THESIS

RELIABILITY OF TMS MEASUREMENTS OF THE MOTOR CORTEX

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY LAURIE CAUSER ENTITLED RELIABILITY OF TMS MEASUREMENTS OF THE MOTOR CORTEX BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

RELIABILITY OF TMS MEASUREMENTS OF THE MOTOR CORTEX

BACKGROUND: Transcranial magnetic stimulation (TMS) was introduced in 1985 and has been used to study the human motor system through a variety of applications including single pulse, paired pulse and repetitive pulse stimulation parameters. Paired pulse TMS studies assess motor cortical excitability, in which the first (conditioning) stimulus (CS) modifies the response to the second (test) stimulus (TS) (Maeda, Gangitano, Thall, & Pascual-Leone, 2002). The time between pulses, or the interstimulus interval, is the distinguishing factor between the application of paired pulse TMS to investigate intracortical inhibition (ICI) or intracortical facilitation (ICF). Studies of cortical excitability using paired pulse TMS can provide novel insights into the pathophysiology of various neurological and psychiatric disorders (Maeda, et al., 2002) and have begun to be utilized as outcome measures to document changes in cortical excitability in response to repetitive TMS. The stability of the muscle responses known as motor evoked potentials (MEPs) elicited in response to paired pulse stimulation has not been well documented in the literature to date. As such, the primary goal of this study was to establish the test-retest reliability of two paired pulse measures of the motor cortex, ICI and ICF, in two muscle representations; first dorsal interossei (FDI) and abductor pollicis brevis (APB). METHODS: Fifteen healthy individuals, age 19-37 years

old, participated in two identical testing sessions held exactly one week apart from each other. Four different types of stimulation (CS, TS, 2ms, and 15ms) were delivered over the motor cortex 20 times in a random order. The corresponding MEPs were recorded and their size were documented using two common methods found in the literature; area under the curve and peak to peak amplitude. RESULTS: Reliability was determined using intra-class correlation coefficients (ICCs). Poor reliability was documented in both methods of analysis; whether twenty trials or ten trials were averaged, and even still after normalizing data, with ICCs ranging from (-.508 - .347). CONCLUSION: Additional studies investigating the test-retest reliability of paired pulse measures of the motor cortex need to be conducted to document the stability of MEPs. Potential sources of variation in MEPs size include electrode placement variation, stimulation intensity changes, coil placement variability, state of the overall nervous system, and the state of the individual muscle (contracted/relaxed). Until the reliability of paired pulse stimulation is established, researchers should use caution linking the changes in the size of MEPs in response to paired pulse stimulation to interventions, disease, or other external factors.

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Introduction

Transcranial magnetic stimulation (TMS) is a safe, non-invasive method of investigating the motor cortex. A very brief, high intensity electric current is passed through a well-insulated wire coil held over the scalp, setting up a perpendicularly directed magnetic field, which passes relatively unimpeded through the layers of tissue and bone over the cortex (Harris-Love & Cohen, 2006). If current amplitude, duration, and direction are appropriately directed over the primary motor cortex, TMS will depolarize cortical neurons and generate action potentials (Rothwell, et al., 1999). This results in the activation of descending corticospinal neurons which activate motoneurons, resulting in motor unit recruitment (Kamen, 2004) .

TMS techniques are typically used for three different purposes: to modulate cortical excitability, to evaluate the behavioral or physiologic consequences of temporarily suppressing or enhancing the excitability of focal brain regions, and to measure various aspects of brain function such as intracortical inhibition, intracortical facilitation, interhemispheric inhibition, and cortical excitability (Harris-Love & Cohen, 2006). The intended purpose dictates whether TMS is delivered in single, paired, and/or repetitive pulses, with the majority of TMS sessions involving a combination of at least two of these stimulation parameters.

One method of application is the deliverance of a set of TMS pulses to the same cortical region through a single coil. In this neuropysiological technique, the first

subthreshold conditioning stimulus modifies the response to the second suprathreshold test stimulus. Muscle responses elicited by the paired pulse stimulation are used to investigate intracortical inhibition and intracortical facilitation, which depend upon the intensity and the interval between the stimuli (Kujirai, et al., 1993). Paired pulse paradigm applications have been cited extensively in publications seeking to document changes in cortical excitability, offering novel insights into the pathophysiology of various neurological and psychiatric disorders (Maeda, et al., 2002). Despite a large number of studies applying these measures, reliability data are lacking. Without sufficient data documenting the baseline variability that can be attributed to uncontrollable mechanisms of the nervous system in healthy populations, the source of variation remains unclear as to how much of the variability in cortical excitability can be confidently attributed to disease or therapeutic interventions.

Paired pulse TMS

The cortical motor output is the net result of the interplay between multiple systems that exert excitatory and inhibitory influences on the corticospinal neurons (Chen, et al., 2008). This interplay, or balance, between excitatory and inhibitory influences affords humans their refined motor control. From this, the term intracortical (cortico-cortical) inhibition and facilitation arose, altogether defined as 'intracortical excitability' (Cantello, Tarletti, & Civardi, 2002). Paired pulse TMS may be used to investigate these facilitatory and inhibitory mechanisms (Chen, et al., 2008). In paired pulse TMS, the standard protocol is the deliverance of a subthreshold conditioning (CS) stimulus followed by a suprathreshold test stimulus (TS). The CS is enough to activate cortical neurons, but small enough so that no descending influence on the spinal cord can

be detected and there is no MEP (Hallett, 2007). Conversely, the TS is a supratheshold stimulus that consistently evokes a MEP.

The time between pulses, or the interstimulus interval, is the distinguishing factor between the application of paired pulse TMS to investigate intracortical inhibition (ICI) or intracortical facilitation (ICF). ICI is explored by delivering two pulses to the same hemisphere at short (1 – 5ms) interstimulus intervals (Rossini & Rossi, 2007). The resulting MEP has a smaller amplitude than was elicited by the test stimulus alone. This inhibited response is believed to be mediated by y-aminobutyric acid(GABAergic inhibition) at the intracortical level (Fisher, Nakamura, Bestmann, Rothwell, & Bostock, 2002; Reis, et al., 2008; Roshan, Paradiso, & Chen, 2003). GABA is the main inhibitory transmitter in the central nervous system (Stein, Stoodley, 2006).

ICF is explored by delivering two pulses to the same hemisphere at longer (6 – 20ms) interstimulus intervals (Rossini & Rossi, 2007). The resulting amplitude of the MEP is increased in comparison to the MEP elicited by the test stimulus alone (Rossini & Rossi, 2007). The NMDA receptor antagonist dextromthorphan reduces ICF (Ziemann, Chen, Cohen, & Hallett, 1998), suggesting that glutamate plays a role in mediating ICF (Chen, et al., 2008). Glutamate is the main excitatory transmitter in the central nervous system (Stein, Stoodley, 2006).

Single pulse TMS

The determination of cortical motor threshold (MT) using single pulse TMS is a necessary aspect of paired pulse protocols as MT is used to determine both the subthreshold conditioning stimulus and suprathreshold test stimulus. MT reflects the

global excitability of the motor pathway, including large pyramidal cells, cortical excitatory and inhibitory interneurons, and spinal motoneurons (Weber & Eisen, 2002).

When applied to the motor cortex at appropriate stimulation intensity, single - pulse TMS produces a contralateral muscle response that can be recorded with surface electromyography. This muscle response is known as a motor evoked potential (MEP). MT is defined as the lowest level of stimulation required to elicit an MEP in 50% of TMS trials (Pascual-Leone, et al., 1998). The size of the MEP depends on complex interactions between the cortical motor neuronal system and the anterior horn cell, reflecting the sum of upper and lower motor neuron activity (Weber & Eisen, 2002). MEP size is usually measured as peak to peak amplitude (Weber & Eisen, 2002) or as area under the curve (Kamen, 2004). There is considerable trial-to-trial variability of MEP size (Truccolo, Ding, Knuth, Nakamura, & Bressler, 2002) so rather than using individual trials as dependent measures, the mean amplitude of a group of trials has been found to be reliable (Kamen, 2004). Using mean scores has the effect of increasing reliability estimates, as means are considered better estimates of true scores, theoretically reducing error variance (Portney & Watkins, 2000).

Repetitive TMS

Paired pulse stimulation parameters such as ICI and ICF are often used to document the effects of repetitive TMS (rTMS) protocols, which include the deliverance of a series or trains of magnetic pulses in a repetitive fashion. rTMS stimulation parameters include coil orientation, frequency, intensity, and train duration. When applied to the motor cortex at a low (1 Hz) frequency, cortical excitability is decreased (Chen, et al., 1997) and can outlast the period of stimulation (Muellbacher, Ziemann,

Boroojerdi, & Hallett, 2000). The depression of the excitability of the stimulated region may occur via long term synaptic depression (Rossini & Rossi, 2007). Conversely, high (>5 Hz) frequency application results in increased cortical excitability that can also outlast the period of stimulation (Peinemann, et al., 2004) by saturating the inhibitory capacity of the cortical network (Wassermann & Lisanby, 2001) via longterm synaptic potentiation (Rossini & Rossi, 2007).

Clinical Relevance of TMS

The development of paired and repetitive TMS protocols has allowed investigators to explore and influence inhibitory and excitatory interactions of various motor and non-motor cortical regions within and across cerebral hemispheres (Reis, et al., 2008). Paired pulse TMS has been used in a wide variety of studies investigating ICI and ICF in patients with amyotrophic lateral sclerosis (ALS), cerebellar disease, dementia, multiple sclerosis (MS), Parkinson's disease, stroke, epilepsy, and migraines (Chen, et al., 2008). Multiple studies involving a large number of individuals with myelopahty, ALS, and MS have demonstrated diagnostic utility of TMS measures (Chen, et al., 2008). Promising results, with potential clinical utility, have been documented in a variety of studies involving smaller number of individuals with cerebellar disease, dementia, facial nerve disorders, movement disorders, stroke, epilepsy migraine, and chronic pain (Chen, et al., 2008). Before the results of these smaller studies can be used for diagnostic purposes, follow-up studies with larger samples sizes are needed to confirm initial findings.

Research has demonstrated that ICI is atypical in clinical populations. Individuals with ALS had a reduced or absent ICI when compared to age-match group of healthy

control subjects (Yokota, Yoshino, Inaba, & Saito, 1996; Ziemann, et al., 1997) as did individuals with other movement disorders such as Parkinson's Disease, coritobasal degeneration, dystonia, cortical myoclonus, and tourette's syndrome (Cantello, 2002). A reduction in ICI was documented in patients with Alzheimer's disease (Di Lazzaro, et al., 2004; Di Lazzaro, et al., 2002; Liepert, Bar, Meske, & Weiller, 2001), while ICI was reported as normal in one study (Pepin, Bogacz, de Pasqua, & Delwaide, 1999). Reduced ICI has been documented in patients with MS (Caramia, et al., 2004).

The application of rTMS to treat disease symptoms, improve functional deficits, and/or modify cortical excitability are also under investigation. Paired pulse TMS, specifically ICI and ICF, are sometimes utilized to measure pre-post effects of rTMS. In addition to current interest in investigating rTMS as a potential therapy for stroke survivors to promote reorganization and improve response to conventional treatments (Talelli, Greenwood, & Rothwell, 2006), researchers are exploring the therapeutic application of rTMS as a treatment for depression, schizophrenia, Parkinson's disease, task related dystonia (writer's cramp), tic disorder, and epilepsy (Wassermann & Lisanby, 2001).

Reliability

One limitation of the application of paired pulse TMS as a diagnostic tool or outcome measure is the lack of reliability data related to paired pulse TMS applied over multiple testing sessions. Considering that reliability is the extent to which a measurement is consistent and free from error (Portney & Watkins, 2009), the lack of such data raises several importation questions. How can the neurophysiological effects of rTMS be determined when the test retest reliability has yet to be clearly established?

How can differences documented in clinical populations with compromised nervous systems be confidently attributed to disease when the test retest reliability in healthy populations has not been documented? Clearly the test retest reliability of MEPs elicited in response to paired pulse TMS needs to be investigated in healthy populations to establish a baseline understanding of the variability that can be attributed to uncontrollable mechanisms of the nervous system.

The usefulness of a measurement in clinical research and decision making depends on the extent to which clinicians can rely on data as accurate and meaningful indicator of an attribute (Portney & Watkins, 2009). Using a measure for which reliability has not been established limits the strength of conclusions made. One can not have confidence in the data collected, nor can rational conclusions be drawn from those data if reliability is unknown (Portney & Watkins, 2009). Researchers may falsely attribute variable changes to interventions or experimental procedures when in reality the changes are a result of the measure's inability to document reproducible data. Studies which expect a change in MEP size resulting from an intervention, disease, or experimental procedure can not clearly demonstrate the change to be a result of the intervention if the measure has not been proven to be reliable.

A limited number of studies, with small sample sizes, have been published that have investigated the test-retest reliability of paired pulse TMS stimulation, with conflicting results using an analysis of variance (ANOVA). A small paired pulse study (n=4) with 3 investigators looked the impact of 'subject', 'session', and 'investigator' on the reproducibility of ICI and ICF in the left first dorsal interosseous (FDI) and found a high rate of variability using an analysis of variance (ANOVA) and coefficients of

variance (Boroojerdi, et al., 2000). In contrast, Maeda et al found that cortical excitability, particularly ICI in the FDI, is reproducible using ANOVA in a sample of ten subjects.

While ANOVA is capable of determining if there is a statistical difference between two or more sample means, it does not describe the proportion of the variance within a data set that can be attributed to each of the independent factors included in the experimental design as intra-class correlation coefficients (ICC) does (Carroll, Riek, & Carson, 2001). To date, there are no published studies that have investigated the test-retest reliability of ICF and ICI using ICCs, which is considered the preferred statistical methodology for assessing reliability (McGraw & Wong, 1996) as it reflects both correlation and agreement between pre- and post-test session findings (Portney & Watkins, 2009).

The purpose of this study is to establish the test-retest reliability of two paired pulse measures of the motor cortex; ICI and ICF in two intrinsic muscle representations, abductor pollicis brevis (APB) and FDI. The studies conducted by Boroojerdi et al. and Maeda et al., both investigated the reliability of muscle responses recorded from one muscle, FDI. This study was designed to compare responses in two muscles to determine whether similar reliability could be found in MEPs evoked in intrinsic muscles or whether one muscle provided more reliable responses than the other. The inherent variability in paired pulse responses evoked in each of these muscles is relevant to future studies as both are frequently utilized in TMS research and can be can be found in currently published literature.

In addition to muscle selection, the method of document MEPs size also varies. Researchers typically report responses as peak to peak amplitude or as area under the curve. In peak to peak amplitude analysis, cursors are set to encompass the peak and trough of the unrectified MEP. In the area under the curve analysis, cursors are placed at the onset and off set of the full-wave rectified MEP. The benefit of the second method is that length of MEP, or the influence of how long the MEP lasts in milliseconds, is reflected in the area measurement. Peak to peak amplitude doesn't reflect the overall size of the MEP, but rather the total height of the response. Time is not a factor in peak to peak amplitude analysis, therefore this method of measurement has the potential to be a more reliable method, while offering a less detailed picture of the MEP response.

The implications of a long lasting MEP indicate potential limitations in inhibitory mechanisms of the nervous system and may be a more appropriate method of analysis to document abnormalities or changes in populations with compromised neurological systems. As it stands, both methods of analysis are currently utilized in paired pulse stimulation studies. While there is the potential that both methods are indeed reliable, whether one method of analysis is more reproducible than the other has yet to be established and is an important aspect of reliability to explore.

This study investigated the reproducibility of MEPs elicited in response to the two types of paired pulse stimulation in healthy individuals with the expectation that these measures will be stable over a period of one week. The experiments in this study sought to utilize ICCs to determine: (1) the test-retest reliability of ICI and ICF in healthy individuals based upon amplitude of MEPs from 20 trials; and (2) the test-retest reliability of ICI and ICF in healthy individuals based upon area under the curve of MEPs

from 20 trials; and (3) the test-retest reliability of ICI and ICF in healthy individuals based upon amplitude of MEPs from 10 trials; and (4) the test-retest reliability of ICI and ICF in healthy individuals based upon area under the curve of MEPs from 10 trials. In doing so, this study will be the first to compare MEPs elicited in response to paired pulse TMS in two intrinsic hand muscles, utilizing two separate methods of analysis for the size of the MEPs, to establish the test retest reliability using what has been described as the preferred index of reliability, or ICC.

Methods

Subjects

Fifteen healthy volunteers (14 female; 1 male) participated in this study with a mean age of 25 (± 6.1) years. Inclusion criteria for study participation included right handed males or females between the ages of 18 – 50 years old. Each participant was required to complete a health screening questionnaire and written informed consent (see Appendix A and B) prior to enrolling in the study in accordance with the policies of the Institutional Review Board at Colorado State University. Potential participants were recruited through flyers circulated on campus at Colorado State University and via online Student FYI posting entitled *Today @ Colorado State*. Exclusion criteria for study participation included: taking medications which may lower the seizure threshold; history of central nervous system illness including but not limited to epilepsy or seizure disorder; mass brain lesions including but not limited to stroke, cancerous and noncancerous tumors, abnormal connnection between blood vessels in the brain; head trauma leading to loss of consciousness of any length; history of drug or alcohol abuse within the past year; implanted devices such as a pacemaker, medication pump, metal plate in skull, metal objects in the eye or skull, cochlear implant, intracardiac lines; personal or family history of heart disease; current pregnancy; history of bi-polar disorder; or left hand dominance.

Experimental procedure

Each subject participated in two identical testing sessions. Each session was conducted during a consistent time of the day (morning, afternoon, or evening), exactly one week apart. Participants were seated comfortably in a dental chair, with a semi-circle pillow placed behind their neck and a rectangular pillow placed underneath their right upper extremity for additional comfort and support. With the Viking II Electromyograph (Nicolet Biomedical, Madison, WI), electromyography (EMG) signals were obtained using passive bipolar surface electrodes placed upon abductor pollicis brevis (APB) and first dorsal interosseous (FDI) in the right upper extremity. The inter-electrode distance was set using tendon-belly set up for both muscles. Bipolar EMG signals were filtered with a bandpass set at 2-10 kHz, rectified and amplified with the Viking II Electromyograph.

A cloth cap was placed on participants head to mark the vertex (Cz) of the head. Cz is determined by measuring the distance between the nasion and inion and the distance between the tragus of each ear and marking their point of intersection. The Magstim 70mm Figure 8 coil has the ability to stimulate focally and was positioned tangentially to the skull, with the handle pointing posteriorly and oriented sagittaly (Appendix C). Focality is normally defined as the cortical area in which the electric field strength exceeds a certain value relative to the maximum (e.g. half the maximum) (Roth, Cohen, Hallett, Friauf, & Basser, 1990). The smaller this area, the steeper the spatial decay of the field strength, in turn indicating a good focality (Thielscher & Kammer, 2004). The cortical area stimulated by a Magstim 70mm Figure 8 coil at level ranging from MT to 120%MT is approximately 3 – 7 cm^2 (Thielscher & Kammer, 2004). The area of stimulation, or motor "hot spot", is defined as the area of the motor cortex where

the largest MEPs are found in response to single pulse TMS and was determined by moving the position of the coil 1-2 cm increments until consistent MEPs were elicited in the APB and FDI. The "hot spot" location was measured in relation to the vertex and recorded to use in the second testing session.

Single pulse TMS was then delivered over the "hot spot" starting at 30% intensity and increasing stimulation intensity in increments of 1 - 5% as needed to determine MT. MT is defined as the lowest stimulation intensity that elicits a discernable motor evoked potential in at least three out of six consecutive stimulations using an oscilloscope gain of 200mV per cm (Wassermann, McShane, Hallett, & Cohen, 1992). Because a slight muscle contraction can affect the size of MEPs and motor threshold determination (Weber & Eisen, 2002), precautions were taken to ensure each muscle was relaxed during the session. These precautions included monitoring the auditory feedback from the Viking II Electromyography, as well as scanning the EMG recordings for indications of muscle contraction such as single motor neurons firing – which are apparent in their small size, repetition, and consistency.

Once MT was determined, it was used to calculate the subthreshold conditioning stimulus (CS) (90% of MT) as well as the suprathreshold testing stimulus (TS) (116% of MT). Each participant received four different types of stimulation, 20 times each, in a randomized order. Using 20 trials has been shown to decrease intersession variability in another paired pulse study (Boroojerdi, et al., 2000). Two single pulse TMS measures, CS and TS, and two paired pulse TMS stimulation, ICI and ICF were used. The first paired pulse stimulation known to reflect ICI, is deliverance of CS followed by the TS

with a 2ms interstimulus interval. In the second paired pulse stimulation known to reflect ICF, the CS is followed by the TS with a 15ms interstimulus interval (Appendix D).

Data collection

Data collection and analysis was completed by the same person. The MEPs evoked in response to the four types of stimulation (CS, TS, 2ms, 15ms) were recorded. Cursors were set to encompass the time that the MEP began and ended for both muscles, and the peak to peak amplitude of the unrectified MEP and the area of the full-wave rectified MEP were then measured in each of the 20 trials (McDonnell, Ridding, & Miles, 2004).

Statistical analysis

To assess the test-retest reliability of paired pulse stimulation, intraclass correlation coefficient (ICC) were used to compare pre- and post-values for each measure, with ICC $\geq$ .75 indicating good reliability (Portney & Watkins, 2000). ICC values range from 0.00 to 1.00, and a negative intraclass correlation occurs when between-group variation is less than within-group variation, indicating some third (control) variable has introduced nonrandom effects on the different groups (Garson, 2008). When a negative ICC is obtained, the value can not be considered valid (Portney & Watkins, 2009).

ICC analysis expresses the ratio of the variance between subjects over the total variance of the group (McDonnell, et al., 2004). ICC is calculated using variance estimates obtained through an analysis of variance, reflecting both degree of correspondence and agreement among ratings (Portney & Watkins, 2009). ICCs were calculated based upon a Two Way Random, Absolute Agreement, Model. A Two Way

Random Model (Model 2) includes systematic error, assumes that each subject was assessed by the same raters, and assumes these raters were randomly sampled from the population of raters (Weir, 2005). Absolute Agreement compares both the consistency between trials and the agreement between ratings (Weir, 2005).

Consistency type was not selected as it tests solely for consistent ratings, the values do not have to agree (Ricard, 2010). The One Way Model (Model 1) was not selected as it assumes each subject is assessed by different raters than other subjects nor was the Two Way Mixed Model (Model 3) as it assumes each subject was assessed by the same raters which are the only raters of interest, and a fixed effect (Ricard, 2010).

Results

Table 1 summarizes participant demographics and MT. Motor threshold values varied slightly over the two sessions as expected. The average values of MEP size, along with their standard deviation, for each session are reported in Tables 2-9, based upon the corresponding hypothesis. As expected overall, ICF trials produced larger mean MEPs than TS only trials; ICI produced smaller mean MEPs than TS only trials; and CS responses average little to no MEP response.

Averages which did not follow the usual pattern of size were highlighted or marked with an asterisk. Some variability in responses can be attributed to uncontrollable changes in the nervous system, so these unusual responses were classified as either "expectable" or "unexpected" depending upon the frequency of their occupancy and/or size. For example, trace MEPs recorded in response to the CS in a variety of sessions were unexpected. If the averaged CS stimulus responses represented a response size of less than1% of the averaged TS response, or a trace MEP was elicited 10% or less of the trials (two or less out of 20 trials or one or less out of 10 trials) in two or less of the twenty trials (10% or less) CS data were marked with an asterisk and considered to be "expectable". If the averaged CS stimulus responses represented a size of greater than 1% of the averaged TS response, or a trace MEP was elicited in greater than 10% of the trials, CS data were highlighted in blue and considered to be "unexpected". All ICI and

ICF mean averages that did not follow the usual pattern of size when compared to TS average response size were highlight in blue and considered to be "unexpected".

The Averaged Measure ICC values are reported for each hypothesis, with $P > .05$ for all ICC values, indicating the effect is not significant and raising suspicion regarding the validity of the ICC (Portney & Watkins, 2009).

Table 1. Demographic and MT data for participants

Participant	Sex	Age (years)	Motor Threshold 1	Motor Threshold 2
PP4	F	25	60	56
PP5	F	19	55	54
PP6	M	37	55	56
PP7	F	26	40	38
PP8	F	23	45	46
PP9	F	18	47	50
PP10	F	22	54	53
PP11	F	19	63	63
PP12	F	33	40	44
PP13	F	23	42	44
PP14	F	18	44	43
PP15	F	20	50	51
PP16	F	30	53	51
PP17	F	30	50	51
PP18	F	32	45	45
Range	1M;14F	19 - 37	40 - 63	38 - 63
AVG		25.00	49.53	49.67
SD		6.09	7.07	6.40
CV			14.27%	12.88%

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Note: Motor threshold values are a percentage of total stimulator output

Hypothesis One Results

Hypothesis One sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon amplitude of MEPs from 20 trials, with the expectation that results would be stable over a period of one week in both FDI and APB. Poor reliability was found in both muscle representations.

The average amplitude of 20 MEPs from FDI elicited in response to CS were 0.004mV (±0.006), TS were 1.011mV (±0.651), 2ms were 0.322mV (±0.271), and 15ms were 1.901mV (±1.163). Specific data including mean responses and corresponding standard deviations from each individual session can be found in Table 2. ICCs calculated based upon 20 amplitude trials from FDI were 0.009 (p=0.445) for ICI and 0.185 (p=0.137) for ICF.

The average amplitude of 20 MEPs from APB elicited in response to CS were 0.002mV (±0.007), TS were 0.510mV (±0.358), 2ms were 0.150mV (±0.174), and 15ms were 1.1441mV (±0.936). Specific data including average MEP values of APB amplitude measurement, along with their standard deviation, for each session are reported in Table 3. ICCs calculated based upon 20 amplitude trials from APB were - 0.173 (p=0.691) for ICI and -0.343(p=0.954) for ICF.

Table 2 also highlights unexpected results in mean FDI amplitude findings. In PP9 second session, the average ICF response is the same size as the average TS response (.265mV and .270mV respectively). TS responses were consistently elicited in each of the 20 trials and ranged from 0.100 - 0.400mV, with the exception of two outliers that registered 1.100mV each. ICF responses were consistently elicited in each of the 20

trials and ranged 0.100 – 0.700mV with no recorded outliers. It appears the two outlying TS MEPs increased the average TS amplitude MEP to greater than the ICF average.

In PP11 first session, the average ICF response is similar to the ICI average response (0.865mV and 0.890mV respectively). ICI responses were consistently elicited in each of the 20 trials and ranged from 0.100 - 1.700mV. ICF responses were consistently elicited 19 trials and ranged from 0.100 – 4.900mV. It appears the one missing data point was enough to drag the average ICF responses to a value equal to that of the average ICI response.

In PP11 second session, the average ICI was documented to be less than the ICF value as expected but greater than the averaged MEPs evoked in response to the TS . Responses were consistently elicited in all 20 of the TS, ICI, and ICF trials , ranging from 0.200 – 2.100mV, 0.200 – 2.900mV, and 0.100 – 4.000mV respectively. The cause of the unexpected results remain unclear..

In PP12 first and second session, the average ICF was found to be smaller than the averaged TS responses. In PP12 first session, only one ICF trial elicited no response, with the other 19 responses ranging from 0.200 – 4.100mV. In contrast 5 ICI trials elicited no response, and the remaining 15 responses ranged in values from 0.100 – 1.100mV while each of the 20 TS trials elicited a response ranging from 0.300 - 4.100mV. This information does not offer a clear understanding of why ICF was found to be smaller than the average TS response, but raises the question that the average ICI amplitude may be disproportionately low due to the lack of MEP elicited in 5 trials. In PP12 second session, ICF responses were consistently elicited in each of the 20 trials and ranged from 0.100 - 3.500mV. While 7 ICI responses were missing, the 13 responses

ranged in value from 0.100 – 0.900mV and TS responses were consistently elicited in each of the 20 trials and ranged from 0.100 – 2.500mV. Again, this information does not offer a clear understanding of why ICF was found to be smaller than the average TS response, but raises the question that the average ICI amplitude may be disproportionately low due to the lack of MEP elicited in 7 trials.

In PP18 second session, the average ICF response was found to be smaller than TS average. ICF responses were recorded in 19 trials, with values ranging from 0.100 – 3.200mV, ICI responses were recorded in 18 trials, with values ranging from 0.100 – 1.400mV, and 19 values were recorded in TS trials with values ranging from 0.100 – 3.700mV. Two TS values were recorded as 3.700mV and are potential outliers that increased the overall average of the TS.

Table 2. Hypothesis One. Mean FDI Amplitude (mV) of 20 Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.005*	0.022	0.795	0.714	0.035	0.067	1.690	0.857
PP4.2	0.015	0.037	0.520	0.639	0.040	0.082	2.155	1.077
PP5.1	0.000	0.000	1.385	1.201	0.580	0.526	2.830	2.147
PP5.2	0.000	0.000	1.190	1.002	0.615	0.572	2.805	0.952
PP6.1	0.000	0.000	3.595	2.466	0.440	0.839	6.335	3.508
PP6.2	0.005*	0.022	2.005	2.195	0.210	0.554	2.805	2.411
PP7.1	0.015	0.037	0.665	0.689	0.040	0.094	2.440	1.292
PP7.2	0.000	0.000	0.120	0.101	0.060	0.182	0.500	0.773
PP8.1	0.000	0.000	1.115	0.786	0.360	0.619	2.540	1.322
PP8.2	0.000	0.000	1.340	1.192	0.345	0.372	2.695	1.917
PP9.1	0.000	0.000	0.270	0.298	0.120	0.106	0.265	0.173
PP9.2	0.000	0.000	0.320	0.161	0.260	0.216	0.360	0.139
PP10.1	0.010*	0.031	1.075	0.805	0.200	0.371	2.010	1.276
PP10.2	0.005*	0.022	0.870	0.738	0.110	0.234	1.460	0.830
PP11.1	0.000	0.000	0.995	0.944	0.890	0.782	0.865	1.199
PP11.2	0.005*	0.022	0.700	0.579	1.030	0.821	1.695	1.125
PP12.1	0.005*	0.022	1.825	1.120	0.335	0.406	1.520	1.311
PP12.2	0.000	0.000	1.120	0.686	0.160	0.237	1.025	0.880
PP13.1	0.000	0.000	1.010	0.962	0.440	0.535	1.905	1.617
PP13.2	0.005*	0.022	0.760	0.436	0.145	0.182	2.805	0.883
PP14.1	0.000	0.000	0.435	0.176	0.065	0.067	1.185	1.020
PP14.2	0.000	0.000	0.720	0.522	0.105	0.193	1.270	0.953
PP15.1	0.005*	0.022	0.870	1.064	0.705	0.502	2.745	2.066
PP15.2	0.025	0.044	1.020	1.368	0.225	0.245	2.035	1.988
PP16.1	0.005*	0.022	0.685	0.580	0.125	0.257	1.650	1.951
PP16.2	0.000	0.000	1.055	1.161	0.365	0.413	1.020	1.099
PP17.1	0.000	0.000	1.475	1.347	0.760	0.934	2.375	0.955
PP17.2	0.000	0.000	1.270	0.934	0.595	0.487	2.425	0.725
PP18.1	0.000	0.000	0.470	0.535	0.205	0.338	0.495	0.373
PP18.2	0.000	0.000	0.650	0.405	0.085	0.104	1.125	0.658
Group	0.003	0.007	1.011	0.651	0.322	0.271	1.901	1.163

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Table 3 also highlights unexpected results in mean APB amplitude findings. In PP7 second session, the average ICF response was half the size of the average TS response. TS responses were recorded in 16 trials, ranging from 0.100 – 3.500mV. ICI responses were recorded in 6 trials, with values ranging from 0.100 – 0.600mV. ICF responses were recorded in 15 trials, with values ranging from 0.100 – 1.400mV. Seeing that a comparable amount of responses were not elicited in response to TS and ICF (4 and 5 respectively), the source of variation remains unclear why the average ICF response was smaller than the average TS response.

Average TS, ICI, and ICF responses were documented to be similar in PP9 second session, 0.074, 0.075, and 0.65mV respectively. These low findings may be the result of missing data points as only 11 values, ranging from 0.100 – 0.400mV, were recorded in response to TS; 8 values were recorded in response to ICI, ranging in value from 0.100 – 0.400mV; and 13 values, ranging from 0.100 – 0.100mV were recorded in response to ICF. Such a high rate of lack of responses and small amplitude size throughout stimulation types indicate there may have been a problem with the stimulation site or coil orientation.

In PP11 second session, the average TS response was found to be smaller than the ICI average response. This discrepancy appears to be the result of an outlier value elicited in response to ICI valued at 3.900mV, which has increased the average value of ICI responses. The remainder of the 19 responses range in value from 0.100 – 1.500mV, with 3 trials that elicited no response to ICI.

In PP12 second session, the average TS and ICF are similar (0.255 and 0.250mV respectively). The cause of this unexpected result is unclear as neither TS nor ICF are

missing responses, with TS responses ranging from 0.100 – 1.600mV and ICF responses ranging from 0.100 to 1.200mV.

In PP18 first session displays a similar problem to PP12 second session in that the average TS and ICF responses are similar (0.140 and 0.130 respectively. The cause of this result is unclear. TS is missing two data points, with the remaining 18 responses ranging from 0.100 – 0.400mV. ICF responses were elicited in 19 trials, with values ranging from 0.100 – 0.300mV.

Table 3. Hypothesis One. Mean APB Amplitude (mV) of 20 Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.000	0.000	0.305	0.366	0.050	0.224	1.175	1.042
PP4.2	0.000	0.000	0.375	0.299	0.020	0.089	1.865	1.088
PP5.1	0.000	0.000	0.530	0.298	0.430	0.503	1.030	0.575
PP5.2	0.000	0.000	0.810	0.707	0.685	0.949	2.700	1.350
PP6.1	0.000	0.000	0.990	1.012	0.090	0.129	2.595	2.151
PP6.2	0.000	0.000	0.390	0.692	0.015	0.037	1.000	1.100
PP7.1	0.040*	0.157	1.545	1.550	0.045	0.089	3.365	1.774
PP7.2	0.000	0.000	0.600	0.983	0.075	0.152	0.315	0.556
PP8.1	0.000	0.000	0.640	0.573	0.155	0.170	2.480	1.544
PP8.2	0.000	0.000	0.370	0.495	0.065	0.081	1.125	0.842
PP9.1	0.000	0.000	0.080	0.089	0.040	0.050	0.085	0.059
PP9.2	0.000	0.000	0.074	0.093	0.075	0.125	0.065	0.049
PP10.1	0.000	0.000	0.250	0.233	0.030	0.057	1.010	1.063
PP10.2	0.005*	0.022	0.155	0.100	0.045	0.076	0.605	0.679
PP11.1	0.000	0.000	0.885	1.015	0.475	0.549	1.260	1.243
PP11.2	0.000	0.000	0.595	0.798	0.605	0.915	0.665	0.623
PP12.1	0.000	0.000	0.525	0.520	0.065	0.059	0.590	0.664
PP12.2	0.000	0.000	0.255	0.289	0.040	0.050	0.250	0.244
PP13.1	0.000	0.000	0.225	0.275	0.170	0.205	0.390	0.415
PP13.2	0.000	0.000	1.060	1.094	0.175	0.171	2.880	1.105
PP14.1	0.000	0.000	0.270	0.189	0.105	0.128	0.905	0.798
PP14.2	0.000	0.000	0.285	0.193	0.130	0.175	0.605	0.537
PP15.1	0.000	0.000	0.855	0.594	0.090	0.141	2.520	1.290
PP15.2	0.000	0.000	0.670	0.470	0.070	0.117	1.075	0.788
PP16.1	0.000	0.000	0.135	0.176	0.030	0.080	0.325	0.672
PP16.2	0.000	0.000	0.055	0.089	0.040	0.075	0.125	0.215
PP17.1	0.000	0.000	0.960	0.536	0.180	0.154	1.310	0.908
PP17.2	0.000	0.000	0.825	0.789	0.285	0.436	1.335	0.970
PP18.1	0.000	0.000	0.140	0.105	0.110	0.121	0.130	0.073
PP18.2	0.005	0.022	0.460	0.250	0.105	0.105	0.540	0.492
Group	0.000	0.001	0.510	0.358	0.150	0.174	1.144	0.936

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Hypothesis Two Results

Hypothesis Two sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon area under the curve of MEPs from 20 trials, with the expectation that the results would be stable over a period of one week in both FDI and APB. Poor reliability was found in both muscle representations

The average area of 20 MEPs from FDI elicited in response to CS were 0.009mVms (±0.012), TS were 3.253mVms (±2.162), 2ms were 1.033mVms (±0.949), and 15ms were 6.642mVms (±4.307). The average values of MEP FDI area measurement, along with their standard deviation, for each session are reported in Table 4. ICCs calculated based upon 20 area trials from FDI were - 0.007 (p=0.470) for ICI and 0.214 (p=0.110) for ICF.

The average area of 20 MEPs from APB elicited in response to CS were 0.007mVms (±0.027), TS were 1.987mVms (±1.379), 2ms were 0.581mVms (±0.761), and 15ms were 4.679mVms (±3.562). The average MEP values of APB area measurement, along with their standard deviation, for individual sessions are reported in Table 5. ICCs calculated based upon 20 area trials from APB were - 0.234 (p=0.741) for ICI and -0.353 (p=0.913) for ICF.

Table 4 also highlights unexpected results in the mean FDI area findings. In PP9 first session, the average ICF response is smaller than the average TS response. This may be due in part to two outlier values recorded in response to TS that may have increased the overall average of TS responses. The outliers were valued at 3.00 and 2.500mVms, with the remaining 18 values ranging from 0.200 – 1.200mVms. ICF also elicited

responses to each of the 20 trials, with values ranging from 0.200 – 1.600mVms, with no outliers noted.

PP11 second session resulted in ICI average response greater than the averaged TS response. This may be due in part to two outlier values recorded in response to ICI, which were valued at 10.100 and 11.700mVms. The remaining 18 values ranged from 0.700 – 8.300mVms. Neither TS nor ICI were missing MEP responses, and TS responses ranged from 0.500mVms – 7.600mVms.

PP12 first and second session resulted in ICF average responses that were less than those elicited in response to the TS. Neither PP12 sessions were missing TS responses, with PP first session TS values ranging from 0.800 – 10.500mVms and PP12 second session TS values ranging from 1.000 – 6.700mVms. PP12 first session was missing one ICF response, which may have accounted for the decreased averaged ICF response. The recorded 19 ICF responses ranged in value from 0.600 – 13.000mVms. PP12 second session ICF responses were elicited in all 20 trials, with values ranging from 0.300 – 11.700mVms. The source of the discrepancy between TS and ICF in the second PP12 session remains unclear.

PP18 first session also resulted in an average ICF area that was smaller than the average TS area. Neither stimulations were missing data points, but this may be due in part to two outlier values elicited in response to TS, with values of 6.200 and 6.700mVms. The remaining 18 TS values ranged from 0.500 – 3.900mVms. ICF values also ranged from 0.500 – 3.900mVms.

Table 4. Hypothesis Two. Mean FDI Area (mVms) of 20 Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.015*	0.067	2.915	3.027	0.150	0.204	6.545	3.827
PP4.2	0.015*	0.037	1.760	2.448	0.090	0.162	9.095	4.984
PP5.1	0.000	0.000	4.325	3.788	1.835	1.725	9.270	7.642
PP5.2	0.000	0.000	3.515	2.921	1.775	1.704	8.800	3.309
PP6.1	0.000	0.000	11.955	9.450	1.450	2.990	23.645	14.647
PP6.2	0.010*	0.045	6.240	7.133	0.640	1.523	9.490	8.619
PP7.1	0.040	0.075	1.745	1.989	0.120	0.191	6.390	3.696
PP7.2	0.000	0.000	0.305	0.265	0.200	0.558	1.525	2.746
PP8.1	0.005*	0.022	4.015	2.849	1.250	2.416	8.710	4.725
PP8.2	0.005*	0.022	5.995	4.871	1.435	1.628	11.795	8.229
PP9.1	0.000	0.000	0.755	0.744	0.310	0.265	0.690	0.393
PP9.2	0.010*	0.045	0.910	0.382	0.725	0.500	0.960	0.413
PP10.1	0.030*	0.098	3.150	2.299	0.535	0.999	6.725	4.880
PP10.2	0.010	0.045	2.585	2.149	0.315	0.678	4.725	2.690
PP11.1	0.000	0.000	4.055	3.967	3.320	3.115	3.900	6.638
PP11.2	0.010*	0.045	2.845	2.206	4.000	3.220	8.240	6.278
PP12.1	0.010*	0.045	4.665	2.927	0.855	0.985	4.045	3.661
PP12.2	0.000	0.000	3.000	1.842	0.450	0.686	2.765	2.779
PP13.1	0.000	0.000	3.000	2.923	1.330	1.464	6.235	5.526
PP13.2	0.010*	0.045	2.090	1.215	0.460	0.490	9.225	3.136
PP14.1	0.010*	0.031	1.560	0.722	0.255	0.287	5.165	5.108
PP14.2	0.000	0.000	2.300	1.752	0.365	0.652	4.840	3.944
PP15.1	0.015*	0.049	2.790	3.559	2.160	1.742	9.175	7.072
PP15.2	0.050	0.095	3.500	4.827	0.770	0.831	7.355	7.378
PP16.1	0.010*	0.045	2.025	1.638	0.415	0.741	6.035	8.181
PP16.2	0.000	0.000	3.820	4.164	1.380	1.518	3.845	4.190
PP17.1	0.000	0.000	4.400	4.562	1.990	2.753	7.245	3.640
PP17.2	0.000	0.000	3.770	3.129	1.605	1.313	7.410	2.780
PP18.1	0.000	0.000	1.525	2.022	0.575	1.043	1.400	1.205
PP18.2	0.010*	0.031	2.075	1.348	0.235	0.243	4.015	2.933
Group	0.006	0.015	3.253	2.162	1.033	0.949	6.642	4.307

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Table 5 also highlights unexpected results in mean APB area findings. In PP7 second session, ICF was found to be less than TS. TS average was missing 4 responses, while 3 outliers were found valued at 7.600, 10.200, and 13.900mVms, and remaining responses ranged from 0.200 – 2.900mVms. ICF was missing one data point with the remaining responses ranging from 0.100 – 7.900mVms. It would appear the outliers in response to TS increased the average.

PP9 session two resulted in similar TS, ICI and ICF responses (0.250, 0.275, and 0.230 respectively. Responses to 3 TS trials were nil, with the remaining 17 responses ranging from 0.100 – 0.400mVms. Responses to 3 ICI trials were nil, with the remaining 17 responses ranging from 0.100 – 0.600mVms. ICF trials yielded 20 responses ranging from 0.100 - 0.600mVms. Given this information , the cause of the unexpected average responses remains unclear.

PP12 session two and PP18 session one resulted in ICF average response that are smaller than the TS average response without a clear indication why. In PP12 session two, neither TS nor ICF trials were missing response and both TS and ICF had outliers of 5.200 and 5.300 respectively. TS responses ranged in value from 0.200 – 2.600mVms and ICF responses ranged in value from 0.200 – 2.200mVms. The cause of smaller ICF responses in PP12 session two is unclear. PP18 session one is similar in that only one TS response is missing, with the remaining values ranging from 0.200 – 1.800mVms. ICF values range from 0.100 – 1.600mVms, with no missing responses. Again, the cause of smaller ICF values in PP18 session one is unclear.

Table 5. Hypothesis Two. Mean APB Area (mVms) of 20 Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.000	0.000	1.200	1.597	0.235	0.934	4.745	4.334
PP4.2	0.005	0.022	1.585	1.456	0.100	0.288	8.435	5.984
PP5.1	0.000	0.000	1.810	1.079	1.480	1.803	3.415	2.193
PP5.2	0.005*	0.022	2.710	2.629	2.320	3.684	9.370	5.176
PP6.1	0.000	0.000	4.300	4.338	0.310	0.467	10.365	8.468
PP6.2	0.005*	0.022	1.835	3.251	0.050	0.089	5.175	5.856
PP7.1	0.150	0.467	4.770	5.097	0.175	0.275	11.955	6.069
PP7.2	0.000	0.000	2.170	3.911	0.245	0.508	1.070	1.981
PP8.1	0.005*	0.022	2.320	2.143	0.465	0.550	10.160	6.510
PP8.2	0.000	0.000	1.465	2.199	0.225	0.257	4.840	3.635
PP9.1	0.000	0.000	0.315	0.291	0.135	0.099	0.320	0.214
PP9.2	0.000	0.000	0.250	0.254	0.275	0.286	0.230	0.108
PP10.1	0.005*	0.022	0.870	0.832	0.105	0.209	4.015	4.372
PP10.2	0.010*	0.045	0.480	0.298	0.150	0.242	2.170	2.914
PP11.1	0.000	0.000	4.805	5.896	2.315	2.964	7.025	9.173
PP11.2	0.000	0.000	2.965	4.215	3.215	5.882	3.395	3.828
PP12.1	0.000	0.000	2.240	2.464	0.260	0.182	2.455	3.026
PP12.2	0.000	0.000	0.915	1.170	0.120	0.120	0.850	1.128
PP13.1	0.000	0.000	0.595	0.724	0.530	0.633	1.080	1.116
PP13.2	0.000	0.000	3.490	4.076	0.540	0.526	10.510	4.727
PP14.1	0.011*	0.032	1.065	1.153	0.420	0.580	5.280	7.471
PP14.2	0.000	0.000	0.885	0.701	0.415	0.654	3.195	4.146
PP15.1	0.000	0.000	3.465	2.385	0.305	0.533	10.015	4.961
PP15.2	0.000	0.000	2.935	2.465	0.205	0.328	4.710	3.779
PP16.1	0.000	0.000	0.490	0.678	0.150	0.242	1.330	2.701
PP16.2	0.000	0.000	0.255	0.233	0.245	0.503	0.520	0.909
PP17.1	0.000	0.000	3.620	2.100	0.555	0.551	4.955	3.660
PP17.2	0.000	0.000	3.250	3.302	1.070	1.934	5.645	4.703
PP18.1	0.000	0.000	0.570	0.504	0.385	0.489	0.470	0.422
PP18.2	0.010*	0.045	1.990	1.152	0.430	0.391	2.665	2.459
Group	0.007	0.031	1.987	1.379	0.581	0.761	4.679	3.562

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Hypothesis Three Results

Hypothesis Three sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon amplitude of MEPs from 10 trials, with the expectation that the results would be stable over a period of one week in both FDI and APB.. Poor reliability was found in both muscle representations

The average amplitude of 10 randomly selected MEPs from FDI elicited in response to CS were 0.004mV (±0.008), TS were 1.001mV (±0.665), 2ms were 0.347mV (±0.300), and 15ms were 1.973mV (±1.247). Specific data including mean FDI responses from 10 randomly selected trials, along with their standard deviations, are reported for each session in Table 6. ICCs calculated based upon 10 randomly selected amplitude trials from FDI were 0.105 (p=0.319) for ICI and 0.006 (p=0.361) for ICF.

The average amplitude of 10 randomly selected MEPs from APB elicited in response to CS were 0.000mV (±0.002), TS were 0.472mV (±0.337), 2ms were 0.146mV (±0.163), and 15ms were 1.144mV (±0.905). Specific data including mean APB responses from 10 randomly selected trials, along with their standard deviations, are reported for each session in Table 7. ICCs calculated based upon 10 randomly selected amplitude trials from APB were -0.016 (p=0.457) for ICI and -0.508 (p=0.921) for ICF.

Table 6 and Table 7 also highlights unexpected results in the mean FDI amplitude and mean APB amplitude findings, but because these are 10 randomly selected trials from the full 20 trials, please see the unexpected results described in Table 2 and Table 3 respectively.

Table 6. Hypothesis Three. Mean FDI Amplitude (mV) of 10 Random Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.000	0.000	0.830	0.710	0.050	0.071	1.850	0.916
PP4.2	0.030	0.048	0.600	0.643	0.040	0.084	2.060	1.201
PP5.1	0.000	0.000	1.130	0.800	0.660	0.534	3.400	2.104
PP5.2	0.000	0.000	0.970	0.826	0.590	0.674	2.920	1.107
PP6.1	0.000	0.000	3.230	2.991	0.190	0.242	6.200	3.605
PP6.2	0.010*	0.032	2.100	2.506	0.280	0.721	3.380	2.029
PP7.1	0.020	0.042	0.410	0.381	0.060	0.126	2.490	1.705
PP7.2	0.000	0.000	0.120	0.079	0.030	0.067	0.530	0.872
PP8.1	0.000	0.000	1.140	0.824	0.540	0.850	2.430	1.590
PP8.2	0.000	0.000	1.410	0.692	0.540	0.403	3.610	2.289
PP9.1	0.000	0.000	0.250	0.314	0.140	0.135	0.260	0.184
PP9.2	0.000	0.000	0.320	0.169	0.310	0.296	0.360	0.143
PP10.1	0.010*	0.032	1.160	0.962	0.230	0.316	2.230	1.119
PP10.2	0.010*	0.032	0.980	0.888	0.190	0.314	1.460	1.033
PP11.1	0.000	0.000	0.830	1.025	1.050	0.610	0.660	0.813
PP11.2	0.010*	0.032	0.700	0.622	1.020	0.649	1.090	0.852
PP12.1	0.010*	0.032	2.180	1.210	0.310	0.381	1.470	1.702
PP12.2	0.000	0.000	1.310	0.599	0.090	0.099	1.160	0.746
PP13.1	0.000	0.000	0.730	0.607	0.610	0.689	1.590	1.193
PP13.2	0.000	0.000	0.940	0.497	0.120	0.162	2.920	0.719
PP14.1	0.000	0.000	0.430	0.189	0.040	0.052	0.890	0.572
PP14.2	0.000	0.000	0.530	0.403	0.040	0.052	1.250	1.044
PP15.1	0.000	0.000	0.880	1.060	0.680	0.535	3.080	2.199
PP15.2	0.020	0.042	0.550	0.692	0.260	0.272	1.940	2.180
PP16.1	0.000	0.000	0.620	0.594	0.200	0.353	2.150	2.651
PP16.2	0.000	0.000	1.430	1.454	0.370	0.377	0.970	1.050
PP17.1	0.000	0.000	1.780	1.624	0.810	0.711	2.490	1.108
PP17.2	0.000	0.000	1.450	1.083	0.700	0.521	2.380	0.808
PP18.1	0.000	0.000	0.420	0.509	0.180	0.305	0.550	0.433
PP18.2	0.000	0.000	0.590	0.401	0.080	0.079	1.420	0.767
Group	0.003	0.008	1.001	0.665	0.347	0.300	1.973	1.247

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Table 7. Hypothesis Three. Mean APB Amplitude (mV) of 10 Random Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.000	0.000	0.300	0.183	0.000	0.000	1.210	1.141
PP4.2	0.000	0.000	0.360	0.303	0.040	0.126	2.000	1.184
PP5.1	0.000	0.000	0.470	0.258	0.520	0.639	1.290	0.617
PP5.2	0.000	0.000	0.610	0.761	0.600	0.745	2.760	1.384
PP6.1	0.000	0.000	0.670	0.640	0.060	0.097	2.150	1.879
PP6.2	0.000	0.000	0.300	0.400	0.020	0.042	1.100	1.187
PP7.1	0.010*	0.032	0.850	0.974	0.030	0.095	3.220	2.021
PP7.2	0.000	0.000	0.250	0.276	0.110	0.202	0.400	0.678
PP8.1	0.000	0.000	0.410	0.423	0.220	0.220	2.830	1.914
PP8.2	0.000	0.000	0.530	0.660	0.100	0.082	1.130	0.850
PP9.1	0.000	0.000	0.040	0.052	0.040	0.052	0.080	0.063
PP9.2	0.000	0.000	0.056	0.053	0.110	0.166	0.070	0.048
PP10.1	0.000	0.000	0.250	0.172	0.040	0.070	1.020	1.018
PP10.2	0.000	0.000	0.130	0.048	0.050	0.053	0.790	0.889
PP11.1	0.000	0.000	1.180	1.274	0.580	0.658	1.530	1.489
PP11.2	0.000	0.000	0.680	1.038	0.330	0.450	0.370	0.333
PP12.1	0.000	0.000	0.520	0.476	0.070	0.067	0.370	0.340
PP12.2	0.000	0.000	0.320	0.397	0.030	0.048	0.280	0.329
PP13.1	0.000	0.000	0.130	0.142	0.190	0.247	0.350	0.504
PP13.2	0.000	0.000	1.330	1.433	0.220	0.199	2.490	0.805
PP14.1	0.000	0.000	0.290	0.120	0.060	0.097	0.800	0.490
PP14.2	0.000	0.000	0.330	0.157	0.160	0.222	0.600	0.485
PP15.1	0.000	0.000	0.710	0.489	0.110	0.160	2.330	1.268
PP15.2	0.000	0.000	0.600	0.540	0.040	0.070	0.830	0.780
PP16.1	0.000	0.000	0.160	0.212	0.010	0.032	0.470	0.938
PP16.2	0.000	0.000	0.090	0.110	0.050	0.097	0.170	0.298
PP17.1	0.000	0.000	1.070	0.542	0.140	0.126	1.400	0.816
PP17.2	0.000	0.000	0.880	0.873	0.260	0.353	1.430	1.002
PP18.1	0.000	0.000	0.120	0.123	0.110	0.137	0.110	0.057
PP18.2	0.000	0.000	0.510	0.281	0.090	0.099	0.730	0.638
Group	0.000	0.000	0.472	0.337	0.146	0.163	1.144	0.905

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Hypothesis Four Results

Hypothesis Four sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon area under the curve of MEPs from 10 trials, with the expectation that the results would be stable over a period of one week in both FDI and APB. Poor reliability was found in both muscle representations

The average area of 10 randomly selected MEPs from FDI elicited in response to CS were 0.008mVms (±0.014), TS were 3.249mVms (±2.263), 2ms were 1.108mVms (±1.045), and 15ms were 6.847mVms (±4.622). Specific area data including mean FDI responses from 10 randomly selected trials, along with their standard deviations, are reported for each session in Table 8. ICCs calculated based upon 10 randomly selected area trials from FDI were 0.192 (p=0.210) for ICI and 0.131 (p=0.275) for ICF.

The average area of 10 randomly selected MEPs from APB elicited in response to CS were 0.004mVms (±0.015), TS were 1.870mVms (±1.479), 2ms were 0.552mVms (±0.676), and 15ms were 4.698mVms (±3.564). Specific area data including mean APB responses from 10 randomly selected trials, along with their standard deviations, are reported for each session in Table 9. ICCs calculated based upon 10 randomly selected area trials from APB were -0.007 (p=0.447) for ICI and -0.057 (p=0.895) for ICF.

Table 8 and Table 9 also highlight unexpected results in the mean FDI area and mean APB area findings, but because these are 10 randomly selected trials from the full 20 trials, please see the unexpected results described in Table 4 and Table 5 respectively.

Table 8. Hypothesis Four. Mean FDI Area (mVms) of 10 Random Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.000	0.000	3.040	3.040	0.190	0.197	7.420	4.366
PP4.2	0.030	0.048	2.190	2.714	0.090	0.166	8.260	5.110
PP5.1	0.000	0.000	3.580	2.576	2.100	1.807	11.120	7.533
PP5.2	0.000	0.000	2.830	2.330	1.760	2.017	9.250	3.854
PP6.1	0.000	0.000	11.260	11.828	0.530	0.698	23.140	15.251
PP6.2	0.020*	0.063	6.810	8.392	0.800	1.954	11.070	6.723
PP7.1	0.060	0.084	1.050	1.073	0.150	0.268	6.600	4.757
PP7.2	0.000	0.000	0.300	0.163	0.100	0.170	1.680	3.149
PP8.1	0.010*	0.032	4.180	3.007	1.970	3.309	8.150	5.592
PP8.2	0.000	0.000	6.510	3.254	2.260	1.735	15.640	9.918
PP9.1	0.000	0.000	0.670	0.855	0.360	0.350	0.670	0.437
PP9.2	0.000	0.000	0.870	0.362	0.870	0.673	0.970	0.460
PP10.1	0.020*	0.063	3.350	2.688	0.600	0.845	7.430	4.188
PP10.2	0.020*	0.063	2.840	2.570	0.530	0.925	4.840	3.427
PP11.1	0.000	0.000	3.350	4.261	3.780	2.157	2.610	3.279
PP11.2	0.020*	0.063	2.690	2.288	3.960	2.560	4.990	4.737
PP12.1	0.020*	0.063	5.580	3.081	0.780	0.884	3.870	4.650
PP12.2	0.000	0.000	3.580	1.689	0.240	0.272	3.070	2.096
PP13.1	0.000	0.000	2.080	1.713	1.800	1.845	5.070	3.700
PP13.2	0.000	0.000	2.630	1.406	0.410	0.486	9.630	2.688
PP14.1	0.000	0.000	1.540	0.830	0.140	0.117	3.680	2.625
PP14.2	0.000	0.000	1.670	1.196	0.150	0.232	4.700	4.373
PP15.1	0.000	0.000	2.850	3.591	2.100	1.769	10.370	7.741
PP15.2	0.030	0.048	1.800	2.349	0.950	0.981	7.040	8.208
PP16.1	0.000	0.000	1.820	1.715	0.610	1.021	8.490	11.097
PP16.2	0.000	0.000	5.130	5.185	1.410	1.457	3.610	3.880
PP17.1	0.000	0.000	5.570	5.466	1.960	1.867	7.800	4.413
PP17.2	0.000	0.000	4.380	3.628	1.890	1.354	7.380	3.289
PP18.1	0.000	0.000	1.420	2.036	0.530	0.879	1.590	1.388
PP18.2	0.000	0.000	1.910	1.259	0.220	0.199	5.280	3.628
Group	0.005	0.014	3.249	2.263	1.108	1.045	6.847	4.622

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Table 9. Hypothesis Four. Mean APB Area (mVms) of 10 Random Trials

	CS		TS		2ms		15ms	
Subject.Session	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**	**AVG**	**SD**
PP4.1	0.000	0.000	1.140	0.869	0.030	0.048	4.680	4.502
PP4.2	0.010*	0.032	1.520	1.372	0.170	0.403	9.240	7.615
PP5.1	0.000	0.000	1.630	0.929	1.760	2.305	4.270	2.424
PP5.2	0.000	0.000	1.970	2.769	1.900	2.958	9.650	5.175
PP6.1	0.000	0.000	2.820	2.990	0.210	0.335	9.330	7.889
PP6.2	0.010*	0.032	1.360	1.836	0.060	0.107	6.360	6.050
PP7.1	0.080	0.114	2.590	3.411	0.140	0.341	11.300	6.963
PP7.2	0.000	0.000	0.750	0.889	0.340	0.679	1.380	2.494
PP8.1	0.010	0.032	1.490	1.714	0.660	0.724	11.730	8.088
PP8.2	0.000	0.000	2.160	2.949	0.310	0.251	4.910	3.641
PP9.1	0.000	0.000	0.160	0.097	0.130	0.095	0.280	0.204
PP9.2	0.000	0.000	0.200	0.141	0.360	0.372	0.250	0.135
PP10.1	0.000	0.000	0.900	0.742	0.170	0.275	4.070	4.144
PP10.2	0.000	0.000	0.400	0.189	0.180	0.204	3.050	3.912
PP11.1	0.000	0.000	6.450	7.145	2.960	3.590	8.950	11.932
PP11.2	0.000	0.000	3.510	5.440	1.770	3.291	1.690	1.635
PP12.1	0.000	0.000	2.170	2.081	0.270	0.142	1.350	1.421
PP12.2	0.000	0.000	1.170	1.490	0.110	0.120	0.980	1.527
PP13.1	0.000	0.000	0.340	0.425	0.600	0.804	1.010	1.356
PP13.2	0.000	0.000	4.550	5.439	0.620	0.575	8.550	3.113
PP14.1	0.000	0.000	0.970	0.508	0.190	0.202	3.930	4.174
PP14.2	0.000	0.000	0.960	0.675	0.540	0.880	3.040	3.588
PP15.1	0.000	0.000	3.030	2.154	0.350	0.633	9.330	4.919
PP15.2	0.000	0.000	2.590	2.918	0.120	0.155	3.590	3.697
PP16.1	0.000	0.000	0.540	0.744	0.090	0.160	1.930	3.774
PP16.2	0.000	0.000	0.360	0.280	0.320	0.699	0.700	1.278
PP17.1	0.000	0.000	4.000	2.076	0.400	0.429	5.180	2.999
PP17.2	0.000	0.000	3.560	3.822	0.940	1.632	6.140	4.915
PP18.1	0.000	0.000	0.510	0.540	0.400	0.604	0.370	0.287
PP18.2	0.000	0.000	2.290	1.294	0.460	0.458	3.700	3.114
Group	0.003	0.015	1.870	1.479	0.552	0.676	4.698	3.564

Note: Data marked with * indicates expectable findings. Data highlighted in grey indicates unexpected findings.

Table 10. Raw vs Normalized Averaged Measure Intraclass Correlation Coefficients

Raw ICCs	FDI 2ms	FDI 15ms	APB 2ms	APB 15ms
Hyp 1 (20AMP)	0.009	0.185	-0.173	-0.343
Hyp2 (20Area)	-0.007	0.214	-0.234	-0.353
Hyp3 (10AMP)	0.105	0.060	-0.016	-0.508
Hyp 4 (10Area)	0.192	0.131	-0.007	-0.057

Normalized ICCs	FDI 2ms	FDI 15ms	APB 2ms	APB 15ms
Hyp 1 (20AMP)	0.101	-0.053	0.252	-0.123
Hyp2 (20Area)	0.046	-0.049	0.177	-0.145
Hyp3 (10AMP)	0.347	-0.173	0.342	-0.315
Hyp 4 (10Area)	0.332	-0.078	0.347	-0.305

Discussion

The aim of this study was to establish the reproducibility of different methods of analysis of MEPs elicited in response to paired pulse TMS in two hand muscles. Overall, CS, TS, ICI, and ICF characteristics followed a usual pattern of size. ICI resulted in a MEP with a smaller amplitude than was elicited by the TS alone, while ICF resulted in a MEP with an increased amplitude in comparison to the MEP elicited by the TS alone. However, poor test retest reliability was found regardless of the method of analysis or number of trials averaged. Overall, raw data indicated that higher test retest reliability could be established from MEPs elicited from FDI than APB while normalized data indicated ICI findings had increased reliable when compared to ICF data. Comparing results based upon peak to peak analysis versus amplitude analysis did not offer a clear indication of one method provided higher reliability than the other.

The experiment for Hypothesis One sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon amplitude of MEPs from 20 trials. We generally found that ICF had higher reliability in both FDI and APB than ICI. This is in contrast to findings of Maeda et al (2002), whose data indicated that not ICF, but ICI may be reproducible.

Interestingly, APB ICC for both ICI and ICF were negative, indicating that a third control variable introduced nonrandom effects. Responses to TMS are sensitive to various factors that may be difficult to control across experimental sessions, including

precise replication of the position of the recoding electrodes thus different populations of muscle fibers and cortico-spinal cells may be studied across experimental sessions (Carroll, et al., 2001). Muscle response's recorded from FDI and APB have been found to be more susceptible to electrode placement variability than more proximal muscles found in the forearm. Intrinsic hand muscles are smaller, have a higher density of motor units, and a comparatively more complex neural representation than forearm muscles (Malcolm, et al., 2006); with a greater number of cortico-motoneuronal connections (Brasilneto, McShane, Fuhr, Hallett, & Cohen, 1992). One method used to minimize the effects of electrode placement variability is known as normalizing data. This "normalizing" occurs by expressing the average ICI and ICF values over the average TS values. Please see Table 10 which displays ICC coefficients based upon normalized data related to each of the four hypotheses.

The experiment for Hypothesis Three sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon amplitude of MEPs from 10 trials. The data indicated similar findings as Hypothesis One, indicating that already low ICC values are not improved by increasing the number of trials used to calculate the mean response. This is in direct contrast to previous studies that have documented decreased variability when trials were increased from 5- 20 (Boroojerdi, et al., 2000).

The experiment for Hypothesis Two sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon area under the curve of MEPs from 20 trials. We generally found that area under the curve was less reliable than amplitude measurement. An intraclass correlation coefficients study that assessed the reliability of alternate methods of analysis of MEPs including the two methods used in this study

(peak to peak amplitude and area under the curve) found poor reliability of MEP measures in FDI over time regardless of the method used (McDonnell, et al., 2004). Single pulse stimulation was used in this study at 110% and 120% of MT and MT was defined as the TMS intensity that elicited MEPs in 5 out of 10 trials (McDonnell, et al., 2004).

Again, ICI and ICF ICCs based upon MEPs elicited in APB were negative raising question to the possibility of that a third control variable introduced nonrandom effects, as was ICI based upon MEPs elicited in FDI. When data was normalized, FDI ICI ICC and APB ICI ICC became positive, while APB ICF remained negative.

The experiment for Hypothesis Four sought to determine the test-retest reliability of ICI and ICF in healthy individuals based upon area under the curve of MEPs from 10 trials. The data indicated similar findings as Hypothesis Two. These results indicate that already low ICC values are not improved by increasing the number of trials used to calculate the mean response, which is in direct contrast to previous studies that have documented decreased variability when trials were increased from 5- 20 (Boroojerdi, et al., 2000).

An unresolved issue in trying to improve the quality of the data is the number of TMS pulses applied to generate a set of baseline parameters, it has to be considered that assessment of baseline measures itself might influence the very same measures even during the probing phase of an experiment (Paulus, et al., 2008). In this study, it was necessary to determine the motor hot spot and motor threshold prior to collecting and recording MEPs in response to the four different stimuli. The influence, if any, establishing these baseline parameters had on the data that was collected is unknown.

The CS, TS, ICI, and ICF measures were calculated based upon the stimulation intensity determined to be representative of MT. MT is a global measure of corticospinal excitability and depends on the excitability of axons activated by the TMS pulse, as well as the excitability of synaptic connections at both the cortical and spinal level (Paulus, et al., 2008). Whether or not measuring MEPs to a single TMS intensity is objective, reliable and valid as a sole measure of excitability remains unclear, as there are rather few studies of the stability of single-pulse MEPs over repeated sessions, especially over hours and even days (Paulus, et al., 2008). Wassermann (2002) examined MT for the FDI and APB and concluded that there is a wide variability in MT over time, while other studies have found that motor threshold had a low coefficient of variation (Maeda, et al., 2002) (Wolf, et al., 2004).

Upon establishing MT, careful determination of CS is an equally important factor in a paired pulse TMS study. While unexpected CS response findings did not offer conclusive information suggesting the CS was too strong to be considered subthreshold, it is noteworthy. Alternative reasons why MEPs were elicited in response to a subthreshold stimulus include changes in the muscle state (contracted vs. relaxed), changes in the arousal level of the person, or unknown physiological changes in the nervous system.

Considering that increasing the CS intensity to motor threshold or above results in less suppression or even facilitation (Kujirai, et al., 1993), ensuring the CS is at a subthreshold level is imperative. A study investigating corticocortical inhibition in the human motor cortex in ten healthy subjects found that the intensity of both conditioning and test stimulus influenced the amount of suppression, with the best suppression

(inhibition) seen with a small CS of 0.7-0.9 times MT in a relaxed muscle, with maximum suppression of the test response occurred with a conditioning intensity of 0.8 times threshold (Kujirai, et al., 1993). Increasing the conditioning strength even further resulted in less suppression, and at suprathreshold intensities, the suppression was replaced by facilitation.

In healthy populations that require lower percentage of TMS stimulation maximum output, it may be beneficial to define CS in the more conservative range of 0.8 times MT when considering the effect it has on ICI. The average MT for subjects over both sessions in this study was 49.6% of maximum stimulator output, giving us a CS that averaged 5% less than MT. Had we defined CS as 0.8 times MT instead of 0.9 times MT our CS on average would have been 10% less than MT.

Interestingly, the determination of CS as it relates to ICF has different implications. The same study mentioned above investigated the effect of conditioning intensity at the longer interstimulus interval of 15 ms (ICF) and found at this interval, test responses were facilitated when the strength of the conditioning stimulus was 0 9 times threshold or above (Kujirai, et al., 1993).

We attempted to control some of the factors that are known to affect variability such as the level of alertness (Kiers, Cros, Chiappa, & Fang, 1993), muscle relaxation (Thickbroom, Byrnes, & Mastaglia, 1999), and attention to the stimulus (Hess, Mills, & Murray, 1987) by hosting sessions in a quiet room with participants seated in a comfortable position and provided with EMG feedback. The state of excitability of the participants may also vary across sessions because of factors that are difficult to control for such as prior activity, diurnal (daily activity) variations, and prior consumption of

food or drugs (Carroll, et al., 2001) such as coffee or alcohol that could potentially influence measures (Paulus, et al., 2008).

Limitations and recommendations

Our sample is limited by not only size, but it does not allow examination of the effect of handiness, as our experimental design included right handed participants only. Recruitment of male participants was difficult, of the 62 people who contacted the researcher to participate in the study, only nine were males. Of those nine males, three were not enrolled due to sustaining a head injury that resulted in the loss of consciousness for any amount of time, one was over the 50 year age maximum, and three did not return phone calls after making initial contact expressing interest in the study. Female subject's motor cortical excitability varies with their menstrual cycle. Because the menstrual cycle may account for significant variability (>20%) in intracortical inhibition, it represents a substantial potential confound that should be controlled for in paired-pulse TMS studies that include menstruating women (Smith, et al., 1999). Future studies interested in investigating reliability of paired pulse TMS should consider monitoring subject's menstrual cycle and schedule sessions accordingly.

Our results are limited to the methodological differences that define our study such as the type of coil, stimulation parameters, and two distal muscles selected for investigation in this study. Our findings highlight the need for continued research regarding the reproducibility of MEPs elicited in response to paired pulse stimulation as these results have particular relevance for studies of patients with neurological and psychiatric disorders aimed at assessing differences or studying the course of a disease or treatment outcomes (Maeda, et al., 2002).

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Appendix A: Health history questionnaire

NEUROREHABILITATION RESEARCH LABORATORY
COLORADO STATE UNIVERSITY
CONFIDENTIAL HEALTH HISTORY QUESTIONNAIRE

The information obtained in this questionnaire will be kept confidential and in a secure area.
Please do NOT write your name on these forms.

STUDY_____ **DATE**_____ **SUBJECT ID #**_____

Reviewed by :_____

Age_____ **Birthday**_____ **Gender**_____

HAND PREFERENCE

Are you right or left handed? RIGHT ☐ LEFT ☐
Which hand do you write with? RIGHT ☐ LEFT ☐

Do you use one hand for some activities and the other for other activities? YES ☐ NO ☐
If Yes, please explain:

GENERAL MEDICAL HISTORY

Do you have any current medical conditions? YES ☐ NO ☐
If Yes, please explain:

Have you had any major illnesses in the past? YES ☐ NO ☐
If Yes, please explain:

Have you ever been hospitalized or had surgery? YES ☐ NO ☐
If Yes, please explain: (include date and type of surgery, if possible)

Have you ever had a seizure? YES ☐ NO ☐
If Yes, please explain:

Have you been diagnosed with epilepsy? YES ☐ NO ☐
If Yes, please explain:

Do you have a family history of epilepsy? YES ☐ NO ☐
If Yes, please explain:

Have you ever had a head injury resulting in the loss of consciousness for any amount of time? YES ☐ NO ☐
If Yes, please explain:

Have you ever lost consciousness for any reason? YES ☐ NO ☐
If Yes, please explain:

Are you or do you believe you could be pregnant? YES ☐ NO ☐

GENERAL MEDICAL HISTORY (continued)

Have you had an alcohol or drug abuse problem within the past year? YES ☐ NO ☐

Do you have a history of heart disease? YES ☐ NO ☐
If Yes, please explain:

Have you ever had a fainting episode? YES ☐ NO ☐
If Yes, please explain:

Have you ever been diagnosed with any of the following?

Traumatic Brain Injury	YES ☐	NO ☐
Parkinson's disease	YES ☐	NO ☐
Dementia	YES ☐	NO ☐
Alzheimer's disease	YES ☐	NO ☐
Cerebral Palsy	YES ☐	NO ☐
Brain Tumor	YES ☐	NO ☐
Bipolar disorder or other psychiatric condition	YES ☐	NO ☐
If Yes, please explain:		
Other neurological disorder	YES ☐	NO ☐
If Yes, please explain:		

MEDICATIONS

Are you currently taking any medications?	YES ☐	NO ☐
If Yes, please explain:		

Medication	Reason	Times taken per Day	Taken for how long?

IMPLANTED DEVICES AND METAL*

Do you have a pacemaker?	YES ☐	NO ☐
Do you have an implanted medication pump?	YES ☐	NO ☐
Do you have an implanted deep brain stimulator?	YES ☐	NO ☐
Do you have any other type of implanted device? (excluding dental implants)	YES ☐	NO ☐
Do you have any implanted metal in your upper body? (excluding dental work)	YES ☐	NO ☐
Do you have any metal piercings in your upper body? (piercings will need to be removed)	YES ☐	NO ☐

**If you answered "YES" to any questions under IMPLANTED DEVICES AND METAL, please explain here:*

Appendix B: Consent form

Consent to Participate in a Research Study
Colorado State University

TITLE OF STUDY: ***Reliability of TMS measurements of the motor cortex.***

PRINCIPAL INVESTIGATOR: Matt Malcolm, PhD, OTR
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(970) 491-2646
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CO-PRINCIPAL INVESTIGATOR: Laurie Causer
Department of Occupational Therapy
Colorado State University
(970) 491-3444
laurie.causer@gmail.edu

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH?

You are a right-handed adult man or woman aged 18 – 50 years. You will not be allowed to participate in this study for any of the following reasons:

1. **Are taking medications which may lower the seizure threshold such as (tricyclic) antidepressants. Seizure threshold is a term that refers to a person's susceptibility to seizures. A person with a lowered seizure threshold has an increased risk of having a seizure.**
2. **Have a history of central nervous system (CNS) illness. CNS illness include:**
    a. **epilepsy or seizure disorder**
    b. **mass brain lesions, which is an area of damaged tissue in the brain resulting from, but not limited to, stroke, cancerous and noncancerous tumors, or abnormal connnection between blood vessels in the brain.**
    c. **head trauma leading to loss of consciousness of any length**
3. **Have a history of drug or alcohol abuse within the past year**
4. **Have an implanted pacemaker or medication pump, metal plate in skull, metal objects in the eye or skull, or inner ear (cochlear) implant**
5. **Have monitoring lines that have been surgical implated within your heart (intracardiac lines) or significant history of heart disease**
6. **Are pregnant**
7. **Have a family history of heart disease**
8. **History of bi-polar**
9. **Are left handed**

WHO IS DOING THE STUDY?

This study is part of a combined effort between Matt Malcolm, PhD, Patti Davies, PhD, and Laurie Causer in the Department of Occupational Therapy at Colorado State University.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to establish the test- retest reliability of two of the most frequently used TMS measurements; paired-pulse TMS and a single pulse TMS measurement known as the recruitment curve. Test-retest reliability is important to show that results are consistent.

Paired-pulse TMS is two magnetic pulses delivered to the brain. These two magnetic pulses can be delivered close together or further apart. When these two pulses are delivered close together - with 2 milliseconds (2 thousandths of one second) between the first and second magnetic pulse, it is used to study intracortical inhibition. One way to think about intracortical inhibition is that weaker intracortical inhibition makes it easier for the messages from the brain to pass down the rest of your body. When these two pulses are delivered further apart – with 15 milliseconds (15 thousandths of one second) between the first stimulation, it is used to study intracortical facilitation. One way to think about intracortical facilitation is that weaker intracortical facilitation makes it challenging for the messages from the brain to pass down to the rest of your body.

The recruitment curve is created by delivering a single pulse of TMS, starting at a low stimulation intensity and slowing increasing the stimulation intensity in increments of five. The stimulation intensity is compared to the muscle response that it created. This provides information about the strength of the intracortical (within the brain) connections and the corticospinal (from your brain to your spine) connections.

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?

The study will take place on the Colorado State University campus in Dr. Malcolm's NeuroRehabilitation Research Laboratory located next to (directly east of) the Occupational Therapy building. The study will include two visits one week apart from each other. Each session should last 1 – 1.5 hours for a total of 3 hours.

WHAT WILL I BE ASKED TO DO?

During both sessions, you will be seated comfortably in a chair. At the beginning of the first session, we will place two EMG electrodes over two muscles in your hand,, for a total of four EMG electrodes. These electrodes are what allow your muscle activity to be recorded. Next, magnetic stimulation (pulses) will be applied over your head for up to one hour. The investigator will use a magnetic coil shaped like a figure eight to deliver magnetic pulses over the designated spot on your scalp. During this time you will feel a mild to moderate tapping sensation. You should NOT feel any discomfort during this process, but please let us know if you feel any discomfort for any reason. Both EMG and TMS are non-invasive, so these techniques do not involve puncturing the skin. You will then be asked to come back one week later for the second session. This process will be repeated the exact same way during your second session.

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY?

You will complete a confidential health questionnaire which will help determine if you are eligible for the study. You will not be allowed to participate in this study for any of the following reasons:

1. **Are taking medications which may lower the seizure threshold such as (tricyclic) antidepressants. Seizure threshold is a term that refers to a person's susceptibility to seizures. A person with a lowered seizure threshold has an increased risk of having a seizure.**
2. **Have a history of central nervous system (CNS) illness. CNS illness include:**
    a. **epilepsy or seizure disorder**
    b. **mass brain lesions, which is an area of damaged tissue in the brain resulting from, but not limited to, stroke, cancerous and noncancerous tumors, or abnormal connnection between blood vessels in the brain.**
    c. **head trauma leading to loss of consciousness of any length**
3. **Have a history of drug or alcohol abuse within the past year**
4. **Have an implanted pacemaker or medication pump, metal plate in skull, metal objects in the eye or skull, or inner ear (cochlear) implant**
5. **Have monitoring lines that have been surgical implated within your heart (intracardiac lines) or significant history of heart disease**
6. **Are pregnant**
7. **Have a family history of heart disease**
8. **History of bi-polar**
9. **Are left handed**

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

Transcranial magnetic stimulation (TMS) is the administration of magnetic pulses delivered to the brain.

- **TMS may cause a seizure in individuals who have a history of seizures or epilepsy. For this reason, any individual who has experienced a seizure or has a history of epilepsy will be excluded from this study.**
- **TMS may interfere with implanted devices such as a heart pacemaker or other metal implants in the upper body. For this reason, individuals with a heart pacemaker or other implanted device in their upper body will be excluded from the study. The project staff will ask you if you have a history of seizures or epilepsy, and if you have a pacemaker or other implanted metal device.**
- **During the TMS procedure, you will feel a mild to moderate "tapping" on your scalp. This should not be painful. If this becomes uncomfortable for any reason, please notify the researchers so that we may stop the procedure.**
- **For some individuals, TMS may cause a mild headache. These headaches typically occur due to stimulation of the scalp muscles. These headaches are usually short lasting and may respond well to mild analgesics (for example, Tylenol). If you develop a headache that is too uncomfortable during the testing session, please notify the project so that the TMS procedure may be stopped.**

Please inform us if you have a history of fainting in the doctor's office or other locations when excited or if you suffer from a sodium or nutritional deficiency.

It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

ARE THERE ANY BENEFITS FROM TAKING PART IN THIS STUDY?

There are no direct benefits in participating in this study, but we hope you will gain more knowledge on your brain activity and function.

DO I HAVE TO TAKE PART IN THE STUDY?

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

WHAT WILL IT COST ME TO PARTICIPATE?

There are no financial costs to participate in this study.

WHO WILL SEE THE INFORMATION THAT I GIVE?

We will keep private all research records that identify you, to the extent allowed by law. Your information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private. This is the only form where your name will be recorded. All other paper and computer documentation pertaining to you will be labeled using a number code. This code will be used to identify you in association with your research data. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information or what that information is. You should know, however, that there are some circumstances in which we may have to show your information to other people. For example, the law may require us to show your information to a court.

CAN MY TAKING PART IN THE STUDY END EARLY?

Your participation in the study could end in the unlikely event that you are unable to tolerate the study procedures.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY?

Yes, you will receive a $20 stipend for taking part in this study.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH?

The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

WHAT IF I HAVE QUESTIONS?

Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigators, Laurie Causer at (970) 491-3444. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, IRB Senior Administrator at 970-491-1655 or janell.barker@research.colostate.edu. We will give you a copy of this consent form to take with you.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing _4_ pages.

_____ _____

Signature of person agreeing to take part in the study Date

_____

Printed name of person agreeing to take part in the study

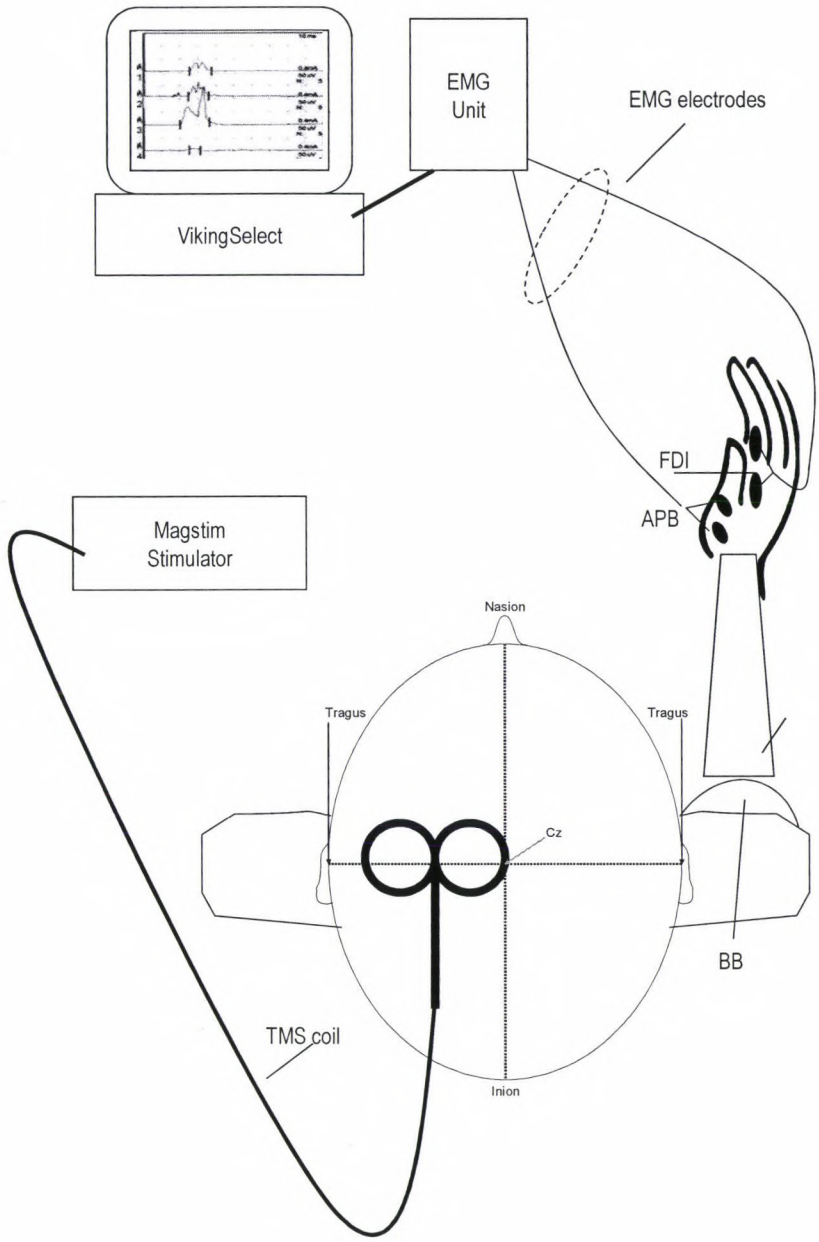
_____ _____

Name of person providing information to participant Date

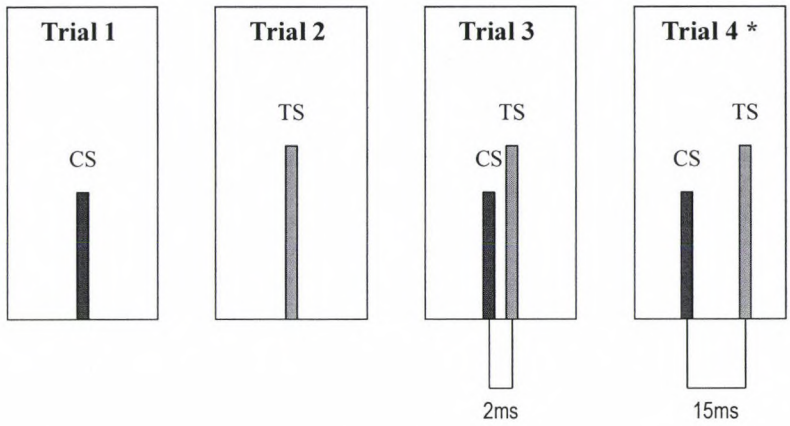
_____ _____

Signature of Research Staff Date

Appendix C: TMS data collection set up



Appendix D: Stimulation deliverance



* Continued in random order for total of 80 trials

Appendix E: TMS data recording form

NeuroRehabilitation Research Laboratory

TMS Evaluation Form

Session (circle): First Second

MT= _____ Hot Spot: L: ___(cm) A/P: ___(cm) Date: _____

TS (116%)= _____ Vertex: N/I: ___(cm) I-A: ___(cm) Subject Code: _____

CS (90%)= _____

ISI			
Trial #	ISI	File #	Gain
1	CS		
2	TS		
3	2ms		
4	15ms		
5	2ms		
6	TS		
7	CS		
8	TS		
9	CS		
10	15ms		
11	2ms		
12	15ms		
13	2ms		
14	15ms		
15	2ms		
16	TS		
17	CS		
18	TS		
19	CS		
20	15ms		
21	2ms		
22	15ms		
23	2ms		
24	15ms		
25	TS		
26	CS		
27	CS		
28	TS		
29	TS		
30	CS		
31	2ms		
32	15ms		
33	2ms		
34	15ms		
35	2ms		
36	15ms		
37	CS		
38	CS		
39	TS		
40	TS		

ISI			
Trial #	ISI	File #	Gain
41	CS		
42	TS		
43	2ms		
44	15ms		
45	2ms		
46	TS		
47	CS		
48	TS		
49	CS		
50	15ms		
51	2ms		
52	15ms		
53	2ms		
54	15ms		
55	2ms		
56	TS		
57	CS		
58	TS		
59	CS		
60	15ms		
61	2ms		
62	15ms		
63	2ms		
64	15ms		
65	TS		
66	CS		
67	CS		
68	TS		
69	TS		
70	CS		
71	2ms		
72	15ms		
73	2ms		
74	15ms		
75	2ms		
76	15ms		
77	CS		
78	CS		
79	TS		
80	TS		