Assessing the agreement and consistency of absolute and relative corticospinal stimulus response curves in healthy young adults

by

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Stimulus response curves (SRC) use the relationship between transcranial magnetic stimulation (TMS) intensity and motor evoked potential (MEP) amplitude to determine the input-output properties of the corticospinal system. SRCs can be constructed with stimulation intensities based on the absolute stimulator capacity or relative to motor threshold (MT), but the two methods have not been directly compared.

PURPOSE: To determine whether SRC parameters (MEP_{max}, V_{50}, and slope) produced by absolute and relative SRCs are similar and consistent.

METHODS: Thirty (15W, age: 27.0±6.3y, height: 171.9±8.9cm, weight: 80.2±19.3kg) young, healthy individuals completed absolute (5-100% of stimulator output in 5% increments) and relative (65-160% of active MT (AMT) in 5% increments) SRCs of the rectus abdominis (RA), vastus lateralis (VL), and first dorsal interosseous (FDI) during submaximal voluntary isometric contractions of each target muscle. Two single TMS pulses were delivered at each intensity in a randomized order with the mean MEP fit to a Boltzmann sigmoidal equation to derive MEP_{max}, V_{50}, and slope. Absolute agreement and consistency of the SRC parameters were determined with intraclass correlation coefficients, Cronbach's alphas, and Bland-Altman plots. A secondary analysis examined differences in AMT, physical activity, maximal voluntary isometric contraction force, and baseline EMG activity among participants who had one successful SRC (absolute or relative only) using independent samples t-tests.

RESULTS: Absolute and relative SRCs displayed good absolute agreement and consistency for MEP_{max} and V_{50} in all muscles, but poor agreement and consistency for slope. Bland-Altman plots showed greater variance for slope with larger mean differences for each muscle compared with MEP_{max} and V_{50}. Individuals who successfully fit an absolute SRC in the RA had lower AMTs and higher physical activity levels. No such between-group differences were found for the other measures or muscles.

DISCUSSION: Absolute and relative SRCs produce similar values for the MEP_{max} and V_{50} but not slope. Additionally, AMT and physical activity level may influence the success of RA SRCs depending on the SRC technique. Researchers should consider these factors when selecting an SRC method.

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1.0 Introduction

Transcranial magnetic stimulation (TMS) is a form of non-invasive brain stimulation that can be used to assess corticospinal system function and structure in humans and thereby advance our understanding of the bidirectional relationship between the brain and the body (1-6). When TMS is applied over the primary motor cortex with a sufficiently strong magnetic field, the resultant electric current can activate corticospinal neurons and cause the target muscle contralateral to the coil to produce a motor evoked potential (MEP), which can be detected with surface electromyography (EMG) (1-3). MEPs are absent when stimulation is applied at subthreshold intensities but once a threshold is reached, increases in TMS intensity result in progressively larger MEP amplitudes until a saturation point occurs. The input-output properties of the corticospinal system are formally examined with stimulus response curves (SRC).

By plotting MEP amplitudes as a function of TMS intensity using a Boltzmann sigmoidal equation (2, 7), SRCs characterize prominent aspects of corticospinal system function. There are four major aspects of an SRC: the EMG activity present at baseline (EMG_{base}), the slope of the linear portion of the curve, the size of the MEP produced at the stimulation intensity where the MEPs reach a maximal plateau (MEP_{max}), and the stimulation intensity that produces an MEP equal to 50% of MEP_{max} (V_{50}). Together, these parameters reflect the physiological strength of corticospinal pathways and each parameter represents a distinct property of corticospinal excitability (CSE) (1).

The MEP_{max} is the value at which MEP amplitudes saturate and no longer increase with increasing stimulation intensity, and it represents the activation of all target motor neurons accessible to TMS. However, MEP_{max} is smaller than a compound muscle action potential induced

by peripheral nerve stimulation due to: 1) the inability to synchronously activate all motor neurons in the corticospinal tract; and 2) the incomplete excitation of spinal neurons that innervate the target muscle (8). V_{50} is the midpoint between EMG_{base} and MEP_{max} and provides information on the stimulation intensity at the most sensitive (steepest) region of the SRC (9), with steeper slopes due to innervation of motor neurons with larger excitatory postsynaptic potentials (i.e., stronger corticospinal projections) (10).

The stimulation intensities used to produce SRCs are typically selected using one of two methods: 1) predetermined intensities based on maximal stimulator output (absolute SRC); and 2) intensities based on individual (relative SRC) motor thresholds (MT). Both methods apply various stimulation intensities in a systematic fashion (e.g., 5% increments of maximum stimulator output or MT), and given that a valid MEP_{max} estimate can be obtained, produce a sigmoidal curve when MEP values are plotted as a function of stimulator output. While both techniques are commonly used, absolute and relative SRCs have never been directly compared, and it is unknown whether the estimates obtained from each technique are similar or consistent.

In addition to potential differences in parameter estimates with the use of absolute or relative SRC techniques, there is growing interest in the corticospinal properties of muscles (e.g., postural and lower extremity) implicated in various neurological disorders. These muscles frequently have higher MTs (lower CSE) compared with traditional target muscles of the hand (e.g., first dorsal interosseous or abductor pollicis brevis) (4, 5, 11, 12). Given the sensitivity of SRC parameter estimates to absolute differences in CSE, the range of stimulation intensities needed to produce valid SRC parameter estimates may be unfeasible for some individuals. Thus, absolute and relative SRCs may be limited by maximal stimulator output intensity. In addition, because relative SRC stimulation intensities depend on individual MTs, muscles with lower MTs

(higher CSE) may not provide valid SRC parameter estimates if the resultant stimulation intensities are insufficient to reach a saturation point.

1.1 Literature Review

In the mid 1980s, Anthony Barker discovered the use of a pulsed magnetic field to non-invasively stimulate the human motor cortex, now a widely used technique in both clinical and research fields termed transcranial magnetic stimulation (TMS) (6). This discovery was a major advance in neurophysiology because it allowed for the functional assessment of the corticospinal tract without some of the disadvantages of the prior method, transcranial electrical stimulation (TES). TES includes careful and specific placement of the electrodes on the scalp and the application of direct current (6, 13). Compared with peripheral nerves, electrical stimulation of the motor cortex requires higher intensities to pass through the highly resistive skull, and such currents activate pain fibers in the scalp (2, 6). In the case of TMS, approximately the same intensity of magnetic stimulation is needed for the motor cortex and peripheral nerves, and magnetic fields pass through the scalp unimpeded with greater spatial resolution (6). Thus, in addition to being non-invasive and easy to use, TMS is painless and relatively focal.

1.1.1 Technical Principles of Transcranial Magnetic Stimulation

TMS uses electromagnetic induction to stimulate the cortex. Following Faraday's principle, an electric current passing through a wire coil produces a magnetic field. A rapidly fluctuating electric current in the TMS coil generates a magnetic field perpendicular to the

direction of the wiring within the coil. The rapidly fluctuating magnetic field induces an electric current in the brain that is parallel to the current passing through the coil but in the opposite direction.

1.1.2 Stimulation of the Motor Cortex

When TMS is applied over the motor cortex, the induced electric current trans-synaptically depolarizes motor neurons, causing descending volleys in the corticospinal tract. These volleys travel to peripheral nerves and can be recorded outside the muscle as electromyographic (EMG) MEPs. TMS recruits motor units in the same way they are recruited physiologically, from smallest to largest in accordance with the size principle (13, 14).

Corticospinal volleys have been studied through epidural recordings at the cervical spinal cord (15-17). Direct activation of the motor neuron is referred to as a D-wave, while I-waves, which have longer latencies at intervals of 1.2-2.0ms, are produced via trans-synaptic depolarization of motor neurons (18, 19). The excitation of the interneurons leads to the activation of the motor neurons. Unlike TES which preferentially produces D-waves (direct activation), TMS at a suprathreshold intensity produces multiple I-waves and *potentially* D-waves (16, 17). Hence, with TMS, motor neurons are primarily activated trans-synaptically.

1.1.3 Motor Evoked Potentials

Action potentials are induced in spinal motor neurons if descending corticospinal volleys are strong enough to surpass depolarization thresholds (19). At the periphery, an MEP can be recorded from the activated muscle, which represents the sum of the excitatory and inhibitory

influences on the corticospinal tract. Assessment of MEPs is generally how CSE is determined in humans. Different features of MEPs, such as the amplitude, area under the curve, and latency can be analyzed for further information about the corticospinal tract. The MEP amplitude (negative to positive peak, known as peak-to-peak amplitude) is the most examined aspect, and the resulting amplitude from a single pulse of TMS is dependent on external and internal factors. A higher intensity TMS pulse (external factor) generally increases the size of the MEP (1, 13, 19). Additionally, the integrity of the corticospinal tract and influence of different neurological states (internal factor) can affect MEP amplitude, either decreasing or increasing them (20-23). Furthermore, MEPs can be facilitated by increasing the excitability of the motor neuron pool, such as through voluntary contraction of a target muscle (7, 19).

1.1.4 Stimulus Response Curves

As mentioned above, the size of the MEP increases with stimulation intensity. When this relationship is plotted, the result is a sigmoidal curve. The curve begins as a flat line which then sharply increases as the TMS intensity reaches motor threshold and continues to linearly increase until a plateau is reached at higher intensities. Using the Boltzmann sigmoidal equation, parameter estimates can be produced and include the MEP_{max}, V_{50}, and slope. These parameters offer further insights into CSE. The MEP_{max} is often studied as a measure of CSE as it shows the size of the MEP at suprathreshold intensities and when, in theory, all of the motor neurons accessible to TMS are activated (8). However, the V_{50} and slope are known as more sensitive regions of the SRC and can reflect more subtle changes in CSE that the MEP_{max} may not indicate (9, 10, 24). These parameters, assessed separately or together, can be used to examine CSE across and within individuals.

1.1.4.1 Use of Stimulus Response Curves

The SRC has been a commonly used tool for CSE assessment since the late 1990s (7, 25, 26). Also referred to as an input-output curve, the SRC describes the relationship between TMS intensity (input) and MEP amplitude (output). In general, the SRC is performed by stimulating the area of the motor cortex that produces the largest and most consistent MEP responses in a target muscle (i.e., the hotspot) over a wide range of incremented (e.g., 5%) stimulation intensities. Stimulation intensity can be determined based on stimulator output (absolute SRC) or the individual's MT (relative SRC).

1.1.4.2 Factors Influencing Stimulus Response Curves

Originally, the input-output properties of corticospinal circuits were not well-understood (7, 26). Early research used a linear model until it was determined that sigmoidal models (e.g., a Boltzmann equation) provided a better fit for relating corticospinal responses (as measured by motor-evoked potentials) to TMS (7, 10, 26-28). In addition to establishing that input-output relationships for upper and lower extremity muscles are sigmoidal (7), the slope of the SRC is influenced by the ability to recruit upper and lower motor neurons as well as differences in membrane excitability as a result of historical or current physiological activity (7, 29). In the latter case, endogenous physiological activity (e.g., voluntary muscle contraction) increases MEP size across SRC stimulation intensities, thus increasing the value of SRC parameters such as EMG_{base} and MEP_{max}, and reduces the variability of electromyographic responses (29).

SRC parameters are muscle specific. Altering TMS intensity has a different effect on MEP recruitment depending on the muscle as well as the individual (10). Generally, SRCs are steeper for intrinsic hand muscles than lower extremity muscles (5, 10). Furthermore, the MEP_{max} (or

plateau point), is reflective of excitatory and inhibitory influences on the corticospinal tract, rather than a maximal response to a fully excitatory corticospinal volley (7).

Age also influences SRC parameter estimates. In the FDI, MEP_{max} amplitude and peak slope do not differ by age, but older individuals require a larger stimulation intensity to reach MEP_{max} (30). In addition, MEP amplitudes increase more slowly in response to incremental stimulation from MT to V_{50}, resulting in a larger V_{50} value and a rightward curve shift compared with younger individuals (30). Thus, while MEP_{max} is similar, the rate of slope change is slower for older individuals and a greater stimulation intensity is needed to reach V_{50} and MEP_{max} (30).

Pharmacological studies confirm that SRCs are sensitive to ion-gated (Na^+ and Ca^{2+}) channel properties, as well as GABAergic and monoaminergic activity. Lorazepam, a positive allosteric modulator of $GABA_A$ receptors, reduces MEP amplitudes at intermediate to high (e.g. 30-100% output and 100-150% MT) TMS intensities (27). Lamotrigine, a Na^+ and Ca^{2+} channel inhibitor, also suppresses SRC-derived estimates of CSE, while D-amphetamine, an indirect agonist of the dopaminergic-noradrenergic system, facilitates SRC CSE estimates (27). These drugs influence MEP amplitudes across SRC intensities, yet MT and TMS-based measures of cortico-cortical inhibition and facilitation were only affected in some cases, which suggests that SRCs may be more sensitive to drug-induced changes in CSE (27).

1.1.4.3 Reliability of Stimulus Response Curves

The reliability of SRCs for upper extremity muscles ranges from moderate to excellent (31-33). For the FDI, intraclass correlation coefficients (ICC) vary between 0.60 and 0.94 for the MEP_{max} and between 0.63 and 0.84 for the V_{50} (24, 33). The slope parameter generally demonstrates poorer agreement with ICCs from 0.60 to 0.77 (24, 33). Furthermore, the abductor pollicis brevis, extensor digitorum communis, and flexor carpi radialis muscles produce moderate

to good test-retest reliability for the slope parameter specifically (31). SRC parameters for lower extremity muscles such as the tibialis anterior also possess moderate to good reliability (ICC = 0.73-0.78). In addition, MEP_{max} and slope have relatively good reliability when assessed by the same TMS operator in the same day, and moderate reliability when examined between two different TMS operators (11). Moreover, between sessions conducted by the same operator four weeks apart, the tibialis anterior showed good reliability for MEP_{max} and slope (11).

Because traditional SRC procedures are relatively time-consuming, researchers have assessed the ability to produce valid and reliable SRCs with shorter interstimulus intervals (ISI) and the delivery of fewer TMS pulses. Although most studies perform SRCs with ISIs above five seconds (5, 11, 31, 33, 34), SRCs done with ISIs of at least 1.4 seconds produce reliable MEP_{max}, V_{50}, and slope estimates for the FDI, abductor digit minimi, and biceps brachii, with no differences between ISIs of 1.4 and four seconds (32). Furthermore, reliable SRCs of the FDI can be plotted with as little as 40 TMS pulses. Specifically, ICC values for MEP_{max}, V_{50}, and slope estimates produced by two SRC sessions on the same day (that used only 40 pulses with 20 intensities at 5% increments in duplicate) were similar to the corresponding ICC values when assessed with 90 pulses between three sessions across three separate days (24, 33). As such, reliable SRC parameter estimates can be obtained with more efficient protocols (i.e., 40 versus 90 pulses).

1.2 Stimulus Response Curve: Methodological Considerations

SRCs measure CSE by characterizing the relationship between TMS electromagnetic induction (input) and MEP amplitude (output): as TMS intensity increases, so does MEP size. A larger current in the TMS device induces a larger current in the brain, which depolarizes less

excitable cortical (upper) motor neurons, spinal (lower) motor neurons, and the muscle fibers these neurons innervate (motor units). As measured by electromyography (EMG), the recruitment of less excitable motor units results in larger motor-evoked potentials (MEP) (19). Compared with other measures such as MT, SRCs are preferentially used to assess CSE because the characterization of MEPs at various intensities below, at, and above MT can provide a more robust picture of corticospinal system function.

Given that SRCs are designed to characterize the input-output properties of the corticospinal system, it is critical that the range of stimulation intensities include those that: 1) produce no responses (subthreshold); 2) adequately sample linear aspects of the response curve; and 3) surpass the point of maximal MEP responses (saturation). One general challenge for the SRC technique is the difficulty of obtaining valid parameter estimates in muscles with higher MTs, such as lower extremity and postural muscles. These muscles often have much higher MTs (> 75%) than hand muscles (typically < 50%) (4, 11, 27, 35). Thus, efforts to assess CSE in individuals or muscles with higher MTs may be limited by stimulator outputs that do not permit MEP saturation at the upper end of the SRC range.

For relative SRCs, stimulation intensities are based on individual MT (e.g., 65-160% MT), meaning the range must include intensities substantially below and above MT in order to include non-responses and the saturation point. However, MTs vary among individuals, within the same individual, and among muscles (36, 37). Clinical factors and conditions can also influence MT (37, 38); individual MTs can vary by more than 25% in certain clinical populations, such as in individuals with depression (36). Additionally, CSE assessments are affected by modifiable and non-modifiable intrinsic (e.g., sex, physical activity, history of injury) and extrinsic (e.g., TMS device and operator) factors (4, 37, 39-41). The large amount of variability in MT presents a

challenge for relative SRCs if the required stimulation intensities cannot consistently produce valid parameter estimates. In addition to being limited in muscles with higher MTs, the limited range of intensities used for relative SRCs may also be problematic for muscles with low MTs. For example, if a muscle has a MT of 35% and a relative SRC is performed at intensities from 65% to 160% of MT, the highest stimulation intensity delivered to that muscle would be 56%. In this case, given that MEP_{max} is meant to reflect the point of MEP saturation, the resultant SRC parameter estimates may be compromised by stimulator intensities that were too low to induce maximal MEP responses.

For muscles with lower MTs, issues with procedural standardization are eliminated with absolute SRCs, which are based on absolute stimulator output (e.g., 5-100% SO). In addition, because absolute SRCs do not require MT estimation, they are a more efficient way to assess CSE across or within individuals over time. Nevertheless, when compared with absolute SRCs, relative SRCs use a narrower window of stimulation intensities centered around the MT and may thus be more sensitive to subtle differences in corticospinal input-output properties, particularly V_{50} and slope. Finally, whether a relative or absolute SRC technique is used, fixed (hardware) limitations on upper stimulation intensities (i.e., 100% SO) can invalidate parameter estimates when CSE is low (e.g., trunk muscles, individuals with stroke).

The application and results of relative and absolute SRCs are used interchangeably, but it is unknown whether these two approaches produce similar estimates of corticospinal system input-output properties. Given that SRC procedures are more time intensive than traditional CSE assessments, one approach is typically used. However, without direct evidence to support the assumption that differences between absolute and relative SRCs are trivial, it is potentially problematic to use or interpret the results of these methods interchangeably. If such curves produce

different parameter estimates, one method may be advantageous in terms of sensitivity, efficiency, or robustness.

1.3 Problem Statement

Although SRCs are widely used to characterize corticospinal function in humans, it is unknown whether parameter estimates are influenced by the choice of technique (i.e., absolute versus relative). Additionally, since muscles with higher MTs may not facilitate MEP saturation (i.e., valid MEP_{max} estimates), there is a need to determine the SRC parameters that are impacted by unfulfilled (i.e., %MT stimulation intensities being limited by maximal stimulator output) or poorly fit SRC parameters. Specifically concerning relative SRCs, muscles with low MTs may not produce accurate parameter estimates due to a condensed range of stimulation intensities.

1.4 Study Purpose

The purpose of this investigation was to determine whether absolute and relative SRC parameter estimates (MEP_{max}, V_{50}, and slope) are similar and consistent.

1.5 Specific Aim and Hypothesis

1.5.1 Aim

Examine the absolute agreement and consistency of SRC methods (absolute versus relative) on MEP_{max}, V_{50}, and slope for upper extremity (FDI), axial (RA), and lower extremity (VL) muscles.

Hypothesis: Absolute and relative SRCs will produce similar MEP_{max}, V_{50}, and slope estimates in all muscles based on intraclass correlation coefficients, Cronbach's alpha (≥ 0.75), and visual assessment of Bland-Altman plots and limits of agreement.

1.6 Study Significance

Stimulus response curves (SRC) are commonly used to characterize corticospinal system function and structure (1, 2, 24). SRC stimulation intensities are based on absolute stimulator output or individual MT estimates. While both techniques are accepted as effective and valid, there is a need to determine whether absolute and relative SRC procedures and results can be used and interpreted interchangeably. Moreover, technical or methodological limitations in stimulation intensity may make certain SRC procedures unfeasible in muscles with particularly low (absolute and relative) or high (relative) CSE.

2.0 Methods

2.1 Study Design

The study used motor cortex (M1) transcranial magnetic stimulation (TMS) to assess the similarity and consistency of absolute and relative corticospinal stimulus response curves (SRC) in the right rectus abdominus (RA), vastus lateralis (VL), and first dorsal interosseous (FDI). Thirty (N = 30) participants completed a total of three visits, including a consent/familiarization visit, a visit to acquire magnetic resonance images (MRI) of brain structure, and an experimental visit with TMS. During the experimental visit participants completed: 1) questionnaires to assess status and compliance with experimental controls; 2) transcranial magnetic stimulation (TMS) assessments to determine individual motor thresholds (MT) for each muscle; and 3) absolute and relative SRCs for each muscle in a randomized counterbalanced order.

2.2 Participants

2.2.1 Recruitment

Thirty (15 women) healthy, young, physically active adults participated in this study. Participants were recruited via posted flyers, word-of-mouth, and Pitt+Me. Interested individuals contacted the research study team for a screening to determine eligibility. All participants provided

written informed consent. The study was approved by the University of Pittsburgh's Institutional Review Board.

2.2.2 Inclusion Criteria

Individuals had to meet the following eligibility requirements: between 18 and 40 years of age, right-handed and legged, perform at least 120 minutes of physical activity per week, and have normal or corrected normal vision.

2.2.3 Exclusion Criteria

Participants were excluded from the study for the following reasons:

- History of neurological, cardiovascular, or any other major disorders
- History of epilepsy, seizure, or sleep disorder
- Contraindications to transcranial magnetic stimulation (TMS) or magnetic resonance imaging (MRI)
- Any musculoskeletal injury that would limit ability to perform the physical study requirements
- History of alcohol or substance abuse
- Current use of CNS active, ANS active, seizure threshold-lowering, anti-inflammatory, ototoxic, or anabolic hormonal substances
- Pregnant
- Claustrophobic
- Unable to produce a motor-evoked response to single pulse TMS at stimulation intensity ≤75%
- Weight ≥300lbs (MRI restrictions)

2.3 Instrumentation and Procedures

2.3.1 Participant Demographics and Experimental Controls

During the consent/familiarization visit, participants completed surveys and assessments of age, height, weight, and physical activity (Tegner Activity Level). Prior to testing, participants confirmed compliance with the following experimental controls: no vigorous physical activity 48h prior, no alcohol or analgesic drug use 24h prior, and no caffeine ingestion 12h prior. Additionally, participants reported their sleep quality, soreness, fatigue, stress (Holmes-Rahe Life Stress inventory), and mood state (Profile of Mood State Short Form).

2.3.2 Sensor Placement

After initial study questionnaires were completed, electromyographic (EMG) activity was recorded with wireless active Ag differential parallel-bar sensors (Delsys Inc, Natick, MA). The sensors were placed on the right rectus abdominis (RA), vastus lateralis (VL), and first dorsal interosseous (FDI) in accordance with SENIAM guidelines (42). Sensor locations were prepared by shaving the area and lightly exfoliating the skin with tape and isopropyl alcohol swabs to remove excess hair, oils, and skin.

2.3.3 Transcranial Magnetic Stimulation

Single pulse TMS was delivered using a 96mm curved double coil (Jaltron LLC, Waltham, MA) and biphasic stimulator (Super Rapid^2, The Magstim Company Ltd., Carmarthenshire, UK).

During all TMS procedures, frameless stereotactic neuronavigation (Brainsight v2.4, Rogue Research, inc., Montreal, Quebec, Canada) and an infrared camera (Polaris Vicra, Northern Digital Inc., Waterloo, Ontario, Canada) were used in combination with individual structural and functional MRI (BOLD signals during target muscle contractions) to improve stimulation accuracy and consistency. Reflective markers and cranial landmarks (nasion, inion, right and left periauricular areas) were used to co-register each participant to their structural brain images in Montreal Neurological Institute (MNI) space.

2.3.3.1 Motor Hotspot

After sensor placement, participants performed four 3-5s maximal voluntary isometric contractions (MVC) of the trunk flexors (RA, bilateral), knee extensors (VL, bilateral), and finger abductor (FDI, unilateral, right hand). A rest period of approximately one minute was given between attempts. The MVC with the largest force output and least amount of variation during the plateau (determined visually by the TMS operator) was used to calculate active contraction intensity for the subsequent TMS procedures.

The area of the scalp that produces the largest and most consistent motor evoked potentials (MEP) in a target muscle (motor hotspot) was determined while participants performed sustained isometric contractions of target muscles (bilateral for VL and RA, unilateral for FDI) at 15% of MVC with visual feedback. Hotspots were determined by placing the coil over the scalp area (near the vertex) contralateral to the target muscle, with the coil oriented parallel to the longitudinal fissure (RA and VL) or 45-degrees posterolateral to the longitudinal fissure (FDI) to create a posterior-anterior/anterior-posterior current along the precentral gyrus.

2.3.3.2 Motor Threshold

The active motor threshold (AMT) was incrementally determined during submaximal (15% of MVC) contractions of the target muscle (bilateral for VL and RA, unilateral for FDI) based on the minimum stimulation intensity needed to produce an MEP in the target muscle using the parameter estimation by sequential testing (PEST) (i.e., maximum likelihood regression) (43). For the PEST procedure, stimulation boundaries were set between 40% and 100% of stimulator output and the presence of an MEP was visually confirmed by the TMS operator.

2.3.3.3 Stimulus Response Curves

Two SRC procedures were completed: absolute and relative. Each SRC included 40 single TMS pulses with two pulses delivered at each stimulation intensity. The pulses were delivered in eight sets of five pulses with approximately 5s between each pulse. Absolute SRCs had stimulation intensities between 5% and 100% of maximum stimulator output and relative SRCs had stimulation intensities between 65% and 160% of AMT. Both SRCs had pulses delivered in randomized 5% increments. During the SRCs, participants maintained submaximal contractions at 15% of MVC in the target muscle(s) (bilateral for VL and RA, unilateral for FDI) and were given 30s of rest between each set. 80 pulses (40 for each procedure) were delivered to each target muscle and individuals received 240 pulses (40 pulses per procedure x two procedures x three muscles) in total. Target muscle order was randomized, and the order of SRC method was randomized and counterbalanced such that half of the participants completed the absolute SRC procedure first and the other half completed the relative SRC procedure first. The two MEPs at each stimulation intensity were averaged and fit to a Boltzmann sigmoidal curve using least squares regression. MEP_{max}, V_{50}, and slope were calculated from the Boltzmann sigmoid and used for analysis. The Boltzmann sigmoidal equation is as follows:

$$\mathrm{MEP(V)} = \mathrm{EMG_{base}} + \frac{\mathrm{MEP_{max}}}{1 + e^{\frac{V50-V}{S}}}$$

2.4 Data Analysis

EMG activity was sampled at 2,000Hz with a gain of 500 and 20-450Hz bandpass filter. Trunk flexion was measured with an isokinetic dynamometer (Biodex System 4, Biodex, Shirley, NY, USA). Knee extension and finger abduction force were recorded with load cells (Interface Inc., Scottsdale, AZ, USA) and sampled at 2,000Hz and gain of 500. MEP amplitudes were defined as peak-to-peak EMG amplitude from 15-65ms post-stimulus.

2.5 Statistical Analysis

Agreement and consistency of relative and absolute SRCs were determined separately for each parameter estimate (MEP_{max}, V_{50}, and slope) with intraclass correlation coefficients (ICC) and Cronbach's alphas. ICCs were run using a two-way single random model with an absolute agreement definition. ICCs and Cronbach's alphas of 0.75 or greater were considered indicative of good consistency (44). Absolute agreement was also visualized with Bland-Altman plots and assessed by evaluating the limits of agreement (LOA), defined as the mean difference ± 1.96 times the standard deviation (45).

3.0 Results

3.1.1 Participants

Thirty (15 women) individuals participated in the study. Absolute and relative stimulus response curves (SRC) of the rectus abdominis (RA) and vastus lateralis (VL) were assessed in 29 participants. For the first dorsal interosseous (FDI), 28 participants were assessed. Four participants were unable to complete SRCs for all three muscles due to time constraints. After removing participants with poor quality fits for one or both SRCs (as determined by visual analysis and best-fit value confidence intervals), 14, 13, and 19 participants were included in the analysis of RA, VL, and FDI SRCs, respectively. Demographic data for the included participants are summarized in **Table 1**. Additionally, information on participant handedness, footedness, sleep quality, stress, muscle soreness, and mood is provided in **Supplementary Tables 1-2**.

Table 1. Characteristics of participants included in primary analysis

Measure	Rectus Abdominus	Vastus Lateralis	First Dorsal Interosseous
N	14	13	19
Men	5	6	9
Women	9	7	10
Age (y)	29.1 ± 5.8	27.1 ± 6.5	27.5 ± 6.5
Height (cm)	169.8 ± 8.0	171.8 ± 9.3	172.7 ± 9.4
Weight (kg)	82.5 ± 17.8	77.2 ± 20.5	78.2 ± 16.2
AMT (%)	62.4 ± 10.2	58.8 ± 9.0	43.9 ± 6.2
Physical Activity	5.9 ± 2.1	6.2 ± 1.8	5.7 ± 2.1

3.1.2 Agreement and Consistency of Stimulus Response Curve Parameters

Regardless of muscle, relative and absolute SRC MEP_{max} and V_{50} estimates displayed high agreement and consistency, while slope displayed poor to moderate agreement and consistency (**Table 2**). Bland-Altman analysis showed that for each muscle and parameter, one data point was outside the LOA (RA MEP_{max}, V_{50}, and slope = 7.14%; VL MEP_{max}, V_{50}, and slope = 7.69%; FDI MEP_{max}, V_{50}, and slope = 5.26%, **Figure 1**). In this case, absolute agreement was considered acceptable.

Table 2. Intraclass correlation coefficients and Cronbach's alphas of rectus abdominis, vastus lateralis, and first dorsal interosseous MEP_{max}, V_{50}, and slope

Muscle	Parameter	ICC	Cronbach's Alpha
Rectus Abdominis	MEP_{max}	0.989	0.994
	V_{50}	0.939	0.966
	Slope	0.231	0.729
Vastus Lateralis	MEP_{max}	0.966	0.982
	V_{50}	0.907	0.948
	Slope	0.397	0.654
First Dorsal Interosseous	MEP_{max}	0.889	0.939
	V_{50}	0.929	0.967
	Slope	0.042	0.143

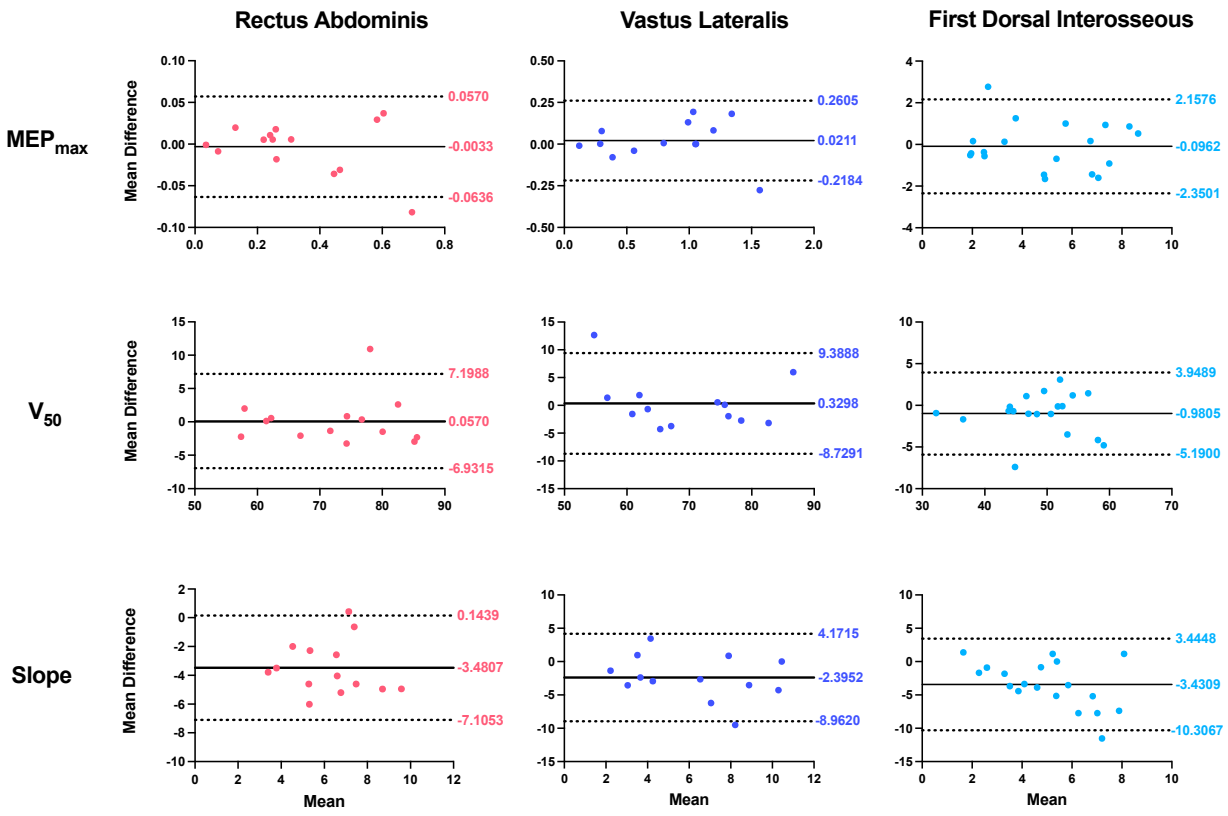

Figure 1. Absolute agreement of rectus abdominis, vastus lateralis, and first dorsal interosseous MEP_{max}, V_{50}, and slope for absolute and relative stimulus response curves

3.1.3 Secondary Analysis of Incomplete Relative Stimulus Response Curves

Six (RA) and five (VL) participants were unable to complete the relative SRC due to limitations in stimulation intensity, respectively. These participants had higher AMTs in the target muscles (RA mean = 72.7±4.7%; VL mean = 67.2±4.9%) compared with the other participants (RA mean = 54.8±4.3%; VL mean = 53.5±5.6%) and were thus unable to receive stimulation at higher relative SRC intensities (125-160% AMT). To assess the agreement and consistency of

SRC parameters between absolute and relative SRCs without these individuals, ICCs, Cronbach's alphas, and Bland-Altman plots were run on the remaining eight participants included in the analysis of SRC parameter estimates for the RA and VL muscles.

The MEP_{max} and V_{50} had good agreement and consistency for both muscles (RA MEP_{max}: ICC = 0.983, Cronbach's alpha = 0.990; RA V_{50}: ICC = 0.967, Cronbach's alpha = 0.983; VL MEP_{max}: ICC = 0.964, Cronbach's alpha = 0.961; VL V_{50}: ICC = 0.815, Cronbach's alpha = 0.886; **Table 3**). The VL slope parameter had poor agreement and consistency (ICC = 0.262, Cronbach's alpha = 0.525; **Table 3**), and the RA slope parameter had similarly poor agreement (ICC = 0.303) but with high consistency (Cronbach's alpha = 0.905; **Table 3**). For the RA and VL MEP_{max} and VL V_{50}, all but one data point (12.5% of all data points) were within the Bland-Altman LOA (**Figure 2**). For RA and VL Slope and RA V_{50}, all data points were within the LOA (**Figure 2**).

Table 3. Intraclass correlation coefficients and Cronbach's alphas of rectus abdominis and vastus lateralis MEP_{max}, V_{50}, and slope without incomplete relative stimulus response curves

Muscle	Parameter	ICC	Cronbach's Alpha
Rectus Abdominis	MEP_{max}	0.983	0.990
	V_{50}	0.967	0.983
	Slope	0.303	0.905
Vastus Lateralis	MEP_{max}	0.964	0.961
	V_{50}	0.815	0.886
	Slope	0.262	0.525

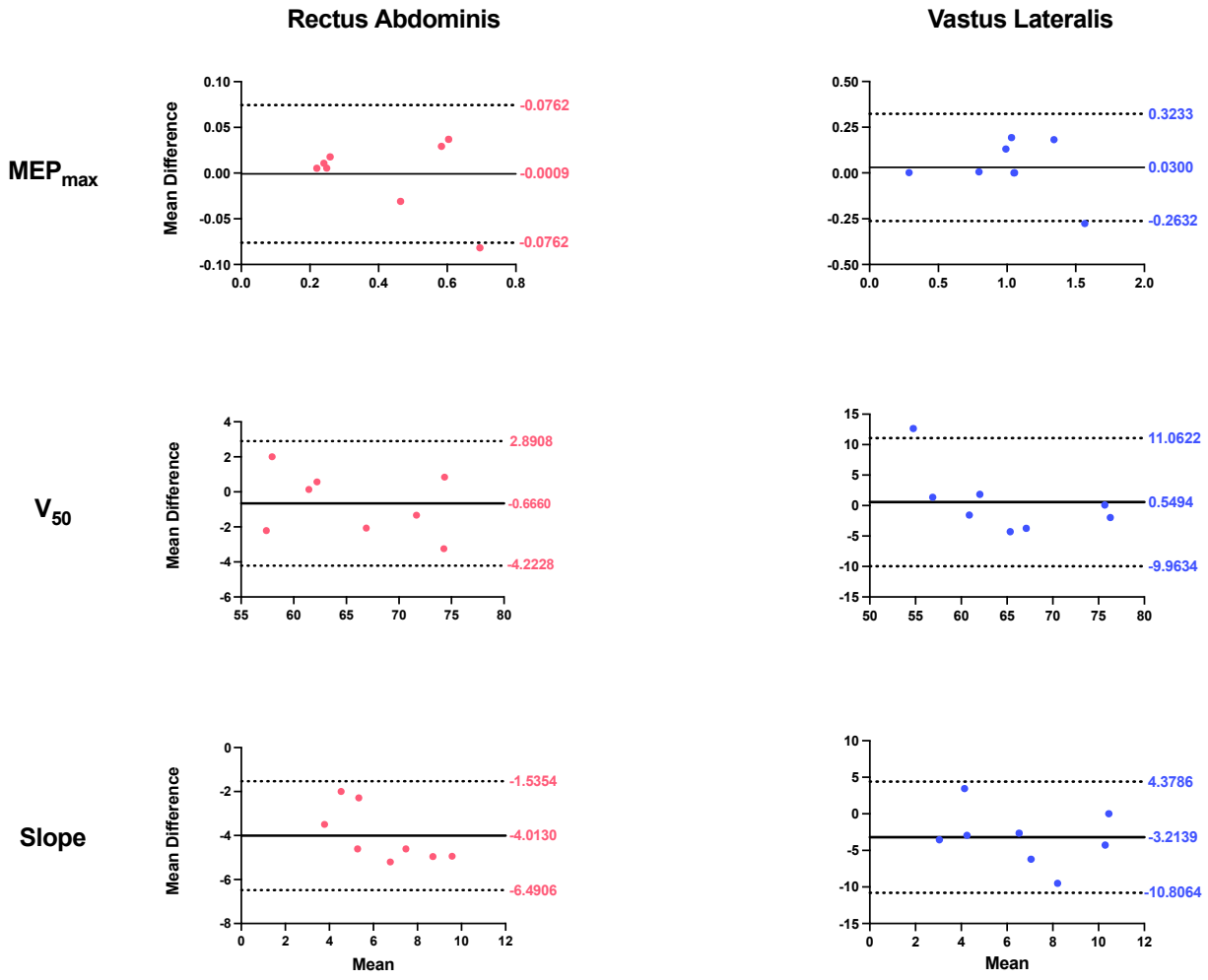

Figure 2. Absolute agreement of rectus abdominis and vastus lateralis absolute and relative MEP_{max}, V_{50}, and slope without incomplete relative stimulus response curves

To determine if differences in AMT, physical activity level, maximal voluntary isometric contraction (MVC) force, and EMG_{base} might explain the ability to produce valid SRCs, these factors were compared in included and excluded participants using independent samples t-tests. As expected, in the RA, AMT was higher in the excluded group (included = 54.8±4.3%, excluded = 72.7±4.7%, $p < 0.001$; **Table 4**). There were no differences in physical activity levels, MVC

force, or EMG_{base} values (physical activity: included = 7.0±1.2, excluded = 4.7±2.4, p = 0.068; MVC force: included = 85.3±69.6Nm, excluded = 69.6±52.0Nm, p = 0.586; EMG_{base}: included = 0.041±0.026 = mV, excluded = 0.028±0.021mV, p = 0.353; **Table 4**). Similarly, VL AMT was higher in the excluded group (included = 53.5±6.6%, excluded = 67.2±4.9%; p = 0.002; **Table 4**), but the groups did not differ in terms of physical activity levels, MVC force, or EMG_{base} (physical activity: included = 6.3±2.2, excluded = 6.2±1.1, p = 0.963; MVC force: included = 384.8±94.5N, excluded = 348.4±234.8N, p = 0.689; EMG_{base}: included = 0.087±0.036 = mV, excluded = 0.095±0.106mV, p = 0.846; **Table 4**).

Table 4. Characteristics of participants with and without complete rectus abdominis and vastus lateralis relative stimulus response curves

Muscle	Measure	Included	Excluded	p-value
Rectus Abdominis	AMT (%)	54.8 ± 4.3	72.7 ± 4.7	< **0.001**
	Physical Activity Level	7.0 ± 1.2	4.7 ± 2.4	0.068
	MVC Force (Nm)	85.3 ± 69.6	69.6 ± 52.0	0.586
	EMG_{base} (mV)	0.041 ± 0.026	0.028 ± 0.021	0.353
Vastus Lateralis	AMT (%)	53.5 ± 6.6	67.2 ± 4.9	**0.002**
	Physical Activity Level	6.3 ± 2.2	6.2 ± 1.1	0.963
	MVC Force (N)	384.8 ± 94.5	348.4 ± 234.8	0.689
	EMG_{base} (mV)	0.087 ± 0.036	0.095 ± 0.106	0.846

3.1.4 Success of Absolute versus Relative Stimulus Response Curves

When MEP amplitudes are plotted as a function of TMS intensity, the shape of the graph should be a sigmoidal curve. However, in the collected data there were SRCs that did not produce a clear sigmoidal function. If data do not appropriately fit the Boltzmann sigmoidal equation, the resultant parameter estimates may be invalid. Thus, to determine the efficiency of different SRC procedures, it is helpful to compare the frequency of good and poor curve fits among methods and target muscles.

Good SRC curve fits were most often found for the FDI (N = 28, Absolute = 89%, relative = 75%; **Table 5**). For the RA, 72% of relative SRCs had good-fits, while 62% of absolute SRCs had good-fits (N = 29). Based on curve-fits, SRCs performed worst in the VL: 69% (absolute) and 62% (relative) had good-fits (N = 29; **Table 5**).

Table 5. Percentage of good-fit absolute and relative stimulus response curves

Muscle	Absolute	Relative	Total
Rectus Abdominis	62%	72%	29
Vastus Lateralis	69%	62%	29
First Dorsal Interosseous	89%	75%	28

For some participants, one of the SRC methods produced a good-fit curve, while the other produced a poor fit. After visual assessment of the SRCs (i.e., the curves reached a visible plateau) and parameter estimate values, participants were sub-grouped based on whether they had a

successful absolute or relative curve for each muscle. Independent samples t-tests were then used for between-group comparisons of AMT, physical activity level, MVC force, and EMG_{base}.

Four participants had a good-fit for the RA absolute SRC (only) and eight had a good-fit for the RA relative SRC. The absolute-only group had a lower AMT than the relative-only group and a higher physical activity level score, but no differences in MVC force or EMG_{base} (**Table 6**). For the VL and FDI, there were no differences between the absolute-only groups and relative-only groups for AMT, physical activity level, MVC force, or EMG_{base} (**Table 6**).

Table 6. Participant characteristics of those with good fits for absolute-only or relative-only rectus abdominis, vastus lateralis, and first dorsal interosseous stimulus response curves

Muscle	Measure	Absolute-only	Relative-only	p-value
Rectus Abdominis N = 12	AMT (%)	56.3 ± 4.3	67.1 ± 6.9	**0.017**
	Physical Activity Level	8.3 ± 1.5	5.9 ± 0.9	**0.008**
	MVC Force (Nm)	83.6 ± 73.7	98.0 ± 60.0	0.724
	EMG_{base} (mV)	0.040 ± 0.030	0.022 ± 0.015	0.190
Vastus Lateralis N = 12	AMT (%)	57.6 ± 7.6	63.6 ± 9.4	0.248
	Physical Activity Level	6.2 ± 2.6	5.0 ± 2.2	0.476
	MVC Force (N)	491.9 ± 160.0	352.1 ± 140.5	0.148
	EMG_{base} (mV)	0.068 ± 0.037	0.085 ± 0.042	0.473
First Dorsal Interosseous N = 8	AMT (%)	43.8 ± 6.6	40.0 ± 9.9	0.540
	Physical Activity Level	7.3 ± 1.0	6.0 ± 1.4	0.190
	MVC Force (N)	22.9 ± 8.7	22.2 ± 14.4	0.931
	EMG_{base} (mV)	0.893 ± 0.506	1.466 ± 0.201	0.185

4.0 Discussion

The aim of this study was to assess the agreement and consistency of two common stimulus response curve (SRC) techniques: which determine stimulation intensities based on predefined stimulation intensities (absolute) or individual motor thresholds (MT). The two SRC methods were compared in terms of parameter estimates used to assess corticospinal excitability (CSE), including MEP_{max}, V_{50}, and slope. SRCs of the right rectus abdominis (RA), vastus lateralis (VL), and first dorsal interosseous (FDI) muscles were evaluated in thirty individuals (15 women). The MEP_{max} and V_{50} parameters had high agreement and consistency for all muscles, however the slope had only poor to moderate levels of agreement and consistency. These results were similar after removing individuals who could not produce maximal values on the relative SRC due to higher MTs, except for the RA slope parameter, which showed better consistency. Additionally, in comparing the characteristics of individuals who had a successful absolute or relative SRC, MT was lower and physical activity higher in the absolute-only group compared with the relative-only group for the RA, but there were no differences among groups in the VL or FDI, nor for MVC force or EMG_{base} generally.

4.1.1 Absolute versus Relative Stimulus Response Curves

In all muscles, absolute and relative SRCs showed good agreement and consistency for MEP_{max} and V_{50} (**Table 2**). Intraclass correlation coefficients (ICC) and Cronbach's alphas were all > 0.75, with most > 0.90 (except for FDI $MEP_{max,}$ ICC = 0.889). Contrary to our hypothesis that all parameters and muscles would have ICCs and Cronbach's alphas > 0.75, the slope

parameter displayed poor to moderate agreement and consistency in all muscles. Bland-Altman plots were used to assess absolute agreement among absolute and relative SRC parameter estimates, with data between the limits of agreement (LOA) considered to agree. For each muscle and parameter, one data point was outside the LOA, and due to the small sample size (RA = 14, VL = 13, FDI = 19), one data point was equal to more than 5% of the data. Nevertheless, visual analysis confirmed condensed data and a small mean difference for MEP_{max} and V_{50} estimates, while slope displayed more spread and a higher mean difference. These results confirm the similarity of MEP_{max} and V_{50} estimates obtained from the different common SRC techniques, and the limited interchangeability of slop estimates.

ICCs, Cronbach's alphas, and Bland-Altman plots were reexamined for the RA and VL after removing participants with incomplete SRCs due to high MTs (inadequate stimulator intensities) to determine if the inclusion of such individuals was consequential. MEP_{max} and V_{50} maintained good agreement and consistency for both muscles (**Table 3**); the VL slope showed low ICC and Cronbach's alpha, and RA slope maintained a low ICC but improved Cronbach's alpha (0.905). Thus, while participants with complete relative RA SRCs did not display good agreement between methods for the slope parameter, the slopes were consistent, indicating that the two methods did not produce similar slopes for the same participant, but the slope parameters were correlated in an additive manner across all participants (44). After the removal of participants with incomplete relative SRCs, Bland-Altman plots were similar to the original analysis for RA and VL MEP_{max} and VL V_{50}, with one data point outside the LOA (**Figure 2**). Additionally, the mean differences of these plots were small, indicating a high level of agreement. For RA and VL slope and RA V_{50}, all the data points were within the LOA (**Figure 2**), but the slope ICCs indicate low agreement. With the decrease in sample size, the LOA increased for the RA and VL slope, which

explains why all the data are within the LOA. In addition, by examining the mean differences, the RA V_{50} had a small value which is consistent with the ICC value, while the slopes for the RA and VL had similar mean differences to the original analysis including participants with higher motor thresholds (**Figure 2**).

As indicated by the high level of agreement and consistency for each muscle, MEP_{max} and V_{50} are unaffected by the choice of SRC method. The slope, however, is affected by SRC methodology. This is likely due to the fact that the slope is more influenced by the whole range of stimulation intensities than MEP_{max} and V_{50}, which are only impacted by the higher stimulation intensities. The MEP_{max} is the size of the MEP at the plateau/saturation point (24) and is determined by only the upper end of the SRC (i.e., higher stimulation intensities). Since the V_{50} is directly related to MEP_{max}, as it is the stimulation intensity needed to produce an MEP half the size of MEP_{max} (24), it, too, is primarily dictated by the upper end of the curve.

In this study, the mean AMTs increased from FDI to VL to RA (**Table 1**). The calculated stimulation intensity range for relative SRCs would therefore be 40-99% for the RA, 38-94% for the VL, and 29-70% for the FDI. Thus, for the RA and VL, the upper range of stimulation intensities used for the relative SRC procedure was similar to that of the absolute SRC, and since the MEP_{max} and V_{50} are primarily influenced by higher intensities, it is not surprising that the two methods produced similar estimates for these parameters. Conversely, in the FDI, although the highest intensity used for the relative SRC (70% SO) was substantially lower than that of the absolute SRC (100% SO), MEP_{max} may be reached at much lower intensities compared with the RA and VL. Accordingly, a lower stimulation intensity did not substantially affect MEP_{max} and V_{50} estimates, which were similar between the two techniques. Conversely, given that slope is influenced by the entire range of stimulation intensities and the absolute (5%) and relative (30-

40%) SRCs started at substantially different intensities, relative SRCs could be expected to produce a larger slope, as there will be a quicker and steeper increase from the initial subthreshold portion of the curve to the suprathreshold linear portion. Examination of the mean parameter estimate values for the absolute and relative SRCs confirmed that relative SRCs had a larger slope than absolute SRCs, (up to 100%), while MEP_{max} and V_{50} values were similar (**Supplementary Table 3**).

As part of a secondary analysis, participant characteristics were compared to determine if factors other than AMT might influence the ability to produce a successful relative SRC. We compared the participants who were included in the secondary ICC, Cronbach's alpha, and Bland-Altman analysis with those who produced incomplete relative SRCs due to high MTs. As expected, RA and VL AMT were greater in participants with incomplete curves, but physical activity level, MVC force, and EMG_{base} were similar (**Table 4**). Thus, out of the factors examined, AMT was the only measure that influenced whether an individual was able to appropriately complete the relative SRC. Although individuals with complete relative RA SRCs had a tendency to report higher physical activity levels, the result was not statistically significant (**Table 4**).

4.1.2 Characteristic Differences for Successful Absolute versus Relative Stimulus Response Curves

A successful SRC will fit the Boltzmann sigmoidal equation, and when the MEP amplitudes are plotted in relation to TMS intensity, the graph should appear as a sigmoidal curve. In some cases, for a given muscle, participants produced only one SRC (absolute or relative) with a good fit. As part of a secondary analysis, participants were grouped based on whether they successfully produced an absolute *or* relative SRC and compared to determine if any characteristic

differences in AMT, physical activity level, MVC force, and EMG_{base} might explain higher or lower success for each SRC method. For the RA, AMT and physical activity influenced SRC success. Participants who were part of the absolute-only group had lower AMTs (higher CSE) and higher physical activity levels compared with the relative-only group (**Table 6**). Nevertheless, AMT and physical activity were not different between groups for the VL and FDI, nor did MVC force or EMG_{base} differ between groups in any muscle (**Table 6**).

The mean AMT of the absolute-only group for the RA was 56.3±4.3%, so the calculated relative SRC range of intensities for the mean AMT was 36-90% of SO. Hence, the relative SRCs for this group ended at a lower stimulation intensity compared to the absolute SRC which went all the way to 100% of SO. The absolute-only participants had poor relative SRCs because the curves lacked a clear sigmoidal shape as a result of a submaximal MEP_{max} estimate. One potential reason for this is that compared with the maximal absolute SRC intensity (100% SO), maximal relative SRC stimulation intensities were approximately 10% lower (~90% SO), and MEP values were still submaximal at this point. For the relative-only group, the mean AMT was 67.1±6.9%, and the calculated relative SRC range of intensities was 44-100 (*107*)% of SO. Based on the upper end of stimulation intensities, there was no difference between the absolute and relative SRCs. In this case, the poor fit for the absolute curve could be due to a factor that influenced the curve at lower stimulation intensities.

4.2 Limitations

CSE is a dynamic measure that is influenced by numerous factors (36, 37, 39). To minimize the influence of potential confounds, participants were asked to limit certain activities and

behaviors, such as caffeine ingestion, alcohol, and physical activity. Additionally, there were multiple TMS operators, and variability among them could introduce differences in MT estimates that influence (relative) SRC data. Lastly, although there were 30 participants overall, only participants who had good absolute *and* relative SRC fits were included in the main analysis, which was thus limited to relatively small sample sizes (14 for RA, 13 for VL, 19 for FDI).

4.3 Future Research

SRCs are commonly used in clinical settings to evaluate CSE. While this study used young, healthy, physically active adults, future efforts should assess the comparative reliability of different SRC techniques in individuals with neurological disorders or injuries. Furthermore, the SRCs were collected while participants performed isometric submaximal contractions. CSE is often assessed with participants in a resting state, especially in clinical populations. Thus, future studies may examine potential differences between absolute and relative SRCs at rest. Additionally, the results of this study indicate that absolute and relative SRC methods produce similar MEP_{max} and V_{50} estimates, but slopes differ. More detailed investigation into factors that influence slope estimates would be beneficial. Although we were able to determine that the slope parameter differs between the two SRC methods, this study does not elucidate whether one method may be more appropriate than the other. Research examining how different extrinsic and intrinsic factors influence the slope for both absolute and relative SRCs could help to clarify which method better characterizes the slope parameter.

4.4 Conclusion

We compared the agreement and consistency of absolute and relative SRC parameter estimates in upper, axial, and lower extremity muscles. Although SRC parameter estimates from the Boltzmann sigmoidal equation are often used to assess CSE, the absolute and relative SRC methods have never been directly compared. Researchers generally select one of the methods under the assumption of equivalent results. We produced absolute and relative SRCs for three different muscles in healthy young adults and examined the consistency and agreement of SRC parameter estimates for MEP_{max}, V_{50}, and slope. In all muscles, absolute and relative SRCs had high consistency and absolute agreement for MEP_{max} and V_{50}, but low consistency and absolute agreement for slope. Participants were evaluated to see if there were characteristic differences between those who had good curve fits for absolute *or* relative SRCs. For the VL and FDI, the two groups did not differ in terms of AMT, physical activity level, or EMG_{base}. For the RA, the absolute-only group had a lower AMT and higher physical activity level than the relative-only group. Overall, each technique produced similar MEP_{max} and V_{50} values but different slopes. Thus, if SRC slope is a critical endpoint, the SRC technique should be carefully considered. Furthermore, when examining corticospinal circuits associated with axial muscles, individual MT and physical activity levels may influence the ability to produce valid SRC parameter estimates.

Appendix A Supplementary Tables

Supplementary Table 1. Participant handedness, footedness, sleep quality, muscle soreness, and life stress

Handedness	Footedness	Sleep Quality	Muscle Soreness	Life Stress
750.0 ± 83.0	701.7 ± 204.9	3.6 ± 0.5	13.3 ± 19.8	0.2 ± 0.4

> 0 – right side dominant, = 0 – ambidextrous, < 0 – left side dominant

Supplementary Table 2. Participant mood state

Vigor	Depression	Confusion	Tension	Anger	Fatigue
8.0 ± 4.3	0.1 ± 0.4	0.4 ± 1.0	0.9 ± 1.6	0.1 ± 0.4	0.9 ± 1.9

Supplementary Table 3. Mean parameter estimate values for absolute and relative stimulus response curves

Measure	Rectus Abdominis	Vastus Lateralis	First Dorsal Interosseous
Absolute MEP_{max} (mV)	0.325 ± 0.199	0.832 ± 0.456	4.9 ± 2.4
Relative MEP_{max} (mV)	0.328 ± 0.208	0.811 ± 0.457	5.0 ± 2.4
Absolute V_{50} (%)	72.5 ± 10.0	69.7 ± 9.8	48.2 ± 7.0
Relative V_{50} (%)	72.4 ± 9.9	69.4 ± 10.9	49.1 ± 7.0
Absolute Slope	4.5 ± 1.9	5.0 ± 2.9	3.3 ± 1.8
Relative Slope	8.0 ± 2.1	7.4 ± 3.7	6.8 ± 3.2

Appendix B Consent Form

University of Pittsburgh
School of Health and Rehabilitation Sciences
Department of Sports Medicine and Nutrition
Neuromuscular Research Laboratory

Neuromuscular Research Lab
3830 South Water St
Pittsburgh, PA 15203
Email: nmrllab@upmc.edu
http://www.pitt.edu/~neurolab/

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Using Non-Invasive Brain Stimulation to Enhance Physiological Resilience

PRINCIPAL INVESTIGATOR
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SOURCE OF SUPPORT: Department of Defense

Key Considerations

- This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before you decide whether to participate.
- Your participation is voluntary. You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits.
- You may or may not benefit as a result of participating in this study. Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.
- You will be provided with any new information that develops during the study that may affect your decision whether to continue to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

1. Why is this research being done?

You are invited to participate in a study to determine if non-invasive brain stimulation can be used to adjust hormones that play an important role in physical performance and responses to stress. Hormonal responses to stimulation have been observed before, but likely resulted from the unintentional stimulation of brain areas that influence the adrenal gland, a more recent discovery. The purpose of this study is to 1) determine the best way to find the areas of the brain we want to stimulate, 2) test responses to different kinds of stimulation, and 3) study responses to repeated sessions of brain stimulation. The study will be divided into two smaller studies. If you decide to participate in the first study (Study 1), you will complete five (5) experimental visits over a one- to two-month period. If you participate in the second study (Study 2), you will complete six (6) experimental visits over a two-week period. If you participate in both studies, you will complete a total of nine experimental visits (the first two visits are the same for both studies and only need to be done once). Participation in Study 1 and 2 will be separated by no less than two weeks (time to complete analysis of Study 1) and as much as 10 months depending on when you participate and how long it takes us to complete Study 1. Depending on whether you participate in one or both studies, the time commitment will be between 15 and 31 hours and take between six weeks and 12 months (see section 5 below).

2. Who is being asked to participate in the research study?

We will enroll up to 2000 people. Participants will be healthy, right-handed/legged men and women aged 18 to 40 years with normal or corrected-normal vision. We are looking for people who do at least 120 minutes of exercise per week and are comfortable with maximal exercise, transcranial magnetic stimulation (TMS), magnetic resonance imaging (MRI), and blood draws. Individuals must weigh less than 300lb due to MRI restrictions.

3. What will happen if I take part in this study?

All tests will occur at the University of Pittsburgh Neuromuscular Research Laboratory (NMRL) and Brain Imaging Data Generation and Education (BRIDGE) Center:

NMRL	BRIDGE Center – Mellon Institute
3860 South Water St	4400 Fifth Ave
Pittsburgh, PA 15203	Pittsburgh, PA 15213
412-240-0460	412-268-7140

The main aspect of this study is transcranial magnetic stimulation (TMS), a technology used to stimulate the brain. A coil (**Figure 1**) is placed on your head above the areas of the brain we intend to stimulate. When turned on, the coil produces a strong but brief magnetic field (i.e. pulse) that activates small areas of your brain. TMS can be administered in single pulses or repetitively (rTMS; when one or more pulses are delivered every second). Single-pulse TMS is used to assess communication between the brain and muscles and responses to rTMS, which is used to increase or decrease brain activity for around one hour.

Changes in brain activity can have important clinical benefits for certain mental health disorders, but in this study, the goal is to see if changes in brain activity can improve physical performance, change hormones that play an important role in stress and brain function, and improve responses to physical stress from maximal exercise in healthy individuals. You are encouraged to ask any questions you have about the study procedures. The study you are about to review will take 1-4 hours per visit.

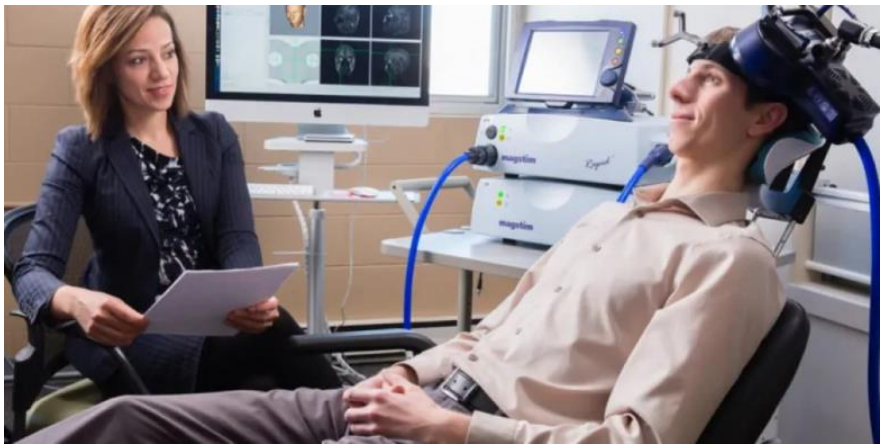


Figure 1. Set-up with rTMS in the reclining chair

When you first visit the NMRL, before you volunteer to participate in this study, you will be asked to complete forms for: 1) TMS safety, 2) MRI safety, 3) medical history, 4) physical activity readiness, 5) physical activity, and 6) handedness. The forms will help us confirm your eligibility and determine if there are any issues that might make it unsafe for you to participate.

You will not be selected to participate if you have:

- History of epileptic events, seizures, or sleep disorders
- Cannot provide a physician in the event of abnormal findings on the MRI
- Implanted medical device, shrapnel or metal object in the body, including dental retainer and copper IUD
- History of adverse reactions to TMS
- Claustrophobia
- Are or may be pregnant
- History of neurological, cardiovascular, or other major disorder (s)
- Current brain injury, psychiatric, or mental health disorder (s)
- History of alcohol or substance abuse
- Injuries or physical limits that prevent or make maximal exercise unsafe
- Currently use any medication with the following properties
    - Acts on the central nervous system
    - Lowers seizure threshold
    - Can damage hearing (ototoxic)
    - Anti-inflammatory
    - Hormonal substances, except for birth control
- Do not respond to or are uncomfortable with transcranial magnetic stimulation
- Weigh more than 300lbs

If you are still interested and eligible to participate, we will explain or demonstrate the study procedures and give you the opportunity to ask questions and review the consent form. If you would like to review or discuss the study procedures privately or with others, you are encouraged to take the consent form with you and return if you choose to participate or contact us with any other questions. If you provide informed consent, you will receive TMS to confirm that you respond and are comfortable with the procedure, and complete the maximal exercise test. We will also shave and mark areas of the skin on your stomach, legs, chest, and fingers so that we may place sensors that measure muscle activity, breathing, and sweating. We will then schedule the remaining visits.

Before the first visit, we will ask to you record your diet over the past 24hr so that you can repeat the same diet 24hr before every remaining visit. You will be asked to avoid physical activity or exercise two days prior to each visit. These requests are made to minimize possible effects of nutrition, soreness, or fatigue on performance or responses to TMS. Between and prior to each visit, you will also be asked (and reminded) to:

1. Avoid changes in medication use
2. Maintain a similar diet and exercise regimen
3. No alcohol, drug, or analgesic use within 24hr

4. Similar amount and timing of sleep the evening before
5. No caffeine within 12hr
6. Don’t eat within 2hr

At the beginning of each visit, you will fill out forms about your sleep quality, mood, stress, and soreness. We will test a urine sample to confirm you are not dehydrated and administer a pregnancy test (if applicable). If you are dehydrated, we will ask you to drink water that we will provide. Each visit will need to be scheduled at a similar time of day (±2hr), and visits will be separated by 1-4 (Study 1) or 2 days (Study 2). Menstrual cycle timing can affect responses to TMS, so unless you are on a monophasic contraceptive (keeps hormones at same level throughout each month), you will be asked to report your menstrual cycle timing and we will schedule your visits during the first 15 days of menses.

Consent Visit: Familiarization

If you decide to participate in this study and provide informed consent, we will use hypoallergenic tape to place sensors on your skin so that we can measure signals from your leg, stomach, back, and finger muscles. We will mark the locations of the sensors for consistent placement every visit. We will apply single pulse TMS to make sure you are comfortable with the procedures and that we can obtain responses from the target muscles. You will then complete maximal strength tests for the legs, stomach muscles, and fingers. Finally, you will practice the maximal cycle ergometer test for familiarity. We will then schedule the remaining visits. This visit will take about 1.5 hours.

Visit 1: Locating the Stimulation Targets Based on Brain Images

During the first visit, you will travel to the BRIDGE center so that we may take pictures of your brain’s structure and function with magnetic resonance imaging (MRI). This painless technique uses strong magnetic fields and radio waves to generate images of the human body. We will also tape sensors to your stomach, legs, chest, and fingers to measure muscle activity, breathing, and sweating. First, we will reconfirm your responses to the MRI safety form to reduce any inconvenience if you are not eligible. You will then be asked to complete the forms about your sleep quality, mood, stress, and soreness.

Before the MRI procedure, we will explain the experimental procedures, which will involve you lying still on a bed with your eyes closed (7-10min) or following instructions presented on a screen, which will include 1) flexing your stomach muscles, 2) flexing your leg muscles, 3) flexing your hand muscles and 4) staring at a point on a screen (10min total). You will be given time to practice each activity.

During the MRI session, you will be exposed to a magnetic field and radio waves. You will hear repetitive tapping sounds and be required to wear earplugs or headphones to reduce the noise. The entire visit will take about 1 hour.

Visit 2: Locating the Stimulation Targets Based on TMS

For the second visit, you will go to the NMRL and we will use single-pulse TMS to locate the areas of the brain we want to stimulate. First, you will tell us whether you can hear TMS pulses while white noise is played through earplugs, and we will adjust the loudness until you no longer hear the TMS pulses. This sound will be played during all visits whenever we use TMS. The dB level of the sound will be less than or equal to 85 dB SPL (equivalent to a food blender, heavy traffic or a noisy restaurant). Next, we will use hypoallergenic tape to place sensors on your skin so that we can measure signals from the skin and muscle. In addition, we will put sensors on your skin that measure your heart rate. Next, we will perform several tests with TMS to find the locations we are going to stimulate during the remaining visits. Images from the first visit will be used to help us find each area, with some initial trial and error. We will then see how the muscles respond to single-pulse TMS at different intensities and stimulation locations. All single-pulse TMS tests will be performed during light muscle contractions in a seated position. Finally, you will perform the maximal cycling exercise test again for additional familiarity. This visit will take about 2-2.5 hours and require up to 720 single TMS pulses.

Whenever single-pulse TMS is used, you will wear earplugs to reduce the clicking sound created by the coil, which is similar to that of an MRI scanner. When we stimulate areas of the brain that control movement, it often makes the target muscles twitch. Also, because we stimulate the scalp, each pulse creates a tapping sensation (similar to somebody tapping you on the head using their finger) and may produce responses in the face, including twitches and eye blinks. Twitches are normal and painless, but can cause scalp soreness and headaches, which are not the result of brain stimulation, but tightness in scalp muscles not used to being activated this way. In addition, responses depend on stimulation intensity: at the lowest intensities, you may feel nothing. As intensity increases, the machine will become louder, tapping sensations on your scalp will grow stronger, and twitches in your muscles will get larger and extend to other muscles.

Visits 3-6 (Study 1): Optimizing the Stimulation Protocol

If you participate in Study 1, you will be randomly assigned to one of two groups: each will receive a different rTMS protocol. You will complete a total of 3 visits, each with a different stimulation target, but otherwise identical. Visits will be separated by at least 2 days (up to three weeks based on menstrual cycle timing) and each visit (at the NMRL) will take up to 4 hours depending on the stimulation protocol.

1. Place Sensors and Cannula for Blood Draws

After the initial procedures, we will use hypoallergenic tape to place sensors on your skin so that we can measure activity in the muscles and skin. The skin will be prepared by removing dead skin with adhesive tape and cleansing with alcohol swabs. Sensors will be placed on your stomach, legs, back, and index fingers. The location of

each sensor will be marked with temporary ink so that we can quickly place sensors on the remaining visits. You will also wear the chest harness so that we can measure your heart and breathing rates, as well as skin temperature. A trained phlebotomist will insert a flexible plastic tube (Teflon cannula) into a vein on the inner side of your forearm to allow for multiple blood draws. A saline solution will be put into the line to prevent clogging. The first blood draw will be taken after cannula placement.

2. Stimulation Target and Intensity

While you produce light contractions in a seated position, we will confirm the location of the stimulation targets and determine the intensity for repeated stimulation (rTMS). To determine rTMS intensity, we will first apply single pulses and then adjust the intensity until we find the lowest intensity that produces consistent responses in the right leg and stomach muscles. We will also measure the size of responses in each muscle to stimulation at a standard intensity.

3. Test Battery

A test battery will be completed at different times during the visit, including right before rTMS, immediately after rTMS, and 30-, 60-, and 90- minutes after rTMS. The test battery will include blood samples, surveys of mood, and sensor measurements of sweating, heart rate, breathing rate, skin temperature, and blood pressure. In addition, we will deliver 10 single TMS pulses to each of the three targets. Each test battery will take around 10 minutes and require 30 single TMS pulses (150 total) and ~14mL of blood (~84mL total).

4. Repetitive Transcranial Magnetic Stimulation

Repetitive TMS (rTMS) will be delivered to one of three brain areas while you relax in a seated position in a reclining chair with built-in support to keep your head still (**Figure 1**). Stimulation may or may not produce muscle twitches (small and brief contractions like eye winking) and will take either 40 or 190 seconds, depending on the protocol. Information from the sensors will be monitored throughout and you will be asked to remain quiet and still unless you feel any negative effects from stimulation. You will also wear the noise making headphones at the intensity that was determined at visit 2.

5. Maximal Exercise

Immediately after the post rTMS test battery, you will complete a maximal lower-extremity cycling exercise test (same as familiarization visit) to assess anaerobic power. The test will begin with a 2-3-minute warm-up. You will then pedal up to maximum speed. Resistance will be added, and you will pedal as fast as possible for 30 seconds. Two sets will be performed, with 2-minutes rest between sets. Information from the sensors (i.e., sweating, heart rate, breathing, skin temperature) will be monitored throughout.

6. Wait Period

After the post-exercise test battery, you will rest in a seated position for approximately one hour. An additional test battery will be administered every 30 minutes (2 total).

Study 2: Responses to Repeated Stimulation

If you participate in Study 2, you will be randomly assigned to one of two groups with the same stimulation protocol and target, but one group will receive sham stimulation. Visits will be separated by two days and each visit will take up to 4 hours depending on the stimulation protocol. Study 2 will be nearly identical to Study 1 except that there will be one fewer test battery (no test battery 90min after rTMS) and two fewer blood draws (4 x ~15mL = ~60mL per visit).

4. How many times will I be stimulated?

Depending on which study or studies you complete, you will receive a different number of TMS pulses, as detailed in the table below. These are estimates.

Study	Name	Subthreshold Excitatory	Subthreshold Inhibitory
1	Visit 1	0	0
	Visit 2	720	720
	Visit 3-6	1000 x 3	1000 x 3
	Total	**3,720**	**3,720**

Study	Name	Pulses
2	Visit 1	0
	Visit 2	840
	Visit 3-7	1000 x 4
	Total	**4,720**

5. How long will I be in the study?

The time and duration of the study will depend on whether you participate in Study 1, Study 2, or both studies. Each visit will last 1-4 hours, as described below:

Study	Name	# of Visits	Visit Time (Hr)	Total Time (Hr)
1	Locate Targets	2	1-2.5	3.5
	Optimized Stimulation	3	4	12
			Total	**15.5**

Study	Name	# of Visits	Visit Time (Hr)	Total Time (Hr)
2	Locate Targets	2	1-2.5	3.5
	Repeated Stimulation	4	4	16
			Total	**19.5**

Study	Name	# of Visits	Visit Time (Hr)	Total Time (Hr)
Both	Locate Targets	2	1-2.5	3.5
	Optimized Stimulation	3	4	12
	Repeated Stimulation	4	4	16
			Total	**31.5**

6. What are the risks, side effects, and discomforts of this research study?

There may be adverse events or side effects that are currently unknown and some of these risks could be permanent, severe or life-threatening. If you experience a life-threatening event, a medical doctor will not be in the facility; 911 will be called. To ensure your safety, the investigators will take every precaution to prevent adverse events and minimize risks. These precautions include screening, familiarization, proper instructions, safe and validated protocols, optimized testing procedures, and close supervision.

Risks of Transcranial Magnetic Stimulation (TMS)

TMS is non-invasive and considered safe. There are conservative research guidelines for the use of TMS in healthy and clinical populations and our examinations fall within those guidelines. The safety of chronic rTMS for brain tissue was confirmed in animals. In the few human case studies performed, no undesirable changes were found in the brain. Nevertheless, TMS carries several risks you should carefully consider.

- **TMS and Metal Objects or Devices**: If you have any metallic objects or implanted devices in close contact with TMS coils, we cannot perform TMS. Examples include cochlear implants, deep brain or vagus nerve stimulators, cardiac pacemakers, spinal cord stimulators, shrapnel, buckshot, and aneurism clips. It is very important that you notify us of any metal objects, devices, or implants in your body before TMS is used.

- **rTMS and Seizure**: Seizure is a serious acute adverse event, but the risk is very small, and almost all reported incidents occurred when TMS use did not conform to current safety guidelines. Sleep deprivation and proconvulsant medications (drugs that stimulate alertness and may place brain at risk for overactivation) may increase this risk. In the rare instance when seizure or fainting occurs, it is usually of short duration, does not require drug treatment, causes no durable effects, and does not increase your risk of such events in the future. Nevertheless, if a seizure does occur, you may lose driving privileges for up to one year. During rTMS, we will monitor muscle activity to ensure that the effects of stimulation do not extend beyond the

target brain regions. If muscle activity spreads to non-target muscles, rTMS will be stopped immediately and you will be released from the study.

- **TMS and Scalp Soreness/Headaches**: Stimulation can cause brief local pain below the sites of stimulation due to soreness from neck or head muscle contraction. Mild headaches, local pain, neck pain, and toothache may occur. These effects are brief and typically limited to the first TMS session.

- **TMS Noise**: Similar to MRI, TMS produces loud clicking sounds. Prior to any TMS procedure, we will provide you with protective earplugs. As a precautionary measure, TMS will not be performed if you use drugs that can damage the ears (e.g., furosemide, gentamicin, cisplatin).

- **rTMS and vasovagal repsonses:** rTMS procedures can also result in lightheadedness, nausea, fainting, or vomiting (vasovagal responses). If you experience a vasovagal response and symptoms have not begun to improve after 15-20 minutes, 911 will be called and you will be released from the study.

- **rTMS Mental Effects**: rTMS can produce undesired mental events, including an increase or decrease in performance on tasks involving correct responses, reaction times, or detection. Hormonal changes may also affect mental functions and cause fatigue, mood changes, irritability, or anxiety. The effects are usually brief and minor.

- **TMS and Pregnancy**: If you are or are trying to get pregnant, the effects of TMS on a fetus are unknown and, therefore, we will not perform the examination at this time. We will ask you to indicate whether you are, might be, or are trying to get pregnant.

- **rTMS Coil Heat**: When the intensity, duration, and frequency of stimulation increase, more heat is generated in the coil. As a consequence, the coil may get warmer, but not to the point of posing any risk to you.

- **rTMS Regulatory Status**: While rTMS is FDA approved for other indications, the safety guidelines were not developed with this type of deep brain stimulation and the risks are unknown.

You may have stimulation stopped at any time if you feel uncomfortable. We will work closely with you and carefully monitor your responses during all TMS tests.

By consenting, you agree to:

- Answer the TMS safety and study intake forms accurately;
- Communicate the experience of any discomfort during TMS and rTMS tests

Risks of Magnetic Resonance Imaging (MRI)

There are no known significant risks with MRI because the magnetic field strengths used are believed to be without harm. There are conservative guidelines for

radio frequency magnetic field exposure and our examinations fall within them. We believe these are safe and less hazardous than an x-ray computed tomography examination (CT scan).

A call button and an intercom are provided so that you may have the scan stopped at any time during the study.

- **MRI and metallic objects**: If a person has a cardiac pacemaker or a certain type of metallic clip or prostheses in their body (i.e., an aneurysm clip in the brain); if a person has worked with metal or had a piece of metal removed from the eye (s); or if a person has shrapnel, bullets, or buckshot in their body. As metallic objects may be strongly attracted to the magnet, it is very important that you notify us of any metal objects, devices or implants in or on your body before you enter the magnet room.

  All other metallic objects must also be removed from you prior to entering the magnet room or approaching the magnet, to prevent them from becoming a projectile or being pulled by the magnet. This includes keys, jewelry, pocketknives, money clips, paper clips, safety pins, hairpins, and barrettes. In addition, objects such as watches, credit cards, and hearing aids could be damaged in the presence of the magnetic field. A locker will be provided for you to secure your valuables.

- **MRI and Pregnancy**: If you are or are trying to get pregnant, the effects of the scan on a fetus are unknown and, therefore, we will not perform the examination.

- **MRI and heating**: There is a risk of heating of metal objects such as wires from exposure to radio waves. Please report any heating/burning sensation immediately. You may have the scan stopped at any time if this occurs using the call button.

- **MRI and Muscle Twitches**: There is a possibility that you will experience a localized twitching sensation due to the magnetic field changes during the scan. This is not unexpected and should not be painful. However, you may have the scan stopped at any time if this occurs using the call button.

- **MRI and Dizziness**: Dizziness and nausea may occur momentarily when your head is moved in or out of the tunnel of the magnet. The sensation should disappear quickly. If not, you may discontinue the scan at any time.

- **MRI and Claustrophobia**: You may experience claustrophobia, i.e. the fear of having no escape and being closed in. You may discontinue the scan at any time.

- **MRI and Incidental Findings**: The images collected in this study are intended for research. The imaging protocols are not designed to examine medical conditions and BRIDGE Center staff are not trained to evaluate your images. Your brain images will not be routinely examined by a clinical radiologist and you should not rely on this MRI to detect or screen for brain abnormalities. There is a remote

possibility that something unusual or different about your brain that has nothing to do with the research may be noticed by the researchers involved in this study or BRIDGE Center staff. For example, this could be a structural abnormality. If this happens, we may share your scans with a radiologist who will review them at no charge to you. If the radiologist suggests you obtain further tests, the Principal Investigator will attempt to contact you with this recommendation. You will be responsible for following up with your physician, and if you or your physician requests copies of your brain images from this study, they will be provided to you. At the investigator's discretion, you may view your brain images and receive copies of them. However, you should be aware that brain structures within the normal population are highly variable, and that it is difficult to draw any conclusions from your images. You should also be aware there is a potential you could experience some distress or discomfort from viewing your own images.

By consenting, you agree to:

- Answer the MRI safety form accurately; tell investigators about any metal in your body
- Not bring any metal devices into the scanning room without staff approval

Risks of Physical Activity

- When performed correctly, physical activity is a low-risk activity in healthy individuals. However, risks exist, including fatigue, soreness, dizziness, lightheadedness, fainting, nausea, and vomiting. Additional risks associated with physical activity include the possibility of falls, muscle strains or pulls of the involved musculature, muscle spasms or strains, and in extremely rare instances, muscle, ligament, or tendon damage. There is also a slight risk of cardiopulmonary overexertion. We will make all exercises as safe as possible through screening, familiarization, instruction, practice, and supervision by experienced testing personnel. All lab personnel are CPR and automated external defibrillator (AED) certified, and there is an AED in the laboratory. However, <u>in the case of a life-threatening event, a medical doctor will not be in the facility; 911 will be called.</u>

Risks of Blood Draws

- There are slight risks associated with blood draws and indwelling cannulas including localized soreness, ecchymosis (blood under the skin), and in extremely rare cases, infection. Blood draw procedures can also result in lightheadedness, nausea, fainting, or vomiting (vasovagal responses). To reduce any risks associated with blood draws, all research personnel conducting blood draws will be well-trained phlebotomists. If you experience a vasovagal response and symptoms have not begun to improve after 15-20 minutes, 911 will be called and you will be released from the study.

Risks of Body Sensors

- Sensors placed on your skin with tape, gel, or temporary adhesives can cause discomfort when pressed tightly against the skin. The electrode application process

involves the use of scrubbing to remove dead skin, and an electrode gel, adhesive, or tape, which may cause temporary discomfort and skin itchiness or redness.

Risks of Breach of Confidentiality

- Breach of confidentiality is a risk of any research study. To minimize this risk, all data is kept in locked cabinets and password protected computers without any information that could link you to your data. Any information that could be used to identify you will be destroyed after 7 years and any remaining data will be anonymous.

Risks of Repeated Visits

- Multiple visits could cause financial or time-related inconveniences.

7. What benefits can I expect from being in the study?

There are no direct benefits of participation. This study will determine if non-invasive brain stimulation can be used to improve physical performance and stress responses by regulating hormone activity. In addition, we will test the effect of repeated sessions of brain stimulation. You will learn about modern brain science and performance testing techniques. You may better understand several aspects of your physical performance. At your request, we will review your data with you after testing and explain our observations.

8. If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be notified if any new information we learn during this research study may cause you to change your mind about continuing to participate in the study.

9. Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

None of the services or procedures you receive during this research study will be billed to you or your health insurance. If you receive a bill or believe your health insurance has been billed for something that is part of the study, notify a member of the research team or UPMC Patient Billing Services.

10. Will I be paid if I take part in this research study?

Compensation will be provided based on the number of visits you complete. If you only participate in study 1, there will be 5 visits, resulting in $250 compensation. If you only participate in study 2, there will be 6 visits and you will receive $350. If you participate in both studies, you will not complete the first two visits twice, and will therefore be asked to come to the laboratory 9 times and get compensated $700 for your time. If you are unable to make it to study visits without additional funds please speak with the study team as reimbursement for travel may be available.

Study 1*		Study 2		Both Studies	
Consent/Familiarization	$10	Consent/Familiarization	$10	Consent/Familiarization	$10
Visit 1	$15	Visit 1	$15	Visit 1	$15
Visit 2	$50	Visit 2	$50	Visit 2	$50
Visit 3	$50	Visit 3	$50	Visit 3	$50
Visit 4	$50	Visit 4	$50	Visit 4	$50
Visit 5	$75	Visit 5	$75	Visit 5	$75
		Visit 6	$100	Visit 6	$100
				Visit 7	$100
				Visit 8	$100
				Visit 9	$150
Total	**$250**	**Total**	**$350**	**Total**	**$700**

Since you are being compensated for your participation in this study, your name, address, and social security number will be released to the Accounting Office. If the total reimbursement for your participation in research is greater than $600 in a year, this will be reported to the Internal Revenue Service (IRS) as income.

11. Who will pay if I am injured as a result of taking part in this study?

There is no compensation for injury for participation in this study.

12. Who will know about my participation in this research study?

Per University of Pittsburgh policy all research records must be maintained for at least 7 years following final reporting or publication of a project. All records related to your involvement in this research study will be stored in a locked file cabinet or password-protected computer and any information about you will be kept as private as possible. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission.

13. Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study.Authorized representatives of the University of Pittsburgh Office of Research Protections and the Department of Defense may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study. Authorized representatives of the UPMC hospitals or other affiliated health care providers may have access to your identifiable information (which may your identifiable medical record information) for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (such as laboratory tests, diagnostic procedures) associated with research study

participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and (3) for internal hospital operations (i.e. quality assurance).

The data from this study may be shared with other investigators; however, only data without identifiers (such as your name) will be shared. We will protect your privacy and the confidentiality of your research records, as described in this document, but cannot guarantee the confidentiality of your research records, including information obtained from your medical records, once your personal information is disclosed to others outside UPMC or the University. In unusual cases, the investigators may be required to release identifiable information related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

14. Is my participation in this research study voluntary?

Your participation in this research study is entirely voluntary. You may want to discuss this study with your family and friends and your personal physician before agreeing to participate. If there are any words you do not understand, feel free to ask us. The investigators will be available to answer your current and future questions.

Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

15. May I withdraw my consent for participation in this research study?

You can, at any time withdraw from this research study; you can also withdraw your authorization for us to use your identifiable medical information for the purposes described above. This means that you will also be withdrawn from further participation in this research study. Any identifiable research or medical information obtained as part of this study prior to the date that you withdrew your consent will continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw from this research study, you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form. Your decision to withdraw from this study will have no effect on your current or future relationship with the University of Pittsburgh.

16. If I agree to participate in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers if, for example, you are unable or unwilling to perform the required tasks or upon the unlikely development of a neurological or cardiovascular disorder. The co-investigators have the right to withdraw you from this study if you develop a muscular, ligament or bone injury. Any injury will be determined by the co-investigators through questioning and physical examination. You may be removed for signs of intolerance to TMS, including severe headaches, dizziness, nausea, or hearing loss.

17. Status of Investigational Devices

The TMS device (Magstim stimulator) used in this study is considered substantially equivalent to electrical devices (like TENS units) and cleared through the FDA 510 (k) premarket notification process. One of the TMS coils (Jaltron Curved Double coil) is an investigational device limited by federal law to investigational use. The MRI scanner (3T Prisma) is cleared through the FDA 510 (k) premarket notification process.

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator (s) listed on the first page of this consent document at the telephone number (s) given.

I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator. I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations that occurred during my participation. By signing this form I agree to participate in this research study. A copy of this consent form will be given to me.

_____

Participant's Name (Print)

_____

Participant's SignatureDate

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual (s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual (s) have about this study have been answered, and we will always be available to address future questions, concerns or complaints as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

Appendix C Screening Form

1. Demographics

Sex: M / F

Date of Birth ____/_____/_____ (Exclude if older than 40)

Height _____

Weight _____ (Exclude if >300lbs)

Best way to contact you:

- Phone _____

- Email _____

2. Handedness/footedness

a. Are you right-handed?

Yes

No (Exclude if NO)

b. Are you right footed? (e.g. if you were to kick a ball would you use your right foot most often?)

Yes

No (Exclude if NO)

3. Physical Activity

a. How many days per week do you engage in physical activity? _____

b. How many minutes do you spend engaged in physical activity on these days? _____

Total minutes of exercise per week _____

(Total of days per week x minutes/day less than 120 minutes- Exclude)

4. Are you currently taking any prescription medications, or over-the-counter medications?

☐ No

☐ Yes (if yes, please list)

IF yes:

Are any of these drugs used to treat a psychological disorder or diagnosis?

☐ Yes (exclude)

☐ No

Would any of these drugs alter your hormones (with the exception of birth control)?

☐ Yes (exclude)

☐ No

Are any of these drugs an antibiotic, antiviral or antifungal medication?

☐ Yes (Wait until medication is stopped prior to enrollment)

☐ No

Are any of these drugs used to treat acne?

☐ Yes (obtain PI approval prior to enrollment)

☐ No

5. Have you used any recreational drugs more than 2 times per month in each of the previous 6 months?

☐ Yes

☐ No

a. Is cannabis the only recreational drug you have used more than 2 times per month in the past 6 months?

☐ Yes

☐ No (If No exclude)

b. Are you willing to not use cannabis for 24hr before each visit?

☐ Yes

☐ No (exclude)

6. Do you frequently experience vasovagal responses, such as feeling faint, paleness, nausea, dizziness, in response to blood draws or exercise?

☐ Yes (Exclude)

☐ No

7. MRI Safety Screening

a. Have you had a prior surgery or operation?

☐ No

☐ Yes (if yes, please list below)

_____

_____

b. Do you have non-removable electronic, mechanical or magnetic implants (e.g., metal screws, etc.) anywhere in your body?

☐ No

☐ Yes (if yes, please list below) (If yes Exclude)

_____

_____

c. Have you been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)?

☐ No

☐ Yes (if yes, please list below) (If yes review with PI prior to enrollment)

_____

_____

d. Do you have any intravascular stents, filters, aneurism clips or shunts?

☐ No

☐ Yes (if yes, please list below) (If yes Exclude)

_____

e. Do you have a cardiac pacemaker?

☐ No

☐ Yes (If yes Exclude)

f. Do you have a cochlear implant?

☐ No

☐ Yes (If yes Exclude)

g. Do you have any non-removable body piercings?

☐ No

☐ Yes (If yes Exclude)

h. Do you have any large tattoos?

☐ No

☐ Yes (if yes, please list locations below) (If yes review with PI)

_____

i. Have you ever done any welding, grinding, or cutting of metal in your lifetime?

☐ No

☐ Yes (if yes, please describe how much, if you wore eye protection, and every had an injury while working with metal) (If yes review with PI)

_____

j. Do you have a medical history of developing seizures?

☐ No

☐ Yes (Exclude if Yes)

k. Do you have a history of claustrophobia or discomfort with confined spaces?

☐ No

☐ Yes (Exclude if Yes)

l. Are you able to tolerate loud noises for sustained periods of time?

☐ No (explain) (If No review with PI)

☐ Yes

m. Have you experienced any problem related to a previous MRI examination or MR procedure?

☐ No

☐ Yes (if yes, please describe below) (If yes review with PI)

_____

n. Do you currently have an ear infection or any ear pain?

☐ No

☐ Yes

If yes, wait for enrollment until condition is resolved.

o. Do you currently wear hair extensions, a weave or wig?

☐ No

☐ Yes

If yes, are your extensions/weave/wig held in place with metallic clips, threads, or another metallic item that cannot be removed for the experiment?)

☐ Can be removed

☐ Cannot be removed (If cannot be removed Exclude)

p. Females only: Are you pregnant?

☐ No

☐ Yes (If Yes Exclude)

q. Females only: Do you have an IUD?

☐ No

☐ Yes (if yes, please list below which one) (3T compatible IUDs are: Mirena and Liletta)

_____

r. Do you currently have braces?

☐ No

☐ Yes (please indicate below) (If yes exclude)

_____

s. Do you have a permanent retainer?

☐ No

☐ Yes (If yes exclude)

_____

t. Do you wear glasses?

☐ No

☐ Yes

8. Do you wear contacts?

☐ No

☐ Yes

a. If YES to glasses or contacts: Is your corrected vision normal (20/20)?

☐ No (If No Exclude)

☐ Yes

9. Are you able to hold still for over an hour?

☐ No (If no Exclude)

☐ Yes

10. Do you currently have a cold or allergies that result in sneezing or coughing?

☐ No

☐ Yes

If yes, wait for enrollment until condition is resolved.

11. Other medical history

a. Do you have a history of epilepsy, seizure or sleep disorders?

☐ No

☐ Yes (If Yes Exclude)

b. Do you have a history of any other major disorder or chronic condition (e.g, cardiovascular or neurological disorder)?

☐ No

☐ Yes (If Yes Exclude)

c. Are you comfortable performing maximal exercise?

☐ No (If No Exclude)

☐ Yes

d. Do you currently have any musculoskeletal injuries or physical limitations?

☐ No

☐ Yes (If Yes Exclude)

If inclusion criteria are met: Based on your answers, it appears you may be eligible to participate in this study. Would you like to schedule a time to come to the NMRL to complete the screening/enrollment process?

Date Scheduled ____/_____/_____ Time ___:____

If you have any questions or concerns, please feel free to contact me. My name is [name] and I can be reached at [phone number] and/or [email address].

If PI review is needed: Based on your answers it appears you may be eligible to participate in this study. However, our PI will need to review some of your answers prior to continuing. We'll call you back in 24-48 hours to let you know if you are eligible.

If you have any questions or concerns, please feel free to contact me. My name is [name] and I can be reached at [phone number] and/or [email address].

If any exclusion criteria are met: Based on your answers, it appears that you are not eligible to participate in this study at this time. Could we contact you again if we have other study opportunities?

☐ No ☐ Yes

If you have any questions or concerns, please feel free to contact me. My name is [name] and I can be reached at [phone number] and/or [email address)

For Staff Completion:

Date of phone screening: ____/_____/_____

Screening Result: ☐ Eligible Enrollment Scheduled

☐ Eligible-Declined participation

Reason for declining _____

☐ Excluded

Reason for exclusion _____

Staff completing phone screening:

Print Name _____

Signature _____

Appendix D Questionnaires

Edinburgh Handedness Inventory

Please mark the box that best describes which hand you use for the activity in question

	Always Left	Usually Left	No Preference	Always Right	Usually Right
Writing					
Throwing					
Scissors					
Toothbrush					
Knife (without fork)					
Spoon					
Match (when striking)					
Computer mouse					

Waterloo Footedness Questionnaire

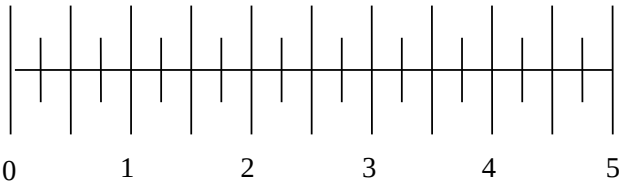
Answer each of the following question as best you can. Please do not simply check one box for all questions, but imagine yourself performing each activity in turn, and then mark the appropriate answer. If necessary, stop and simulate the activity.

	Always Left	Usually Left	Equal	Always Right	Usually Right
1. Which foot would you use to kick a stationary ball at a target straight in front of you?					
2. If you had to stand on one foot, which foot would it be?					
3. Which foot would you use to smooth sand at the beach?					
4. If you had to step up onto a chair, which foot would you place on the chair first?					
5. Which foot would you use to stomp on a fast-moving bug?					
6. If you were to balance on one foot on a railway track, which foot would you use?					
7. If you wanted to pick up a marble with your toes, which foot would you use?					
8. If you had to hop on one foot, which foot would you use?					
9. Which foot would you use to help push a shovel into the ground?					
10. During relaxed standing, people usually put most of their weight on one foot, leaving the other leg slightly bent. Which foot do you put most of your weight on first?					
11. Is there any reason (i.e. injury) why you have changed your foot preference for any of the above activities?	YES	NO	(circle one)		
12. Have you ever been given special training or encouragement to use a particular foot for certain activities?	YES	NO	(circle one)		

13. If you answered YES for question 11 or 12, please explain:

Subject Number: _____ Date: _____

Sleep Quality



No sleep Best sleep

1- Very light sleep
2- Poor sleep quality
3- Moderate quality sleep
4- Very good sleep quality

Hours of sleep last night: _____

Where did you sleep? _____

What Kind of Surface did you sleep on?

CIRCLE:

BED

SOFA

OUTDOORS

CARPET

HARDWOOD

WATERBED

Holmes-Rahe Life Stress Inventory **Subject Number: _____** **Date: _____**

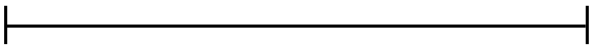
*Circle the associated number next to each of these life events that has happened to you during the previous **month***

#	Event
1	Death of spouse
2	Divorce
3	Marital Separation from mate
4	Detention in jail or other institution
5	Death of a close family member
6	Major personal injury or illness
7	Marriage
8	Being fired at work
9	Marital reconciliation with mate
10	Retirement from work
11	Major change in the health or behavior of a family member
12	Pregnancy
13	Sexual Difficulties
14	Gaining a new family member (i.e.. birth, adoption, older adult moving in, etc)
15	Major business readjustment
16	Major change in financial state (i.e.. a lot worse or better off than usual)
17	Death of a close friend
18	Changing to a different line of work
19	Major change in the number of arguments w/spouse (i.e.. either a lot more or a lot less than usual regarding child rearing, personal habits, etc.)
20	Taking on a mortgage (for home, business, etc..)
21	Foreclosure on a mortgage or loan
22	Major change in responsibilities at work (i.e. promotion, demotion, etc.)
23	Son or daughter leaving home (marriage, attending college, joined mil.)
24	In-law troubles
25	Outstanding personal achievement
26	Spouse beginning or ceasing work outside the home
27	Beginning or ceasing formal schooling
28	Major change in living condition (new home, remodeling, deterioration of neighborhood or home etc.)
29	Revision of personal habits (dress manners, associations, quitting smoking)
30	Troubles with the boss
31	Major changes in working hours or conditions
32	Changes in residence
33	Changing to a new school
34	Major change in usual type and/or amount of recreation
35	Major change in church activity (i.e.. a lot more or less than usual)
36	Major change in social activities (clubs, movies,visiting, etc.)
37	Taking on a loan (car, tv,freezer,etc)
38	Major change in sleeping habits (a lot more or a lot less than usual)
39	Major change in number of family get-togethers
40	Major change in eating habits (a lot more or less food intake, or very different meal hours or surroundings)
41	Vacation
42	Major holidays

Subject_____ Date_____ Visit_____ Time Point _____

Muscle Pain/Soreness Data Sheet

Draw a vertical line corresponding to the pain/soreness that you have as a result of the exercise protocol.



No pain/ soreness

Pain/soreness as bad as it could be

Subject Number: _____ **Date:** _____

Shortened Profile of Mood States

Below is a list of words that describe feelings people have. Please read each one carefully; then mark ONE circle under the answer to the right which best describes HOW YOU FEEL RIGHT NOW.

The numbers refer to these phrases:

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

	0	1	2	3	4
Tense					
Angry					
Worn Out					
Unhappy					
Lively					
Confused					
Peeved					
Sad					
Active					
On edge					
Grouchy					
Blue					
Energetic					
Hopeless					
Uneasy					
Restless					
Unable to Concentrate					
Fatigued					
Annoyed					

	0	1	2	3	4
Discouraged					
Resentful					
Nervous					
Miserable					
Cheerful					
Bitter					
Exhausted					
Anxious					
Helpless					
Weary					
Bewildered					
Furious					
Full of Pep					
Worthless					
Forgetful					
Vigorous					
Uncertain about things					
Bushed					

Tegner Activity Level Score

Please indicate in the spaces below the HIGHEST level of activity that you participated in BEFORE YOUR INJURY and the HIGHEST level you are able to participate in CURRENTLY.

BEFORE INJURY: LEVEL_____ CURRENT: LEVEL_____

Level	Activity
10	Competitive sports- soccer, football, rugby (national elite)
9	Competitive sports- soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball
8	Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing
7	Competitive sports- tennis, running, motorcars speedway, handball Recreational sports- soccer, football, rugby, bandy, ice hockey, basketball, squash, racquetball, running
6	Recreational sports- tennis and badminton, handball, racquetball, down-hill skiing, jogging at least 5 times per week
5	Work- heavy labor (construction, etc.) Competitive sports- cycling, cross-country skiing, Recreational sports- jogging on uneven ground at least twice weekly
4	Work- moderately heavy labor (e.g. truck driving, etc.)
3	Work- light labor (nursing, etc.)
2	Work- light labor Walking on uneven ground possible, but impossible to back pack or hike
1	Work- sedentary (secretarial, etc.)
0	Sick leave or disability pension because of knee problems

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