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Differential Effects of Low-Frequency rTMS on Motor Performance, Cortical Excitability and Inhibition in Aged Healthy Participants

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Abstract Cover Page

Differential Effects of Low-Frequency rTMS on Motor Performance, Cortical Excitability and Inhibition in Aged Healthy Participants

By

Lauren Edwards

B.S., University of Arizona, 2016

Advisor: Michael Borich, DPT/Ph.D.

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Abstract

Differential Effects of Low-Frequency rTMS on Motor Performance, Cortical Excitability and Inhibition in Aged Healthy Participants

By Lauren Edwards

Low frequency repetitive transcranial magnetic stimulation (LF-rTMS) of the primary motor cortex (M1) alone, or when combined with sensorimotor training, can induce changes in neuronal activity at both the stimulated location and in remotely connected cortical areas. Level B evidence supports the use of LF-rTMS over the contralesional M1 for the treatment of post-stroke hemiparesis, an effect that may be mediated through interhemispheric connections with the non-stimulated ipsilesional M1. However, the effects of LF-rTMS are variable and the biological substrate underlying these therapeutic effects remains controversial. Furthermore, despite the highest prevalence of stroke in individuals above 65 years old, many current rTMS studies in healthy adults consist of younger cohorts. The objective of the present study is to characterize the modulatory effects of LF-rTMS on motor performance and cortical excitability, in both the stimulated and non-stimulated M1, and between each M1, in an older healthy population. Twenty right-handed healthy older adults (60 ± 7.2 years old, 13 females) with normal cognition and brain structure underwent 3 LF-rTMS experiments where rTMS was applied at 1 Hz (15 minutes, 900 pulses) over the left M1. Three different intensities of rTMS were used: 80% of resting motor threshold (RMT), 90% of RMT, and sham stimulation using a placebo coil. To evaluate the effects of LF-rTMS on M1 excitability, motor evoked potentials (MEPs) were collected from the extensor carpi ulnaris muscle before and after administration of LF-rTMS. Single- and paired-pulse TMS paradigms were used to assess general corticospinal excitability, intracortical inhibition and inter-hemispheric inhibition. A unimanual pointing task was used to assess motor performance of each hand. Results demonstrated that LF-rTMS did not significantly impact excitability or inhibition of either the stimulated or non-stimulated M1. LF-rTMS did differentially impact motor performance by hand with the contralateral hand showing no rTMS-related effects, but the ipsilateral hand having a reduction in improvement after LF-rTMS at 90% of RMT. A single administration of LF-rTMS does not significantly modify cortical excitability and has modest effects on motor performance in older adults, which is important for LF-rTMS application in the treatment of stroke.

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Introduction

Stroke Overview

Stroke is defined as an infarction of central nervous system tissue attributable to ischemia or hemorrhage, based on neuropathological, neuroimaging, and/or clinical evidence of permanent injury [1]. Stroke is the fourth leading cause of death and remains the number one leading cause of long-term adult disability [2]. Furthermore, the loss of productivity after stroke currently costs the United States an average of $33.9 billion per year and is expected to reach $56 billion by 2030 [3], making stroke a public health crisis. A primary contributor to persistent disability after stroke is incomplete motor recovery [4]. Spontaneous biological recovery of motor function occurs during the first months after stroke [5], underlying a current emphasis on intensive early intervention, although results are often mixed and complex [6]. Despite intensive therapy, upper extremity impairment resolves up to 70% of baseline function for a given patient with some patients showing even less recovery than predicted [7]. Most stroke survivors are left with a limited ability to perform skilled hand movements necessary for daily functioning [8]. To reduce disability after stroke, there is a need to improve our understanding of the neuronal network physiology necessary to regain skilled functional hand use.

Normal sensorimotor control of voluntary movement

The primary motor cortex (M1) plays a critical role in the execution of voluntary movements. Upper extremity movement execution is particularly dependent on descending output from M1 through the spinal cord to upper limb muscles. Pyramidal neurons in layer V have axons that are bundled together as a significant portion of the corticospinal tract (CST), where 85-90% of the fibers decussate in the medullary pyramids to provide control to the hand contralateral to the hemisphere of the M1 [9]. The remaining fibers, approximately 10-15%, maintain ipsilateral

projections and control trunk and proximal musculature [10]. Of the M1 cortical axons that terminate in the spinal cord, some of these projections indirectly influence movements by synapsing onto interneurons in the intermediate zone [11], whereas direct control arises from the cortico-motoneuronal (CM) cells that terminate monosynaptically on α-motoneurons in the ventral horn of the spinal cord [12]. These α-motoneurons innervate skeletal muscles that control contralateral muscle contractions, and subsequently, voluntary movements [11, 13].

The strongest direct projections from M1 to α-motoneurons terminate on motoneurons that innervate hand muscles allowing for direct and individualized control of fingers required for complex and skilled hand movements [14]. A lesion of CST axonal fibers that specifically causes loss in individualized finger function [15, 16] is the leading cause of motor disability in stroke, reiterating the importance of this connection from M1 to the α-motoneurons innervating muscles of the hand. While CST is the largest contributor to skilled hand movements, there are other pathways, such as the reticulospinal tract, that offer additional contributions to certain aspects of hand function (see [17] for review). The topographical organization of M1 demonstrates a larger spatial representation for the hand reflecting the relative importance of the output from CM cells to hand muscles [18]. The populations of CM cells in M1 fire differentially based on the actions of the target muscle (i.e. if the target muscle is used as an agonist vs an antagonist) to allow for a variety of functional uses of the hand [19]. Within these populations, individual neurons can be tuned to preferentially code for single or multiple fingers or more proximal joints [20], and the kinematics of a movement, such as direction, force, and speed are also encoded [21-23]. This level of specification in M1 neuronal tuning allows for the execution of an extensive repertoire of complex hand movements.

The left and right M1s are connected via the corpus callosum, which allows for interhemispheric inhibition (IHI), a mechanism that plays a critical role in motor control. IHI allows for the lateralization of information processing and integration such that one M1 does not interfere with the other [24]. Inhibiting the ipsilateral M1 (iM1) improves unilateral task performance. It has been postulated that the suppression of iM1 excitatory activity decreases IHI from the iM1 onto the contralateral M1 (cM1) [25]. While it had previously been thought that the cM1 only is activated during unilateral movements [26], more recent studies have also reported evidence of iM1 activation [27, 28]. Bilateral activation of M1s during unilateral movements is thought to be related to the level of complexity of the motor task, such that more demanding tasks involve an increase in iM1 activation even after controlling for muscle activity of the non-performing hand [29]. Bilateral M1 activation and excitability is further complicated after the brain has undergone stroke as stroke induces changes in neural activity that can influence the activation and interaction of the hemispheres during the production of movement

Abnormal sensorimotor control of voluntary movement after stroke

An infarction in the middle cerebral artery (MCA) is the most common type of stroke [30]. Given the MCA supplies cortical and subcortical sensorimotor regions, stroke in this vascular territory has a greater likelihood of affecting sensorimotor control of movements. Therefore, our research focuses exclusively on MCA strokes affecting the CST although strokes in other vascular territories may also impact sensorimotor integration [31]. There are dynamic processes post-stroke that change as a function of time and affect the neurophysiology of sensorimotor integration. Time post-stroke is defined in phases: hyper-acute (0-24 hours); acute (1-7 days); early subacute (7 days-3months); late subacute (3-6 months); and chronic (>6 months) [32]. Initial neuronal cell death in the lesion core leads to both structural and functional disconnection with brain regions outside the

primary area of infarct [33]. Motor recovery occurs, in part, from spontaneous biological recovery where the lesioned brain tissue transitions from a state of acute injury marked by cell death and inflammation, towards recovery via molecular and cellular changes (i.e. increased neuronal sprouting, dentritic branching, and growth factors) leading to increased neuronal excitability and experience-dependent plasticity lasting ~3 months post-stroke [5]. Most post-stroke functional recovery occurs rapidly in the early sub-acute phase and the magnitude of improvement slows down in the late sub-acute phase [34]. In the chronic phase post-stroke, motor recovery trajectories plateau, but remain modifiable [35], with less than 20% of patients experiencing full recovery of upper extremity motor function [36].

Upper extremity paresis is the most predominant motor impairment after MCA stroke, which is likely due to a lesion in the CST that serves skilled hand movements [37]. Paresis can contribute to deficits in both the initiation and termination of voluntary movements of the wrist [38]. Other motor deficits include spasticity and impaired motor control [39], with 85% of patients in the chronic phase post-stroke still possessing residual motor deficits [34]. Common somatosensory modalities affected after stroke are tactile sensation, proprioception, and stereognosis [40]. It has been recently reported that 62% of acute stroke patients demonstrated deficits in their ability to locate their hand and arm in space [41]. Deficits in proprioception have direct implications on motor control as information about the arm and hand are necessary for proper movement and important for improving sensorimotor function after stroke [42]. Due to the reliance of the motor system on sensory information for movement optimization, sensory impairments are expected to have motor repercussions. Similarly, sensory deficits can occur in cases of ischemic lesions confined to the M1 motor pathway that do not directly damage

somatosensory afferents [43], suggesting that sensorimotor integration can be disrupted even in the absence of somatosensory afferent pathway lesions, which will have behavioral manifestations.

Clinically, sensorimotor deficits are usually described in terms of separate assessments of either sensory or motor deficits. Sensory and/or motor deficits after stroke have been routinely measured using observer-based clinical scales either focused on measuring level of impairment, with scales such as the Fugl-Meyer Assessment [44] and Nottingham Sensory Assessment [45], or focused on measuring level of function with the Wolf Motor Function Test [46] and the Jebsen Taylor Hand Test [47]. However, there are several limitations of standard observer-based clinical assessments including: decreased reliability and sensitivity compared to objective assessments, lack of precision with non-continuous data, and greater susceptibility to floor and ceiling effects of performance [48]. Therefore, there is a need for objective assessments to better characterize not only post-stroke sensorimotor behavioral deficits, but also characterize the brain circuitry underlying behavior.

Stroke alters the connections between the M1s and their involvement in control of movements, particularly in the control of the paretic hand. In the post-stroke subacute phase, there is a shift towards more bilateral M1 activation for paretic hand movement control, even during the performance of simple tasks. However, when patients have good recovery in the chronic phase, their M1 activation pattern is more lateralized, comparable to healthy controls [49]. The M1 with the stroke lesion, called ipsilesional M1, has been shown to have reduced excitability compared to the M1 contralateral to the lesion, called the contralesional M1 [50]. This asymmetry in excitability is thought to create a disparity in IHI such that the contralesional M1 excessively inhibits the ipsilesional M1, which would be expected to have behavioral consequences. In 2004, a published study measured the extent of IHI between the contralesional and ipsilesional M1s as chronic stroke

patients prepared to perform a simple motor task. It was determined that patients with abnormally high IHI from contralesional onto ipsilesional M1 close to movement onset also had poor motor performance [51]. This was the beginning of the narrative that contralesional M1 should be inhibited to better aid recovery of stroke patients. However, the role of the contralesional M1 in stroke motor recovery is still quite controversial. While some studies have shown that abnormal contralesional M1 excitability negatively impacts recovery [51-54], other studies have demonstrated a supportive role of increased contralesional M1 excitability in recovery outcomes [55-58]. Therefore, results are currently inconclusive in determining the key factors that underlie contralesional M1's role in motor recovery. The advent and use of non-invasive brain stimulation (NIBS) has helped not only characterize pertinent factors like cortical excitability, but NIBS brings forth the possibility to modulate the abnormal balance between M1s after stroke.

Investigating neural mechanisms of sensorimotor control in humans with non-invasive brain stimulation

Transcranial magnetic stimulation (TMS) has become a powerful tool to non-invasively stimulate the primary motor cortices and provide objective assessments about the physiological state of the corticospinal output [59]. TMS is applied using activation of an electromagnetic coil that delivers electric charge to produce a current in the wire circuitry that induces a magnetic field [60]. This current flows parallel to the field of stimulation coil when the coil is placed tangentially onto the scalp overlaying M1 [61]. The magnetic field passes through the skull to produce an electric current proportional to the time rate of change of the magnetic field that subsequently depolarizes the cell membranes [60]. The current induced produces a descending volley with different components: the D wave represents direct activation of the fast conduction pyramidal tract neurons in layer V of M1, whereas the I waves represent indirect, transsynaptic activation of

the pyramidal tract neurons [62]. It is postulated that TMS excites the superficial pyramidal neurons in Layers II and III of the cortex as they are the most excitable elements and the main source of inputs to the corticospinal neurons in Layer V, the main output of the motor cortex. This creates the initial I wave, called I1 wave, that is approximately 1 millisecond after the faster D wave [62]. At higher intensities, later volleys appear called late I-waves and are believed to represent a more complex circuitry including cortical interneurons. At higher intensities, there's also the chance to evoke D waves as the pyramidal track neurons are stimulated directly [63]. Once these neurons are stimulated above threshold and the signal is propagated along the CST down to the peripheral motor nerves to elicit a contraction in contralateral muscles called a motor evoked potential (MEP). A MEP is reflective of corticospinal excitability due to both cortical and spinal-segmental contributions. The amplitude of a MEP is measured peak-to-peak using electromyography and is the result of cortical descending volleys. The latency of a MEP is reflective of the conduction time of the CST [64]. The shape of the coil and its placement on the skull determines the properties of the electromagnetic field, and therefore, the characteristics of the evoked MEPs. When the coil is oriented 45° to the midline, TMS induces a posterior-to-anterior (PA) current flow that preferentially activates intracortical circuits within M1 [65]. PA current flow produces the largest MEPs at the lowest thresholds through the activation of I waves [62, 63].

TMS allows for an objective assessment of M1 net excitability and the integrity of the corticospinal tract [66] that is useful as we aim to better understand the role of contralesional M1 after stroke. We examine cortical excitability measured by stimulus response curves (SRC), intracortical inhibition measured by short-interval intracortical inhibition (SICI), and resting interhemispheric inhibition (rIHI) in both the contralesional and ipsilesional M1s. An SRC

measures MEP amplitude growth as a function of increasing stimulus intensity, which is related to the size and number of volleys. A study found that lorazepam, a gamma-aminobutyric acid $(GABA)_A$ receptor-positive allosteric modulator, significantly reduced the SRCs suggesting that GABA influences the corticospinal system likely via the regulation of late I waves amplitudes [67, 68]. The paired-pulse SICI paradigm pairs an early subthreshold conditioning stimulus (CS) pulse with a suprathreshold test stimulus (TS) pulse at a variety of interstimulus intervals (ISIs). ISIs that are less than 5 ms typically lead to TS suppression. Mechanistically, a subthreshold CS suppresses the recruitment of descending volleys that will be elicited by the TS, specifically the late I waves [69]. This suppression is mediated by $GABA_A$ receptors, which late I-waves have an increased sensitivity to [70]. Inhibition can also be measured between the M1s using rIHI paradigm. rIHI pairs a suprathreshold CS over one M1 that precedes a TS over the other M1 at a variety of ISIs. Inhibition from the CS occurs via the corpus callosum that suppresses the TS amplitude via suppression of late I waves; I1 and D waves are not affected by the CS [71] likely due to transmission time of the transcallosal route. It is hypothesized that IHI may be induced by the same neuronal populations that contribute to long-interval GABAB-mediated intracortical inhibition, although the results are inconclusive. While a long-interval ISI like 40 ms has proven to be mediated by $GABA_B$ receptor mediated inhibition, it is not clear what mediates rIHI at an ISI of 10 ms [72]. However, evidence does support that SICI and rIHI are mediated by different neuronal populations [73, 74] making it more likely that $GABA_B$ receptor-mediated inhibition has a predominant role in rIHI. Ultimately, the probe of rIHI allows us to characterize the inhibitory balance between M1s, and this balance could be potentially modulated using repetitive trains of TMS.

Single TMS pulses delivered at repeated intervals lead to the development of repetitive TMS (rTMS) such that instead of assessing the excitability of the brain, rTMS can induce changes that outlast the stimulation period. A review evaluating the use of combined TMS and electroencephalography (EEG) studies to capture the physiological post-effects of single session rTMS demonstrated that the effects generally last ~1 hour although the direction of effects, whether they lead to facilitation or suppression, are not easily sorted [75]. Nonetheless, the ability for changes to outlast stimulation and potentially bring forth persistent changes in behavior provides unique therapeutic potential in the treatment of neurological disorders such as stroke. However, the exact understanding for how these phenomena are even mediated is not entirely known. The effect of rTMS is largely dependent upon parameters such as intensity and duration of stimulation. The most important parameter is stimulation frequency where low-frequency (LF) stimulation ($\leq$ 1 Hz) is believed to be inhibitory and high-frequency stimulation ($\geq$ 5 Hz) is generally thought to be excitatory to M1.

It is widely believed that rTMS-induced changes in neuronal activity are mediated by long-term potentiation (LTP) and long-term depression (LTD)-like mechanisms [76]. LTP in M1 is considered a primary synaptic process involved in the experience-dependent plasticity that underlies motor learning [77-81]. At the synaptic level, a bidirectional range of dynamic modifiability exists, such that a synapse experiences a limited amount of synaptic strengthening (LTP) or reduction in strength (LTD) [82]. Cortical LTP is largely mediated through activation of N-methyl-D-aspartate (NMDA) receptors at glutamatergic synapses [83]. In addition to glutamatergic synapse contributions to experience-dependent plasticity, GABA synaptic modifiability is another important contributor to plasticity. GABA is the main inhibitory

neurotransmitter in the brain [84], and transient reductions in GABAergic inhibition have been shown to be necessary for LTP induction [84-86].

LTD induction is mediated by an increase in GABAergic inhibition and long-lasting depression of NMDA-mediated glutamatergic synaptic transmission in response to LF rTMS [87]. rTMS is believed to induce synaptic plasticity through LTP/LTD-like mechanisms because it shares similar outcomes: the effects outlast the stimulation; the frequency is the biggest indicator for results; and that prior stimulation and/or physiological activity impact responsiveness to rTMS [88]. However, because of the limited number of human studies, additional evidence is needed to provide a direct link between rTMS effects and synaptic LTP/LTD mechanisms. Cervical epidural recordings did demonstrate that 1 Hz rTMS above threshold does not impact the I1-wave, but does reduce the size of the late I-waves [89]. This suppression was correlated with the change in the MEP amplitude demonstrating that rTMS does have an impact on the excitability of the circuitry [89]. For more in-depth discussion about potential mechanisms of rTMS, including alternatives to LTP/LTD, see [90] for a detailed review.

Level B evidence currently exists for the use of rTMS in the treatment of stroke. A recent review analyzed 7 meta-analyses conducted to measure the efficacy of rTMS applied to motor recovery post-stroke. It was found that while LF-rTMS over the contralesional hemisphere seems to be the most promising (compared to high-frequency stimulation over ipsilesional M1), there are still conflicting results that require further comprehensive evaluation [91]. Another promising benefit of rTMS is the ability for it to induce changes not only at the site of stimulation, but to have modulatory effects on the functional connectivity of larger cortical networks in both healthy controls [92, 93] and stroke patients [94, 95]. These effects can be further capitalized on with the addition of motor training to the rTMS protocols [96]. While the motor training addition has had

success in some individual stroke studies [97-99], a systematic review analyzing the added benefit of rTMS to behavioral training of the upper extremity across 11 studies, did not show any extra benefit from the training by itself [100]. Many uncertainties remain about the patients who will benefit from which protocols still exist, making the field unable to fully elucidating the therapeutic potential of LF-rTMS in the treatment of stroke.

Similar to the uncertainty of effects of rTMS in stroke, the effects of rTMS on the intact brain remain unclear. It is difficult to compare rTMS efficacy across studies due to differences in parameter settings (frequency, intensity, number of pulses); lack of studying comprehensive effects of rTMS (only examining the stimulated M1, lack of behavioral measures); and the use of varying paradigms to assess the same fundamental outcome measures (i.e. single pulse for excitability at various intensities or sizes versus a SRC). A detailed summary of rTMS studies in healthy individuals is provided in Table 1. Even when studies have used similar parameters, the results were variable. Furthermore, the majority of the foundational studies establishing the effects of rTMS in a healthy brain have been conducted in younger adults and therefore the impact of aging has not been adequately accounted for. Given that the incidence of stroke increases with age with an increased prevalence of strokes occurring in individuals over 65 years old [101], there remains a need to have a comprehensive assessment of rTMS neuromodulatory effects in an aged healthy population representing the typical age of the stroke population. The goal of our present study to characterize the comprehensive modulatory effects of LF-rTMS on motor performance, cortical excitability, and inhibition in an older healthy population.

Table 1 – A detailed summary of rTMS studies evaluating healthy controls[1]

Study	Frequency (Hz)	Intensity (%RMT)	# of Pulses	Participant Information	Outcome Measures	Primary Findings

Chen et al. 1997 [102]	0.1, 0.9,	105, 115	360, 810	N=14 RHand (10 males, mean age 44.7 years old; range 27-65)	Stimulated LM1 MEP amplitude of APB muscle and finger-tapping speeds	0.9 Hz led to ↓ excitability. No changes in motor performance.
Ziemann et al. 1998 [103]	0.1	120	180	N=5 RHand and 2 LHand (7 males, mean age 28.9 ± 8.4 years old)	Stimulated LM1 MT, MEP amplitude, ICI, and ICF of APB	No effect on any of the variables.
Wassermann et al. 1998 [104]	1	Individualized to produce small (50-500 μV) MEPs	900	N=11 RHand (6 males; mean age 38 years old; range 23-65)	Stimulated LM1 and non-stimulated RM1 Recruitment Curves of FDI	↓ in non-stimulated RM1 excitability via slope decrease. No change in single intensity MEP amplitude.
Siebner et al 1999 [105]	1	90	1800	N=11 RHand (7 males, mean age 40 years old; range 24-65)	Stimulated LM1 RMT, AMT, SRC, LICI, ICI, ICF	No effect on any of the variables.
Maeda et al 2000 [106]	1, 10, 15 or 20	90	240 and 1600	N=36 RHand	Stimulated LM1 MEP amplitude at 120% for excitability using 10 pulses in APB muscle.	For 240 pulses, 1 Hz ↓ excitability by 4.4%. 1600 pulses of 1 Hz ↓ excitability by 34%.
Maeda et al 2000 [107]	1, 10, 20	90	240	N=20 RHand (11 females; mean age 26.3 years old; range 20-41)	Stimulated LM1 MEP amplitude at 120% for excitability using 10 pulses in APB muscle	↓ in excitability for 1 Hz rTMS. ↑ in excitability for 20 Hz rTMS.
Fierro et al 2001 [108]	1, 7	100, 115, 130	30 trials (5 trains x 2 frequencies x 3 intensities) separated by intervals of 1-2 min	N=8 (5 males, age range 24-43 years old)	Stimulated LM1 Intracortical inhibition measured by silent periods recorded from active APB	↓ in intracortical inhibition. Amplitude of facilitated MEPs was unchanged.
Gerschlager et al 2001 [109]	1	90 AMT	1500 (increments of 300 separated	N=8 (6 males, mean age 29.5	Stimulated LM1 MEP amplitude of FDI	No effect.

			by 1 min)rmt	±4.2 years old)		
Fitzgerald et al 2002 [110]	1	115, 85	900	N=9 (8 males, mean age 32.9 ±6.4 years old; range 25-44)	Stimulated LM1 RMT and AMT, MEP size evoked with suprathreshold stimulation, CSP, CI and CF with ppTMS in APB	↓ in excitability (via RMT) and 115% RMT reduced MEP amplitude. No effect on CSP or CI/CF.
Romero et al 2002 [111]	1	90	600	N=20 RHand (12 males; mean age 29 years old, range 20–46)	Stimulated LM1 MEP amplitude at 120% RMT, SICI (2 ms) and ICF (10 ms) of the contralateral FDI.	↓ in intracortical facilitation. ↓ in excitability (MEP amplitude) in training blocks that were 10-25 min after rTMS. Intracortical inhibition unchanged.
Sommer et al 2002 [112]	1	90	900 monophasic and biphasic	N=10 (6 males; mean age 25.7 years old, range 19–31)	Stimulated LM1 MEP amplitude (1mV response). Recording MEPs every 180 rTMS pulses.	↓ in excitability (MEP amplitude) after monophasic rTMS only.
Munchau et al 2002 [113]	1	70, 80 and 90 AMT	1200	N=13 RHand (10 males, mean age 34.2 ± 4.7 years old)	LM1 stimulated RMT, AMT, ICI/ICF, CSP, and MEP amplitude at 120% AMT of FDI	No effect on any of the variables.
Modugno et al 2003 [114]	1	90	900	N=14 (10 males; age range 24-36 years old)	MT, MEP amplitude, CSP, SICI (3 ms)/LICI, ICF of right FDI	↓ in intracortical inhibition 16-30 minutes after rTMS. All other variables unchanged.
Gilio et al 2003 [115]	1	Individualized to elicit a left-to-right IHI of 70%	900	N=10 RHand (5 males, mean age 30 years old; range 20-39)	Stimulated LM1 and non-stimulated RM1 MEP amplitude, SICI (ISI of 2	↓ in Left-right IHI and ↑ in non-stimulated RM1 MEP amplitude.

					and 4ms), ICF (9 and 12 ms), Left-right IHI (10 ms), CSP and ISP recorded from FDI	All other variables unchanged.
Plewnia et al 2003 [116]	1	115	800	N=8 RHand (mean age 23.9 ± 2.4)	Stimulated LM1 and non-stimulated RM1 MEP amplitudes at 140 and 180% MT; (2 ms, TS 140% MT, CS 80%) and ICF (10 ms)	↓ LM1 excitability ↓ in intracortical inhibition in the non-stimulated RM1. No change in excitability in RM1.
Stinear and Byblow 2004 [117]	1	Individualized calculations with active threshold	1200 (4 blocks of 300)	N=7 RHand (5 males; mean age 47 years old, range 37–56)	Stimulated LM1 Active threshold for excitability, and SICI of the dominant hand FDI.	↓ in excitability in using active threshold ↑ CSP. Intracortical inhibition unchanged..
Khedr Gilio Rothwell 2004 [118]	0.6 paired rTMS	80% AMT	500	N=12 RHand (6 males; mean age 35.5 ± 8.43 years old; range 18–44)	Stimulated LM1 AMT, RMT, MEP recruitment curve, ICI at different ISI, CSP of the right FDI with rTMS on the LM1.	↓ in excitability and ↑ in intracortical inhibition and cortical silent period.
Kobayashi et al 2004 [119]	1	90	600	N=16 (12 males, mean age 29.6 ± 3.6 years old; range 25–35)	Stimulated LM1 MT, MEP amplitude, long and short paired pulse, motor performance.	↑ in ipsilateral hand motor performance. ↑ in intracortical facilitation and ↓ in intracortical inhibition.
Brighina et al 2005 [120]	1	90	900	N=8 (5 females, mean age 30.4 ± 4.3 years old)	Stimulated LM1 MT, amplitude of TS alone and then SICI (2 ms) and ICF (10 ms) of the contralateral APB.	↓ in intracortical inhibition. No change in excitability.

Daskalakis et al 2006 [121]	1, 10, 20, Priming (but focusing on 1Hz)	90	900	N=12 RHand (9 males; mean age 40.2 ± 12.7 years old; range 22–61)	Stimulated LM1 MT, MEP amplitude, SICI, and ICF. Recorded from the contralateral FDI.	↑ in CSP. No change in intracortical inhibition or excitability.
Dafotakis et al 2008 [25]	1	100	600	N=9 (7 males; mean age 27 ± 6 years old; range 22–61)	Stimulated LM1 and RM1 in different experiments measuring motor performance metrics.	RM1 stimulation ↑ ipsilateral hand hand tapping frequency. LM1 stimulation ↑ finger and hand tapping and grasp aperture.
Houdayer et al 2008 [122]	1 and 20	90 and 115 for 1 Hz; 90 for 20 Hz	1800 for 1 Hz and 40 trains of 40 pulses for 20 Hz	N=26 (14 females; mean age 28.5 ± 6.7 years old; range 19–41)	Stimulated LM1 recruitment curves assessed bilaterally of FDI.	No change for 1 Hz at 90% RMT. ↓ in cortical excitability of stimulated M1 for 1 Hz at 115% RMT.
Kobayashi 2010 [123]	1	90	600	N=16 (12 males; mean age: 29.6 ± 3.6 years old; range 25-35)	Stimulated LM1 MEP amplitude, ICI/ICF of FDI and a finger pressing task.	↑ in motor performance of the ipsilateral hand. ↑ in intracortical facilitation. ↓ in intracortical inhibition. No change in excitability.
Chen et al 2015 [124]	1	90	M1 (1200); PMC (1200), and 600 pulses each for PMC + M1	N=10 (7 females; mean age: 25.2 ± 5.4 years old)	Stimulated LM1 SICI, ICF, CSP of right FDI.	↑ in intracortical inhibition and cortical silent period. No effects on ICF.
Chen et al 2018 [125]	1	90	900	N=21 RHand (13 females; mean age 26.1 +- 5.2 years old)	SICI using differing ISIs and CS intensities that were optimized per participant.	No change in intracortical inhibition.

[1] In the case that a study stimulated other regions besides M1, or compared a patient population with healthy controls, please note that such studies are outside the scope of this table. Only information about healthy

controls undergoing rTMS on M1 are included in this table. LM1, left primary motor cortex; RM1, right primary motor cortex; MEP, motor evoked potential; RHand, right handed; APB, abductor pollicis brevis; FDI, first dorsal interosseous; CSP, cortical silent period; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; IHI, interhemispheric inhibition; TS, test stimulus; CS, conditioning stimulus; ISI, interstimulus interval.

Chapter I – Neuromodulation as a probe of excitability and motor performance in healthy aged individuals

Introduction

Repetitive transcranial magnetic stimulation (rTMS) has been routinely used to non-invasively modulate human brain activity. Low-frequency rTMS (LF-rTMS) has been shown to transiently reduce corticospinal excitability of the stimulated primary motor cortex (M1) [89, 102], and to also change neuronal activity in remotely connected cortical areas including the non-stimulated M1 [119]. The changes in the non-stimulated M1 is thought to occur through effects on interhemispheric inhibition (IHI) mediated by direct transcallosal projections from the stimulated M1 onto local, inhibitory circuits in the non-stimulated M1 [73, 126]. The impact on IHI makes LF-rTMS potentially useful in stroke where the pathophysiology is typified by to an imbalance of M1 excitability between the contralesional and ipsilesional M1. The idea is to use LF-rTMS to reduce the hyperexcitability of the contralesional M1 and in turn, reduce the inhibition exerted on the hypoexcitable ipsilesional M1. The functional implication, and overarching goal of this therapeutic neuromodulation approach, is ultimately to improve recovery of motor function of the affected, paretic upper extremity. The application of LF-rTMS to the contralesional M1 corresponds to the paretic hand being the hand ipsilateral to stimulation in healthy adults. Therefore, ipsilateral upper extremity motor performance after LF-rTMS in healthy adults is of particular interest for the comparison of effects of LF-rTMS on the paretic upper extremity.

LF-rTMS in healthy adults has been shown to impact motor performance such that, following stimulation, there is motor improvement in the hand ipsilateral to LF-rTMS on simple motor tasks [25] and an improvement in the hands both ipsilateral and contralateral to the stimulation on motor tasks with higher demand levels [127]. The proposed mechanism of LF-rTMS is thought to alter synaptic plasticity through the induction of long-term depression (LTD)-like changes of neuronal synapses via changes mediated by N-methyl-D-aspartate (NMDA) glutamate receptors [128]. Complementarily, there are also changes affecting γ-aminobutyric acid (GABA)-mediated inhibitory interneuron activity [128, 129] that results in an overall shift in balance between excitatory and inhibitory circuitry towards a decrease in excitatory synaptic transmission. Single- and paired-pulse TMS paradigms, along with motor tasks measuring changes in performance, have been used to assess changes after rTMS stimulation but with mixed results in humans. For example, some studies have shown a suppression of intracortical inhibition in the stimulated M1 [119], whereas other studies have shown either no change [111, 121], or an increase [118, 124], in intracortical inhibition (see Table 1). The results in the literature are highly variable in healthy participants, which further complicates understanding the use of LF-rTMS in a heterogenous population such as stroke.

There are a number of differences across study parameters that make it difficult to compare rTMS efficacy (see above). Of high importance, differences in the aging nervous system could reduce the efficacy of rTMS. Previous studies have demonstrated that changes in excitability and inducing plasticity in response to paired associative stimulation is reduced in an older population [130]. As adults age, there is greater bilateral activation during unilateral movement speculated to be due, in part, to age-related degeneration of the corpus callosum linked to reduced IHI between the M1s [131]. Older adults have slower conduction velocity for corticospinal axons [132], age-

related atrophy of gray matter [133], and there is evidence for GABAergic decline in the aging motor system that could all lessen the ability for neuromodulation [134]. However, limited studies have investigated the effects of neuromodulation strategies in older adults without neuropathology.

The primary objective of the present study is to characterize the modulatory effects of LF-rTMS on motor performance and cortical excitability in both the stimulated and non-stimulated M1, and between each M1 in an older healthy population. Based on the previous literature, the effects of LF-rTMS on motor improvement were expected to be most pronounced in the hand ipsilateral to stimulation, while having no significant effect on the contralateral hand. We expected to see a reduction in excitability in the stimulated hemisphere and an increase in excitability in the non-stimulated M1. These changes in excitability were expected to be mediated by changes in IHI such that inhibition would decrease from the stimulated M1 onto the non-stimulated M1. This comprehensive approach will allow us to identify potential therapeutic targets and inform how to optimally utilize LF-rTMS in stroke.

Materials and Methods

Overview of Experimental Set-Up

Six experiments were carried out on separate days to determine the effect of left M1 (LM1) LF-rTMS (1Hz) on excitability of the stimulated and non-stimulated M1, IHI, and performance accuracy on a novel skilled hand motor task [119]. In three of the six experiments the effect of the different interventions (1 Hz rTMS at 80%MT, 90%MT and sham) were tested on M1 excitability measures. M1 excitbaility and subsequent inhibition were indexed by stimulus response curves (SRCs) and short-interval intracortical inhibition (SICI) and IHI, indexed by resting IHI (rIHI), of both the stimulated and non-stimulated M1 were tested with the participants at rest. In the

remaining three experiments the effect of different intensities for LM1 LF-rTMS on participant's performance on a unimanual joystick task [135] was tested (see below for details). The order of experiments was randomized and separated by at least >24 hours with participants being blinded to the experimental condition and purpose of the stimulation. The study was approved by Emory's Institutional Review Board.

Subjects

Twenty subjects (13 females, 7 males, aged 60 ± 7.2 years) provided consent and were included in the study. All subjects met the following inclusion criteria: age 50-80 years old, right-handedness determined by Edinburgh Handedness Inventory [136], normal magnetic resonance image (MRI) of the brain absent of pathology confirmed by a neurologist, normal cognitive functioning determined by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) [137], no history of neurological disorders, no contraindications to TMS [60], no intake of CNS active drugs that could confound results of the study, ability to elicit a measurable motor evoked potential (MEP) response (> 0.2 mV) and ability to meet accuracy threshold for performance of a unimanual joystick task (see below).

Motor Task

The motor task was designed as a unimanual pointing task that allowed parametric variation of the level of demand on precision imposed by the task through change in target size [29, 127, 135]. Specifically, subjects were asked to manipulate a joystick to move a cursor into target squares of different sizes and locations on a computer screen (Fig. 1). The task was administered using Presentation software (www.neurobs.com) with data stored on the computer for offline data analysis. According to the speed-accuracy tradeoff in Fitts' law, increasing the level of demand on

accuracy will increase movement times [138]. In the present experiment, participants were given a short, predefined time of 2 seconds to complete the task. Given the time constraint, participants were expected to have poorer accuracy for smaller targets [29, 127, 135].

Before and immediately following LM1 LF-rTMS (described in detail below), participants were tested on the pointing task [135]. Participants were comfortably seated in a dental chair that was ~165 cm in front of a computer monitor (29 x 51 cm) displaying the targets for the joystick task. The base of the joystick was attached to a cushioned tablet that rested comfortably on the participant's laps. Participants were instructed to manipulate the joystick using their thumb and middle fingers with their hand resting on the base of the joystick and their arm supported by foam cushions. The manipulation of the joystick also required wrist extension/flexion movements of 5° in addition to the finger movements. Subjects were asked to move a cursor into a target square displayed on the computer screen. Target squares were one of four sizes: small (5.3 x 5.3 mm), medium (9.3 × 9.3 mm), large (13.2 × 13.2 mm), or x-large (17.2 × 17.2 mm).

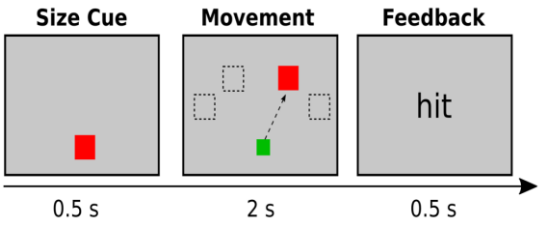


Figure 1. Pointing task depiction. Each trial begins with the display of a red target that serves as a cue informing the size of the target. At 500 ms, the red cursor disappears and a green cursor and white target box appear. The green cursor must be in the home position of (0,0 x y coordinates). The target box can appear in 1 of 4 potential locations. Participants then have 2000 ms to move the cursor into the target box before receiving feedback on accuracy performance i.e. hit or miss.

At the beginning of each trial a red square of the same size as the upcoming target was presented for 500 ms providing advance information about the target size. After the presentation of the cue, the target square and a green cursor were presented for 2 s. The cursor position on the screen represented real-time feedback about the joystick position. With the joystick in its neutral position, the cursor was

located in the center of the screen (x, y coordinates of 0, 0) which was defined as the home position. The target square appeared in one of four spaces: 30, 60, 300, and 330° of the upper halves of the monitor. Participants had a predefined time of 2000 ms to move the cursor from a home position into the target square as quickly as possible and maintain their position until feedback was given, denoted as a "hit" or "miss" (Fig. 1). If the center of the cursor was inside the target square, the accuracy requirements were met. A successful trial was indicated as a "hit" if the accuracy requirements were met and the cursor was located in the home position (x, y coordinates of 0, 0) at the time of the go signal. Failing to meet any of these criteria was defined as an unsuccessful trial and indicated as a "miss."

Prior to the main experiments, participants were trained on the pointing task to ensure inclusion into the study. Participants performed 1-3 training runs (1 run = three blocks comprised of 21 trials/target size; 252 trials per run) of the task to achieve a minimum accuracy of 50% for the largest target, our minimum criteria for inclusion. Participants who performed at this level or better in the first run, continued training until performance improved on the next smallest target size or the maximum number of three runs was completed [135].

TMS Measures of M1 Excitability and Interhemispheric Inhibition

Before and immediately following LM1 LF-rTMS (described below), TMS measures of M1 excitability and rIHI were collected. All measures were obtained in the stimulated LM1 and non-stimulated right M1 (RM1). Electromyographic (EMG) activity (bandpass: 3 Hz to 1 kHz) of the extensor capri ulnaris (ECU) muscle was recorded with surface electrodes (11-mm diameter) in a belly-tendon montage with a 5-cm distance between electrodes using a customized data acquisition program in LabVIEW (LabVIEW, National Instruments, CA, USA). The active electrode was

placed over the motor point of the ECU muscle and the reference electrode proximal to it. Raw EMG was sampled at a frequency of 5 kHz and stored for offline analysis.

Single and paired-pulse TMS were applied using a figure-of-8 shaped coil (70-mm wing diameter) using 2 Magstim 200 stimulators connected via a Bistim module (Magstim Company, UK). The coil was positioned perpendicular to the midline of the skull, oriented 45° to midline to induce a PA current flow that preferentially activates neurons within M1 [65]. The coil targeted the optimal location to produce the largest MEP in the ECU, termed the hotspot. The TMS coil position for stimulation of the hot spot was registered to a reconstructed MRI of each participant's brain using a frameless neuronavigation system (BrainSight, Rogue Research, Montreal, Canada) to ensure accuracy and precision in coil position for targeting the M1 ECU hotspot during all measurements. We measured resting motor threshold (RMT) in the hotspot using the adaptive method of Parameter Estimation by Sequential Testing (PEST) [139].

After identification of the RMT, single-pulse TMS at increasing intensities was used to obtain the stimulus response curve (SRC) [140, 141]. Stimulation began subthreshold at 5% maximum stimulator output (MSO) below the participants' RMT. Subthreshold intensity was confirmed by the absence of any measurable MEP in 10 trials. If a MEP was seen at this intensity, the intensity was decreased again by 5% MSO until there was absence of any MEPs. Intensities were then increased in 5% increments to at least 80% MSO, or until the MEP's size no longer increased with increasing intensity. For each administered intensity, 10 stimuli were given with an interstimulus interval (ISI) of 5s.

Paired-pulse TMS was used to measure intracortical inhibitory networks using short-interval intracortical inhibition (SICI) paradigm [142]. In this paradigm a subthreshold condition

pulse (CS, set at 60% or 80% RMT) preceded a suprathreshold test pulse (TS, 120% RMT) at an ISI of 2ms [143]. We used two different intensities of CS to explore the inhibitory effect of the CS in more detail. Single TS and CS pulses and paired CS-TS pulses were administered in a pseudo-randomized order of sets of 5 TS pulses (10 TS total), 2 CS pulses at 80% RMT(4 CS total) and paired CS-TS pulses (10 paired CS-TS pulses for each CS intensity total). Single CS pulses at 80% MT were given to confirm that stimulation was subthreshold. The timing and sequence of stimuli were controlled through customized software (LabVIEW, National Instruments, CA, USA).

For rIHI, two single Magstim 200 stimulators (Magstim Company, UK) were used and stimulation was administered through two figure-of-eight coils (5 and 7-cm diameter). The smaller coil was selected because of the spatial limitation of a participant's head to accommodate the two coils. The TS was applied through the smaller 5-cm coil because it provides more focal stimulation and subsequent higher spatial resolution. Due to the spatial restrictions of some subject's head size and to ensure similar coil position in all subjects, the CS coil was oriented Lateral-Medial (LM) while the TS coil was oriented in the PA position in all subjects [144]. While participants were at rest, CS and TS were administered to the ECU hot spot of either M1. The intensity of CS and TS were adjusted to produce a 1.0 to 1.5 mV MEP response. Ten paired pulses were applied at ISI of 2 and 10 ms and intermixed with 10 single TS pulses. Single TS pulses and paired pulses were administered in a pseudo-randomized order of sets of 5 single TS pulses (10 TS total) or paired pulses (10 paired pulses at each ISI total). IHI was measured from stimulated M1 to non-stimulated M1, and vice versa with the order being randomized across experimental days.

LM1 LF-rTMS Protocol

The LM1 LF-rTMS protocol has previously been described in detail [127]. Participants were comfortably seated in a dental chair. A tool balancer anchored from the ceiling was used to help with the application of rTMS by offsetting the weight of the coil. Surface EMG was recorded from the right ECU muscle throughout the stimulation process and monitored at a sensitivity of 0.01 mV/div to ensure the subject's muscle relaxation. This sensitivity was also used to confirm subthreshold stimulation based on the absence of MEPs with stimulation. The air-cooled figure-of-8-shaped coil (70-mm wing diameter) or sham air-cooled figure-of-8 coil (70-mm wing diameter) connected to a Magstim Rapid^2 (Magstim Company, UK) were used to apply LM1 LF-rTMS (see above for description of coil placement) to the ECU hotspot of the left M1. At the beginning of each experiment, the RMT was determined for the rTMS coil using PEST [139]. RTMS was applied at 1-Hz frequency for a total of 900 pulses, 15 minutes, to the LM1 at either 80% (rTMS80%) or 90% (rTMS90%) RMT or sham (intervention).

Data Analysis

Motor Task

For the pointing task, the primary outcome measure was accuracy, defined as the number of correct movements expressed as percentage of all movements (% hits) for each target size. The % hits were calculated for left and right hand depending on the different conditions: target size (S, M, L, XL), intervention (rTMS80%, rTMS90%, sham) and time point (pre- or post-intervention). Movement time (MovT) was defined as the time from target presentation until the center of the cursor reached the target and was a secondary outcome measure. Only trials constituting a hit were included in the MovT analysis. For 12 of the 20 subjects (n = 12), MovT was determined using continuously collected trajectory data detailing online cursor position, termed $MovT_{cont}$. For the

remaining subjects (n = 8), individuals were instructed to press a button located on the top of the joystick with their index finger to indicate that the cursor had reached the target [135], termed $MovT_{disc}$. $MovT_{cont}$ and $MovT_{disc}$ were analyzed separately for the right and left hand depending on the different conditions: target size (S, M, L, XL), intervention (rTMS80%, rTMS90%, sham) and time point (pre- or post-intervention).

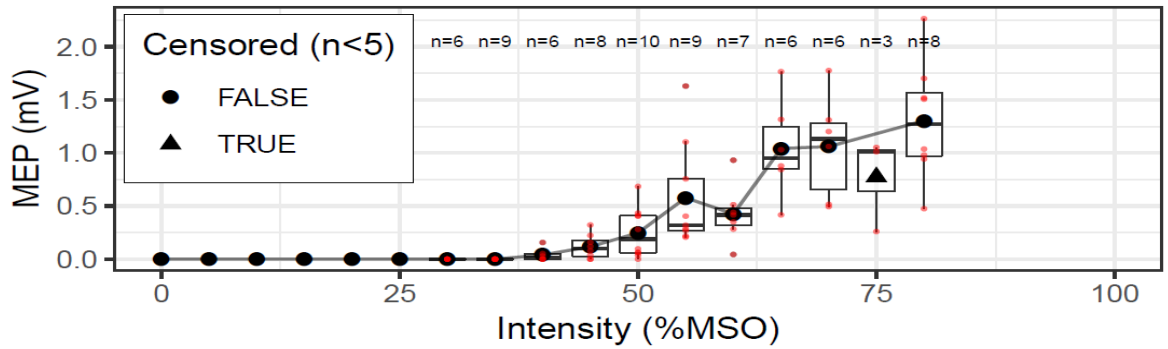
EMG Analysis

EMG data were analyzed in LabVIEW. EMG recordings were visually inspected and trials with increased EMG background activity, i.e. amplitudes exceeding 50 uV in the in the 20 ms preceding the TMS pulse, were excluded from further analysis. A minimum of 5 of the 10 trials were required for calculation of the mean and SD of peak-to-peak MEP amplitudes at each intensity per subject, otherwise the data point was coded as a missing data and were censored out of the data set (see Figure 2A) [56, 57, 127, 145]. Outliers were defined as exceeding a boundary defined by the 75th percentile of the sample + 3 times the interquartile range.

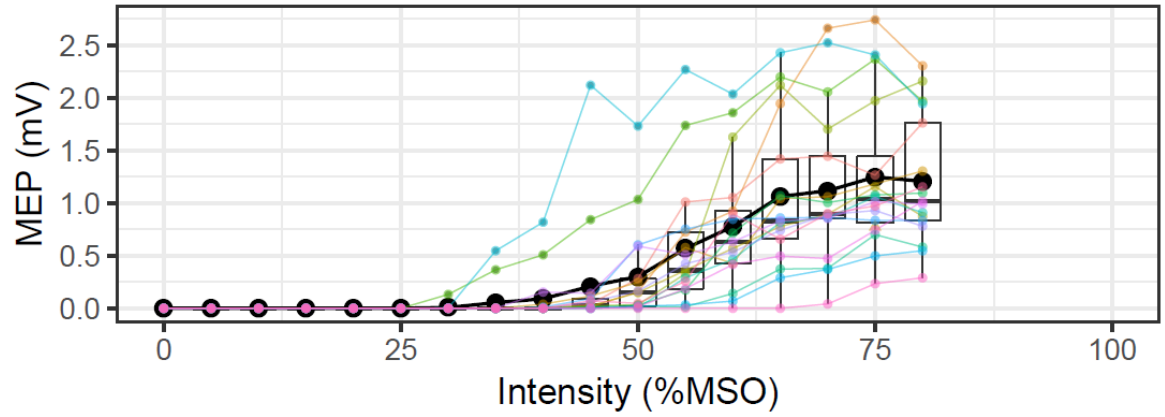
TMS Measures of M1 Excitability and Interhemispheric Inhibition

SICI was expressed as the ratio of the mean conditioned MEP amplitude (CS-TS) to the mean test MEP amplitude (TS alone). Three observations were discarded from this analysis due to their identification as outliers. rIHI was expressed as the ratio of the mean conditioned MEP amplitude (CS + TS) to the mean test MEP amplitude (TS alone).

A) Step 1



B) Step 2



C) Step 3

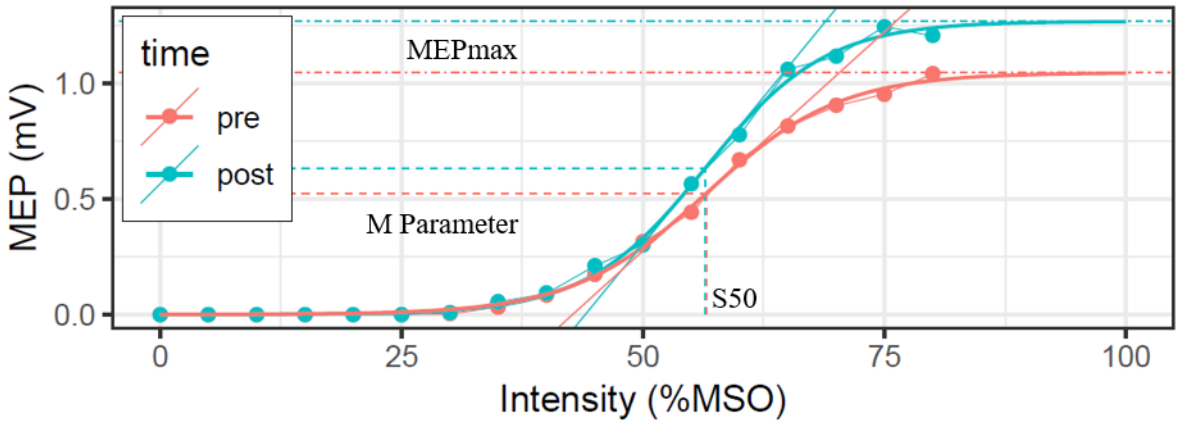


Figure 2. Extraction of curve parameters from SRCs. (A) Single subject: For each intensity 10 TMS pulses were applied and EMG recorded from ECU muscle (n=10). Trials that did not have at least 5 trials per data point were excluded. The number of trials for each intensity were reduced accordingly (for example n= 5 for intensity of 35% MSO) and mean was calculated. For intensities with less than 5 trials, the data point was not included in the further analysis (intensity of 75%, n= 3). The mean, and box- and whisker plot is indicated. Individual trials are indicated in red. (B) Group data (n=20): mean SRC curve is indicated in black. The mean, and box- and whisker plot is indicated. Individual SRCs are displayed in different colors. (C) A curve was fitted through the group mean SRC curves for pre (red) and post (green) intervention the group (n=20). The curve parameter MEPmax, M parameter and S50 are indicated with dotted lines.

For analysis of SRCs, Step 1 consisted of the MEP amplitudes being plotted as a function of stimulus intensity (Fig. 2A) ranging from intensities below each subject's RMT to 80% MSO. The data point required a minimum of 5 trials to be 'true' and are included in for further analysis. Data points below the level of measurable MEP amplitudes were set at 0. Then, a 3-parameter sigmoid function Boltzmann equation, that fits the averaged data points by using the Levenberg-Marquardt least mean-squares algorithm [141, 146-148] was applied to each SRC. Using the Boltzmann function, three parameters were extracted from each SRC: MEPmax, S50, and M. Here, MEPmax represents the maximum MEP amplitude, S50 represents the stimulation intensity (in % MSO) needed to evoke 50% of the maximum MEP amplitude, and M represents the slope parameter. While all model fits converged, in some cases such individual fits were problematic. For example, measurable MEP amplitudes for some subjects were only observed towards high stimulator intensities and did not reach a maximum before reaching 100% of MSO. In such cases, the MEPmax asymptote from the model fit could end up far larger than any measured data point [141]. To account for the variation in maximum stimulation intensity per each participant, we only included values measured up to 80% MSO and extrapolated the values 80-100% MSO from the curves. Given the inability to correctly model each individual SRC, a jackknife, or leave-one-out approach was adopted. A composite SRC profile was generated by averaging the mean MEP responses across subjects at each stimulus input, ranging from 0% to 80% MSO. In Step 2, twenty such profiles were generated, each time excluding one subject from the average. Finally, a Boltzmann model was fit to each composite profile, resulting in twenty sets of parameter triplets (indexed by the subject left out) per hemisphere, intervention, and time point [141]. To calculate the effects of the different interventions (rTMS90%, rTMS80%, sham) on curve parameter estimates, delta curve estimates were calculated (ΔMEPmax-estimate, ΔS50-estimate and ΔM-

estimate) by subtracting each pre-intervention curve estimate (MEPmax, S50, and M) from the corresponding post-intervention estimates matched across hemisphere, intervention and the indexing participant. A jackknife correction to t-score (divided by n-1) was applied and the jackknife-adjusted p-values were used for statistical comparison.

Statistical Analysis

Statistical significance was assessed at the .05 level in all cases and mean values are shown ± SD. There were no corrections for multiple comparisons. All statistical analyses were conducted using R (R Core Team, 2017) or JMP13 software (SAS Institute, Cary NC).

Motor Task

As left- and right-hand motor performance may be affected differently by the interventions, the effect on accuracy was examined for each hand separately using mixed model ANOVAs. The dependent variable was accuracy and the independent variables were the interventions (rTMS80%, rTMS90%, sham), target size (small, medium, large, xlarge), and time (pre- or post-intervention). To account for the possibility that the button pushes for indication of movement time in the one group ($MovT_{dis}$) introduced a difference in the accuracy measures we also ran mixed model ANOVAs for each movement time group ($MovT_{cont}$ vs $MovT_{dis}$) as the independent variable. The effect of the intervention on MovT was analyzed separately for $MovT_{cont}$ and $MovT_{dis}$. Each measure was examined for each hand separately using a mixed model ANOVA with subject as the random factor. The dependent variables were $MovT_{cont}$ and $MovT_{dis}$ for their respective ANOVAs and the independent variables were intervention, target size, and time.

SRC

The effect of the interventions on the delta curve estimates (ΔMEPmax-estimate, ΔS50-estimate and ΔM-estimate) were examined using separate mixed model ANOVAs with each delta curve estimate as a dependent variable with hemisphere (stimulated or non-stimulated M1) and intervention (rTMS80%, rTMS90%, sham) as the independent variables. Subjects were included as a random factor in the model. The pre-intervention curve estimates were included as a covariate. The use of the jackknife approach led to an artificial reduction in error variance that created unnaturally large t-values so it was corrected by dividing the t-value by (N-1) where n=20.

SICI

In the primary analysis, a mixed effects ANOVA was used to test the effect of the interventions at the different intensities on SICI. SICI was the dependent variable and the hemisphere (stimulated or non-stimulated M1), intervention (rTMS80%, rTMS90%, sham), time (pre- or post-rTMS) and CS intensity (60% RMT, 80% RMT) were the independent variables. In a secondary analysis, a one-way ANOVA was used to test for differences of pre-intervention SICI across different days. Intervention was the independent variable and pre-intervention SICI the dependent variable. The effect of pre-intervention on post-intervention SICI was tested using a mixed effect ANOVA with pre-intervention SICI, hemisphere (stimulated or non-stimulated M1), CS intensity (60% RMT, 80% RMT) and intervention (rTMS80%, rTMS90%, sham) as independent variables and SICI post-intervention as the dependent variable.

rIHI

The primary analyses used a mixed effects ANOVA to analyze the effect of the interventions on rIHI. rIHI was the dependent variable and the hemisphere (stimulated or non-stimulated M1), interventions (rTMS80%, rTMS90%, sham), and time (pre- or post-intervention) were the independent variables. In a secondary analysis, a one-way ANOVA was used to test for differences of pre-intervention rIHI across different days. Intervention was the independent variable and pre-intervention rIHI was the dependent variable. We also tested whether intervention-related changes in rIHI were dependent on pre-intervention rIHI using a mixed effect ANOVA. Pre-intervention rIHI, hemisphere (stimulated or non-stimulated M1), and intervention (rTMS80%, rTMS90%, sham) were independent variables and post-rTMS rIHI was the dependent variable.

Results

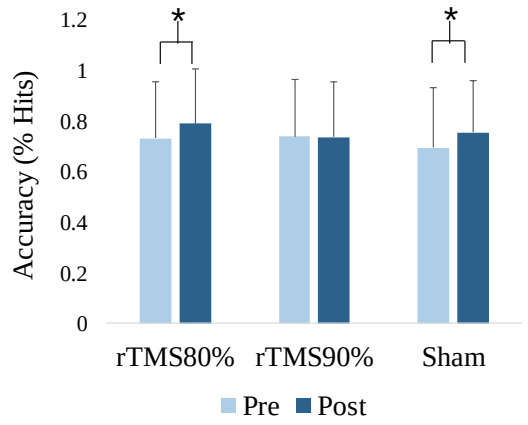


Figure 3. Differential effects of LM1 LF-rTMS on left hand accuracy. Values are means ± SD. **Participants were significantly more accurate on the motor task (regardless of target size) following the rTMS80% and sham rTMS**

LM1 LF-rTMS-related effects on Motor Performance

For the group data analysis, data from all 20 subjects were included. Of the 20 subjects, 18 had complete data sets for all three rTMS conditions. In one subject, the data for the sham and rTMS 80% conditions were not collected because of reported migraine, and in one subject the data for the sham condition was not collected because of scheduling conflict (n = 20 for rTMS90%, n = 19 for rTMS80%, and n = 18 for sham rTMS).

Evaluating effects of rTMS on left hand (ipsilateral to stimulated M1), results from the mixed model ANOVA showed significant effects for each of the main variables: Intervention ($p = 0.039$), Target size, ($p < 0.0001$) and Time ($p = 0.0003$). There was a significant interaction between intervention and time ($p = 0.021$) on performance accuracy. There were no other significant interaction effects. Post hoc analysis using planned contrasts demonstrated that accuracy significantly improved following the intervention for both the rTMS80% ($p= 0.002$) and sham ($p = 0.002$) conditions, but not rTMS90% (Fig. 3). For the right hand, as expected, there were statistically significant effects for the main variable target size ($p < 0.0001$), with no other main variables or interactions reaching the level of statistical significance (see Appendix A, Table 2 for report of results of each model). Due to not having a significant effect for the intervention nor the interaction of intervention x target size, post-hoc analyses were not performed for right hand accuracy.

To determine whether changes in accuracy were influenced by corresponding changes in movement time, $MovT_{cont}$ and $MovT_{disc}$ were analyzed separately (see Methods

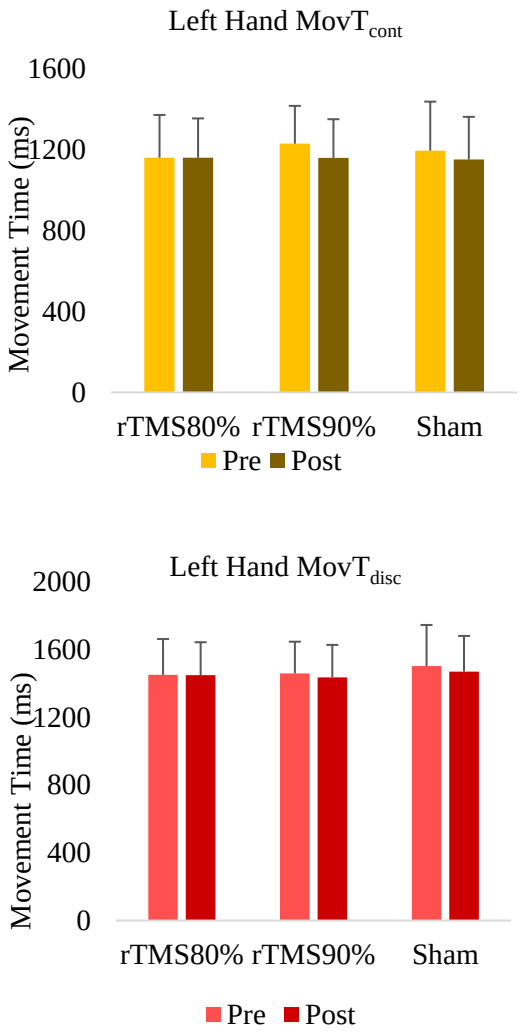


Figure 4. Changes in movement time of the left hand after LM1 LF-rTMS. MovT (ms) collapsed across target sizes is displayed pre- and post-intervention for all intensities (sham, rTMS80, rTMS90) for both the MovT continuous (MovTcont) and discrete (MovTdisc) groups. **Movement time did not significantly increase post LM1 LF-**

regarding differences in task characteristics). Results showed that MovT did not increase significantly post-intervention in either group ($MovT_{cont}$ and $MovT_{disc}$) for either hand (left or right) suggesting that improvements in left hand accuracy following stimulation were not due to a change in movement time (Fig. 4).

LM1 LF-rTMS-related effects on SRC

The results from the mixed model ANOVA testing the effects of LF-rTMS on SRC extracted parameters revealed no significant main or interaction effects. Group SRC data are illustrated in Figure 5 and statistical outputs are summarized in Table 2.

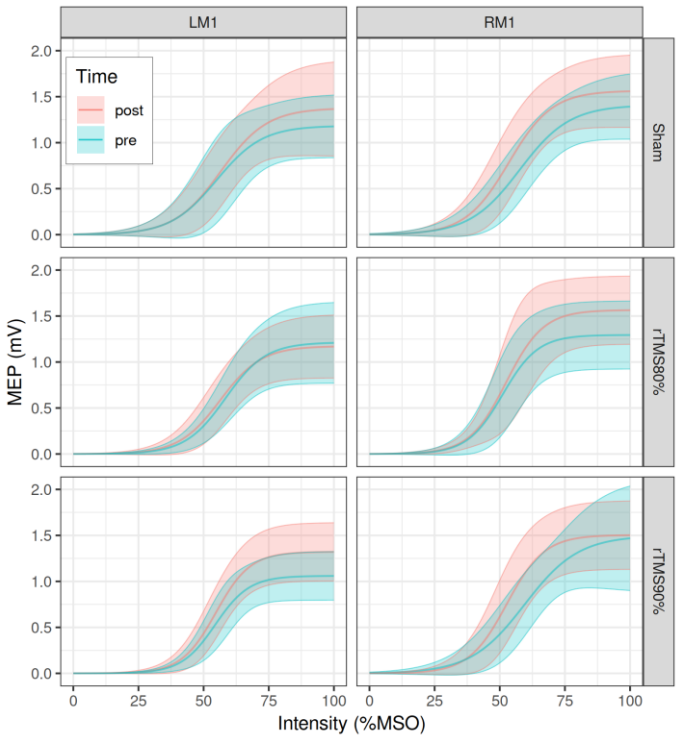


Figure 5. Illustration of group stimulus response curves by LF-rTMS interventions. SRCs pre- and post-intervention with % maximum stimulator output (MSO) plotted against MEP amplitude (mV) with ± 1 inflated SD (shaded area) for TMS stimulation on LM1 (left panel graphs) and RM1 (right panel graphs). The LM1 LF-rTMS intensities (sham, rTMS80, rTMS90) are displayed horizontally. MEP amplitudes above 80% MSO were estimated from the calculated MEPmax. **There were no significant LF-rTMS-related effects on SRC-curve parameter estimates**.

LM1 LF-rTMS-related effects on SICI

The mixed model ANOVA testing the effects of the interventions on SICI of LM1 and RM1 showed significant effects for the main variables of CS intensity ($p = 0.0007$) and hemisphere ($p = 0.014$) with LM1 having less intracortical inhibition (higher ratios) than RM1 (SICI expressed as ratio CS-TS/TS alone: LM1: 0.75 ± 0.05, RM1: 0.68 ± 0.05). Inhibition was stronger with CS of 80% RMT (SICI expressed as ratio: 0.66 ± 0.05) when compared to the inhibitory effect of CS of 60% RMT (SICI expressed as

ratio CS-TS/TS alone: CS80%MT: 0.66 ± 0.05, CS60%MT: 0.77 ± 0.05) (see Table 2). No significant main or interaction effects were observed for intervention (Fig. 6) or time variables (see Table 2).

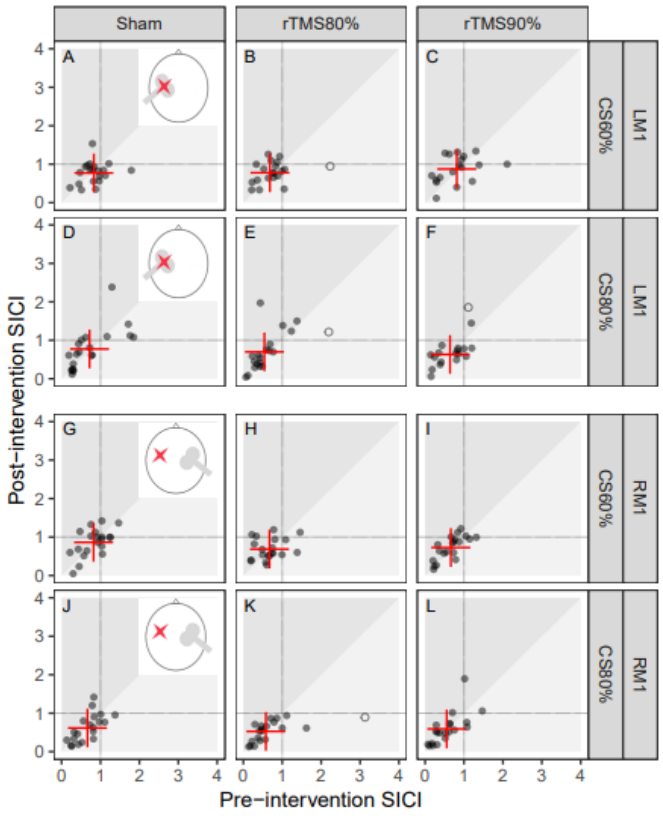


Figure 6. Effects of LM1 LF-rTMS on short-interval intracortical inhibition (SICI). All figures A-L show the pre-intervention values (x-axis) and post-intervention values (y-axis) expressed each as a ratio of the conditioned test stimulus to the unconditioned test stimulus. Individual data points are plotted as filled circles; outliers are unfilled circles. The red crosses represent averaged group data with outliers excluded. In each figure, dark grey shading represents a decrease in inhibition post-intervention; light grey shading represents an increase; the diagonal line represents no change. The interventions (sham, rTMS 80, and rTMS 90) are displayed vertically and the parameters of SICI are displayed horizontally for the left and right primary motor cortex (L M1, R M1). The intensity of the conditioning stimulus (CS) was either 60%MT or 80%MT. **There were no significant LF-rTMS-related effects on SICI**.

To evaluate if SICI pre- intervention was similar across different days of testing, a mixed effect ANOVA was calculated where hemisphere (stimulated or non-stimulated M1), CS intensity (60% RMT, 80% RMT) and intervention (rTMS80%, rTMS90%, sham) were the independent variables and SICI pre-intervention was the dependent variable. No significant main or interaction effects were observed confirming that pre intervention SICI was comparable across different days of testing. We also tested whether pre-intervention SICI had an effect on the magnitude of intervention related effects on post-intervention SICI [121]. A mixed model ANOVA with pre-intervention SICI, hemisphere (stimulated or non-stimulated M1), CS intensity (60% RMT, 80% RMT) and intervention

(rTMS80%, rTMS90%, sham) as independent variables and SICI post intervention as the dependent variable demonstrated no statistically significant effect.

LM1 LF-rTMS-related effects on rIHI

A mixed effects ANOVA with hemisphere, time and LM1 LF-rTMS as independent variable and rIHI as the dependent variable demonstrated an effect of hemisphere on rIHI ($p < 0.0001$). Inhibition from LM1 on RM1 was greater than inhibition from RM1 on LM1 (IHI expressed as ratio (CS-TS)/TS alone: LM1 on RM1: 0.60 ± 0.04, RM1 on LM1: 0.76 ± 0.04). The main effect of intervention and interaction effects between the variables were not statistically significant (Fig. 7).

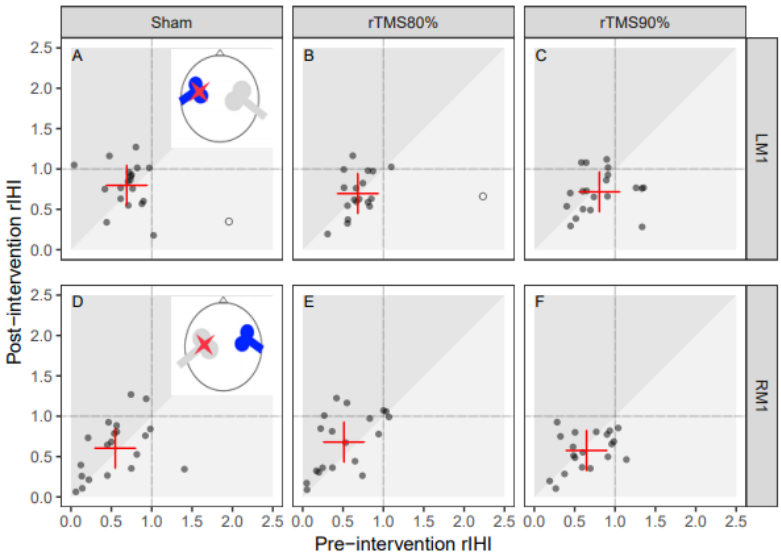


Figure 7. Effects of LM1 LF-rTMS on resting interhemispheric inhibition (rIHI). All figures A-F show the pre-intervention (x- axis) and post-intervention (y- axis) values expressed each as a ratio of the conditioned test stimulus to the single test stimulus. Individual data points are plotted as filled circles; outliers are represented unfilled circles. The red crosses represent averaged group data with outliers excluded. In each figure, dark grey shading represents a decrease in inhibition post-intervention; light grey shading represents an increase; the diagonal line represents no change. The interventions (sham, rTMS80, and rTMS90) are displayed vertically and the location of where the TS was placed is displayed horizontally: left and right primary motor cortex. **There were no significant LF-rTMS-related effects on rIHI**.

In a secondary analysis, the one-way ANOVA demonstrated that rIHI values were similar across the different experimental days (see Table 2). A mixed effect ANOVA with pre-intervention rIHI, hemisphere (LM1 or RM1), and intervention as the independent variables and rIHI post-intervention as the dependent variable demonstrated effects for pre-intervention rIHI ($p= 0.029$) and a significant interaction between hemisphere and pre-intervention rIHI ($p = 0.004$). This

indicates that pre-intervention rIHI had an effect on post-intervention rIHI values depending on whether M1 was stimulated or not. There were no significant interactions between intervention and pre-intervention rIHI, or any other variables (see table 2), indicating that intervention-related effects were independent of pre-intervention rIHI.

Discussion

The purpose of this study was to comprehensively assess the effects of LF-rTMS on motor performance and cortical excitability of both the stimulated and non-stimulated M1 in an older healthy adult population. Although LF-rTMS did not significantly modulate cortical excitability in either M1 or inhibition between M1s, LF-rTMS at 90% RMT modestly impeded task performance in the hand ipsilateral to stimulation delivery. These findings suggest that LF-rTMS does not modify cortical excitability in aged healthy adults but can impact motor performance.

LM1 LF-rTMS-related effects on Motor Performance

Motor performance was differentially impacted by LF-rTMS depending on the performing hand. The left hand, ipsilateral to LM1 LF-rTMS, showed improvements in task accuracy for rTMS80% and sham conditions only. Given the similar improvement post-sham, it is likely that rTMS80% did not have a neuromodulatory effect on motor performance. Instead, the improvement following these two conditions could be a practice effect from performing the motor task prior to stimulation. The neuromodulatory effect of LF-rTMS appeared to rTMS90% blocking performance improvement post-stimulation. The difference in effects between rTMS90% and rTMS80% could be reflective of the thresholds of inhibitory interneurons thought to be targeted by LF-rTMS such that 80% of RMT was too low for significant activation of these interneuron populations. Previous

studies have also demonstrated a change in performance of the ipsilateral hand, except LF-rTMS90% had a faciliatory effect and improved motor performance [25, 119, 127]. The studies inferred improvement was due to altering interhemispheric inhibition: LF-rTMS decreased the excitability of the stimulated M1, which lessened its inhibition on, and increased the excitability of, the non-stimulated M1 to allow for greater motor performance. Of the studies, only one assessed and supported this model by showing intracortical inhibition was suppressed and intracortical facilitation was enhanced after LF-rTMS in the non-stimulated M1 [119]. However, in our current study, we did not observe significant changes in excitability of the non-stimulated M1, nor was intracortical inhibition suppressed which could explain the lack of facilitatory effect of rTMS90% on ipsilateral hand performance. Given the differences in neurophysiology, it is reasonable for our LF-rTMS90% stimulation to have a different impact that has led to a modest reduction in ipsilateral motor performance improvement compared to the other intervention conditions.

Right hand task performance, contralateral to the LM1 LF-rTMS, showed no rTMS-related effects. If LF-rTMS resulted in a decrease in excitability of the stimulated M1, it would be plausible to expect behavior of the contralateral hand to be negatively impacted. However, LF-rTMS has shown to not be deleterious for motor performance of the contralateral hand [25, 149], making our results aligned with previous findings. LF-rTMS has only shown to be deleterious during motor learning of the contralateral hand as it interrupts early consolidation of the motor task [123, 149]. Given that our participants were trained on the motor task prior to meet inclusion criteria, we are confident we probed LF-rTMS effects on motor performance and not motor learning; our lack of LF-rTMS related changes would also support the likelihood of probing motor performance. The first study to show a bilateral increase in performance of both the ipsilateral and

contralateral hand after LF-rTMS arose from our lab using the same motor task. Previously, we found an increase in motor performance after LF-rTMS90% of the medium sized target only (before intervention: 82.89 ± 9.94% accuracy, after: 89.22 ± 6.38%) [127], which is in contrast to our current study results of no effect. In the current study, we collapsed across target sizes during analysis but in observing the medium sized target, did not find improvement (before intervention: 83.71 ± 9.2% accuracy, after: 81.70 ± 1.2%). This difference in results could be related to the subtle difference in age of participants; the previous study's mean age of participants was 55 ± 11.34 years and the current mean age was 60 ± 7.2 years. There are age-related effects on neurophysiology during motor performance [150], but the slight difference in age between the studies is less likely to have substantial impacts. It is more likely that the effect size of our previous study was modest and not able to be replicated in a larger group of participants (previous study n = 12, current study n = 20) and likely there was additional variability added due to variability in inter-individual motor performance. Presently, we conclude that LF-rTMS does not have effects on motor performance of the contralateral hand.

LM1 LF-rTMS-related effects on Cortical Excitability

LF-rTMS is traditionally believed to have an inhibitory effect resulting in a reduction in excitability of the stimulated M1 [102, 107], and subsequent increase in excitability of the non-stimulated M1 due to shifts in interhemispheric inhibition [104, 115], yet we did not find significant LF-rTMS-related changes in excitability of either the stimulated or non-stimulated M1. The first potential explanation for differences in outcomes could be different study parameters (i.e. frequency, intensity, number of pulses), which can vary substantially across studies. However, several previous studies have employed the same parameters as those in the present study: 1 Hz LF-rTMS stimulation at 90% resting motor threshold (RMT) for 900 pulses. For measuring

changes of cortical excitability, Sommer et al., [112] found a significant reduction in MEP amplitude when applying single pulses at 120% RMT after rTMS, but several studies found no effect on MEP amplitude with the same parameters [114, 120, 121, 125]. It should be noted the difference in measuring excitability. Previous studies have used changes in MEP amplitude administered at a single stimulation intensity (typically 120% RMT) as their measure of excitability [102, 106, 107, 111]. Post-stimulation, a decrease in MEP amplitude with the intensity held constant demonstrated the neurons were less excitable and would therefore require an increased electrical current to produce the same sized MEP response pre-stimulation. In comparison, we utilized a different measure of excitability by capturing a stimulus response curve that details MEP amplitude as a function of TMS intensity. Fitting an SRC by a Boltzmann sigmoidal function allows for the extraction of three parameters: the slope, stimulus intensity needed to reach 50% of the maximum amplitude (S50), and the plateau (MEPmax) [141, 147]. These parameters allow for more sensitive measures of corticospinal excitability, such as reflecting the recruitment gain of the CST, changes in cortical motor maps, and degree of transsynaptic excitability [140, 141].

The evaluation of SRC parameters has given more insight to the effects of LF-rTMS. A study by Houdayer et al. found that the slope of SRCs was increased in the stimulated M1 after LF-rTMS at 115% RMT in young healthy adults [122]. An increase in slope reflects enhancement of transsynaptic excitability, which would represent an increase in corticospinal excitability in stimulated M1 [140], which is the opposite effect one would expect. However, neither the S50 nor MEPmax parameters were affected so the effect of the slope is thought to be associated with higher recruitment gains in the corticospinal pathway. Higher recruitment gains are a marker of increased RMT when S50 and MEPmax are held constant. An increase in motor threshold would therefore

be representative of a decrease in net excitability. This study illustrated the complex effects of LF-rTMS that can be captured using SRCs that would not have been captured by only measuring MEP amplitude at a single intensity. In looking over our extracted parameters, our SRC parameters did show a similar trend?? towards a potential increase in stimulated M1's excitability, such that LF-rTMS at 90% was held more constant for the slope and S50 parameters, but the MEPmax was increased after stimulation (see Appendix A, Fig. 8). However, with the substantial amount of inter-individual variability (Fig. 5), no significant differences in excitability were observed. Substantial inter-individual variability of the modulatory effects of LF-rTMS has been observed extensively [105, 106, 111] and are attributable to a variety of reasons including cortical morphometry, pre-intervention network connectivity determining potential for modifiability [50], circadian rhythms [151] or simply differences in the dynamics of the nervous system [152]. Therefore, due to the large variability, we likely were underpowered to detect changes in excitability.

LM1 LF-rTMS-related effects on Intracortical Inhibition

Intracortical inhibition, as measured by SICI, was not shown to be affected by LF-rTMS. One study showed a decrease in intracortical inhibition in the stimulated M1 after LF-rTMS [114], and the attributable difference was likely the use of an ISI of 3 ms (instead of 2 ms) in the SICI paradigm [120, 121, 125]. Several studies using an ISI of 2 ms, similar the present study, found no LF-rTMS related changes in SICI. Though our results align with many past studies, we also recognize the potential effect of our population being significantly older as well. SICI is mediated by $GABA_A$ergic inhibition [68, 153] which has been shown to decline with age. This decline is associated with reduced modulation of inhibition during motor performance, suggesting that the ability to modulate $GABA_A$ergic inhibition is further diminished in older adults [134]. Therefore,

it is likely that SICI is less modifiable in our studied population. Another factor impacting modifiability is homeostatic plasticity. Homeostatic plasticity refers to the concept that synapses only have a target range of synaptic modifiability such that neural processes will maintain synaptic strength within the target range [82, 154]. SICI responds differently to rTMS depending on baseline values: subjects with less baseline intracortical inhibition had larger rTMS-related effects than subjects with higher baseline intracortical inhibition who are closer to the upper-limit of their modifiable range [121]. Our results indicated that pre-rTMS SICI was comparable across different days of testing and therefore did not confound our results. Accumulating evidence suggests that LF-rTMS does not impact $GABA_A$ergic inhibition in older adults.

LM1 LF-rTMS-related effects on interhemispheric inhibition

The lack of rTMS-related changes in rIHI demonstrated that LF-rTMS is not affecting the inhibitory balance between the M1s. These results are not surprising given the lack of changes in excitability and inhibition of both M1s that were expected to be mediated by rIHI. Pal et al. demonstrated the ability of LF-rTMS to reduce rIHI in both directions, but with a predominance from the stimulated to non-stimulated M1 [155]. A potential explanation of the varied results in this present study is the intensity used for LM1 LF-rTMS. Our study used subthreshold intensities, 80% and 90% RMT whereas Pal et al. used 115% RMT with otherwise similar stimulation parameters. It is reasoned that using higher intensity stimulations may lead to the additional activation of neuronal excitatory circuitry in the non-stimulated hemisphere [110, 115] that are likely not activated with our subthreshold parameters. It is also possible that repeated stimulation of interhemispheric fibers could reduce their effectiveness, such that the non-stimulated M1 is less inhibited and it could produce an increase in excitability [115]. An increase in non-stimulated M1 as a result of a decrease in rIHI from the stimulated to non-stimulated have been shown as well

[115]. Given our lack of changes found in the excitability of the non-stimulated M1, this further supports the possibility that our stimulation parameters were too low to activate the necessary neuronal circuitry. As with SICI, there is some evidence that the level of interhemispheric inhibition prior to stimulation may predict or mediate rTMS-related effects [115], which could explain our lack of results. However, our findings indicate that pre-intervention rIHI was similar across days and therefore did not confound our findings.

Limitations

Based on the evidence presented, LF-rTMS90% seems to suppress motor performance improvement in older adults. This creates a new question about the impact of motor task performance on M1 excitability prior to the LF-rTMS. There is the potential that motor performance primed M1 and subsequently altered its response to LF-rTMS. Our study is limited due to measuring excitability and motor performance on different days. All excitability and inhibitory measures were measured at rest. Therefore, we can only postulate what effects LF-rTMS might have had in a motor system that was active immediately preceding stimulation. Additionally, many studies in the literature have relied upon different outcomes metrics for corticospinal excitability and intracortical inhibition, including using changes in RMT or active motor threshold; averaged MEP amplitude at a given stimulus intensity – usually 120% RMT; long interval intracortical inhibition; and/or cortical silent period (see Table 1). Therefore, though the broader terms are the same, i.e. “corticospinal excitability,” there are differences in what exactly is being measured. Our study lacked any common metrics and our unique approach for analyzing the stimulus response curves means we have no direct comparison with past study findings, leaving us to infer how our results align with the literature.

Conclusion

In summary, the results of our present study did not show consistent neuromodulatory effects on cortical excitability using LF-rTMS at 90% or 80% RMT in older healthy adults. LF-rTMS at 90% had modest effects on reducing motor performance gains of the hand ipsilateral to stimulation in older healthy adults. These findings have clinical implications given that many neurologic conditions, such as stroke and Parkinson's disease, increase in prevalence with aging. A follow-up study should include a cohort of younger adults to test the hypothesis that the lack of clear neuromodulatory effects currently presented is a function of a less modifiable nervous system due to aging. If the results found presently are a result of aging, future studies should evaluate the impact of LF-rTMS at suprathreshold intensities in an attempt to produce greater modulatory effects that can subsequently be leveraged in the treatment of stroke.

Discussion

The ultimate goal of our work was to establish findings in healthy older controls to be leveraged in future studies to ultimately determine the therapeutic potential of LF-rTMS in motor recovery after stroke. We did not observe significant modification of excitability, intracortical inhibition, or interhemispheric inhibition after LF-rTMS, which could be due in part to the reduced modifiability of the nervous system in older adults. The dynamic changes after stroke potentially provide a nervous system that is more readily modifiable. The hyperexcitability of contralesional M1 [156], the hypoexcitability of ipsilesional M1 [56], the reduction of IHI from ipsilesional M1 onto contralesional M1 at rest [57], and the increase of IHI from contralesional onto ipsilesional M1 during movement [51] all provide instances of abnormality altering where the nervous system is in its range of modifiability. The brains of individuals with stroke could therefore be more susceptible to modification by LF-rTMS. As mentioned previously, LF-rTMS over the

contralesional hemisphere has had some positive results in modulating contralesional M1 excitability, but there are still conflicting results that require further comprehensive evaluation [91].

A secondary finding in our study was the significant effect of CS intensity on the TS. More inhibition was for CS of 80% of RMT compared to CS of 60% of MT, which is consistent with previous reports that demonstrated the intensity of CS will dictate the predomination of inhibitory or excitatory circuitries [57]. In a typical SICI paradigm with healthy adults, the conditioning stimulus (CS) has an inhibitory effect on the test stimulus. On the contrary, in the early subacute phase post-stroke, increased CS intensities lead to facilitation in the contralesional M1. This is hypothesized to be due to a down-regulation of $GABA_A$ receptors, which had broader implications in the balance of inhibition between the hemispheres such that abnormal SICI and rIHI were correlated in patients with cortical lesions [57]. Our present findings reiterate that even in healthy controls, subthreshold intensities of different magnitudes can still produce differential effects. In addition to differences observed in CS intensities, we also observed differences in LF-rTMS stimulation intensity for its impact on motor performance.

LF-rTMS only had modulatory effects on motor performance of the hand ipsilateral to stimulation, that would correspond to the paretic hand in stroke for LF-rTMS over the contralesional M1. Diverging from previous literature [25, 119, 127], LF-rTMS90% had a suppressive effect on motor performance of the ipsilateral hand. In stroke, LF-rTMS of the contralesional M1 was found to improve kinematics of motor performance of the paretic hand, but that was correlated with a reduction in excitability of contralesional M1 [157, 158]. Our results bring up the concern that LF-rTMS could have potentially deleterious effects to behavior if stimulation is not enough to cause substantial changes in excitability of the stimulated and non-

stimulated M1 to shift towards facilitation of motor performance. In probing the neuromodulatory effects of LF-rTMS in stroke, it is necessary to have a comprehensive assessment, such as the one presented, to better understand the differential LF-rTMS effects observed in stroke patients.

Ongoing Studies – Assessments Post-Stroke

Now that we have a semblance of understanding of the effects of rTMS in a healthy older population, we will use this data for age-matched comparison with an ongoing study of the effects of LF-rTMS of the contralesional M1 post-stroke that uses comparable stimulation parameters, data collection procedures, and primary outcome measures. Given that some stroke patients lack a measurable response to TMS in the ipsilesional M1, we aimed to minimally measure the contralesional M1 pre- and post-rTMS stimulation. If patients had an ipsilesional MEP response, excitability and inhibitory measures were taken bilaterally along with rIHI. Another limitation is that stroke patients may lack the ability to perform the aforementioned motor task with their paretic hand due to loss of motor function. Due to the range of motor ability post-stroke, we have chosen to capture simpler ballistic wrist extensions as the primary behavioral outcome. The wrist extensions are a simpler task that have increased sensitivity for detecting more subtle improvements measured through electromyographic activity compared to the motor task that requires complex, dexterous fine motor skills. If patients have the ability to play the motor task, it will be measured in addition to the ballistic wrist movements. All of these assessments are measured longitudinally across two time points: the early subacute phase (1-month post-stroke ± 2 weeks) and the chronic phase (6 months ± 2 weeks) post-stroke due to differences in the contralesional and ipsilesional M1s based on time post-stroke. The ultimate goal is to probe the neuromodulatory effects of LF-rTMS of contralesional M1 and its role in motor performance across the phases of stroke recovery.

Future Direction – A Sensorimotor Approach

As mentioned previously, the execution of skilled hand movements is dependent on M1 descending projections but also requires sensory information processing and sensorimotor integration. Representations of the external environment must be generated from visual, proprioceptive, and tactile input [159], and these representations are combined with internal representations of the motor system, such as hand position, to create an internal model [160]. Both external and internal representations have inherent variability that can be reduced by incorporating input from multiple sensory modalities [161]. Therefore, a discussion about motor performance and motor recovery after stroke inherently relies upon the integration of sensory and motor modalities.

Successful multisensory integration contributes to execution of a motor command that results in the desired movement outcome. For instance, if the goal is to button a shirt, the internal model should include the position of the button and buttonhole and starting position of the hand. These positions are determined by visual, proprioceptive, and tactile information that will be processed through the posterior parietal cortex (PPC; primarily processing visual information [162]) and the primary somatosensory cortex (S1; primarily processing proprioceptive, tactile [163], and nociceptive [164] information). Sensory information associated with the manipulation of the button will also be provided creating a sensorimotor feedback loop. The relevant sensory information is then relayed to M1, where a motor command is generated. This internal model will also be influenced by prior motor execution that contributes to the development of an efferent copy of the motor output [165]. Using this information, an internal model includes predictions about expected sensory feedback resulting from the generated movement [166]. In this example, if the button is not at the correct angle required for it to go through the button hole, or if the hand is in

the incorrect starting position, the sensory reafferent information occurring in response to movement will not align with the predicted feedback generated from the efference copy [165]. Therefore, the predicted sensory consequence will be updated, the model adapted, and subsequently, the error will be corrected by adjusting the motor command [167].

There is interest in evaluating the PPC due to its potential contributions specifically during visually guided motor tasks like the task employed in Chapter 1. As previously postulated, an improvement in accuracy on the pointing task could be due to integration occurring in the PPC that has bilateral projections to both M1s [127]. The PPC is comprised of Brodmann Area (BA) 5, 7, 39 and 40 in the human brain and is anatomically connected to motor areas M1 and premotor cortex (PMC) via the superior longitudinal fasciculus (SLF) [168, 169]. Although the PPC is not traditionally considered a primary part of the cortical motor network, it is involved in motor execution with populations of neurons that are motor dominant, in addition to populations that are visual dominant, or a combination of the two [170]. Non-human primate studies have demonstrated dense reciprocal PPC-M1 connections between the rostral strip of PPC and the medial lateral portion of M1 [171]. Furthermore, regions of the PPC have distinct and direct pathways and networks with prefrontal motor cortical regions organized in functional zones [172], which demonstrates the level of specific information the PPC can provide to the motor network. While PPC is thought to primarily influence M1 through polysynaptic connections with the PMC [173], support for monosynaptic projections from PPC to M1 has also been published [174]. Additionally, it has been shown that PPC has disynaptic connections with hand motoneurons in the dorsal horn and intermediate zone of the spinal cord in non-human primates [175], further suggesting potential contributions of PPC in the control of hand movements.

The PPC is a multisensory association area functioning to integrate different sensory modalities from visual, somatosensory, prefrontal and auditory inputs [176]. The PPC has abundant reciprocal connections with sensory areas and is functionally parcellated such that the rostral portion of PPC is connected to somatosensory and motor regions, and the caudal portion of PPC has connections with visual and auditory regions [177]. The necessary inputs to PPC for sensorimotor processing needed for skilled hand movements include direct reciprocal inputs from the dorsomedial visual area that allows for continuous visual motion analysis necessary for interacting with the environment [178-180] (see [162] for review). Sensory inputs to BA 5 primarily come from somatosensory area S2 and the parietal ventral area, along with weaker inputs from S1 [177]. All three regions provide pertinent sensory information to PPC about proprioceptive and tactile activity of hand movements [181, 182] that are important for sensorimotor integration used in hand exploration and object discrimination [183]. Inputs to BA 5 are important as BA 5 is responsible for visuomotor transformations [184], making the PPC-M1 connection important for visuomotor control and visual spatial processing [185, 186]. PPC combines sensory signals about visual and kinematic reference frames into complex sensorimotor representations that are relayed to M1 to optimize motor commands [187]. PPC neurons are not only involved in control and error correction of a movement once initiated, but are important for movement planning to achieve a motor goal [188, 189], as neuronal firing also encodes movement intention [190]. Lesions in the rostral portion of PPC result in difficulty with shaping the fingers prior to grasping an object [185], further demonstrating an important role for PPC during the sensorimotor integration required for successfully performing goal-directed hand movements.

Future directions will benefit from assessing the connectivity between M1 and PPC during movement performance in both healthy aged adults and stroke patients. Given the ability of PPC

to convey information for motor planning during task performance [191] and to increase the excitability of the ipsilateral M1 through applying a conditioning stimulus to the PPC in healthy adults [192], the PPC-M1 connection has the potential to be a therapeutic target in increasing motor performance after stroke.

Conclusion

This research aimed to characterize the modulatory effects of LF-rTMS on motor performance, cortical excitability in both the stimulated and non-stimulated M1, and between each M1 in an older healthy population. This study addressed a gap in the literature by testing LF-rTMS effects in an older population, which can be useful for comparison to a variety of neurologic conditions primarily affecting older adults. Our work provided a comprehensive assessment by testing the impact of LF-rTMS on excitability and inhibition of the stimulated and non-stimulated M1, with bilateral behavioral assessments in one study design. The field generally considers LF-rTMS to be inhibitory in nature, but many studies, including ours, do not support this effect. Given that our work exclusively examines older adults, our findings question the capacity for of LF-rTMS to modulate cortical excitability in an aging nervous system. However, we do show evidence in modulation of motor behavior.

Our work supports the application of LF-rTMS applied at 90% rTMS to modestly be used to suppress motor performance improvement in older healthy adults. This creates the new possibility that increasing the intensity used could potentially increase the magnitude of behavioral effects and bring forth more significant effects in modulating cortical excitability as well. Furthermore, this work sets the stage for comparison with stroke patients to probe the neuromodulatory effects of LF-rTMS in a lesioned brain. If we can continue to show an effect of LF-rTMS on motor behavior and better understand its neuromodulatory effects, we can help

provide neurobiological evidence that if applied therapeutically, could ultimately improve motor recovery after stroke.

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Appendix A

Table 2. Statistical Results

LHand Accuracy Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Group	1	0.87	0.36
Intervention	2	3.26	0.04*
Target Size	3	339.52	<.0001*
Timepoint	1	13.29	0.0003*
Intervention x Target Size	6	0.78	0.59
Intervention x Timepoint	2	3.87	0.022*
Target Size x Timepoint	3	0.16	0.92
Intervention x Target Size x Timepoint	6	0.59	0.74
LHand Accuracy Post-Hoc Contrast Test	**t**	**p**	
rTMS80%, pre vs post	3.172	0.002	

rTMS90%, pre vs post	-0.14	0.89	
Sham, pre vs post	3.19	0.0002	
RHand Accuracy Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Group	1	2.04	0.17
Intervention	2	0.34	0.71
Target Size	3	246.92	<.0001*
Timepoint	1	3.12	0.08
Intervention x Target Size	6	0.48	0.83
Intervention x Timepoint	2	1.40	0.25
Target Size x Timepoint	3	0.40	0.75
Intervention x Target Size x Timepoint	6	0.39	0.89
LHand $MovT_{cont}$ Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Target Size	3	142.75	<.0001*
Timepoint	1	10.04	0.0017*
Intervention	2	2.21	0.11
Intervention x Target Size	6	2.26	0.0386*
Intervention x Timepoint	2	3.05	0.0494*
Intervention x Target Size x Timepoint	6	0.98	0.44
RHand $MovT_{cont}$ Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Target Size	3	226.14	<.0001*
Timepoint	1	3.15	0.08
Intervention	2	4.87	0.0085*
Intervention x Target Size	6	0.69	0.66
Intervention x Timepoint	2	4.34	0.0141*
Intervention x Target Size x Timepoint	6	0.66	0.68
LHand $MovT_{disc}$ Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Target Size	3	37.14	<.0001*
Timepoint	1	2.27	0.13
Intervention	2	3.84	0.0238*
Intervention x Target Size	6	0.26	0.95
Intervention x Timepoint	2	0.51	0.60
Intervention x Target Size x Timepoint	6	0.37	0.90
RHand $MovT_{disc}$ Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Target Size	3	37.51	<.0001*
Timepoint	1	0.63	0.43
Intervention	2	2.30	0.10
Intervention x Target Size	6	0.87	0.52
Intervention x Timepoint	2	0.59	0.56
Intervention x Target Size x Timepoint	6	0.93	0.48
SRC Parameter M	**DF**	**t/19**	**2-tail p**
Hemisphere	60.66	-0.57	0.58
Intervention rTMS80%	47.93	-0.41	0.69

Intervention rTMS90%	96.02	1.06	0.30
Intervention rTMS80% x Hemisphere (l)	41.71	0.30	0.77
Intervention rTMS90% x Hemisphere (l)	37.48	-0.26	0.80
Pre-Intervention	27.56	0.58	0.57
SRC Parameter MEPmax	**DF**	**t/19**	**2-tail p**
Hemisphere	90.05	-0.33	0.74
Intervention rTMS80%	98.16	-0.48	0.64
Intervention rTMS90%	85.70	-0.09	0.93
Intervention rTMS80% x Hemisphere (l)	105.46	-0.87	0.39
Intervention rTMS90% x Hemisphere (l)	102.23	0.61	0.55
Pre-Intervention	82.86	0.41	0.69
SRC Parameter S50	**DF**	**t/19**	**2-tail p**
Hemisphere	90.31	1.97	0.06
Intervention rTMS80%	103.81	0.22	0.83
Intervention rTMS90%	101.37	-0.62	0.54
Intervention rTMS80% x Hemisphere (l)	109.33	-0.11	0.91
Intervention rTMS90% x Hemisphere (l)	108.20	0.04	0.97
Pre-Intervention	110.22	0.22	0.83
SICI Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Hemisphere	1	6.15	0.0135*
Timepoint	1	0.01	0.91
Intervention	2	1.78	0.17
CS Intensity	1	11.62	0.0007*
Intervention x Timepoint	2	0.07	0.94
rIHI Fixed Effect Tests	**DF**	**F Ratio**	**Prob > F**
Hemisphere	1	17.76	<.0001*
Timepoint	1	0.00	1.00
Intervention	2	0.07	0.93
Intervention x Timepoint	2	1.15	0.32

Figure 8. Effect of LM1 rTMS on Curve parameters extracted from SRC.

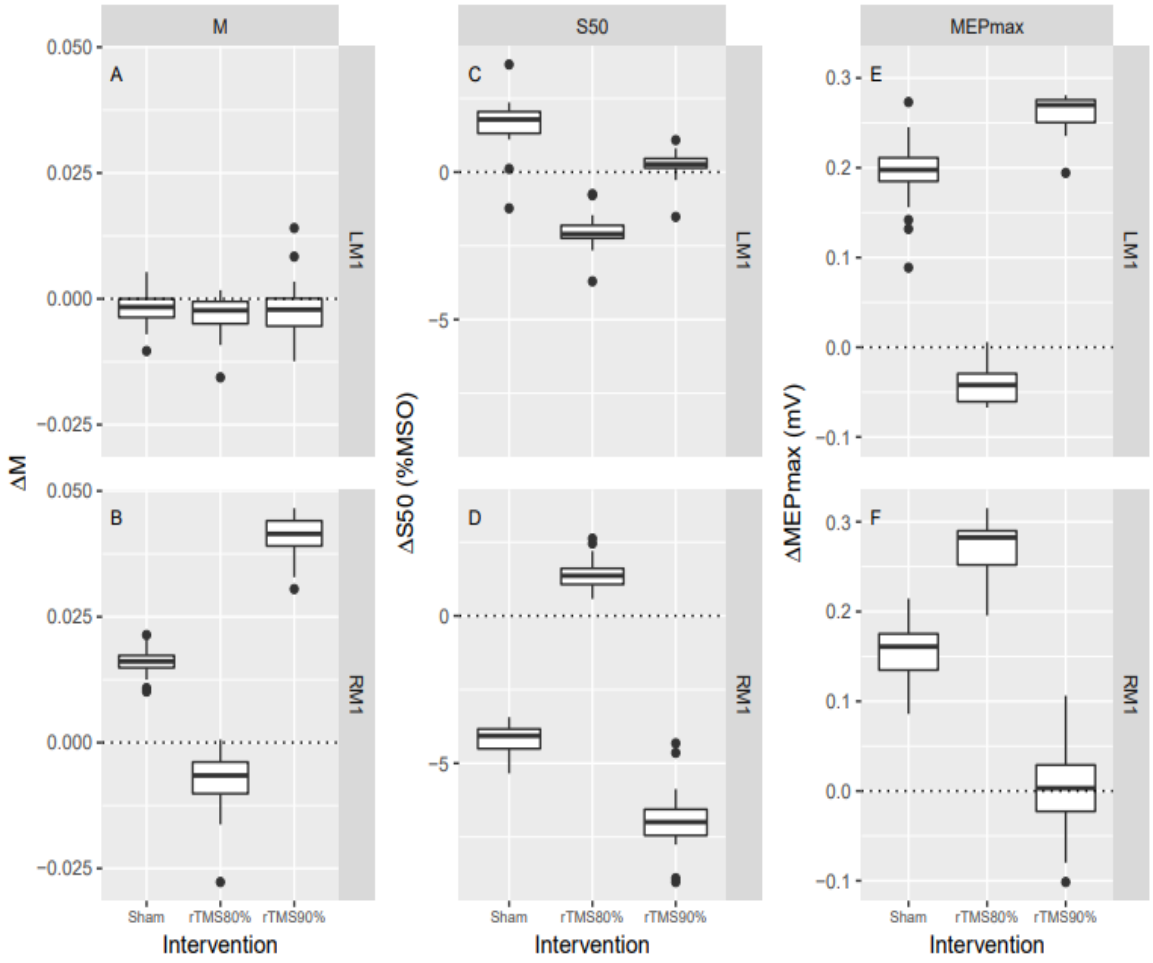

Figure 8. Effect of LM1 rTMS on Curve parameters extracted from SRC. Box and whisker plots display the ΔSRC parameters were measured as the difference (post-intervention – pre-intervention) of: slope-parameter ΔM (A, B), inflection point ΔS50 (C, D) and Δ MEPmax (E, F) calculated for the LM1 rTMS intensities (sham, rTMS80%, rTMS90%). Values >0 indicates an increase in the parameter, =1 indicates no change, and <1 indicates a decrease in the parameter.