INVESTIGATION OF NEURAL MECHANISMS OF GRIP RELAXATION

by

Binal Motawar

A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

in Health Sciences

at

The University of Wisconsin-Milwaukee

May 2016

ABSTRACT

INVESTIGATION OF NEURAL MECHANISMS OF GRIP RELAXATION

by

Binal Motawar

The University of Wisconsin-Milwaukee, 2016
Under the Supervision of Professor Na Jin Seo

Neural mechanisms for grip relaxation are relatively unknown and understudied, as compared to mechanisms for grip initiation. Yet, termination of motor activity is as important as initiation in daily function. This knowledge gap presents incomplete understanding of neural control of hand function and its impairment with aging and neurologic disorders. The purpose of this dissertation was to identify and examine neural mechanisms of grip relaxation in healthy young adults, with aging, and in chronic stroke survivors. A series of experiments in healthy young adults showed that the relaxation from a maximum power grip was mediated by increase in the short-interval intracortical inhibition (SICI). The role of spinal motor excitability modulation for grip relaxation was refuted, in contrast to previous literature for the leg muscle. These data from healthy young adults suggest that the grip relaxation time is a cortically mediated active process. Additionally, these studies also showed that the neural mechanism of grip relaxation is comparable for the dominant and the nondominant hand in healthy young adults. The next step was to identify any delays in relaxing from a grip in healthy older adults. Assessment of the effects of aging on the role of SICI showed that the delayed grip relaxation time in older adults was accompanied by reduced modulation of SICI for grip relaxation. The cortical silent period and H reflex did not explain delays in grip relaxation observed in older

adults. Another series of experiments showed that the chronic stroke survivors and age-matched control adults demonstrated comparable modulations of SICI, cortical silent period, corticomotor excitability, and H reflex. Yet, the paretic hand of the stroke survivors was significantly delayed in relaxing from a grip. Correlation and regression analysis showed that the stroke-related delayed grip relaxation time may be explained by increased spasticity, reduced somatosensation, paretic grip weakness relative to the nonparetic, strength of the corticospinal connections and interhemispheric inhibition. An intervention aimed to modulate cortical excitability and interhemispheric inhibition, Active Passive Bilateral Therapy, was employed but was found to be not effective in modulating grip relaxation time and interhemispheric inhibition after a one-time 20-minute session, warranting a longer treatment time. In summary, this dissertation investigated neural mechanisms of grip relaxation and contributes to the general body of knowledge regarding neural control of hand movements.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This work was supported by the American Heart Association, the Clinical and Translational Science Institute of Southeast Wisconsin, the American Society of Biomechanics, and the UWM College of Health Sciences Graduate Student Research Grant.

Chapter 1. Introduction

Terminating a muscle contraction is an important aspect of motor control, yet neural mechanisms of muscle relaxation are not as well understood as initiating and maintaining a muscle contraction. Deficits in temporal modulation of muscle activity and associated force is crucial for many daily activities such as objects manipulation [1, 2] and reaching [3]. Inefficient grip force scaling [4, 5] and poorly coordinated movement [5] can be explained by temporal disturbances of muscle activation. Although neural correlates of initiation of muscle contraction have been well-studied, our understanding of neural mechanisms of termination of muscle contraction remains limited. The overall goal of this dissertation is to expand the current knowledge of neural correlates of a hand muscle relaxation following a maximum grip.

The brain plays an active role in muscle relaxation [6-9]. Imaging studies have shown that muscle relaxation is preceded and accompanied by activation of the primary and supplementary motor areas of the brain in healthy young adults [7, 9, 10]. A limitation of brain imaging research is that the excitatory or inhibitory nature of the brain activity cannot be determined by brain scans alone. Muscle relaxation may be mediated by corticospinal activation of spinal inhibitory interneurons in order to turn off ongoing muscle activity [11-14]. Alternatively, muscle relaxation may be mediated by activation of cortical inhibitory circuits to turn off ongoing excitatory activity of corticospinal neurons.

To examine nature of the brain activity, transcranial magnetic brain stimulation (TMS) has proven useful. TMS researchers study excitatory and inhibitory nature of the brain activation by varying stimulation parameters such as stimulation intensity, pairing of stimuli, and

interstimulus interval, owing to differences in thresholds and numbers of synapses involved in various excitatory and inhibitory circuits. TMS research has shown that muscle relaxation was accompanied by increased short-interval intracortical inhibition (SICI)[15], which may lead to withdrawal of corticospinal input [8, 16].

The drawback of these studies is that intracortical and spinal motor neuronal activity changes with background muscle activity [17]. Hence, it cannot be denied that changes found in SICI and/or spinal excitability in above mentioned previous research may have been confounded by continuous reduction in the muscle activity during muscle relaxation. Therefore, the first aim of this dissertation was to examine the role of SICI and spinal excitability in grip relaxation while controlling for the background muscle activity.

Considering the active involvement of the central nervous system (CNS) in muscle relaxation described above, it is possible that the grip relaxation time is prolonged by aging and movement disorders caused by CNS pathology. General slowing of reaction time associated with degeneration and slowing of the nervous system takes place with aging [18-22] . However, effects of aging on the grip relaxation time and its neural mechanisms have not been documented. Therefore, the second aim of this dissertation was to examine the effects of aging on muscle relaxation time and associated neural mechanisms.

It is not a surprise that delayed termination of muscle activity accompanies movement disorders such as stroke [16, 23, 24], Parkinson's disease [25], and dystonia [26, 27]. Post-stroke motor deficits are of interest due to wide-spread of occurrence of stroke. A stroke occurs every

40 seconds in the U.S. [28]. Stroke is also a leading cause of long-term disability in the U.S. Among impairments that occur after stroke, hand impairment is particularly persistent and difficult to recover from. Almost 60% of chronic stroke survivors suffer from hand impairment [29]. Especially, impaired ability to grasp and release objects [23, 24] severely limits stroke survivors' activities of daily living.

Active control of muscle activity is crucial for movement coordination [30], in addition to the passive phenomena of spasticity and muscle tone. After stroke, delay is substantially more pronounced during muscle relaxation than initiation of muscle contraction [23]. Yet, muscle relaxation post stroke has not been much studied. The third aim of this dissertation was to examine the effects of stroke on muscle relaxation time and associated neural mechanisms.

In summary, the following three specific aims were put forward to achieve the objective of determining neural mechanisms associated with timely muscle relaxation.

Aim 1: To examine the role of SICI and spinal excitability in grip relaxation.
This aim was accomplished by comparing SICI and spinal excitability during grip relaxation to those during sustained contraction with matching background muscle activity in healthy young adults.
Hypothesis 1: SICI increases and spinal excitability is suppressed during grip relaxation compared to sustained contraction with matching background muscle activity in healthy young adults.

Aim 2: To examine the effects of aging on the grip relaxation time and modulations of SICI and spinal excitability for grip relaxation.

Hypothesis 2: Older adults exhibit longer grip relaxation times and lesser modulation of SICI and spinal excitability.

Aim 3: To examine neural mechanisms of delayed grip relaxation in chronic stroke survivors.

Hypothesis 3.1: Chronic stroke survivors have lesser modulation of SICI and spinal excitability during grip relaxation in the paretic hand compared to their nonparetic and age-matched control hands.

Hypothesis 3.2: Imbalanced interhemispheric inhibition is associated with longer grip relaxation times in chronic stroke survivors.

Chapter 2. Aim 1: To examine the role of SICI and spinal excitability in grip relaxation

1. Introduction

Termination of muscle contraction is an important aspect of motor control. Precise temporal modulation of muscle activity and associated limb force is necessary for many daily activities such as object manipulation [1, 2] and reaching [3]. Delays in initiation and termination of muscle activity can lead to inefficient grip force scaling during grip-and-lift tasks [4, 5] and poor timing and coordination of movement [5]. Indeed, delays in initiation and termination of muscle activity often characterize motor deficit in Parkinson's disease [25], dystonia [26, 27], and stroke [16, 23]. Yet, neural mechanisms for muscle relaxation have been relatively understudied.

1.1 Short-interval intracortical inhibition for grip relaxation

The active role of the brain in muscle relaxation has been demonstrated in previous studies [6-9]. Imaging studies have shown that voluntary muscle relaxation is preceded and accompanied by activation of primary and supplementary motor areas [7, 9, 10]. However, how these brain activities lead to the cessation of spinal motoneuron activity and mediate muscle relaxation remains unclear. One potential mechanism is the activation of intracortical inhibitory circuits. Specifically, muscle relaxation may be mediated by increased intracortical inhibition [15], leading to withdrawal of excitatory corticospinal input [8, 16] . Alternatively, muscle relaxation may be mediated by corticospinal activation of spinal inhibitory interneurons [11-14].

Two previous studies examining the role of short interval intracortical inhibition (SICI) in muscle relaxation have reported conflicting results [14, 15]. Both studies found changes in SICI after a relaxation cue and approximately 20 to 70 ms prior to the termination of first dorsal interosseous (FDI) muscle activity. However, Buccolieri et al. [15] found that SICI increased for FDI relaxation, whereas Begum et al. [14] found that SICI decreased for relaxation.

Such disparity in the findings could be due to different experimental settings such as the level of muscle activity just prior to stimulation and different transcranial magnetic stimulation (TMS) intensities. For instance, SICI decreases with an increasing level of muscle activity [17]. Thus, increase in SICI observed during muscle relaxation in Buccolieri et al. [15] may have been due to decreasing level of muscle activity, and may not represent modulation of SICI specific to a person's intention to relax. In Begum et al. [14], the level of muscle activity during relaxation just prior to stimulation is unknown and thus incomparable to that during the baseline SICI measurement. Therefore, in the present study, to address this issue, we compared SICI between grip relaxation and SICI during sustained contraction with matching background muscle activity. In addition, different stimulation intensities were used to evoke SICI in the two studies. Begum et al. [14] used 90% and 110%-120% of the active motor threshold (AMT) as the conditioning and test stimulus intensities respectively (conditioning stimulus intensity = 35% ± 5% of the maximum stimulator output (MSO), test stimulus intensity = 46% ± 6% MSO). Facilitation can occur when both conditioning and test stimulus intensities are close to AMT [31]. Thus, it is possible that the results by Begum et al. [15] may have been contaminated by cortical facilitation and therefore lacked inhibition during relaxation. On the other hand, Buccolieri et al. [15] used conditioning stimulus intensities of 80% and 100% of AMT, while the test stimulus intensity was large enough to produce approximately 1 mV of peak-to-peak motor evoked potential (MEP)

amplitude (conditioning stimulus intensity = 35% ± 5% MSO and test stimulus intensity = 59% ± 16% MSO). This large test stimulus intensity is consistent with other previous studies eliciting SICI [17, 32, 33]. Therefore, in the present study, we used stimulation intensities of 90% AMT for conditioning stimulation and stimulus intensity large enough to evoke 1 mV peak-to-peak amplitude MEP in at rest.

1.2 Spinal motor excitability for grip relaxation

In healthy adults, spinal motor neuron excitability, specifically the Ia reflex loop assessed by H-reflex [34, 35], was shown to decrease during relaxation of the Soleus muscle in the leg [13]. However, in the upper extremity, spinal motor neuron excitability for grip relaxation has not been studied.

1.3 Contralateral differences in motor control

The differences between the dominant and nondominant hands are well-documented in regards to preferred use [36], motor unit firing behavior [37], muscle fiber composition [38], and structural and functional differences at the spinal and supraspinal level [39-41]. Therefore, it is possible that the dominant and nondominant hands may have different neural mechanisms for grip relaxation. For example, contraction time and half-relaxation time were shorter in the dominant vs. nondominant leg muscles in soccer players [42]. However, there is currently no evidence for differences in the dominant and nondominant hand muscle relaxation.

Towards this end, this chapter describes investigation of modulations of SICI and spinal motor excitability during relaxation from a grip. A novel protocol was developed to control for background muscle activation to tease out changes in SICI and H reflex specific to the intent of muscle relaxation while not being confounded by the muscle activity level. This unique protocol consisted of comparing SICI and H reflex during relaxation from a power grip with that during sustained power grip at the similar level of muscle activity. We hypothesized that the SICI would increase whereas H reflex would decrease during grip relaxation compared to those during sustained contraction. The modulations of SICI and H reflex were examined in both the dominant and nondominant hands of right-handed healthy young adults to examine the effect of side. Power grip was used for its functional prevalence and for the potential to extend the developed protocol to patient populations who may have abilities to perform only power grips but not precision pinch grips. The flexor digitorum superficialis (FDS) muscle was examined because of its major role in power grip and functional activities of the hand [43, 44].

This aim was accomplished through three separate studies. In Study 1, we examined SICI at 70%, 80%, and 90% into the relaxation period and at their matching sustained contractions. No change in SICI was observed with progression of relaxation from 70% to 90% of the relaxation period. To demonstrate dynamic changes of SICI with relaxation, Study 2 was conducted to describe SICI changes over a wider range of the relaxation period, namely at 25%, 50% and 75% into muscle relaxation. In Study 3, H reflex was examined at 25%, 50% and 75% into grip relaxation and matching sustained contraction.

2. Methods

2.1 Study 1: Role of SICI for 70%, 80% and 90% into grip relaxation

2.1.1 Subjects

A total of 20 healthy right-handed subjects (mean±SD age = 25±6 years, 9 females) participated in this study. Subjects did not have any neurological and musculoskeletal disorders at the time of this study based on their self-disclosure. Handedness was determined using the Edinburgh handedness inventory [45]. One subject was tested for both hands, while all the rest subjects were tested for only one hand. A total of 11 dominant hands (age 24±5 years, 3 females) and 10 nondominant hands (age 27±6 years, 6 females) were tested. The sample size per hand is presented in Table 1. All subjects signed an informed consent form approved by the Institution Review Board.

Table 1. Subject numbers across tested hands in Study 1.

	Dominant only tested	Nondominant only tested	Both hands tested	Dominant total	Nondominant total
Study 1 (70-90%)	10	9	1	11	10

2.1.2 Procedure

SICI at 70%, 80%, and 90% into the relaxation from a voluntary power grip was quantified and compared to the SICI during a sustained power grip. First, to decide the time to elicit SICI, each hand's muscle relaxation period was determined. Second, SICI during relaxation from a maximal voluntary isometric power grip was determined in Experiment 1. SICI

during a sustained voluntary isometric power grip at a comparable muscle activity level was determined in Experiment 2 (to control for background muscle activity that affects SICI). Experiment 1 was immediately followed by Experiment 2 on the same day. One subject who was tested for both hands came for two days of testing (one day for each hand). For the right hand testing, the left motor cortex was stimulated using TMS. For the left hand testing, the right motor cortex was stimulated using TMS.

2.1.2.1 Measurement of grip relaxation time

Subjects were seated on a height-adjustable chair in front of a table with a cylindrical handle and a computer screen on top (Figure 1). The chair height and handle location were adjusted so that at rest, subjects had their fingers comfortably around the handle in a grasping posture, with the shoulder flexed at approximately 20°, the elbow flexed at approximately 100°, the forearm resting on the table in the midprone position, and the wrist in neutral posture. The contralateral hand and forearm were resting on a pillow on their lap. Electromyography (EMG) for the FDS muscle was recorded using adhesive Ag-AgCl bipolar surface electrodes (Bortec Biomedical Ltd., Calgary, Alberta, Canada) placed on the skin overlying the FDS muscle according to the literature [46].

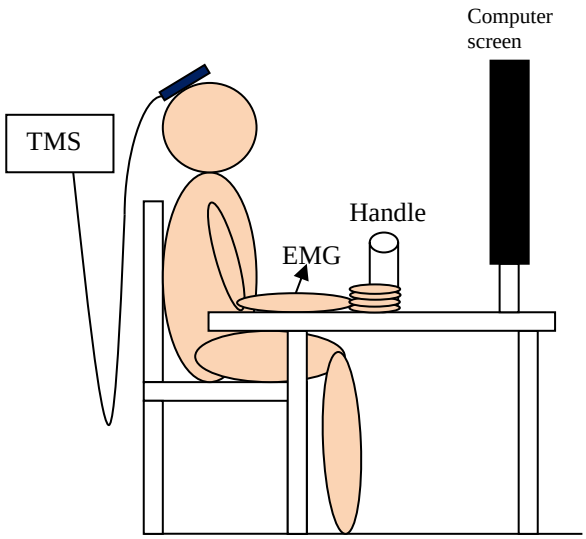


Figure 1: Experimental set up for Studies 1 and 2

In the posture described above, subjects were instructed to relax, and then grip the handle as hard as they could upon hearing a computer-generated sound, maintain the grip for the duration of the sound, and relax as quickly as possible upon termination of the sound. Subjects were instructed to stay relaxed for the next 4 s (). The sound lasted for 4 s. The subjects were instructed not to contract muscles in other limbs. The subjects were also instructed not to open their fingers during grip relaxation. The EMG was recorded at 2 kHz throughout the grip-and-relax trial, using NI BNC 2021 (National Instruments Corp., Austin, Texas, USA).

The grip relaxation time was determined as the time interval between when the sound ended and when the FDS muscle activity decreased to its precontraction baseline level (). Specifically, the root mean square (RMS) values of EMG data with a 20-ms moving window were obtained using a custom-made LabVIEW program (National Instruments Corp., Austin, Texas, USA). The baseline EMG level was determined as the mean of RMS EMG data for a 3 s

period immediately before the sound. The FDS muscle activity was determined to have decreased to the baseline level when the FDS RMS EMG was less than the mean + 3 standard deviations (SD) of the baseline EMG level for at least 50 ms after the sound ended [23].

Subjects had several practices until they became familiarized with the grip-and-relax trial. After practice, subjects performed 5 grip-and-relax trials. The mean relaxation period of these 5 trials determined the subject's muscle relaxation period. The average (±standard deviation, SD) grip relaxation time across all subjects of Study 1 was 416 ± 184 ms. The mean ± SD grip relaxation time for the dominant hand and nondominant hand was 329 ± 123 ms and 495 ± 198 ms, respectively (two-sample t-test $p<0.05$).

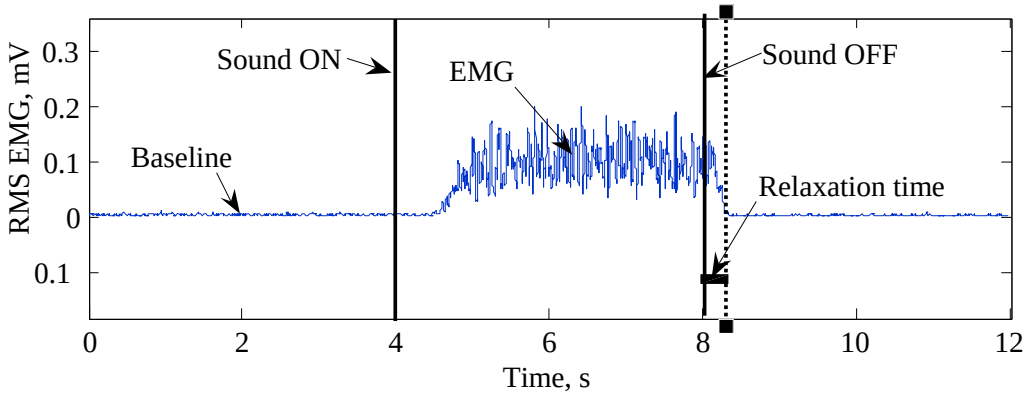


Figure 2: RMS EMG during a single grip-and-relax trial for determination of the muscle relaxation period

2.1.2.2 Experiment 1: SICI during relaxation

SICI during the relaxation phase of the grip-and-relax trials was determined using TMS (The Magstim Company Ltd, Wales, UK) in Experiment 1. A 70 mm figure of eight coil was placed over the 'hotspot' of the motor cortex representing the contralateral FDS muscle (approximately 6 cm anterolateral to vertex of the skull) (Figure 1). The handle of the coil was postero-lateral at an approximately 45° angle to the midsagittal plane. The coil was held in

position by an adjustable stand and the subjects rested their head on a chin rest (not shown in Figure 1) to ensure that they were relaxed and the coil position was not disturbed during the experiment. Coil position was checked regularly throughout the data collection session.

The paired pulse technique was used to determine SICI [33]. The test stimulus intensity was set at the % MSO that evoked peak-to-peak nonconditioned MEP amplitude of 1 mV in the resting FDS muscle. Mean test stimulus intensity (± standard deviation, SD) across all subjects in Study 1 was 188 ± 57% of AMT. To evoke a conditioned MEP, the suprathreshold test stimulus was preceded by a subthreshold conditioning stimulus with a 2 ms interstimulus interval (ISI). The subthreshold conditioning stimulus intensity was set at 90% of the AMT. These conditioning and test stimulus intensities with the ISI of 2 ms were chosen to minimize contamination of SICI by intracortical facilitatory pathways [32]. SICI was determined using Equation 1, as used by Coxon et al. [47].

$$SICI = 100 * \left(1 - \frac{conditionedMEP}{nonconditionedMEP}\right) \qquad \textit{Equation 1}$$

The AMT was determined as the %MSO that evoked a peak-to-peak MEP amplitude of 100 μV, at least 5 times in response to 10 stimuli while the person was maintaining the RMS EMG at 10% of the maximum voluntary contraction (MVC), according to Rossini et al. [48]. The muscle activation level at 10% MVC was successfully achieved by providing subjects with visual feedback on the computer screen and verbal feedback by examiners. The screen showed real-time RMS EMG along with a target line. Subjects were instructed to match their real-time EMG to the target during determination of the AMT.

SICI was determined at 70%, 80% and 90% into each subject's muscle relaxation period following a maximum power grip (after the sound ended) during the grip-and-relax trials (Figure 3 A, B). SICI was evoked at these times because changes in SICI were expected at approximately 80% into the muscle relaxation period according to previous studies [14, 15]. Ten conditioned and ten nonconditioned MEPs were evoked at these three stimulation times in a random order. Mean values of ten conditioned and ten nonconditioned MEPs were used to compute SICI (Equation 1).

2.1.2.3 Experiment 2: SICI during sustained contraction

SICI during sustained contraction at comparable muscle activity levels was determined. Specifically, the three background EMG levels at 70%, 80%, and 90% into the muscle relaxation period in Experiment 1 were targeted. The background EMG level was determined as the mean RMS EMG for a 20 ms period immediately before the stimulus (Figure 3 B). Subjects were instructed to maintain the target EMG level using visual feedback on the computer screen and verbal feedback by the examiners. Stimulation was delivered while subjects were holding the target EMG level (Figure 3 C, D). At each of the three background EMG levels, nonconditioned and conditioned MEPs were evoked 10 times to determine SICI (Equation 1).

Trials were discarded if they did not have background EMG levels within the mean ± SD of the Experiment 1 background EMG levels. This was to ensure that muscle activity levels were similar during relaxation (Experiment 1) and sustained contraction (Experiment 2) to permit comparisons of SICI. The same motor units are expected to be active for both Experiments 1 and 2, as motor units follow an orderly recruitment and de-recruitment (i.e., the motor units recruited

at low forces are de-recruited at the similar low forces, and motor units recruited at high forces are de-recruited at high forces [49].

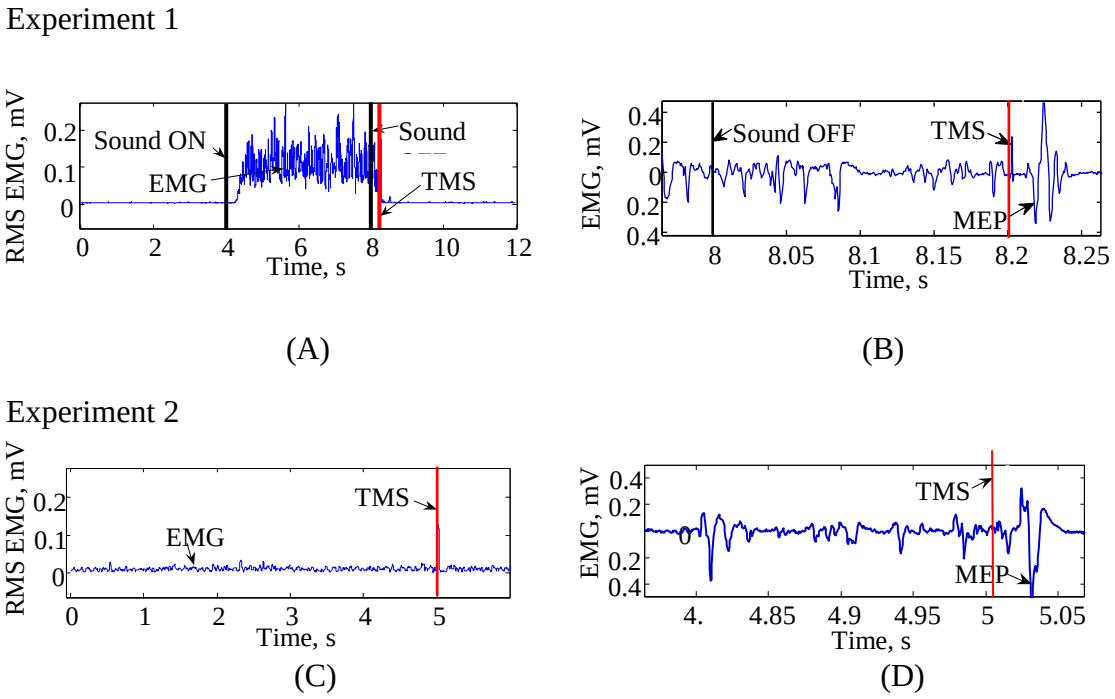


Figure 3: TMS delivery during grip relaxation and sustained contraction. (A) RMS EMG for the entire grip-and-relax trial in Experiment 1. Stimulation was delivered during relaxation after grip. (B) Raw EMG during relaxation, showing MEP (note different time scales). (C) RMS EMG showing a sustained contraction trial in Experiment 2. (D) Raw EMG showing the stimulation timing and MEP at the comparable background FDS RMS EMG during Experiment 2.

2.2 Study 2: Role of SICI for 25%, 50% and 75% into grip relaxation

2.2.1 Subjects

A total right-handed 20 subjects (24±5 years, 8 females) participated in this study. Subjects did not have any neurological and musculoskeletal disorders at the time of this study based on their self-disclosure. Handedness was determined using the Edinburgh handedness inventory [45]. Twelve subjects were tested for both hands. A total of 20 dominant hands (24±5

years, 8 females) and 12 nondominant hands (25±7 years, 6 females) were tested in this study. The sample size per hand is presented in Table 2. All subjects signed an informed consent form approved by the Institution Review Board. A total of 11 subjects who participated in Study 2 had also participated earlier in Study 1.

Table 2. Subject number distribution across hands in Study 2.

	Dominant only	Nondominant only	Both hands	Dominant total	Nondominant total
Study 2 (25-75%)	8	0	12	20	12

2.2.2 Procedure

To observe dynamic changes of SICI with relaxation, the same protocol as Study 1 was repeated except that SICI was measured at 25%, 50% and 75% into muscle relaxation. Study 2 was conducted in case changes of SICI for the FDS muscle relaxation from power grip may have occurred earlier than 70% into the muscle relaxation period examined in Study 1. In addition to SICI measurements during relaxation and sustained contraction, SICI during maximum power grip and at rest were recorded to obtain a complete picture of the SICI changes with relaxation. In addition to the FDS EMG, the antagonist EMG from the extensor digitorum communis (EDC) muscle was recorded to examine if SICI was influenced by the EDC activity. The FDS and EDC muscle activity was normalized to their respective mean maximum EMG values during 5-10 maximum power grip (MVC). The maximum EMG value of a single MVC trial was computed as the maximum of the RMS EMG using a 20ms moving window over 2-3 s of a 4 s long MVC. The Mean (± SD) relaxation time across all subjects of Study 2 was 469 ± 176 ms. The mean ±

SD grip relaxation times for the dominant and nondominant hands were 468 ± 186 ms and 470 ± 167 ms, respectively (two-sample t-test, $p>0.05$). Mean test stimulus intensity (± SD) across all subjects of Study 2 was 198% (± 39%) AMT.

2.3 Study 3: Role of H reflex for grip relaxation

2.3.1 Subjects

A total 25 right-handed subjects participated in this study. Eleven subjects were tested for both hands. Subjects did not have any neurological and musculoskeletal disorders at the time of this study based on their self-disclosure. Handedness was determined using the Edinburgh handedness inventory [45]. A total of 20 dominant hands (25±5 years, 10 females) and 16 nondominant hands (24±5 years, 10 females) were tested for Study 3. Table 3 presents sample size per hand. Four subjects participated in all three studies. One subject participated in Studies 1 and 3 only. Two subjects participated in Studies 2 and 3 only. All subjects signed an informed consent form approved by the Institution Review Board.

Table 3. Subject number distribution across hands in the H reflex study.

	Dominant only	Nondominant only	Both hands	Dominant total	Nondominant total
study 3 (25-75%)	9	5	11	20	16

2.3.2 Procedure

The spinal level motor circuit's excitability was assessed as the H-reflex/M-wave ratio. The spinal motor circuit excitability during relaxation from a voluntary maximal power grip was

compared to that during a sustained power grip in Study 3, for the dominant and nondominant hands of healthy young adults. Grip relaxation time was determined first as described in Study 1. The mean grip relaxation time across all subjects of Study 3 was 489 ± 155 ms. The mean ± SD grip relaxation times for the dominant and nondominant hands were 497 ± 162 ms and 480 ± 150 ms, respectively (two-sample t-test, $p>0.05$). Next, spinal motor circuit excitability at 25%, 50% and 75% into relaxation from a maximal voluntary isometric power grip was determined in Experiment 1, followed by the assessment of spinal motor circuit excitability during a sustained voluntary isometric power grip at a comparable muscle activity level in Experiment 2 (to control for background muscle activity that affects spinal motor circuit excitability). Both Experiments 1-2 took place one after another, in the same day.

To determine H/M ratio, H-reflex and M-wave were elicited in separate trials in a random order at 25%, 50% and 75% into relaxation, by stimulating the median nerve in the cubital fossa [34]. The median nerve was stimulated using a single pulse electric stimulation (square pulse width of 1 ms) delivered through bipolar surface electrodes (3 cm inter-electrode distance with cathode proximal).

The stimulation of the median nerve (Figure 4 A) was confirmed by resulting paraesthesia in the lateral 3.5 fingers. After securing the stimulating electrodes firmly, the stimulation intensity was increased gradually to evoke H-reflex and M-wave. The stimulation intensities required to elicit i) Mmax and ii) H reflex peak-to-peak amplitude similar to 15% peak-to-peak amplitude of M-max were determined at rest. The mean ± SD stimulation intensity across all subjects for eliciting Mmax was 17.4 ± 7.9 mA and for H reflex was 8.3 ± 5.1 mA. FDS responses to these two stimulation intensities were recorded at 25%, 50% and 75% into grip relaxation (Figure 4 B). Ten trials to record responses for each of the two stimulation intensities

at each timing were collected in a random order. The ratio of H-reflex amplitude to M-wave amplitude at 25-75% into relaxation determined H/M ratio during relaxation. In addition, the H/M ratio during sustained contraction was determined at the matching background FDS muscle activity using visual feedback using the similar method described Study 1 Experiment 2. EDC muscle activity was also monitored for signs of finger opening online and recorded for offline data analysis.

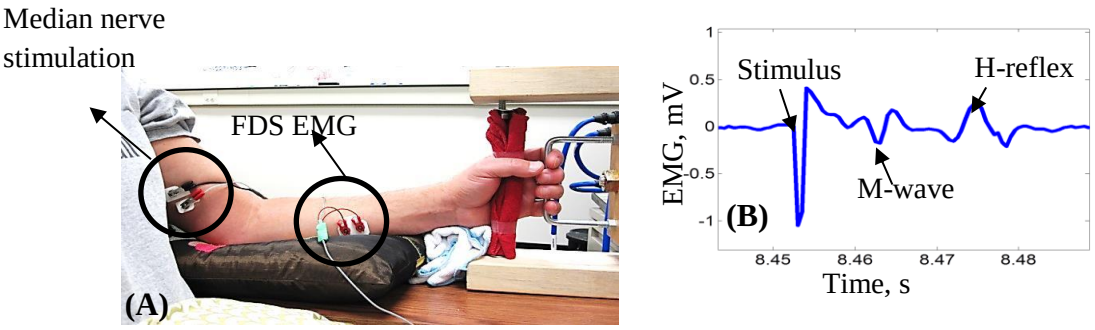


Figure 4: H reflex measurement. (A) Median nerve stimulation and EMG recording while examining spinal motor neuron excitability. (B) FDS EMG trace showing stimulus artifact followed by the M wave and H-reflex.

2.4 Statistical Analysis

As the primary analysis, mixed model ANOVA was performed for the main and 1st order interaction effects of two within-subject variables (contraction condition: relaxation vs. contraction, stimulation time with three levels: 70%, 80% and 90% in Study 1 and 25%, 50% and 75% for Studies 2 and 3) and one between-subject variable (side: dominant vs. nondominant) on the response variable of SICI or normalized H reflex for each Study.

To examine if contraction condition-specific changes in SICI could have been confounded by the FDS background activity and nonconditionedMEP amplitude and if contraction condition-specific changes in H reflex could have been confounded by the FDS

background activity [17], the same mixed model was used to examine if the FDS background muscle activity (for Studies 1-3) and peak-to-peak MEP amplitude (for Studies 1 and 2) changed with contraction condition, time, side, and their interactions. Furthermore, another mixed model ANOVA was performed for the main and interaction effects of the contraction condition, time and side on the background EDC EMG level to examine how the EDC EMG level changed during relaxation (25-75% into relaxation in Study 2 and 3), and to confirm that the subjects did not increase the EDC activation during the progress of relaxation in effort to extend/open their fingers.

In addition, for Study 2, two mixed models were used to examine main and interaction effects of time (one for mid-grip vs. 25% into relaxation and another for 75% into relaxation vs. rest), side (dominant vs. nondominant) and their interaction on the SICI. Additional mixed models (main effects and interaction between time and side) were used for the nonconditioned MEP, FDS background EMG, and EDC background EMG to describe the complete time course of the grip relaxation. All statistical analyses were performed using SPSS software (version 20, IBM, Armonk, NY). The significance level was set at 0.05.

3. Results

3.1 Study 1 (SICI at 70-90% into relaxation)

SICI assessed in FDS was 36% greater across the three relaxation times (mean ± 95% confidence interval (CI) = 37.4% ± 6%) than during the sustained active contraction at comparable muscle activity levels (27.4% ± 2.9%) (Figure 5 A). The primary ANOVA showed a significant main effect for contraction condition (relaxation vs. sustained contraction) on SICI ($p = 0.035$). The main effect of time ($p = 0.779$) and the interaction between time and contraction

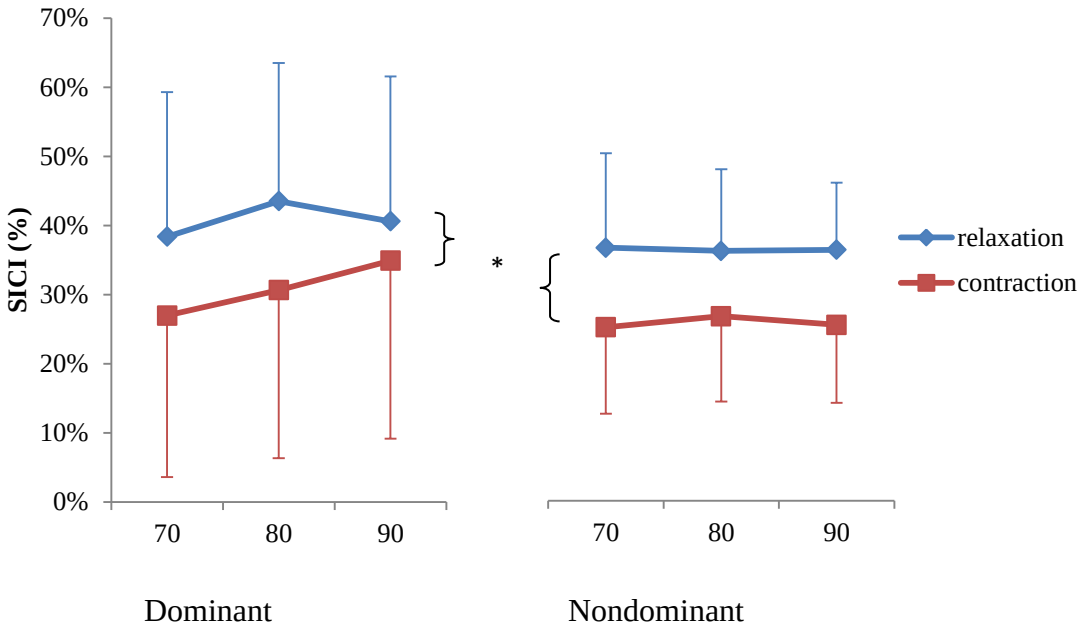
condition ($p = 0.641$) were not found to significantly affect SICI. Additionally, SICI was not affected by side (dominant vs. nondominant, $p=0.054$) and interactions of side with time and side and contraction condition ($p>0.929$).

To examine if the greater SICI during relaxation may have been resulted from task-specific changes in the nonconditioned MEP and/or the background FDS EMG, further analysis was performed as follows. Mean nonconditioned MEP amplitudes were not significantly different between the two contraction conditions (main effect of contraction condition $p=0.992$, Figure 5 B). Mean peak-to-peak amplitudes of nonconditioned MEP decreased with progression of muscle relaxation in Experiment 1 and with decreasing muscle activity in Experiment 2 (Figure 5 B, time main effect $p=0.011$). Mean nonconditioned MEP was not affected by side ($p=0.273$) and any 1^{st} order interactions among time, contraction condition, and side ($p>0.362$).

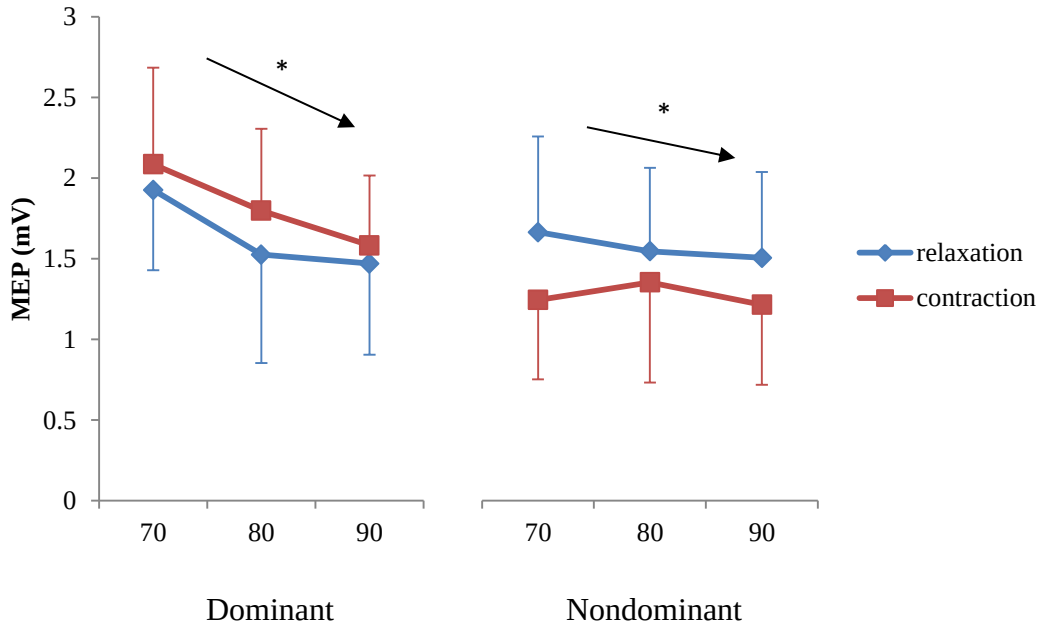
Background FDS EMG amplitudes were similar between the two contraction conditions (main effect of contraction condition $p=0.210$, Figure 5 C). The background FDS EMG decreased with time ($p<0.001$), and this reduction was greater in the dominant hand than the nondominant hand (significant interaction between time and side $p<0.001$). The dominant hand also exhibited greater background FDS EMG overall (11.9±2.3 %MVC in the dominant hand vs. 4.6±1.3 %MVC in the nondominant hand, $p=0.011$). The interaction between side and contraction condition, and time and contraction condition did not significantly affect the background FDS EMG ($P>0.115$).

To summarize, SICI increased during grip relaxation compared to sustained contraction at matching muscle activity, for both dominant and nondominant hands. This relaxation-specific increase in SICI was not accompanied by changes in MEP and/or background FDS muscle

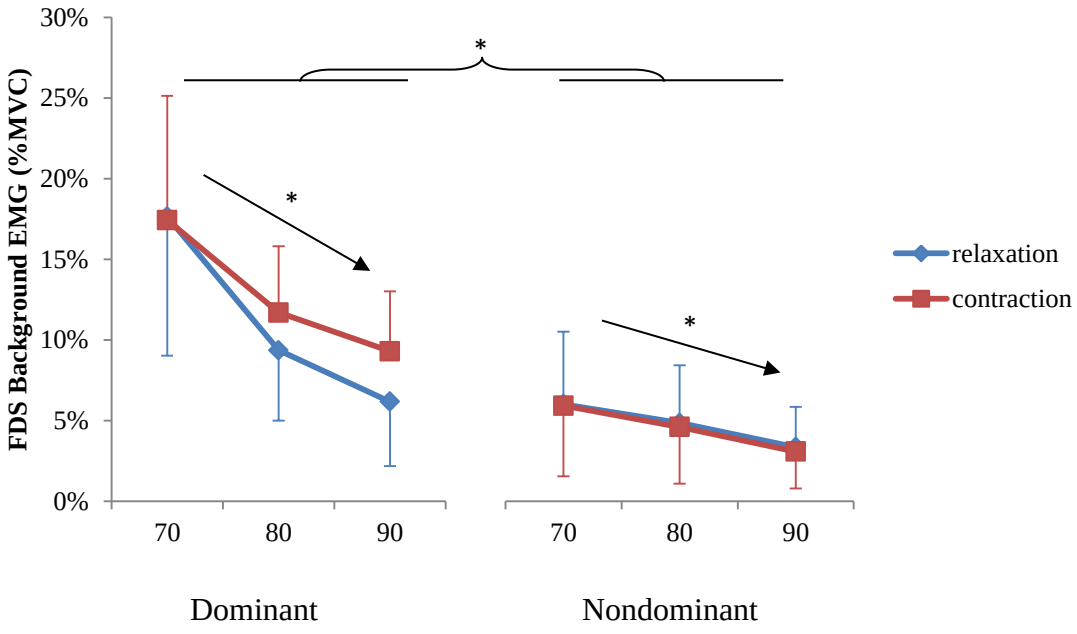
activity specific to relaxation. Therefore, the relaxation-specific increase of SICI during grip relaxation in our study suggests the cortical control of grip relaxation in both hands.



(A)



(B)



(C)

Figure 5: Study 1 results. (A) Mean ± 95%CI SICI obtained during 70%, 80% and 90% into the relaxation period during the grip-and-relax trials (Experiment 1) and sustained contraction trials at the matching background EMG levels (Experiment 2) in dominant and nondominant hands. (B) Mean ± 95%CI nonconditioned MEPs obtained during 70%, 80% and 90% into the muscle relaxation period (Experiment 1) and during sustained contraction at the matching background EMG levels (Experiment 2) for both hands. (C) Mean ± 95%CI FDS background EMG during relaxation and matching contraction for both hands.

3.2 Study 2 (SICI at 25-75% into relaxation, mid-grip and rest)

3.2.1 SICI at 25-75% into relaxation

The primary result is that SICI assessed in the FDS muscle was, on average, 34% greater during muscle relaxation (mean ± 95%CI= 29% ± 6% when pooled for the three times) than during sustained contraction at the matching background muscle activity (21% ± 6%) (main effect of contraction condition p=0.038, Figure 6 A), consistent with Study 1. Different from Study 1, SICI increased with the progression of relaxation (main effects of time, p<0.001). SICI was greater in the nondominant hand (29±9% in the nondominant hand vs. 23±5% in the

dominant hand, main effect of side, $p<0.001$). SICI was not significantly affected by any of the 1st order interactions between contraction condition, time, and side ($p>0.507$).

To examine if this relaxation-specific increase in SICI was influenced by changes in the noncoditioned MEP and/or the background FDS muscle activity, the further analyses were performed. The results of these further analyses are as follows. Consistent with Study 1, mean peak-to-peak amplitudes of nonconditioned MEP were not different between the two contraction conditions ($p>0.05$). The mean peak-to-peak amplitudes of nonconditioned MEP decreased with progression of muscle relaxation in Experiment 1 and with decreasing FDS activity in Experiment 2 (Figure 6 B, main effect of time $p<0.001$). The noncontioned MEP was not affected by any other variables including side and any 1st order interactions between time, side and contraction condition ($p>0.471$). Also consistent with Study 1, the background FDS EMG amplitudes were similar between the two contraction conditions in Study 2 (Figure 6 C). The main effect of contraction condition ($p = 0.465$) and the interaction between contraction condition and stimulation time ($p=0.934$) were not significant. Background FDS EMG amplitudes decreased as the relaxation progressed and as the target muscle activity level of the sustained contraction decreased ($p<0.001$ for the main effect of time). The background FDS EMG was also not affected by side and any 1st order interactions between time, contraction condition and side ($p>0.061$). These findings confirm that the FDS background EMG was well-controlled between the two contraction conditions in this study and that the main finding of SICI increase for relaxation as compared to sustained contraction is not confounded by the background FDS EMG amplitudes or the nonconditioned MEP amplitudes.

To examine if subjects activated the EDC muscle in order to extend the fingers while relaxing their FDS muscles, another mixed ANOVA was used. The background EDC EMG

amplitudes decreased with the progression of muscle relaxation in Experiment 1 and decreasing muscle activity target levels in Experiment 2 ($p<0.001$, Figure 6 D), similarly with the FDS EMG amplitudes (Figure 6 C). The background EDC EMG was greater for the dominant hand ($p=0.032$), and comparable between the two tasks ($p=0.068$). The 1st order interaction effects of hand, task and time on the EDC background EMG were all not significant ($p>0.170$). This finding confirms that the subjects did not open their fingers into extension following their instructions, as evidenced by decreasing EDC background EMG with the progression of relaxation.

In summary, SICI was greater during relaxation compared to contraction with matching FDS background EMG for both dominant and nondominant hands. Analysis of secondary response variables suggested that the relaxation-specific increase of SICI was not confounded by the FDS background EMG and nonconditioned MEP amplitude. Additionally, the EDC EMG recoding confirmed that the subjects did not extend their fingers while relaxing from the power grip and that the relaxation of the FDS was not influenced by increased activation of the EDC and associated reciprocal inhibition. The increase in SICI during relaxation, while the background FDS EMG and nonconditioned MEP were controlled, suggests the role of SICI in grip relaxation in young adults.

3.2.2 SICI from mid-grip to 25% into relaxation and from 75% into relaxation to rest

A separate mixed model showed that the SICI during relaxation significantly increased from mid-grip to 25% into grip relaxation (main effect of time $p = 0.027$) (Figure 6 A). The main effect of side ($p=0.773$) and the interaction between time and side ($p=0.949$) did not significantly affect SICI for the analysis involving mid-grip and 25% into grip relaxation only. SICI did not

show a significant change between 75% into grip relaxation and rest (main effect of time, $p=0.063$), and did not depend on the side ($p=0.387$) and interaction between side and time ($p=0.254$). Together, the results showed that for both hands, SICI had increased by 25% into grip relaxation time after the signal to relax, and SICI continued to increase from 25% to 75% into grip relaxation, however SICI remained similar from 75% into grip relaxation to rest.

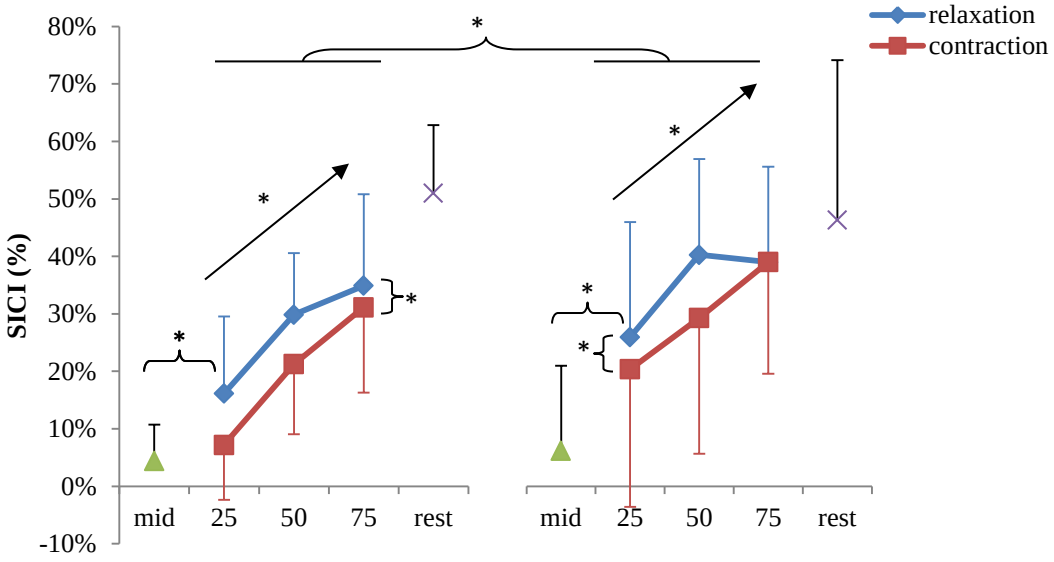
The mixed model analyzing changes in the nonconditioned MEP between mid-grip and 25% into grip relaxation showed no significant effects of time, side or time and side interaction ($p>0.437$). The nonconditioned MEP decreased from 75% into grip relaxation to the resting level ($p<0.001$), but was not affected by side or interaction between side and time ($p>0.806$). This analysis suggests that the nonconditioned MEP started to decrease during 25-75% into grip relaxation and continued to decrease even after 75% into grip relaxation into rest.

The background FDS EMG significantly decreased from mid-grip to 25% into grip relaxation (main effect of time, $p<0.001$) in both sides (insignificant main effect of side and interaction between side and time, $p>0.409$). The FDS background EMG at 75% into relaxation was greater than at rest in both hands (main effect of time $p=0.012$, insignificant main effect of side and interaction effect between time and side $p>0.301$).

Likewise, the EDC background activity decreased from mid-grip to 25% into relaxation (main effect of time, $p=0.018$) in both hands (main effect of hand, $p=0.179$, interaction between hand and time, $p=0.862$). The background EDC EMG was significantly greater at75% into relaxation compared to resting level in both hands (main effect of time, $p=0.001$, main effect of hand, $p=0.124$ and interaction between hand and time $p=0.107$). This finding confirms that the

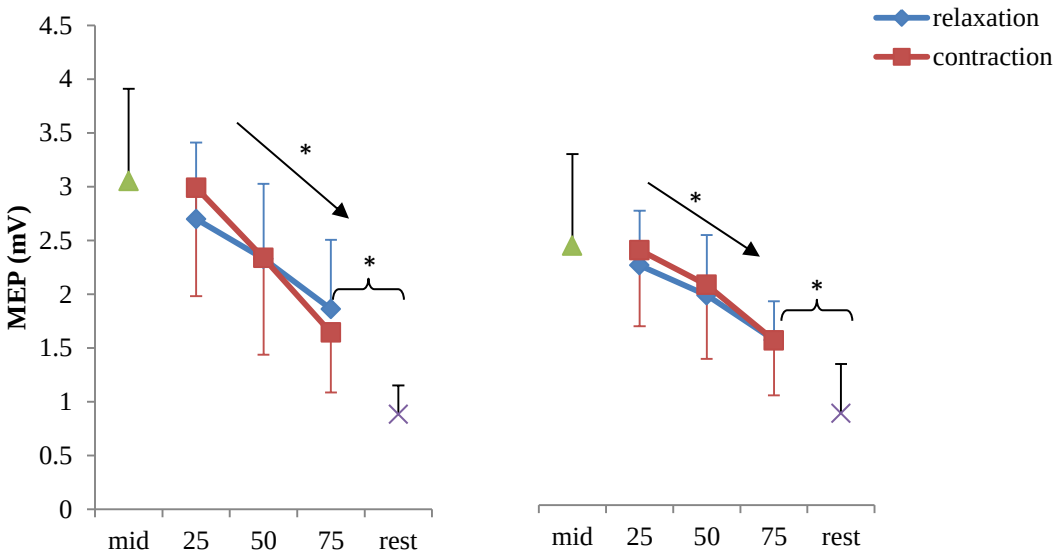
subjects did not open their fingers into extension following their instructions, as evidenced by decreasing EDC background EMG with the progression of relaxation.

In summary, the complete time course of relaxation can be described as both FDS and EDC muscle activities decreasing from mid-grip to 25%-75% of grip relaxation time and to rest. SICI increased from mid-grip to 75% of grip relaxation time, while the nonconditioned MEP decreased from 25% of grip relaxation to rest. This slight delay in the time period of changes in the nonconditioend MEP as compared to SICI may suggest that for relaxation, SICI increases first, followed by MEP decrease, as in the previous study [15]. Alternatively, the insignificant changes in high SICI levels (toward rest) and in high nonconditioned MEP levels (during maximum grip) may be due to the ceiling effect.

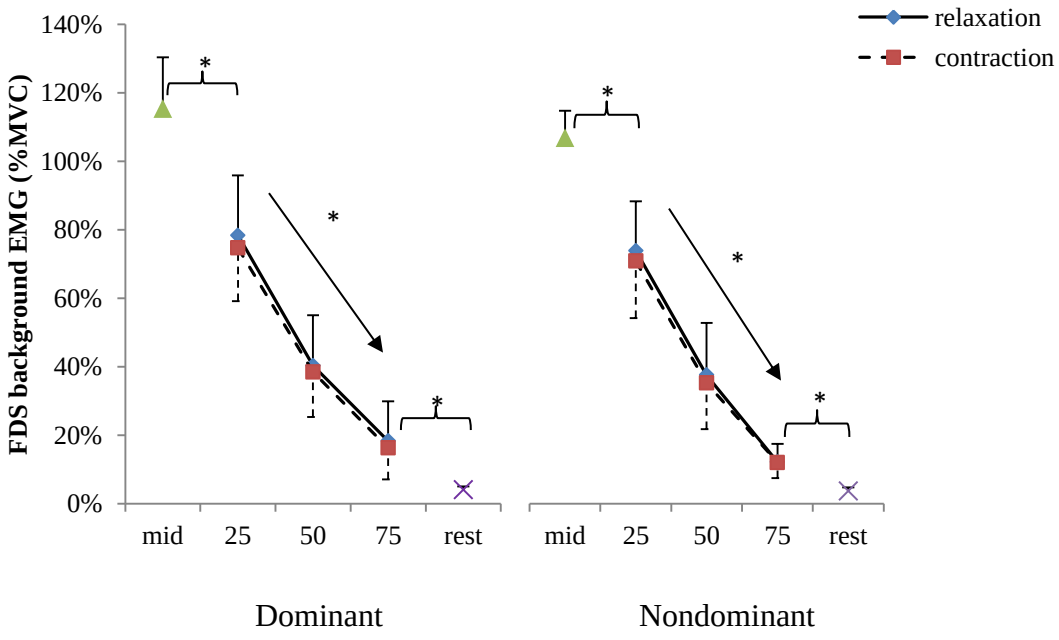


Dominant Nondominant

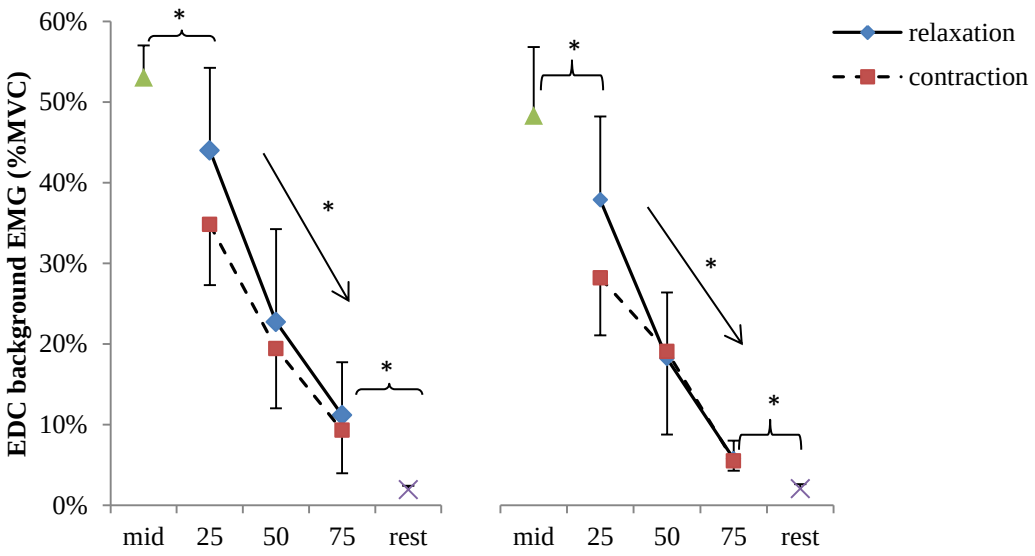
(A)


relaxation
contraction
MEP (mV)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
mid
25
50
75
rest
*
*
*
*
Dominant
Nondominant

(B)


relaxation
contraction
FDS background EMG (%MVC)
140%
120%
100%
80%
60%
40%
20%
0%
mid
25
50
75
rest
*
*
*
*
*
*
Dominant
Nondominant

(C)



(D)

Figure 6: Study 2 results. (A) Mean ± 95%CI SICI obtained in Study 2 during mid-grip, 25%, 50%, and 75% into the relaxation period, and rest (after the relaxation was achieved) during the grip-and-relax trials (Experiment 1) and sustained contraction trials at the matching background EMG levels for 25%-75% relaxation (Experiment 2). Both dominant and nondominant hands's data are shown. (B) Mean ± 95%CI nonconditioned MEPs. (C) Mean ± 95%CI FDS background EMG. (D) Mean ± 95%CI EDC background EMG showing consistent decrease in the EDC EMG with time.

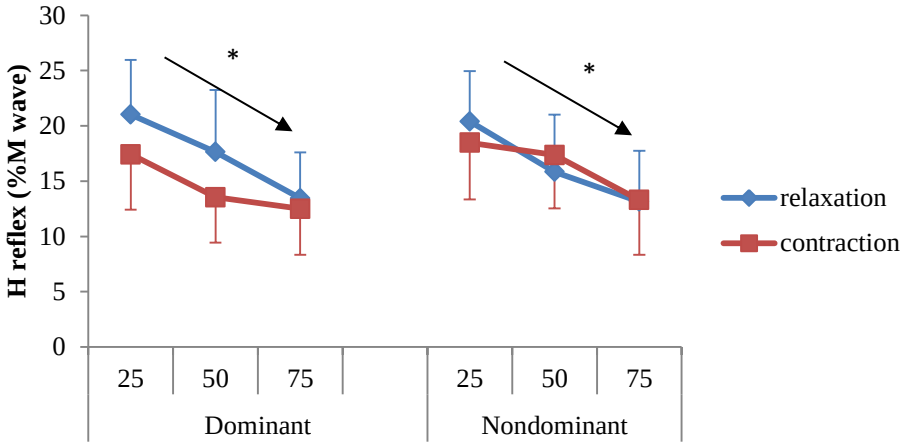
3.3 Study 3 (H reflex at 25-75% into relaxation)

H reflex (%M wave) did not significantly differ between the grip relaxation and sustained contraction task in both hands (Figure 7 A, main effect of contraction condition, $p=0.169$, interaction between side and contraction condition, $p=0.195$). H reflex levels decreased with progression of time during grip relaxation in Experiment 1 and with reduction in matching background muscle activity level in Experiment 2 in both hands (main effect of time, $p<0.001$, insignificant interaction between time and side, $p=0.942$, and between time and contraction

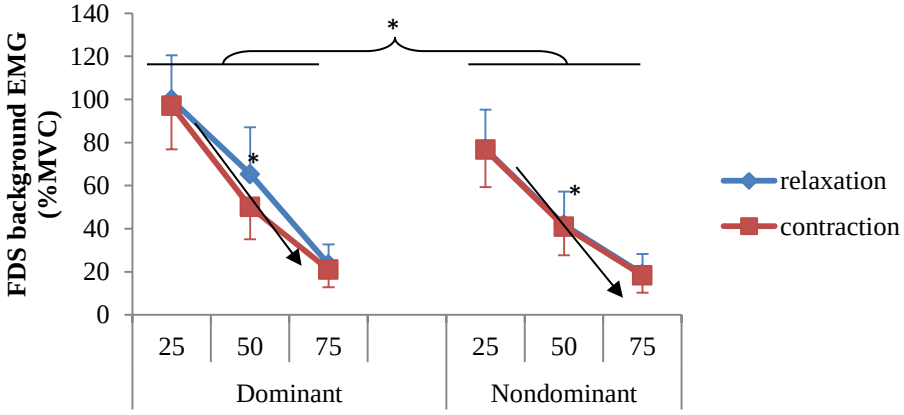
condition, p=0.659). The H reflex levels were similar between the dominant vs. nondominant hand (main effect of side, p=0.356).

To examine if the H reflex result could have been influenced by differences in the background FDS EMG, another mixed model ANOVA was performed for the background FDS EMG. The results are as following. The background FDS EMG did not differ between the two contraction conditions (Figure 7 B, main effect of contraction condition p=0.298). The interaction between side and contraction condition were not significant (p=0.381). The background FDS EMG decreased with progression of time in Experiment 1 and matching sustained contraction in Experiment 2 in both hands (main effect of time, p<0.001, interaction between side and time, p=0.102, interaction between contraction condition and time, p=0.630). The background FDS EMG levels were 30% greater for the dominant hand (59.5±8.5%MVC in the dominant hand vs. 45.6±7.2%MVC in the nondominant hand, main effect of side p=0.001).

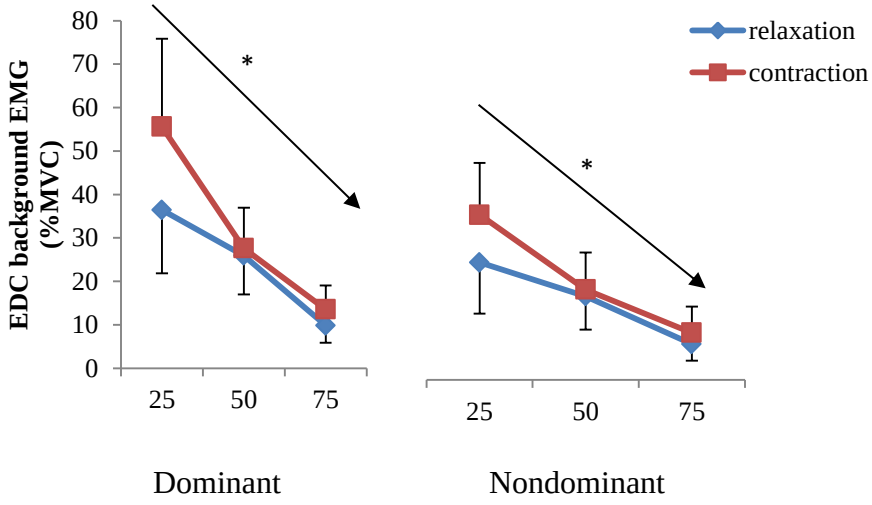
To examine if the H reflex result could have been influenced by increased activation of the antagonist EDC muscle during relaxation, another mixed model ANOVA (contraction condition, time, side, and their interactions) was performed for the EDC EMG. The background EDC EMG was smaller during the grip relaxation task compared to the sustained contraction with matching FDS EMGs (Figure 7 C, main effect of contraction condition p=0.042). The background EDC EMG decreased with progression of relaxation in Experiment 1 and decreasing contraction target levels in Experiment 2 (main effect of time p<0.001). The dominant hand had overall greater activation of the EDC muscle than the nondominant hand (29 ± 6% MVC vs. 20 ± 4%MVC, main effect of side p=0.009). The 1st order interactions among contraction condition, time, and side did not significantly affect the background EDC EMG (p>0.137).


H reflex (%M wave)
30
25
20
15
10
5
0
*
*
25
50
75
25
50
75
Dominant
Nondominant
relaxation
contraction

(A)


FDS background EMG (%MVC)
140
120
100
80
60
40
20
0
*
*
*
25
50
75
25
50
75
Dominant
Nondominant
relaxation
contraction

(B)


EDC background EMG (%MVC)
80
70
60
50
40
30
20
10
0
*
*
25
50
75
25
50
75
Dominant
Nondominant
relaxation
contraction

(C)

Figure 7: Study 3 results (A) Mean ± 95% CI H reflex (%M wave) at 25%, 50% and 75% into relaxation and matching sustained contraction in the dominant and nondominant hands. H reflex was not significantly different between the grip relaxation and sustained contraction tasks. H reflex decreased with time during grip relaxation (Experiment 1) and matching muscle contractions (Experiment 2) ($p<0.05$). H reflex levels did not significantly differ between the dominant and nondominant hands. (B) Mean ± 95% CI FDS background EMG (%MVC) during grip relaxation and sustained contraction conditions in both hands. The background FDS EMG did not differ between the two contraction conditions ($p>0.05$). The background FDS EMG decreased with progression of relaxation and decreasing muscle activation ($p<0.05$). (C) Mean background EDC muscle activity during grip relaxation and sustained contraction with matching FDS activity. The background EDC EMG differed between the two contraction conditions ($p>0.05$). The EDC activity showed gradual and significant decrease in the muscle activity with the progression of grip relaxation ($p<0.05$).

In summary of Study 3, the main finding was that the H reflex did not show relaxation-specific changes in both hands of young adults. Secondary analysis suggests that this finding of no involvement of H reflex modulation for relaxation was not confounded by the background FDS activity, since the background FDS activity remained consistent for the two contraction conditions. In addition, subjects did not increase activity in the antagonist EDC while relaxing from the grip, suggesting that the subjects did not open their fingers as instructed during the relaxation task. The EDC EMG was lower during relaxation than sustained contraction especially at 25% into relaxation (Figure 7 C), which could have reduced reciprocal inhibition and increased H reflex for the FDS muscle as seen in the slight increase of H reflex for relaxation as compared to sustained contraction at 25% into relaxation (Figure 7 B). However, such an effect was not substantial to yield a statistical significance in H reflex, likely because reciprocal inhibition circuits are suppressed during co-contraction in order to ensure high excitability in motor neurons for both muscles [50]. Also, an increase in H reflex during relaxation compared to sustained contraction would have been the opposite phenomenon of the original hypothesis.

Therefore, the hypothesis that H reflex would be reduced during relaxation compared to sustained contraction was not supported by the data.

As an overall summary for Studies 1-3, the series of three studies in healthy right-handed young adults demonstrated that cortical inhibition (SICI) increased while corticomotor excitability (nonconditioend MEP) and spinal motoneuron excitability (H reflex) remained the same for grip relaxation compared to sustained grip at the matching muscle activity level. The increase in SICI for grip relaxation was due to the intent to relax, not confounded by background FDS muscle activity, nonconditioned MEP, or antagonistic (EDC) muscle activity. In fact, as intended by the study design and instructions provided, the background FDS muscle activity was well controlled between the two conditions and the EDC muscle activity did not increase during the grip relaxation. This finding of relaxation-specific SICI increase without change in MEP and H reflex was consistent for both dominant right and nondomninant left hands. Grip relaxation time was also not significantly different between the sides (two-sample t-test $p=0.980$ with the mean grip relaxation time$\pm$SE = 458.7±55.9 ms for the dominant hand and 457.6±57.4 ms for the nondominant hand, averaged across all fourty eight subjects in the three studies).

4. Discussion

4.1 Modulation of SICI for grip relaxation

The objective of Study 1 and Study 2 was to determine the role of short interval intracortical inhibition (SICI) in grip relaxation by comparing SICI for the FDS muscle during a voluntary relaxation from a unilateral isometric power grip with the SICI during sustained isometric power grip at comparable levels of FDS activation. The main finding of the present

study was that SICI was, on average, 35% greater during voluntary relaxation from a power grip than sustained power grip at comparable muscle activity levels in healthy adults (from both Studies 1 and 2; Figure 5 A and Figure 6 A). Comparable levels of FDS muscle activity between the two conditions were achieved using visual feedback in the present study. In addition, nonconditioned MEP amplitudes for the FDS muscle and the antagonistic EDC muscle activity were found to be comparable between the two conditions, suggesting that changes in SICI were not due to differences in the background FDS muscle activity level, nonconditioned MEP, or the antagonistic EDC muscle activity level. These results suggest that activation of short-interval intracortical inhibitory circuits may assist with grip muscle relaxation, without active modulation of the corticomotor excitability (MEP) per given muscle activity level.

Our results are in agreement with Buccolieri et al. [15], despite the methodological differences (unilateral vs. bilateral relaxation, relaxation from a 100% vs. 20% MVC, power grip vs. index finger abduction). Although power grip may involve direct corticospinal pathways to a lesser degree than fine motor control, increase in SICI was observed with relaxation from power grip in the present study. It may be because motor cortical excitability changes for power grip and fine motor control similarly but with different magnitudes [51]. Our finding is in line with previous brain imaging studies that demonstrated increased activation of the M1 and supplementary motor areas during voluntary muscle relaxation [7-10]. Elevation of SICI during relaxation may be able to cease the ongoing muscle contraction through reduced corticospinal excitatory output.

The increased inhibition during muscle relaxation observed in the present study and Buccolieri et al. [15] is not in agreement with the other previous study [14], possibly due to the different stimulation parameters. Begum et al. [14] found reduced SICI during muscle relaxation.

Both conditioning and test stimulus intensities close to AMT could result in contamination of SICI by intracortical facilitation [31]. In contrast to Begum et al. [14], the present study and Buccolieri et al. [15] used a large test stimulus intensity to minimize contamination of SICI by intracortical facilitatory circuits [15, 17, 33, 47]. The test stimulation intensities used in the present study are also near the acceptable range (110-150% RMT) to evoke SICI [52]. Based on the relationship between AMT and RMT (AMT = 0.82 RMT) [53], the test stimulation intensity used in our study (188 ± 57% AMT for Study 1 and 198 ± 39% AMT for Study 2) may be interpreted as 158% RMT. Therefore, the stimulation parameters used in the present study appear to be acceptable for the current investigation of SICI according to the available evidence.

A remote possibility exists that the visual cue provided to subjects to relax muscle in Begum [14] may have triggered a different brain mechanism compared to auditory cues provided in the present study and Buccolieri et al. [15]. However, literature demonstrating different effects of sound and visual stimuli on M1 excitability is unavailable. Although cortical excitability can be suppressed by unexpected loud, startling auditory stimulation (greater than 80 dB) [54], the sound used in the present study was not startling (approximately at 60 dB) and was expected as the subjects knew in advance that the initiation and cessation would be cued by the sound. Therefore, it is unlikely that the greater SICI during relaxation than during sustained contraction was caused by the sound used in the present study.

The corticomotor excitability reduced with the muscle relaxation, in agreement with the previous literature [14, 15]. However, our study was the first to control for the changes in the corticomotor excitability due to the background muscle activity. While the nonconditioned MEP decreased with decreasing FDS muscle activity following a power grip, the nonconditioned MEP did not differ by the intent to relax or sustain a contraction, unlike SICI. These findings suggest

that the active component inducing muscle relaxation is modulation of SICI, not MEP at the cortical level.

Lastly, the increased SICI for relaxation reported in the present study may not have been caused by the antagonist (EDC) muscle activity. The EDC muscle was active during maximum grip, as co-contraction is typically observed in power grip [44, 51, 55]. The EDC EMG decreased during grip relaxation in the same manner with the FDS EMG (Figure 6 D). Since antagonist muscle coactivity increases SICI [56], if the change in SICI over the progression of relaxation observed in our study were due to the EDC activity, reduced EDC EMG with relaxation would have resulted in reduction of SICI with relaxation. Instead, SICI increased with the progression of relaxation in Study 2, supporting the conclusion that the increase in SICI for grip relaxation is due to increased excitability of intracortical inhibitory circuits, not due to the antagonist activity.

4.2 Changes in SICI with progression of relaxation

SICI was the lowest (mean=5%) during the maximum power grip, increased gradually from the mid-grip to 25% to 75% into relaxation, and was the highest (mean=50%) during rest in Study 2 (Figure 6 A). When examined only during the 70% to 90% of the relaxation period in Study 1, such changes in SICI were not apparent (Figure 5 A), suggesting that the increase of SICI occurs as early as 25% of the relaxation period and plateaus at or before 70% of the relaxation period.

The nonconditioned MEPs decreased with the progression of relaxation from 25% to 90% of the relaxation period and to rest (in both Studies 1 and 2), indicating that the associated

changes in the corticomotor excitability continue to occur even after SICI has reached a plateau. This order of increase in SICI followed by decrease in MEP is consistent with the previous study [15], hinting that increased SICI could have resulted in decreased MEP. However, within the matched background FDS muscle activities, no difference in nonconditioned MEPs was observed between the two contraction condition (relaxation vs. sustained contraction). Thus, the decrease in nonconditioned MEP with time may simply be due to reduced background FDS muscle activity [57, 58]. The insignificant change in the nonconditioned MEP from the maximum grip to 25% into the relaxation (Figure 6 B) could be due to the ceiling effect.

SICI increased as the target contraction level decreased in Study 2, Experiment 2. This was expected because increased muscle activity leads to decreased SICI [17]. Such a pattern of increase in SICI with decreased target contraction level was not seen in Study 1. It is possible that the three muscle activity levels used in Study 1were not very different from each other in magnitude (12%, 8% and 6% MVC for the three target background FDS EMG) to result in statistically significant changes in SICI.

4.3 H reflex is maintained for grip relaxation

Our finding of stable spinal motor excitability during grip relaxation compared to sustained grip at matching FDS muscle activity is somewhat different from the previous studies concerning the soleus muscle [12, 13, 59]. Both our study and the previous studies showed that H reflex decreased during muscle relaxation in healthy young adults. However, the previous studies showed that the H reflex in the soleus muscle, an antigravity postural muscle in the leg, during relaxation was decreased to the level that is even lower than H reflex at the resting state

[13]. In contrast, the present study showed that the H reflex level in the FDS muscle during relaxation was comparable to H reflex level during sustained contractions (presumably higher than H reflex level at rest). The motor control of the hand and leg muscles may be distinct from each other because of their different functional demands. It is likely that the leg muscles may have greater spinal control through reflex circuits contributing to the maintenance of balance and posture as well as gait, a function not shared by hand muscles. The hand muscles are known to have greater cerebral control that likely contributes to the hand's ability to perform fine motor tasks with great precision [60, 61]. Thus, it is possible that the spinal circuits may play a lesser role for hand muscle control compared to leg muscles owing to their different functional roles and neural connectivity. Specifically, relaxation from contraction may be under greater cortical control for hand muscles than leg muscles.

The background FDS activity could not have confounded with the H reflex results of no difference between the two contraction conditions, since the background FDS EMG was not different between the two contraction conditions. Also, the background EMG activity of both the FDS and EDC muscles gradually decreased with progression of relaxation, suggesting that our subjects did not open their hand in order to relax. While the EDC activity was greater during relaxation than the contraction task, it is unlikely that this relative increase in EDC activity confounded the H reflex results since reciprocal inhibition circuits are suppressed during co-contraction in order to ensure high excitability in motor neurons for both muscles [50], and even if reciprocal inhibition was active, it did not result in suppression of H reflex against the hypothesis.

4.4 Similar behavior for the dominant and nondominant hands

This was the first study to examine differences in the grip relaxation time in the dominant and nondominant hands. The grip relaxation time was comparable between two hands, contrary to a previous finding in the vastus lateralis muscle in soccer athletes [42]. Soccer athletes predominantly use their dominant leg for kicking and maneuvering the ball, hence it is possible that their interlimb differences are more pronounced than general population studied in the present study. Consequently, the neural substrates related to grip relaxation were not different between the dominant right hand and the nondominant left hand.

5. Conclusion

The present study demonstrated that cortical inhibitory circuits (SICI) increased its activation, while corticomotor excitability (MEP) and spinal motor excitability (H reflex) remained the same, during voluntary grip muscle relaxation compared to during sustained contraction at the comparable FDS muscle activity levels, in both dominant and nondominant hands of right-handed healthy young adults. The increase in SICI for grip relaxation was due to the intent to relax, not confounded by background FDS muscle activity, nonconditioned MEP amplitude, or antagonistic (EDC) muscle activity. The results suggest that hand muscle relaxation is primarily mediated by the cortical inhibitory mechanism as opposed to the spinal mechanism and that SICI is a general phenomenon that helps initiate and maintain progressive relaxation by resultant withdrawal of descending corticospinal drive. This finding suggests that inhibitory intracortical pathways play an important role in mediating hand muscle relaxation. The functional implication of this finding is that disturbances of the cortical inhibitory pathways

may lead to difficulties in terminating the voluntary muscle relaxation or “letting go” such as after stroke [23, 24].

Chapter 3. Aim 2: To examine the effects of aging on the grip relaxation time and modulations of SICI and spinal excitability for grip relaxation

1. Introduction

Movements become slow with aging. The ability to rapidly start and swiftly execute a movement is slower in older adults [18-22]. Not only movement initiation, but also prompt termination of a hand movement is important for activities of daily living. For example, failure to terminate finger flexors' activation while releasing a spoon may require greater antagonist activation to open the hand to release the spoon. Unwanted muscle activity and failure to terminate such activity in a timely manner also impair the quality of dynamic movement such as reaching and walking, and hamper movement efficiency and energy expenditure. As such, delays in muscle relaxation considerably impair function in stroke [23, 24, 62], dystonia [26] and Parkinson's [25]. Despite the functional significance of grip muscle relaxation, whether grip relaxation is delayed with aging is unknown. Additionally, while aging-related changes in skeletal muscles have been shown [63, 64], potential cortical neural correlates of prolonged grip relaxation with aging have not been examined.

Muscle relaxation is accompanied by activation of the dorsolateral prefrontal cortex, primary, supplementary and pre-supplementary motor areas in healthy young adults [9, 65]. This activity in the motor cortex is inhibitory in nature, as evidenced by increased short-interval intracortical inhibition (SICI) in M1 during muscle relaxation [15, 66]. This increased intracortical inhibition may be responsible for decreased spinal motor excitability during muscle relaxation in the soleus muscle in young adults [12, 13, 59].

Such increase in SICI needed for timely muscle relaxation may decline with aging. Older people exhibit decreased level of SICI at rest [67-69]. More importantly, older adults cannot *modulate* SICI as much, as evidenced by the reduced ability to decrease SICI to initiate and maintain muscle contractions [68-72]. In addition to intracortical inhibition, older adults were shown to have reduced modulation of spinal excitability, as seen in soleus H reflex during walking in older adults [73] and during muscle relaxation in patients with upper motor neuron lesion [59]. These changes in neurophysiology which may ultimately affect their limb function. While aging-related reduction in modulation of SICI during movement preparation and execution was noted, aging-related changes in modulation of SICI during *muscle relaxation* are unknown.

This study examined timely grip muscle relaxation in young vs. older adults and the effects of aging on intracortical inhibition. We hypothesized that grip muscle relaxation is delayed in older adults and that delayed grip relaxation in older adults is associated with lesser increase of SICI during relaxation. In addition to SICI, we also analyzed cortical silent period (GABA-Bergic intracortical inhibition as opposed to GABA-Aergic SICI) as an exploratory examination. Furthermore, we examined if delayed grip relaxation was accompanied by altered modulation of spinal motoneuron excitability assessed by H reflex during grip relaxation in older adults. In examination of SICI, cortical silent period, and H reflex, both dominant and nondominant hands' data were collected, since they differ in regards to the motor unit firing behavior [37] and structural and functional differences at the spinal and supraspinal level [39-41] and may have altered neural mechanisms for grip relaxation with aging.

2. Methods

2.1 Subjects

Data from a total of 40 young (mean and standard deviation of 25±5 years old, ranging from 18 to 37 years old, 19 females) and 21 older (57±6 years old, ranging from 47 to 68 years old, 12 females) adults were obtained for the study. All young subjects' data are from Chapter 2. These subjects did no additional activities than required during Chapter 2. All subjects were right-handed, as determined by the Edinburgh Inventory [45]. Both dominant right and nondominant left hands were tested. All subjects were healthy and did not have any known neurological and orthopedic disorders affecting the upper limb. Subjects were also screened for contraindications to Transcranial Magnetic Stimulation (TMS, used to assess SICI and cortical silent period) and electrical nerve stimulation (used to assess H reflex) [74].

While relaxation time was recorded for all participants, TMS (for SICI and cortical silent period) and H reflex data were not obtained from all participants due to difficulty in subject retention and difficulty in obtaining H reflex from the flexor digitorum superficialis (FDS) muscle. Specifically, TMS SICI data were obtained from 20 young subjects (25±5 years old, 8 females) and 20 older subjects (57±6 years old, 11 females). Cortical silent period data were obtained from 15 young (24±5 years old, 5 females) and 15 older subjects (57±6 years old, 7 females) who had also participated in the SICI experiment. No additional experiments were conducted for the cortical silent period examination. Only those who exhibited cortical silent periods during the SICI experiment were included for the cortical silent period data. H reflex data were obtained from 25 young (26±6 years old, 14 females) and 9 older (59±5 years old, 4 females) subjects. Although a total of 20 older adults were screened, only 9 older adults

exhibited H reflex in the FDS muscle. The number of subjects from whom data were obtained for the left and the right hand for each test is indicated in Table 4. All subjects signed an informed consent form approved by the Institutional Review Board.

Table 4. Subject distribution for the aging experiment. The number of subjects tested for the right dominant and left nondominant hands for SICI, cortical silent period and H reflex in both age groups. Grip relaxation time was recorded for all subjects who participated in either the TMS (SICI and cortical silent period) or H reflex testing.

	Young (n=40 total)					Older (n=21 total)				
	Dominant only	Nondominant Only	Both hands	Dominant total	Nondominant total	Dominant only	Nondominant only	Both hands	Dominant total	Nondominant total
Grip relaxation time (n=40 young & 21 older)	17	10	13	30	23	8	5	8	16	13
SICI (n=20 young & 20 older)	10	9	1	11	10	8	4	8	16	12
Cortical silent period (n=15 young & 15 older)	8	6	1	9	7	6	4	5	11	9
H reflex (n=25 young & 9 older)	9	5	11	20	16	3	3	3	6	6

2.2 Procedure

The effect of aging on grip relaxation time, SICI [33], cortical silent periods, and H reflex [34, 35] were examined in both hands of young and older adults. The TMS (including SICI and cortical silent periods) test and H reflex test were conducted on separate days; two hands were examined on separate days as well. Grip relaxation time was measured at the beginning of each testing day.

Grip relaxation time was examined following a maximum power grip as described in Chapter 2 (Figure 8). SICI was examined at 70%, 80% and 90% into grip relaxation and during matching sustained grip to control for muscle activity dependent changes in SICI, as described previously in Chapter 2 Study 1 (Figure 9). The cortical silent period was examined at 70% into grip relaxation and during sustained contraction with matching background FDS EMG. The portion of the data from the SICI trials used to obtain nonconditioned MEP amplitudes was used for cortical silent period measurement. The reason for examining the cortical silent period only at 70% into grip relaxation is to prevent interference of the lack of EMG at the complete muscle relaxation in correct computation of the cortical silent period. The cortical silent period was defined as the period of EMG silence. The starting point of the cortical silent period was at the end of nonconditioned MEP, and the endpoint was defined as return of EMG activity, all visually determined as in previous studies [75, 76]. H reflex was examined only at 80% into grip relaxation in older adults and compared with H reflex at 75% into grip relaxation in young adults obtained in Chapter 2. Since the young adults' H reflex data already collected for Chapter 2 was used, the time discrepancy between the H reflex measurements timing (127 ms difference between 75% in young vs. 80% in older adults) exists. The background EDC EMG was also

recorded to monitor if the subjects increased the EDC muscle activation to open/extend their fingers during relaxation of the power grip during the experiment.

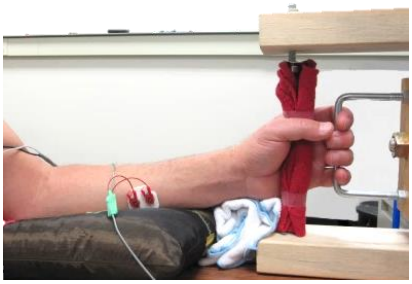


Figure 8: Experimental set-up for grip-and-relax trials. During grip-and-relax trials, subjects isometrically gripped a handle and relaxed upon an audio cue while the EMG was recorded from the FDS and EDC muscle. During sustained grip trials, subjects isometrically gripped the handle to match the FDS EMG level.

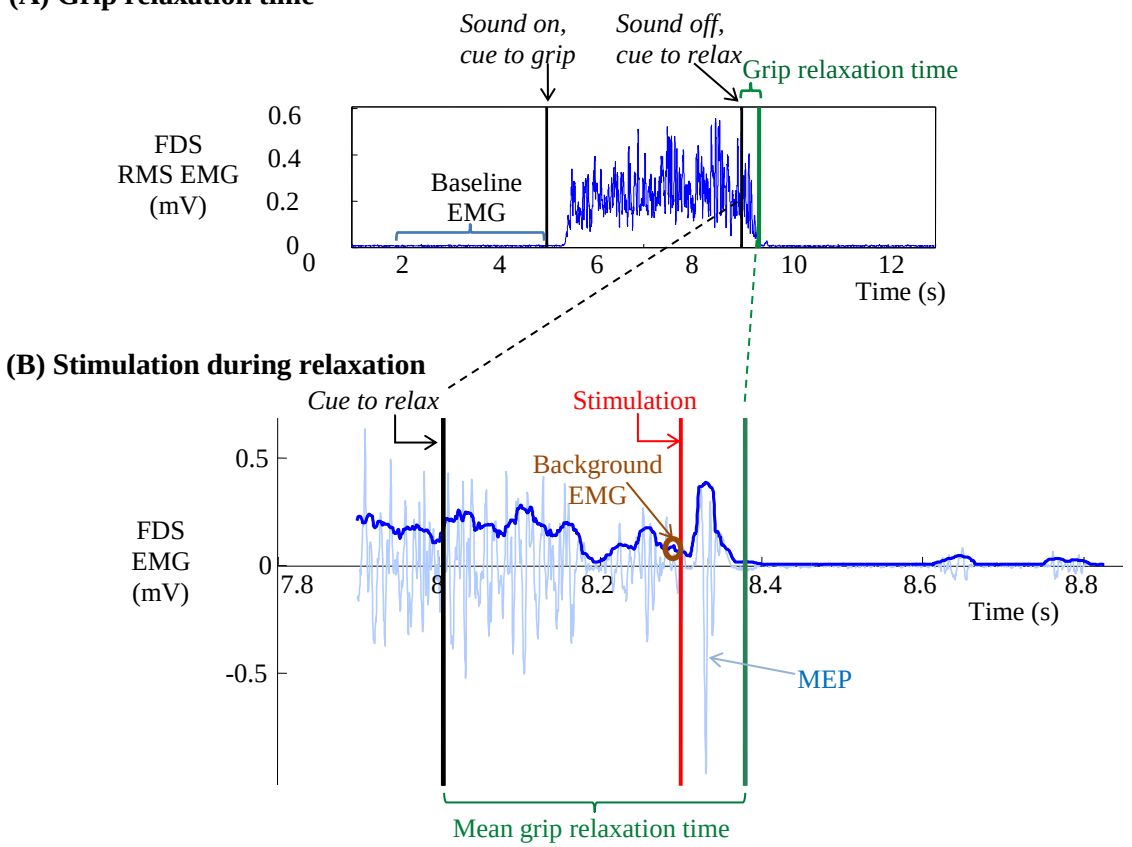


Figure 9: Stimulations during grip relaxation and sustained contraction tasks. (A) To measure grip relaxation time, the subject maximally and isometrically gripped the handle upon the start of a computer generated sound and relaxed the grip upon the termination of the sound. Grip relaxation time was quantified as the time in which the postcontraction FDS RMS EMG fell below mean + 3SD of the precontraction baseline FDS RMS EMG. (B) To measure SICI during relaxation, stimulation was applied at 70%, 80%, or 90% into the subject's mean grip relaxation time during the grip-and-relax trial. The peak-to-peak MEP was used toward computation of SICI. The background RMS EMG during 20 ms immediately before stimulation was obtained during the grip-and-relax trial. The average background RMS EMG was used as a target in the subsequent measurement of SICI during sustained grip. (C) To measure SICI during sustained grip, stimulation was applied while the subject maintained a sustained grip at the target muscle activity level using visual feedback. Example trials from a single subject are shown in this figure. In (B) and (C), lighter EMG traces show raw EMG while thicker traces show RMS EMG. The same protocol was used to obtain H reflex during grip relaxation and sustained grip at a matching EMG level.

2.3 Statistical analysis

The primary statistical analysis for each response variable is described as follows. The effects of aging and hand on grip relaxation time were examined using two-way ANOVA. For SICI, mixed-design ANOVA was used to examine if the level of SICI was affected by the within-subject variables of task (during relaxation vs. during sustained contraction at matching EMG level) and time (70%, 80%, 90% into relaxation), between-subject variables of aging (young vs. older) and hand (dominant vs. nondominant), and their interactions. The factor of interest was the interaction between task and aging, as it indicates whether task-specific modulation of SICI (in this case, for relaxation) is different between the two aging groups. Upon confirmation for the significant task × aging interaction effect, pairwise comparison was performed to examine statistical differences between the two tasks (relaxation vs. sustained contraction) for each group. For the cortical silent period, another mixed model ANOVA was used to examine if the cortical silent period differed between task, aging, hand, and their interactions. For H reflex, mixed-design ANOVA was used to examine if H reflex was affected by task (during relaxation vs. during sustained contraction at the matching EMG level), aging (young vs. older), hand (dominant vs. nondominant), and their interactions. Correlation analysis was used to examine the association between age (years) and grip relaxation time, between age and SICI modulation, between grip relaxation time and SICI modulation, between age and cortical silent period modulation, between grip relaxation time and cortical silent period modulation, between age and H reflex modulation, and between grip relaxation time and H reflex modulation. The modulations of SICI, cortical silent period, and H reflex were quantified by

subtracting SICI averaged across the three time points, cortical silent period, and H reflex during contraction from that during grip relaxation, respectively.

Other secondary analyses were performed as follows. To confirm that SICI, cortical silent periods, and H reflex modulation did not result from different FDS background EMG levels between the two tasks (grip relaxation vs. sustained contraction), mixed-design ANOVAs were used to test if background FDS EMG was different with task, aging, hand, and time during the SICI testing and with task, aging, and hand during the cortical silent period and H reflex testing. Also, since the nonconditioned MEP amplitude may affect SICI [77], another mixed-design ANOVA was used to examine if the nonconditioned MEP amplitude was different with task, aging, hand, and time during the SICI testing. All statistical analysis was performed with SPSS (version 20, IBM, Armonk, NY).

3. Results

3.1 Slowed grip relaxation in older adults

Grip relaxation time was, on average, 29% longer for older adults than young adults (Figure 10). ANOVA results showed that grip relaxation time was significantly different between the two age groups ($p=0.002$). There was no significant effect of hand (dominant vs. nondominant, $p=0.464$) or interaction between aging and hand ($p=0.977$). Grip relaxation time showed significant positive correlation with age (years) of our subjects ($r=0.343$, $p=0.002$. Subjects did not increase activation of the antagonist muscles during grip relaxation, as seen by

both FDS and EDC muscle activities decreasing in a consistent manner for both young and older adults (Figure 11 A, after "Cue to relax"). When the FDS EMG relaxations for young and older adults are overlaid over 0-100% of individuals' relaxation times, similar rates of reduction in the FDS RMS EMG were observed in the two age groups (Figure 11 B).

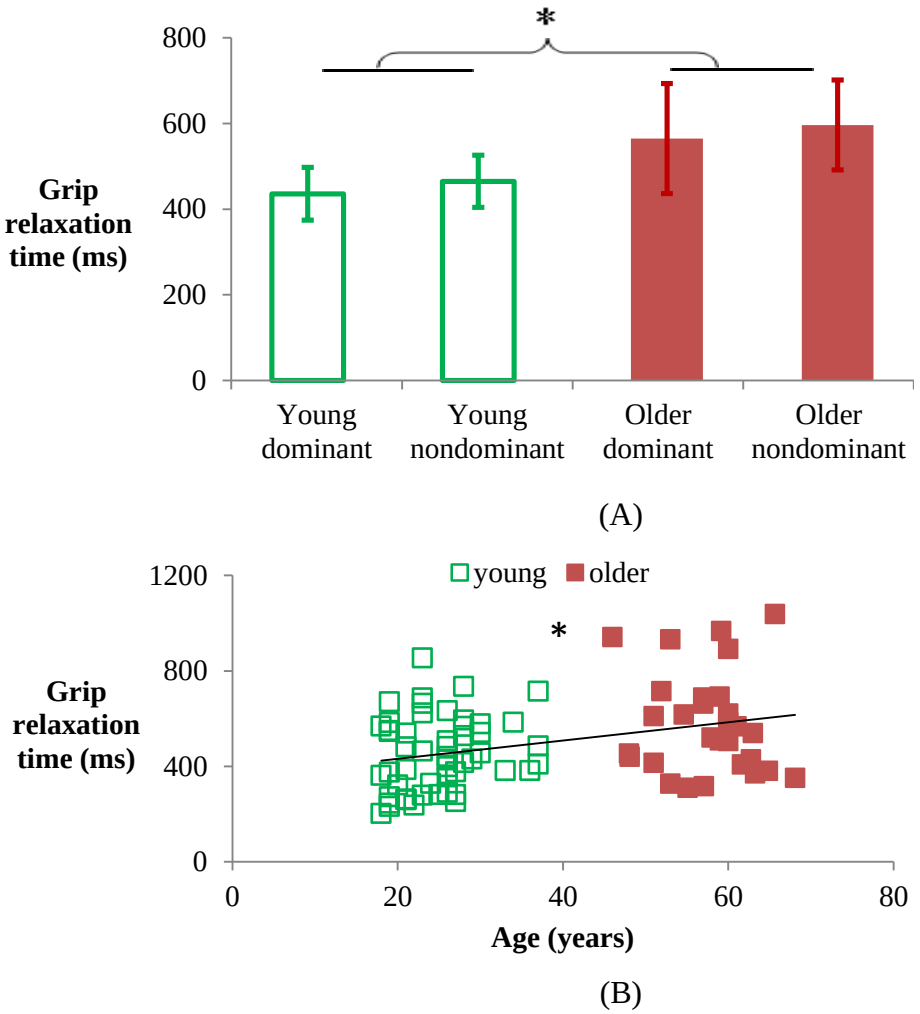
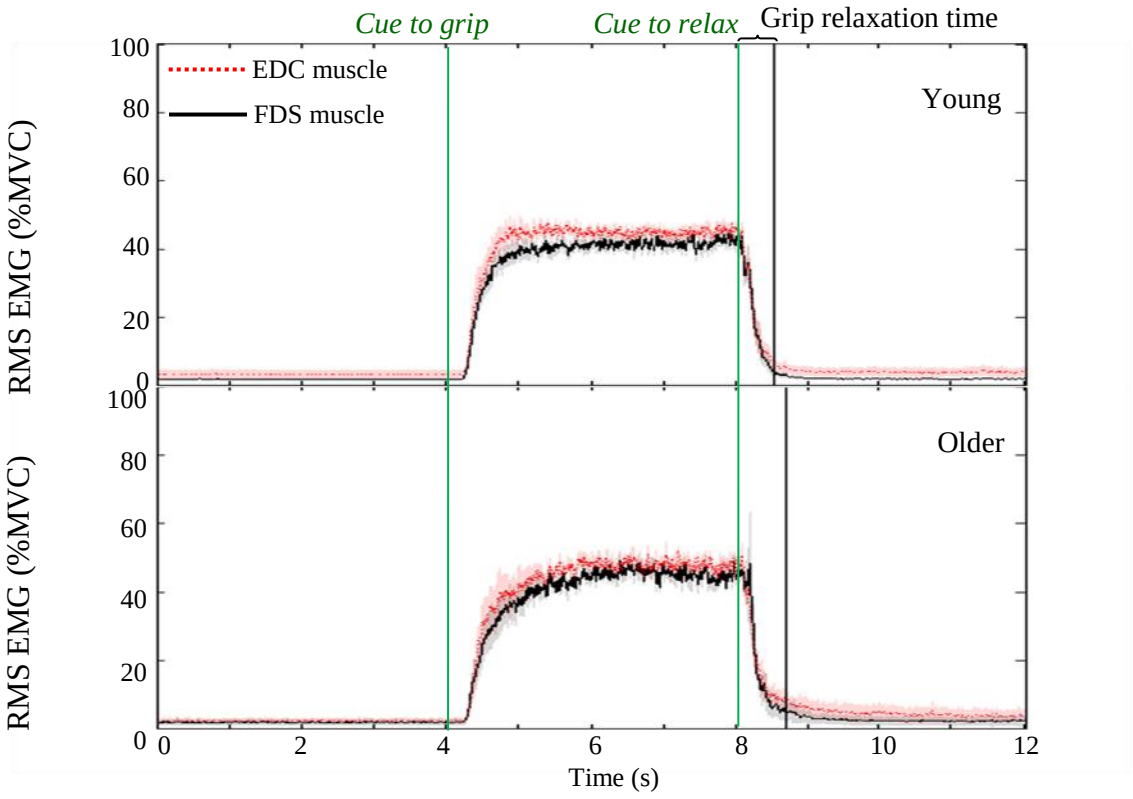
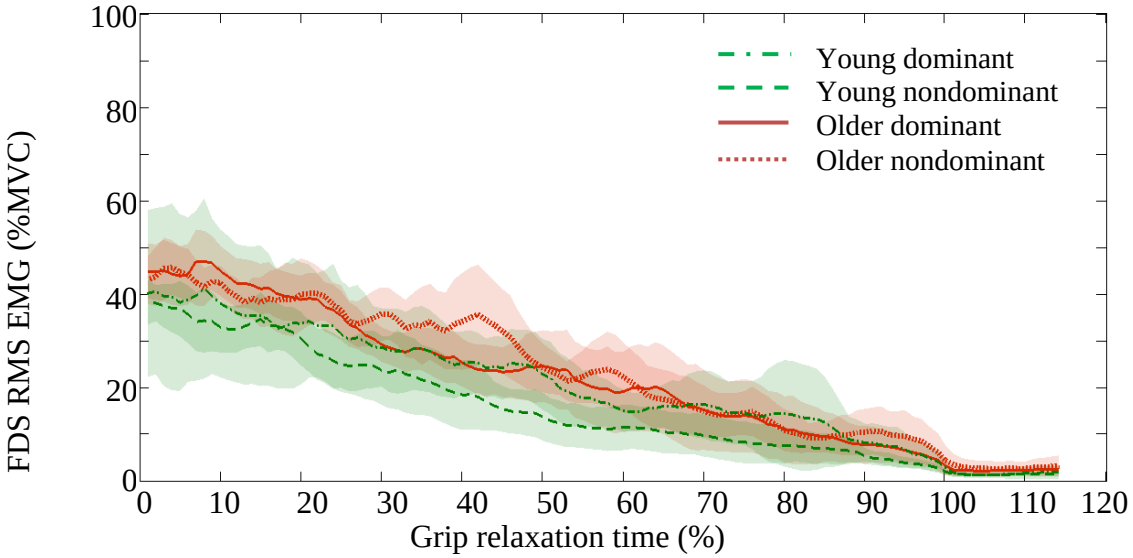


Figure 10: Grip relaxation time with aging. (A) Mean grip relaxation time (ms) for the dominant and nondominant hands of young and older adults. * indicates the main effect of age, $p<0.05$. Error bars indicate 95% confidence intervals. (B) Grip relaxation time significantly increased with age ($r^2=0.1174$, $p<0.05$).



(A)



(B)

Figure 11: Muscle activation patterns with aging. (A) During the grip-and-relax trial, both FDS and EDC muscles were active during grip and decreased during relaxation, similarly for young and older adults. EMGs are expressed as %MVC when MVC was the maximum RMS EMG observed during all grip-and-relax trials. (B) During the grip relaxation time (expressed as 0-100% of individuals' grip relaxation time), FDS EMG decreased similarly for both hands of young and older adults. Shades indicate 95% confidence intervals.

3.2 Lack of increase in SICI during grip relaxation in older adults

While young adults increased SICI during grip relaxation compared to sustained contraction at the matching EMG level by an average of 36%, older adults had an average of 7% decrease in SICI for relaxation (Figure 12 A). The ANOVA results showed that SICI significantly varied by task × aging interaction ($p=0.005$) and time ($p=0.0495$), but not by the task main effect ($p=0.117$), aging main effect ($p=0.939$), hand main effect ($p=0.482$), and other interactions ($p>0.05$). Pairwise comparisons showed that young adults significantly increased intracortical inhibition during grip relaxation compared to sustained grip contraction at matching muscle activity (pairwise comparison $p=0.001$, indicated by * in Figure 12 A). In contrast, older adults failed to increase their intracortical inhibition during grip relaxation compared to sustained grip, as indicated by no significant change in SICI for relaxation in comparison to sustained grip at the matching EMG level (pairwise comparison $p=0.374$, Figure 12 A). The average SICI increased with time (mean±95%CI =30±6%, 34±7% and 35±7%, Figure 12 B), as expected with decreasing background EMG activity with time. SICI modulation significantly decreased with age (Figure 12 C, $r=-0.354$, $p=0.013$), however there was no correlation between the average SICI modulation and grip relaxation time ($r=-0.120$, $p=0.410$).

The secondary statistical analysis results are as follows. The background FDS RMS EMG was not significantly different between the two tasks (relaxation vs. sustained contraction, $p=0.860$). As expected, the background FDS RMS EMG decreased with time ($p<0.001$) (Figure 13 A). The background FDS RMS EMG also did not significantly vary with aging ($p=0.683$),

hand ($p=0.270$), and any of the interactions ($p>0.05$) except for aging and hand interaction ($p=0.032$). The background FDS RMS EMG was greater for the dominant hand than the nondominant hand in young adults (12±2% vs. 5±1%MVC, pairwise comparison $p<0.001$), whereas it was comparable between the two hands in older adults (8±2%MVC vs. 10±2%MVC, pairwise comparison $p=0.096$). In addition, the nonconditioned MEP peak-to-peak amplitudes were not significantly different between the two tasks (relaxation vs. sustained contraction, $p=0.192$, Figure 13 B). The nonconditioned MEP decreased with time ($p<0.001$), as expected with decreased background FDS EMG with time. The nonconditioned MEP did not significantly vary with other main effects of aging ($p=0.245$) and hand ($p=0.688$), as well as interaction between task and aging ($p=0.561$) and any other interactions among task, aging, hand, and time ($p>0.05$) except for interaction between time and hand ($p=0.039$). The decrease of nonconditioned MEP with time was more pronounced for the dominant than the nondominant hand (1.7±0.2, 1.4±0.2, 1.3±0.2 mV for the dominant hand and 1.5±0.3, 1.4±0.2, 1.4±0.2 mV for the nondominant hand at 70%, 80% and 90%, respectively). In summary, these secondary analysis results suggest that the background FDS EMG level was well controlled between the two tasks for both aging groups (Figure 13), and that the finding of aging- and task-dependent SICI modulation was not confounded by different background EMG levels or nonconditioned MEP amplitudes.

3.3 Cortical silent period in grip relaxation

The cortical silent period was similar between the two tasks (Figure 14, main effect of task p=0.874). The lack of task-dependent change was observed in both hands and age groups (interaction between task and aging p=0.571, interaction between task and hand p=0.069). The cortical silent period did not differ between two hands (p=0.122). The cortical silent period was shorter for the young adults compared to the older adults (main effect of aging, p=0.007) consistently with previous findings [67]. The cortical silent period was not affected by other interactions among task, aging and hand (p>0.05). The cortical silent period modulation for grip relaxation was not correlated with age in years (r=-0.095, p=0.532) and grip relaxation time (r=-0.221, p=0.140).

The ANOVA examined the background EMG level of the prestimulation FDS only at 70% timing (used for the cortical silent period measurement) and showed that the background FDS EMG level was well controlled between the two tasks for both age groups (main effect of task p=0.219, aging and task interaction p=0.829, Figure 13 A), suggesting that the finding of lack of cortical silent period modulation was not confounded by different background FDS EMG levels between the two tasks. The FDS background EMG for the cortical silent period was not different between the two aging groups (main effect of aging, p=0.425). The aging and hand interaction for the FDS background EMG was significant (p=0.044), however the pairwise comparisons did not show any significant differences between the dominant and the nondominant sides in both aging groups (p>0.059). The main effect of hand (p=0.458) and interaction between task and hand (p=0.660) were not significant.

In summary, the cortical silent period did not modulate for the grip relaxation in both young and older adults. This finding was not confounded by task-specific differences in the background FDS EMG levels.

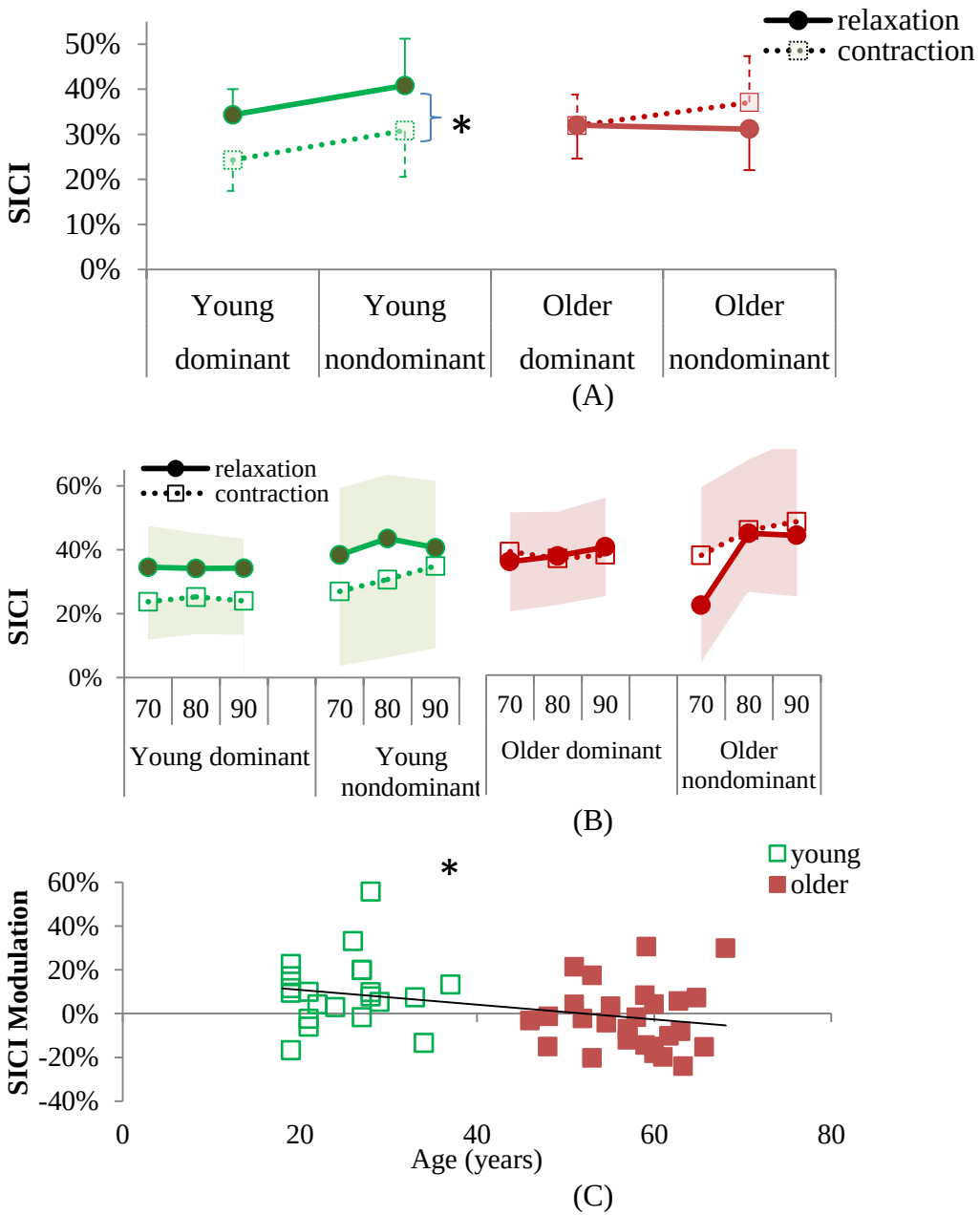


Figure 12: SICI modulation for grip relaxation in aging. (A) Mean SICI during grip relaxation and during sustained grip contraction at matching muscle activity averaged for three time points showed that young adults significantly increased SICI during grip relaxation compared to sustained grip in both hands, while older adults did not significantly modulate SICI ($p=0.005$ for ANOVA task × aging interaction, significant pairwise comparison between the tasks in young adults with $p=0.001$ indicated with *, while $p=0.893$ for pairwise comparison in older adults). (B) Mean SICI for the two tasks are shown separately for the three time points. Error bars/shades in (A)-(B) show upper or lower bound 95% confidence intervals. (C) SICI modulation for grip relaxation significantly reduced with age ($r=-0.354$, $p<0.05$)

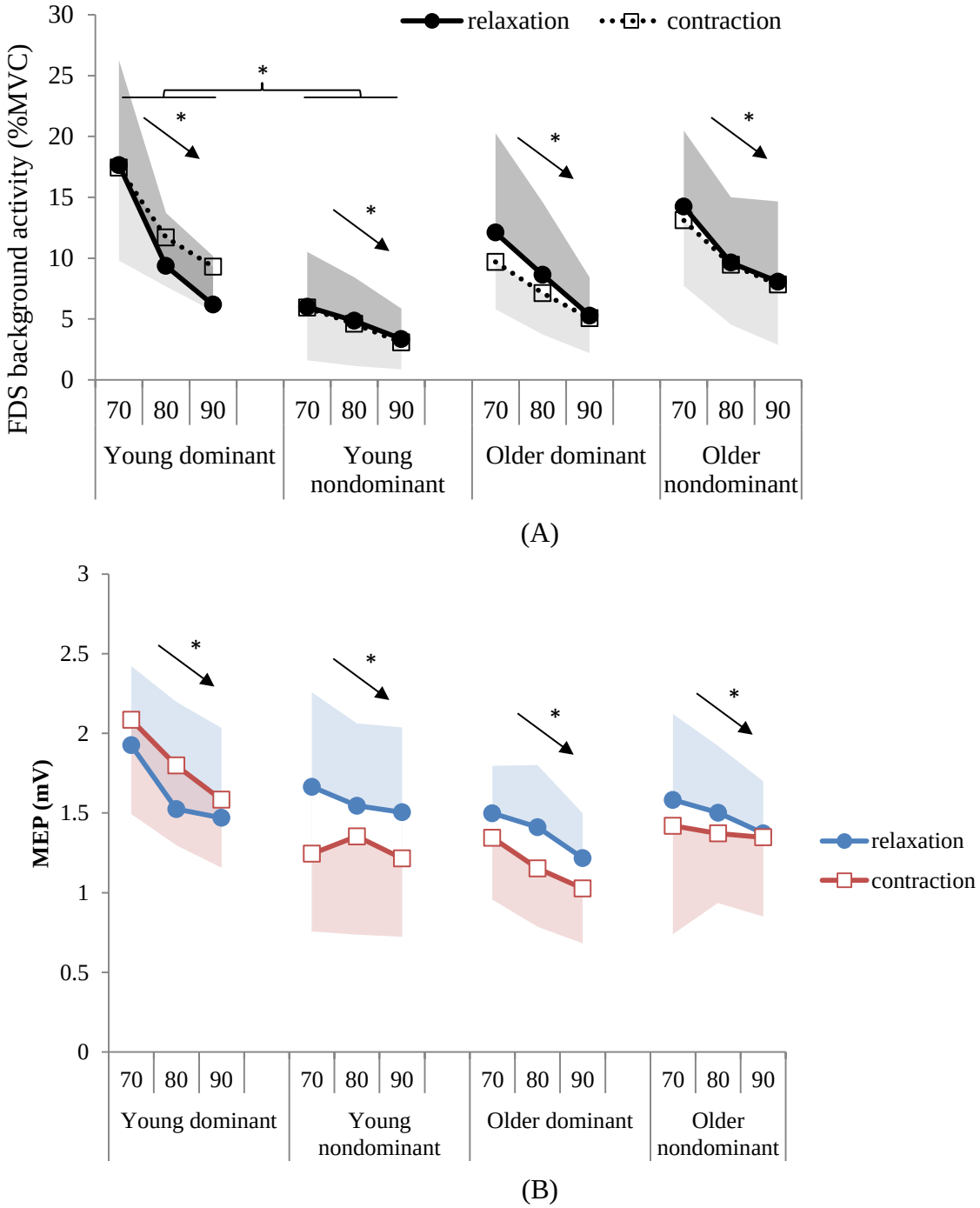


Figure 13: Background FDS and EDC muscle activity during SICI experiement with aging. (A) Mean background FDS EMG levels are shown for both tasks, groups, and hands. The background FDS muscle activity was not statistically different during grip relaxation vs. during sustained grip contraction within each aging group and hand. (B) Mean nonconditioned MEP amplitude for both tasks, groups, and hands. The shades show upper bound 95% confidence intervals for relaxation and lower bound 95% confidence intervals for sustained contraction in both (A) and (B).

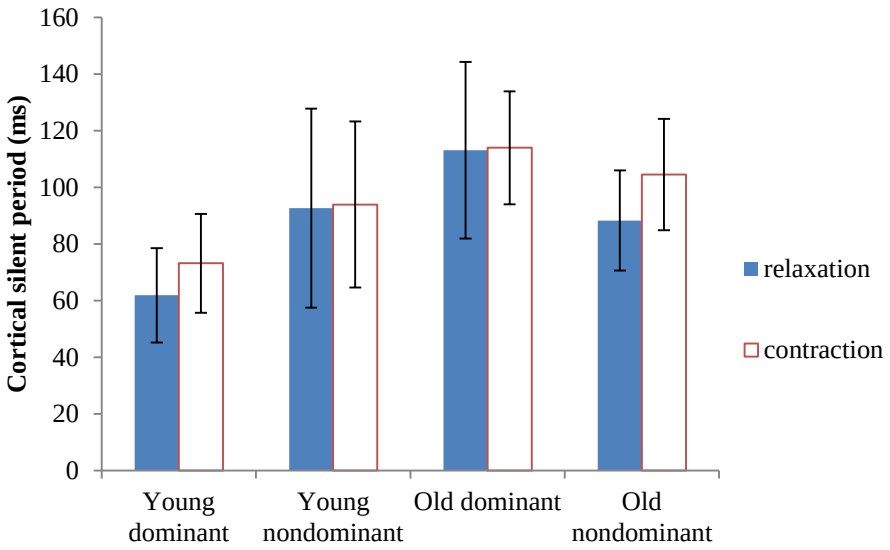


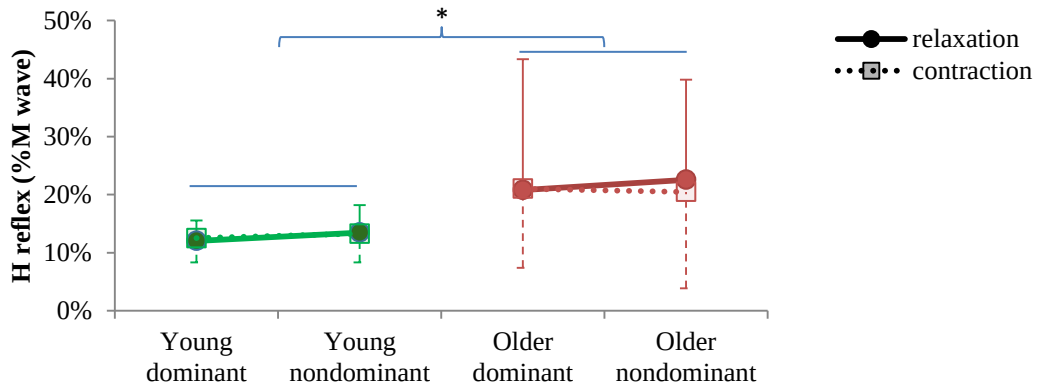
Figure 14: Cortical silent period modulation for grip relaxation in aging. Mean ± 95% CI cortical silent period for the dominant and nondominant hands for both aging groups. The cortical silent period was similar between the two tasks ($p>0.05$) without a significant aging x task interaction ($p>0.05$), suggesting no involvement of the GABA-B inhibitory mechanism in mediating grip relaxation.

3.4 H reflex

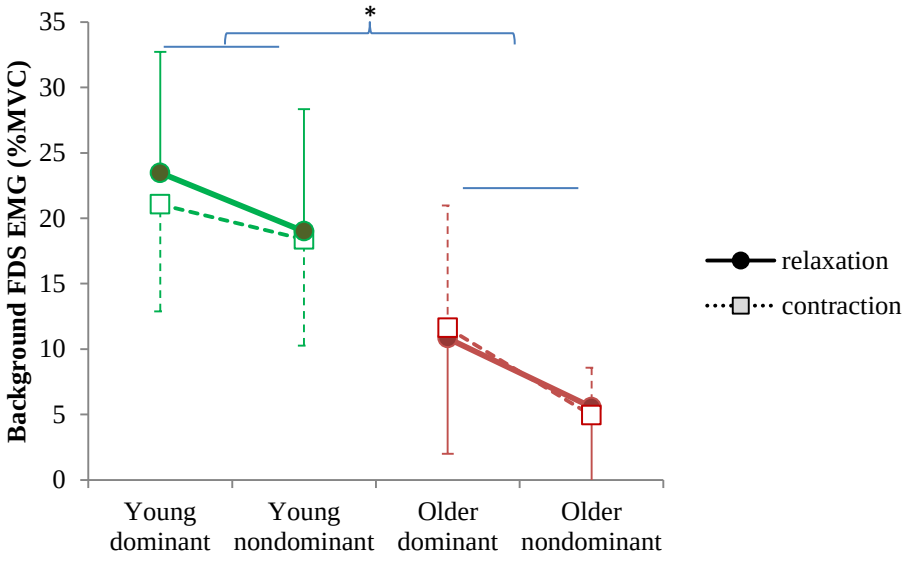
H reflex modulation was not seen for both young and older adults (Figure 15 A). The ANOVA results showed that H reflex was not affected by task ($p=0.853$), hand ($p=0.776$), interaction between aging and task ($p=0.934$), or any other interactions ($p>0.05$). Older adults had overall higher H reflex amplitudes compared to young adults (21±7 vs. 13±2%M wave, $p=0.008$), which was not specific to the relaxation or sustained contraction task. This finding of increased FDS H reflex with is aging new: While reduced H reflex for the Soleus muscle during activity was noted in elderly [73, 78], change in H reflex for the FDS muscle with aging was unknown [73, 78]. H reflex combined for grip relaxation and sustained contraction appears to be elevated in older adults in this study. Both young and older adults maintained spinal motoneuron excitability during grip relaxation compared to sustained grip, as indicated by no task-specific

change in H reflex for both hands in both aging groups. The H reflex modulation was not significantly correlated with the age (in years) ($r=0.069$, $p=0.640$) and grip relaxation time ($r=-0.052$, $p=0.724$).

The secondary statistical analysis for the background FDS EMG for H reflex testing showed that the background FDS RMS EMG was not significantly different between the two tasks (Figure 15 B. 18±5%MVC during grip relaxation vs.17±4 %MVC during sustained grip, $p=0.341$), nor with hand ($p=0.410$), and interactions among task, aging, and hand ($p>0.05$). The mean background FDS RMS EMG was greater for young adults compared to older adults (21±4 vs. 9±3%MVC, $p=0.028$). Yet, within the group, the background FDS EMG level was well controlled between the two tasks. This secondary analysis suggests that relaxation-specific modulation of H reflex (or lack thereof) was not confounded by different background EMG levels between two tasks.



(A)



(B)

Figure 15: H reflex modulation for grip relaxation results in aging. (A) Mean H reflex during grip relaxation and during sustained grip contraction at matching muscle activity. No change in H reflex was observed between relaxation and sustained contraction at matching muscle activity for both groups and both hands. (B) The FDS background EMG was comparable between grip relaxation and sustained contraction across groups and hands. Error bars show upper bound 95% confidence intervals for relaxation and lower bound 95% confidence intervals for sustained contraction.

4. Discussion

The first main finding was that older adults were slower in relaxing their grip compared to young adults in both hands. This finding expands previous knowledge by demonstrating that older adults not only have a slowed reaction time [18, 19, 79] but also a slowed grip relaxation time (Figure 10). The second main finding was that older adults did not increase short-interval intracortical inhibition during grip relaxation as young adults did. This finding was seen for both dominant right and nondominant left hands. This main finding of relaxation-specific increase of SICI only in young adults was not confounded by changes in the background FDS and nonconditioned MEP changes, as they were maintained similar between the relaxation and sustained contraction tasks. While GABA-Aergic inhibition (SICI) increased for grip relaxation in young adults, GABA-Bergic cortical silent period did not exhibit relaxation-specific changes in both aging groups, suggesting that the GABA-Bergic neurons and circuits may not play an important role in grip relaxation. The H reflex did not show grip relaxation specific changes with aging, suggesting that the grip relaxation is predominantly mediated at the cortical level (as shown in Chapter 2) and control of H reflex for grip relaxation does not change with aging. Collectively, these data suggest that control of grip relaxation occurs mainly at cortical level and specifically it involves a GABA-A ergic inhibitory process in young adults. This finding suggests that the delay in grip relaxation with aging is associated with an inability to increase short-interval intracortical inhibition during grip relaxation in older adults. The current study is the first to examine grip relaxation for both young and older adults with neural substrates at both cortical and spinal levels.

Increased GABA-Aergic intracortical inhibition can effectively inhibit corticospinal motor neurons and thus contribute to terminating grip activity in young adults [14, 15, 66]. This study results suggest that older adults' inability to increase the GABA-Aergic cortical inhibitory action during their attempt to relax the muscle may be responsible for slower muscle relaxation time. Older adults' inability to decrease SICI to *initiate* a movement has previously been demonstrated [68-70], associating lack of SICI modulation with the declined performance of functional tasks [70]. Our study expands the previous literature by demonstrating that this lack of SICI modulation exists not only at the movement initiation but also at movement termination, contributing to decline in motor performance in older adults.

This aging-related change in modulation of intracortical inhibition may be related to neural degeneration and decreased white and grey matter volume [80-82]. Specifically, aging decreases the number of dendrites of corticospinal pyramidal cells in the layer V of the motor cortex, which may impact intracortical connectivity [83]. Moreover, structural properties of the GABA-A receptors are affected in addition to reduced GABA content and transport in the aging brain (see Wong [84] for review). It is known that SICI represents activation of GABA-A receptors [85-87]. Thus, it is likely that older adults' inability to increase intracortical inhibition during grip relaxation reflects anatomical and physiological changes in their brain, specifically in the GABA-A circuits. Data from rat show that the GABA-A receptors have reduced affinity in aging brain, but GABA-B receptors are spared [88]. This sparing of GABA-B circuits from aging related changes may explain why the cortical silent period was comparable between two age groups in our study.

It is interesting that this delayed relaxation time and decreased SICI modulation were found for our older adults with ages of 47 to 68 years old, younger than most aging research subjects. This age range may have been relatively understudied in aging research with paucity of data. While behaviorally subtle, these changes in the grip relaxation time and SICI modulation may represent an undiagnosed aging process that may be a precursor of forthcoming aging-related neurologic impairments that become more apparent with further aging.

This study identifies a key neural mechanism of timely muscle relaxation. While this study described differences in neurophysiology and function between older and young adults, causality between neurophysiology and function was not demonstrated. Future studies may explore experimental manipulation of the brain network, such as using rTMS to impair normal function, to reveal direct causal relationships. Future research may also examine interventions to restore SICI modulation in older adults to facilitate timely muscle relaxation and improve movement quality. Such interventions may include neuromodulation by brain stimulation [89-91], operant conditioning [92-94], and GABA agonists [85, 86, 95]. This study only examined the cortical inhibitory and spinal mechanisms and thus does not tease apart the relative contributions of the changes in the skeletal muscles and cortical inhibitory activity on delayed grip relaxation with aging.

5. Conclusion

This study demonstrated that grip relaxation is delayed in older adults and this delay was associated with their inability to increase GABA-Aergic short-interval intracortical inhibition (SICI) during grip relaxation. Other neural substrates examined in this study (GABA-Bergic cortical silent period and H reflex representing the spinal motoneuron excitability) did not appear to play an important role in grip relaxation for both aging groups. This delay in terminating muscle activity, in addition to general aging-related slowness in movement initiation and execution, may contribute to a deterioration of motor control in older adults. Interventions to increase the plasticity of GABA-Aergic inhibitory cortical circuits may be useful in improving muscle relaxation and general motor control in older adults.

Chapter 4. Aim 3: To examine neural mechanisms of delayed grip relaxation in chronic stroke survivors.

1. Introduction

Stroke survivors experience delays in terminating a muscle contraction as well as initiating a muscle contraction [23]. Such temporal disturbance of muscle contraction and relaxation leads to poor quality of movement in grip-release tasks during activities of daily living [23, 24, 62, 96]. While altered neural control of grip muscle activation after stroke has been studied [97], mechanisms of delayed grip relaxation post stroke remain understudied. This knowledge gap is problematic, because timely hand grip release is important for hand function in daily living, and stroke survivors' hand grip delay is more pronounced for relaxation than initiation (5 s long relaxation vs. 2 s long initiation which impose substantial disturbance in day-to-day functional activity of the hand) [23].

At the time of the dissertation proposal defense, the known knowledge was as follows: Neural mechanisms of timely muscle relaxation in neurologically-intact adults included increased brain activity [7-9]. Specifically, short-interval intracortical inhibition (SICI) for the target muscle increased during relaxation from a muscle contraction [26, 65, 66]. It was thought that this increase in intracortical inhibition may mediate muscle relaxation by reducing corticospinal output and/or by influencing the spinal motor excitability through spinal inhibitory interneurons in neurologically-intact adults [11-14, 25, 59]. Stroke survivors exhibit reduced SICI at rest [87, 97-99] and impaired ability to decrease SICI during initiation of muscle contraction [97] compared to age-matched healthy controls. Increased SICI is seen with motor recovery in chronic stroke [98-100]. In addition, abnormal hyperexcitability of spinal motor

neurons is a prominent feature of stroke [101, 102]and increased H reflex was linked with longer relaxation times in upper motor neuron lesion patients [59]. Furthermore, stroke survivors exhibit excessive interhemispheric inhibition from the nonlesioned hemisphere to the lesioned hemisphere, which was associated with delayed initiation of muscle contraction and poor functional status in stroke survivors [103]. Increased interhemispheric inhibition reduces intracortical inhibition at rest in healthy adults [104], which leads to the possibility that excessive inhibition of the lesioned hemisphere after stroke may suppress the intracortical inhibitory circuits in the lesioned hemisphere and subsequently delay muscle relaxation.

Based on this knowledge base that was available at the time, the original hypotheses were formed as (1) chronic stroke survivors have lesser modulations of SICI and spinal excitability during grip relaxation in the paretic hand compared to their nonparetic and age-matched control hands (Study 1); and (2) imbalanced interhemispheric inhibition is associated with longer grip relaxation times in chronic stroke survivors (Study 2). Unfortunately, our own work (Chapter3) revealed that neurologically-intact older adults (with similar ages with chronic stroke survivors studied in this aim) do not modulate SICI, MEP, nor H reflex specific to grip relaxation compared to sustained contraction at the matching background muscle activity [105] (Chapter 3). This new knowledge suggests that SICI, MEP, and H reflex modulation is not a proper biomarker to explain delayed grip relaxation post stroke. However, such new information was not available at the time of conducting the study for this aim. Thus, we present the two studies as they were originally planned, although stroke-specific difference is no longer anticipated in these neural measurements taken. Newly, we added Study 3 to explore any other post-stroke characteristics that may explain delayed relaxation time in chronic stroke survivors.

2. Study 1: To examine the role of SICI and H reflex in delayed grip relaxation in chronic stroke survivors

2.1. Introduction

The primary objective of this study was to examine the role of intracortical inhibition for the flexor digitorum superficialis muscle (FDS) in stroke-related delayed grip relaxation. We hypothesized that the stroke survivors' delayed grip relaxation is associated with inability to increase the intracortical inhibition for grip relaxation. To accomplish this objective, short-interval intracortical inhibition (SICI) was examined using Transcranial Magnetic Stimulation (TMS) in the affected and unaffected hands of chronic stroke survivors and healthy age-matched controls. Secondary analyses for the cortical control measured using TMS included cortical silent period to assess function of GABA-Bergic intracortical inhibition, and peak-to-peak amplitude, area and latency for the nonconditioned MEP to assess corticomotor excitability. We also examined H reflex (spinal motor excitability) to identify effects of stroke on spinal motor contribution to grip relaxation. These cortical and spinal neural substrates were examined during grip relaxation vs. sustained grip at similar background muscle activity to isolate grip relaxation-specific changes that are not confounded by the background muscle activity [66] as in Chapters 2-3. We also examined if reciprocal inhibition was involved in delayed FDS relaxation time by examining the EDC EMG.

2.2. Methods

2.2.1 Subjects

A total of 25 chronic survivors (mean±SD age = 58±9 years old, 11 females) and 21 age-matched neurologically-intact adults (57±6 years old, 12 females) participated. The age-matched control subjects are same as the older adults in Chapter 3. All the control subjects were right handed per Edinburgh handedness inventory [45]. The study involved 4 sessions on separate days per subject (2 hands x 2 testing for TMS and H reflex each). Our stroke survivors were relatively high functioning (mean±SD Chedoke [106] hand section score = 5±2 out of 7, Fugl-Meyer [107] wrist and hand section score = 18±7 out of 24, and spasticity of the wrist flexors assessed by the Modified Ashworth Score [108] = 1±2 when transformed to a linear scale of 0-5). In addition to motor assessments, sensory function was assessed by using the 2 point discrimination test and the Monofilament test scores for the index and thumb fingertips [109, 110]. The index and thumb scores were averaged for the sensation scores. All subjects except one (S6) came back for more testing sessions when eligible. All subjects were screened for the presence of MEP and H reflex on both sides. All subjects signed an informed consent form authorized by the Institutional Review Board.

The detailed demographic information for individual stroke subjects is described in Table 5. Empty cells in the Table 5 indicate absence of data or knowledge. Subjects' participation in each study is indicated by "x". S6 was not available to come to more sessions of the research, indicated by "NA".

Table 5. Demographic and clinical information for stroke survivors

Sub	Paretic hand	Age	Sex	Pre-stroke handedness	Time since stroke (years)	GRT (ms)	Paretic Grip force (N)	Chedoke (0- 7)	MAS (0-4)	FM (0-24)	SWMF score	2-point (mm)	Lesion Type	SICI		CSP		H reflex	
														A	UA	A	UA	A	UA
S1	Left	60	F	Left	6.9	1001		5	4	15	6.65	11	Ischemic	x	x		x	x	x
S2	Right	63	M	Right	6.6	542		7	0	24	2.83	3	Hemorrhagic	x	x	x	x		
S3	Left	50	F	Right	1.7	799	67	7	0	22	3.61	6	Ischemic	x	x	x	x	x	x
S4	Right	74	F	Right	2.2	634	202	7	0	24	3.61	5.5	Ischemic	x	x	x	x	x	x
S5	Left	53	M	Right	3.7	1492		3	2	8	6.65	15	Ischemic		x		x	x	x
S6	Right	56	F	Right		1859	122						Hemorrhagic	x	NA	x	NA		
S7	Right	59	M	Right	2.9	1243	218	7	0	23	2.83	4.5	Ischemic	x	x	x	x		
S8	Left	53	M	Right	0.7	1565	231	7	0	24	3.61	4	Ischemic	x	x		x	x	x
S9	Right	56	M	Right	6.3	550		2	1+	14			Ischemic		x		x	x	x
S10	Right	47	M	Right	1.6	773	279	7	0	23	3.61	3.5	Unknown	x	x		x		
S11	Right	60	F	Right	4.2	603	241	7	0	22	3.61	5	Unknown	x	x	x	x		
S12	Right	47	F	Left	13.3	567	286	4	0	20	3.61	4	Ischemic	x	x	x	x		
S13	Right	67	F	Right	3.8	766		2	3	5	3.61	15	Ischemic		x		x	x	
S14	Right	53	F	Left	9.8	645	219	7	0	24	3.61	5	Ischemic	x	x	x	x		
S15	Left	60	M	Right	12.4	437	329	7	0	22	3.61	6	Ischemic	x	x	x	x		
S16	Left	66	M	Right	1.1	1367	126	5	1	15			Unknown	x	x	x	x		
S17	Left	39	F	Right	2.6			9	4	9			Unknown		x		x		
S18	Left	61	M	Right	9.4	460	154	7	0	21	3.61	5	Unknown		x	x	x		
S19	Left	64	M	Left	24	210	171	7	0	23			Unknown	x	x	x	x		
S20	Left	63	M	Left	0.6	606	252	7	0	24			Unknown	x	x		x		
S21	Right	80	M	Left	1.6			2	4	10			Ischemic		x		x		
S22	Left	46	F	Right	5.5	2127	25	4	4	16	6.65	14.5	Unknown	x	x		x		
S23	Left	68	M	Right	2.8	691	50	7	0	24	3.22	2	Unknown	x	x		x		
S24	Right	56	M	Right				2	1	9			Unknown		x		x		
S25	Right	46	F	Right	13.4			3	0	19			Unknown		x		x		

GRT=grip relaxation time, Chedoke=the hand section of the Chedoke-McMaster Stroke Assessment, MAS=Modified Ashworth Scale, FM=hand and wrist section of the Fugl-Meyer assessment, SWMF=Seimmes Weinsten Monofilament Score, A=Affected hemisphere, UA=Unaffected hemisphere, NA=Not available to continue the study.

2.2.2 Procedure

The effect of stroke on modulations of the TMS measures including SICI [33] and modulation of H reflex [34, 35] were examined in both hands of stroke survivors and age-matched control subjects. The same procedures were followed as in Chapter 3. Specifically, the TMS and H reflex tests were conducted on separate days; two hands were examined on separate days as well. Grip relaxation time was measured at the beginning of each testing day. The role of SICI modulation during 70-90% into grip relaxation and H reflex modulation during 80% of grip relaxation compared to sustained contraction at matching FDS EMG activity were examined. Additionally, grip force data was also recorded during the maximum power grip through a load cell (MC3A-100, AMTI, Watertown, MA) mounted on the gripping handle (Figure 16). Grip force for each grip-and-relax trial was computed as the maximum force during the 4-second power grip. Grip force data was used to examine the effects of stroke on grip force and association between grip force and grip relaxation time.

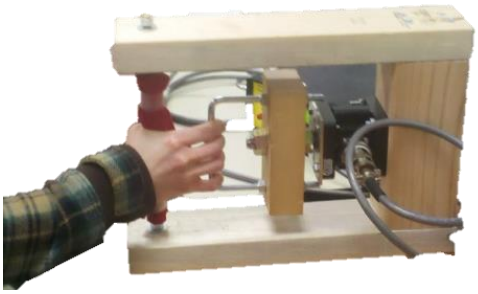


Figure 16: Load cell attached to the grip handle in order to record grip force data.

From the TMS testing designed primarily for obtainment of SICI, all other TMS measures were derived. Specifically, the cortical silent period was obtained from the portion of the SICI trials involving collection of nonconditioned MEP data at 70% into grip relaxation and during sustained contraction at that matching level of FDS EMG. The cortical silent period was defined as the period of EMG silence after the end of nonconditioned MEP until return of EMG

activity, determined visually. The corticomotor excitability was assessed by nonconditioned MEP peak-to-peak amplitude, nonconditioned MEP area, and nonconditioned MEP latency. Nonconditioned MEP data collected during the SICI examination during relaxation and during sustained contraction was used to obtain the MEP peak-to-peak amplitudes, MEP area, and MEP latency. The MEP area was defined as area under the rectified nonconditioned MEP curve. The MEP beginning and end was determined visually for the area calculations. The MEP latency was computed as the time between the stimulation and beginning of the nonconditioned MEP, determined visually. Stimulation intensities used in eliciting nonconditioned and conditioned MEP was used to examine if SICI results were confounded by differences in the stimulation intensities. AMT measured during SICI experiment was used as a measure to compare the cortical motor excitability across hands.

The reciprocal inhibition was examined by analyzing relative activation of the agonist (FDS) and the antagonist (EDC) muscles. Two variables were examined: 1) the relaxation time for the FDS and EDC muscles and 2) EMG amplitude (%MVC) for each muscle in three phases: pregrip (1.5-1 s before the grip cue during the grip-and-relax trial), midgrip during the 2-2.5 s out of the 4 s grip of the grip-and-relax trial), and over relaxation time. The EDC data was collected only in a subset of our subjects (12 control dominant hands, 9 nonparetic and 6 paretic hands of stroke survivors.

2.2.3 Statistical analysis

A one-way ANOVA was used to examine the grip relaxation time and grip force in 4 hand types (paretic, nonparetic, control dominant, control nondominant). Correlation between the grip relaxation time and grip force was examined.

For SICI, mixed model ANOVA determined the effects of the between-subject variable of hand (paretic, nonparetic, control dominant, control nondominant), and within-subject variables of task (relaxation vs. contraction) and time (70%, 80%, and 90% into the relaxation) and their 1st order interactions on SICI. The stimulation intensities used to evoke SICI were examined by two separate one-way ANOVAs to examine the main effect of hand on the test stimulus intensity and conditioning stimulus intensity (90% AMT).

For H reflex, another mixed model ANOVA was used to examine the main and interaction effects of hand and task on H reflex. The same model was applied to the cortical silent period as well. Separate mixed model ANOVAs determined the effects of hand, task, and time on the MEP peak-to-peak amplitude, MEP area and MEP latency. The same model was applied to the background FDS EMG to see if the background FDS EMG could have had a confounding effect on hand and task related changes in SICI, H reflex, MEP peak-to-peak amplitude, MEP area, MEP latency, and cortical silent period if there were any. The differences in the motor threshold (AMT) across 4 hands were examined by another one-way ANOVA.

In addition, correlation analysis was performed to examine if grip relaxation time correlated with grip force and measures of neurophysiology (modulations of SICI, cortical silent period, MEP peak-to-peak amplitude, MEP area, MEP latency, motor threshold and H reflex).

The data for the relative antagonistic muscle activity (reciprocal inhibition) was examined in the following ways. The relaxation time was examined for the main and interaction effects of hand (control, nonparetic and paretic hands) and muscle (FDS, EDC) using a mixed-model ANOVA. To examine if the activity of EDC relative to FDS was different during the grip-and-relax trial, another mixed model ANOVA was used to examine the effects of the two within-

subject variables (muscle: FDS vs EDC, time: pregrip, midgrip, relaxation phase) and one between-subject variable of hand (control, nonparetic, paretic) on the muscle EMG values (%MVC).

The grip force data was also used to assess if our subjects showed fatigue (reduced grip force over time) during the Experiment 1. Correlation between grip force and progression of Experiment 1 (0-100%) was examined in all four hands.

2.3. Results

2.3.1 Paretic FDS was the slowest to relax and the weakest

Grip relaxation time was significantly different among the 4 hands (main effect of hand, $p=0.030$). The mean relaxation time was the longest for stroke survivors' affected hands (Figure 17, mean grip relaxation times were 902±232 ms for stroke paretic hand, 625±174 ms for stroke survivors' nonparetic hand, 596±105 ms for control nondominant and 565±128 ms for control dominant hands). Tukey post-hoc showed that the paretic hand of stroke survivors was slower than the dominant hand of control subjects ($p<0.049$). All other Tukey post-hoc comparisons were not significant ($p>0.085$).

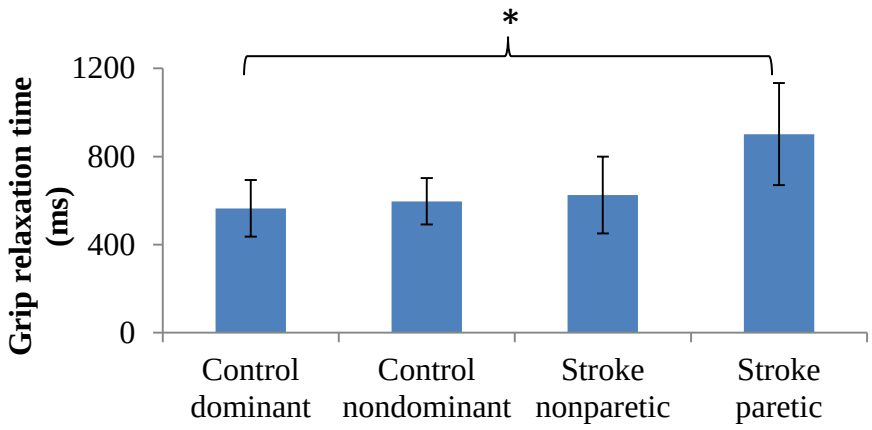
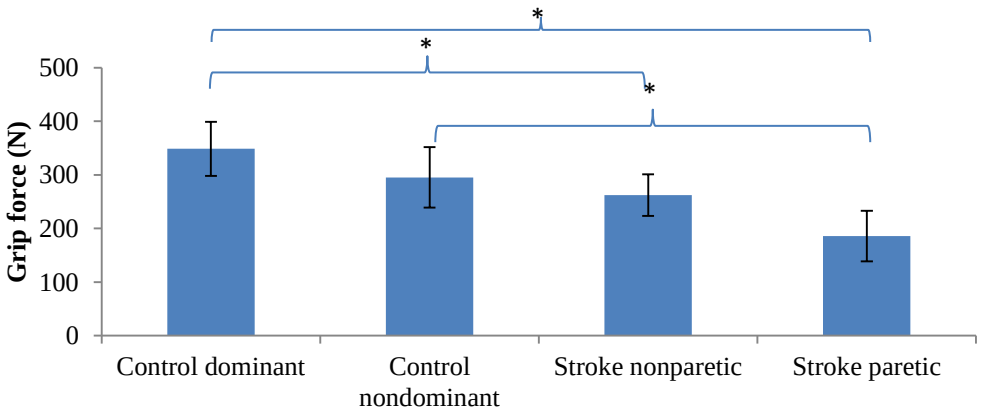


Figure 17: Effect of stroke on grip relaxation time. Stroke survivors' affected hand had the longest mean grip relaxation time. * indicates stroke affected hands being slower than the control dominant hands with Tukey posthoc at $p<0.05$. Error bars show 95% confidence interval.

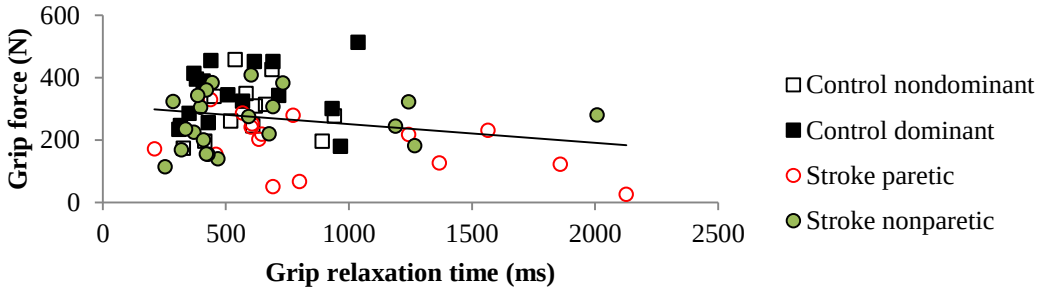
The mean maximum grip force was significantly different among the four hand types (main effect of hand type, $p<0.001$, Figure 18 A). The stroke survivors' paretic hand was, on average, the weakest compared to the dominant and nondominant hands (Tukey posthoc, $p<0.05$). The mean maximum grip force showed a trend of negative correlation with the grip relaxation time (Figure 18 B), however it did not reach the significant level for all hands combined ($r=-0.232$, $p=0.061$) and for the paretic hand only correlation analysis ($r=-0.463$, $p=0.071$).

All hands showed fatigue as seen by reducing grip force with the progression of Experiment 1 duration (Figure 18 C, $r=-0.934$ for the control dominant hand, $r=-0.908$ for the control nondominant hand, $r=-0.673$ for nonparetic hand of stroke survivors, $r=-0.762$ for the paretic hand of stroke survivors, and $p<0.0001$ for all correlations). To examine if the four hands fatigued differently, a one-way ANOVA was used to examine the fatigue. The fatigue was defined as the grip force during the last 10% of the Experiment 1 normalized to the grip force during the first 10% of the Experiment 1. All 4 hands showed comparable fatigue (main effect of

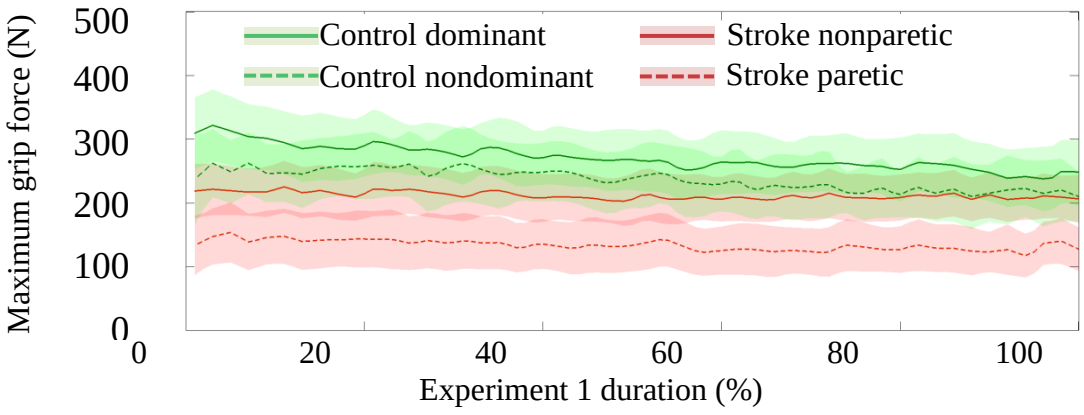
hand p=0.267). This finding suggests that the neurophysiological results of this study were not complicated by the fatigue, at least during Experiment 1.



(A)



(B)



(C)

Figure 18: Grip force results. (A) The maximum grip force was lowest for the paretic hand of the stroke survivors. (B) Grip force was not significantly correlated with the grip relaxation time. (C) All four hands showed similar trend of maximum grip force production during the grip-and-relax trials with progression of Experiment 1 consisting grip-and-relax trials. Error bars/shades indicate 95% confidence interval.

2.3.2. No abnormal modulation of intracortical inhibition for grip relaxation after stroke

2.3.2.1 SICI

SICI did not show task-specific changes for grip relaxation for all hand types. SICI was comparable between grip relaxation and sustained contraction tasks in all hands (Figure 19 A, main effect of task $p=0.439$, interaction between hand and task $p=0.572$). SICI was comparable across all hand types (main effect of hand $p=0.278$). SICI increased with progression of time during grip relaxation and sustained contraction for all hand types (Figure 19 B, main effect of time $p=0.031$, interaction between hand and time $p=0.313$). Moreover, SICI was not affected by the interactions between task and time ($p=0.489$). The SICI modulation (subtraction of SICI during contract from SICI during relaxation, averaged across the three time points) was not correlated with grip relaxation time for all hands combined and also for paretic hands only (Figure 19 C, $r=0.165$, $p=0.172$ for all hands combined, and $r=-0.052$, $p=0.837$ for the paretic hand only).

Further analysis examined if the background FDS EMG confounded the SICI results. The results showed that the background FDS EMG was comparable between two tasks (Figure 19 D, main effect of task $p=0.230$, interaction between hand and task $p=0.398$, interaction between task and time $p=0.698$). The background FDS EMG was different across all 4 hands (main effect of hand $p<0.001$). The nondominant hand of control individuals had the highest mean background FDS EMG (10.4±2.1%MVC in the nondominant hand, 8.0±1.9 %MVC in the dominant hand, 6.5±0.9%MVC in the nonparetic hand, and 8.4±1.2%MVC in the paretic hand). The Bonferroni-corrected pairwise comparison showed a significant difference only between the dominant and nondominant hands of control adults' background FDS activity ($p<0.001$). The Bonferroni-corrected pairwise comparison also showed that the background FDS EMG for the paretic hand

was similar to other hand types ($p>0.457$). The background FDS activity decreased with progression of relaxation and decreasing target muscle activity levels during sustained contraction task, as expected (main effect of time $p<0.001$). This reduction in EMG was hand-dependent (interaction between hand and time $p=0.015$), with the paretic hand showing the least mean reduction (9.6±2.2%MVC at 70% time point, 7.3±1.9%MVC at 80% time point, and 8.3±2.1%MVC at 90% time point combined for both tasks).

We also examined if the test and conditioning stimulation intensities were different across the hand types, since SICI may increase with higher stimulation intensities [52]. The test stimulation intensities used to evoke the nonconditioned MEPs were not significantly different between the 4 hand types (Figure 20 A, main effect of hand $p=0.214$, control dominant 75±8, control nondominant 67±7, stroke nonparetic 65±7, stroke paretic 70±7%MSO). The conditioning stimulation intensity (90% AMT) was also not different across hands (Figure 20 B, main effect of hand $p=0.252$, control dominant 37±7, control nondominant 30±4, stroke nonparetic 36±5 and stroke paretic hand 38±7 %MSO). This finding suggests that the SICI and corticomotor excitability results presented in this study were not influenced by differences in the stimulation intensity among the four hand types.

2.3.2.2 Cortical silent period

Cortical silent period was comparable among the four hand types (Figure 21 A, $p=0.151$), and two tasks ($p=0.694$). The interaction between hand and task on cortical silent period was also not significant ($p=0.629$). The task-specific modulation in the cortical silent period (cortical silent period during contraction subtracted from that during relaxation) was not correlated with grip relaxation time (Figure 21 B, $r=0.011$, $p=0.939$ with all hands included; $r=0.093$ $p=0.775$ only for the paretic hands).

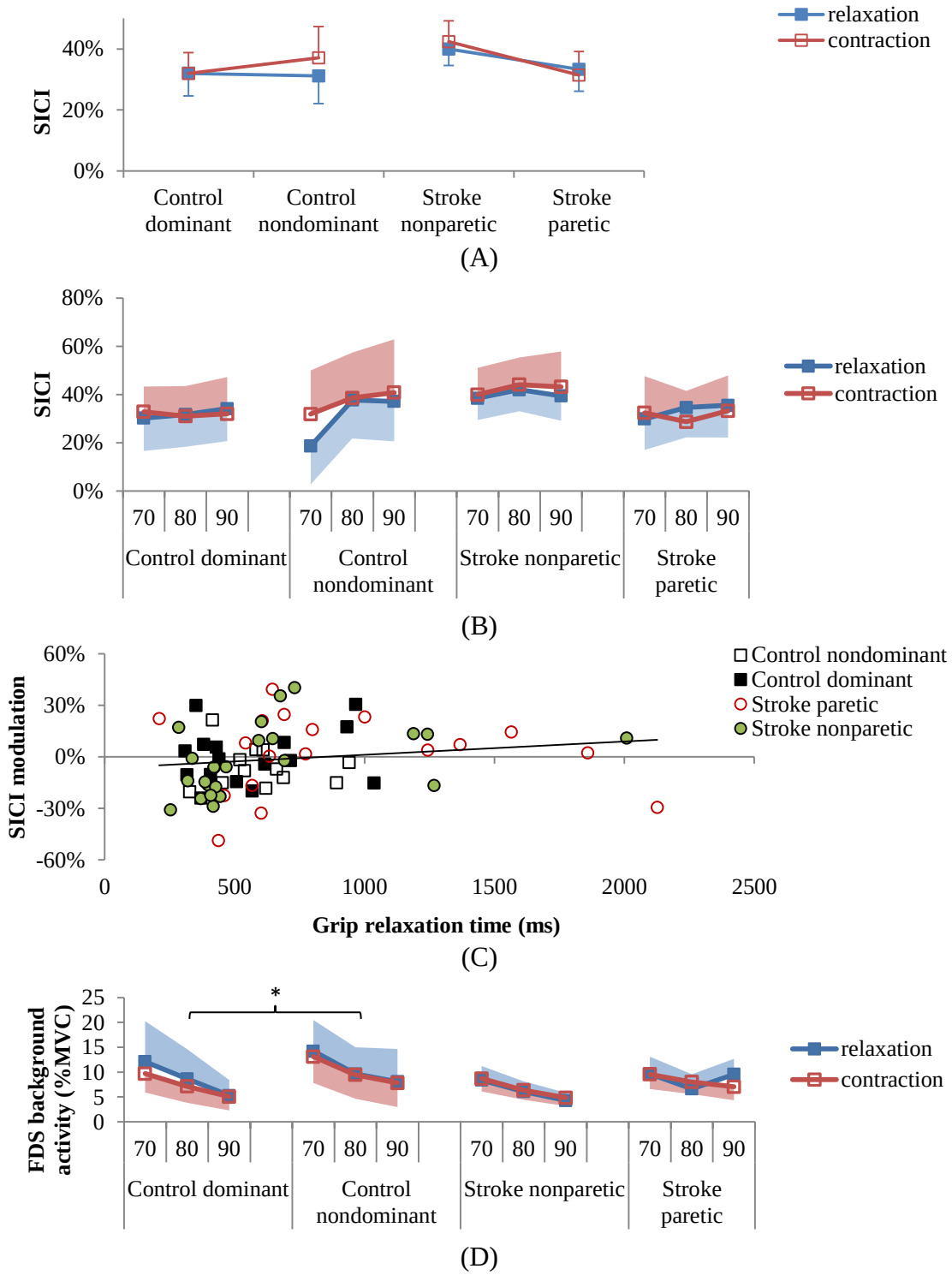
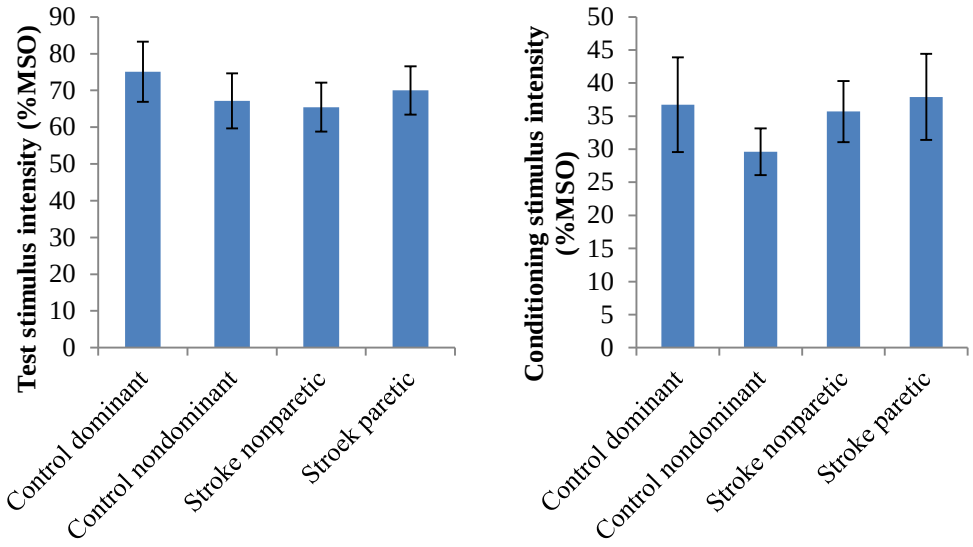
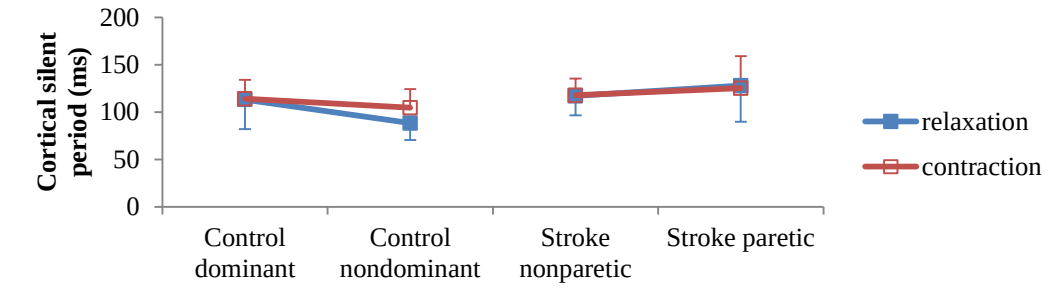


Figure 19: SICI results. (A) SICI did not change for grip relaxation in all hand types (both the main effect of task and hand x task with $p>0.05$). (B) SICI increased with progression of muscle relaxation and with decreasing muscle activity in both tasks (grip relaxation and sustained contraction with matching background FDS EMG). (C) SICI modulation was not correlated with grip relaxation time. (D) Background FDS EMG decreased with progression of relaxation and during matching sustained contractions ($p<0.05$). Figures present mean values with error bars/shades indicating 95% confidence intervals.

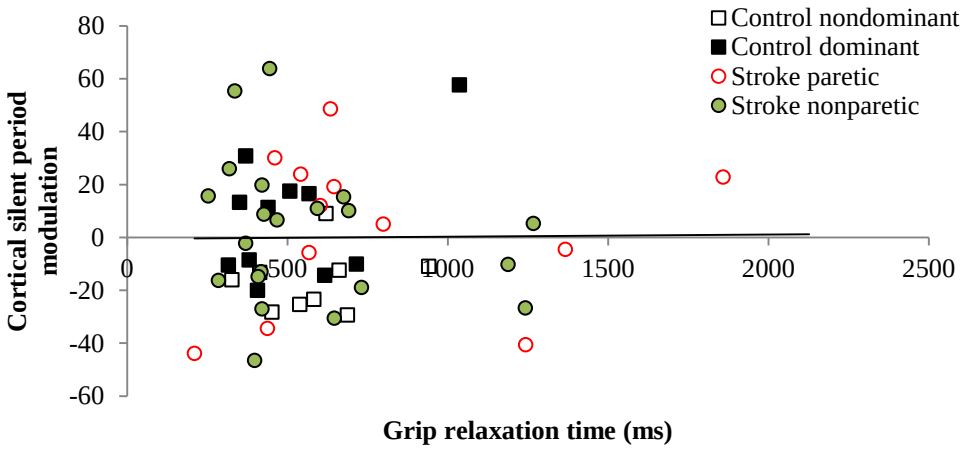


(A) (B)

Figure 20: Stimulation intensities across four hand types. (A) The test stimulation intensity to evoke nonconditioned MEP was comparable between the four hand types (p=0.214). (B) The conditioning stimulation intensity (90% AMT) used to evoke conditioned MEP during SICI examination was comparable between the four hand types (p=0.252). Error bars show 95% confidence interval.



(A)



(B)

Figure 21: Cortical silent period results. (A) The cortical silent period for nonconditioned FDS MEP did not change with hand, task or their interaction. (B) The task-dependent change in the cortical silent period was not correlated with the grip relaxation time. Error bars show 95% confidence interval.

2.3.3 No abnormal modulation of cortical excitability for grip relaxation after stroke

2.3.3.1 Peak-to-peak MEP amplitude

The nonconditioned MEP peak-to-peak amplitude was not affected by task (p=0.089) and interaction between task and hand (p=0.242) (Figure 22 A&B). The peak-to-peak amplitude of nonconditioned MEP was different across the 4 hands (Figure 22 A&B, main effect of hand p=0.006). Pairwise comparison showed that the nonconditioned MEP was significantly smaller

for the paretic hand compared to the nonparetic hand (0.9±0.1mV in the paretic hand vs. 1.3±0.1mV in the nonparetic hand with posthoc pairwise comparison p=0.005). The nonconditioned MEP values for the dominant and nondominant hands were not significantly different than paretic hand (1.3±0.1mV in the dominant hand and 1.4±0.2mV in the nondominant hand). The nonconditioned MEP peak-to-peak amplitude decreased with progression of time during muscle relaxation and with decreasing muscle activation in sustained contraction (Figure 22 B, main effect of time p=0.005, time x hand interaction p=0.903). Modulation of the MEP peak-to-peak amplitude (MEP peak-to-peak amplitude during relaxation – MEP peak-to-peak amplitude during sustained contraction, averaged across the three time points) was not correlated with the grip relaxation time (Figure 22 C, r=-0.178, p=0.141 with all hands included; r=-0.177, p=0.483 only for the paretic hands).

2.3.3.2 MEP area

The area under the rectified nonconditioned MEP curve was not different across 4 hand types and between 2 tasks (Figure 22 E&F, main effect of hand p=0.666, main effect of task p=0.330, and hand x task p=0.531). Nonconditioned MEP area decreased with progression of muscle relaxation and reduced background FDS muscle activity (Figure 22 E, main effect of time p<0.001). First order interactions between hand and time, and task and time did not significantly affect the nonconditioned MEP area (P>0.05). Modulation of the nonconditioend MEP area (MEP area during relaxation-MEP area during contraction) was not correlated with grip relaxation time (Figure 22 F, r=-0.134, p=0.269 with all hands included; r=-0.176, p=0.484 only for the paretic hands).

2.3.3.3 MEP latency

The latency for nonconditioned MEPs was different across 4 hand types (Figure 23 A, main effect of hand p=0.001). The paretic hand FDS had the longest mean latency compared to other hands (16.6±0.3ms in the paretic hand vs. 15.0±0.3ms in the nonparetic hand, Bonferroni-corrected pairwise comparison p=0.002, paretic hand vs. 14.5±0.3ms in the nondominant hand, p=0.033, and paretic hand vs. 14.8±0.3ms in the dominant hand, p=0.058), consistent with literature [111]. The latency was not affected by any other variables including task, time, and 1st order interactions among hand, task and time (Figure 23 B, p>0.05). The task-dependent modulation of the nonconditioned MEP latency (nonconditioned MEP latency during relaxation-nonconditioned MEP latency during contraction) was not correlated with grip relaxation time (Figure 23 C, r=0.027, p=0.829 with all hands included; r=0.277, p=0.281 only for the paretic hands).

2.3.3.4 Active Motor threshold

The stimulation intensity for AMT (a measure of cortical excitability) was not significantly different across hands (main effect of hand p=0.252, control dominant 41±8, control nondominant 33±4, stroke nonparetic 40±5, stroke paretic 42±7%MSO). The AMT was not correlated with the grip relaxation time (Figure 24, r=-0.027, p=0.825 for all hands included, r=0.267, p=0.187 only for the paretic hands).

In summary of investigation of the cortical excitability, paretic hands showed overall reduced excitability as seen by the smaller average peak-to-peak MEP amplitude and longer average MEP latency. However, modulation of the cortical excitability of the paretic hand motor

area (MEP peak-to-peak amplitude and MEP latency) was not associated with delayed grip relaxation time in the paretic hand.

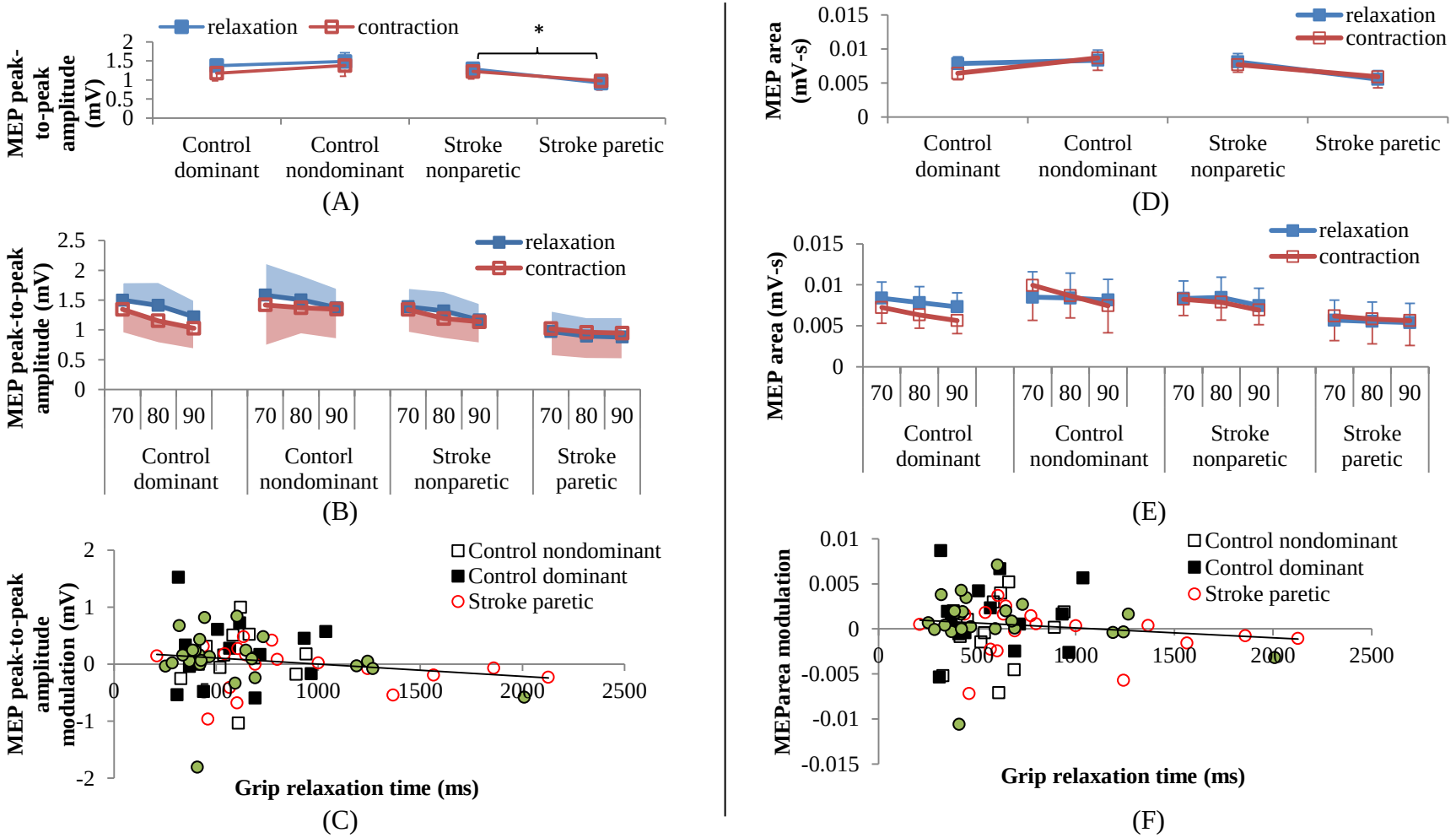


Figure 22: peak-to-peak MEP amplitude and MEP area results. (A) MEP peak-to-peak amplitude was not significantly different between the two tasks and by the task and hand interaction. (B) MEP peak-to-peak amplitude decreased with progression of relaxation and with decreasing FDS background muscle activity. (C) The task-dependent modulation in the MEP peak-to-peak amplitude averaged across the three time points was not correlated with grip relaxation time. (D) The MEP area was not significantly different between the two tasks and by the task and hand interaction (E) The MEP area decreased with progression of relaxation and decreasing background FDS muscle activity. (F) The task-dependent modulation in the MEP area was not correlated with grip relaxation time. Error bars/ shades show 95% confidence interval.

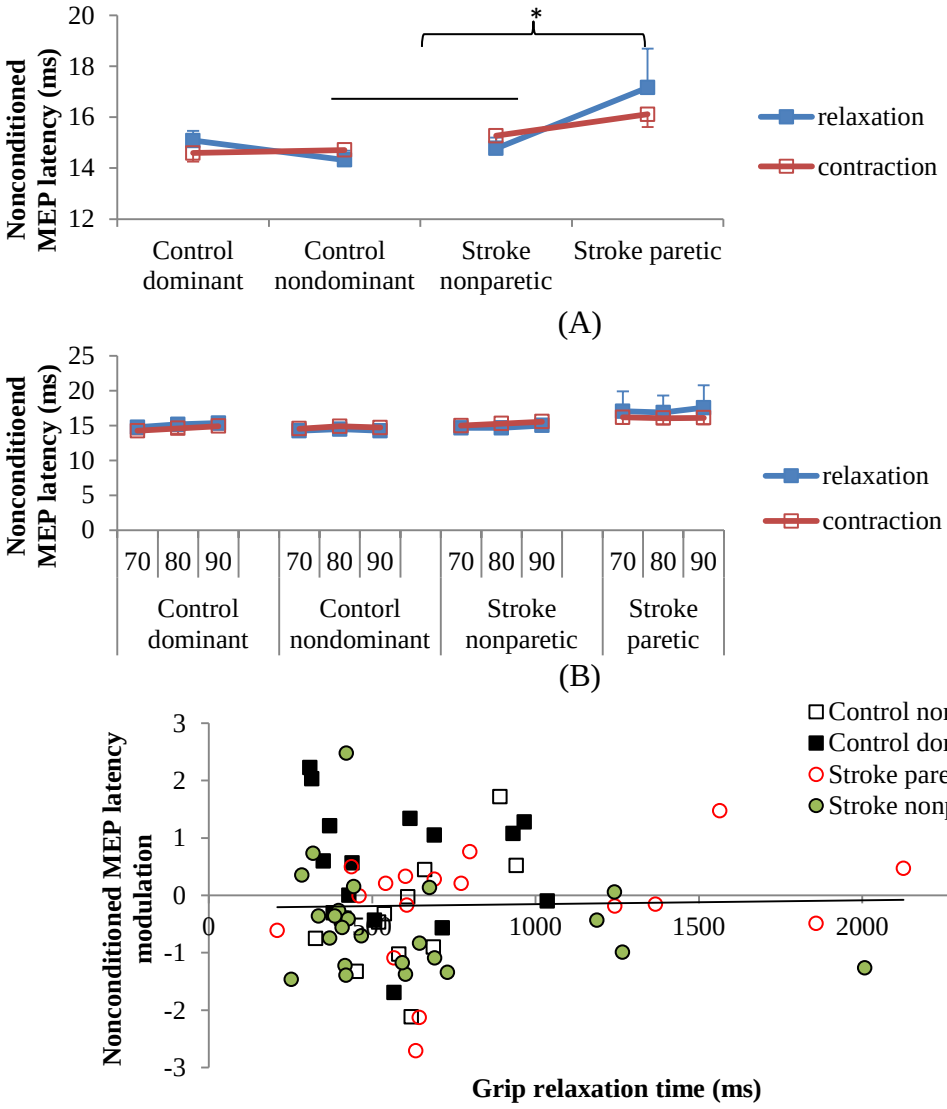


Figure 23: MEP latency results. (A) The MEP latency did not differ between the two tasks for all hands. The mean MEP latency was the longest in the paretic hand. (B) The MEP latency did not vary with progression of relaxation and decreasing levels of background FDS muscle activity during contraction. (C) MEP latency modulation was not correlated with the grip relaxation time. Error bars show 95% confidence interval.

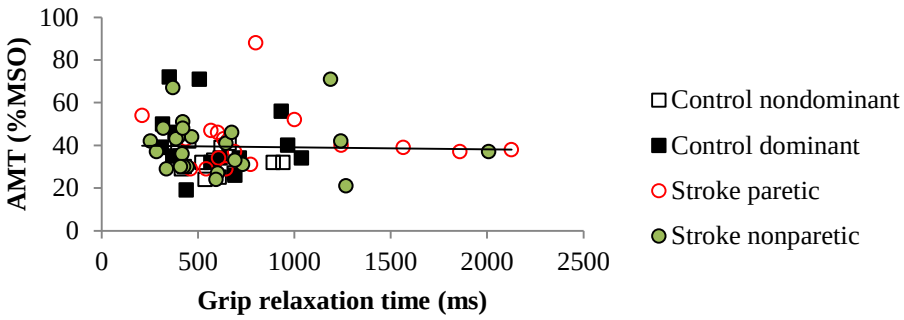


Figure 24: AMT was not correlated with grip relaxation time.

2.3.4 No abnormal modulation in H reflex for grip relaxation after stroke

H reflex was not significantly modulated for grip relaxation in all hands (Figure 25 A, main effect of task p=0.453, interaction between hand and task p=0.929). H reflex was not significantly different across hands (p=0.132). The background FDS EMG for H reflex experiment did not significantly differ between the two tasks in all hands (Figure 25 B, main effect of task p=0.996, interaction between hand and task p=0.909). The background FDS EMG was similar across the four hand types (p=0.142). H reflex modulation (H reflex during relaxation-H reflex during contraction) was not correlated with grip relaxation time (Figure 25 C, r=-0.138, p=0.509 for all hands combined, r=-0.018, p=0.969 for the paretic hands only).

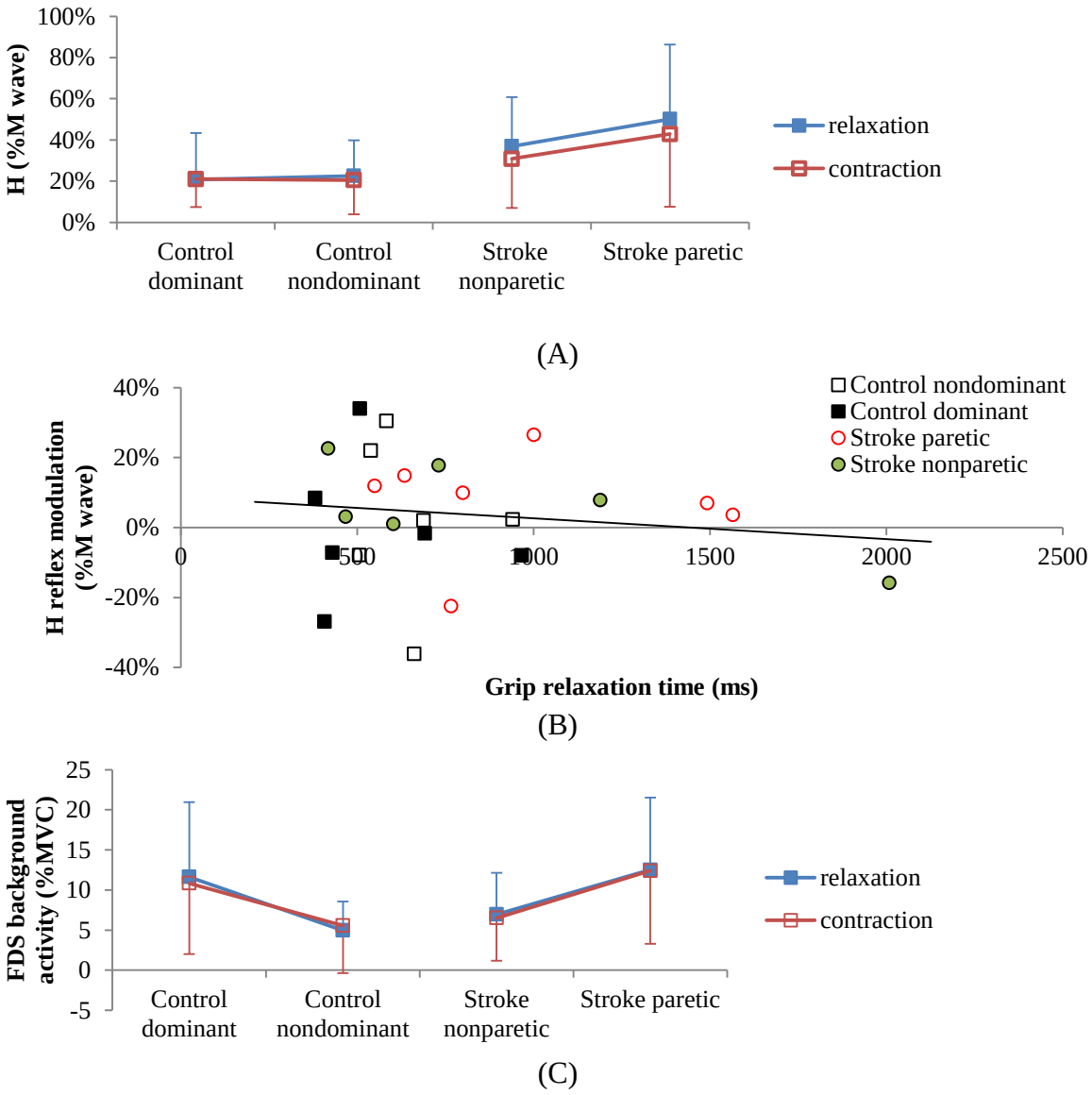


Figure 25: H reflex results. (A) No changes in H reflex depending on hand, task or their interaction (B) H reflex modulation was not correlated with the grip relaxation time (C) Background FDS EMG was comparable between hands and tasks. Error bars show 95% confidence interval.

2.3.5 No abnormal antagonistic muscle activity during delayed grip relaxation

As further analysis, EMG traces were examined to see if relative antagonist (EDC) and agonist (FDS) muscle activation may be different during grip-and-relax trials post stroke which may contribute to delayed grip relaxation. Such imbalanced muscle activation may be associated

with post-stroke difference in reciprocal inhibition or abnormal reciprocal facilitation [112-114]. This analysis was performed in a subset of subjects who had EDC data (n=12 for dominant hand of control subjects, 0 for control nondominant hand; 9 nonparetic and 6 paretic hands). Two variables were examined: 1) relaxation time for each muscle and 2) EMG amplitude for each muscle in three phases: pregrip (1.5-1 s before the grip cue during the grip-and-relax trial), midgrip during the 2-2.5 s out of the 4 s max grip of the grip-and-relax trial), and over each muscle's relaxation time. The muscle relaxation time was not different for each muscle in all hands (mean±SD, 663±326 ms FDS relaxation time vs. 561±387 ms EDC relaxation time averaged across all three hand types, Figure 26, main effect of muscle $p=0.707$, muscle and hand interaction $p=0.215$). The muscle relaxation time was not affected by the hand type (main effect of hand $p=0.495$). For the EMG amplitudes, EDC had overall 23% more activity than FDS (main effect of time $p<0.001$). The overall muscle activity was different between the three phases ($p<0.001$). The main effect of hand and all interactions among hand, phase, and muscle were not significant ($p>0.106$), suggesting that the greater overall EDC activity was not hand- or phase-specific. In other words, there was no EDC/FDS imbalance specific to the relaxation period for the paretic hand (Figure 26).

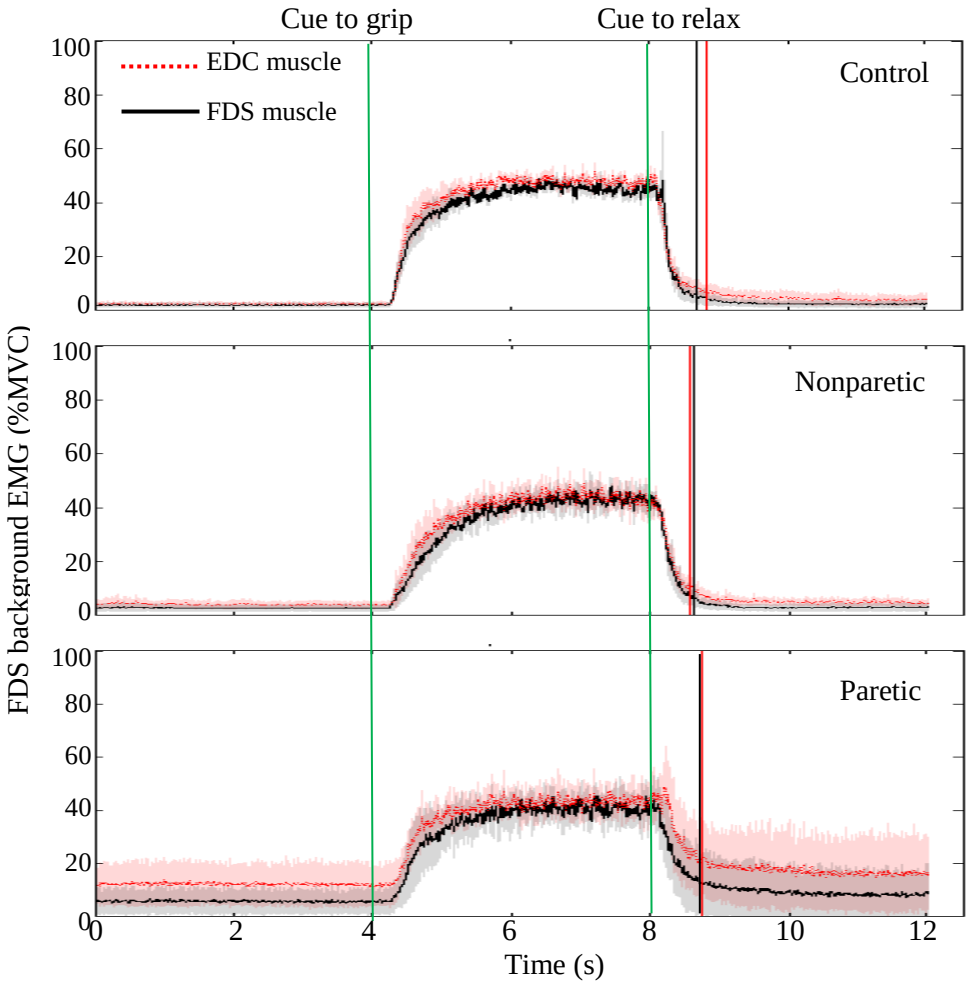


Figure 26: FDS and EDC EMG during grip-and-relax trials. EDC relaxation time was comparable to FDS relaxation time in all hands. EDC activation relative to FDS activation was comparable throughout the grip-and-relax trial for all hands. Error shades show 95% confidence interval.

2.4. Discussion

This is the first study to examine modulation of neural substrates specific for grip relaxation in stroke survivors. The main finding of this study is that stroke-specific delayed grip relaxation in the paretic hand was not explained by altered modulations of SICI, cortical silent period, corticomotor excitability, and H reflex. All the neurophysiologic measures studied in this

study did not explain stroke-specific delay in the stroke survivors' paretic grip relaxation times. In fact, there were no modulation of the intracortical inhibition (GABA-Aergic SICI, GABA-Bergic cortical silent period), cortical excitability (MEP peak-to-peak amplitude, MEP area, MEP latency) and spinal motor excitability (H reflex) for grip relaxation in all hand types (dominant and nondominant hands of age-matched control subjects as well as nonparetic and paretic hands of stroke subjects). Yet, paretic hands were, on average, the slowest in relaxing from a maximum power grip.

While no task-specific modulation was observed in all hand types, some of the measures significantly differed across the hand types. Specifically, the peak-to-peak MEP amplitude was smaller and the MEP latency was longer for the paretic hand compared to other hands. These findings of altered peak-to-peak MEP amplitude and MEP latency post stroke are consistent with literature [111]. Therefore, our study suggests that MEP peak-to-peak amplitude and MEP latency may be related to delays in grip relaxation time in stroke survivors, which was further examined in Study 3.

The background FDS EMG was comparable between the two tasks. Additionally, all hand groups fatigued with the progression of experiment, however no differential pattern of fatigue was observed across the four hand types. These findings suggest that the above results of lack of modulation of SICI, cortical silent period, corticomotor excitability and H reflex were not influenced by differences in the background muscle activation or muscle fatigue.

Alpha motor neuron excitability may be affected by activation of the antagonist muscles through reciprocal inhibition [115]. During grip relaxation, the EDC muscle activity was not different for the paretic hand of stroke survivors compared to their nonparetic hand or age-

matched controls. Similar EDC and FDS muscle activation patterns suggest that the relative activation of EDC was comparable across all hand types. After stroke, the reciprocal inhibition was shown to decrease from the extensor carpi radialis (ECR) to the flexor carpi radialis (FCR) muscle in the upper limb [116, 117] and from the tibialis anterior (TA) to the soleus [112-114] in the lower limb. Therefore, it is possible that while paretic hands show normal level of EDC activity, the EDC muscle activity's influence on the FDS activity may be reduced after stroke via reduced reciprocal inhibition. However, reciprocal inhibition was not directly assessed in our subjects, and we cannot confirm the postulation.

3. Study 2: To assess the effects of Active Passive Bilateral Therapy (APBT) on grip relaxation time and interhemispheric inhibition

3.1. Introduction

Two cerebral hemispheres of the healthy human brain are known to inhibit each other's activity in order to prevent movements in the unintended side during unilateral movement [118]. This characteristic is known as interhemispheric inhibition, which can be assessed by using paired stimulation of both hemispheres using TMS [119]. Interhemispheric inhibition is balanced in neurologically-intact adults (i.e., the magnitude of the interhemispheric inhibition from the left to the right hemisphere is approximately the same as that from the right to left hemisphere). Interhemispheric balance after stroke is impaired, with decreased inhibition from the affected to the unaffected hemisphere and increased inhibition from the unaffected to the affected hemisphere at rest [120] and during the pre-movement phase of the affected first dorsal interosseous muscle [103] compared to age-matched control adults, leading to net inhibition of the affected hemisphere.

Greater interhemispheric imbalance is associated with lower intracortical inhibition in the inhibited hemisphere in healthy adults [104]. Thus, it is possible that the greater interhemispheric inhibition from the contralesional hemisphere reduces the affected hemisphere's intracortical inhibition [120, 121]. However, the role of interhemispheric imbalance on delayed muscle relaxation has not been investigated.

This study examined the effect of the modulation of the interhemispheric inhibition on grip muscle relaxation time in chronic stroke survivors. To modulate interhemispheric inhibition,

we used the Active Passive Bilateral Therapy (APBT). APBT involves mirror symmetric bilateral hand movements at the wrist joints, with active movements by the nonparetic hand that mechanically results in mirror symmetric passive movements in the paretic hand [122]. APBT was chosen, because APBT has been shown to improve hand function per Fugl-Meyer score by restoring interhemispheric balance and increasing the cortical excitability of the affected hemisphere after 4 weeks of APBT in chronic stroke survivors [122]. The APBT resulted in a faster recovery per Action Research Arm Test scores when tested during and after 4 weeks of APBT in acute stroke survivors [123]. A control condition was required because APBT involves passive repeated stretching of the paretic muscles and it is known that passive repeated stretching of the paretic muscles results in prolonged relaxation time [23]. Thus, we developed the Unilateral Passive Therapy (UPT), involving only passive movements for the paretic hand induced by a motor while the nonparetic hand rests. The working hypothesis was that grip muscle relaxation time and interhemispheric inhibition to the affected hemisphere decrease after ABPT, compared to UPT.

3.2. Methods

3.2.1 Subjects

A total of 10 chronic stroke survivors were recruited, however only 8 chronic stroke survivors completed both of the two sessions required for the study (first 8 rows in the Table 6). Only these 8 subjects were included in the analysis unless noted (aged 62±5 years old, 3 females). The subjects were screened for contraindications to TMS. All subjects signed informed consent approved by the Institution Review Board.

3.2.2 Approach

This study involved two sessions of testing for each subject. One session was for APBT, and another session was for UPT. The two sessions occurred on two different days. The order of APBT and UPT sessions was randomized. Each testing session involved 20-minute intervention (either APBT or UPT) with immediate pre- and post-intervention measurements of the FDS muscle relaxation time and interhemispheric inhibition (IHI). The session length of 20 minutes was chosen because the previous longitudinal studies used 20-minute [123] and 10-15 minute APBT intervention a day [122]. The pre- and post-intervention included measurements of the muscle relaxation time and the interhemispheric inhibition for both hands. The FDS relaxation time was measured before the IHI during the pre-intervention measurements and after the IHI during the post-intervention measurements. Each testing session was approximately 3 hours long.

3.2.2.1 Intervention

APBT and UPT machines include two hand plates (Figure 27). The two hand plates in the APBT apparatus are linked mechanically in a way that when one hand moves in flexion (active hand), the APBT machine moves the other hand in flexion to the same range (passive hand). The nonparetic hand served as the active hand to induce passive movements of the paretic hand at the wrist. While APBT and UPT employed wrist movement, the FDS muscle is involved maximally in wrist flexion as well as power grip.

Both the interventions were 20 minutes long (15 minutes exercise with 5 minute rest interspersed). There were total 4 rest periods of 75 seconds each, given after every 3 minutes of

movement. Each intervention included 900 movements (450 flexions, 450 extensions). For both APBT and UPT, movements (either flexion or extension) were conducted at 1 Hz. For APBT, a metronome was used to regulate the rhythm of the movement at 1 Hz. The UPT movement frequency was maintained at 1 Hz by Labview-generated commands sent to the driver motor moving the paretic hand plate. Subjects were encouraged to move over a 40 degree range of motion (20 degrees of flexion and extension each from the neutral wrist posture) during APBT. The movement range was set from 20 degrees of wrist flexion to 20 degrees of wrist extension for the UPT protocol as well. The motor of the UPT device generated a loud noise; therefore subjects wore a noise-cancelling headphone during both intervention sessions in order for consistency.

Table 6. Demographic and clinical information for stroke survivors in Study 2.

Subj	Affec-ted hand	Age	Sex	Pre-stroke handedness	Time since stroke (years)	Chedoke (0- 7)	MAS (0-4)	FM (0-24)	SWMF score	2-point (mm)	Lesion type
S16	Left	66	M	Right	1.1	5	1	15	-	-	Unknown
S4	Right	74	F	Right	2.2	7	0	24	3.61	5.5	Ischemic
S19	Left	64	M	Left	24	7	0	23	-	-	Unknown
S18	Left	61	M	Right	9.4	7	0	21	3.61	5	Unknown
S22	Left	46	F	Right	5.5	4	4	16	6.65	14.5	Unknown
S23	Left	68	M	Right	2.8	7	0	24	3.22	2	Unknown
S15	Left	60	M	Right	12.4	7	0	22	3.61	6	Ischemic
S14	Right	53	F	Left	9.8	7	0	24	3.61	5.5	Ischemic
S20	Left	63	M	Left	0.6	7	0	24	-	-	Unknown
S26	Left	87	M	Right	2.2	3	2	4	-	-	Unknown

Chedoke=the hand section of the Chedoke-McMaster Stroke Assessment, MAS=Modified Ashworth Scale, FM=hand and wrist section of the Fugl-Meyer assessment, SWMF=Seimmes Weinsten Monofilament Score, 2-point=2-point discrimination score.

Figure 27: APBT and UPT devices. (A) Active Passive Bilateral Therapy (APBT) included passive mirror symmetric movements of the paretic wrist caused by active movement of the nonparetic wrist and (B) Unilateral Passive Therapy (UPT) included passive movement of the paretic wrist with the resting/stationary nonparetic wrist.

3.2.2.2 Pre and post evaluation

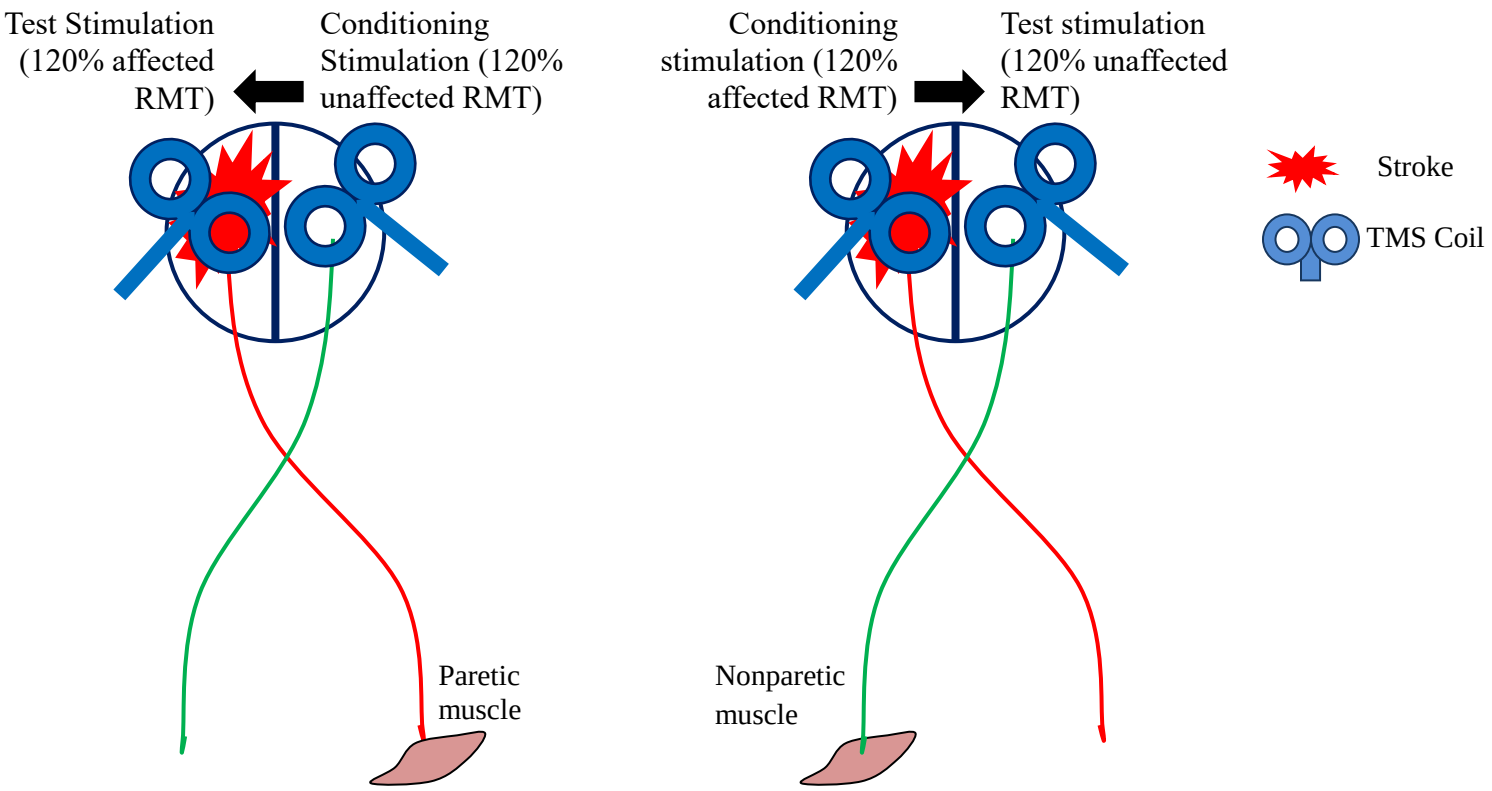
Muscle relaxation time and interhemispheric inhibition for the FDS muscle were measured immediately before and after either the APBT or UPT. The FDS relaxation time was measured before the IHI during the pre-intervention measurements and after the IHI during the post-intervention measurements. This was done because both measurements required a different setup. The FDS muscle relaxation time was examined as described in Chapters 2 and 3, except that relaxation from an isometric MCP joint flexion was examined instead of relaxation from an isometric maximum power grip. The reason for this variation was that the APBT involves wrist flexion-extension. We decided against using the power grip in this experiment, because of involvement of multiple muscles during a maximal power grip, in contrast to wrist flexion-extension. Instead, a simple metacarpophalangeal (MCP) joint flexion task was used in order to isolate/maximize the FDS activation.

Interhemispheric inhibition for both hemispheres was determined by delivering TMS on each hemisphere's "hotspot" for the contralateral FDS muscle using two 70-mm figure of eight

coils independently connected to two TMS stimulators separately, (Figure 28). Paired pulse protocol with a 10 ms interstimulus interval was used to quantify interhemispheric inhibition in the FDS muscle, following literature [103, 119]. This protocol includes evoking (1) nonconditioned MEP by delivering a test stimulus on the test hemisphere for which interhemispheric inhibition is being investigated and (2) evoking a conditioned MEP by delivering a conditioning stimulus on the contralateral hemisphere (interhemispheric inhibition generating hemisphere), 10 ms before the test stimulus to the test hemisphere [119]. The average conditioned and nonconditioned MEPs were used to quantify interhemispheric inhibition (IHI) as per Equation 2.

$$IHI = 100 * \left(1 - \frac{conditionedMEP}{nonconditionedMEP}\right) \qquad \textit{Equation 2}$$

To obtain IHI for the paretic side, test stimulation was delivered at 120% of the affected hemisphere's resting motor threshold (RMT) to the affected M1 and conditioning stimulation was delivered at the 120% unaffected hemisphere's RMT to the unaffected M1 [124] (Figure 28). RMT was determined as the minimum %MSO that evokes peak-to-peak MEP amplitude of at least 50 μV in 5 out of 10 times [48]. Similarly, to obtain IHI for the nonparetic side, the test stimulation was delivered at the unaffected hemisphere's 120% RMT to the unaffected M1, whereas the conditioning stimulation was delivered to the affected M1 with the intensity of the affected hemisphere's 120% RMT. Mean of the MEPs obtained in ten single pulse stimulations and ten paired pulse stimulations delivered in a random order of four blocks of five stimuli each was used to quantify IHI.



(A) Interhemispheric inhibition in the lesioned hemisphere from the nonlesioned hemisphere

(B) Interhemispheric inhibition in the nonlesioned hemisphere from the lesioned hemisphere

Figure 28: Depiction of the interhemispheric inhibition measurements (A) in the lesioned hemisphere from the nonlesioned hemisphere and (B) in the nonlesiodne hemisphere from the lesioned hemisphere. The nonconditioned MEP in the target hemisphere evoked by the test stimulation was compared to the conditioned MEP evoked by conditioning the test stimulation in the target hemisphere by stimulation of the contralateral hemisphere.

The apparatus used for the IHI evaluation as well as the FDS muscle relaxation time is shown in Figure 29. During IHI evaluation, subjects maintained a moment of 0.4 Nm (through visual feedback on the computer screen) of MCP flexion in the hand contralateral to the target hemisphere (where the test stimulation was given), because IHI can be easily examined during contraction [103]. The other hand was at rest during the IHI measurements. We used a custom-made apparatus to perform the above mentioned MCP flexion task (Figure 29). In this apparatus, the forearm was supported and secured in a mid-prone position with the elbow at 90 degrees flexion and the wrist neutral. The MCP joints were flexed at 45 degrees and the fingers were secured in between two hand plates. The subjects were asked to press against the hand plate with their fingers in the flexion direction, while contracting the FDS muscle. A load cell was attached below the hand plates and the MCP joint was placed at the center axis of the load cell such that the load cell measured the MCP flexion/extension moment.

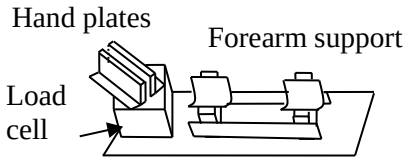


Figure 29: Device used in the IHI experiments to test the FDS muscle during MCP joint flexion

3.2.3 Statistical Analysis

Two repeated measures ANOVA was used to identify the effects of therapy (APBT vs. UPT), time (pre- vs. post-intervention) and side (the paretic vs. nonparetic hand for FDS muscle relaxation time, and the lesioned vs. nonlesioned hemisphere for IHI) on the muscle relaxation time and IHI. Additional repeated measures ANOVA was run to test the variability of the pre-intervention IHI measurement (main and interaction effects of side and day). Association between the muscle relaxation time and IHI was examined by (1) regression for the pre-

intervention muscle relaxation time using IHI of each side and (2) regression between the change in the paretic relaxation time and interhemispheric inhibition before and after intervention of both sides). The alpha was set at 0.05. The statistical analysis was performed using SPSS (v. 20, IBM, Armonk, NY).

3.3. Results

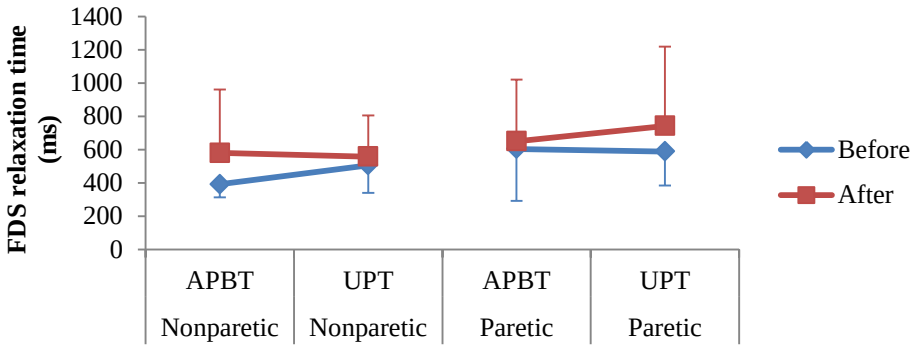
The muscle relaxation time was not affected by either of the APBT or UPT movement therapies (main effect of time, p=0.265, Figure 30 A). This lack of increase in the muscle relaxation time after the passive stretching may be because our subjects are relatively higher functioning compared to the previous study [23]. The muscle relaxation time was also not affected by the type of intervention (Figure 30 A, main effect of therapy, p=0.400, interaction between therapy and time, p=0.867). The muscle relaxation time was not different between the two sides (main effects of side, p=0.202, interaction between side and therapy, p=0.943, interaction between side and time, p=0.770). The muscle relaxation time was not affected by therapy x time x hand (p-0.189). When only paretic hand data was included in the ANOVA, the FDS relaxation time was not affected by the type of therapy (main effect of therapy p=0.640, main effect of time p=0.244 and the time x therapy interaction p=0.360).

The IHI was not affected by both types of intervention (Figure 30 B, main effect of time p=0.347, main effect of therapy, p=0.667, interaction between therapy and time, p=0.065). The IHI was also not different between two sides (main effect of side, p=0.725, interaction between side and therapy, p=0.616, interaction between side and time, p=0.279). The interaction of side x therapy x time were not significant (p>0.05). The pre-intervention IHI measurement was not

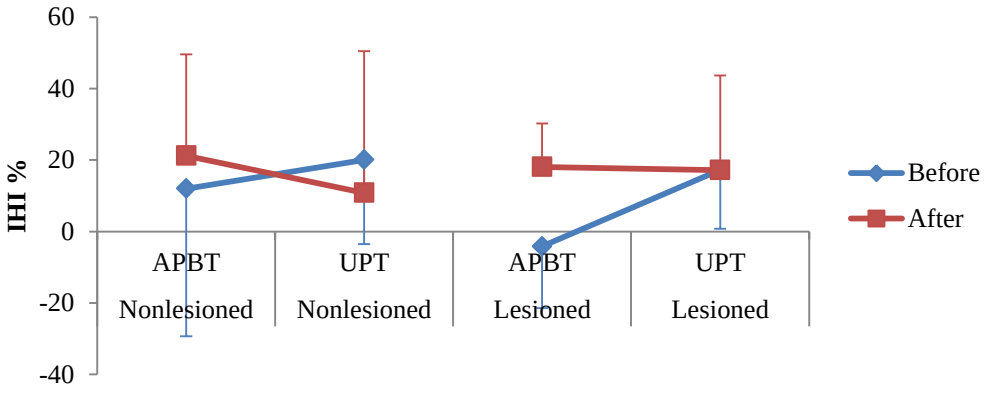
different between the two days and sides (main effects of day, p=0.205, main effect of side, p=0.428, interaction between day and side, p=0.582).

Regression model to predict the paretic FDS relaxation time with IHI in the paretic and nonparetic hemisphere showed that the none of the pre-intervention IHI significantly predicted the pre-intervention paretic FDS relaxation time (n=10, IHI in the paretic hemisphere p=0.644, IHI in the nonparetic hemisphere p=0.0644). The pre-intervention IHI and relaxation time were averaged across the two days. Similarly, the change in the paretic FDS relaxation time (post-intervention – pre-intervention values) was not explained by pre- or post-intervention IHI (n=8, pre-intervention IHI in the affected hemisphere p=0.648, pre-intervention IHI in the unaffected hemisphere p=0.395, and post-intervention IHI in the affected p=0.757 and unaffected hemisphere p=0.690). The pre- and post-intervention IHIs were averaged across the two days.

In summary, both muscle relaxation time and IHI were not affected by the APBT or UPT intervention. Based on the regression analysis, muscle relaxation time was not associated with IHI. The muscle relaxation time and IHI were comparable between the paretic and nonparetic sides in this subject population.

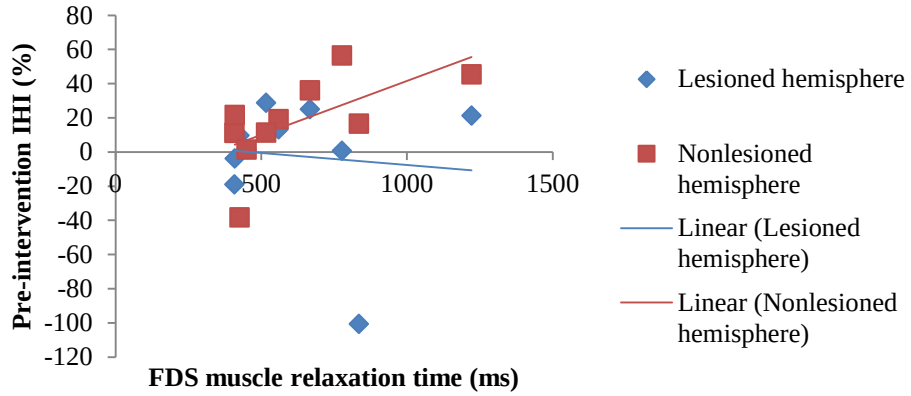


(A)

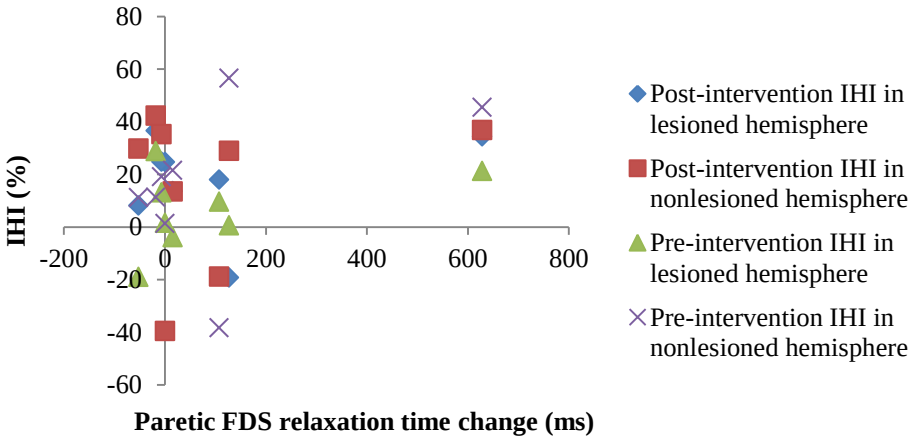


(B)

Figure 30: Effects of APBT and UPT on grip relaxation time and IHI. (A) Muscle relaxation times did not significantly change with APBT or UPT (B) IHI in each hemisphere did not significantly change with APBT or UPT. Error bars show 95% confidence interval.



(A)



(B)

Figure 31: IHI was not associated with paretic FDS relaxation time. (A) Pre-intervention paretic muscle relaxation time and IHI (averaged for the two days) were not significantly associated (n=10). (B) Changes in the muscle relaxation time and IHI averaged for the two days were not associated (n=8). Error bars show 95% confidence interval.

3.4. Discussion

The results of this study showed that the single-session 20-minute APBT did not change IHI and muscle relaxation time. The reasons for no change in IHI with APBT in the present study unlike the previous studies [122, 123] are discussed as follows.

First, the present study used a single 20-minute session APBT on stroke survivors, while previous literature showed changes in IHI, improvement in clinical assessment of motor function in chronic stroke survivors after 4 weeks of APBT intervention in stroke survivors [122, 123]. Only one study used the APBT intervention for a single 20-minute session and examined its effects on neurophysiological measurements, however this study was conducted in healthy young adults [125]. In that study, the APBT intervention increased corticomotor excitability of the paretic hemisphere, decreased long-interval intracortical inhibition in the passive hemisphere, and reduced interhemispheric inhibition in the passive from the active hemisphere [125]. Thus, APBT may have the capacity to change corticomotor excitability and IHI after a single session, but only in healthy adults.

Second, the present study used the APBT as a stand-alone intervention, not combined with any other traditional therapies, unlike previous studies. In the pilot APBT study in chronic stroke survivors, APBT was used without hand rehabilitation [126]. One-month of APBT use improved Fugl-Meyer score and had a trend of increased cortical map in the lesioned hemisphere and significant reduction in the cortical map in the nonlesioned hemisphere [126]. This may have been due to balancing of the between-hemisphere corticomotor excitability, however interhemispheric inhibition was not examined in this study. In the subsequent investigation involving intervention of APBT after stroke, the APBT intervention was used as a priming technique and was found effective in modulating interhemispheric inhibition and cortical excitability in the lesioned hemisphere [122, 123]. Other forms of motor tasks such as manipulating wooden blocks or upper limb rehabilitation (physical therapy and occupational therapy) followed APBT in both studies [122, 123]. The APBT intervention along with traditional hand therapy increased IHI from the affected to the unaffected hemisphere in chronic

stroke survivors [122] and in acute phases of stroke recovery [123]. The present study used the APBT as a stand-alone intervention and did not show any changes in the IHI or muscle relaxation time. Our findings suggest that a single-session of the APBT intervention alone without traditional rehabilitation may not produce changes in IHI in chronic stroke survivors.

Third, the method of assessing IHI differed between the present study and the previous studies. The two previous studies with APBT in stroke survivors examined the IHI with the ipsilateral silent period technique in the ECR muscle [122, 123]. In this technique, duration of the inhibition of ongoing ECR muscle activity in response to a single-pulse TMS to the ipsilateral M1 was measured [122, 123]. In contrast, the present study used the paired pulse method for the FDS muscle, in which IHI in the target FDS was examined by test stimulation to the contralateral M1 and a conditioning stimulation to the ipsilateral M1 [119]. Exact mechanisms for each form of IHI (paired pulse vs. ipsilateral silent period) are unknown [127]. Both forms of IHI are mediated at least in part through corpus callosum as both are reduced in patients with corpus callosum lesions [128, 129]. Some evidence suggests that the IHI at 10ms interstimulus interval and ipsilateral silent period may be controlled through different population of neurons because IHI at 10ms interstimulus interval was found in response to TMS current induced in all directions, whereas ipsilateral silent period was maximum with TMS current in anteromedial direction [130]. In summary, the APBT may affect neurons specific to the ipsilateral silent period, but not the paired-pulse IHI.

In summary, this study suggests that a single-session of 20 minute APBT may not induce changes in the paired-pulse IHI and muscle relaxation time in chronic stroke survivors. A longer intervention duration, as done previously, may be more suitable to examine the effects of APBT on IHI and muscle relaxation time in stroke survivors [122, 123].

3.5. Conclusion

The main goal of this study was to examine the effects of a single session of mirror symmetric active passive bilateral therapy, APBT on interhemispheric inhibition and the FDS muscle relaxation time. The interhemispheric inhibition measured by the paired pulse protocol was not affected by the APBT intervention. The FDS muscle relaxation time was not affected by the APBT intervention, as well. These results suggest that a single session of APBT may not be effective in modulating IHI and muscle relaxation time. A longitudinal study with APBT priming may be a more suitable approach to test the effects of APBT.

4. Study 3: Regression analysis for paretic muscle relaxation time

4.1 Introduction

This study describes potential post-stroke characteristics that may contribute to delayed relaxation time in chronic stroke survivors based on literature reviews and also explores such relationships using a regression model with previously collected data. All the data used in this regression analysis was previously presented in Study 1 and 2.

(1) Interhemispheric inhibition

Excessive interhemispheric inhibition to the lesioned hemisphere may explain delayed initiation of muscle contraction after stroke [103]. This imbalance in brain activation is associated with poor functional status of stroke survivors [103]. Increased interhemispheric inhibition reduces cortical excitability at rest in healthy adults [104]. It is possible that excessive inhibition of the lesioned hemisphere after stroke may contribute to the difficulty in terminating a muscle contraction through suppressing the cortical excitability and intracortical inhibitory circuits in the lesioned hemisphere. However, our data from Study 2 did not show any significant association between the paretic FDS muscle relaxation time and the IHI in each hemisphere. We explored if IHI could help explain delayed paretic muscle relaxation time when combined with other predictors in regression analysis.

(2) Intracortical inhibitions

Stroke-affected brains exhibit impairments of the GABA-Aergic (SICI) and GABA-Bergic intracortical inhibition circuits (long interval intracortical inhibition, LICI and cortical

silent period). Stroke survivors show reduced SICI and LICI for the paretic first dorsal interosseous (FDI) muscle in acute and chronic stages [97, 98] and reduced SICI for the paretic extensor digitorum communis muscle (EDC) at rest compared to nonparetic muscles in chronic stage [99]. In the early stages of recovery (at 3 months post-stroke), low level of SICI and LICI for the paretic FDI muscle at rest is associated with poor gross and fine motor control in the paretic arm and hand (Action Research Arm Test and Nine-Hole Peg Test) [98]. In chronic stages of recovery, low SICI in the paretic EDC muscle continues to be correlated with poor motor function (Fugl Meyer Upper Extremity and Stroke impairment assessment set [99]). In addition to low SICI at rest, stroke survivors are unable to decrease SICI during initiation of FDI muscle contraction, in contrast to age-matched healthy controls [97], which may explain delayed grip muscle initiation after stroke. The GABA-Bergic intracortical inhibition, cortical silent period, is increased in acute stroke in the paretic abductor digiti minimi compared to the nonparetic and healthy control muscles [131] and FDI muscle at rest [132, 133]. In the chronic stages, shortened cortical silent periods are observed only in hands with spasticity [134]. Our own work (Chapter 4 Study 1) showed that grip muscle relaxation was not accompanied by changes in intracortical inhibitions (either SICI or CSP) in both neurologically-intact adults and stroke survivors. We explored if the overall level of SICI and/or CSP is associated with paretic grip relaxation times.

(3) Cortical excitability

Stroke results in reduced excitability of motor cortex as evidenced by reduced peak-to-peak amplitude of MEP. The MEP size in the hand muscles in the acute phase of stroke has prognostic value for motor recovery in the chronic phase [135]. Our work showed that

modulation of MEP amplitude (peak-to-peak and area) specific to muscle relaxation did not explain delayed paretic FDS muscle relaxation time after stroke, but the MEP peak-to-peak amplitude (for the nonconditioned MEP) was found to be lower for the paretic hand with prolonged relaxation time (Chapter 4, Study 1). In addition, MEP latency was longer for the paretic hand. Thus, we explored if the overall reduced corticomotor excitability may explain delayed grip relaxation time in the paretic hand.

(4) Spasticity

Spasticity is characterized by hyperactive stretch reflex and increased muscle tone. Factors influencing spasticity are summarized below as they may affect one's ability to relax their muscles.

Spinal motoneuron excitability (H reflex)

Spasticity may be influenced by the excitability of the stretch reflex arc, assessed by H reflex. Hyperexcitability of spinal motor neurons is a prominent aftereffect of stroke, mainly in the flexors in the upper limb and extensors in the lower limb [101, 102, 136-139]. Stroke survivors have increased excitability of the spinal motor circuits in the paretic compared to the nonparetic flexor carpi radialis (FCR) muscles at rest [102, 137-139]. The FCR H reflex measurements are shown to be reliable tools for measuring spinal excitability after stroke [102, 139]. In lower extremity, paretic Soleus H reflex is elevated at rest [138], during pedaling [140] and walking [141] compared to nonparetic muscles. Reduced modulation of paretic Soleus H reflex during phases of pedaling was correlated with motor impairment [140].

However, our own work showed that the FDS H reflex did not change for the grip relaxation task compared to the sustained contraction with matching background activity in both stroke survivors and age-matched healthy control adults (Chapter 4 Study 1). In addition, there were no hand-specific changes in the overall H reflex excitability. The purpose to include H reflex data in the regression analysis is to see if H reflex helps explain delayed paretic FDS muscle relaxation time when other neural mechanisms are also taken into consideration in a regression analysis.

Reticulospinal pathways

After stroke, the spinal motor neurons exhibit sustained firing even after synaptic input is reduced or removed [16, 142]. Stroke reduces the inhibitory control of the brain stem from the corticoreticular pathways, giving rise to a hyperactive brainstem and resultant low tonic serotonergic drive from the brainstem to spinal motor neurons through reticulospinal pathways [143]. This low tonic serotonergic drive keeps spinal motor neurons closer to threshold [144]. These membranous electrophysiological changes make spinal motor neurons easier to activate and difficult to deactivate, resulting in the sustained firing of spinal motor neurons even after synaptic inputs to the motor neurons has been removed or reduced. Shortening of paretic FDS muscle relaxation time upon administering serotonin antagonist supports the role of tonic serotonergic drive affecting spinal motor neurons and prolonging paretic FDS muscle relaxation times in stroke [62]. The low-level serotonergic drive may be associated with delayed grip relaxation in stroke survivors (Figure 32).

Presynaptic inhibition

Other factors affecting spinal motor neuron activity are are reduced presynaptic inhibition and reciprocal inhibition in the affected side [57, 136, 145]. Post-stroke reduced presynaptic inhibition has been argued to be a non-specific correlate of motor impairment as a result of corticospinal lesion or reorganization of spinal circuitry following stroke [138, 145], because (i) reduced presynaptic inhibition is not correlated with presence or degree of spasticity [138, 145] (ii) reduced presynaptic inhibition is observed on the unaffected side of stroke survivors as well [145], and (iii) reduced presynaptic inhibition is observed in other movement disorders such as writer's cramp [146] and Parkinson's disease [147]. In summary, literature suggests that reduced presynaptic inhibition is not a causative factor for spasticity and spinal reflex hyperexcitbility after stroke.

Reciprocal inhibition

Stroke survivors have lower reciprocal inhibition from the tibialis anterior (TA) to the Soleus muscle in the paretic lower limb [112-114]. This lower reciprocal inhibition from the antagonist muscles is linked to slower walking speed [114]. In the upper limb, reciprocal inhibition from the ECR to the FCR muscle is reduced in the paretic hand after stroke [116, 117]. Our work showed that the EDC antagonist muscle activity was not different in the paretic hand compared to nonparetic and age-matched control muscles during grip and relax (Chapter 4, Study 1). Yet, reduced reciprocal inhibition of the agonist muscles from the antagonist muscles may contribute to prolonged muscle relaxation time in stroke survivors.

To summarize spasticity's role in grip relaxation, spasticity is a complex phenomenon. Exact mechanisms of spasticity still remain unknown. We included the clinical measure of

spasticity (Modified Ashworth score, MAS) as well as measures most commonly used and relevant to spasticity (H reflex and co-contraction level) in this correlation/regression analysis.

(5) Muscles

Muscle biopsies suggest that the paretic vastus lateralis muscle has a greater proportion of fast-twitch muscle fibers compared to the nonparetic side and age-matched control subjects [148, 149]. Fast-twitch muscle fibers are associated with anaerobic metabolism and accumulate lactate and CO_2 as a byproduct of metabolism during contractions [149, 150]. Acidosis as a result of fatiguing exercise may slow down the muscle relaxation [151, 152]; however it does not explain slower muscle relaxation times in an *unfatigued state* in chronic stroke survivors, unless chronic stroke survivors' muscles are in a chronically fatigued state. To avoid acidosis induced delayed grip relaxation, we provided frequent rests during our experiments. Literature suggests that several muscular changes occur after stroke which may contribute to delayed muscle relaxation after stroke [149]. No data specific to muscles were collected in Studies 1-2, other than the maximum grip force. Thus we included maximum grip force measures in the correlation/regression analysis.

(6) Somatosensation

Stroke survivors suffer from reduced somatosensation on the paretic side [153, 154]. These sensations are deemed crucial in providing sensory feedback in gripping [4, 155]. It is possible that decreased sensory feedback affects temporal acuity of termination of muscle contraction.

In summary, stroke-related delayed paretic FDS muscle relaxation time may be affected by a myriad of neural factors. In this study, we first examined if relaxation time is a reliable measure in chronic stroke survivors by intraclass correlation. This study also explored if these neural factors are associated with delayed grip relaxation in chronic stroke survivors. Specifically, the factors of 1) abnormal interhemispheric inhibition modulation in the lesioned hemisphere, 2) the level of intracortical inhibition (GABA-Aergic SICI and GABA-Bergic cortical silent period), 3) cortical motor excitability, 4) abnormal H reflex modulation and reduced reciprocal inhibition, and 5) Clinical spasticity measure, 6) somatosensation in chronic stroke survivors, 7) reduced grip force, were explored in delayed grip relaxation in chronic stroke survivors.

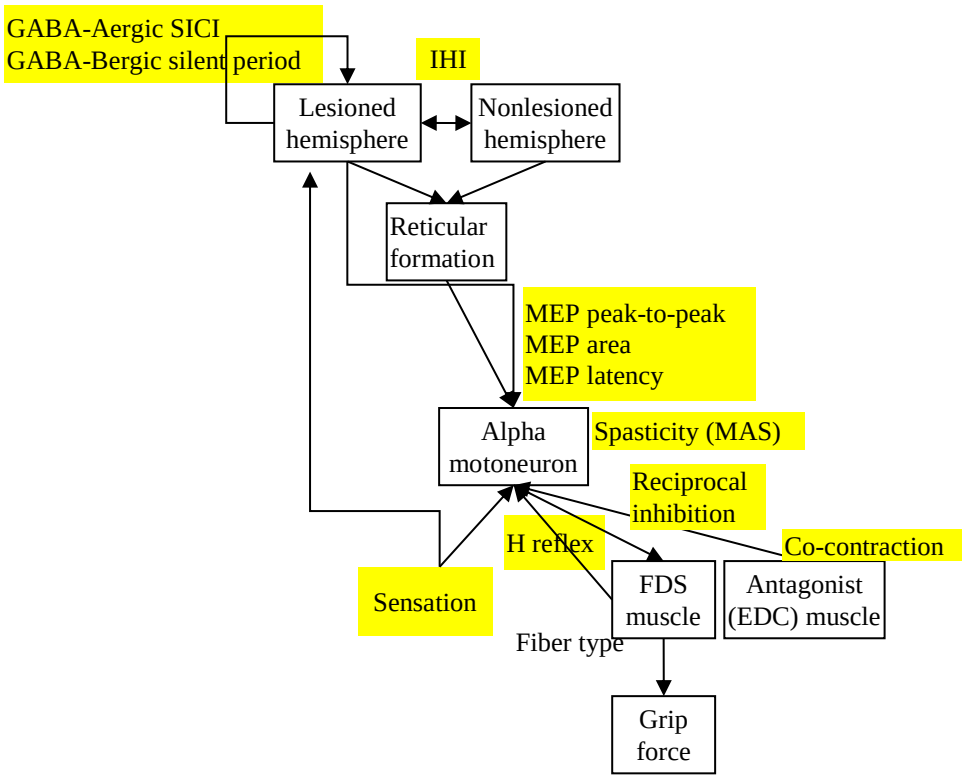


Figure 32: Schematic representation of potential neuromechanisms involved in delayed grip relaxation after stroke.

4.2 Method

4.2.1 Subjects

Data in Studies 1-2 were used in this analysis. No new subjects or data collection were involved.

4.2.2 Procedure

4.2.2.1 Muscle relaxation time reliability

Reliability of the FDS muscle relaxation time was measured using intraclass correlation coefficient (ICC). The relaxation time data was sorted by date, and ICC was examined for the relaxation time measured on day 1 vs. day 2. Two ICC value were of interest (one for paretic FDS muscle relaxation time from the grip-and-relax task in Study 1, and one for FDS relaxation time from the MCP flexion task in Study 2). Subjects who had completed at least 2 relaxation time measurements on separate days in Chapter 4 Study 1 and 2 were included. From Study 1, a total of 7 older adults (6 dominant hands, 5 nondominant hands, 3 subjects for both hands) were included. A total of 6 stroke survivors were included (6 nonparetic, 4 paretic hands, all 4 subjects in the paretic hand assessment were also part of the nonparetic hand relaxation time reliability assessment). From Study 2, total of 8 stroke survivors were included in the ICC analysis (8 paretic and 8 nonparetic hands).

4.2.2.2. Correlation and regression analysis

Correlation analysis was first performed to examine if paretic FDS muscle relaxation time (averaged across all studies) is associated with measures of spasticity (MAS transformed to

0-5 scale), motor function (Chedoke and Fugle-Meyer), paretic grip force, grip asymmetry (paretic/nonparetic grip force), sensory function (Monofilament score or mono, and 2-point discrimination score or 2pt), and neurophysiological variables as listed below. The co-contraction (FDS(%MVC)/EDC(%MVC) observed during 4-sec maximum paretic grip), H reflex, active motor threshold (AMT), test stimulation intensity (%MSO that evoked 1 mV MEP peak-to-peak amplitude), MEP peak-to-peak amplitude (MEPp2p), MEP area, MEP latency (of only nonconditioned MEP for the three MEP measures), SICI, and cortical silent period (CSP) of the paretic side (averaged across two tasks of paretic FDS muscle relaxation time and sustained contraction and timings) from Study 1 and interhemispheric inhibition (pre-intervention data only, IHIp for the lesioned hemisphere, IHInp for the nonlesioned hemisphere, averaged for sessions) from Study 2 were used.

Only the variables with R^2adj of more than 1% were included in the subsequent regression analysis. Since we did not have enough sample size with all the predictors present, therefore several combinations of predictors were run. The combinations with multicollinearity (variance inflation factor VIF of >5) or R^2adj of less than 80% were not further considered. The combinations that resulted in the highest R2adj with a significant overall regression ($p<0.05$) were sought.

4.3 Results

4.3.1 Muscle relaxation time reliability

Intraclass correlation coefficient (ICC) for all 4 hands from Study 1 was 0.65, which is medium. The cutoff point for ICC to be good is 0.7. The FDS muscle relaxation time for all 2

hand types from the Study 2 had intraclass correlation of 0.713, which is good. The paretic FDS muscle relaxation time from the Study 2 also had a good ICC of 0.780. Overall, medium-good reliability was observed for the muscle relaxation time measurement using FDS EMG.

4.3.2 Correlation analysis for paretic FDS muscle relaxation time

The results of correlation analysis between the paretic FDS muscle relaxation time and each of the examined predictors are shown in Table **7**. The MAS (Modified Ashworth Scale, grip weakness (paretic/nonparetic grip force), moniofilaments and two-point discrimination scores were significantly correlated with the paretic FDS muscle relaxation time independently (Figure 33). Coactivation (FDS/EDC activity) during grip, the average H reflex value, motor cortical excitability as measured by AMT, stimulation intensity to evoke 1 mV MEP in a resting muscle, nonconditioned MEP latency, average SICI, and IHI in the lesioned hemisphere individually explained <1% of variance in the paretic FDS muscle relaxation time (italicized in the Table **7**) and thus were not further considered in the following regression analysis.

Table 7. Correlation analysis results. Bold predictors significantly correlated with the paretic FDS muscle relaxation time p<.05. Predictors in italics had R^2adj<1%, suggesting no role in the paretic FDS muscle relaxation time. The rest of the predictors had R^2adj>1% but were not significantly correlated with the paretic FDS muscle relaxation time.

Predictor	n	R2-adj (%)	p-value	Coeff
MAS	**21**	**23.56**	**0.015**	**139.4**
Chedoke	21	2.50	0.234	
Fugl-Meyer	21	4.42	0.181	
Paretic grip force (N)	16	8.74	0.141	
Grip weakness (Paretic grip force/nonparetic grip force)	**15**	**21.19**	**0.048**	**-510**
Monofilament	**16**	**31.71**	**0.014**	**189.1**
2-point discrimination	**16**	**22.32**	**0.037**	**48.9**
Coactivation (FDS/EDC) during grip	*9*	*0*	*0.908*	
H reflex	7	11.68	0.238	
AMT (%MSO)	*17*	*0*	*0.875*	

Test stimulation intensity	*17*	*0*	*0.392*	
MEP latency (ms)	*18*	*0*	*0.430*	
MEP peak-to-peak amplitude (mV)	18	6.11	0.166	
MEP area (mV-s)	18	5.13	0.185	
SICI	*18*	*0*	*0.376*	
CSP	15	1.58	0.289	
IHIp	*10*	*0*	*0.965*	
IHInp	10	20.7	0.105	

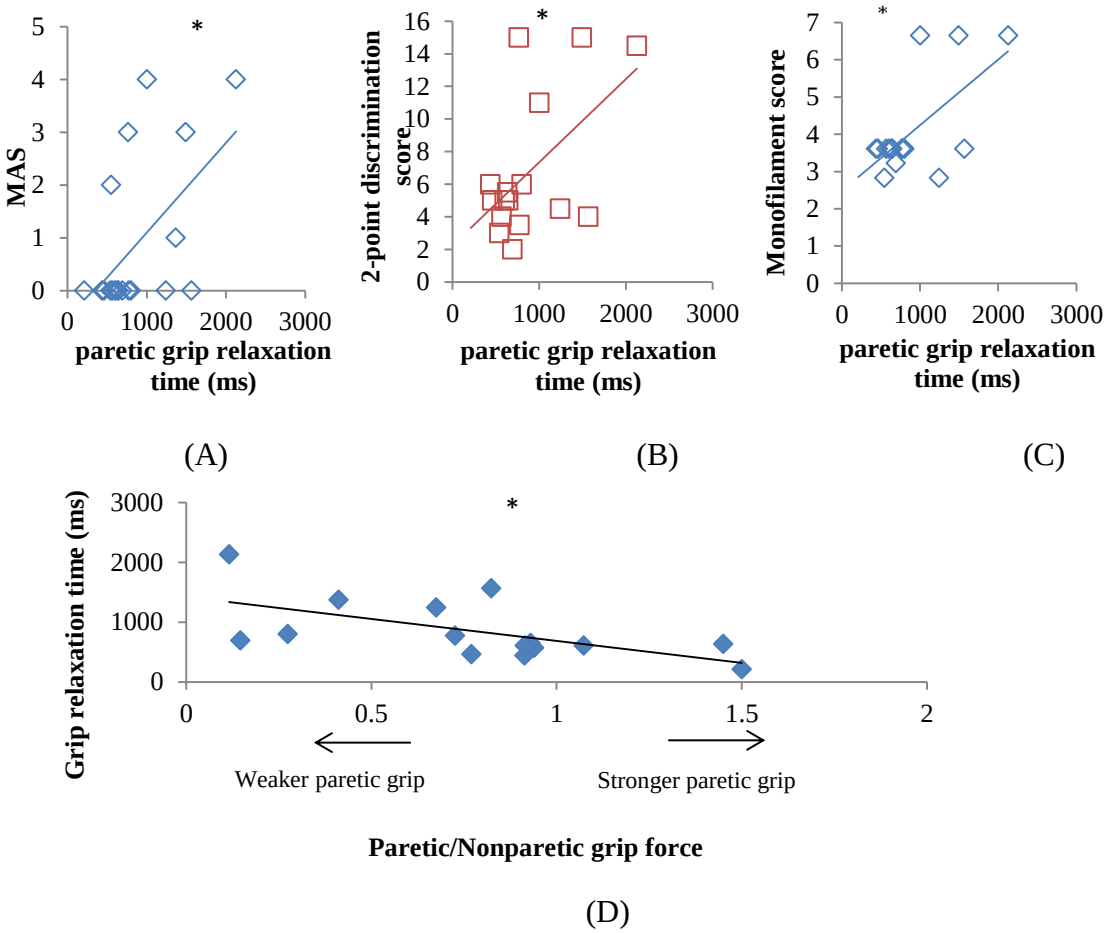


Figure 33: Significant correlations of spasticity, sensation and grip force asymmetry with paretic FDS muscle relaxation time. (A) Paretic FDS muscle relaxation time was positively correlated with MAS. (B) Paretic FDS muscle relaxation time significantly correlated with the 2-point discrimination score. (C) Paretic FDS muscle relaxation time showed significant correlations with the Monofilament scores. (D) Paretic FDS muscle relaxation time was negatively correlated with the grip force weakness (paretic/ nonparetic grip force ratio, the ratio of less than 1 indicating weaker paretic grip force, greater than 1 indicating stronger paretic grip force compared to the nonparetic).

4.3.3 Regression analysis for paretic FDS muscle relaxation time

The results of regression analysis between the paretic FDS muscle relaxation time and the examined predictors are shown in Table 8. The predictors that explained less than 1% of the variance of the paretic relaxation time by themselves (italicized in the correlation table (Table **7** above) were not included in the regression analysis. In Table 8, non-grayed models are of interest, because they showed R^2adj>80% and overall p<0.05. Light-grayed models are those with R^2adj<80% or overall p>0.05. Dark-grayed combinations of predictors represent those with VIF>10 or no result, and these models were deemed inadequate. For instance, since the monofilament scores and two point discrimination test scores were highly correlated, they could not be in a regression model together. Thus, sensation was represented in the regression model using the monofilament score, since the monofilament score predicted the paretic relaxation time better than the two point discrimination score in the correlation analysis (Table **7**). Likewise, MEP area and MEP peak-to-peak amplitude were correlated, and could not be in a regression model together.

The best combinations of the predictors that resulted in the highest R^2adj are yellow-highlighted. The best combinations suggest that 5 variables explain approximately 90% of the variability in the paretic FDS muscle relaxation time. These 5 variables were: spasticity (MAS), sensation (monofilament score), grip force imbalance (paretic maximum grip force/nonparetic maximum grip force), MEP peak-to-peak amplitude for test stimulation for the paretic FDS, and interhemispheric inhibition from the paretic to the nonparetic hemisphere. Unfortunately, regression with all 5 predictors could not be performed due to low sample size. The likely conclusion is that these 5 variables altogether predict the paretic hand's relaxation time.

Table 8. Regression analysis results. Most useful combinations to predict the paretic FDS muscle relaxation time are highlighted in yellow. Dark-grayed models were influenced by multicollinearity. Light-grayed models explained <80% of the variance in the paretic FDS muscle relaxation time and/or had nonsignificant models. Non-greyed models explained >80% of the variance in the paretic FDS muscle relaxation time with an overall significance. Among them, the two models that explained the most variance in the paretic relaxation time were found and highlighted in yellow.

Predictors	n	R2-adj,%	p-value (regression, predictor 1, predictor 2, …)	VIF	Coeff (Constant, predictor 1, predictor 2, …)
MAS, Chedoke, FM	21	19.39	0.086 (0.031, 0.556, 0.929)	<=5.68	
MAS, grip weakness	15	36.76	0.025 (0.063, 0.296)	1.33	
Mono, 2pt	16	27.40	0.049 (0.688, 0.183)	2.52	
MAS, mono, 2pt	16	21.35	0.123	<=10.47	
MAS, mono	16	27.01	0.051 (0.759, 0.301)	4.09	
MAS, 2pt	16	20.61	0.088 (0.418, 0.894)	6.36	
MAS, grip weakness, mono	12	27.83	0.161 (all >.05)	<=20.05	
Mono, grip weakness	12	18.43	0.162 (all>.05)	1.19	
MAS, mono, H	6	0.89	0.531 (all >0.05)	<=2.54	
MAS, MEPp2p	17	24.71	0.054 (0.039, 0.459)	1.08	
MAS, MEPp2p, CSP	14	56.20	0.010 (0.005, 0.625, 0.606)	<=1.23	
MAS, grip weakness, MEPp2p, CSP,	13	68.35	0.008 (all >0.05)	<=1.98	
MAS, MEPp2p, MEParea, CSP	14	70.73	0.003 (0.001, 0.048, 0.037, 0.659)	<=29.67	
Mono, 2pt, MEPpeak-to-peak, CSP	11	30.04	0.203 (all >0.05)	<=19.80	
Mono, MEPp2p, CSP	11	33.10	0.712 (all >0.05)	<=1.28	
MAS, mono, MEPpeak-to-peak, CSP	11	64.02	0.034 (0.038, rest >0.05)	<=11.31	
MAS, IHInp	10	84.38	0.001 (0.001, 0.359)	1.27	
Mono, IHInp	6	89.51	0.016 (0.014, 0.907)	1.65	
2pt, IHInp	6	73.19	0.065 (0.062, 0.521)	1.39	
Mono, grip weakness, IHInp	6	91.77	0.049 (0.028, 0.309, 0.446)	<=3.97	(355.2,-284,-4.12)
MAS, mono, IHInp	6	93.63	0.038 (all >0.05)	<=75.94	
Mono, 2pt, IHInp	6	95.88	0.025 (0.053,0.141,0.451)	<=31.36	
MAS, MEPp2p,	8	88.28	0.082 (all >0.05)	<=237.32	

MEParea, CSP, IHInp					
MAS, MEPp2p, IHInp	9	88.18	0.003(0.002, 0.555, 0.464)	<=1.40	276.1, -39.9, 1.70
MAS, MEPp2p, CSP, IHInp	8	85.76	0.036(0.026, 0.968, 0.492, 0.507)	<=1.92	
MAS, grip weakness, MEPp2p, IHInp	9	87.71	0.011 (0.007, rest >0.05)	<=1.86	
MAS, grip weakness, MEPp2p, CSP, IHInp	8	87.02	0.090 (all>0.05)	<=5.36	
Mono, MEPp2p, CSP, IHInp	5	N/A	N/A	<=10.80	
MAS, grip weakness, MEP2p2, CSP, IHInp	8	87.02	0.090 (all >.05)	<=5.36	
Mono, grip weakness, MEPp2p, CSP	10	44.32	0.145 (all>.05)	<=1.79	
Mono, grip weakness, MEPp2p, IHInp	6	84.84	0.259 (all>.05)	<=10.94	
2pt, MEPp2p, IHInp	6	76.44	0.138 (all>0.05)	<=1.64	
2pt, grip weakness, MEPp2p, IHInp	6	67.43	0.422 (all>0.05)	<=12.67	
MAS, Mono, grip weakness, MEPp2p, IHInp	5	N/A	N/A	<=3901	

4.3 Discussion

Correlation analysis showed that the paretic FDS muscle relaxation time was associated with spasticity in the finger flexor muscles and impaired sensation in the hand, as well as grip force asymmetry. These variables by themselves explain only 21-32% of the variance in the paretic muscle relaxation time. However, when added together with neural factors of MEP peak-to-peak amplitude and IHI, these variables explained approximately 90% of the variance in the paretic FDS muscle relaxation time. Thus, these 5 behavioral and neural measurements appear to be associated with the paretic FDS muscle relaxation time (Figure 34).

Spasticity and relaxation time association was observed previously in lower limb of children with cerebral palsy [156]. Spasticity is associated with hyperactive spinal stretch reflex

[115, 157, 158]. The excitability of the spinal stretch reflex is mediated by excitability of the muscle spindles, afferent fibers, intraspinal networks affecting the alpha motor neuron and supraspinal control [34, 115, 157, 158]. Muscle spindle activation is comparable between paretic muscles after stroke and healthy controls at rest and during contraction [159, 160]. H reflex is conventionally used to examine the excitability of the spinal stretch reflex arc [34, 35, 161]. However, H reflex modulation and mean H reflex was comparable between paretic, nonparetic and control FDS muscle in the present study. Additionally, H reflex had a R^2adj value of less than 1%, suggesting that the FDS H reflex is not associated with paretic FDS muscle relaxation time. Another abnormal neural mechanism linked with stroke-related hyperactive alpha motor neurons is abnormal reciprocal facilitation. After stroke, the reciprocal inhibition decreases from the TA to the soleus muscle [112-114] in the lower limb, and from the ECR to the FCR in the upper limb [116, 117]. Our study did not directly assess reciprocal inhibition, but our study showed that the EDC muscle activity during grip relaxation was not different for the stroke survivors compared to age-matched controls. Not only that, the FDS/EDC coactivation ratio had a R^2adj value of less than 1%, suggesting no association between coactivation and muscle relaxation time in our subjects. Our investigation of spinal mechanisms of grip relaxation suggests that the spinal reflex arc and co-contraction control may not be significantly linked to the delayed paretic muscle relaxation time in stroke survivors.

Previous research points towards hyperactivity of the reticulospinal tract as a major mechanism for spasticity [16, 115, 142, 157, 158]. The reticuslospinal tract is partially under control of higher centers via corticoreticular pathway. Stroke lesion may affect the corticoreticular pathway due to its proximity to the pyramidal fibers in the corona radiata and internal capsule [115, 162]. Lesion of the corticoreticular tract decreases the inhibitory effects of

the dorsal (or medullary) reticulospinal tract onto the alpha motor neuron [115, 162]. Additionally, ventral (or ponticular) reticulospinal tract does not receive cortical input, and has a excitatory effect on the alpha motor neuron [115, 162]. Reticulospinal tracts are known to exert excitatory effect on the flexor muscles and inhibitory effect on the extensor muscles in the upper limb [16, 163], which coincides with clinical presentation of the paretic upper limb post stroke. Primate research shows that the reticulospinal connections for the flexor muscles in the upper limb strengthen following stroke [143], hence spasticity is observed in the upper limb flexors. The hyperactivity of the reticulospinal pathway creates a low level serotoneric drive on the spinal motor neurons [164]. This tonic drive may result in sustained discharge of the spinal motor neurons even after synaptic input decreases or ends [16, 144, 164, 165]. This drive may also create spontaneous discharge after stroke [164]. Such changes in the spinal motor neuron activation profile as a result of reticulospinal hyperactivity may lengthen the paretic muscle relaxation time (Figure 34). Supporting evidence comes from a previous study that showed shortening of the muscle relaxation time after administering serotonin antagonist in stroke survivors [23].

Delayed paretic FDS muscle relaxation time was associated with reduced tactile sensation (Figure 34). Sensation from the skin and peripheral sensory system is crucial for hand function as it provides sensory feedback to perform a task correctly and efficiently. In particular, the role of sensation has been described as important for maintaining and modulating grip force before [166, 167]. Given the time scale of the muscle relaxation time (a few hundred ms), it is plausible that people utilize somatosensation to inform the progress of muscle relaxation and use that sensory feedback to facilitate timely muscle relaxation. And impairment in such sensory

feedback after stroke may prolong the muscle relaxation process. This is the first study to identify the role of sensation in relaxing from a maximum grip.

Stronger paretic grip force in relation to the nonparetic grip force was associated with shorter paretic FDS muscle relaxation times per correlation analysis. Greater paretic/ nonparetic grip force ratio has been indicated as a biomarker for good motor recovery after stroke [168].

In addition to the clinical measures of spasticity and sensation, the paretic FDS muscle relaxation time was further explained by addition of corticomotor excitability (MEP peak to peak amplitude) and interhemispheric inhibition, which showed promise in predicting the paretic FDS muscle relaxation time in the regression analysis. Lower corticospinal excitability was associated with longer paretic muscle relaxation times. The direction of the interhemispheric inhibition was unclear in this study. One regression model (with sensation, grip weakness and IHInp) showed a negative trend of interhemispheric inhibition with the paretic muscle relaxation time, while the other regression model (with spasticity, MEPp2p, and IHInp) showed a positive correlation of the interhemispheric inhibition with the paretic FDS muscle relaxation time.

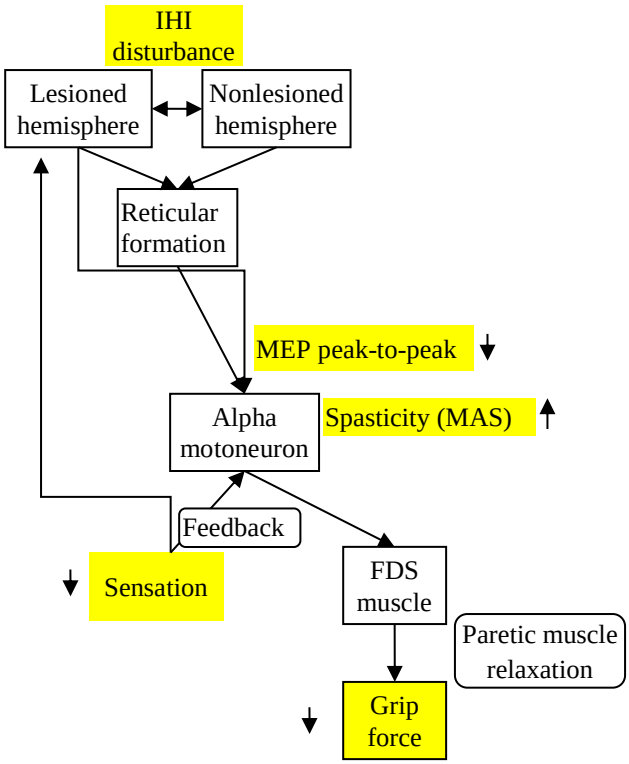


Figure 34: The interhemispheric inhibition, M1 excitability, spasticity, sensation and grip asymmetry may predict the paretic FDS muscle relaxation time.

4.4 Conclusion

The corticospinal excitability and the interhemispheric inhibition did not explain the paretic muscle relaxation time on their own in correlation analysis, but when combined with the clinical measures of spasticity and somatosensation and the behavioral measure of grip force weakness, explained almost 90% of the variance in the paretic FDS muscle relaxation time. To summarize, the correlation and regression analysis showed that the delayed paretic FDS muscle relaxation time may depend on a combination of interhemispheric inhibition, lower corticospinal excitability, reduced sensation, spasticity, and motor weakness in the paretic hand.

Chapter 5. Dissertation summary

Grip relaxation is an important function for activities of daily living. This dissertation developed a novel protocol to examine the role of various neurophysiologic measures in grip relaxation time, while controlling for the confounding effects of background muscle activation.

One of the main finding of this dissertation is that the grip relaxation time is a cortically mediated process in healthy young adults. A series of studies testing SICI through a span of contraction and relaxation phases in Chapter 2 showed that healthy young adults increased SICI in order to relax their muscles from a contraction. Investigation of H reflex showed that the H reflex excitability remained unchanged for grip relaxation, suggesting that the spinal motor excitability did not play a significant role in timely grip relaxation in healthy young adults. A study involving healthy young and older subjects in Chapter 3 showed that the older adults had a lack of modulation of SICI for grip relaxation. This lack of SICI modulation appears to explain older adults' longer grip relaxation times.

Relaxation time is further delayed in chronic stroke survivors. Investigation of neural mechanisms of grip relaxation in chronic stroke survivors in Chapter 4 revealed that intracortical inhibitions, mediated by GABA-A (SICI) and GABA-B (cortical silent period), did not explain the delayed grip relaxation in stroke survivors. Spinal motor excitability as measured by H reflex also did not explain the delays in grip relaxation after stroke. There was no apparent evidence suggesting the role of abnormal antagonist muscle activity interfering with the grip relaxation time in stroke survivors, either. This dissertation also tested the efficacy of a mirror symmetric Active Passive Bilateral Therapy, APBT, on IHI and muscle relaxation time. A single session of the APBT tested on 8 stroke survivors was not found to affect IHI and muscle relaxation time,

warranting a longer intervention period as in previous longitudinal studies with a larger sample size in future research.

Correlation and regression analyses showed that the longer grip relaxation time was significantly associated with increased spasticity, poor somatosensation, and grip weakness. Addition of neurophysiologic measures of the strength of the corticospinal connections and interhemispheric inhibition to the regression model enhanced the model's ability to explain approximately 90% of the variance in delayed muscle relaxation in the paretic grip muscle, although these neurophysiologic measures by themselves were not significantly correlated with the paretic muscle relaxation time.

In summary, this dissertation introduced a novel protocol to examine the neural mechanisms of grip relaxation. This dissertation showed that the grip relaxation time is an active cortical process mediated by GABA-Aergic intracortical inhibition (SICI) in healthy young adults. Reduced modulation of SICI may explain why grip relaxation takes longer in older adults. However, the same approach did not yield strong evidence of neurophysiologic correlates for delayed muscle relaxation in chronic stroke survivors. Instead, correlation and regression analysis suggests that post-stroke delayed grip relaxation may be associated with spasticity, reduced somatosensation, grip weakness, strength of the corticospinal connections, and interhemispheric inhibition.

Limitations

Limitations of this research are as follows. In Chapter 4, despite the sample size of 26, our stroke survivors were relatively high functioning based on clinical assessment scores. It is

because only stroke survivors who showed MEP in response to TMS were eligible to participate in the studies and they tend to be higher functioning than those who do not show MEP. This constraint limits the generalizability of our data to only high functioning chronic stroke survivors. In addition, there is a wide heterogeneity among chronic stroke survivors with different lesion locations and clinical symptoms. This research lumped all chronic stroke survivors in a single group and was limited in the effort to categorize stroke survivors based on their lesion or clinical characteristics. Although this dissertation was the first-ever attempt to tease out the neuromechanisms for grip relaxation at the cortical and spinal levels in healthy adults and in stroke survivors, other potential mechanisms remained unstudied. For instance, neuromechanisms at the brainstem level were not studied largely due to limitations in available tools. Persistent inward current has been proposed as a factor in spasticity via sustained motor neuron firing even after the synaptic input stops [142, 164] and may be relevant to delayed muscle relaxation post stroke, but was not examined within this dissertation.

Future directions

Future research may utilize the new knowledge obtained in this dissertation toward better understanding of muscle relaxation and development of interventions to improve hand function among older adults and stroke survivors. For example, future research may investigate all proposed potential neuromechanisms simultaneously to explain stroke-related delayed grip relaxation. The proposed neuromechanisms include spasticity, sensation, muscle weakness, cortical excitability and IHI. In addition, future research may involve a larger sample of stroke survivors with a wider distribution of hand function and categorize them according to the lesion

characteristics, as different neuromechanisms may contribute to delayed muscle relaxation more strongly than others depending on their lesion characteristics. Ultimately, new knowledge may serve as the foundation to develop interventions to improve hand motor function among older adults and to help motor recovery and facilitate muscle relaxation in chronic stroke survivors in the future.

References

1. Kutz, D.F., et al., *Detection of changes in grip forces on a sliding object.* Journal of Neuroscience Methods, 2007. **166**(2): p. 250-258.
2. Moerchen, V.A., J.C. Lazarus, and K.G. Gruben, *Task-dependent organization of pinch grip forces.* Exp Brain Res, 2007. **180**(2): p. 367-76.
3. Kimura, T. and H. Gomi, *Temporal development of anticipatory reflex modulation to dynamical interactions during arm movement.* J Neurophysiol, 2009. **102**(4): p. 2220-31.
4. Nowak, D.A., J. Hermsdorfer, and H. Topka, *Deficits of predictive grip force control during object manipulation in acute stroke.* J Neurol, 2003. **250**(7): p. 850-60.
5. Nowak, D.A., et al., *Dexterity is impaired at both hands following unilateral subcortical middle cerebral artery stroke.* Eur J Neurosci, 2007. **25**(10): p. 3173-84.
6. Dimitrov, B., *Brain potentials related to the beginning and to the termination of voluntary flexion and extension in man.* International Journal of Psychophysiology, 1985. **3**(1): p. 13-22.
7. Terada, K., et al., *Movement-related cortical potentials associated with voluntary muscle relaxation.* Electroencephalogr Clin Neurophysiol, 1995. **95**(5): p. 335-45.
8. Rothwell, J.C., K. Higuchi, and J.A. Obeso, *The offset cortical potential: an electrical correlate of movement inhibition in man.* Mov Disord, 1998. **13**(2): p. 330-5.
9. Toma, K., et al., *Activities of the primary and supplementary motor areas increase in preparation and execution of voluntary muscle relaxation: an event-related fMRI study.* J Neurosci, 1999. **19**(9): p. 3527-34.
10. Pope, P.A., et al., *Cortical control of muscle relaxation: a lateralized readiness potential (LRP) investigation.* Clin Neurophysiol, 2007. **118**(5): p. 1044-52.
11. Schieppati, M. and P. Crenna, *Excitability of reciprocal and recurrent inhibitory pathways after voluntary muscle relaxation in man.* Exp Brain Res, 1985. **59**(2): p. 249-56.
12. Schieppati, M. and P. Crenna, *From activity to rest: gating of excitatory autogenetic afferences from the relaxing muscle in man.* Exp Brain Res, 1984. **56**(3): p. 448-57.
13. Schieppati, M., A. Nardone, and M. Musazzi, *Modulation of the Hoffmann reflex by rapid muscle contraction or release.* Hum Neurobiol, 1986. **5**(1): p. 59-66.
14. Begum, T., et al., *Cortical mechanisms of unilateral voluntary motor inhibition in humans.* Neurosci Res, 2005. **53**(4): p. 428-35.
15. Buccolieri, A., G. Abbruzzese, and J.C. Rothwell, *Relaxation from a voluntary contraction is preceded by increased excitability of motor cortical inhibitory circuits.* J Physiol, 2004. **558**(Pt 2): p. 685-95.
16. Kamper, D.G., et al., *Relative contributions of neural mechanisms versus muscle mechanics in promoting finger extension deficits following stroke.* Muscle Nerve, 2003. **28**(3): p. 309-18.
17. Ortu, E., et al., *Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex.* J Physiol, 2008. **586**(Pt 21): p. 5147-59.
18. Fozard, J.L., et al., *Age differences and changes in reaction time: the Baltimore Longitudinal Study of Aging.* J Gerontol, 1994. **49**(4): p. P179-89.
19. Der, G. and I.J. Deary, *Age and sex differences in reaction time in adulthood: results from the United Kingdom Health and Lifestyle Survey.* Psychol Aging, 2006. **21**(1): p. 62-73.

20. Wolkorte, R., J. Kamphuis, and I. Zijdewind, *Increased reaction times and reduced response preparation already starts at middle age.* Front Aging Neurosci, 2014. **6**: p. 79.
21. Langan, J., et al., *Functional implications of age differences in motor system connectivity.* Front Syst Neurosci, 2010. **4**: p. 17.
22. van de Laar, M.C., et al., *Lifespan changes in motor activation and inhibition during choice reactions: a Laplacian ERP study.* Biol Psychol, 2012. **89**(2): p. 323-34.
23. Seo, N.J., W.Z. Rymer, and D.G. Kamper, *Delays in grip initiation and termination in persons with stroke: effects of arm support and active muscle stretch exercise.* J Neurophysiol, 2009. **101**(6): p. 3108-15.
24. Chae, J., et al., *Delay in initiation and termination of muscle contraction, motor impairment, and physical disability in upper limb hemiparesis.* Muscle Nerve, 2002. **25**(4): p. 568-75.
25. Grasso, M., L. Mazzini, and M. Schieppati, *Muscle relaxation in Parkinson's disease: a reaction time study.* Mov Disord, 1996. **11**(4): p. 411-20.
26. Buccolieri, A., et al., *Muscle relaxation is impaired in dystonia: a reaction time study.* Mov Disord, 2004. **19**(6): p. 681-7.
27. Yazawa, S., et al., *Abnormal cortical processing of voluntary muscle relaxation in patients with focal hand dystonia studied by movement-related potentials.* Brain, 1999. **122 (Pt 7)**: p. 1357-66.
28. Roger, V.L., et al., *Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. 188-97.
29. Wade, D.T., et al., *The hemiplegic arm after stroke: measurement and recovery.* J Neurol Neurosurg Psychiatry, 1983. **46**(6): p. 521-4.
30. Bhakta, B.B., et al., *Use of botulinum toxin in stroke patients with severe upper limb spasticity.* J Neurol Neurosurg Psychiatry, 1996. **61**(1): p. 30-5.
31. Di Lazzaro, V., et al., *Intracortical origin of the short latency facilitation produced by pairs of threshold magnetic stimuli applied to human motor cortex.* Exp Brain Res, 1999. **129**(4): p. 494-9.
32. Peurala, S.H., et al., *Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF).* Clin Neurophysiol, 2008. **119**(10): p. 2291-7.
33. Kujirai, T., et al., *Corticocortical inhibition in human motor cortex.* J Physiol, 1993. **471**: p. 501-19.
34. Knikou, M., *The H-reflex as a probe: pathways and pitfalls.* J Neurosci Methods, 2008. **171**(1): p. 1-12.
35. Palmieri, R.M., C.D. Ingersoll, and M.A. Hoffman, *The hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research.* J Athl Train, 2004. **39**(3): p. 268-77.
36. De Luca, C.J., M.A. Sabbahi, and S.H. Roy, *Median frequency of the myoelectric signal. Effects of hand dominance.* Eur J Appl Physiol Occup Physiol, 1986. **55**(5): p. 457-64.
37. Adam, A., C.J.D. Luca, and Z. Erim, *Hand Dominance and Motor Unit Firing Behavior*. Vol. 80. 1998. 1373-1382.
38. Tanaka, M., M.J. McDonagh, and C.T. Davies, *A comparison of the mechanical properties of the first dorsal interosseous in the dominant and non-dominant hand.* Eur J Appl Physiol Occup Physiol, 1984. **53**(1): p. 17-20.

39. Tan, U., *The H-reflex recovery curve from the wrist flexors: lateralization of motoneuronal excitability in relation to handedness in normal subjects.* Int J Neurosci, 1989. **48**(3-4): p. 271-84.
40. Yakovlev, P.I. and P. Rakic, *Patterns of decussation of bulbar pyramids and distribution of pyramidal tracts on two sides of the spinal cord.* Trans. Am. Neurol. Assoc., 1966. **91**: p. 366-367.
41. Toga, A.W. and P.M. Thompson, *Mapping brain asymmetry.* Nat Rev Neurosci, 2003. **4**(1): p. 37-48.
42. Alvarez-Diaz, P., et al., *Comparison of tensiomyographic neuromuscular characteristics between muscles of the dominant and non-dominant lower extremity in male soccer players.* Knee Surg Sports Traumatol Arthrosc, 2014.
43. Kaufmann, R.A., et al., *Biomechanical analysis of flexor digitorum profundus and superficialis in grip-strength generation.* Am J Orthop (Belle Mead NJ), 2007. **36**(9): p. E128-32.
44. Long, C., 2nd, et al., *Intrinsic-extrinsic muscle control of the hand in power grip and precision handling. An electromyographic study.* J Bone Joint Surg Am, 1970. **52**(5): p. 853-67.
45. Oldfield, R.C., *The assessment and analysis of handedness: the Edinburgh inventory.* Neuropsychologia, 1971. **9**(1): p. 97-113.
46. Basmajian, J.V., *Biofeedback: Principles and Practice for Clinicians*. 3rd ed. 1989, Baltimore: Williams & Wilkins.
47. Coxon, J.P., C.M. Stinear, and W.D. Byblow, *Intracortical inhibition during volitional inhibition of prepared action.* J Neurophysiol, 2006. **95**(6): p. 3371-83.
48. Rossini, P.M., et al., *Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee.* Electroencephalogr Clin Neurophysiol, 1994. **91**(2): p. 79-92.
49. De Luca, C.J., et al., *Behaviour of human motor units in different muscles during linearly varying contractions.* J Physiol, 1982. **329**: p. 113-28.
50. Nielsen, J. and Y. Kagamihara, *The regulation of disynaptic reciprocal Ia inhibition during co-contraction of antagonistic muscles in man.* The Journal of Physiology, 1992. **456**: p. 373-391.
51. Flament, D., et al., *Task dependence of responses in first dorsal interosseous muscle to magnetic brain stimulation in man.* J Physiol, 1993. **464**: p. 361-78.
52. Garry, M.I. and R.H. Thomson, *The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states.* Exp Brain Res, 2009. **193**(2): p. 267-74.
53. Ngomo, S., et al., *Comparison of transcranial magnetic stimulation measures obtained at rest and under active conditions and their reliability.* J Neurosci Methods, 2012. **205**(1): p. 65-71.
54. Furubayashi, T., et al., *The human hand motor area is transiently suppressed by an unexpected auditory stimulus.* Clin Neurophysiol, 2000. **111**(1): p. 178-83.
55. Schieppati, M., C. Trompetto, and G. Abbruzzese, *Selective facilitation of responses to cortical stimulation of proximal and distal arm muscles by precision tasks in man.* Journal of Physiology, 1996. **491**(2): p. 11.

56. Christova, M., et al., *Dependence of intracortical inhibition and facilitation on the level of CO-activity of antagonist muscles.* Comptes Rendus de l'Academie Bulgare des Sciences, 2003. **56**(9): p. 77.
57. Pierrot-Deseilligny, E. and D. Burke, *The Circuitry of the Human Spinal Cord: Spinal and Corticospinal Mechanisms of Movement*. 2012: Cambridge University Press.
58. Kojima, S., et al., *Modulation of the cortical silent period elicited by single- and paired-pulse transcranial magnetic stimulation.* BMC Neurosci, 2013. **14**: p. 43.
59. Schieppati, M., M. Poloni, and A. Nardone, *Voluntary muscle release is not accompanied by H-reflex inhibition in patients with upper moto neuron lesions.* Neurosci Lett, 1985. **61**(1-2): p. 177-81.
60. de Noordhout, A.M., et al., *Corticomotoneuronal synaptic connections in normal man: an electrophysiological study.* Brain, 1999. **122 (Pt 7)**: p. 1327-40.
61. Brouwer, B. and P. Ashby, *Corticospinal projections to upper and lower limb spinal motoneurons in man.* Electroencephalogr Clin Neurophysiol, 1990. **76**(6): p. 509-19.
62. Seo, N.J., et al., *Effect of a serotonin antagonist on delay in grip muscle relaxation for persons with chronic hemiparetic stroke.* Clin Neurophysiol, 2009. **122**(4): p. 796-802.
63. Lexell, J., *Human aging, muscle mass, and fiber type composition.* J Gerontol A Biol Sci Med Sci, 1995. **50 Spec No**: p. 11-6.
64. Ohlendieck, K., *Proteomic Profiling of Fast-To-Slow Muscle Transitions during Aging.* Front Physiol, 2011. **2**: p. 105.
65. Spraker, M.B., D.M. Corcos, and D.E. Vaillancourt, *Cortical and subcortical mechanisms for precisely controlled force generation and force relaxation.* Cereb Cortex, 2009. **19**(11): p. 2640-50.
66. Motawar, B., et al., *Contribution of intracortical inhibition in voluntary muscle relaxation.* Exp Brain Res, 2012. **221**(3): p. 299-308.
67. Oliviero, A., et al., *Effects of aging on motor cortex excitability.* Neuroscience Research, 2006. **55**(1): p. 74-77.
68. Marneweck, M., A. Loftus, and G. Hammond, *Short-interval intracortical inhibition and manual dexterity in healthy aging.* Neurosci Res, 2011. **70**(4): p. 408-14.
69. Heise, K.F., et al., *The aging motor system as a model for plastic changes of GABA-mediated intracortical inhibition and their behavioral relevance.* J Neurosci, 2013. **33**(21): p. 9039-49.
70. Papegaaij, S., et al., *Aging causes a reorganization of cortical and spinal control of posture.* Front Aging Neurosci, 2014. **6**: p. 28.
71. Fujiyama, H., et al., *Age-related differences in corticospinal excitability and inhibition during coordination of upper and lower limbs.* Neurobiol Aging, 2012. **33**(7): p. 1484 e1-14.
72. Peinemann, A., et al., *Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex.* Neurosci Lett, 2001. **313**(1-2): p. 33-6.
73. Raffalt, P.C., T. Alkjaer, and E.B. Simonsen, *Changes in soleus H-reflex during walking in middle-aged healthy subjects.* Muscle Nerve, 2014.
74. Rossi, S., et al., *Screening questionnaire before TMS: An update.* Clinical Neurophysiology, 2011. **122**(8): p. 1686.
75. van Kuijk, A.A., et al., *Definition dependent properties of the cortical silent period in upper-extremity muscles, a methodological study.* Journal of NeuroEngineering and Rehabilitation, 2014. **11**(1): p. 1-9.

76. Groppa, S., et al., *A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee.* Clin Neurophysiol, 2012. **123**(5): p. 858-82.
77. Sanger, T.D., R.R. Garg, and R. Chen, *Interactions between two different inhibitory systems in the human motor cortex.* The Journal of Physiology, 2001. **530**(2): p. 307-317.
78. Scaglioni, G., et al., *Plantar flexor activation capacity and H reflex in older adults: adaptations to strength training.* J Appl Physiol (1985), 2002. **92**(6): p. 2292-302.
79. Smith, C.D., et al., *Critical decline in fine motor hand movements in human aging.* Neurology, 1999. **53**(7): p. 1458-61.
80. Sherwood, C.C., et al., *Aging of the cerebral cortex differs between humans and chimpanzees.* Proceedings of the National Academy of Sciences, 2011. **108**(32): p. 13029-13034.
81. Sullivan, E.V. and A. Pfefferbaum, *Diffusion tensor imaging and aging.* Neuroscience & Biobehavioral Reviews, 2006. **30**(6): p. 749-761.
82. McGinnis, S.M., et al., *Age-related changes in the thickness of cortical zones in humans.* Brain Topogr, 2011. **24**(3-4): p. 279-91.
83. Nakamura, S., et al., *Age-related changes of pyramidal cell basal dendrites in layers III and V of human motor cortex: A quantitative Golgi study.* Acta Neuropathologica, 1985. **65**(3-4): p. 281-284.
84. Wong, T., *Aging of the Cerebral Cortex.* McGill Journal of Medicine, 2002. **6**(2): p. 104-113.
85. Di Lazzaro, V., et al., *GABAA receptor subtype specific enhancement of inhibition in human motor cortex.* J Physiol, 2006. **575**(Pt 3): p. 721-6.
86. Di Lazzaro, V., et al., *Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans.* J Physiol, 2005. **564**(Pt 2): p. 661-8.
87. Ziemann, U., et al., *Pharmacological control of facilitatory I-wave interaction in the human motor cortex. A paired transcranial magnetic stimulation study.* Electroencephalogr Clin Neurophysiol, 1998. **109**(4): p. 321-30.
88. Turgeon, S.M. and R.L. Albin, *GABAB binding sites in early adult and aging rat brain.* Neurobiol Aging, 1994. **15**(6): p. 705-11.
89. Stagg, C.J., et al., *Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy.* J Neurophysiol, 2009. **101**(6): p. 2872-7.
90. Stetkarova, I. and M. Kofler, *Differential effect of baclofen on cortical and spinal inhibitory circuits.* Clin Neurophysiol, 2013. **124**(2): p. 339-45.
91. Boros, K., et al., *Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans.* Eur J Neurosci, 2008. **27**(5): p. 1292-300.
92. Arduin, P.J., et al., *"Master" neurons induced by operant conditioning in rat motor cortex during a brain-machine interface task.* J Neurosci, 2013. **33**(19): p. 8308-20.
93. Wolf, S.L. and R.L. Segal, *Conditioning of the spinal stretch reflex: implications for rehabilitation.* Phys Ther, 1990. **70**(10): p. 652-6.
94. Tenteromano, L., et al. *Operant conditioning of the ankle dorsiflexor motor evoked potential (MEP) in people with and without CNS damage: Changes MEP size and silent period*. in *Society for Neuroscience 42ndAnnual Meeting,* . 2012. New Orleans, LA.
95. Misgeld, U., M. Bijak, and W. Jarolimek, *A physiological role for GABAB receptors and the effects of baclofen in the mammalian central nervous system.* Prog Neurobiol, 1995. **46**(4): p. 423-62.

96. Motawar B, S.N. *Role of inhibitory motor cortical pathways during voluntary grip relaxation in chronic stroke survivors*. in *Society for Neuroscience*. 2012. New Orleans.
97. Hummel, F.C., et al., *Deficient intracortical inhibition (SICI) during movement preparation after chronic stroke.* Neurology, 2009. **72**(20): p. 1766-72.
98. Swayne, O.B., et al., *Stages of motor output reorganization after hemispheric stroke suggested by longitudinal studies of cortical physiology.* Cereb Cortex, 2008. **18**(8): p. 1909-22.
99. Honaga, K., et al., *State of intracortical inhibitory interneuron activity in patients with chronic stroke.* Clin Neurophysiol, 2012.
100. Fujiwara, T., et al., *Modulation of cortical and spinal inhibition with functional recovery of upper extremity motor function among patients with chronic stroke.* Restor Neurol Neurosci, 2015.
101. Krakauer, J.W., *Arm function after stroke: from physiology to recovery.* Semin Neurol, 2005. **25**(4): p. 384-95.
102. Phadke, C.P., et al., *Upper-extremity H-reflex measurement post-stroke: reliability and inter-limb differences.* Clin Neurophysiol, 2012. **123**(8): p. 1606-15.
103. Murase, N., et al., *Influence of interhemispheric interactions on motor function in chronic stroke.* Ann Neurol, 2004. **55**(3): p. 400-9.
104. Daskalakis, Z.J., et al., *The mechanisms of interhemispheric inhibition in the human motor cortex.* J Physiol, 2002. **543**(Pt 1): p. 317-26.
105. Motawar, B. and N.J. Seo. *Aging-related changes in neural function for grip relaxation: intracortical inhibition and spinal motoneuron excitability*. in *7th World Congress of Biomechanics*. 2014. Boston, MA.
106. Gowland, C., et al., *Measuring physical impairment and disability with the Chedoke-McMaster Stroke Assessment.* Stroke, 1993. **24**(1): p. 58-63.
107. Fugl-Meyer, A.R., et al., *The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance.* Scand J Rehabil Med, 1975. **7**(1): p. 13-31.
108. Bohannon, R.W. and M.B. Smith, *Interrater reliability of a modified Ashworth scale of muscle spasticity.* Physical therapy, 1987. **67**(2): p. 206-207.
109. Krumlinde-Sundholm, L. and A.C. Eliasson, *Comparing tests of tactile sensibility: aspects relevant to testing children with spastic hemiplegia.* Dev Med Child Neurol, 2002. **44**(9): p. 604-12.
110. Bell-Krotoski, J., S. Weinstein, and C. Weinstein, *Testing sensibility, including touch-pressure, two-point discrimination, point localization, and vibration.* J Hand Ther, 1993. **6**(2): p. 114-23.
111. Traversa, R., et al., *Mapping of motor cortical reorganization after stroke. A brain stimulation study with focal magnetic pulses.* Stroke, 1997. **28**(1): p. 110-7.
112. Crone, C., et al., *Appearance of reciprocal facilitation of ankle extensors from ankle flexors in patients with stroke or spinal cord injury.* Brain, 2003. **126**(Pt 2): p. 495-507.
113. Crone, C., L.L. Johnsen, and J. Nielsen, *Reciprocal inhibition in hemiplegic patients--a longitudinal study.* Suppl Clin Neurophysiol, 2000. **53**: p. 187-91.
114. Bhagchandani, N. and S. Schindler-Ivens, *Reciprocal inhibition post-stroke is related to reflex excitability and movement ability.* Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology, 2012. **123**(11): p. 2239-2246.
115. Trompetto, C., et al., *Pathophysiology of spasticity: implications for neurorehabilitation.* BioMed research international, 2014. **2014**.

116. Phadke, C.P., C.T. Robertson, and C. Patten, *Upper-extremity spinal reflex inhibition is reproducible and strongly related to grip force poststroke.* Int J Neurosci, 2015. **125**(6): p. 441-8.
117. Artieda, J., P. Quesada, and J.A. Obeso, *Reciprocal inhibition between forearm muscles in spastic hemiplegia.* Neurology, 1991. **41**(2 (Pt 1)): p. 286-9.
118. Duque, J., et al., *Intermanual Differences in movement-related interhemispheric inhibition.* J Cogn Neurosci, 2007. **19**(2): p. 204-13.
119. Ferbert, A., et al., *Interhemispheric inhibition of the human motor cortex.* J Physiol, 1992. **453**: p. 525-46.
120. Butefisch, C.M., et al., *Relationship between interhemispheric inhibition and motor cortex excitability in subacute stroke patients.* Neurorehabil Neural Repair, 2008. **22**(1): p. 4-21.
121. Shimizu, T., et al., *Motor cortical disinhibition in the unaffected hemisphere after unilateral cortical stroke.* Brain, 2002. **125**(Pt 8): p. 1896-907.
122. Stinear, C.M., et al., *Priming the motor system enhances the effects of upper limb therapy in chronic stroke.* Brain, 2008. **131**(Pt 5): p. 1381-90.
123. Stinear, C.M., et al., *Bilateral priming accelerates recovery of upper limb function after stroke: a randomized controlled trial.* Stroke, 2014. **45**(1): p. 205-10.
124. Harris-Love, M.L., et al., *Interhemispheric Inhibition in Distal and Proximal Arm Representations in the Primary Motor Cortex.* Journal of Neurophysiology, 2007. **97**(3): p. 2511-2515.
125. Byblow, W.D., et al., *Mirror symmetric bimanual movement priming can increase corticomotor excitability and enhance motor learning.* PLoS One, 2012. **7**(3): p. e33882.
126. Stinear, J.W. and W.D. Byblow, *Rhythmic bilateral movement training modulates corticomotor excitability and enhances upper limb motricity poststroke: a pilot study.* J Clin Neurophysiol, 2004. **21**(2): p. 124-31.
127. Perez, M.A. and L.G. Cohen, *Interhemispheric inhibition between primary motor cortices: what have we learned?* The Journal of Physiology, 2009. **587**(4): p. 725-726.
128. Meyer, B.U., S. Roricht, and C. Woiciechowsky, *Topography of fibers in the human corpus callosum mediating interhemispheric inhibition between the motor cortices.* Ann Neurol, 1998. **43**(3): p. 360-9.
129. Li, J.-Y., P.-H. Lai, and R. Chen, *Transcallosal inhibition in patients with callosal infarction.* Journal of Neurophysiology, 2013. **109**(3): p. 659-665.
130. Chen, R., D. Yung, and J.-Y. Li, *Organization of Ipsilateral Excitatory and Inhibitory Pathways in the Human Motor Cortex.* Journal of Neurophysiology, 2003. **89**(3): p. 1256-1264.
131. Braune, H.J. and C. Fritz, *Transcranial magnetic stimulation-evoked inhibition of voluntary muscle activity (silent period) is impaired in patients with ischemic hemispheric lesion.* Stroke, 1995. **26**(4): p. 550-3.
132. van Kuijk, A.A., et al., *How salient is the silent period? The role of the silent period in the prognosis of upper extremity motor recovery after severe stroke.* J Clin Neurophysiol, 2005. **22**(1): p. 10-24.
133. Liu, H. and S.S. Au-Yeung, *Reliability of transcranial magnetic stimulation induced corticomotor excitability measurements for a hand muscle in healthy and chronic stroke subjects.* J Neurol Sci, 2014. **341**(1-2): p. 105-9.

134. Oozumi, T., et al., *Inhibitory period following motor potentials evoked by magnetic cortical stimulation.* Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 1991. **85**(4).
135. Bembenek, J.P., et al., *The prognostic value of motor-evoked potentials in motor recovery and functional outcome after stroke – a systematic review of the literature.* Functional Neurology, 2012. **27**(2): p. 79-84.
136. Katz, R., *Presynaptic inhibition in humans: A comparison between normal and spastic patients.* Journal of Physiology-Paris, 1999. **93**(4): p. 379-385.
137. Pizzi, A., et al., *Evaluation of upper-limb spasticity after stroke: A clinical and neurophysiologic study.* (0003-9993 (Print)).
138. Lamy, J.-C., et al., *Impaired efficacy of spinal presynaptic mechanisms in spastic stroke patients.* Brain, 2009. **132**(3): p. 734-748.
139. Stowe, A.M., et al., *A pilot study to measure upper extremity H-reflexes following neuromuscular electrical stimulation therapy after stroke.* Neurosci Lett, 2013. **535**: p. 1-6.
140. Schindler-Ivens, S., et al., *Soleus H-reflex excitability during pedaling post-stroke.* Exp Brain Res, 2008. **188**(3): p. 465-74.
141. Cho, S.-H. and J.-H. Lee, *Comparison of the Amplitudes of the H-reflex of Post-stroke Hemiplegia Patients and Normal Adults during Walking.* Journal of Physical Therapy Science, 2013. **25**(6): p. 729-732.
142. Mottram, C.J., et al., *Origins of abnormal excitability in biceps brachii motoneurons of spastic-paretic stroke survivors.* J Neurophysiol, 2009. **102**(4): p. 2026-38.
143. Zaaimi, B., et al., *Changes in descending motor pathway connectivity after corticospinal tract lesion in macaque monkey.* Brain, 2012. **135**(Pt 7): p. 2277-89.
144. Harvey, P.J., et al., *5-HT2 receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury.* J Neurophysiol, 2006. **96**(3): p. 1158-70.
145. Aymard, C., et al., *Presynaptic inhibition and homosynaptic depression.* Brain, 2000. **123**(8): p. 1688-1702.
146. Nakashima, K., et al., *Reciprocal inhibition between forearm muscles in patients with writer's cramp and other occupational cramps, symptomatic hemidystonia and hemiparesis due to stroke.* Brain, 1989. **112**(3): p. 681-697.
147. Lelli, S., M. Panizza, and M. Hallett, *Spinal cord inhibitory mechanisms in Parkinson's disease.* Neurology, 1991. **41**(4): p. 553-6.
148. De Deyne, P.G., et al., *Muscle molecular phenotype after stroke is associated with gait speed.* Muscle Nerve, 2004. **30**(2): p. 209-15.
149. Hafer-Macko, C.E., et al., *Skeletal muscle changes after hemiparetic stroke and potential beneficial effects of exercise intervention strategies.* J Rehabil Res Dev, 2008. **45**(2): p. 261-72.
150. Street, D., J. Bangsbo, and C. Juel, *Interstitial pH in human skeletal muscle during and after dynamic graded exercise.* The Journal of Physiology, 2001. **537**(Pt 3): p. 993-998.
151. Allen, D.G., G.D. Lamb, and H. Westerblad, *Skeletal muscle fatigue: cellular mechanisms.* Physiol Rev, 2008. **88**(1): p. 287-332.
152. Cady, E.B., et al., *The metabolic causes of slow relaxation in fatigued human skeletal muscle.* The Journal of Physiology, 1989. **418**: p. 327-337.

153. Bowden, J.L., G.G. Lin, and P.A. McNulty, *The prevalence and magnitude of impaired cutaneous sensation across the hand in the chronic period post-stroke.* PLoS One, 2014. **9**(8): p. e104153.
154. Hughes, C.M.L., et al., *Upper Extremity Proprioception in Healthy Aging and Stroke Populations, and the Effects of Therapist- and Robot-Based Rehabilitation Therapies on Proprioceptive Function.* Frontiers in Human Neuroscience, 2015. **9**: p. 120.
155. Nowak, D.A., S. Glasauer, and J. Hermsdorfer, *How predictive is grip force control in the complete absence of somatosensory feedback?* Brain, 2004. **127**(Pt 1): p. 182-92.
156. Tammik, K., et al., *Quadriceps femoris muscle voluntary force and relaxation capacity in children with spastic diplegic cerebral palsy.* Pediatr Exerc Sci, 2008. **20**(1): p. 18-28.
157. Li, S. and G.E. Francisco, *New insights into the pathophysiology of post-stroke spasticity.* Frontiers in Human Neuroscience, 2015. **9**: p. 192.
158. Mukherjee, A. and A. Chakravarty, *Spasticity mechanisms - for the clinician.* Front Neurol, 2010. **1**: p. 149.
159. Wilson, L.R., et al., *Evidence for fusimotor drive in stroke patients based on muscle spindle thixotropy.* Neurosci Lett, 1999. **264**(1-3): p. 109-12.
160. Wilson, L.R., et al., *Muscle spindle activity in the affected upper limb after a unilateral stroke.* Brain, 1999. **122**(11): p. 2079-2088.
161. Møller, A., *Neural Plasticity and Disorders of the Nervous System*. 2010: Cambridge University Press.
162. Standring, S., et al., *Gray's anatomy: the anatomical basis of clinical practice.* American Journal of Neuroradiology, 2005. **26**(10): p. 2703.
163. Davidson, A.G. and J.A. Buford, *Motor outputs from the primate reticular formation to shoulder muscles as revealed by stimulus-triggered averaging.* J Neurophysiol, 2004. **92**(1): p. 83-95.
164. Mottram, C.J., et al., *Origins of spontaneous firing of motor units in the spastic-paretic biceps brachii muscle of stroke survivors.* J Neurophysiol, 2010. **104**(6): p. 3168-79.
165. Powers, R.K., D.L. Campbell, and W.Z. Rymer, *Stretch reflex dynamics in spastic elbow flexor muscles.* Annals of Neurology, 1989. **25**(1): p. 32-42.
166. Augurelle, A.-S., et al., *Importance of Cutaneous Feedback in Maintaining a Secure Grip During Manipulation of Hand-Held Objects.* Journal of Neurophysiology, 2003. **89**(2): p. 665-671.
167. Seo, N.J., et al., *Use of visual force feedback to improve digit force direction during pinch grip in persons with stroke: a pilot study.* Archives of physical medicine and rehabilitation, 2011. **92**(1): p. 24-30.
168. Boissy, P., et al., *Maximal grip force in chronic stroke subjects and its relationship to global upper extremity function.* Clin Rehabil, 1999. **13**(4): p. 354-62.

Curriculum Vitae

Binal Motawar

EDUCATION			
INSTITUTION AND LOCATION	DEGREE	MM/YYYY	FIELD OF STUDY
Maharaja Sayajirao University of Baroda, Baroda, Gujarat, India	B. Physio-therapy	09/2002-09/2007	Physical Therapy
East Carolina University, Greenville, NC, USA	M.S	01/2008-05/2010	Exercise Sport Science
University of Wisconsin-Milwaukee, Milwaukee, WI, USA	Ph.D.	09/2010-current	Health Sciences

Positions and Employment

2006-2007	Intern Physiotherapist, S.S.G. Hospital, Baroda, Gujarat India
2007	Physiotherapist, Om Sai Physiotherapy Clinic, Baroda, Gujarat, India
2008-2009	Graduate teaching and research assistant, East Carolina University, Greenville, NC Classes: (1) Yoga (2) Structural Kinesiology
2010	Assistant teacher, Easter Seals UCP North Carolina, Greenville, NC
2011	Graduate teaching assistant, University of Wisconsin, Milwaukee, WI Classes: Lab for (1) Engineering Drawing (2) Neuromechanics
2012-13	Supplemental instructor/ Tutor, University of Wisconsin, Milwaukee, WI Classes: (1) Anatomy & Physiology I (2) Anatomy & Physiology II.
2014	Physical therapist, Easy Living Home Health Agency, Germantown, WI
2010-2015	Graduate research assistant, University of Wisconsin, Milwaukee, WI
2015-present	Physical Therapist, Easy Living Home Health Agency, Germantown, WI
2015-present	Physical Therapist, Vesta Therapy, Wauwatosa, WI

Awards:

2010	Nominated for the Best Master's Thesis Award, East Carolina University
2011	Graduate Student Travel Award, University of Wisconsin-Milwaukee
2011	Chancellor's Award, University of Wisconsin-Milwaukee
2012	Graduate Student Travel Award, University of Wisconsin-Milwaukee
2012	Chancellor's Award, University of Wisconsin-Milwaukee
2012	Honorary Student Award, Asian Faculty and Staff Association, University of Wisconsin-Milwaukee
2012	Third place, The IEEE Milwaukee Section 2012 Poster Competition

2013	Third place, CHS Spring 2013 Research Symposium, University of Wisconsin-Milwaukee
2013	Third place, CHS Fall 2013 Research Symposium, University of Wisconsin-Milwaukee
2014	Summer Chancellor's Award, University of Wisconsin-Milwaukee
2014	Travel award, 38th Annual Meeting of the American Society of Biomechanics in conjunction with the 7th World Congress of Biomechanics, Boston, MA, USA
2014	Chancellor's Award, University of Wisconsin-Milwaukee

Volunteer activities:

2007	Leprosy surgery and rehabilitation camp, Government of Gujarat, Baroda, India
2007	Physiotherapist, Krishna Pediatric Physiotherapy Center, Baroda, India
2008	Camp Counselor, Camp Whole Heart, Arapahoe, NC
2010	Volunteer, Easter Seals UCP North Carolina, Greenville, NC
2013	Judge, Nicolet High School Science and Engineering Fair, Glendale, WI
2013	Residence Hall Tutor, Panther Academic Support Services, University of Wisconsin-Milwaukee, Milwaukee, WI
2013, 2015	Judge, Badger State Science Fair, Milwaukee, WI
2012-15	Judge, University of Wisconsin-Milwaukee Undergraduate Research Symposium, Milwaukee, WI, USA
2012-present	Physical Therapist, Free health clinic at City on a Hill, Milwaukee, WI
2014-present	Big Sister, Big Brothers Big Sisters of Metro Milwaukee, Milwaukee, WI

Professional licenses:

2007-present	Physiotherapist	Indian Association of Physiotherapists, India
2011-2014	Physical Therapist	New York State Department of Education, NY
2012-present	Physical Therapist	Department of Safety and Professional Services, WI
2015-present	Physical Therapist	Washington State Department of Health, WA

Continued education:

2005	Pain management-the combination approach of physiotherapy and yoga
2005	On normal and abnormal EMG-NCV study
2005	Bronchial asthma-integrated approach
2005	Radiology for physiotherapists
2006	Workshop on comprehensive approach to low back ache syndrome
2006	Upper and lower quarter mulligan's concept course
2007	Neurodevelopment training for pediatric patients
2007	Evidence based physiotherapy & clinical practice guideline

2007	Ankle and foot disorders and management
2013	Myofascial Release
2015	Ethics for Rehabilitation Professionals
2015	Advanced Ethics for the Healthcare Professionals

Peer-review activities:
Experimental Brain Research, Neuroscience Research

Peer reviewed journal publications:

1. **Motawar, B.**, Stinear, J., Lauer, A., Ramakrishnan, V., Seo, N.J. (2016). Delayed grip relaxation and altered modulation of intracortical inhibition with aging, *Experimental Brain Research; accepted.*
2. Seo, N.J., Kumar, J., Hur, P., Crocher, V., **Motawar, B.**, Lakshminarayanan, K. (2015). Development and usability evaluation of low-cost hand and arm virtual reality rehabilitation games, *Journal of Rehabilitation Research & Development; accepted.*
3. Seo, N.J., Enders, L.R., **Motawar, B.**, Kosmopoulos, M. (2015). Digit force deviation correlates with upper extremity function scores in chronic stroke survivors, *Journal of Biomechanics; 48(2), 383-7.*
4. Hur, P., **Motawar, B.**, Seo, N.J. (2013) Muscular responses to handle perturbation with different glove condition. *Journal of Electromyography and Kinesiology; 24(1), 159-64.*
5. **Motawar, B.**, Hur, P., Stinear, J., Seo, N.J. (2012). Contribution of intracortical inhibition in voluntary muscle relaxation. *Experimental Brain Research; 221(3), 299-308.*
6. Hur, P., **Motawar, B.**, Seo, N.J. (2012). Hand breakaway strength model – Effects of glove use and handle shapes on a person's hand strength to hold onto handles to prevent fall from elevation. *Journal of Biomechanics; 45(6), 958-64.*
7. Howatson, G., Taylor, M., Rider, P., **Motawar, B.**, McNally, M., Solnik, S., DeVita, P., Hortobágyi, T. (2011). Ipsilateral motor cortical responses to TMS during lengthening and shortening of the contralateral wrist flexors. *European Journal of Neuroscience, 33(5), 978-90.*

International/ National peer-reviewed conference abstracts:

1. **Motawar, B.**, Seo, NJ. (2014). Aging-related changes in neural function for grip relaxation: intracortical inhibition and spinal motoneuron excitability. *7th World Congress of Biomechanics, Boston, MA.*
2. Arunkumar, J., Hur, P., **Motawar, B.**, Seo, NJ. (2013). Low-cost virtual reality game for upper limb rehabilitation using Kinect and P5 glove. *Annual conference of American Society of Biomechanics 2013, Omaha, NE.*
3. **Motawar, B.**, Seo, N.J. (2012). Modulation of the intracortical inhibition for grip relaxation in chronic stroke survivors. *The 42nd Annual Meeting of the Society for Neuroscience*, New Orleans, LA.
4. Sotelo, N., **Motawar, B**., Seo, N.J. (2012). Modulation of the intracortical inhibition

during grip relaxation. *13th RCMI International Symposium on Health Disparities*, San Juan, Puerto Rico.

5. **Motawar, B.**, Hur, P., Seo, N.J. (2011). Effects of Gloves with Different Coefficients of Friction on Fall Recovery During Simulated Ladder Falls. *Annual conference of American Society of Biomechanics 2011, Long Beach, CA.*
6. Hur, P., **Motawar, B.**, Seo, N.J. (2011). Effects of Glove and Ladder Rung Design on Prevention of Ladder Fall. *Annual conference of American Society of Biomechanics 2011, Long Beach, CA.*
7. Hortobagyi, T., **Motawar, B.**, McNally, M., Rider, P. & DeVita, P. (2010). Spinal excitability in contralateral muscle is similar during shortening and lengthening of the wrist flexors. Medicine and Science in Sports and Exercise (suppl.), 42, s413.

Regional/ University symposia presentations:

1. **Motawar, B.**, Seo, NJ. (2014). Aging-related changes in neural function for grip relaxation: intracortical inhibition and spinal motoneuron excitability. *Spring 2014 CEAS Poster Competition at University of Wisconsin-Milwaukee.*
2. **Motawar, B.**, Seo, N.J. (2013). Effect of aging on intracortical mechanisms for grip relaxation in the nondominant hand. *CHS Fall 2013 Research Symposium at University of Wisconsin-Milwaukee.*
3. **Motawar, B.**, Seo, N.J. (2013). Investigation of spinal motoneuron excitability during grip relaxation in healthy young adults. 4th Annual Milwaukee Regional Research Forum, Milwaukee, WI.
4. **Motawar, B.**, Seo, N.J. (2013). Interlimb differences in the spinal motoneuron excitability during grip relaxation. *Milwaukee area Society for Neuroscience 2013 Meeting.*
5. **Motawar, B.**, Seo, N.J. (2013). Interlimb differences in the spinal motoneuron excitability during grip relaxation. *CHS Spring 2013 Research Symposium at University of Wisconsin-Milwaukee.*
6. **Motawar, B.**, Seo, N.J. (2013). Interlimb differences in the spinal motoneuron excitability during grip relaxation. *Spring 2013 CEAS Poster Competition at University of Wisconsin-Milwaukee.*
7. **Motawar, B.**, Seo, N.J. (2012). Role of inhibitory motor cortical pathways during voluntary relaxation following a maximal power grip in chronic stroke survivors. *Neuromechanics Symposium*, Chicago, IL
8. **Motawar, B.,** Seo, N.J., (2012). Role of inhibitory motor cortical pathways during voluntary grip relaxation in chronic stroke survivors. *IEEE Milwaukee Section 2012 Larry Hause Student Design Poster Competition*
9. **Motawar, B.,** Seo, N.J., (2012). Role of inhibitory motor cortical pathways during voluntary grip relaxation in chronic stroke survivors. *Fall 2012 CHS Poster Competition at University of Wisconsin-Milwaukee.*
10. **Motawar, B.,** Seo, N.J., (2012). Role of inhibitory motor cortical pathways during voluntary grip relaxation in chronic stroke survivors. *Fall 2012 CEAS Poster Competition at University of Wisconsin-Milwaukee.*
11. Hill, R., McNally, M., **Motawar, B.,** Rider, P., DeVita, P., and Hortobágyi, T. (2011). Unilateral eccentric and concentric exercise produces non-specific reductions in spinal

excitability in the contralateral homologous plantarflexors. *Proceedings of the Human Movement Science Symposium at University of North Carolina, Chapel Hill, NC.*

12. Hill, R., McNally, M., **Motawar, B.**, Rider, P., DeVita, P., and Hortobágyi, T. (2011). Effect of repeated unilateral eccentric and concentric exercise on spinal excitability in the contralateral homologous plantarflexors. *Proceedings of the 5th Annual Research and Creative Achievement Week at East Carolina University, Greenville, NC*.
13. Hill, R., McNally, M., **Motawar, B.**, Rider, P., DeVita, P., & Hortobágyi, T. (2010). Task Specific Effects of Unilateral Eccentric and Concentric Exercise on Spinal Excitability of the Contralateral Homologous Plantar Flexors. *Proceedings of 4th Annual Research and Creative Achievement Week.*
14. **Motawar, B.**, McNally, M., Rider, P., DeVita, P., & Hortobágyi, T. (2009). Modulation of H-reflex response to voluntary contraction of the homologous muscle in the contralateral limb. *Proceedings of the 3rd Annual ECU Research and Creative Achievement Week, 3, 109.*
15. Slye, A., Rider, P., Solnik, S., Moscicki, B., Gomez, J., **Motawar, B.**, Steinweg, K., DeVita, P., and Hortobágyi, T. (2008). Age-related differences in muscle coactivation during treadmill locomotion. *Proceedings of the 2nd Annual ECU Research and Creative Achievement Week.3, 87*.

Invited talks:

1. **Motawar, B.** (2015) Breakfast Research Talk, *UWM Research Foundation*
2. **Motawar, B.**, Seo, N.J. (2013) Grip relaxation after stroke, *Clinical and Translational Science Research Seminar*

Support:

1. Mechanisms and novel rehabilitation methods for prolonged muscle relaxation following stroke, (Motawar: Research Assistant, Seo: Mentor), *the Clinical and Translational Science Award (CTSA) Program,* 2012-2013, ($13713+ benefits).
2. Identification of neural mechanisms for the delayed grip relaxation in chronic stroke survivors, Graduate Student Grant-in-Aid, American Society of Biomechanics ($2000)
3. Identification of neural mechanisms for the delayed grip relaxation in chronic stroke survivors, Graduate student research grant, 2012-2013 CHS Student Research Grant, University of Wisconsin-Milwaukee ($2000).