on impaired hand motor function in chronic stroke

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An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Neuroscience
2018

Abstract

The study of Hebbian-type repetitive transcranial magnetic stimulation
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By Julianne Jasmine Freeman

More effective stroke motor rehabilitation strategies are needed considering that the majority of the six million stroke survivors in the United States suffer long-term motor impairment. Repetitive transcranial magnetic stimulation (rTMS) is a promising tool to enhance stroke motor recovery; however, high intra-individual variability in the efficacy of current rTMS strategies remains a concern. To move toward the development of more effective rTMS strategies, we studied the effect of a novel form of rTMS, called Hebbian-type rTMS ($rTMS_{Heb}$), on patients with impaired hand motor function suffering chronic stroke involving the primary motor cortex (M1) and/or the corticospinal tract (CST).

The role of M1 and CST in supporting affected hand function has not been previously studied in humans in great detail. Therefore, to better understand the neural substrates supporting impaired motor function of the distal upper extremity in chronic stroke, we determined the relationship between hand and wrist motor function with M1 and its associated CST (n=18, 10M, 61.78 ± 11.89 years). We report that the magnitude of corticospinal output from M1 of the lesioned hemisphere is most likely associated with the extent of impaired hand, but not wrist, motor function.

Next, the effect of $rTMS_{Heb}$ on training-related motor improvement was determined in a double-blinded, placebo controlled study. Twenty patients suffering chronic stroke completed five days of wrist motor training to improve distal UE motor function and were randomized to receive either $rTMS_{Heb}$ (n=10, 6M, 62.6 ± 12.0 years) or sham ($rTMS_{sham}$, n=10, 4M, 59.7 ± 10.9 years) during training. Exploratory analysis revealed that $rTMS_{Heb}$ may prolong the retention of training-related hand motor improvement compared to $rTMS_{sham}$.

In conclusion, by restricting our studied population to patients whose infarct included the primary motor system, we examined the role of the surviving tissue of M1 and CST in supporting hand and wrist motor function after stroke. We conclude that M1 output supports impaired hand function and that targeting M1 with $rTMS_{Heb}$ could be of benefit when considering rehabilitative treatment for patients with chronic stroke of M1 and/or CST.

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Table of Contents

List of Figures

List of Tables

CHAPTER 2

CHAPTER 3

Abbreviations

ADL	activities of daily living
AFNI	Analysis of Functional NeuroImages
AIP	anterior parietal area
AUC	area under the curve
CMA	cingulate motor area
CMAd	dorsal cingulate motor area
CMAr	rostral cingulate motor area
CMAv	ventral cingulate motor area
CST	corticospinal tract
CS	conditioning stimulus
DTI	diffusion tensor imaging
DWI	diffusion weighted image
ECU	extensor carpi ulnaris
EMG	electromyography
EPSP	excitatory postsynaptic potential
FA	fractional anisotropy
FAS	functional ability score
fMRI	functional magnetic resonance imaging
FOV	field-of-view
ISI	interstimulus interval
JTT	Jebsen Taylor Test

L	left
LTP	long-term potentiation
LTD	long-term depression
LQ	laterality quotient
M	male
MAL	Motor Activity Log
MEP	motor evoked potential
MRC	Medical Research Council
MRI	magnetic resonance imaging
MSO	maximum stimulator output
M1	primary motor cortex
N/A	not applicable
PMC	premotor cortex
PMd	dorsal premotor cortex
PMv	ventral premotor cortex
PPR	posterior reach area
R	right
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
rMT	resting motor threshold
ROI	region of interest
rTMS	repetitive transcranial magnetic stimulation
rTMS	repetitive transcranial magnetic stimulation
SRRR	Stroke Recovery and Rehabilitation Roundtable

SRC	stimulus response curve
SMA	supplementary motor area
S1	primary somatosensory cortex
SICI	short-interval intracortical inhibition
TE	time echo
TI	time inversion
TMS	transcranial magnetic stimulation
TR	time repetition
TS	test stimulus
UE	upper extremity
WMFT	Wolf Motor Function Test

CHAPTER 1:
Introduction

1.1 Stroke

Stroke, a neurological disorder in which disrupted blood flow leads to cell death in the brain, has a major economic and social impact on the United States. Currently six million adults over the age of twenty are reported to have suffered a stroke in the United States (Mozaffarian et al., 2015). As the aging population of the United States grows, the incidence of stroke is predicted to increase (Fang et al., 2014). Combined with a general decline in stroke-related death attributed to improved medical techniques, the number of stroke survivors is predicted to grow by almost 3.4 million people over the next decade (Fang et al., 2014; Mozaffarian et al., 2015).

1.2 Potential outcomes of stroke

Potential outcomes after stroke are broad and the deficits can span multiple domains including language, motor, memory and attention (Corbetta et al., 2015). However, the type and degree of impairment strongly depend upon the region of disrupted blood flow and subsequent infarct location. The two major arterial sources from which branches deliver blood and glucose to the brain are the right and left internal carotid arteries and the right and left vertebral arteries (Kandel et al., 2013a). The internal carotid arteries supply the anterior aspects of the cerebral hemispheres and the vertebral arteries supply the brain stem and posterior aspects of the cerebral hemispheres. Once the internal carotid artery penetrates the dura, the internal carotid artery branches into the anterior cerebral artery and middle cerebral artery (Kandel et al., 2013a). The anterior cerebral artery supplies blood to the frontal cortex and corpus callosum while the middle cerebral artery primarily supplies blood to the most of the cortex, including the frontal,

parietal, temporal and some aspects of the occipital lobe (Kandel et al., 2013a). Smaller branches of the middle and anterior cerebral arteries supply blood to deeper, subcortical structures of the brain (Kandel et al., 2013a). Importantly, multiple arteries can perfuse a single region of the brain; therefore, blood flow must be sufficiently restricted for cell death to occur.

Ischemic and hemorrhagic stroke are the two main types of stroke with the main difference being the method in which the blood supply is disrupted (Kandel et al., 2013b). In ischemic stroke, blood flow becomes occluded but the vessel remains intact; however, in hemorrhagic stroke, the blood vessel ruptures and causes blood to pool in the surrounding tissue (Kandel et al., 2013b). Stroke survivors in the United States more frequently suffer from ischemic (87%) than hemorrhagic stroke (13%) (Mozaffarian et al., 2015). Blockage of the middle cerebral artery is a common cause of ischemic stroke and many stroke survivors suffer infarcts involving the cortical and/or subcortical motor system (Kandel et al., 2013a). Consequently, long-term motor impairment is common after stroke of this type (Corbetta et al., 2015). This thesis will focus on motor impairment of the upper extremity (UE) after ischemic stroke.

1.3 Upper extremity motor recovery after stroke

Treatment of UE motor deficits is important because UE motor deficits can severely compromise, or even cause a loss in independence for activities of daily living, (ADL) and contribute toward a diminished quality of life (Carod-Artal et al., 2000; van Mierlo Maria et al., 2017). In a study of 118 patients suffering from stroke, 78.9% reported a loss of independence in ADL immediately after stroke and almost half (49.8%) remained dependent for ADL over year post-stroke (Carod-Artal et al., 2000). When UE

function can be improved, better overall physical and psychosocial quality of life outcomes are reported (Carod-Artal et al., 2000; Barker and Brauer, 2005; Nichols-Larsen et al., 2005a).

Improvement in motor function can be characterized as either true motor recovery or compensation (Levin et al., 2009; Bernhardt et al., 2017). True motor recovery is defined as regaining the original patterns of movement that were evident prior to stroke and compensation is defined as the substitution of original patterns of movement with alternative movements derived from the remaining motor function (Levin et al., 2009; Bernhardt et al., 2017). While both true motor recovery and compensation lead toward improvement in motor function and involve cortical relearning processes, only true motor recovery requires neural repair (Levin et al., 2009; Bernhardt et al., 2017). The discrimination between compensation and true motor recovery is beyond the scope of this dissertation.

Rehabilitative therapy is currently the only treatment available to target UE motor deficits after stroke (Winstein et al., 2016). Rehabilitative therapy is not standardized across hospitals but typically includes physical and occupational therapy in the United States (Miller et al., 2010). Physical and occupational therapists assess all aspects of motor function including, but not limited to, strength, endurance, range of motion and sensory loss and will work with the patients to regain lost motor function. They also help patients relearn skills needed for ADL, such as personal grooming, dressing and preparing meals. The functional gains associated with rehabilitative therapy depend upon the level of initial impairment as patients with more mild motor deficits improve faster than those with more severe motor deficits (Nakayama et al., 1994). Other factors also

influence the outcome of rehabilitation, such as the duration and intensity of therapy, comorbidities, socioeconomic factors, location of stroke and mental health (Kwakkel et al., 2006; Kwakkel, 2009; Buma et al., 2013).

The most intense rehabilitative therapy is typically administered in the first few weeks after stroke onset and is based on evidence that the rate of recovery slows as time progresses (Duncan et al., 1992; Krakauer, 2005). In fact, the most dramatic improvement in motor function typically occurs in the first thirty days post-stroke (Nakayama et al., 1994; Kwakkel et al., 2006; van Kordelaar et al., 2014). The time period after stroke can be categorized into three phases: acute, subacute and chronic and were defined at the 2017 annual meeting of the Stroke Recovery and Rehabilitation Roundtable (SRRR) (Bernhardt et al., 2017). According to the SRRR, the acute phase is the first seven days after stroke, the subacute phase is seven days to six months after stroke and the chronic phase is anytime six months after stroke (Bernhardt et al., 2017).

Converging lines of evidence suggest that the increased rate of recovery in acute and early subacute stroke is associated with a critical period of increased gene expression and associated neuroplasticity (Cramer, 2008; Murphy and Corbett, 2009; Dromerick et al., 2015). Specifically, increased levels of growth-associated proteins and cell-cycle proteins were found in the peri-infarct tissue within 24 hours of cortical damage in rodent models of ischemic stroke (Comelli et al., 1993; Kleim et al., 2003). Other rodent studies have reported increased angiogenesis, synaptogenesis and dendritic branching, even after a month following ischemic stroke (Buma et al., 2013). For instance, increased rate of cortical dendritic spine formation was observed in rats after ischemic stroke (Brown et al., 2007). While the greatest increase occurred 1-2 weeks after stroke, the elevated rate

of dendritic spine formation was still detected after six weeks (Brown et al., 2007). With time, the rate of dendritic remodeling of neurons in the peri-infarct tissue decreases leading to a slower rate of motor recovery in later months and years (De Roo et al., 2008).

However, the brain remains plastic even in the chronic phase of stroke as indicated by evidence generated with transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI). For example, the excitability of the primary motor cortex (M1), as measured by the peak-to-peak amplitude of a TMS-evoked motor evoked potential (MEP), increases in parallel to training-related improvement in motor function in patients suffering from chronic stroke (Liepert et al., 2000a; Sawaki et al., 2014). Other studies have reported a change in task-related brain activity, as measured by functional magnetic resonance imaging (fMRI), after UE motor improvement in the chronic phase of stroke (Cramer et al., 2002; Ward et al., 2003a; Page et al., 2009). One study even found that the reduction in task-related activity of several brain regions, including M1 and PMC, was linearly associated with UE motor recovery when patients were evaluated weekly up to 6-12 months post-stroke, suggesting that the change in brain organization was associated with functional motor improvement (Ward et al., 2003a). Together these findings demonstrate that, despite evidence from animal models that neural plasticity is reduced in the chronic phase of stroke, the brain remains plastic and patients remain capable of improving UE motor function albeit at a slower rate than in earlier phases (Page et al., 2004).

1.4 Neural substrates supporting motor recovery after stroke

An important step in the development of therapeutic strategies is the identification of the neural substrates that support UE motor recovery. Although a greater understanding of these neural substrates in humans has been reached by studying the healthy and diseased brain, the neural substrates are still not completely understood. The use of fMRI in human patients after stroke identified an early and widespread increase in neural activity associated with UE movement that progressively reduces over time (Ward et al., 2003a; 2003b; Cramer, 2004). The affected brain regions can include but are not limited to M1, the premotor cortex (PMC), the supplementary motor area (SMA), the primary somatosensory cortex (S1), portions of the parietal lobe, thalamus and cingulate motor areas (CMAs) (Ward et al., 2003a; 2003b; Cramer, 2004). In patients who attain complete functional recovery, the hyperactivation returns to a level similar to that of healthy, age-matched subjects (Ward et al., 2003b). However, neural activation remains abnormally high even in the chronic phase of stroke for patients suffering with long-term UE motor deficits (Ward et al., 2003b; Nair et al., 2007). The extent to which neural reorganization in these brain regions contributes toward functional recovery of the UE is unclear. Lesion location, lesion size and level of initial motor impairment have been identified as contributing factors toward the variability in neural reorganization in rodents, monkeys and humans (Cramer, 2004; Dancause, 2006; Dancause et al., 2006; Touvykine et al., 2015).

In this dissertation, we consider motor recovery in patients with mild to moderate upper extremity motor deficits suffering an ischemic stroke to the primary motor system (M1 and its associated corticospinal tract (CST) fibers). For this type of stroke, the

surviving tissue of M1 and/or the associated corticospinal tract (CST) have been identified as important substrates for UE motor recovery in humans (Liepert et al., 2000a; Sawaki et al., 2008), monkeys (Nudo et al., 1996b; Dancause et al., 2005) and rats (Jones et al., 2009). Here I will present a brief overview of the cortical motor system with a focus on the anatomy and function of M1 as well the evidence that M1 plasticity is functionally relevant toward stroke motor recovery.

1.5 Cortical motor system

The cortical motor system is composed of densely intra- and inter-hemispherically connected brain regions including M1, PMC (dorsal and ventral), SMA, portions of the parietal lobe and CMAs.

1.5.1 Primary motor cortex

1.5.1.1 Organization

M1 is five-layered agranular cortical structure located on the precentral gyrus of the frontal lobe, just anterior to the central sulcus in Brodmann's Area (BA) 4. Approximately 70-80% of the neurons in M1 are excitatory, pyramidal neurons with the remaining 20-30% of neurons being interneurons (DeFelipe and Farinas, 1992; Markram et al., 2004). Although interneurons can be excitatory or inhibitory, the majority of M1 interneurons are inhibitory with the majority being GABAergic basket cells (Markram et al., 2004). During gross dissection of the cortex, M1 can be distinguished from nearby cortical structures by the high concentration of large excitatory pyramidal Betz cells in layer V. Although other secondary motor areas also contain Betz cells, M1 contains the densest population of these cells in the brain (Rivara et al., 2003).

M1 receives bilateral inputs from other cortical motor structures including PMC, SMA, cingulate motor areas, BA2 and BA1 of S1 and portions of the parietal cortex as determined by injection of horseradish peroxidase into M1 of the monkey (Muakkassa and Strick, 1979; Lu et al., 1994) (Fig. 1.1). In addition, inter- and intrahemispheric efferents of M1 are sent to other cortical and subcortical structures including contralateral M1, PMC, SMA, parietal lobe, thalamus, striatum and brainstem. However, the primary output of M1 is through the CST (Dum and Strick, 1991). The axons of Betz cells and of other excitatory pyramidal cells located in layer V exit M1 through the CST, descend through the posterior limb of the internal capsule, through the cerebral peduncle and into the brain stem (Amaral, 2013). At the level of the medulla, approximately 90% of the axons decussate and descend contralaterally until synapsing in the ventral horn of the spinal cord contralateral to the pyramidal cell somata (Amaral, 2013). Depending upon the origin of the axonal projections, some axons are more likely to decussate than others, with axons originating from the M1 hand motor region decussating more frequently than axons of M1 trunk motor region (Amaral, 2013). Axons that do not decussate synapse in the ventral horn of the spinal cord ipsilateral to the pyramidal cell somata (Amaral, 2013). In primates, many of the CST projections originating from the M1 hand motor area form monosynaptic connections upon alpha motor neurons supplying the muscles of the hand and fingers (Lemon, 1997). CST projections that do not project directly upon alpha motor neurons, synapse upon local inhibitory and excitatory interneurons that form a broad premotoneuronal network to integrate converging signaling pathways (Lemon et al., 2004).

Finally, M1 is topographically organized into neural representations called motor maps. Two primary theories were first proposed to describe the functional significance of M1 motor maps (Keller, 1993). The first theory argued that voluntary movements are represented in the cortical maps and is based on evidence that a single neuron can project to several motoneuron pools (Keller, 1993). The second theory stated that each motor map represents an individual muscle of the body and is based on evidence that stimulation to a discrete region of the cortex can cause the contraction of a single muscle (Keller, 1993). In the early 2000s, overwhelming evidence led to the development of a new theory of convergence and divergence where M1 neurons across a distributed region of the cortex can converge upon motoneurons that control a single muscle, but a single pyramidal neuron can also diverge onto multiple motoneurons connected to different muscles through axonal collaterals (Schieber, 2002). In accordance with this theory, a motor map supporting a single muscle can overlap with another motor map supporting another muscle and a single pyramidal neuron can be associated with multiple motor maps (Schieber, 2002).

An important characteristic of M1 motor maps is that they are modifiable (plastic) and can be altered by behavioral experience or environmental insult (Rioult-Pedotti et al., 1998; 2000). Evidence that long-term potentiation (LTP) -like mechanisms are at least one of the neural processes supporting M1 plasticity was initially generated by a series of studies by Rioult-Pedotti and colleagues. In the first study, training-related plasticity in M1 of the healthy rat was identified as evoked field potentials, recorded from micropipettes inserted into layers II and III, were larger in the trained than untrained M1 (Rioult-Pedotti et al., 1998). A follow-up study, evaluating the role of LTP in supporting

M1 plasticity, found that less LTP and more long-term depression (LTD) could be generated in the trained than untrained M1 (Rioult-Pedotti et al., 2000). Because the range of synaptic change is fixed according to the model of synaptic modification, the reduction in LTP and increase in LTD suggested that LTP-like mechanisms had already occurred in the trained M1 (Rioult-Pedotti et al., 2000). Although neurons driving the change in field potential size were not identified, the LTP-like mechanisms most likely occurred along the horizontal projections of pyramidal neurons as these projections are found in high concentration in layers II and III in M1 (Rioult-Pedotti et al., 1998; 2000; Amaral, 2013). Together, these findings suggest that LTP-like mechanisms likely underlie training-related M1 plasticity and support the hypothesis that M1 reorganization, such as the change in size of a motor map, is at least partially supported by the exposure of latent synapses that occur after a change in synaptic strength associated with LTP (Rioult-Pedotti et al., 1998; 2000).

1.5.2.2 Functional relevance toward voluntary movement

Based on evidence from in vivo single-unit recordings from M1 pyramidal neurons of the awake healthy monkey during arm reach movements, M1 helps generate and execute movement of the UE. Specifically, more than half of neurons in the hand motor region of M1 exhibit movement-related activity as defined as a close temporal relationship between an increase in neuronal firing rate and the onset of arm movement (Weinrich et al., 1984). Further, neurons exhibiting movement-related activity can encode one or more distinct aspects of movement including acceleration, direction, and/or force of UE movement (Weinrich et al., 1984; Riehle and Requin, 1989; Kalaska and Crammond, 1992; Riehle and Requin, 1995). For instance, one study identified a

population of M1 movement-related neurons that encoded both movement direction and force (Riehle and Requin, 1995). Other M1 pyramidal neurons, whose activity was more closely related to the delay period between movement cues or the signal to begin movement, exhibited set- and signal-related activity, respectively (Weinrich et al., 1984). Although movement-, set- and signal-related activity is not unique to M1, M1 contains a greater percentage of neurons expressing movement-related activity than the surrounding motor areas. For instance, 85% of M1 pyramidal neurons express movement-related activity compared to only 65% of PMC pyramidal neurons in the healthy non-human primate (Weinrich et al., 1984). M1 also may encode different aspects of movement than other motor regions as M1 contains a smaller percentage of directionally-selective neurons than the PMC, but a greater percentage than the parietal and somatosensory cortex (Riehle and Requin, 1989). Another study found M1 expressed a greater percentage of acceleration-selective neurons than the PMC (Weinrich et al., 1984). Although histological analysis revealed that the sites of M1 microstimulation used to study movement-, set-, and signal-related activity had a high concentration of large layer V pyramidal neurons, the cortical layer from which the studied neurons originated was not recorded and the output destination to which the studied neurons projected was not determined (Weinrich et al., 1984; Riehle and Requin, 1989; Kalaska and Crammond, 1992; Riehle and Requin, 1995). Therefore, more research is needed to understand how movement-, set-, and signal-related neurons of M1 interact with other brain regions to support UE movement.

1.5.2 Premotor cortex

The PMC is one of the cortical areas that provides the strongest cortical inputs to M1 (Muakkassa and Strick, 1979; Lu et al., 1994). The PMC composes part of BA6 and, in the human, expands across the anterior lip of precentral sulcus and the posterior regions of the middle and superior frontral gyri (Kantak et al., 2012). The PMC has two primary subdivisions, the dorsal PMC (PMd) and ventral PMC (PMv), which each have distinct cytoarchitecture and extrinsic connectivity (Kantak et al., 2012). Although in the monkey, the PMd can be further subdvided into areas F2 and F7 and PMv can be further subdivided into areas F4 and F5, additional subdivisions of PMd and PMv in the human are unclear (Kantak et al., 2012).

One of the roles of the PMd and PMv is thought to be in movement preparation as these regions contain a high concentration of set- and signal-related neurons (Weinrich and Wise, 1982; Weinrich et al., 1984). In fact, the PMd and PMv express more set- and signal-related neurons than movement-related neurons, suggesting that PMC is more closely involved in motor planning than motor execution (Weinrich and Wise, 1982; Weinrich et al., 1984). Further, movement-related activity of the PMd and PMv typically occurs prior to the onset of movement-related activity in M1 in the monkey, prompting some to suggest that the delay between PMC and M1 activity is temporally consistent with the hypothesis that the PMC passes information about motor plans to M1 prior movement onset (Weinrich and Wise, 1982; Weinrich et al., 1984).

Differences in the afferent and efferent projections of PMd and PMv in the monkey suggest that the functions of PMd and PMv in motor preparation are distinct, although the prisice roles of each are highly debated (Kurata, 1991; He et al., 1995; Dum

and Strick, 1996). Injection of horseradish perixodase into the cervical enlargement of the spinal cord revealed that the PMd expresses denser corticospinal projections than PMv (He et al., 1995). Although fewer corticospinal projections of the PMd enter the ventral horn of the spinal cord than M1, this evidence still suggests that the PMd has at least some ability to act directly on motoneurons innervating the hand and arm (He et al., 1995; Dum and Strick, 1996). On the other hand, the PMv gives rise to the densest projections to M1 than any other cortical areas, including the PMd, and may be responsbile for passing set- and signal-related activity to M1 during movement preparation (Lu et al., 1994).

1.5.3 Supplementary motor cortex

SMA also sends projections to M1 and the spinal cord (He et al., 1995). SMA can be found in the medial portion of BA6 which lies on the superior frontal gryus, just anterior to the leg motor regions of M1. The medial portion of BA6 can be subdivided into SMA proper and pre-SMA (Picard and Strick, 1996; Nachev et al., 2008). The precise function of SMA proper and pre-SMA in motor control is debated but differences in their afferents, thalamic input, activity patterns during movement and responsiveness to somatosensory stimuli suggest that SMA proper and the pre-SMA support distinct aspects of movement (Picard and Strick, 1996; Nachev et al., 2008).

One proposed function of the pre-SMA is to support cognitive control, which includes the ability to flexibly switch between movements or to inhibit an inappropriate motor response (Nachev et al., 2008). This finding would be consistent with the dense reciprocal connections between the pre-SMA and portions of the prefrontal cortex that are known to support exective functioning (Jürgens, 1984). The pre-SMA does not

contain a large number of neurons that give rise to corticospinal projections or cortico-cortical connections with M1 further suggesting a less direct role in movement execution (Dum and Strick, 1991; He et al., 1995).

On the other hand, SMA proper contains dense, somatotopically organized projections to the spinal cord and M1 that evoke movements of the head, forelimbs or hindlimbs when stimulated (Dum and Strick, 1991; He et al., 1995; Dum and Strick, 2002). Further, a significant portion of the corticospinal terminations from SMA proper (11%) synapse within the ventral horn of the lower cervical spinal cord and allow for monosynaptic control upon motoneurons innervating muscles of the hand (Dum and Strick, 1991). The involvement of SMA proper in movement generation and execution is unclear but appears dependent upon task complexity and cognitive demand (Thaler et al., 1988; 1995; Shima and Tanji, 1998a; Nachev et al., 2008). Some speculate that the SMA supports the initiation of movement in the absence of environmental cues (Thaler et al., 1995; Shima and Tanji, 1998a). As evidence, lesion to SMA proper disrupts movement in tasks when the monkey is required to self-generate a motor response, but not in tasks when the movement is externally cued (Thaler et al., 1995). Further, the SMA appears particularly important in the generation of sequential movements as monkeys who receive muscimol injections to the SMA proper cannot perform a previously trained sequences of movement without being cued for each successive movement (Shima and Tanji, 1998b).

1.5.4 Cingulate motor areas

The CMAs are found on the medial wall of the superior frontal gyrus and, based on differences in cytoarchitectonics, can be divided into three distinct motor fields

including the rostral CMA (CMAr), the dorsal CMA (CMAd) and the ventral CMA (CMAv) (Picard and Strick, 1996). The CMAs lie on the cingulate sulcus with CMAd on the dorsal bank in BA 6c and CMAv on the ventral bank in BA 23 (Picard and Strick, 1996). CMAr lies anterior to CMAd and CMAv, also on the cingulate sulcus, but spans both banks in BA 24c (Picard and Strick, 1996). Although the CMAs have been studied in the greatest depth in the monkey, there is fMRI evidence that similar fields exist in the human (Amiez and Petrides, 2014).

The precise role of CMAs in motor control is unclear, but may be involved in reward-motivated behaviors based on evidence derived from single cell recordings of the awake monkey (Shima and Tanji, 1998a). For example, when monkeys voluntarily performed one of two rewarded motor behaviors, a subpopulation of CMAr neurons increased in activity prior to movement during trials in which the reward was reduced and the alternative movement was selected (Shima and Tanji, 1998a). Further, the CMA is also part of the limbic system, which is a well-described system of brain structures that support emotion, motivation and arousal (Devinsky et al., 1995). This combined evidence suggests that at least the CMA may be responsible for conveying information about motivation and reward to M1, especially given that the CMAd and CMAv have the second most dense cortico-cortical projections to M1 of the cortical motor structures (Lu et al., 1994). The CMAr, CMAd and CMAv also project directly to the spinal cord, but few of their corticospinal projections innervate the ventral horn and consequently, likely have a more indirect role in motor control than other cortical structures (Dum and Strick, 1996).

1.5.5 Parietal cortex

Finally, M1 also receives dense afferent bilateral projections from the regions of parietal lobe (Jones et al., 1978) which is thought to be associated with the transmission of higher-ordered sensory signaling, including visuomotor transformations (Cohen and Andersen, 2002; Fogassi and Luppino, 2005). Examples of two regions in the parietal lobe that are involved in motor control include the posterior reach region (PPR) and the anterior intraparietal area (AIP) of the intraparietal sulcus. In the monkey activity of the PPR has been associated with the visual transformation of space and body representation during arm reach movements while activity of the AIP has been associated with the planning of hand grasp movements (Cohen et al., 2002). For instance, single-cell recording of the monkey parietal lobe identified a population of AIP neurons that were preferentially active during the manipulation of a specific object with the hand (Sakata et al., 1995). However, activity of some of these neurons, termed "visual and motor" neurons, depended not only on the type of object being handled, but the visual condition (light or dark) under which the object was viewed (Sakata et al., 1995). Combined these findings indicate that the AIP contains neurons that are capable of integrating visual information for use in object manipulation. As additional evidence for this observation, monkeys have difficulty moving their hands into the correct orientation to grasp an object when AIP activity is disrupted with an injection of muscimol (Gallese et al., 1994). Follow-up research indicated that the visuomotor integration may be used toward motor planning as a portion of the "visual and motor" neurons exhibited set-related activity (Murata et al., 1996). A role of the PPR and AIP in visuomotor integration is also consistent with evidence that the parietal lobe receives auditory, visual and touch

information from afferent bilateral signaling pathways through the thalamus and S1 (Avendaño et al., 1985; Jbabdi et al., 2007).

1.5.6 Descending motor tracts

Although the cortical motor system was described here in great depth, the motor-related structures of the brain are not restricted to the cortex. Multiple descending pathways are involved in motor control and allow motor commands generated in the brain to be delivered to the spinal cord. These tracts can include, but are not limited to, the interstitiospinal, tectospinal, reticulospinal, bulbospinal, rubrospinal, pontospinal, corticospinal, and/or corticobulbar tracts depending upon the mammalian species (Lemon, 2008).

The CST is composed of descending spinal projections from cortical motor areas including M1, PMd, PMv, SMA, and CMAs. Depending upon the motor region and the level of the spinal cord, a portion of the CST will synapse in the ventral horn and form monosynaptic connections with motoneurons of that spinal region. At the level of the cervical enlargement, where motoneurons related to the hands and fingers are located, corticospinal projections entering the ventral horn are primarily of origin from M1, although some projections also originate from the PMd and SMA (Dum and Strick, 1991; 1996). In the primate, hands and finger-related motoneurons are predominantly innervated by the CST (Dum and Strick, 1991; He et al., 1995). The strong monosynaptic control of the motor cortex upon muscles of the hand and fingers is thought to underlie the higher capacity for fine finger movements in primates compared to other animal species (Lemon, 1997). For instance, in rodents and cats, where independent digit

movement is limited, there is no evidence for the CST to directly synapse upon motoneurons projecting paw or forelimb muscles (Lemon, 1997).

These findings are in stark contrast to motoneurons of the more proximal UE which are innervated by multiple motor tracts including the CST (Lemon, 1997), the reticulospinal tract whose cell bodies are located in the reticular formation (Riddle et al., 2009; Zaaimi et al., 2012) and the vestibulospinal tract whose cell bodies are located in the vestibular nuclei (Markham, 1987). The anatomical differences between the innervation of the proximal and distal UE muscles is particularly important when considering recovery after injury or stroke. For instance, when the CST is permanently lesioned in the monkey, monkeys recover the ability to use the upper limb, likely through functional gains mediated by alternative motor pathways, but are left with an inability to move the fingers independently (Lawrence and Kuypers, 1968; Zaaimi et al., 2012).

1.7 Function of the primary motor cortex in motor skill acquisition

The cortical motor system does not only support voluntary movement of the upper extremity but also participates in motor skill acquisition. Here we will discus the functional relevance of M1 in supporting the improvement of UE motor skill.

The finding that M1 is a crucial substrate for the improvement of upper extremity motor skill was first proposed after morphological changes of M1 pyramidal neurons were detected in normal rats following motor training (Greenough et al., 1985). Specifically, the apical dendrites of layer V pyramidal neurons in M1 contralateral to the trained forelimb were found to have greater number of branches and greater total dendritic length than untrained rodents (Greenough et al., 1985). Further research identified that training-related change to the dendritic structure is not restricted to

pyramidal neurons of layer V, but occurs in pyramidal neurons of almost every layer of M1. Specifically, greater dendritic spinal density and greater wider dendritic spinal width (measured at the widest point of the spine perpendicular to the length) was also found on dendrites located in layer I, II and III of M1 pyramidal neurons after normal rats completed skilled motor training (Withers and Greenough, 1989; Rioult-Pedotti et al., 1998; 2000; Xu et al., 2009). Although there are different types of pyramidal neurons, the subpopulation of pyramidal neurons in which structural changes most often occur was not identified (Withers and Greenough, 1989; Rioult-Pedotti et al., 1998; 2000; Xu et al., 2009). The finding of structural alterations among multiple layers of M1 implies that motor training likely does not only alter the corticospinal output from layer V of M1 but also has the potential to alter the interaction of M1 with other motor regions through horizontal projections from layers II and III.

In addition to changes to structural morphology, functional reorganization of M1 also occurs during upper extremity motor skill acquisition. For instance, the motor representation of the trained limb expands following skilled motor training in rats (Kleim et al., 1998), monkeys (Nudo et al., 1996a) and humans (Elbert et al., 1995). Less intracortical inhibition is also detected in M1 following motor skill learning in humans which is consistent with evidence that functional reorganization of M1 motor representations is associated with LTP-dependent mechanisms that require reduced GABAergic signaling (Smyth et al., 2010). Importantly, functional M1 reorganization is restricted to areas of the cortex engaged in motor training. For instance, the size of the hindlimb motor representation does not change after skilled arm reach training in the rat (Kleim et al., 1998; Molina-Luna et al., 2008). Because change to the dendritic structure

is localized to the motor representation of the trained limb, the change in M1 structure has been proposed to be an important neural mechanism supporting M1 functional reorganization (Nudo et al., 1996b; Kleim et al., 1998; 2002). This conclusion is further supported by evidence that pyramidal cell synaptogenesis precedes the expansion of motor representations during motor training in the rat (Kleim et al., 2004). There is no evidence that the change in structural or functional pyramidal morphology is associated with extraneous variables such as stress or increased motor activity that also occur during skilled motor training. In fact, change in M1 motor representation does not occur after unskilled motor training in rats (Kleim et al., 1998; Molina-Luna et al., 2008) or strength training in humans during which variables such as stress and increased motor activity are also present (Jensen and Marstrand, 2005).

Finally, the precise role of M1 in the formation of a motor memory is still debated. Although improvement in motor skill can still be detected over a year after motor training, training-related structural and functional changes in M1 are thought to be transient and rarely can be detected beyond a few days after training in the healthy animal (Smith et al., 2005). In rats, the expanded motor representation returns to the baseline size within five days after training even though the skilled motor improvement in arm reach persists beyond 27 days (Molina-Luna et al., 2008). In humans, repetitive transcranial magnetic stimulation (rTMS) to M1 only disrupts motor skill acquisition when applied immediately following motor training but not when applied six hours after motor training (Muellbacher et al., 2002b). Based on this evidence, some researchers postulate that a motor memory is first encoded in M1 but is consolidated in other brain regions for long-term retention. However, culminating evidence of long-term M1 structural training-

related changes from research over the past decade leads to speculation that specific aspects of M1 organization may also be important for the retention of a motor skill. In rats, skilled motor training of the forelimb causes an increase in dendritic spinogenesis in layer V pyramidal neurons of M1 (Xu et al., 2009; Zemmar et al., 2014). The higher rate of spine formation is paralleled with an increased rate of spine elimination and; consequently, spinal density returns to baseline levels after training ends (Xu et al., 2009). However, dendritic spines that formed during training are preferentially stabilized during the pruning process (Xu et al., 2009). This finding not only suggests that M1 is capable of long-term structural modification but also that the change in synaptic structure may be associated with motor memory consolidation. More research is needed to understand how structural changes in the M1 pyramidal neurons are associated with long-term motor skill retention.

1.8 Function of the primary motor cortex in stroke motor recovery

M1 reorganization not only supports improvement of UE motor function in healthy adults, but also after stroke. Importantly, M1 reorganization in both the lesioned (ipsilesional) and non-lesioned (contralesional) hemisphere has been observed.

Animal and human experiments have firmly established that use-dependent plasticity of ipsilesional M1 ($M1_{IL}$) supports motor recovery of the affected UE. Reorganization of the peri-infarct $M1_{IL}$ was first identified in 1950 when the thumb motor representation reappeared in adjacent, surviving tissue of the monkey after the original M1 thumb representation was lesioned (Gless and Cole, 1950). Further work determined that the improvement in UE motor function is paralleled with the expansion in the forelimb and/or hand motor representation in monkeys (Castro-Alamancos et al.,

1995; Nudo et al., 1996b) and humans (Liepert et al., 2000a; Wittenberg et al., 2003; Sawaki et al., 2008) and a decrease in intracortical inhibition (Manganotti et al., 2002). Further evidence for the observation that reorganization of $M1_{IL}$ supports UE recovery can be found in studies using fMRI to study the change in task-related M1 activation after stroke. Typically, task-related activation in $M1_{IL}$ is greater after stroke than in healthy subjects but decreases toward more normal activation patterns as UE motor function improves (Ward et al., 2003a).

The functional relevance of contralesional M1 ($M1_{CL}$) reorganization is more controversial than $M1_{IL}$ as scientists disagree as to whether $M1_{CL}$ reorganization is beneficial or maladaptive toward UE motor recovery. The conflicting views can be partly attributed to evidence that $M1_{CL}$ reorganization is not always observed after motor improvement and that the association between $M1_{CL}$ plasticity and affected limb recovery appears to be dependent upon the mechanism by which contralesional plasticity is induced (Jones et al., 2013).

In general, most studies report that $M1_{CL}$ reorganization is beneficial when associated with motor training of the affected UE. For instance, one study reported a decrease in the TMS motor map area of hand muscle in $M1_{CL}$ following recovery of UE function associated with Constraint-Induced Motor Therapy (Wittenberg et al., 2003). Another study observed a negative linear correlation between the improvement in UE motor function and change in task-related activation of the $M1_{CL}$ when human stroke patients were observed over a period of six months (Ward et al., 2003a). One factor that may influence the benefit of $M1_{CL}$ reorganization on UE motor recovery is the level of initial motor impairment. For instance in one study, when patients with varying levels of

motor impairment were assessed separately, only patients with worse motor function had greater hand motor task-related activity than healthy controls in $M1_{CL}$ (Ward et al., 2003b). Further, an inverse negative correlation between the magnitude of task-related activity in $M1_{CL}$ and UE motor function was only observed in patients with worse motor impairment, suggesting that contralesional reorganization may be of greater benefit for patients with more severe motor deficits (Ward et al., 2003b). This observation may also explain why $M1_{CL}$ reorganization is not always observed after UE motor recovery in patients suffering from less severe motor impairment (Liepert et al., 2000a; Sawaki et al., 2014). In these studies, the organization of the $M1_{CL}$ was stable after UE motor recovery associated with constraint induced therapy (Liepert et al., 2000a; Sawaki et al., 2014). Although one study found a significant anterior shift of the center of gravity after recovery (Sawaki et al., 2014), all other measures of M1 organization collected with TMS including a hand muscle map area, resting motor threshold (rMT), active motor threshold (aMT), silent period and stimulus response curve (SRC) were stable (Liepert et al., 2000a; Sawaki et al., 2014). Other variables that could affect the role of $M1_{CL}$ in supporting UE function could include the phase of stroke, location of the ischemic infarct and patient age (Sawaki et al., 2014).

On the other hand, $M1_{CL}$ reorganization is most often perceived as maladaptive when associated with motor training of the non-affected UE. This concept has been well described in rats. Typically, after lesion to M1, rats demonstrate paw use asymmetry such that the non-affected forelimb is used significantly more frequently than the affected forelimb (Prusky and Whishaw, 1996). If ignored, overuse of the non-affected limb promotes increased dendritic arborization and synaptogenesis in $M1_{CL}$, and prevents

future reorganization of $M1_{IL}$ (Allred et al., 2005; Allred and Jones, 2008). When skilled use of the non-affected limb precedes rehabilitative training of the affected limb, reorganization of $M1_{IL}$ and associated functional improvement of the affected limb does not occur (Jones et al., 2013). The maladaptive effects of $M1_{IL}$ reorganization appear to be mediated through the interhemispheric projections as use of the non-affected hand does not does not impair reorganization of $M1_{IL}$ and associated recovery of the affected forelimb when the corpus callosum is severed (Jones et al., 2013).

1.9 Repetitive transcranial magnetic stimulation

Given the importance of neural reorganization within M1 and associated CST signaling for recovery of upper extremity function after stroke, there is interest in using transcranial magnetic stimulation (TMS) to enhance motor recovery beyond standard rehabilitative therapy alone by facilitating M1 neuroplasticity. TMS is based on the principles of electromagnetic induction and can be used non-invasively to stimulate the surface of the brain. In brief, a magnetic field is created when a strong electric current is passed through the wires of a TMS coil (Epstein, 2008a). A time-varying magnetic field produces an external electric field that penetrates freely through the cranial tissues (Epstein, 2008a). When a TMS pulse of sufficient intensity is applied to M1, the external electric current can activate neurons located within the targeted cortical region (Davey, 2008). Depending upon the TMS intensity, type of TMS coil and diameter of the TMS coil wing(s), the size of the targeted cortical area can be controlled. Typically, lower TMS intensities, a figure-of-eight TMS coil and smaller coil size allow for a more focal TMS application (Epstein, 2008b). The repetitive application of TMS pulses (rTMS) has been identified as a powerful neuromodulator and, when applied to M1 in the context of

stroke rehabilitation, can help patients improve motor function with minimal risk and few side effects (Lefaucheur et al., 2014). There are two commonly-used methods in which rTMS is currently being studied in therapeutic capacity for stroke motor rehabilitation.

The first common method is based on the model of interhemispheric competition. In healthy adults, excitatory projections originating from M1 of each hemisphere cross the corpus callosum and synapse upon inhibitory interneurons of M1 in the opposite hemisphere (Meyer et al., 1998). This system of inhibition allows each M1 to modulate the excitability of M1 in the opposite hemisphere through interhemispheric inhibition (Fig. 1.2A) (Ferbert et al., 1992). Interhemispheric inhibition is thought to be especially important in stabilizing hand movement. As evidence of this, the level of inhibition from M1 ipsilateral to the moving hand onto M1 contralateral to the moving hand is modulated based upon the accuracy required to complete the hand motor task (Wischnewski et al., 2016). However, interhemispheric inhibition often is disrupted after stroke of the middle cerebral artery territory as $M1_{CL}$ becomes hyperexcitable and exerts abnormally high amounts of inhibition onto $M1_{IL}$ (Fig. 1.2B) (Murase et al., 2004). Because abnormal interhemispheric inhibition can impair motor recovery after stroke, there is interest in using rTMS to normalize M1 activity (Murase et al., 2004). This can be accomplished by either increasing activity of $M1_{IL}$ with excitatory high-frequency rTMS (>5 Hz) or by decreasing activity of $M1_{CL}$ with inhibitory low-frequency rTMS (<1 Hz) (Fig. 1.2C, 1.2D). Both types of rTMS improve arm function in subacute and chronic stroke (Mansur et al., 2005; Takeuchi et al., 2005; Liepert et al., 2007; Dafotakis et al., 2008; Kirton et al., 2008; Nowak et al., 2008; Khedr et al., 2009; Grefkes et al., 2010; Conforto et al., 2011; Chang et al., 2014; Khedr et al., 2015; Kim et al., 2015; Lee et al., 2015).

However, the impact of rTMS on the brain typically does not persist beyond a few days (Bäumer et al., 2003). As a result, few studies have demonstrated that high-frequency rTMS or low-frequency rTMS alone can produce long-lasting effects on UE motor function.

The second common method uses rTMS to prime the brain immediately prior to motor training to enhance training-related M1 neuroplasticity. This therapy is based on evidence that recovery-related neuroplasticity is gated by intracortical inhibition in $M1_{IL}$ and that when intracortical inhibition of $M1_{IL}$ is reduced by either high-frequency rTMS (>5 Hz) to $M1_{IL}$ or by low-frequency rTMS (<1 Hz) to $M1_{CL}$, motor recovery can be enhanced (Ziemann and Siebner, 2008). Positive effects have been observed after both the application of inhibitory low-frequency of rTMS to $M1_{CL}$ and the application of excitatory high-frequency rTMS to $M1_{IL}$ prior to motor training (Khedr et al., 2005; Kim et al., 2006; Malcolm et al., 2007; Kim et al., 2010; Koganemaru et al., 2010; Chang et al., 2012; Guan et al., 2017). One study found that 10 Hz rTMS over $M1_{IL}$ enhanced the training-related improvement in movement accuracy on a complex finger motor task and that the improvement in motor skill was associated with an increase in $M1_{IL}$ excitability in chronic stroke (Kim et al., 2006). Another study found that the training-related increase in finger extensor movement was greater when $M1_{IL}$ was primed with 5 Hz rTMS and that patients who received rTMS also experienced a greater training-related increase in $M1_{IL}$ excitability compared to sham (Koganemaru et al., 2010). Of the few studies that have evaluated the long-term efficacy of rTMS priming, motor improvements were observed to persist for several months. One study still detected the effect of rTMS

priming 6-8 weeks post-training and another study detected the effects 3 months post-training (Kim et al., 2010; Koganemaru et al., 2010).

1.10 Limitations of current rTMS interventions

Unfortunately, in addition to questions on the long-term retention of rTMS-related motor improvements, there is also concern over the high inter-individual variability in using rTMS to enhance motor recovery. A possible solution to reduce inter-individual variability could be stratifying patients into groups in order to target cohorts that are most likely to respond to rTMS intervention. For instance, the brain-derived growth factor (BDNF) genotype has been reported to influence the efficacy of rTMS on motor recovery with patients expressing the Val/Val allele having better functional outcomes than patients expressing the Val/Met allele (Chang et al., 2014; Kim et al., 2015). Another study reported that initial motor impairment, stroke location and integrity of the CST should also be considered when stratifying patients for rTMS intervention as patients with less severe motor deficits, subcortical stroke and TMS-evoked MEP response have the greatest benefit from rTMS on UE motor function (Lee et al., 2015). Another often-overlooked factor in determining the efficacy of rTMS is the local state of cortical excitability at the time of rTMS application. A clear example of the dependence of TMS on cortical excitability is that a TMS stimulus of the same intensity will evoke a MEP of larger amplitude when the targeted muscle is contracted as opposed to when the targeted muscle is at rest (Di Lazzaro et al., 1998a). The impact of the cortical excitability on rTMS efficacy has not been systematically and of all the studies that have examined the efficacy of rTMS on stroke motor recovery, only a subset reported the state of M1 excitability before the application of rTMS (Buetefisch et al., 2004b; Khedr et al., 2005;

Takeuchi et al., 2005; Kim et al., 2006; Khedr et al., 2009; Grefkes et al., 2010; Koganemaru et al., 2010; Buetefisch et al., 2011; 2015; Kim et al., 2015). The failure to consider M1 excitability may contribute to the high inter-individual variability in the effect of rTMS on motor recovery.

1.11 Hebbian-type repetitive transcranial magnetic stimulation

Given the limitations of the most common current rTMS interventions, there is interest in studying an alternative form of rTMS called Hebbian-type rTMS ($rTMS_{Heb}$). $RTMS_{Heb}$ differs from other types of rTMS because $rTMS_{Heb}$ is applied over M1 concurrently with motor training. Specifically, $rTMS_{Heb}$ targets movement-related activity of M1 opposite the limb engaged in training by using increases in movement-related electromyography (EMG) to trigger the application of subthreshold rTMS during the training movement (Buetefisch et al., 2011; 2015). $RTMS_{Heb}$ is based on evidence that the pairing the stimulation of cortical afferents (thalamus, callosal, pyramidal tract or somatosensory cortex) with post-synaptic stimulation of the pyramidal tract can facilitate associative LTP or also called Hebbian-type LTP (Baranyi and Feher, 1981; Baranyi et al., 1991). Because the induction of LTP upon horizontal fibers of excitatory pyramidal neurons located in layer II and III of M1 is crucial for motor skill improvement, $RTMS_{Heb}$ may enhance stroke motor recovery by facilitating training-related M1 reorganization (Rioult-Pedotti et al., 1998; 2000). Further, by accounting for the local state of cortical excitability at the time of application, $rTMS_{Heb}$ may have less inter-individual variability than other forms of rTMS. In fact, $rTMS_{Heb}$ is more effective than random rTMS or sham rTMS ($rTMS_{sham}$) in enhancing training-related motor skill improvement and associated

M1 reorganization in healthy subjects but $rTMS_{Heb}$ has not yet been tested in a stroke population (Buetefisch et al., 2011; 2015).

1.12 Summary

In summary, there is great need better for therapeutic strategies for UE motor recovery after stroke, especially for patients in the chronic phase of stroke where motor recovery is slower and more difficult. An important step toward the development of new therapeutic strategies is the identification of neural substrates supporting motor recovery. Here we have described the anatomy and function M1 in great detail and provided evidence that both $M1_{IL}$ and $M1_{CL}$ contribute toward stroke motor recovery of the UE, although the role of the $M1_{CL}$ is less understood. Given that reorganization of M1 is an important neural mechanism supporting of UE motor improvement after stroke, there is interest in using rTMS to improve stroke motor recovery by normalizing M1 activity in either the ipsilesional or contralesional hemisphere. Currently, the two most common forms of rTMS for stroke motor recovery aim to either 1) normalize interhemispheric inhibition from $M1_{CL}$ onto $M1_{IL}$ or 2) reduce intracortical inhibition of $M1_{IL}$ prior to training to improve training-related motor recovery. While there is evidence that both types of rTMS interventions improve UE motor function in some patients with chronic stroke, high inter-individual variability and long-term efficacy remain major challenges for both applications. Thus, while there is a consensus that stroke motor recovery outcomes must be improved, more work is needed to understand the neural substrates supporting UE motor recovery and to develop more effective therapeutic strategies. In response to this gap in knowledge, the aims of this dissertation are to study a specific aspect of UE function, the hand and wrist. Here I study the neural substrates supporting

hand and wrist motor function in the chronic phase of stroke (Aim 1) and test a novel form of rTMS, called $rTMS_{Heb}$, on hand and wrist motor recovery that addresses some of the limitations of the current rTMS techniques (Aim 2).

1.13 Specific Aims

1.13.1 Specific Aim 1

The need for a better understanding of the neural substrates that support motor function after stroke leads to the first aim of the dissertation in which we determine the relationship between the M1 and its associated CST with function of the hand and wrist in patients suffering chronic stroke (Chapter 2). While the critical role of M1 and the CST for hand control has been described in non-human primate stroke models (Nudo and Milliken, 1996; Nudo et al., 1996b), the relationship of M1 and CST with impaired hand motor function not been systematically tested in humans after stroke, as current research on UE motor recovery in humans primarily focuses on the entire arm (Ward et al., 2003a; 2003b; Schaechter et al., 2006; Stinear et al., 2006; Bestmann et al., 2010; Puig et al., 2010; Zhu et al., 2010; Gauthier et al., 2012; Page et al., 2013; Sterr et al., 2013; Stinear et al., 2014; Feng et al., 2015; Quinlan et al., 2015; Park et al., 2016). Given that the neural substrates of the hand and UE are anatomically different, the results on whole-arm UE function cannot be generalized to the hand (Lemon, 2008). To test the hypothesis that $M1_{IL}$ and CST_{IL} are related to impaired function of the hand and wrist, 18 patients suffering from chronic stroke involving the M1 and/or CST were studied (10M, aged 61.78 ± 11.89 years). The Jebsen Taylor Test (JJT) was used to measure hand motor function and peak acceleration of wrist extension movements was used to measure wrist motor function (Jebsen et al., 1969; Buetefisch et al., 2015). TMS-derived stimulus

response curve (SRC) and short-interval intracortical inhibition (SICI) were used to describe function of $M1_{IL}$ and its associated CST_{IL} (Kujirai et al., 1993; Ridding and Rothwell, 1997). M1 cortical thickness and fractional anisotropy (FA) of the CST, derived from structural MRI, were used to measure the structure of M1 and CST, respectively (Kujirai et al., 1993; Ridding and Rothwell, 1997). The relationship between M1 and CST with impaired function of the hand and wrist was determined with linear regression models with the measures of motor function as the dependent variables and the functional and structural measures of M1 and CST as the independent variables.

1.13.2 Specific Aim 2

The need for more effective therapeutic strategies for stroke motor recovery led to the second aim of this dissertation in which we determine the effect of $rTMS_{Heb}$ on training-related motor improvement of the impaired distal UE, specifically of the hand and wrist (Chapter 3). To test the hypothesis that $rTMS_{Heb}$ will enhance training-related motor improvement in patients suffering from chronic stroke to the primary motor system, a double-blind placebo controlled study was conducted. In the study, 20 patients completed five days of wrist motor training that was previously shown to improve motor function of the distal UE (Buetefisch et al., 2015). Patients were randomized to receive either $rTMS_{Heb}$ (n=10, 6M, aged 62.6 ± 12.0 years) or $rTMS_{sham}$ (n=10, 4M, aged 59.7 ± 10.9 years) during motor training. To determine the effect of $rTMS_{Heb}$ on training-related improvement of distal UE function, hand, and wrist motor function was tested before (pre), after the five days of training (p0wks) and 4 weeks after training (p4wks). The Jebsen-Taylor Test (JTT) and the How Well subtest of the Motor Activity Log (MAL How Well) were used to measure hand motor function and the peak acceleration of

ballistic wrist extension movements was used to measure wrist motor function. The primary measure of motor function was the JTT. To determine if $rTMS_{Heb}$ enhanced training-related improvement of other aspects of UE function, whole arm motor function was tested before (pre) to after (p0wks) and 4 weeks (p4wks) after motor training using the Wolf Motor Function Test (WMFT).

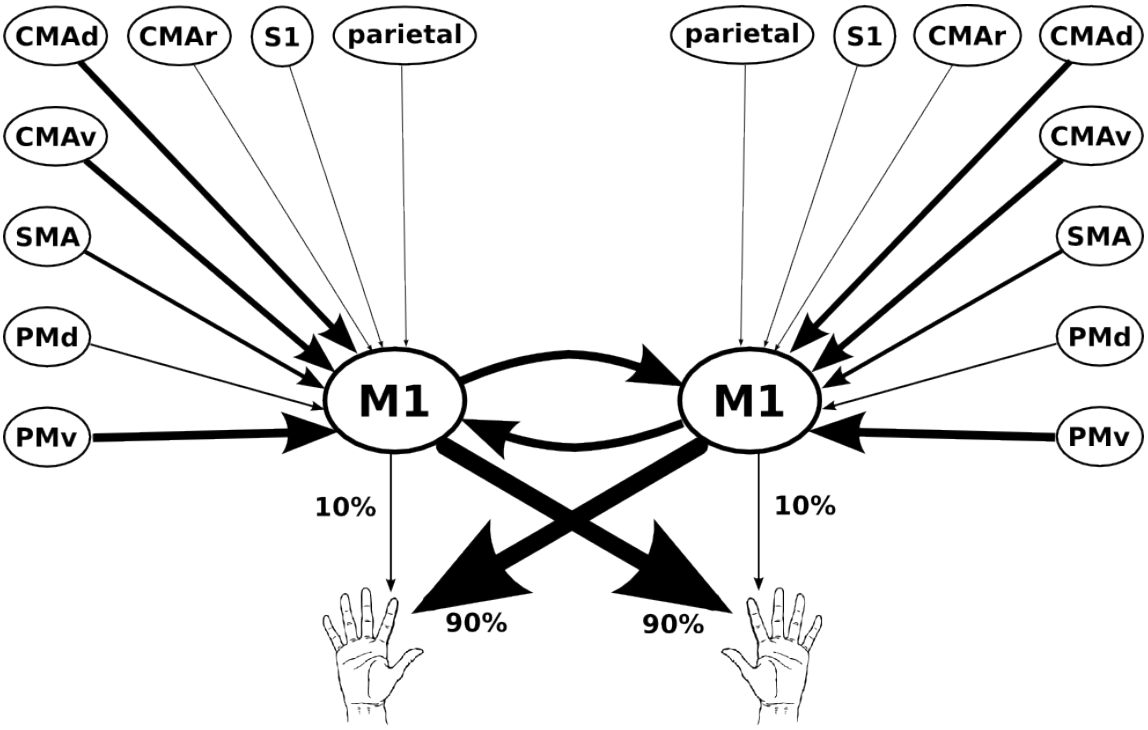

Fig. 1.1 Schematic of the cortical motor areas that innervate the primary motor cortex. The thickness of the line represent the density of the efferent projections to primary motor cortex (M1) with projections from ventral premotor cortex (PMv) being the strongest and projections from rostral cingulate motor area (CMAr), primary somatosensory cortex (S1) and the parietal lobe being the weakest. The strongest output of M1 is to the spine through the ipsilateral (10%) and contralateral (90%) corticospinal tract. At the level of the cervical spinal cord, many M1 projections enter the ventral horn and synapse upon motoneurons innervating muscles of the hand and fingers.

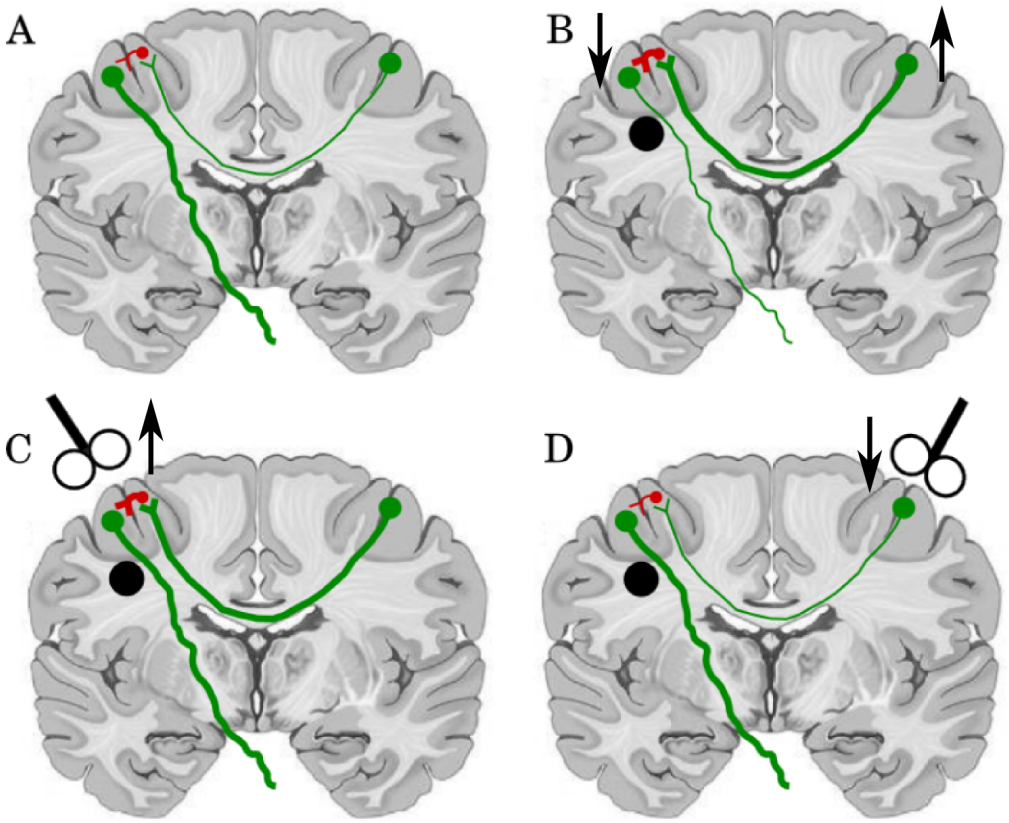

Fig 1.2 Schematic of interhemispheric inhibition.

A) In a healthy brain, excitatory interhemispheric projections (green) from M1 synapse upon inhibitory interneurons (red) of the opposite M1 and modulate the level excitatory corticospinal output (green) during movement. B) After stroke, excitability of $M1_{CL}$ increases and causes interhemispheric inhibition from $M1_{CL}$ onto $M1_{IL}$ to be abnormally high. Abnormality in $M1_{IL}$ function can be attenuated with either C) excitatory high-frequency rTMS to $M1_{IL}$ or D) inhibitory low-frequency rTMS to $M1_{CL}$. Arrows indicate an increase or decrease in motor activity associated with either the ischemic event (B) or rTMS application (C, D)

CHAPTER 2:
Reduced output of primary motor cortex may be related to the extent of impaired hand function in chronic stroke *

* Portions of this chapter are in preparation for manuscript submission

2.1 Introduction

Most ischemic strokes occur in the territory of the middle cerebral artery and impact the integrity of the primary motor system (primary motor cortex (M1) and its corticospinal (CST) projections) (Corbetta et al., 2015). As hand function is mediated through the primary motor system in healthy adults, the hand contralateral to the stroke is often impaired (affected hand) (Lemon, 1997; Lang and Schieber, 2003). Despite rehabilitation treatment, compromised hand function often persists and is one of the most common long-term deficits after stroke (Dromerick et al., 2006).

Rodent and non-human primate studies on paw/hand motor recovery indicate that the primary motor system of the lesioned hemisphere (ipsilesional M1 ($M1_{IL}$) and ipsilesional CST (CST_{IL})) is critical in supporting hand motor function (Dancause and Nudo, 2011). Specifically, expansion of the distal forelimb representation in $M1_{IL}$ is associated with the normalization of hand function in non-human primate stroke models (Nudo et al., 1996b) and recovery of independent finger movements is not observed after lesion of the CST (Zaaimi et al., 2012). Previous studies on hand motor task-related brain activation in humans agree that greater neuronal activity in $M1_{IL}$ is associated with hand motor recovery (Nair et al., 2007). However, details of M1 and CST structure and function as they relate to recovered hand function have not been studied. Identifying these critical aspects of the primary motor system could guide the development of new neuromodulation therapies.

One reason why the association between the primary motor system and hand motor function is not understood in great detail is that stroke recovery research has focused on overall upper extremity (UE) function (Ward et al., 2003a; 2003b; Schaechter

et al., 2006; Stinear et al., 2006; Bestmann et al., 2010; Puig et al., 2010; Zhu et al., 2010; Gauthier et al., 2012; Page et al., 2013; Sterr et al., 2013; Stinear et al., 2014; Feng et al., 2015; Quinlan et al., 2015; Park et al., 2016). Common tests of UE function include the Frenchay Arm Test, Action Research Arm Test, Motricity Index, Upper-Extremity Fugl-Meyer and Wolf Motor Function Test (WMFT). The composite scores derived from these tests more closely describe overall UE function than hand function because the majority of their subtests require only proximal arm function. This distinction is important because the neural substrates of the hand and UE are anatomically different. Specifically, muscles of the UE are innervated by multiple motor tracts such as the CST (Lemon, 1997), the reticulospinal tract (Riddle et al., 2009; Zaaimi et al., 2012) and vestibulospinal tract (Markham, 1987) while muscles of the hand and finger are predominately innervated by only the CST (Lemon, 1997; Zaaimi et al., 2012). When the CST is permanently lesioned in the monkey, monkeys recover the ability to use the upper limb but are left with an inability to move the fingers independently (Lawrence and Kuypers, 1968; Zaaimi et al., 2012). Consequently, conclusions drawn from studies on UE function cannot be generalized to hand and finger function.

Of the few studies that have evaluated the substrates of hand motor function, most evaluated only $M1_{IL}$ and CST_{IL} function (stimulus response curve and/or intracortical inhibition (Ward et al., 2006; Nair et al., 2007) or $M1_{IL}$ and CST_{IL} structure (lesion load and/or M1 thickness (Riley et al., 2011; Jones et al., 2016)). Studies that evaluated both the function and structure of M1 and CST did not test the contribution of $M1_{IL}$ and CST_{IL} separately from M1 and CST of the contralesional hemisphere ($M1_{CL}$, CST_{CL}) (Borich et al., 2015). Since the motor representations in both $M1_{IL}$ and $M1_{CL}$ reorganize after stroke,

the role of $M1_{IL}$ and CST_{IL} cannot be determined from analyses that collapse both hemispheres into a single measure (Allred and Jones, 2008).

Because of their established anatomical functional relationship in non-human primates, we studied 18 patients with chronic stroke involving either M1 and/or the CST and hypothesized that the extent of injury to the function and structure of $M1_{IL}$ and CST_{IL} would be associated with functional impairment in the distal UE, specifically of the hand and wrist. The primary measure of hand motor function was the Jebsen Taylor Test (JTT) and the primary measure of wrist motor function was peak acceleration of ballistic wrist extension movements. Abnormality in hand and wrist motor function was determined by comparing function of the affected hand and wrist to function of the dominant hand and wrist from 18 age-matched healthy subjects. Measures of $M1_{IL}$ and CST_{IL} function and structure were determined using transcranial magnetic stimulation (TMS) and MRI techniques. TMS of M1 results in a synchronized discharge of CST neurons (Amassian and Cracco, 1987; Day et al., 1987). When TMS is applied at increasing intensities, a stimulus response curve (SRC) can be plotted. In the present analysis, we use the Boltzmann function to calculate three parameters of SRC that describe the input-output function of M1 in great detail (Capaday, 1997; Devanne et al., 1997). We also use the paired-pulse TMS technique to study inhibitory neuronal activity in M1 (Kujirai et al., 1993). To determine abnormality in $M1_{IL}$ and CST_{IL} function, TMS measures were compared to 12 age-matched healthy subjects. M1 and CST structure was characterized by the cortical thickness of M1 and fractional anisotropy (FA) of the CST (Basser, 1995; Han et al., 2006). To determine which aspects of the primary motor system support

function of the distal UE in the chronic phase of stroke, correlations between the affected hand or wrist with $M1_{IL}$ and CST_{IL} structure and function were tested.

2.2 Materials and methods

2.2.1 Subjects

18 patients (10M, aged 61.78 ± 11.89 years, Table 2.1, Fig. 1) met the inclusion criteria: (1) single ischemic infarction affecting M1 and/or CST more than 6 months prior to study enrollment, (2) motor deficit in the hand contralateral to the infarct, (3) no other neurological disorder or aphasia (4) no contradiction to TMS or MRI, (4) no intake of medication that interfered with TMS measures, (6) the ability of TMS to elicit a measurable motor evoked potential (MEP) and (7) the ability to give informed consent. Patients were classified as suffering from either cortical or subcortical stroke. A cortical stroke was defined as a lesion that only involved the cortex while a subcortical stroke was defined as a lesion affecting the CST with or without cortical involvement. Co-morbidity was determined from medical records and interview by a board certified neurologist who also determined upper extremity muscle strength and tone using the Medical Research Council (MRC) Scale (Medical Research Council of the United Kingdom, 1976) and the modified Ashworth Scale (Bohannon and Smith, 1987). Cognition was assessed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al., 1998). Handedness was determined by the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects completed written informed consent prior to entering the study. The Institutional Review Board of Emory University approved the study.

Stroke data were compared to data from two groups of age-matched healthy subjects collected in separate studies. The first group's data was used to determine abnormality in the measures of hand function (n=18, aged 62.94 ± 6.98 years) and the second group's data was used to determine abnormality for TMS measures of M1 function (n=12, aged 61.33 ± 5.47 years). The healthy subjects met the same inclusion criteria as the patients except that they had not suffered a stroke and had no motor deficit.

2.2.2 Measures of motor function

The primary measures of function for the affected distal UE included a measure of hand function, the JTT (Jebsen et al., 1969) and a measure of wrist function, peak wrist acceleration during ballistic wrist extension movements (Buetefisch et al., 1995; 2015). Peak wrist acceleration was chosen because wrist acceleration is dependent upon rapid recruitment of M1 pyramidal tract neurons and signaling through the CST (Fromm and Evarts, 1977). Further, extension movements of the distal UE are particularly weak in patients after stroke involving M1 and CST and show poor recovery in non-human primates with CST damage (Zaaimi et al., 2012). Secondary measures of motor function included the Motor Activity Log (MAL) (Uswatte et al., 2006) which was used to determine the impact of compromised hand function on activities of daily living (ADL) and the WMFT (Wolf et al., 1989; 2001) which was used to characterize whole arm UE motor function.

JJT: During the JTT, patients completed seven motor tasks as quickly as possible (capped at 120s) with each hand (Jebsen et al., 1969). The time to complete each motor task was recorded.

Ballistic wrist extension: The kinematic assessment of a ballistic wrist extension movement has been described in detail before (Buetefisch et al., 2015). Briefly, patients executed 5 wrist extension movements with the affected wrist as quickly as possible in response to an auditory cue. Acceleration in two movement planes (extension/flexion; abduction/adduction) was recorded by an accelerometer mounted on the hand. EMG activity (bandpass 3 Hz – 1 kHz) was recorded from the extensor carpi ulnaris (ECU) muscle, which acts as an agonist in the wrist extension movement. Kinematic and EMG signals were sampled at 1 kHz.

MAL: The How Well subtest of the Motor Activity Log (MAL) was used to assess hand function during 30 activities of daily living (ADLs) (Uswatte et al., 2005). Patients reported on a six-point scale how well the affected hand was used during each ADL. The highest score (5) indicated that quality of affected hand movement at the time of questioning was the same as the quality of affected hand movement before the stroke. The lowest score (0) indicated that the affected hand was not used during that activity. In the infrequent case when the patient had not attempted the ADL in the past seven days, a score of 0 was given. If the activity was impossible, for instance combing the hair if the patient was bald, N/A was recorded.

WMFT: For the WMFT, patients completed 15 timed subtests (capped at 120s) with each arm (Whitall et al., 2006). When the affected arm was tested, movement quality was recorded on a five-point functional ability scale (FAS), where the highest score (5) indicated normal movement. Maximum grip strength of each hand was also tested using a hand dynamometer. Maximum arm strength was also tested. For this measure, patients moved their arm from the table to a box with a weighted cuff strapped

to the forearm. The task was repeated after increasing the amount of weight in the cuff by 2 pounds until the patient's maximum or 20 pounds was reached.

2.2.3 Measures of M1 and CST function

EMG activity (bandpass 3 Hz – 1 kHz) was recorded from the ECU muscle with surface electrodes (9mm diameter) in a belly-tendon montage. LabVIEW was used for data acquisition (National Instruments, CA, USA). Raw EMG was sampled and digitized at a frequency of 5 kHz and stored for offline analysis.

TMS was applied over the ECU muscle hotspot of $M1_{IL}$ through a figure-of-eight coil (7cm wing diameter) using two Magstim stimulators connected via a Bistim module (Magstim Company, UK). To ensure accurate positioning of the coil, the TMS coil was registered to an MRI image of the participant's brain using a frameless neuronavigation system (BrainSight software, Rogue Research, Montreal, Canada).

Detailed description of data collection for SRC and short-interval intracortical inhibition (SICI) has been described before (Kujirai et al., 1993; Ridding and Rothwell, 1997). Briefly, for SRC TMS pulses were applied at intensities that ranged from at least the nearest 5% of maximum stimulator output (MSO) below resting motor threshold (rMT) and up to at least 80% MSO in increments of 5% MSO. Ten TMS pulses were applied at each intensity. For SICI, 10 single pulses (TS) at an intensity of 120% rMT were interspersed with 10 paired pulses where the TS was preceded by a conditioning stimulus (CS) intensity of 80% rMT (ISI of 2 ms). SICI was only collected in patients who had MEPs of 200 μV amplitude or greater (Daskalakis et al., 2002).

2.2.4 Measures of M1 and CST structure

Cortical thickness and FA of the CST served as measures of M1 and CST structure, respectively. Images were obtained on a Siemens 3T Trio scanner using a 12-channel head coil. For MPRAGE imaging the following parameters were used: TR=2250ms, TE=4.18ms, TI=900ms, flip angle=9°, 256x256 matrix, FOV=256mm, 176 sagittal slices, resulting in 1mm^3 isotropic voxels. These images were also used to estimate the stroke volume. Diffusion tensor imaging (DTI) data were acquired using a diffusion-weighted EPI sequence with TR=7.7s, TE=90ms, 60 slices, matrix size 102x102 and FOV 204x204 mm, with an isotropic voxel size of 2.0 mm^3. Two averages of 30 non-collinear diffusion directions were collected with a b-value of 1000 s/mm^2, along with a reference B0 image. MR images could not be collected from one stroke patient due to claustrophobia but were collected from remaining 17 out of 18 patients.

2.2.5 Data analysis

2.2.5.1 Measures of motor function

JTT: The JTT raw score (RAW) was calculated by summing the time to complete all but two subtests (writing and simulated feeding). These subtests were omitted because of low test-retest reliability (Stern, 1992). The RAW score was normalized to age- and sex-matched standard scores (STD) that accounted for hand dominance using the formula: (RAW – STD) / (RAW + STD) (Jebsen et al., 1969; Hackel et al., 1992). A normalized score greater than zero indicated abnormal hand function.

Wrist Extension: The peak acceleration of the ballistic wrist extension movements were derived from the first-peak acceleration in the two major movement axes (Buetefisch et al., 2015). Reaction time was defined as time between the movement cue

and onset of movement-related EMG in the ECU muscle. The onset of movement-related EMG was defined as the time when mean EMG of a moving 20 ms time window exceeded mean resting EMG by 3 SD (50 ms time window following the movement cue).

MAL: The score for the How Well subtest of the MAL was calculated by averaging the scores of each ADL. Activities with N/A were excluded from the average score. (Uswatte et al., 2005; 2006).

WMFT: For the WMFT, the mean performance time, mean FAS, maximum arm strength and maximum grip strength were calculated (Wolf et al., 2001). Because performance time decreases non-linearly with improved motor function, a natural logarithmic transformation was applied to each subtest of the WMFT before the mean performance time was calculated (Wolf et al., 2006).

2.5.5.2 Measures of M1 and CST function

Data were analyzed in LabView. Trials with increased EMG background were excluded from further data analysis. For SRC, the mean peak-to-peak MEP amplitudes for each TMS intensity were plotted. The area under the SRC (AUC) was calculated by summing the mean MEP amplitudes evoked by TMS intensities between 35% and 80% MSO. A three-parameter Boltzmann function was fitted to all SRCs that reached a plateau using the Levenberg-Marquard least-squares algorithm to extract three curve parameters: MEPmax, S50 and M parameter (Devanne et al., 1997). The MEPmax is the plateau of the SRC, the S50 is the TMS intensity needed to elicit an MEP of an amplitude corresponding to the inflection point and the M (slope) parameter is proportional to the maximum slope of the SRC independent of MEPmax. For SICI, the mean peak-to-peak MEP amplitude evoked by the paired pulse (CS/TS) was expressed as a percentage of the

mean peak-to-peak MEP amplitude evoked by the TS alone (% MEP). A smaller % MEP indicated greater intracortical inhibition.

2.5.5.3 Measures of M1 and CST structure

M1 thickness: Cortical reconstruction and volumetric segmentation were conducted for the T1 weighted images using the 'recon-all' function of the Freesurfer toolkit version 5.3.0 (http://surfer.nmr.mgh.harvard.edu) (Dale et al., 1999; Fischl et al., 1999). This process includes motion correction, removal of non-brain tissue, automated Talairach transformation, segmentation of cortical and subcortical structures, intensity normalization, model construction of boundary between cortical grey matter -white matter and pial surface, and topology correction, through which the surface area, volume, Gaussian curvature, mean curvature, folding index, thickness, and thickness standard deviation of the cortical and subcortical structures were derived. The processed images were manually inspected and edited for accuracy. Lesions were accounted for by applying edits to the white matter volume to in order to ensure surfaces conformed to the gray-white boundary. Automated parcellation of the primary motor cortex (BA4) into anterior and posterior portions was conducted as part of the FreeSurfer pipeline. This parcellation has shown robust accuracy in its definition of M1 according to cortical folding patterns (Fischl et al., 2008). We used the average cortical thickness for the anterior portions of BA4 (M1) for both hemispheres (ipsilesional and contralesional).

Fractional anisotropy (FA): Diffusion weighted images (DWI) were processed using TRACULA, an automated method available through Freesurfer 5.3.0 (http://surfer,nmr.mgh.harvard.edu). While TRACULA can be used to reconstruct the 18 major white matter pathways, this study focused on the CST. After the initial

reconstruction and labeling of the cortical and subcortical regions through Freesurfer on the T1-weighted images, the tractography consists of three steps: (1) preprocessing; (2) pathway diffusion model validation; and (3) three-dimensional pathway reconstruction (Yendiki et al., 2011). The preprocessing step uses the anatomical segmentation obtained in the reconstruction step to correct for motion, eddy current effects, and B0 distortion in the DWI. The second step of TRACULA uses FSL's (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) bedpost tool to fit the ball-and-stick model of diffusion to the DWI data, returning the reconstructed white matter pathways as outputs. The final step of TRACULA is performed through the fitting of the tracts' shape to the outputs from the second step, and an atlas of healthy manually labeled subjects. We used the weighted mean FA value over the entire CST for each hemisphere.

Lesion volume: Lesion volume was calculated by dividing the volume of the lesion mask, hand-drawn in MRIcron (http://people.cas.sc.edu/rorden/mricron/index.html), by the volume of the whole brain mask extracted during skull stripping in AFNI (Cox, 1996).

2.2.6 Statistical analysis

Measures of motor function: T-tests were used to test abnormality of motor function. Alpha was set to 0.05. The critical value for multiple comparisons was set to 0.005 and was calculated using the Bonferroni method in which alpha was divided by the number of statistical tests (10). Simple linear regression was performed to test the association between wrist and hand function.

Measures of M1 function: To determine abnormality in the TMS measures, unpaired t-tests were used to compare measures of M1 function of the stroke patients

against the same measures of M1 function collected in healthy subjects. Alpha was set to 0.05. The critical value for multiple comparisons was set to 0.008 and was calculated using the Bonferroni method in which alpha was divided by the number of statistical tests (6).

Measures of M1 and CST structure To determine the effect of stroke location on measures of M1 and CST structure, two separate two-way ANOVAs were performed with effect of hemisphere (ipsilesional/contralesional) and stroke location (cortical/subcortical) as independent variables and M1 thickness or CST-FA as the dependent variable. Post-hoc paired t-tests were used to assess significant effects in the ANOVA. Alpha was set to 0.05.

Association between measures of motor function and measures of M1 and CST function and structure: The association between $M1_{IL}$ and CST_{IL} function or structure and function of the affected distal UE were tested with linear regression analysis. Alpha was set to 0.05. The critical value for the Bonferroni test of multiple comparisons was set to 0.0027 and calculated by dividing alpha by the number of linear regression analyses (18). Because age (Kelly-Hayes et al., 2003), post-stroke duration (Jørgensen et al., 1995) and lesion volume (Schiemanck et al., 2005) can affect the variability in distal UE function after stroke, we explored whether the associations between peak wrist acceleration or JTT score and measures of $M1_{IL}$ function or structure became stronger when these variables were controlled for. Separate exploratory multiple linear regression analysis were performed with $M1_{IL}$ as the independent variable, JTT or peak acceleration as the dependent variable and age, post-stroke duration and lesion volume as covariates.

2.3 Results

2.3.1 Measures of motor function

The results of the statistical analyses for distal UE motor function (JTT, wrist extension, MAL) and whole arm UE motor function (WMFT) are summarized in Table 2.2.

JTT: As expected, a two-tailed t-test indicated that hand motor function was abnormal as the normalized JTT of the affected hand was greater than 0 and met criteria for multiple comparisons ($p<0.001$, Fig. 2.2A).

Wrist Extension: Mean peak acceleration ($p<0.001$, Fig. 2.2B) and reaction time ($p=0.055$) were reduced in patients when compared to 18 age-matched healthy subjects in unpaired two-tailed t-tests, indicating abnormality in wrist motor function. However, only the reduction in mean peak acceleration met criteria for multiple comparisons.

MAL: Function of the affected hand was impaired during ADLs as the MAL How Well score was below "healthy" function (score of 5) in a one-sided, two-tailed t-test ($p<0.001$). However, the affected hand still had functional contribution since the MAL How Well score was greater than non-use (score of 0) in a one-sided, two-tailed t-test ($p<0.001$). Both tests met criteria for multiple comparisons.

WMFT: WMFT data (WMFT time, WMFT FAS, WMFT arm strength, WMFT grip strength) were excluded from two patients upon video inspection. For the remaining subjects (n=16), mean performance time, grip strength and arm strength was reduced in the affected arm compared to the non-affected arm in paired, two-tailed t-tests (time: $p=0.002$, arm: $p=0.028$, grip: $p=0.003$). The FAS was also abnormal as indicated by an

FAS below normal (less than 0) in a one-sided, two-tailed t-test ($p<0.001$). All WMFT tests, expect arm strength, met criteria for multiple comparisons.

Simple linear regression analyses were conducted to determine the association between hand and wrist motor function as well as their respective association with use of the hand during ADLs. There was a correlation between hand and wrist function as indicated by a significant association between peak wrist acceleration and the JTT ($p=0.023$, Fig. 2.2C). This finding suggests that function of the wrist is associated with hand motor function. The JTT was also associated with self-reported hand function during ADL as the JTT was correlated with the How Well subtest of the MAL ($p<0.001$).

2.3.2 Measures of M1 and CST function

To determine the impact of stroke on M1 function, the measures of $M1_{IL}$ function (rMT, SRC (AUC, MEPmax, M-parameter, S50), SICI (% MEP)) were compared to measures collected from the dominant hemisphere of 12 healthy age-matched subjects (Table 2.3). Because we expected the rMT, S50 and % MEP (SICI) to be greater and the AUC, MEPmax and M-parameter to be smaller for the stroke than healthy subjects, unpaired one-tailed t-tests were used. The rMT was higher ($p=0.013$) and the AUC was lower ($p=0.021$) for $M1_{IL}$ when compared to healthy M1 indicating a reduction in corticospinal excitability after stroke. Analysis of the curve parameters demonstrated differences in the input-output properties of CST between $M1_{IL}$ and healthy M1. Specifically, the calculated MEPmax was smaller for $M1_{IL}$ than healthy M1 ($p=0.018$). SICI was weaker in $M1_{IL}$ than healthy M1 ($p=0.051$).

2.3.3 Measures of M1 and CST structure

The measures of $M1_{IL}$ and CST_{IL} structure are summarized in Table 2.3. As expected, the lesion volume was smaller in patients with subcortical stroke than cortical stroke (one-tailed t-test, $p<0.01$). For the CST-FA, we found a effect of hemisphere ($p=0.01$, $F(1,13)=8.79$) but not stroke location ($p=0.34$, $F(1,13)=0.98$) or an interaction between hemisphere and stroke location ($p=0.72$, $F(1,13)=0.14$) in a two-way ANOVA. Post-hoc testing with a paired, two-tailed t-test revealed that CST_{IL}-FA was lower than CST_{CL}-FA ($p=0.008$) indicating the structural integrity of CST_{IL} was weaker than of the CST_{CL}. In two-way ANOVA, there was an effect of hemisphere ($p<0.01$, $F(1,15)=10.31$) but not stroke location ($p=0.13$, $F(1,15)=2.53$) on M1 thickness. There was not an interaction between hemisphere and stroke location ($p=0.06$, $F(1,15)=4.23$). In post-hoc testing with a paired, two-tailed t-test, we found that $M1_{IL}$ was thinner than $M1_{CL}$ ($p<0.018$).

2.3.4 Association between M1 and CST function and structure

Linear regression analysis determined that the measures of $M1_{IL}$ function (rMT, AUC, MEPmax, SICI) were not associated with $M1_{IL}$ thickness or CST_{IL}-FA.

2.3.5 Association between M1 and CST function and function of the distal UE

Depending upon the availability of the TMS data, results from 12-18 patients were available to test the relationship between function of the distal UE with function of $M1_{IL}$ and CST_{IL}. Table 2.4 contains the specific number of subjects used in each test and a summary of the results. Neither hand motor function (JTT) nor wrist motor function (peak acceleration) was linearly correlated with any of the measures of $M1_{IL}$ function.

In the exploratory analyses where age, post-stroke duration and lesion volume were held as covariates, the JTT was linearly correlated with greater MEPmax when lesion volume was controlled for alone ($p=0.02$, $R^2=0.41$) or in combination with age and post-stroke duration ($p=0.04$, $R^2=0.42$). There were no other significant associations (Table 2.4).

2.3.6 Association between M1 and CST structure and function of the distal UE

Depending upon the availability of the MRI data, results from 15-17 patients were available to test the relationship between function of the distal UE with structure of $M1_{IL}$ and CST_{IL}. We found $M1_{IL}$ thickness and CST_{IL}-FA were not correlated with either hand motor function (JTT) nor wrist motor function (peak wrist acceleration) (Table 2.4). Exploratory multiple linear regression analysis also failed to demonstrate a correlation between these measures even when age, post-stroke duration and lesion volume were controlled (Table 2.4).

2.4 Discussion

In this study we tested the hypothesis that chronic stroke related injury to $M1_{IL}$ or its CST_{IL} projections is related to function of the distal UE, specifically of the hand and wrist. We found that despite the evidence for the importance of M1 and its corticospinal projection for hand function (Lemon, 2008), the currently employed functional and structural measures were not associated with the variability of impairment in hand or wrist in the primary analysis of the present study. The lack of correlation contradicts our stated hypothesis and the evidence from non-human primate experiments (Nudo et al., 1996b). This discrepancy could be explained by the fact that our patients had measurable MEP in response to TMS of $M1_{IL}$. Considering that the presence of an MEP in response

to TMS of $M1_{IL}$ is dependent on the temporal and spatial summation of descending volleys at the level of the alpha motoneuron pool (Di Lazzaro et al., 1998a), the studied population was biased towards more favorable $M1_{IL}$ function. In our exploratory analysis, we found that MEPmax was associated with affected hand function when lesion volume was controlled. We therefore argue that in chronic stroke patients with a measurable evoked MEP response to TMS, $M1_{IL}$ output, as measured with TMS derived parameter MEPmax, may only be associated with affected hand function depending upon lesion size. However, we did not find any evidence that the current $M1_{IL}$ and CST_{IL} structural measures add in explanation of variability of affected hand or wrist function in the exploratory analysis. While direct comparison to whole arm function is limited, a lack of relationship between $M1_{IL}$ thickness and overall UE function was also reported for patients suffering from chronic stroke (Jones et al., 2016). In longitudinal studies CST_{IL}-FA also lacked additional predictive value in the presence of an $M1_{IL}$ TMS-evoked MEP response (Stinear et al., 2006).

2.4.1 Measures of motor function

In the present study, motor function of hand and the wrist was impaired. These results are consistent with evidence derived from non-human primate stroke models where lesions to the M1 hand area result in abnormal movement kinematics and a loss of function in the hand contralateral to the infarct (Nudo and Milliken, 1996; Dancause et al., 2006). It is also in line with the notion that the integrity of M1 and CST is important for normal hand function in humans (Lang and Schieber, 2003; Schieber et al., 2009).

2.4.2 Measures of M1 and CST function

We found that rMT was higher in $M1_{IL}$ than in healthy M1 which is consistent with other reports comparing rMT between chronic stroke and healthy populations (Buetefisch et al., 2008) and compatible with the findings of rMT being lower in $M1_{IL}$ than $M1_{CL}$ in the chronic phase of stroke (Liepert et al., 2000a; Borich et al., 2015). The hemispheric difference in rMT found in previous studies could be attributed to an abnormal decrease in $M1_{IL}$ excitability or an abnormal increase in $M1_{CL}$ excitability because unilateral stroke causes bi-hemispheric neural reorganization in rodents (Allred and Jones, 2008), non- human primates (Dancause et al., 2005), and humans (Schaechter et al., 2009). Our observation that ipsilesional rMT was abnormally high as compared to healthy M1 rMT suggests that the hemispheric difference in rMT found in previous studies is at least partially the result of an abnormal decrease in $M1_{IL}$ excitability. The mechanistic explanation for this finding is unclear. There is no evidence in the present study to support loss of pyramidal tract neurons and their corticospinal projections as a cause because we found no relationship between $M1_{IL}$ thickness or CST_{IL}-FA and rMT. We also cannot conclude that a higher rMT in $M1_{IL}$ reflects decreased membrane excitability of the targeted pyramidal tract neurons. Although rMT is used as a non-invasive measure of pyramidal tract neuron membrane excitability in healthy humans, the effect of neuronal loss from stroke on the relationship between rMT and membrane excitability is not known (Ziemann, 2004).

When measured at a constant level of motor activity (here, at rest), the three SRC parameters (S50, M-parameter, and MEPmax) completely characterize the input-output relationship of the CST. Therefore, a change in one or more parameters indicates a

change in the input-output relationship in $M1_{IL}$. The abnormally low MEPmax found here suggests that CST output from $M1_{IL}$ was reduced after stroke. The smaller AUC in $M1_{IL}$ when compared to healthy M1 also supports this notion. Reduced CST output with older age has been reported, but cannot explain our findings as the reduction of CST output observed in our sample was statistically different from the CST output in our age-matched control population (Talelli et al., 2008). As the balance between excitatory and inhibitory activity evoked by high TMS intensities determines maximum CST output, a lower MEPmax in $M1_{IL}$ could be associated with either the loss of excitatory neurons or an increase of inhibitory transmission after stroke (Devanne et al., 1997). However, inhibitory transmission was reduced as evidenced by the lower SICI in $M1_{IL}$ than in healthy M1. Other studies also reported SICI to be abnormally low in chronic stroke in patients with motor impairment (Manganotti et al., 2002). Therefore, the loss of excitatory neurons/activity is more likely to be at least one of the factors contributing toward depressed CST output. The similarity between $M1_{IL}$ and healthy M1 in the remaining parameters (M-parameter and S50) indicates that the recruitment characteristics of pyramidal tract neurons were normal (Devanne et al., 1997).

2.4.3 Association between M1 and CST function and function of the distal UE

In the exploratory analysis, we found that a greater MEPmax, reflecting more CST output from $M1_{IL}$, was positively correlated with better hand motor function (JTT) when lesion volume was controlled. As MEPmax is lower in stroke patients than in healthy controls, our results indicate that the normalization of CST output from $M1_{IL}$ could be associated with better hand motor function depending on lesion volume. The other TMS measures (rMT, S50, M-parameter, SICI) were not associated with hand

function. Comparison to prior TMS studies is limited by the fact that MEPmax was not measured in prior studies (Thickbroom et al., 2002; Borich et al., 2015). While not directly comparable, the results of fMRI studies demonstrated more normal task-related BOLD response in M1 in chronic patients with better whole arm function (Ward et al., 2003b). However, this relationship was not observed consistently as other studies on whole arm function did not report the same relationship (Schaechter et al., 2006).

2.4.4 Measures of M1 and CST structure

FA is a well-established measure of CST integrity that quantifies white matter microstructure (Basser, 1995) and is commonly used to detect damage to the CST in chronic stroke (Lindberg et al., 2007; Schaechter et al., 2008). In the current study, we used FA to assess the integrity of the CST in the studied population and derived two major results.

First, we found that CST integrity was lower in the ipsilesional than contralesional hemisphere as indicated by a lower FA value for CST_{IL} than CST_{CL}. Even though many of the patients in the studied population suffered from co-morbid diseases (hypertension, diabetes) that could affect white matter integrity, these diseases would have had a similar probability to affect the CST of each hemisphere (Stenset et al., 2006; Kodl et al., 2008). Therefore, these diseases cannot explain the difference in FA between CST_{IL} and CST_{CL}. It is also unlikely that dominance of the affected hemisphere accounted for the difference in FA values since dominance-related asymmetries in CST-FA have not been detected in healthy adults (Westerhausen et al., 2007). Consequently, it is most probable that the hemispheric difference in FA reflects stroke-related changes to the CST. As stroke-related changes to the brain are the result of both degeneration and

regeneration processes, the mechanisms underlying differences in FA values are likely mixed but cannot be further delineated in the current study (Jones and Jefferson, 2011).

An alternative measure of CST integrity is CST lesion load. Greater CST_{IL} lesion load, calculated from the overlap between the infarct and the CST_{IL}, is associated with poorer UE function after stroke (Zhu et al., 2010; Feng et al., 2015) and has been proposed as a biomarker for UE recovery (Feng et al., 2015). However, given that CST_{IL} lesion load primarily measures direct CST damage (Zhu et al., 2010; Feng et al., 2015) and CST_{IL}-FA captures both direct and indirect CST damage (Lindberg et al., 2007), CST_{IL}-FA was a more appropriate measure of CST_{IL} integrity for the current study.

Finally, we used cortical thickness to assess M1 integrity because M1 thickness is a reliable and replicable measure of M1 structure (Han et al., 2006). Although M1 integrity was worse in the ipsilesional than contralesional hemisphere, we cannot conclude that the $M1_{CL}$ was spared from damage. Because atrophy can occur in functionally connected but distant, brain regions from the ischemic core, the structural integrity of $M1_{CL}$ was likely also impacted after stroke, albeit less severely than $M1_{IL}$, given the dense interhemispheric projections between the motor cortices of opposing hemispheres (Bidmon et al., 1997; Li et al., 1998).

2.4.5 Association between M1 and CST structure and function of the distal UE

We did not find evidence to support our hypothesis that $M1_{IL}$ structure was associated with the function of the affected hand or wrist. To our knowledge this is the first study to test the association of M1 and CST structure with distal UE function of the hand and wrist. While a positive correlation between bilateral precentral gyrus thickness and hand dexterity was reported for patients in the chronic phase of stroke, the thickness

of $M1_{IL}$ and $M1_{CL}$ was not tested separately (Borich et al., 2015). Our results suggest that the correlation reported by Borich et al. was not driven by variation in $M1_{IL}$ thickness, as $M1_{IL}$ thickness was not correlated with hand function in the present study. Our conclusion is similar to reports on whole arm function where $M1_{IL}$ thickness is also not associated with function of the whole arm (Schaechter et al., 2006; Jones et al., 2016).

CST_{IL} structure was also not associated with the function of the affected hand or affected wrist. The lack of an association may be related to the presence of a TMS-evoked MEP in all of the studied patients. Because a TMS-evoked MEP can only occur when the CST has sufficient integrity, we speculate that increases in CST_{IL} integrity are not proportionally related to increases in function of the affected hand and wrist once CST_{IL} is sufficiently intact. This notion is consistent with the finding that the presence of an MEP is predictive of motor recovery in acute stroke patients and that including FA measures in these patients does not add predictive value (Stinear et al., 2006). Further, although we expected integrity of CST_{IL} fibers originating from the $M1_{IL}$ hand area to be associated with function of the hand and fibers originating from the $M1_{IL}$ wrist area to be associated with function of the wrist, the association may have been lost as a result of the non-specificity of the FA measure. The CST is composed of topographically organized groups of fibers descending from multiple cortical motor structures and projecting to the spinal cord (Dum and Strick, 1991). The current FA measure evaluates the integrity of all CST fibers without isolating those that innervate alpha motor neurons of the hand or wrist (Yendiki et al., 2011). Studies on whole arm function have demonstrated a relationship between whole arm function and CST_{IL} structure but the presence of an MEP was not tested (Park et al., 2016).

2.4.6 Limitations

There are few limitations to the current study. First, we were unable to distinguish between dominant and non-dominant hemispheres due to the sample size. Despite this limitation, we identified abnormality in $M1_{IL}$ and CST_{IL} function and structure. Second, a cross-sectional study design did not allow us to distinguish between the regenerative and degenerative processes supporting the manifestation of observed CST_{IL} and $M1_{IL}$ abnormalities (Jones and Jefferson, 2011).

2.5 Conclusions

In our exploratory analysis, we demonstrate that variability in the impaired function of the hand, but not wrist, may be associated with the abnormally low output from $M1_{IL}$ in chronic stroke affecting M1 or the CST but the relationship appears to be dependent upon lesion volume. Although $M1_{IL}$ and CST_{IL} structure was impaired, there was no correlation with the variability of impairment in the hand or wrist. As all study patients had a measurable muscle response to TMS applied to $M1_{IL}$, we argue that structural measures are not associated with the variability of impaired function once M1 and the pyramidal tract function is sufficient for TMS to evoke a MEP. The generation of a measurable MEP requires temporal and spatial summation of TMS-evoked descending volleys that seem to depend on neuronal circuitries that are also crucial for normal hand function. The variability in impaired hand and wrist function may be more strongly related to $M1_{IL}$ and CST_{IL} structure in individuals without measurable MEP responses to TMS but remains to be determined.

Subject	Age	Sex	PSD (months)	Stroke	Edinburgh (LQ)	Dominant Hand	Affected Hand	RBANS (total scale)	MRC (affected UE)	Ashworth	PMH
1	60	M	133	s	-40	L	L	78	4+	3	HTN
2	76	M	18	c	100	R	R	54	4+	0	HLD
3	63	F	18	c	100	R	R	89	4+	0	HTN
4	51	F	9	s	100	R	R	89	4+	1	-
5	61	M	13	c	25	R	R	96	4+	0	-
6	67	F	7	s	100	R	R	118	4+	0	HTN
7	63	M	14	s	100	R	R	89	4+	1	-
8	62	F	10	s	-100	L	L	83	4	0	HTN, DM
9	76	F	17	c	78	R	R	95	4+	0	HTN, HLD, DM
10	78	M	17	c	100	R	L*	108	4	1	-
11	72	F	66	s	100	R	L*	105	3	2	-
12	55	F	10	s	100	R	L*	86	3	2	HTN
13	44	M	18	s	100	R	R	104	4+	0	-
14	66	M	8	c	80	R	L*	94	4+	0	-
15	68	M	53	s	-100	L	L	100	4+	0	-
16	68	M	65	s	80	R	R	72	4+	1	-
17	32	M	84	c	71	R	L*	104	4+	0	-
18	50	F	16	c	100	R	R	85	4+	0	HTN, HLD, pre-DM

Table 2.1. Characteristics of stroke patients

*F = female; M = male; PSD = post-stroke duration; R = right; L = left; * = non-dominant hand was affected; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; UE= upper extremity; c = stroke involved M1 and/or CST (cortical); s = stroke involved CST but not M1 (subcortical); PMH = past medical history. HTN = hypertension, HLD = hyperlipidemia, DM = diabetes mellitus.*

Measures of motor function	**Stroke** aff hand	**Stroke** non-aff hand	**Healthy** dominant hand	**Standard Score**	**Student's t-test** aff/non-aff	**Student's t-test** aff/standard score
JTT, time (contrast ratio)	0.49 ± 0.24 (18)	0.16 ± 0.11 (18)	-	0 = normal function	**p<0.001 t(17)=5.842**	**p<0.001 t(17)=8.656**
WEM, peak acceleration (g)	0.66 ± 0.40 (18)	-	1.38 ± 0.30 (18)	N/A	**p<0.001 t(34)=6.017**	N/A
WEM, reaction time (ms)	221.34 ± 70.41 (18)	-	184.60 ± 34.98 (18)	N/A	**p=0.055 t(34)=1.984**	N/A
MAL, How Well	2.65 ± 1.38 (18)	-	-	5 = normal function	N/A	**p<0.001 t(17)=7.58**
				0 = no contribution	N/A	**p<0.001 t(17)=10.05**
WMFT, Time (log transformed)	1.02 ± 0.73 (16)	0.36 ± 0.17 (16)	-	N/A	**p=0.002 t(15)=3.657**	N/A
WMFT, FAS	3.95 ± 0.70 (16)	N/A	-	5 = normal function	N/A	**p<0.001 t(15)=6.042**
WMFT, Arm Strength (kgs)	13.38 ± 6.76 (16)	16.31 ± 4.05 (16)	-	N/A	**p=0.028 t(15)=2.429**	N/A
WMFT, Grip Strength (kgs)	22.13 ± 11.76 (16)	33.75 ± 12.58 (16)	-	N/A	**p=0.003 t(15)=3.627**	N/A

Table 2.2 Summary of measures describing motor function.

Measures reported as mean ± SD (n), t-tests are reported as t(df)=t-value and F-tests are reported as F(Dfn, DFd)=F-value. *WMFT= Wolf Motor Function Test, MAL= Motor Activity Log, Dfn= degree of freedom for stroke patients, DFd= degree of freedom for healthy subjects.*

M1 function	**Stroke** (ipsi-)	**Stroke** (contra-)	**Healthy**	**T-test** ispi- /contra-	**T-test** ipsi-/healthy
rMT (% MSO)	67.44 ± 19.88 (18)	-	53.17 ± 8.31 (12)	-	**p=0.013 t(28)=2.344**
AUC (mV)	1.63 ± 2.075 (18)	-	3.65 ± 3.11 (12)	-	**p=0.021 t(28)=2.143**
MEPmax (mV)	0.40 ± 0.399 (15)	-	0.87 ± 0.70 (11)	-	**p=0.018 t(24)=2.215**
M-parameter	0.26 ± 0.13 (13)	-	0.28 ± 0.13 (10)	-	p=0.362 t(21)=0.357
S50 (% MSO)	65.07 ± 12.29 (15)	-	58.66 ± 9.62 (11)	-	p=0.073 t(24)=1.503
SICI (% MEP)	78.51 ± 22.30 (10)	-	59.73 ± 27.17 (12)	-	**p=0.051 t(20)=1.710**

M1 structure	**Stroke, All** (ipsi-)	**Stroke, All** (contra-)	**Healthy**	**T-test** ispi- /contra-	**T-test** ipsi-/healthy
Lesion volume (%)	1.11 ± 1.45 (17)	N/A	N/A	-	-
M1 thickness (mm)	2.27 ± 0.27 (17)	2.44 ± 0.20 (17)	-	**p<0.018 t(16)=2.639**	-
FA of CST	0.46 ± 0.06 (15)	0.48 ± 0.04 (15)	-	**p<0.008 t(14)=3.094**	-

Table 2.3 Summary of measures describing M1 and CST function and structure. Measures are reported as mean ± SD (n). *Ipsi= ipsilesional; Contra= contralesional; rMT=resting motor threshold; AUC= area under the stimulus response curve (SRC); SRC parameters: MEPmax (plateau), M- parameter (proportional for the maximum slope), S50 (TMS intensity that evokes an MEP amplitude of half MEPmax); SICI= short-interval intracortical inhibition; M1= primary motor cortex; FA= fractional anisotropy, CST= corticospinal tract; ipsi= ipsilesional; contra=contralesional.*

Dependent Variable	Independent Variable	n	Covariate				
			None	Age	PSD	Lesion Volume	All covariates
Peak wrist Acceleration	rMT	18	p=0.73	-	-	-	-
	AUC	18	p=0.85	p=0.60	p=0.62	p=0.61	p=0.64
	MEPmax	15	p=0.86	p=0.88	p=0.91	p=0.87	p=0.76
	M-parameter	13	p=0.51	p=0.41	p=0.72	p=0.53	p=0.59
	S50	15	p=0.20	p=0.22	p=0.23	p=0.69	p=0.73
	SICI	12	p=0.13	-	-	-	-
	M1 thickness	17	p=0.72	p=0.39	p=0.33	p=0.28	p=0.27
	FA of CST	15	p=0.48	p=0.45	p=0.14	p=0.75	p=0.29
	Lesion volume	17	p=0.58	-	-	-	-
Jebsen Score	rMT	18	p=0.65	-	-	-	-
	AUC	18	p=0.58	p=0.49	p=0.62	p=0.06	p=0.07
	MEPmax	15	p=0.21	p=0.17	p=0.23	**p=0.02**	**p=0.04**
	M-parameter	13	p=0.52	p=0.88	p=0.29	p=0.84	p=0.61
	S50	15	p=0.59	p=0.51	p=0.62	p=0.53	p=0.56
	SICI	12	p=0.12	-	-	-	-
	M1 thickness	17	p=0.81	p=0.76	p=0.85	p=0.71	p=0.73
	FA of CST	15	p=0.83	p=0.87	p=0.30	p=0.72	p=0.17
	Lesion volume	17	p=0.49	-	-	-	-

Table 2.4 Multiple linear regression analysis testing the association between the measures of M1 and CST structure and function with the measures of affected motor function. Each p-value corresponds to the significance of a simple linear regression analysis (no covariate) or multiple linear regression analysis (single covariate) testing the association between the dependent and independent variable. The sample size (n) used for the linear regression analyses of each dependent and independent variable pair is reported. Significant results are bolded ($p<0.05$). *rMT=resting motor threshold; AUC= area under the SRC; SRC parameters= MEPmax, M-parameter, S50; SICI=short-interval intracortical inhibition; M1= primary motor cortex*; *FA= fractional anisotropy; CST= corticospinal tract; PSD= post-stroke duration.*

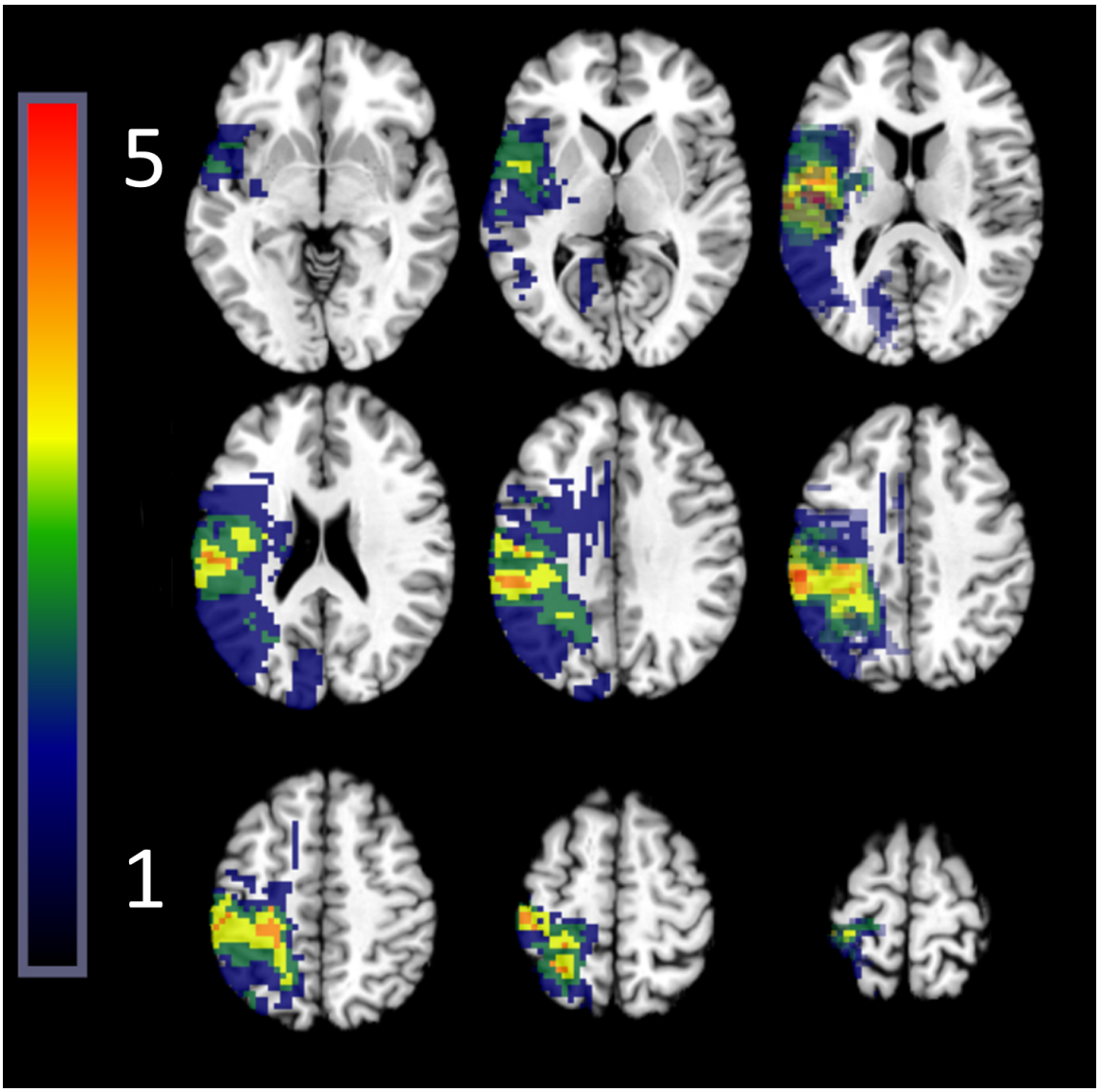

Fig. 2.1 Lesion overlap

Participants' lesions were normalized to standard space and flipped to the left hemisphere for display purposes, shown overlaid on the ch2.better.nii brain distributed with MRIcron. Color indicates the number of participants with lesions in each voxel.

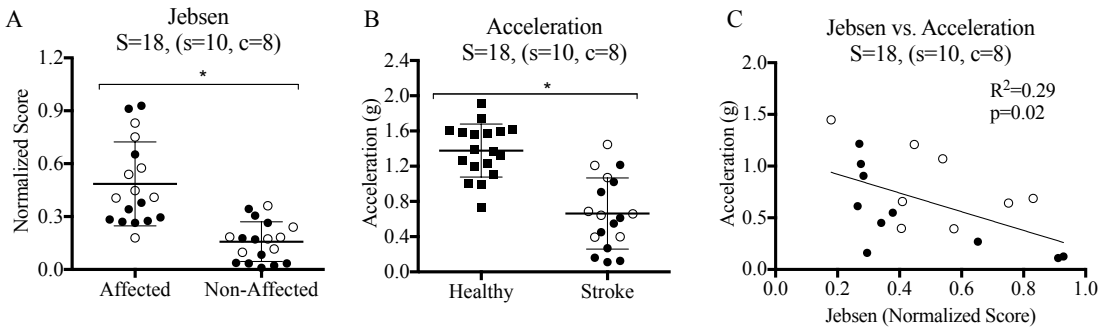

Fig. 2.2 Measures of motor function.

(A, B) Column scatter plots with the mean and SD of peak wrist acceleration and the Jebsen Taylor Test. Data from healthy and stroke subjects were compared using unpaired two-tailed t-tests. Significant results were indicated as * ($p<0.05$). (C) A scatterplot demonstrates the association between peak wrist acceleration and the normalized Jebsen Test score. For the correlation analysis, the R^2 and p-value are given. *Filled circles=stroke involved cortex (c); Open circles=stroke spared the cortex (s).*

CHAPTER 3:
Hebbian-type repetitive transcranial magnetic stimulation may prolong retention of training-related hand motor improvement in chronic stroke

3.1 Introduction

Stroke is the leading cause of long-term disability with over six million adults over the age of 20 years old having suffered a stroke in the United States (Mozaffarian et al., 2015). Because damage to cortical and/or subcortical components of the motor system is common after stroke of the middle cerebral artery, most stroke survivors experience long-term motor impairment, including the upper extremity (UE) (Kandel et al., 2013b; Corbetta et al., 2015). Despite recent advances in stroke motor rehabilitation, UE motor recovery is often incomplete (Nichols-Larsen et al., 2005b; Corbetta et al., 2015). There is interest in using repetitive transcranial magnetic stimulation (rTMS) as a neuromodulatory tool to improve functional outcomes associated with UE motor recovery. Neuromodulation research is based on evidence that UE recovery after stroke is at least partially accomplished through learning-dependent plasticity in the lesioned (ipsilesional) primary motor cortex (M1) in the rodent (Jones et al., 2009), monkey (Nudo and Milliken, 1996; Nudo et al., 1996b) and human (Ward et al., 2003a; 2003b).

One neural mechanism thought to support M1 plasticity is through the induction of associative, or Hebbian-type, long-term potentiation (LTP). Hebbian-type LTP is based on the principles of Hebb's postulate where the simultaneous activation of two systems of cells can cause long-lasting cellular changes such that the two systems of cells become associated and that activation of one cell system will facilitate the other (Hebb, 1949). One of the first descriptions of Hebbian-type LTP was in the hippocampus of the healthy rodent (Keslo et al., 1986; Sastry et al., 1986). Excitatory postsynaptic potentials (EPSPs) of greater amplitude were generated in CA1 of the hippocampus after combining pre- and post-synaptic activity, specifically by pairing the stimulation of CA3

hippocampal axonal output with the depolarization of CA1 hippocampal neurons (Keslo et al., 1986; Sastry et al., 1986). However, Hebbian-type LTP of M1 has also been observed in vivo and in vitro (Baranyi and Feher, 1981; Iriki et al., 1989; Baranyi et al., 1991; Iriki et al., 1991; Hess and Donoghue, 1994; 1995; Hess et al., 1996). For instance, paring pre-synaptic stimulation of cortical afferents (thalamus, callosal, pyramidal tract or somatosensory cortex) with the post-synaptic depolarization of M1 pyramidal neuron facilitated the amplitude of EPSPs evoked in M1of the cat (Baranyi and Feher, 1981; Baranyi et al., 1991). Further, study of the normal rodent found that LTP-like mechanisms occurring along the horizontal projections of pyramidal neurons within layer II/III of M1 support learning-related M1 plasticity after improvement on a paw reach task (Rioult-Pedotti et al., 1998; 2000).

Even though the pairing of pre- and post-synaptic activity in M1 cannot be feasibly tested at the cellular level, there is evidence that Hebbian-type LTP can be generated in the human M1 as well. In the human, TMS applied at sufficiently high intensities can activate pyramidal neurons of M1 as indicated by a measurable corticospinal discharge at the level of the muscle (Rothwell, 1997). By timing the application of TMS and peripheral nerve stimulation to arrive at M1 pyramidal neurons at approximately the same time in a paradigm known as paired associative stimulation (PAS), M1 excitability transiently increases (Stefan et al., 2000). Further, when M1 pyramidal cell input is repetitively stimulated with rTMS in a strict temporal relationship to when M1 pyramidal neurons are most likely to be active (during imagined movement or motor execution), M1 plasticity can also be induced (Buetefisch et al., 2004b; Thabit et al., 2010; Buetefisch et al., 2011; Mrachacz-Kersting et al., 2012; Buetefisch et al.,

2015). Because the PAS-associated increase in M1 excitability is smaller after UE motor training in healthy adults, the change in M1 excitability associated with paired stimulation may share similar LTP-like mechanisms to learning-induced M1 plasticity (Stefan et al., 2006). These studies raise the possibility that Hebbian-type stimulation would be of great benefit to stroke motor recovery by facilitating LTP-like mechanisms associated with M1 plasticity. In fact, Hebbian-type stimulation as delivered by pairing rTMS with training-related activity of M1 ($rTMS_{Heb}$) enhanced motor skill acquisition in healthy adults and induced M1 reorganization after stroke (Buetefisch et al., 2004b; 2011; 2015). The effect of $rTMS_{Heb}$ on motor skill improvement, however, has not yet been tested in a clinical stroke population.

Here in a double-blinded, placebo controlled study we test the hypothesis that the application of $rTMS_{Heb}$ during a wrist-training paradigm known to improve distal UE function will enhance training-related motor improvement of patients suffering from chronic stroke. 20 patients suffering from chronic stroke at least partially involving M1 and/or its associated corticospinal tract (CST) completed 30 minutes of motor training with the affected wrist for five consecutive days. Patients were blinded and randomized to receive either $rTMS_{Heb}$ (n=10) or sham rTMS ($rTMS_{sham}$, n=10) during wrist motor training. To apply rTMS when ipsilesional M1 was most likely engaged in movement-related activity of the wrist, an increase in movement-related EMG recorded from a muscle supporting the training movement triggered rTMS application. To determine the effect of $rTMS_{Heb}$ on training-related improvement of distal UE function, hand and wrist motor function was tested before (pre), after the five days of training (p0wks) and 4 weeks after training (p4wks). The Jebsen-Taylor Test (JTT) and the How Well subtest of

the Motor Activity Log (MAL How Well) were used to measure hand motor function and the peak acceleration of ballistic wrist extension movements was used to measure wrist motor function. The primary outcome measure was the JTT. To determine if $rTMS_{Heb}$ enhanced training-related improvement of other aspects of UE function, whole arm motor function was tested before (pre) to after (p0wks) and 4 weeks (p4wks) after motor training using the Wolf Motor Function Test (WMFT).

3.2 Materials and methods

3.2.1 Subjects

20 patients (10M, 28.55±32.49 years old) participated in the study and met the following criteria: (1) suffered a single ischemic infarction affecting the primary motor output system more than 6 months prior, (2) a motor deficit in the hand contralateral to the infarct, (3) no neurological disorder other than stroke, (4) no contradiction to TMS or MRI, (5) no intake of medication that interfered with TMS measures or motor learning, (6) the ability of TMS to elicit a measurable motor evoked potential and (7) the ability to give informed consent. Co-morbidity was determined from medical records and interview by a board certified neurologist. Upper extremity muscle strength and tone was determined using the Medical Research Council Scale (MRC) and the modified Ashworth Scale (Medical Research Council of the United Kingdom, 1976; Bohannon and Smith, 1987). Cognition was assessed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al., 1998). The Edinburgh Handedness Inventory was used to determine handedness (Oldfield, 1971). Analysis of the Edinburgh Handedness Inventory has been described elsewhere but briefly, a laterality quotient (LQ) was calculated where an LQ greater than 0 indicated that the

patient was right-handed and an LQ less then 0 indicated that the patient was left-handed (Oldfield, 1971). Patients completed written informed consent prior to entering the study. The Institutional Review Board of Emory University approved the study. A schematic of patient inclusion is provided in Fig. 3.1 and a summary of the patient characteristics is presented in Table 3.1.

3.2.2 Experimental design

A schematic of the experimental design is presented in Fig. 3.2A. All patients completed five consecutive days of motor training in a double-blinded, placebo-controlled study. On each day of training, patients completed thirty minutes of motor training (360 wrist extension movements) while receiving $rTMS_{Heb}$ (n=10, 6M, aged 62.6 ± 12.0 years) or $rTMS_{sham}$ (n=10, 4M, aged 59.7 ± 10.9 years). Patients were blinded to the type of rTMS they received and received the same type of rTMS across all five days of training. The effect of rTMS on training-related motor improvement was evaluated by measuring motor function before (pre), after (p0wks), four weeks after (p4wks) motor training.

3.2.3 Motor training

Patients were seated in a dental chair that supported the arm, wrist and hand. On each day of motor training, patients completed 360 auditory-cued ballistic wrist extension movements with their affected wrist (0.2 Hz, 1000 ms jitter). The affected wrist was determined as the wrist contralateral to the lesioned brain hemisphere. During each wrist extension movement, patients attempted to move a cursor from a home position into a target box on a computer screen (Fig. 3.2B). Because movement of the cursor was driven by data collected from a 2-dimensional accelerometer mounted on the dorsum of the

affected hand, the patients had to extend their wrist in the correct direction with sufficient acceleration in order for the cursor to land in the target box. The faster that the patients accelerated the speed of their wrist in extension/flexion direction, the further the cursor moved up/down along the vertical axis of the computer screen, respectively. The faster that the patients accelerated the speed of their wrist in abduction/adduction direction, the further the cursor moved to the right/left along the horizontal axis of the computer screen, respectively. The landing position of the cursor was determined by the maximum (peak) acceleration along the extension/flexion and abduction/adduction axes of the initial wrist movement. If the cursor landed in the target box, an auditory tone was emitted. The goal of the training was to land the cursor in the target box as many times as possible. The location of the target box was customized for each patient based on the peak wrist acceleration observed the beginning of each training session; therefore, the location of the target box could differ between training sessions if peak wrist acceleration improved with training.

This type of training was selected based on evidence that the repetitive execution of ballistic movements improved training-related kinematics and TMS-evoked M1 excitability (Buetefisch et al., 2004b; 2015). To reduce fatigue, the training was broken into 3 blocks of 120 movements with 1-3 minutes of rest between each block. To ensure that the cursor was only driven by movement of the hand and wrist, Velcro straps were placed across the forearm and the training was carefully monitored by research staff. Patients returned their wrist back to a neutral position in the armrest between extension movements.

EMG activity was recorded from the ECU muscle using surface electrodes taped in a belly-tendon montage (9mm diameter, bandpass 3Hz – 1kHz). Raw EMG and accelerometer data was sampled and digitized in LabView (National Instruments) at a frequency of 1 kHz and stored for offline analysis.

3.2.4 Repetitive transcranial magnetic stimulation

Patients were randomized to receive either $rTMS_{Heb}$ (n=10, 6M, aged 62.6 ± 12.0 years) or $rTMS_{sham}$ (n=10, 4M, aged 59.7 ± 10.9 years) during motor training (Table 3.1).

$RTMS_{Heb}$ and $rTMS_{sham}$ were applied over the extensor carpi ulnaris (ECU) hotspot of M1 in the lesioned (ipsilesional) hemisphere ($M1_{IL}$) where a muscle hotspot is defined as the location of M1 that evokes the largest peak-to-peak MEP amplitude in the targeted muscle at the lowest TMS intensity. The rationale for targeting the ECU hotspot over hotspots for other muscles that control extension of the wrist (extensor carpi radialis brevis muscle, extensor carpi radialis longus muscle) was motivated by data that identified changes in the organization of ECU muscle M1 representation after wrist extension training in the healthy adult (Buetefisch et al., 2015). To apply rTMS when M1 pyramidal neurons were most likely to be engaged in movement-related activity, rTMS was triggered by an increase in movement-related EMG activity of the ECU muscle during every second extension movement (Fig. 3.2B). By triggering rTMS at the onset of every second movement, rTMS was applied at a rate of ~ 0.1 Hz (180 stimuli per training session). A rate of 0.1 Hz was selected based on evidence that 0.1 Hz is the most effective rTMS rate to enhance training-related motor improvement in healthy subjects (Buetefisch et al., 2015).

$RTMS_{Heb}$ was applied through a figure-of-eight air-cooled coil (7 cm wing diameter, Magstim Company, UK) at 80% resting motor threshold (rMT). The rMT was defined as the TMS intensity that elicits an MEP with a peak-to-peak amplitude of at least 50 μV in the ECU muscle in five of 10 consecutive trials and was determined prior to hand motor training at the beginning of each training session (Rossini et al., 1994). The intensity of rTMS was selected to be subthreshold (below rMT) to minimize disruption to the training movements. If rTMS evoked an MEP during motor training, the intensity of rTMS was reduced by 1% maximum stimulator output (MSO) until rTMS no longer evoked an MEP. While the $rTMS_{sham}$ coil looked and sounded the same as the $rTMS_{Heb}$ coil, the $rTMS_{sham}$ coil did not produce a large enough magnetic field to activate cortical neurons. To ensure accurate positioning, the rTMS coils were registered to an MRI image of the participant's brain using a frameless neuronavigation system (BrainSight software, Rogue Research, Montreal, Canada). The rTMS coils were held with the handle toward the back at the head and at a 45-degree angle to the midline to produce an anterior-posterior current orthogonal to the central sulcus. This coil position has been identified as most effective to transynaptically stimulate pyramidal neurons of M1 (Mills et al., 1992).

3.2.5 Magnetic resonance imaging

Infarct location was determined by inspection of a structural MPRAGE magnetic resonance image (MRI) obtained on a Siemens 3T Trio scanner using a 12-channel head coil. For MPRAGE imaging the following parameters were used: TR=2250ms, TE=4.18ms, TI=900ms, flip angle=9°, 256x256 matrix, FOV=256mm, 176 sagittal slices, resulting in $1mm^3$ isotropic voxels. Patients were classified as having either a cortical (infarct involved M1 with or without CST involvement) or subcortical (infarct only

involved the CST). Due to claustrophobia, MR images were not collected from one patient and instead lesion location was determined from medical records. MR images were collected from the remaining 17 out of 18 patients (Table 3.1).

3.2.6 Outcome measures

3.2.6.1 Distal UE motor function

JTT: JTT was used to measure hand motor function (Jebsen et al., 1969). Patients completed the seven motor tasks of the JJT as quickly as possible with each hand. The time to complete each of the seven subtests was recorded (capped at 120 s). If a patient could not complete a subtest, the maximum time was recorded (120 s).

Peak wrist acceleration: Peak wrist acceleration of ballistic wrist extension movements, a measure of wrist motor function, was also collected (Buetefisch et al., 2015). To determine peak wrist acceleration, patients executed five auditory-cued ballistic wrist extension movements with the affected hand as quickly as possible. The non-affected hand was not tested. Acceleration in two movement planes (extension/flexion; abduction/adduction) was recorded by an accelerometer mounted on the dorsum of the hand. EMG activity was recorded from the ECU muscle using surface electrodes taped in a belly-tendon montage (9 mm diameter, bandpass 3Hz – 1kHz). EMG and accelerometer data was sampled and digitized in LabView (National Instruments) at a frequency of 1 kHz and stored for offline analysis.

MAL: The How Well subtest of the Motor Activity Log (MAL) was used to assess hand function during 30 activities of daily living (ADL) (Uswatte et al., 2005). Patients reported on a six-point scale how well the affected hand was used during each ADL. The highest score (5) indicated that quality of affected hand movement at the time of

questioning was the same as the quality of affected hand movement before the stroke. The lowest score (0) indicated that the affected hand was not used during that activity. In the infrequent case when the patient had not attempted the ADL in the past seven days, a score of 0 was given. If the activity was impossible, for instance combing the hair if the patient was bald, N/A was recorded.

3.2.6.2 Whole arm motor function

WMFT: Whole arm motor function was quantified using the Wolf Motor Function Test (WMFT), a standardized test of UE function consisting of 15 timed subtests and 2 strength tasks (Wolf et al., 2001). During the WMFT, patients completed each of the 15 timed subtests of the WMFT as quickly as possible with each arm. The time to complete each subtest was recorded (capped at 120 s). If a patient could not complete a subtest, the maximum time was recorded (120 s). The quality of affected arm movement during each subtest was also determined by trained physical therapist on a six-point functional ability score (FAS). The highest FAS (5) indicated that the quality of movement appeared normal. The lowest FAS (0) indicated that the affected arm was unable to be used. In first strength task, patients attempted to move their arm from the table to a box with a weighted cuff strapped to the forearm. The task was repeated after increasing the amount of weight in the cuff by 2 pounds until the patient's maximum or 20 pounds was reached. The maximum weight was determined separately for the affected and non-affected arm. In the second strength task, handgrip of each hand was tested three times with hand dynamometer and the maximum weight (kgs) produced during each attempt was recorded. Patients were given a one-minute rest between each attempt. The administration of the WMFT was video recorded.

3.2.7 Data analysis

3.2.7.1 Distal UE motor function

JTT: The JTT raw score (RAW) was calculated by summing the time to complete all but two subtests (writing and simulated feeding). These subtests were omitted because of their low test-retest reliability (Stern, 1992). The RAW score was normalized to age- and sex-matched standard scores (STD) that accounted for hand dominance using the formula: (RAW – STD) / (RAW + STD) (Jebsen et al., 1969; Hackel et al., 1992). A normalized score greater than 0 indicated abnormal hand function. A separate normalized score was calculated for the affected and non-affected hand.

Peak wrist acceleration: The maximum acceleration of the initial hand movement along the abduction/adduction (max_x) and flexion/extension (max_y) movement axes was calculated and used to derive peak wrist acceleration with the following formula: peak wrist acceleration = $\sqrt{(max_x^2 + max_y^2)}$ (Buetefisch et al., 2000; 2015).

MAL: The score for the How Well subtest of the MAL was calculated by averaging the scores of each ADL. Activities with N/A were excluded from the average score. (Uswatte et al., 2005; 2006).

3.2.7.2 Whole arm motor function

WMFT: For the WMFT, the mean performance time and mean FAS was calculated from the WMFT subtests (Morris et al., 2001; Wolf et al., 2001). Because performance time decreases non-linearly with improved motor function, a natural logarithmic transformation was applied to each subtest of the WMFT before the mean

performance time was calculated (Wolf et al., 2006). The maximum weight (kg) supported by each arm in a single trial was determined for each arm (Morris et al., 2001; Wolf et al., 2001). The maximum force (kg) produced in a single trial was determined for each arm (Morris et al., 2001; Wolf et al., 2001).

3.2.8 Statistical analysis

To determine abnormality in motor function, one-sided t-tests were used for motor tests in which the "healthy" score was known (JJT, MAL). For motor tests in which the function of both arms was tested (WMFT time, WMFT grip strength, WMFT arm strength), paired two-tailed t-tests were used to compare function of the affected and non-affected arm. Unpaired two-tailed t-tests were used to determine if the $rTMS_{Heb}$ and $rTMS_{sham}$ group differed in motor function prior to training (pre).

To determine the effect of $rTMS_{Heb}$ on the training-related change in motor function of the trained (affected) limb, each outcome measure was tested in a separate linear mixed effect model where time and intervention were held as the fixed effects and subject was held as the random effect. We selected a linear mixed effect model instead of a two-way ANOVA because the use of linear mixed effect model reduced the likelihood of type I errors by controlling for random effect associated with subject recruitment (Boisgontier and Cheval, 2016). Alpha was set to 0.05. The critical value for a Bonferroni test of multiple comparisons was set to 0.00714 and was calculated by dividing alpha (0.05) by the number of mixed models (7).

A Bayes factor was also calculated (Dienes, 2014). The Bayes factor is a number indicating the strength of evidence toward supporting the null hypothesis (Bayes factor less than 1) or alternative hypothesis (Bayes factor greater than 1) with the evidence

becoming stronger as the Bayes factor deviates further from 1 (Dienes, 2014). In the present analysis, we calculated a Bayes factor with a normal distribution. We tested the alternative hypothesis that the $rTMS_{Heb}$ group would improve distal UE function by ~15% above the $rTMS_{sham}$ group after motor training (pre to p1wk) which was based on the findings from a previous study on $rTMS_{Heb}$ in healthy adults (Buetefisch et al., 2015). The null hypothesis was that $rTMS_{Heb}$ group would improve distal UE motor function after motor training by a similar percentage as the $rTMS_{sham}$ group.

Finally, we used separate linear mixed effect model for each outcome measure to test specific contrasts of interest in an exploratory analysis. Two contrasts of the model evaluated if the measure changed significantly from pre to p0wks or pre to p4wks in patients who received $rTMS_{Heb}$. Another two contrasts evaluated if the measure changed significantly from pre to p0wks or pre to p4wks in patients who received $rTMS_{sham}$. The final contrast determined if the change in the measure after training differed between patients who received $rTMS_{Heb}$ and patients who received $rTMS_{sham}$.

3.3 Results

3.3.1 Comparison between intervention groups

There was no difference between the intervention groups in motor function of the affected UE prior to motor training (pre) as indicated by an insignificant difference in JTT ($t(18)=1.12$, $p=0.28$), peak acceleration ($t(14)=0.05$, $p=0.96$), MAL How Well score ($t(18)=0.30$, $p=0.77$), WMFT time ($t(16)=0.24$, $p=0.81$), WMFT FAS ($t(16)=0.26$, $p=0.80$), WMFT arm strength ($t(16)=0.58$, $p=0.57$) and WMFT grip strength ($t(16)=0.73$, $p=0.47$) using unpaired two-tailed t-tests.

3.3.2 Distal UE motor function

Motor function data associated with the distal UE (JTT, MAL and peak wrist acceleration) are summarized in Table 3.2. The change in time (mean ± SD) for each subtest of the JTT is reported in Table 3.3. Statistical analysis for measures of the distal UE is presented in Table 3.6 and Table 3.7. Peak wrist acceleration was unable to be calculated from at least one of the three time points (pre, p0wks, p4wks) for five patients because of technical errors that occurred during data collection. Because the mixed model used in the current study requires a complete data set, the patients with corrupted data were not included in statistical analysis.

JTT, time (contrast ratio): Before training (pre), hand motor function was abnormal during the JTT as patients took longer to complete the JTT than healthy age- and sex-matched standards in one-sided two-tailed t-tests (contrast ratio greater than zero for affected hand, $rTMS_{Heb}$: $t(9)=5.22$, $p<0.01$, $rTMS_{sham}$: $t(9)=8.61$, $p<0.01$; and non-affected hand, $rTMS_{Heb}$: $t(9)=4.02$, $p<0.01$, $rTMS_{sham}$: $t(9)=5.17$, $p<0.01$). Although function of both hands was impaired, function of the affected hand was worse prior to training (pre) as both intervention groups took longer to complete the JTT with the affected than non-affected hand ($rTMS_{Heb}$: $t(18)=2.98$, $p<0.01$, $rTMS_{sham}$: $t(18)=4.14$, $p<0.01$).

Function of the affected (training) hand improved after motor training as indicated by a significant fixed effect of time on JTT in a linear mixed effect model (pre to p0wks: $p=0.0071$; pre to p4wks: $p=0.0004$, Fig. 3.3A). The fixed effect of intervention was insignificant. However, the Bayes factor was 1.02 indicating that there was not adequate evidence to reject or accept the null hypothesis that the decrease in JTT time after motor

training was similar between intervention groups. When testing specific contrasts of interest in an exploratory analysis, we found the decrease in JTT time from pre to p0wks was significant for both intervention groups ($rTMS_{Heb}$: p=0.016, $rTMS_{sham}$: p=0.018). However, only patients who received $rTMS_{Heb}$ maintained the improvement in hand function 4 weeks post-training ($rTMS_{Heb}$: p=0.005).

MAL, How Well: Function of the affected hand was impaired during ADLs prior to motor training (pre) as the MAL How Well score was significantly less than the normal score (5) in two-tailed t-tests ($rTMS_{Heb}$: $t(9)=5.24$, $p<0.01$, $rTMS_{sham}$: $t(9)=4.92$, $p<0.01$).

Although the fixed effect of time on MAL How Well score did not reach criteria for multiple comparisons in a linear mixed effect model, there was a tendency for function of the affected hand during ADLs to improve after training (pre to p0wks: p=0.0343; pre to p4wks: p=0.0115, Fig. 3.3B). There was not a significant effect of intervention; however, the Bayes factor was 1.00. When testing specific contrasts of interest in an exploratory analysis, the increase in the MAL How Well score from pre to p0wks did not reach significance for either intervention group. However, the increase in the MAL How Well score from pre to p4wks was significant for patients of the $rTMS_{Heb}$ group (p=0.011).

Peak acceleration (g): There was a tendency for peak wrist acceleration of the trained (affected) wrist to increase after motor training but the fixed effect of time did not meet criteria for multiple comparisons in a linear mixed effect model (pre to p0wks: p=0.0134, pre to p4wks: p=0.0114, Fig. 3.3C). The fixed effect of intervention on peak wrist acceleration was not significant; however, the Bayes factor was 1.02. When specific

contrasts were tested in an exploratory mixed linear effect model, only the $rTMS_{sham}$ group increased peak wrist acceleration from pre to p0wks ($rTMS_{sham}$: p=0.051) and pre to p4wks ($rTMS_{sham}$: p=0.018).

3.3.3 Whole arm motor function

WMFT data (WMFT time, WMFT FAS, WMFT arm strength, WMFT grip strength) were excluded from two patients upon video inspection. The strength of the arm was not tested for one patient. WMFT data are summarized in Table 3.2. The change in time (mean ± SD) for each subtest of the WMFT is reported in Table 3.4. The change in FAS (mean ± SD) for each subtest of the WMFT is reported in Table 3.5. Statistical analysis for the WMFT is presented in Table 3.6 and 3.8.

WMFT, time (log transformed): Motor function of the whole arm also tended to be abnormal prior to motor training (pre) for patients of both intervention groups as the time to complete the WMFT was longer for the affected than non-affected arm ($rTMS_{Heb}$: t(8)=2.23, p=0.06, $rTMS_{sham}$: t(8)=3.13, p=0.01).

Patients improved function of their affected arm on the WMFT after motor training as indicated by a significant fixed effect of time that met criteria for multiple comparisons in a linear mixed effect model (pre to p0wks: p=0.0002, pre to p4wks: p=0.0001, Fig. 3.4A). There was not a significant fixed effect of intervention. In an exploratory model testing specific contrasts of interest, both intervention groups decreased the time to complete the WMFT from pre to p0wks ($rTMS_{Heb}$: p=0.018, $rTMS_{sham}$: p=0.004) and pre to p4wks ($rTMS_{Heb}$: p=0.017, $rTMS_{sham}$: p=0.024).

WMFT, FAS: Patients improved the quality of affected arm function on the WMFT after motor training as indicated by a significant fixed effect of time on WMFT

FAS that met criteria for multiple comparisons in a linear mixed effect model (pre to p0wks: p=0.0003, pre to p4wks: p=0.0033, Fig. 3.4B). The fixed effect of intervention was not significant. When testing specific contrasts of interest in an exploratory mixed model, patients of both intervention groups improved on WMFT FAS from pre to p0wks ($rTMS_{Heb}$: p=0.017, $rTMS_{sham}$: p=0.011), although only patients who received $rTMS_{Heb}$ maintained the improvement for 4 weeks post-training ($rTMS_{Heb}$: p=0.006).

WMFT, arm and grip strength: Patients of both the $rTMS_{Heb}$ or $rTMS_{sham}$ group had weaker arm strength in the affected than non-affected arm prior to motor training (pre), although the difference only reached significance for patients who received $rTMS_{sham}$ ($rTMS_{sham}$: $t(8)=2.57$, p=0.03, $rTMS_{Heb}$: $t(8)=1.55$, p=0.16). Further, patients of both intervention groups also had weaker grip strength in the affected than non-affected arm prior to training (pre) but the difference only reached significance for patients who received $rTMS_{Heb}$ ($rTMS_{sham}$: $t(8)=1.23$, p=0.25; $rTMS_{Heb}$: grip strength: $t(8)=4.40$, $p<0.01$).

Patients did not improve arm or grip strength for the affected UE after motor training, as the fixed effects of time and intervention were not significant in either of the respective linear mixed effect models (Fig. 3.4C, Fig. 3.5D). In the exploratory analysis, there was a tendency for the affected arm to increase in strength after training; however, the increase in arm strength arm only reached significance from pre to p4wks in the $rTMS_{sham}$ group ($rTMS_{sham}$: p=0.042). Grip strength was stable after training for the affected hand of both intervention groups.

3.4 Discussion

The present study evaluated the effect of $rTMS_{Heb}$ on training-related improvement in distal UE motor function in patients suffering chronic stroke involving the primary motor system (M1 and/or CST). Although we did not find evidence that $rTMS_{Heb}$ enhanced training-related improvement of distal UE motor function in our primary analysis, the Bayes factors were close to 1 (range 1.00 to 1.02) indicating that there was insufficient evidence to accept or reject the null hypothesis. We postulate that the insignificant findings may be related to low sample size given that our exploratory analysis provided evidence toward our stated hypothesis and suggest that differences between $rTMS_{Heb}$ and $rTMS_{sham}$ may exist if a larger sample size was used. Specifically, exploratory analysis revealed that $rTMS_{Heb}$ may enhance the retention of training-related hand motor improvement as only patients who received $rTMS_{Heb}$ maintained hand motor improvement on the JTT for 4 weeks post-training. Additional evidence for this observation is provided through analysis of the MAL where the self-reported improvement in the quality of hand use during ADLs was only observed 4 weeks post-training in patients who received $rTMS_{Heb}$. These exploratory findings are consistent with previous reports that the $rTMS_{Heb}$ enhances training-related hand motor improvement in healthy subjects (Buetefisch et al., 2004b; 2015). To confirm that $rTMS_{Heb}$ leads to greater training-related hand motor improvement in a chronic stroke population, the hypothesis could be tested in a study with a larger sample.

3.4.1 $rTMS_{Heb}$ may enhance the retention but not acquisition of hand motor skill

The improvement in motor skill associated with training occurs in two stages where the motor skill is first acquired and then consolidated for longer term retention

(Doyon and Benali, 2005). In our primary analysis, we were unable to conclusively determine the effect of $rTMS_{Heb}$ on either the acquisition or retention of training-related motor improvement for the distal UE. Although the fixed effect of intervention was not significant in any of mixed linear effect models for the measures of distal UE function (JTT, MAL, peak wrist acceleration), the Bayes factors ranged between 1.00 and 1.02, which indicated that the current dataset was insensitive to detecting a difference in the effect of $rTMS_{Heb}$ and $rTMS_{sham}$ on training-related motor improvement. Because the sample size of the current study was based on findings in the healthy adult and motor function after stroke is typically more variable as compared to the healthy adult, a larger sample size may be needed to detect differences between $rTMS_{Heb}$ and $rTMS_{sham}$ after stroke (Freeman et al., 2015).

In our exploratory analysis in which we tested specific contrasts of interest in a mixed model, we identified a potential effect of $rTMS_{Heb}$ on hand motor function (JTT, MAL) that should be further evaluated in a study with a larger sample. Specifically, the exploratory findings suggest that $rTMS_{Heb}$ may enhance the long-term retention of motor improvement associated with motor training as only patients who received $rTMS_{Heb}$, but not $rTMS_{sham,}$ maintained their improvement on the JTT and MAL 4 weeks post-training. $RTMS_{Heb}$ is less likely to improve training-related acquisition of hand motor skill because patients of both interventions needed less time to complete the JTT after five days of training and the decrease in time was not significantly different between the intervention groups. This exploratory finding would be in agreement with other studies that do not report an immediate improvement in motor function after cortical stimulation. In a rodent model of stroke, paired cortical stimulation does not lead to enhanced training-related

improvement in skilled reaching until day 11 of training (Adkins et al., 2008). Although $rTMS_{Heb}$ leads to an immediate enhancement of peak wrist acceleration after a single session of hand motor training in healthy adults (Buetefisch et al., 2004a; 2015), the reduced ability of stroke patients to acquire novel hand motor skills as compared to the healthy adult is well-documented (Winstein et al., 1999). Therefore, the need for stroke patients to complete multiple days of motor training to improve hand motor function is common and could be associated with the delayed effect of $rTMS_{Heb}$ on hand motor recovery (Buetefisch et al., 1995).

The improvement in hand motor function associated with $rTMS_{Heb}$ detected in the exploratory analysis cannot be explained by differences in baseline characteristics as patients in each group were matched for age, sex, post-stroke duration, cognition (RBANS), muscle tone (Ashworth) and motor deficit. Although JTT performance can improve with repeated examination, the test-retest improvement in JTT did not impact the results of the current study as the effect of repeated testing would have affected the $rTMS_{Heb}$ and $rTMS_{sham}$ groups equally (Stern, 1992). Further, the JTT substests with the greatest inter-trial variability (writing and stimulated feeding) were not included in the final JTT score (Stern, 1992). Consequently, we conclude that the improved retention of hand motor function improvement in the $rTMS_{Heb}$ group is most likely associated with the application of $rTMS_{Heb}$ to the $M1_{IL}$ during motor training.

In light of the exploratory evidence that hand motor function was improved by $rTMS_{Heb}$, finding that other aspects of distal UE function, specifically peak wrist acceleration, was not enhanced by $rTMS_{Heb}$ was unexpected. In fact, only the $rTMS_{sham}$ group experienced a significant increase their peak wrist acceleration from pre to p0wks

and pre to p4wks in the exploratory analysis. Because peak wrist acceleration did not differ between intervention groups prior to training (pre), baseline differences in peak wrist acceleration cannot explain the failure of $rTMS_{Heb}$ to enhance the training-related change in wrist motor function. Because the increase peak acceleration of a trained ballistic movement is a well-established phenomena occurring after repetitive practice, the finding that patients of the $rTMS_{Heb}$ group did not have greater peak wrist acceleration after training suggests that the motor training was not as effective for patients of the $rTMS_{Heb}$ than of the $rTMS_{sham}$ group (Muellbacher et al., 2002c; 2002a; Buetefisch et al., 2015). The cause for the reduced efficacy of the motor training is unclear as care was taken to minimize potential differences between intervention groups. The motor training is unlikely to be of insufficient duration to produce an increase in peak wrist acceleration as patients performed 1,800 ballistic wrist movements in current training protocol, which is more than double the amount of training administered in other studies using repetitive ballistic training for stroke motor rehabilitation (Waddell et al., 2014). Instead the difference in peak acceleration could indicate that the $rTMS_{Heb}$ group performed worse on the motor training than $rTMS_{sham}$ group. Because sensory loss was not quantified, the decreased in the quality of training for the $rTMS_{Heb}$ group could be related to a deficit in hand or wrist sensation. Loss of touch sensation in the hand following stroke has been associated with worse functional outcomes (Carey et al., 1993). It is notable that the administration of $rTMS_{Heb}$ was sufficient to overcome any deficits in the quality of motor training as the $rTMS_{Heb}$ group still had a greater improvement than the $rTMS_{sham}$ group in hand function as measured by the JTT and MAL How Well.

The neural mechanism by which $rTMS_{Heb}$ would support motor skill retention is unclear. Study of the non-human primate (Nudo et al., 1996a; 1996b), rodent (Jones et al., 2009) and human (Talelli et al., 2006) indicate that M1 is crucial for the acquisition of motor skill after stroke. However, the precise role of M1 in the retention of a motor memory is debated. Some studies suggest that training-related structural and functional changes in M1 is transient and rarely persist beyond a few days after training despite the persistence of motor skill improvement for over a year post-training (Smith et al., 2005; Molina-Luna et al., 2008). Based on this evidence, some propose that M1 encodes the early motor memory and other brain regions, such as the cerebellum, are responsible for the consolidation of the motor memory for long-term retention (Shadmehr and Holcomb, 1997). Additional evidence for this hypothesis is provided by study of the human cortex where high-frequently rTMS to M1 only disrupts motor skill acquisition when applied immediately following motor training (Muellbacher et al., 2002c). However, recent evidence indicates that motor training promotes long-term structural modification of pyramidal dendrites in M1 and opens the possibility that M1 could also be part of the neural network responsible for long-term motor memory retention (Xu et al., 2009; Zemmar et al., 2014). Given these conflicting hypotheses, $rTMS_{Heb}$ of $M1_{IL}$ could either lead greater retention of motor skill improvement by facilitating the transfer of the motor memory out of $M1_{IL}$ or by supporting structural reorganization of $M1_{IL}$ and thereby facilitating the storage of the motor memory within $M1_{IL}$ itself. In the future, the examination of $M1_{IL}$ excitability with TMS before and after $rTMS_{Heb}$ would provide evidence toward one hypothesis or the other. A greater training-related increase in $M1_{IL}$ excitability after $rTMS_{Heb}$ 4 weeks after training, would support the hypothesis that

$rTMS_{Heb}$ facilitates the storage of the motor memory within $M1_{IL}$ itself, likely through LTP-like mechanisms.

3.4.2 Whole arm motor function improves after wrist motor training

The modified WMFT is a well-established measure of UE motor function with high test-retest reliability for patients after stroke with mild to moderate motor impairment and was used to quantify whole arm function in the current study (Wolf et al., 1989; Taub et al., 1998; Morris et al., 2001). The WMFT is commonly reported as four parts: time (log transformed), FAS, arm strength and grip strength. In our primary analysis we found a significant effect of time on two parts of the WMFT (time and FAS), suggesting that patients improved not only in the effectiveness of UE movement use but also the in the quality of UE movement. The findings of the exploratory analysis further supported these conclusions as specific contrasts of interest identified a decrease in time (log transformed) to complete the WMFT and an increase in WMFT FAS after motor training for both intervention groups.

The WMFT is composed of 15 timed subtests, several of which require function of the distal UE, such as hand and finger, to complete. Even though the motor training focused on an aspect of distal UE function, specifically the wrist, there was a tendency for patients to improve on all subtests and not just on WMFT subtests that require distal UE function. In fact, of the few WMFT subtests on which patients worsened performance, all but one were subtests that required finger function (in bold on Tables 3.4 and 3.5). This observation suggests that the improvement on the WMFT time and WMFT FAS could have not have been driven by only improvement in distal UE function but must also be driven by improvement in more proximal whole arm function.

We speculate that arm motor function improved after motor training as consequence of patients using the affected arm more frequently once use of the affected hand became more useful in daily life (as indicated by an increase on the MAL). Repeated testing of the WMFT may also have contributed toward the improved WMFT performance as a study on the reliability of the WMFT found subjects complete the WMFT subtests 16.8% faster upon the second administration of the WMFT (Morris et al., 2001). Because the arm was not used during motor training, as indicated by careful observation and the presence of Velcro straps which prevented movement of the proximal and forearm, improvement in whole-arm motor function cannot be attributed to the motor training paradigm itself.

Not all aspects of whole arm function improved after motor training. The finding that arm and grip strength minimally changed after training (WMFT, grip strength; WMFT, arm strength) is consistent with previous reports that skill-based motor training does not affect muscle strength (Jensen and Marstrand, 2005).

3.4.3 $rTMS_{Heb}$ may enhance the retention but not acquisition of arm motor skill

The fixed effect of intervention was not significant for any of the linear mixed effect models on the WMFT, suggesting that $rTMS_{Heb}$ does not enhance training-related improvement in whole arm motor function. However, our exploratory analysis indicates that there may be an effect of $rTMS_{Heb}$ on training-related motor improvement that is currently undetectable in our primary analysis. Specifically, in the exploratory analysis we found that only patients who received $rTMS_{Heb}$ maintained the improvement on WMFT FAS after four weeks post-training, indicating that $rTMS_{Heb}$ may enhance retention of training-related whole arm motor improvement.

The mechanism by which $rTMS_{Heb}$ supported whole arm motor improvement is unclear. Although $rTMS_{Heb}$ was applied to the wrist motor region of the brain and theoretically should have only impacted more distal UE function, other studies have demonstrated the feasibility of rTMS to facilitate neuroplasticity in functionally connected but distant cortical regions from the targeted motor area (Rothwell, 2011). For instance, high-frequency excitatory rTMS to the PMC decreased intracortical inhibition as measured with TMS in M1 of the same hemisphere (Münchau et al., 2002a). These findings suggest the possibility of $rTMS_{Heb}$ to facilitate neural plasticity in more proximal UE motor regions in $M1_{IL}$ even though a region associated with distal UE motor function was targeted. Because the improvement in WMFT performance associated with the effect of test-retesting should affect each intervention group to a similar degree, the impact of the repeated testing cannot explain the prolonged retention of arm motor skill associated with $rTMS_{Heb}$ (Morris et al., 2001).

3.4.4 Limitations

Because the studied population was restricted to patients with at least partial damage to the primary motor system (M1 and/or CST), we cannot generalize study results to patients of other types of stroke. However, the general study of rTMS to improve stroke motor recovery traditionally suffers high intra-individual variability. By restricting our studied population to patients with damage to M1 or the CST, we selected a sample that was most likely to benefit from $rTMS_{Heb}$ as $M1_{IL}$ reorganization is crucial for stroke motor recovery in non-human primates with damage to the primary motor system (Nudo and Milliken, 1996; Nudo et al., 1996b). Other studies have found the normalization of

M1 to be associated with better functional recovery outcomes in the human (Ward et al., 2003a; 2003b).

3.5 Conclusions

The exploratory results of this study are in support of the hypothesis that $rTMS_{Heb}$ has the potential to improve stroke motor recovery outcomes by prolonging the retention of the training-related motor improvement. This finding extends the previous findings that $rTMS_{Heb}$ enhances training-related motor performance in healthy adults (Buetefisch et al., 2015) and agrees with previous reports of Hebbian-type stimulation promoting M1 reorganization in patients suffering chronic stroke (Buetefisch et al., 2011). To our knowledge, this is the first evidence suggesting that Hebbian-type stimulation may enhance training-related motor improvement after stroke in humans and should be confirmed in a study of a larger sample size. While we speculate that $rTMS_{Heb}$ supports the consolidation of a motor memory by promoting long-term structural reorganization of M1 through LTP-like processes, the precise neural mechanisms cannot be determined without careful examination of $M1_{IL}$ excitability.

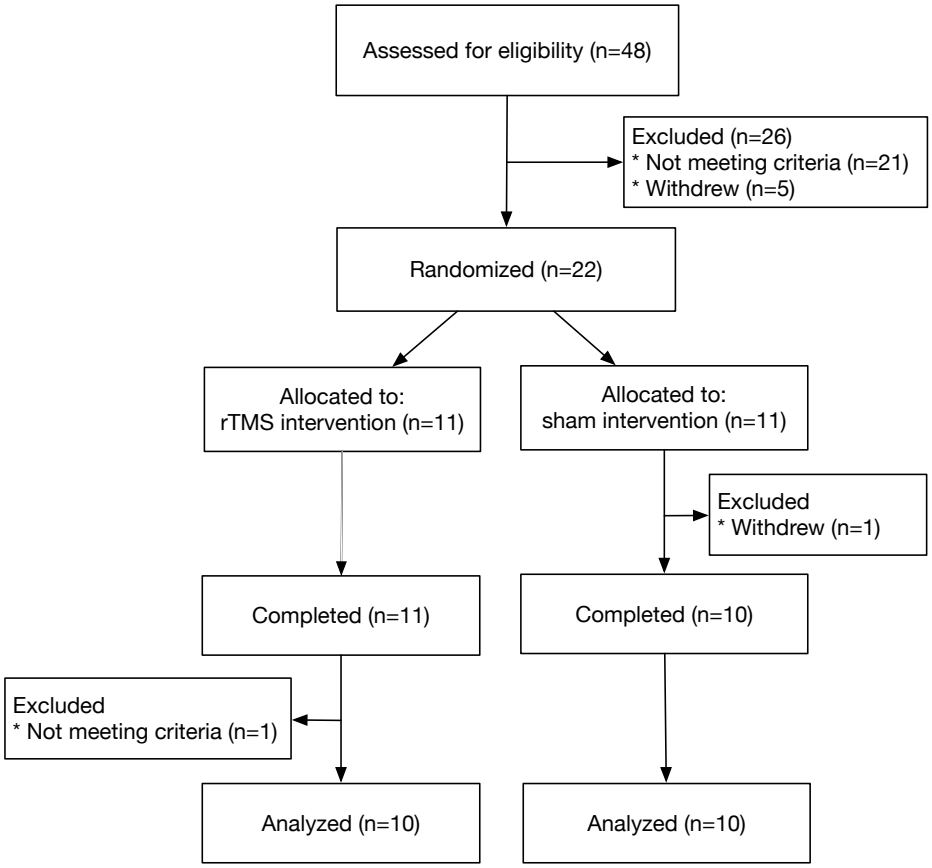

Fig. 3.1 Schematic of patient assessment.

Subject	Age	Sex	PSD (months)	Stroke	Edinburgh (LQ)	Dominant Hand	Affected Hand	RBANS (total scale)	MRC (affected UE)	Ashworth	PMH	rTMS
1	60	M	133	s	-40	L	L	78	4+	3	HTN	Heb
2	76	M	18	c	100	R	R	54	4+	0	HLD	sham
3	63	F	18	c	100	R	R	89	4+	0	HTN	Heb
4	51	F	9	s	100	R	R	89	4+	1	-	sham
5	67	F	7	s	100	R	R	118	4+	0	HTN	Heb
6	63	M	14	s	100	R	R	89	4+	1	-	sham
7	62	F	10	s	-100	L	L	83	4	0	HTN, DM	Heb
8	76	F	17	c	78	R	R	95	4+	0	HTN, HLD, DM	sham
9	78	M	17	c	100	R	L*	108	4	1	-	sham
10	55	F	10	s	100	R	L*	86	3	2	HTN	Heb
11	44	M	18	s	100	R	R	104	4+	0	-	sham
12	66	M	8	c	80	R	L*	94	4+	0	-	Heb
13	68	M	53	s	-100	L	L	100	4+	0	-	Heb
14	68	M	65	s	80	R	R	72	4+	1	-	sham
15	32	M	84	c	71	R	L*	104	4+	0	-	Heb
16	50	F	16	c	100	R	R	85	4+	0	HTN, HLD	sham
17	57	M	8	s	33	R	R	50	4	0		Heb
18	69	F	10	c	100	R	L*	80	4+	1		Heb
19	63	F	43	s	100	R	R	111	4+	0		sham
20	55	F	13	s	100	R	L*	95	4+	0		Heb

Table 3.1. Characteristics of stroke patients. F = female; M = male; PSD = post-stroke duration; R = right; L = left; * = non-dominant hand was affected; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; UE= upper extremity; c = stroke involved cortex (cortical); s = stroke spared the cortex (subcortical); PMH = past medical history; HTN = hypertension, HLD = hyperlipidemia; DM = diabetes mellitus; Heb = $rTMS_{Heb}$; sham = $rTMS_{sham}$.

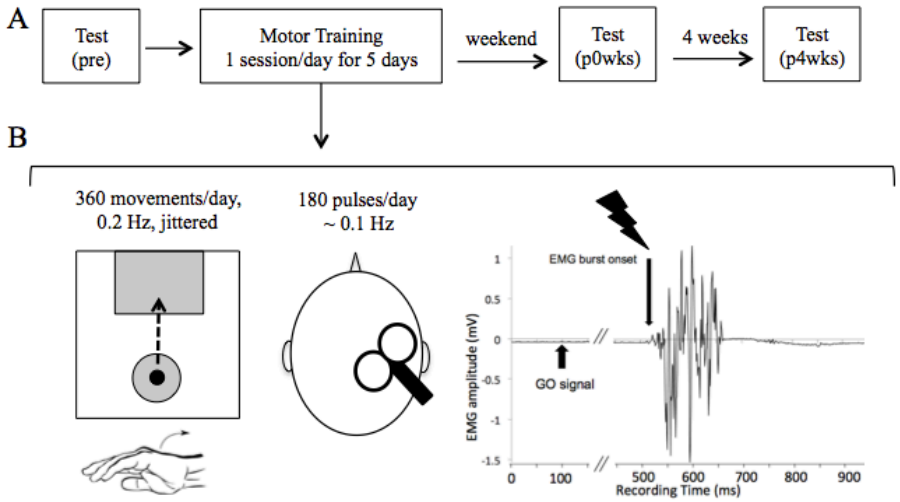

Fig. 3.2 Experimental overview. (A) Schematic of the experimental design (B) Schematic of a training session. During motor training, patients attempted to move a cursor from a home position (circle) into a target box (rectangle) on a computer screen by extending their affected wrist. rTMS was triggered over $M1_{IL}$ by an increase in movement-related EMG of every second wrist extension movement (approximate frequency of 0.1 Hz). An example of EMG recorded during a wrist extension movement demonstrates when the increase in movement-related EMG would have triggered rTMS application (lightning bolt).

Measures	Arm	Pre rTMS	P0wks rTMS	P4wks rTMS	Pre Sham	P0wks Sham	P4wks Sham
JTT, Time (contrast ratio)	Aff	0.38 ± 0.23 (10)	0.34 ± 0.26 (10)	0.31 ± 0.26 (10)	0.49 ± 0.21 (10)	0.45 ± 0.20 (10)	0.44 ± 0.21 (10)
	Non-Aff	0.14 ± 0.11 (10)	0.13 ± 0.11 (10)	0.12 ± 0.11 (10)	0.18 ± 0.11 (10)	0.15 ± 0.08 (10)	0.15 ± 0.07 (10)
WEM, Peak Acceleration (g)	Aff	0.61 ± 0.48 (7)	0.72 ± 0.32 (7)	0.71 ± 0.32 (7)	0.60 ± 0.32 (8)	0.79 ± 0.49 (8)	0.81 ± 0.47 (8)
MAL, How Well (0-5 scale)	Aff	2.91 ± 1.26 (10)	3.22 ± 1.29 (10)	3.53 ± 1.30 (10)	2.73 ± 1.46 (10)	3.04 ± 1.52 (10)	2.85 ± 1.55 (10)
WMFT, Time (log transformed)	Aff	0.87 ± 0.69 (9)	0.73 ± 0.62 (9)	0.69 ± 0.66 (9)	0.94 ± 0.53 (9)	0.76 ± 0.45 (9)	0.77 ± 0.41 (9)
	Non-Aff	0.38 ± 0.17 (9)	0.32 ± 0.19 (9)	0.37 ± 0.18 (9)	0.43 ± 0.14 (9)	0.35 ± 0.13 (9)	0.46 ± 0.21 (9)
WMFT, FAS (FAS)	Aff	4.07 ± 0.57 (9)	4.31 ± 0.55 (9)	4.34 ± 0.54 (9)	3.99 ± 0.74 (9)	4.24 ± 0.60 (9)	4.10 ± 0.51 (9)
WMFT, Arm Strength (kgs)	Aff	13.67 ± 6.73 (9)	14.44 ± 6.54 (9)	14.22 ± 6.72 (9)	11.78 ± 7.01 (9)	14.44 ± 7.32 (9)	13.00 ± 7.57 (9)
	Non-Aff	16.11 ± 3.44 (9)	17.11 ± 4.59 (9)	17.33 ± 4.21 (9)	14.78 ± 5.83 (9)	17.56 ± 4.22 (9)	18.11 ± 3.95 (9)
WMFT, Grip Strength (kgs)	Aff	18.00 ± 11.48 (9)	17.44 ± 12.05 (9)	17.89 ± 10.48 (9)	22.33 ± 13.49 (9)	20.44 ± 9.58 (9)	20.44 ± 12.18 (9)
	Non-Aff	33.11 ± 14.68 (9)	33.33 ± 15.07 (9)	35.22 ± 15.18 (9)	28.00 ± 16.12 (9)	30.00 ± 12.41 (9)	29.67 ± 13.44 (9)

Table 3.2 Summary (mean ± SD (n)) of patient performance on the measures of hand and upper extremity function for the affected limb. Aff = affected limb, NonAff = non-affected limb, JJT = Jebesn-Taylor Test, WEM= wrist extension movements, MAL = Motor Activity Log, WMFT= Wolf Motor Function Test, FAS = functional ability score

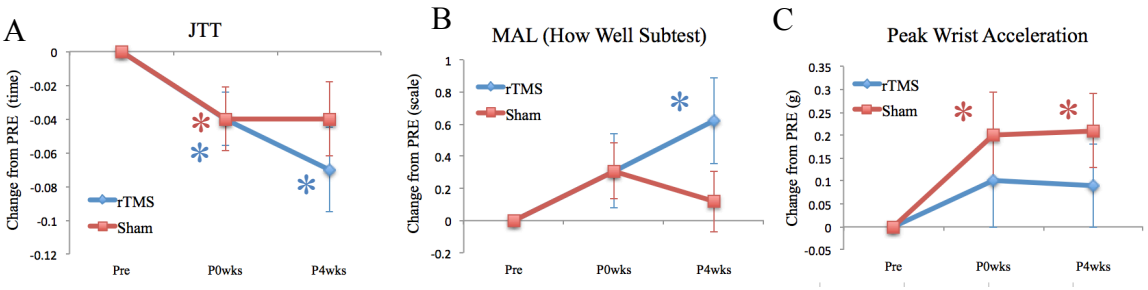

Fig. 3.3 Line graphs depicting the change (mean ± SE) from baseline (pre) for performance of the affected hand on the measures of hand motor function. (A) Jebsen Taylor Test (JTT), (B) How Well subtest of the Motor Activity Log (MAL) and (C) peak wrist acceleration of ballistic wrist extension movements for patients who received either rTMS (red) or sham (blue) intervention. A star (*) indicates that the pre-p0wk or pre-p4wks contrast resulted in p-value <0.05.

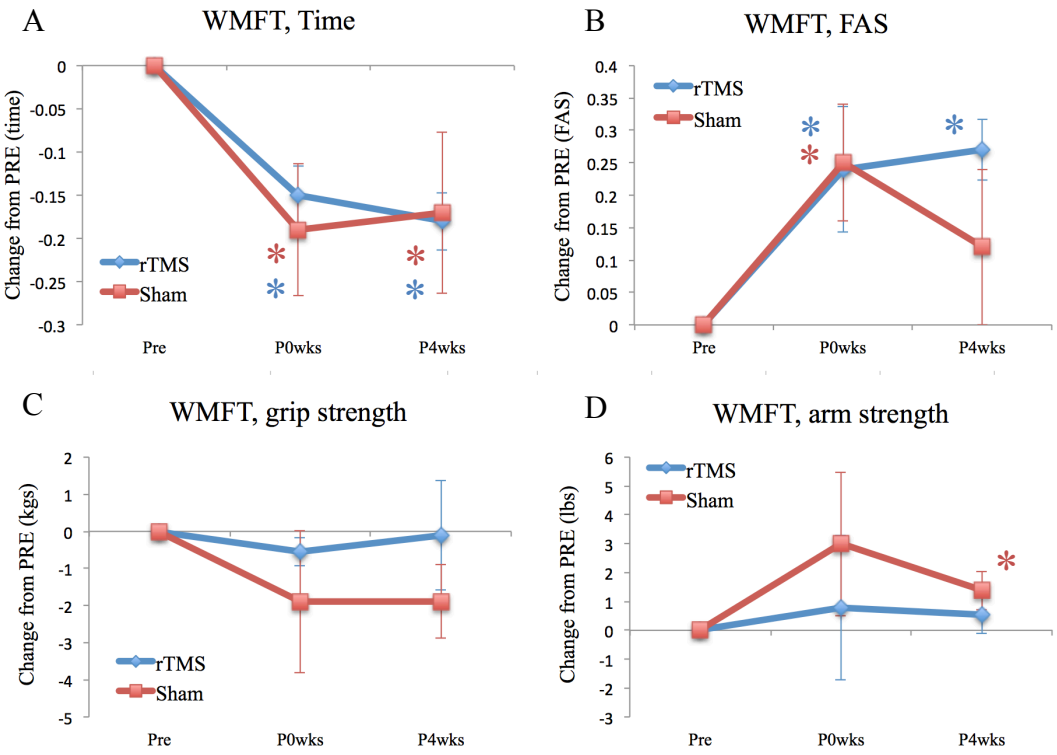

Fig. 3.4 Line graphs depicting the change (mean ± SE) from baseline (pre) in performance of the affected arm on the Wolf Motor Function Test (WMFT). (A) WMFT average score (log transformed), (B) WMFT functional ability score (FAS), (C) WMFT grip strength and (D) WMFT arm strength for the affected arm of patients who received either rTMS (red) or sham (blue) intervention. A star (*) indicates that the pre-p0wk or pre-p4wks contrast of the exploratory analysis resulted in p-value <0.05.

JTT subtests	**Limb**	**Used Hand?**	**Pre to P0wks** rTMS-heb	**Pre to P4wks** rTMS-heb	**Pre to P0wks** rTMS-sham	**Pre to P4wks** rTMS-sham
Q1: writing	Aff	yes	excluded			
Q2: simulated page turning	Aff	yes	-0.08 ± 0.14	-0.10 ± 0.15	-0.05 ± 0.09	-0.08 ± 0.10
Q3: lifting small common objects	Aff	yes	-0.03 ± 0.09	-0.05 ± 0.07	-0.02 ± 0.06	-0.02 ± 0.08
Q4: simulated feeding	Aff	yes	excluded			
Q5: stacking checkers	Aff	yes	-0.05 ± 1.00	-0.10 ± 0.21	-0.02 ± 0.08	-0.05 ± 0.14
Q6: lifting large light objects	Aff	yes	-0.02 ± 0.08	-0.04 ± 0.07	-0.08 ± 0.06	-0.06 ± 0.07
Q7: lifting large heavy objects	Aff	yes	-0.04 ± 0.05	-0.06 ± 0.04	-0.06 ± 0.06	-0.03 ± 0.08

Table 3.3 Summary (mean ± SD) of the change in time (contrast ratio) for the affected arm after training for the subtests of the Jebsen Taylor Test (JTT).

WMFT subtests	Limb	Used Hand?	Pre to P0wks rTMS-heb	Pre to P4wks rTMS-heb	Pre to P0wks rTMS-sham	Pre to P4wks rTMS-sham
Q1: forearm to table	Aff	no	-0.05 ± 0.16	-0.07 ± 0.22	-0.08 ± 0.16	-0.12 ± 0.29
Q2: forearm to box	Aff	no	-0.14 ± 0.32	-0.24 ± 0.39	-0.11 ± 0.33	-0.01 ± 0.23
Q3: extend elbow	Aff	no	-0.12 ± 0.25	-0.07 ± 0.14	-0.11 ± 0.19	-0.08 ± 0.11
Q4: extend elbow (weight)	Aff	no	-0.09 ± 0.30	-0.16 ± 0.47	-0.09 ± 0.19	-0.47 ± 1.42
Q5: hand to table	Aff	no	-0.12 ± 0.25	-0.06 ± 0.18	-0.13 ± 0.16	-0.19 ± 0.17
Q6: hand to box	Aff	no	-0.03 ± 0.27	-0.16 ± 0.23	-0.62 ± 1.41	-0.21 ± 0.23
Q7: weight to box	Aff	no	-	-	-	-
Q8: reach and retrieve	Aff	yes	-0.10 ± 0.22	-0.17 ± 0.17	-0.14 ± 0.19	-0.12 ± 0.40
Q9: lift can	Aff	yes	**0.05 ± 0.22**	**0.26 ± 1.04**	-0.27 ± 0.14	**0.16 ± 1.23**
Q10: lift pencil	Aff	yes	-0.14 ± 0.20	-0.55 ± 0.77	-0.16 ± 0.39	-0.37 ± 0.90
Q11: lift paperclip	Aff	yes	-0.31 ± 0.46	-0.16 ± 0.56	**0.13 ± 1.25**	-0.35 ± 0.77
Q12: stack checkers	Aff	yes	-0.39 ± 0.45	-0.42 ± 0.55	-0.44 ± 0.74	-0.44 ± 1.07
Q13: flip cards	Aff	yes	-0.34 ± 0.40	-0.19 ± 0.18	-0.40 ± 0.78	-0.06 ± 0.34
Q14: grip strength	Aff	yes	-	-	-	-
Q15: turn key in lock	Aff	yes	**0.02 ± 0.55**	**0.07 ± 0.53**	-0.41 ± 1.07	-0.07 ± 0.37
Q16: fold towel	Aff	yes	-0.22 ± 0.55	-0.34 ± 0.66	-0.07 ± 0.20	-0.11 ± 0.17
Q17: lift basket	Aff	yes	-0.20 ± 0.71	-0.36 ± 0.89	**0.25 ± 1.13**	-0.01 ± 0.28

Table 3.4 Summary (mean ± SD) of the change in time (log transformed) for the affected arm after training for the subtests of the Wolf Motor Function Test (WMFT). Bolded font indicates that performance on the subtest worsened after motor training (change from pre was greater than 0).

WMFT subtests (FAS)	**Limb**	**Used Hand?**	**Pre to P0wks** rTMS-heb	**Pre to P4wks** rTMS-heb	**Pre to P0wks** rTMS-sham	**Pre to P4wks** rTMS-sham
Q1: forearm to table	Aff	no	0.33 ± 0.50	0.33 ± 0.50	0.11 ± 0.60	0.22 ± 0.44
Q2: forearm to box	Aff	no	0.00 ± 0.87	0.22 ± 0.67	0.22 ± 0.83	0.00 ± 0.87
Q3: extend elbow	Aff	no	0.33 ± 0.50	0.22 ± 0.44	0.11 ± 0.33	0.11 ± 0.60
Q4: extend elbow (weight)	Aff	no	0.00 ± 0.87	0.00 ± 0.50	0.33 ± 0.50	0.22 ± 0.44
Q5: hand to table	Aff	no	**-0.22 ± 0.44**	**-0.11 ± 0.33**	0.56 ± 0.53	0.33 ± 0.50
Q6: hand to box	Aff	no	0.22 ± 0.44	0.44 ± 0.53	0.56 ± 0.53	0.00 ± 0.87
Q7: weight to box	Aff	no	-	-	-	-
Q8: reach and retrieve	Aff	yes	0.33 ± 0.50	0.33 ± 0.50	0.00 ± 0.00	0.33 ± 0.50
Q9: lift can	Aff	yes	0.00 ± 0.71	0.22 ± 0.83	0.33 ± 0.71	0.22 ± 0.97
Q10: lift pencil	Aff	yes	0.67 ± 0.71	0.44 ± 0.53	0.22 ± 0.67	0.00 ± 0.71
Q11: lift paperclip	Aff	yes	0.67 ± 0.50	0.33 ± 0.50	0.00 ± 1.12	**-0.22 ± 0.97**
Q12: stack checkers	Aff	yes	0.67 ± 0.71	0.56 ± 0.73	0.22 ± 0.67	0.11 ± 0.78
Q13: flip cards	Aff	yes	0.33 ± 0.71	0.33 ± 0.50	0.44 ± 0.73	0.33 ± 0.50
Q14: grip strength	Aff	yes	-	-	-	-
Q15: turn key in lock	Aff	yes	0.00 ± 1.00	0.22 ± 0.97	0.22 ± 0.67	0.11 ± 0.78
Q16: fold towel	Aff	yes	0.22 ± 0.67	0.33 ± 0.50	0.33 ± 0.50	**-0.11 ± 0.78**
Q17: lift basket	Aff	yes	0.00 ± 0.71	0.11 ± 0.78	0.11 ± 0.60	0.11 ± 0.78

Table 3.5 Summary (mean ± SD) of the change in FAS for the affected arm after training for the subtests of the Wolf Motor Function Test (WMFT). Bolded font indicates that performance on the subtest worsened after training (change from pre was less than 0).

Model	Fixed Effect	Estimate	Std Error	df	t-statistic	p-value	Sig.
JTT (Time)	Intervention (rTMS-sham)	-0.1151	0.1008	18	-1.1410	0.2687	
	Time (p1wk-pre)	-0.0426	0.0150	38	-2.8450	0.0071	**
	Time (p5wks-pre)	-0.0585	0.0150	38	-3.9080	0.0004	**
MAL (How Well)	Intervention (rTMS-sham)	0.3459	0.6065	18	0.5700	0.5755	
	Time (p1wk-pre)	0.3090	0.1407	38	2.1950	0.0343	*
	Time (p5wks-pre)	0.3736	0.1407	38	2.6540	0.0115	*
Wrist Extension (peak wrist accel)	Intervention (rTMS-sham)	-0.0500	0.2009	13	-0.2490	0.8075	
	Time (p1wk-pre)	0.1506	0.0571	28	2.6390	0.0134	*
	Time (p5wks-pre)	0.1546	0.0571	28	2.7090	0.0114	*
WMFT (Time)	Intervention (rTMS-sham)	-0.0566	0.2641	16	-0.2140	0.8329	
	Time (p1wk-pre)	-0.1620	0.0384	34	-4.2180	0.0002	**
	Time (p5wks-pre)	-0.1724	0.0384	34	-4.4870	0.0001	**
WMFT (FAS)	Intervention (rTMS-sham)	0.1333	0.2685	16	0.4970	0.6262	
	Time (p1wk-pre)	0.2444	0.0609	34	4.0170	0.0003	**
	Time (p5wks-pre)	0.1926	0.0609	34	3.1650	0.0033	**
WMFT (Arm Strength)	Intervention (rTMS-sham)	1.0370	3.0851	16	0.3360	0.7410	
	Time (p1wk-pre)	1.7222	0.9868	34	1.7450	0.0900	
	Time (p5wks-pre)	0.8889	0.9868	34	0.9010	0.3740	
WMFT (Grip Strength)	Intervention (rTMS-sham)	-3.2960	5.3350	16	-0.6180	0.5450	
	Time (p1wk-pre)	-1.2220	1.0420	34	-1.1730	0.2490	
	Time (p5wks-pre)	-1.0000	1.0420	34	-0.9600	0.3440	

Table 3.6 The estimate, standard error (std error), degrees of freedom (df), t-statistic and p-value for each fixed effect of the linear mixed effect models. The following key for statistical significance (sig.) was used: * = $p<0.05$, ** = $p<0.00714$ (threshold for multiple comparisons).

Model	Contrast	Estimate	Std Error	df	t-statistic	p-value	Sig.
JJT (Time)	both-p0wks-pre	-0.041	0.014	36	-2.961	0.005	*
	both-p4wks-pre	-0.046	0.009	36	-4.994	0.000	*
	rTMS-p0wks-pre	-0.035	0.019	36	-1.789	0.016	*
	sham-p0wks-pre	-0.047	0.019	36	-2.398	0.018	*
	rTMS-p4wks-pre	-0.046	0.013	36	-3.511	0.005	*
	sham-p4wks-pre	-0.046	0.013	36	-3.553	0.078	
	Δ rTMS - Δ sham	-0.011	0.019	36	-0.581	0.565	
MAL (How Well)	both-p0wks-pre	0.309	0.145	36	2.128	0.040	*
	both-p4wks-pre	0.374	0.164	36	2.274	0.029	*
	rTMS-p0wks-pre	0.310	0.205	36	1.511	0.140	
	sham-p0wks-pre	0.308	0.205	36	1.498	0.143	
	rTMS-p4wks-pre	0.622	0.232	36	2.678	0.011	*
	sham-p4wks-pre	0.125	0.232	36	0.537	0.594	
	Δ rTMS - Δ sham	0.495	0.177	36	2.802	0.008	*
Wrist Ext (peak accel)	both-p0wks-pre	0.147	0.070	26	2.107	0.045	*
	both-p4wks-pre	0.150	0.062	26	2.444	0.022	*
	rTMS-p0wks-pre	0.100	0.102	26	0.976	0.338	
	sham-p0wks-pre	0.195	0.096	26	2.042	0.051	*
	rTMS-p4wks-pre	0.089	0.090	26	0.987	0.333	
	sham-p4wks-pre	0.212	0.084	26	2.523	0.018	*
	Δ rTMS - Δ sham	-0.028	0.075	26	-0.375	0.711	

Table 3.7 The estimate, standard error (std error), t-statistic, degrees of freedom (df) and p-value for each contrast of the exploratory linear mixed effect models for the Jebsen Taylor Test (JTT), Motor Activity Log (MAL), and ballistic wrist extension movements (Wrist Ext). The following key for statistical significance (sig.) was used: * = $p<0.05$. Because this analysis was exploratory, a threshold for multiple comparisons was not determined.

Model	Contrast	Estimate	Std Error	df	t-statistic	p-value	Sig.
WMFT (Time)	both-p0wks-pre	-0.162	0.041	32	-3.945	0.000	*
	both-p4wks-pre	-0.172	0.050	32	-3.463	0.002	*
	rTMS-p0wks-pre	-0.145	0.058	32	-2.495	0.018	*
	sham-p0wks-pre	-0.179	0.058	32	-3.084	0.004	*
	rTMS-p4wks-pre	-0.178	0.070	32	-2.522	0.017	*
	sham-p4wks-pre	-0.167	0.070	32	-2.375	0.024	*
	Δ rTMS - Δ sham	-0.045	0.044	32	-1.007	0.322	
WMFT (FAS)	both-p0wks-pre	0.244	0.066	32	3.681	0.001	*
	both-p4wks-pre	0.193	0.064	32	3.031	0.005	*
	rTMS-p0wks-pre	0.237	0.094	32	2.524	0.017	*
	sham-p0wks-pre	0.252	0.094	32	2.682	0.011	*
	rTMS-p4wks-pre	0.267	0.090	32	2.968	0.006	*
	sham-p4wks-pre	0.119	0.090	32	1.319	0.196	
	Δ rTMS - Δ sham	0.163	0.102	32	1.599	0.120	
WMFT (Arm Strength)	both-p0wks-pre	1.722	1.201	32	1.434	0.161	
	both-p4wks-pre	0.889	0.407	32	2.182	0.037	*
	rTMS-p0wks-pre	0.778	1.699	32	0.458	0.650	
	sham-p0wks-pre	2.667	1.699	32	1.570	0.126	
	rTMS-p4wks-pre	0.556	0.576	32	0.964	0.342	
	sham-p4wks-pre	1.222	0.576	32	2.122	0.042	*
	Δ rTMS - Δ sham	1.222	2.374	32	0.515	0.610	
WMFT (Grip Strength)	both-p0wks-pre	-1.222	0.977	32	-1.251	0.220	
	both-p4wks-pre	-1.000	0.885	32	-1.129	0.267	
	rTMS-p0wks-pre	-0.556	1.382	32	-0.402	0.690	
	sham-p0wks-pre	-1.889	1.382	32	-1.367	0.181	
	rTMS-p4wks-pre	-0.111	1.252	32	-0.089	0.930	
	sham-p4wks-pre	-1.889	1.252	32	-1.509	0.141	
	Δ rTMS - Δ sham	0.444	2.561	32	1.740	0.863	

Table 3.8 The estimate, standard error (std error), t-statistic, degrees of freedom (df) and p-value for each contrast of the exploratory linear mixed effect models for each subsection of the Wolf Motor Function Test (WMFT). The following key for statistical significance (sig.) was used: * = $p<0.05$. Because this analysis was exploratory, a threshold for multiple comparisons was not determined.

CHAPTER 4:
Summary and future directions

4.1 Summary of results

The objective of this dissertation was to address the current need to improve stroke motor recovery outcomes by studying function of the distal UE function, specifically the hand and wrist, in patients suffering chronic stroke that at least partially involved the primary motor system (M1 and associated CST). We studied hand and wrist motor function in two aims. First, we evaluated the neural substrates supporting impaired hand and wrist motor function and hypothesized, based on evidence that reorganization of M1 is crucial for hand motor recovery, that the primary motor system would be associated with hand and wrist motor function in the chronic phase of stroke. Second, we tested a form of rTMS, called $rTMS_{Heb}$, on functional recovery of the hand and wrist. We hypothesized that the application of $rTMS_{Heb}$ during a wrist-training paradigm known to improve distal UE function would enhance training-related motor improvement in the hand and wrist for patients suffering from chronic stroke.

The main finding of our first aim was derived from our exploratory analyses and suggested that the magnitude of the corticospinal output from $M1_{IL}$ may be associated with the extent of impaired hand, but not wrist, function depending on lesion volume. We did not find evidence that the measures of M1 and CST structure were related the variability in hand or wrist motor function; although, we hypothesize that this result may be related to the fact that our patients had measurable MEP in response to TMS of $M1_{IL}$. The presence of a TMS-evoked MEP indicates that studied population was biased towards more favorable $M1_{IL}$ and CST_{IL} structural and functional integrity, as the ability for TMS of $M1_{IL}$ to evoke MEP is dependent on the temporal and spatial summation of descending corticospinal volleys at the level of the alpha motoneuron pool (Di Lazzaro et

al., 1998b). Overall, these findings are particularly important for stroke neurorehabilitation research as the role of $M1_{IL}$ and CST_{IL} in supporting affected hand and wrist function has not been previously studied in humans in great detail. Traditionally, human stroke recovery research has focused on whole arm motor function (Ward et al., 2003a; 2003b; Schaechter et al., 2006; Stinear et al., 2006; Bestmann et al., 2010; Puig et al., 2010; Zhu et al., 2010; Gauthier et al., 2012; Page et al., 2013; Sterr et al., 2013; Stinear et al., 2014; Feng et al., 2015; Quinlan et al., 2015; Park et al., 2016), the results of which cannot be generalized to the hand due to anatomical differences in the innervation of UE and hand muscles from the descending spinal projections (Lemon, 2008).

The main finding of our second aim was that $rTMS_{Heb}$ may prolong the retention of training-related increases in hand motor function in patients with chronic stroke but should be verified in a study with a larger sample size. We did not find evidence that $rTMS_{Heb}$ improved the training-related acquisition of motor skill as patients who received $rTMS_{Heb}$ experienced a similar level of motor improvement after training than patients who received $rTMS_{sham}$. Further work is needed to understand the neural mechanism by which $rTMS_{Heb}$ would support motor skill retention, but we speculate $rTMS_{Heb}$ facilitates the neural process involved in transferring the motor memory out of $M1_{IL}$ for consolidation into other brain regions, such as the cerebellum. While $rTMS_{Heb}$ has been previously reported to enhance training-related hand motor improvement in healthy human adults and $M1_{IL}$ reorganization after stroke, this study provides the first evidence that $rTMS_{Heb}$ may also lead to greater training-related hand motor function in a chronic stroke population (Buetefisch et al., 2004b; 2011; 2015).

4.2 Conclusions

In conclusion, this dissertation provides evidence for M1 and CST to support impaired hand, but not wrist, function in patients with sustained damage of the primary motor system after stroke. A limitation of this body of work is that the findings of this dissertation cannot be generalized to stroke of other regions. However, we would argue for the importance of stratifying patients at least partially based on lesion location when considering the development of new rehabilitative techniques, as the neural substrates supporting motor recovery likely vary between stroke of different brain regions (Corbetta et al., 2015). By restricting our studied population to patients whose infarct included the primary motor system, we were able to examine in great detail the functional role the surviving tissue of $M1_{IL}$ and CST_{IL} plays in supporting hand motor function after injury. Further, we conclude that targeting $M1_{IL}$ with $rTMS_{Heb}$ could be of benefit when considering future rehabilitative treatment development for patients with chronic stroke of the primary motor system.

4.3 Future directions

Current research on the theraputic effect of rTMS, including that of this dissertation, has primarily focused on the application of rTMS to M1 based on the crucial role M1 plays in UE motor recovery after stroke in the rodent (Jones et al., 2009), non-human primate (Nudo and Milliken, 1996; Nudo et al., 1996b) and human (Liepert et al., 2000b; Sawaki et al., 2008). Considering evidence that M1 rTMS supports training-related motor improvement by facilitating M1 reorganization (Buetefisch et al., 2004b; 2011; 2015), M1 rTMS may not be the most effective target for patients with extensive M1 damage. In the future, the use of alternative rTMS targets, such as secondary cortical

motor areas, could be studied for these patients. Although secondary motor regions, such as PMC, SMA and posterior parietal lobe, participate as substrates to mediate recovery, their functional role may be influenced by several factors, including but not limited to lesion size (Touvykine et al., 2015), lesion location (Luft et al., 2004) and degree of motor impairment (Johansen-Berg et al., 2002; Ward et al., 2003b). Therefore, these patient characteristics should be considered during the development of alternative rTMS protocols.

For instance, the PMC has gained recognition as an important substrate in mediating UE motor recovery in patients who sustain more severe UE motor impairment (Johansen-Berg et al., 2002; Ward et al., 2003b). Consequently, PMC may be an effective rTMS target for patients experiencing a larger motor deficit. In humans, the PMC composes part of BA6 and expands across the anterior lip of precentral sulcus and the posterior regions of the middle and superior frontral gyri (Kantak et al., 2012). The PMC has two primary subdivisions, the dorsal PMC (PMd) and ventral PMC (PMv), which each have distinct cytoarchitecture and extrinsic connectivity (Kantak et al., 2012). In the macque monkey, the PMd and PMv can be even further subdivided with PMd including areas F2 and F7 and with PMv including areas F4 and F5 (Kantak et al., 2012). However, because the human cortex has not been described in as much detail as the non-human primate, further subdivisions of PMd and PMv in the human are unclear.

We argue that study of the PMC as an alternative rTMS target should begin in PMd based on anatomical and functional evidence that the PMd is better developed for supporting motor control in patients with cortical damage. For instance, PMv contains far fewer corticospinal projections than PMd (He et al., 1995), causing some to speculate that

PMv primarily supports motor control by modulating the corticospinal discharge of other motor structures (Quessy et al., 2016). On the other hand, the dense corticospinal projections of PMd would allow the PMd to bypass cortical damage by projecting directly to the spinal cord (He et al., 1995). Further, because the PMd projects bilaterally to the spinal cord and has less lateralized task-related activity than M1, both the ipsilesional and contralesional PMd could serve as rTMS targets (Kuypers and Brinkman, 1970). This would also allow for the contralesional PMd to be targeted when precentral gyrus is damaged.

Evidence for the participation of PMd and PMv in motor recovery after stroke comes from study of the human and non-human primate. In squirrel monkeys, large lesions to M1 led to expansion of the distal forelimb area of the PMv (Frost et al., 2003). Further, the size of the PMv expansion was proportional the size of M1 lesion (Frost et al., 2003). In a separate study, anatomical changes in the PMv architecture, including the expansion of PMv terminal fields in M1, were found to parallel the neurophysiological increase in distal forelimb area size after M1 lesion (Dancause et al., 2005). However, when the M1 lesion was smaller (less than 50% of M1), the distal forelimb area of PMv reduced in size regardless of the lesion placement with M1 (Dancause et al., 2006). Together this evidence suggests that the PMv, as a functionally-connected region of M1, is capable of undergoing substantial anamical and functional change after M1 lesion, although the degree of PMv plasticity may be related to M1 lesion size.

Further study of PMd and PMv reorganization has provided compelling evidence that adaptive plasticity of PMd and PMv is functionally relevant to UE motor recovery. For instance, inhibition of PMv and PMd with bilateral muscimol injection with M1

lesion transiently worsened motor impairment in macaque monkeys (Liu and Rouiller, 1999). Further, the reaction time of individualized finger movement was slowed when PMd activity of the ipsilesional or contralesional hemisphere was temporarily disrupted with high intensity TMS in patients suffering chronic stroke of variable lesion location and size (Johansen-Berg et al., 2002; Fridman, 2004; Lotze et al., 2006; Takeuchi et al., 2007). Together, these findings suggest that involvement of PMd during hand motor function after stroke is functionally significant and provides evidence against the hypothesis that the increase in bilateral PMC activity observed during UE motor function after stroke is maladaptive (Weiller et al., 1992; Seitz et al., 1998; Johansen-Berg et al., 2002). Because the slowing of visually-cued finger movement was proportional to the degree of motor impairment in the human after stroke, PMd may acquire a larger role in UE motor recovery in patients with more severe motor impairment (Johansen-Berg et al., 2002). As additional evidence, a negative correlation has been reported between hand motor task-related activity and UE motor function in many regions including the PMd and PMv (Ward et al., 2003b). Altogether, these findings demonstrate that recovery-related reorganization of PMd intracortical networks is functionally relevant, especially in patients with greater motor deficit, and could potentially be targeted by PMd rTMS to support stroke motor recovery.

A possible challenge in the development of a PMd rTMS protocol is the difficulty in identifying the optimal region of the PMd to target. In M1, the optimal coil positon for rTMS is determined by locating the cortical region that evokes the MEP of largest peak-to-peak amplitude in the muscle of interest at the lowest TMS intensity (hotspot) (Buetefisch et al., 2004b; 2011; 2015). Because PMC has a similar somatotopic

organization to M1, the identification of hotspots for specific hand or UE motor regions in the PMC is also possible (Godschalk et al., 1995). However, the higher intensities needed to determine motor hotspots of PMC are not always comfortable for patients (Münchau et al., 2002b). To avoid using TMS intensities that are not well tolerated, most studies requiring TMS of the PMd use coordinates derived from PET imaging of the brain where the hand motor region of the PMd is approximately 2 cm anterior and 1 cm medial to the hand motor region of M1 (Fink et al., 1997). Alternatively, the location of peak hand motor-task activity, dervied from a region of interest (ROI) analysis of the PMd with fMRI, has been previously used with succcess as an rTMS target in a stroke population (Lotze et al., 2006). The use of an ROI to guide the rTMS coil position may be especially important for patients with large cortical stroke where an M1 hotspot is not always identified easily.

In conclusion, there are many directions that could be taken in the development of alternative rTMS targets for specialized cohorts of patients, including the study of PMd as a rTMS target for patients with more severe motor deficits. This line of work would build upon the exisitng literature, including this dissertation, on the efficacy of rTMS in stroke motor recovery and would be a step toward the development of alternative rTMS protocols for patients in which M1 rTMS is not feasble.

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