Investigating the Test-Retest Reliability of Motor Cortex Excitability Using Transcranial Magnetic Stimulation

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Declaration

I declare that this thesis is my own account of my research and contains as its main content
work that has not previously been submitted for a degree at any tertiary educational
institution.

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21/10/19
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Abstract

The coordination of movement, from the process of deciding how to move to the accurate execution of that movement, is what allows for successful interaction with the environment. The study of the motor cortex provides insight into what constitutes normal and abnormal patterns of movement. Research using a non-invasive brain stimulation technique called transcranial magnetic stimulation (TMS) has suggested that intracortical facilitation, that is facilitatory neural activity in the primary motor cortex, plays an important role in motor control. One form of facilitation known as short-interval intracortical facilitation (SICF), can be measured using a paired-pulse protocol of TMS. However, the reliability of this protocol has yet to be established. The current study aimed to investigate the test-retest reliability of paired-pulse TMS to measure SICF in healthy younger adults ($N = 16$). In addition, the current study explored the relationship between SICF and manual dexterity as measured by the Purdue pegboard test. Results indicated excellent test-retest reliability of SICF magnitude. Finally, SICF magnitude was found to be positively associated with right-hand performance in the Purdue pegboard test. Taken together, the findings of the current study suggest that SICF can be reliably measured by TMS across different sessions. The current study contributes to the literature suggesting that SICF is important for motor control. Further, this understanding of the role of SICF in the healthy brain could provide avenues for future research to examine SICF in people with movement or neurological disorders affecting motor control.

Keywords: short-interval intracortical facilitation, transcranial magnetic stimulation, primary motor cortex
Investigating the Test-Retest Reliability of Motor Cortex Excitability Using Transcranial Magnetic Stimulation

Many of us only think of motor control in the context of abnormal movement or movement disorders such as Parkinson’s disease, dystonia or essential tremor. However, motor control is crucial in all forms of movement, from simple tasks such as reaching and grasping for a glass of water, to more complex tasks such as walking or running. The coordination of movement, from the process of deciding how to move to the accurate execution of that movement, is what allows for successful interaction with the environment (Latash, Levin, Scholz & Schöner, 2010). The ability to regulate movement and manipulate objects is referred to as motor control (Latash et al., 2010; Martin, 2005). Despite the knowledge that motor control serves a crucial role in everyday life, the neural mechanisms that underlie motor control have yet to be fully understood. By understanding the mechanisms underlying motor control in the healthy brain, we can begin to understand the causes of movement disorders and abnormal patterns of movement.

Structurally, the primary motor cortex (M1) is situated in the frontal lobe of the brain and plays a role in the execution of movement (Seidler et al., 2010). M1 contains representations of every human muscle, organised somatotopically (Marieb & Hoehn, 2019). Larger areas of cortical brain matter are devoted to the control of muscles requiring more complex and fine motor movements such as hands and fingers (Marieb & Hoehn, 2019). The two hemispheres of M1 are responsible for the motor representations of the two sides of the body, with the left M1 responsible for representations of the right side of the body and right M1 responsible for the left side of the body (Marieb & Hoehn, 2019). Neural signals descend from M1 along a white matter motor pathway known as the corticospinal tract (Seidler et al., 2010). The corticospinal tract is the primary route in controlling voluntary muscle activity in
the body and is therefore fundamental in the execution of coordinated movement (Marieb & Hoehn, 2019).

Within the hand representation in M1, there are both inhibitory and facilitatory neuronal circuits, referred to as intracortical inhibition and intracortical facilitation, respectively. Intracortical inhibition refers to activity from inhibitory neuronal circuits in M1 suppressing activity in the corticospinal tract (Kujirai et al., 1993). Intracortical facilitation refers to activity from facilitatory neuronal circuits in M1 increasing activity in the corticospinal tract (Chen et al., 2008). Intracortical inhibition and facilitation are both important in motor control, as they are both involved in the planning, controlling and execution of movement (Chen et al., 2008). Thus, understanding intracortical inhibition and facilitation is crucial in understanding motor control.

Transcranial magnetic stimulation (TMS) has emerged as an effective tool for assessing the excitability of both inhibitory and facilitatory processes occurring in M1 (Chen et al., 2008; Hallett, 2007). TMS is a non-invasive brain stimulation technique that has been used to understand human motor physiology and motor cortex function (Hallett, 2007). TMS is a safe and painless stimulation procedure that has been used in both research and clinical settings since the 1980s (Barker, Jalinous & Freeston, 1985). TMS involves generating a high-intensity electrical current through a coil consisting of copper wiring, insulated by a plastic case (Chen et al., 2008; Hallett, 2007). When a pulse is delivered through the coil held over the scalp, the electrical current produces a magnetic field that passes through the scalp and skull and induces current flow in the underlying brain tissue (Di Lazzaro et al., 2004). This current flow sends a wave of electrical activity, referred to as the descending volley, down the corticospinal tract towards the spinal cord (Di Lazzaro et al., 1998).

Figure 1, from Klomjai, Katz and Lackmy-Vallée (2015), outlines the mechanism of action of TMS on M1. A single TMS pulse applied over the hand representation of M1 will
activate neuronal circuits, sending action potentials down the corticospinal tract, and will activate the hand muscles (Hallett, 2007). This activation or “twitch” of the hand muscles is known as a motor evoked potential (MEP), which is measured by recording electromyography (EMG) activity from electrodes placed on the muscle(s) of interest (Di Lazzaro et al., 2004). Peak-to-peak MEP amplitudes provide a measure of corticospinal excitability, which is the level of activity of the pathway from the point of stimulation to the muscle from which the MEP is recorded. Whilst a single pulse of TMS can explore cortical excitability, different TMS protocols can be used to explore facilitatory and inhibitory processes occurring in M1.

Figure 1. The mechanism of action of transcranial magnetic stimulation (TMS) applied over the motor cortex. TMS over the motor cortex preferentially activates interneurons, leading to
electrical volleys descending along the corticospinal tract. The excitation of interneurons following TMS leads to activation of the target muscle evoking a motor-evoked potential (MEP). The amplitude of the MEP provides a measure of corticospinal excitability. Image adapted from “Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS)”, by W. Klomjai, R. Katz and A. Lackmy-Vallée (2015), Annals of Physical and Rehabilitation Medicine, 58(4), 209. Copyright 2015 by Elsevier Masson. Adapted with permission.

Intracortical facilitation can be measured with a TMS protocol called paired-pulse TMS. Paired-pulse TMS to measure facilitation involves delivering an initial suprathreshold pulse (enough to elicit a MEP) followed by a subthreshold pulse (insufficient to elicit a MEP) occurring 1-5 milliseconds later (Ziemann et al., 1998). The MEP produced using the paired-pulse protocol of TMS is greater than the MEP produced by a single pulse alone. The facilitation of the MEP can be seen in Figure 2A, which shows the increased level of amplitude of the MEP following paired-pulse TMS compared to single-pulse TMS. This increased activity is thought to arise from the activation of facilitatory processes from the first pulse that are still active at the time of the second pulse (Kujirai et al., 1993). This form of facilitation is referred to as short-interval intracortical facilitation (SICF). SICF is quantified by expressing the MEP elicited by single-pulse TMS as a ratio of the MEP elicited by paired-pulse TMS. Studies have shown three distinct SICF peaks. As can be seen in Figure 2B, the three peaks occur when paired pulses are separated by interstimulus intervals (ISIs) of (1) 1.3-1.7 milliseconds (ms), (2) 2.3-2.7 ms, and (3) 4.1-4.5 ms (Clark, Loftus & Hammond, 2011; Opie, Cirillo & Semmler, 2018; Peurala, Müller-Dahlhaus, Arai, & Ziemann, 2008; Van den Bos et al., 2018; Ziemann et al., 1998). Facilitation is strongest at the first peak, decreases at the second peak, as is the lowest at the third peak (Figure 2B). There is evidence to suggest that SICF originates at the cortical level, with the peaks of facilitation thought to
be the results of interactions between activation by the two stimuli of facilitatory circuits in M1 (Di Lazzaro et al., 1998; Ziemann et al., 1998).

Figure 2. Panel A shows the motor evoked potential (MEP) following single-pulse and paired-pulse TMS. Stimulus 1 (S1) represents a single suprathreshold stimulus eliciting a MEP. S1 coupled with a subthreshold stimulus 2 (S2) represents the paired-pulse protocol required to elicit short-interval intracortical facilitation (SICF). The MEP following paired-pulse stimulation is greater than that of a single pulse alone. Panel B shows SICF as a function of interstimulus interval, showing three distinct peaks of facilitation as well as two troughs.
troughs where no facilitation occurs. The x-axis indicates the time between the first and second stimulus in milliseconds (ms), referred to as the interstimulus interval (ISI). The y-axis indicates SICF as a ratio of mean paired-pulse MEP amplitude to mean single-pulse MEP amplitude. A ratio greater than 1.0 indicates facilitation, a ratio of 1.0 indicates no facilitation, and a ratio of less than 1.0 indicates inhibition.

The TMS literature suggests that SICF is important in manual dexterity and might be associated with the pathophysiology of movement disorders. For example, Clark et al. (2011) explored the relationship between SICF and manual dexterity of both younger and older adults. Paired-pulse TMS was used to measure SICF at all three peaks of facilitation, whilst the Purdue pegboard test was used to measure manual dexterity. The results showed a significant positive relationship between the magnitude of the second SICF peak and manual dexterity, suggesting that increased SICF at the second peak reflects better manual dexterity.

Further, Cattaneo et al. (2005) explored the relationship between SICF and aspects of motor control such as planning and execution of manual dexterity tasks. Paired-pulse TMS was used to measure SICF whilst individuals prepared to make a grasping movement with both hands. The results showed that modulation of SICF, particularly at the second peak, is positively associated with the planning of grasping movement with both hands (Cattaneo et al., 2005). Thus, measurements of SICF may elucidate mechanisms involved in motor control of the hand muscles.

Studies investigating SICF in individuals with neurological disorders have found an association between SICF and abnormal movement (Ho, Lee, Nithi, Palace & Mills, 1999; Mori et al., 2013; Ni, Bahl, Gunraj, Mazzella & Chen, 2013). People with Parkinson’s disease show greater SICF at all three peaks than controls without Parkinson’s disease (Ni et al., 2013). In addition, patients with multiple sclerosis show reduced SICF magnitude in comparison to controls without multiple sclerosis (Ho et al., 1999; Mori et al., 2013). Whilst the research in this area is limited, it does suggest that SICF might be useful to understand the
pathophysiology of movement disorders. Despite its potential clinical utility in understanding both normal and abnormal patterns of movement, there is limited research suggesting that SICF is a reliable measure of facilitatory processes occurring in M1.

Currently, no studies have assessed the test-retest reliability of SICF across different sessions. Single-pulse and paired-pulse measures of other facilitatory and inhibitory processes have shown varying levels of test-retest reliability. Measures of short-acting and long-acting intracortical inhibition using well established paired-pulse TMS protocols have shown excellent and good test-retest reliability respectively (Biabani, Farrell, Zoghic, Eganb & Jaberzadeh, 2018; de Goede & van Putten, 2017). Only one study has assessed the reliability of paired-pulse TMS measures of intracortical facilitation (Biabani et al., 2018). The study assessed a form of facilitation known as intracortical facilitation (ICF), which is distinct from SICF. (To measure ICF, a subthreshold first pulse is followed by a suprathreshold second pulse separated by an ISI of 8-30 ms (Biabani et al., 2018)). Test-retest reliability for ICF was found to be poor (Biabani et al., 2018). To date, no published studies have investigated the test-retest reliability of SICF. As such, it remains to be seen whether paired-pulse TMS protocols can reliably assess SICF.

Due to the lack of evidence indicating the test-retest reliability of SICF, the utility of TMS to help understand motor control is limited and may lead to the misinterpretation of findings in both research and clinical settings. Establishing the test-retest reliability of SICF will provide a reliable measure for future research to examine the role of SICF in the context of the neural control of movement. In addition, the reliability of SICF will provide a reliable measure for assessments and treatments of abnormal or disordered movement that involve facilitatory processes. Thus, the present study aimed to investigate the test-retest reliability of paired-pulse TMS measuring SICF. SICF was assessed using well established single-pulse and paired-pulse TMS protocols delivered to M1 in two identical sessions. Of interest was the
difference in the magnitude of SICF and the ISIs at which SICF was maximal between sessions. Two hypotheses were generated, namely: (1) that SICF will demonstrate excellent test-retest reliability among participants for both sessions; (2) that the ISIs that produce maximal SICF will be consistent between sessions within individuals. In addition, if SICF was found to have excellent test-retest reliability, the present study sought to explore the relationship between SICF and manual dexterity (as measured by the Purdue pegboard test).

Methods

Sample Size Justification

The determination of a suitable sample size was based on the sample sizes used in previous research assessing the test-retest reliability of intracortical processes in M1 using paired-pulse TMS. Biabani et al. (2018) used the most similar study design to the present study, but instead assessed the test-retest reliability of short-acting inhibition occurring in M1. Biabani et al. (2018) used two identical sessions separated by one week. Their study found significant and strong test-retest reliability of intracortical inhibition using 15 participants. The present study recruited 16 participants.

Participants

Participants consisted of 11 female and 5 male young adults ($M_{age} = 24$, $SD_{age} = 5.4$, age range: 18-35). Participants comprised mostly undergraduate psychology students enrolled at Murdoch University ($n = 10$), who signed up to the experiment through an online study portal and were remunerated with credit towards their studies. Other participants were recruited from the wider community and were not remunerated for their participation ($n = 6$). In accordance with the Declaration of Helsinki, informed consent was obtained by all participants (Appendix C). Further, participants were informed that they were able to withdraw from the study at any point during the study procedure.
All participants were screened with the TMS safety screening questionnaire (Appendix B). Items on the safety screening questionnaire include whether the participant: was pregnant; had a history of epilepsy or seizures; had brain surgery; suffered a concussion within the last six months; had any underlying neurological conditions; or was currently taking medications that affect the central nervous system. Participants were excluded if they responded “Yes” to any of the exclusion criteria outlined in the safety screening questionnaire. Participants were also excluded if the TMS intensity required to elicit a MEP was high – above 85% of machine stimulator output ($n = 1$). Participants were also excluded if they could not maintain total relaxation of the hand muscles ($n = 1$). Participants were also screened for their hand dominance, as research suggests significant differences in intracortical processes between people who are left and right-hand dominant (Cirillo, Rogasch & Semmler, 2010). A 10-item Edinburgh Handedness inventory was used during the screening procedure to determine the handedness of participants (Appendix E; Oldfield, 1971). The test produces a score of handedness on a scale of -100 (indicating extreme left-handedness) to 100 (indicating extreme right-handedness), with scores between -40 and 40 indicating ambidexterity. Individuals with a score below 40 were thus excluded ($n = 1$). All individuals who participated in the study were right-handed ($M$ score = 92, $SD$ = 5.6).

**Neurophysiological Procedure**

**Electromyography (EMG).** Participants were seated in a height-adjustable chair with their arms resting on the armrest of the chair or a cushion placed on their lap. EMG activity was recorded from the first dorsal interosseous (FDI) of the right-hand muscle. The FDI was chosen because it is a muscle important for the abduction of the index finger and for fine motor movements such as precision grasping and object manipulation (Clark et al., 2011). The skin was prepared by cleaning the hand with an alcohol solution and course gauze. EMG recording electrodes were placed on the FDI, with an active recording electrode over the
belly and a reference electrode over the tendon. Ground electrodes were placed on the elbow and wrist of the participant to reduce background electrical noise – such as noise from the main power supply - contaminating the EMG signal. The data obtained from the EMG signal were recorded and digitised at a sampling rate of 5000Hz using a CED 1401 analogue-to-digital converter (Cambridge Electronic Design, Cambridge, UK), and amplified by a magnitude of 1000 and band pass filtered (20-1000Hz) using a CED 1902 amplifier (Cambridge Electronic Design, Cambridge, UK). All EMG measures were obtained during the resting state and participants were asked to keep their hand and arm as still as possible. Participants were also asked to refrain from falling asleep or talking. MEP amplitudes are larger when an individual is talking or moving, whilst sleep can result in reduced MEP amplitudes (Hallett, 2007). EMG activity was visually inspected throughout the session to determine whether there was any voluntary muscle activity.

**Transcranial magnetic stimulation.** All TMS pulses were delivered from a MagStim 200² stimulator (Magstim Co., Whitland, UK) using a figure of eight coil 90 mm in diameter, which was connected to a BiStim module. The coil was manually placed and held over the left primary motor cortex with the handle pointing away from the midline of the skull at an angle of 45 degrees. The positioning of the coil was intended to induce posterior-anterior current flow in the brain which has been shown to preferentially activate facilitatory interneurons (Hallett, 2007; Opie et al., 2018). The optimal site for eliciting MEPs from the FDI was found through systematic delivery of suprathreshold (enough to elicit a MEP) single TMS pulses to the left M1. This process involved a combination of lateral, medial and posterior movements with the coil over the participant’s scalp. The final site was determined based on the site on the scalp that produced the most consistent and largest MEPs in the FDI. A washable marker was then used to mark the spot on the scalp which enabled the
experiment to reliably re-place the coil on the site throughout the session. All subsequent pulses of TMS were delivered at this site.

Two TMS intensities were required to set the SICF experimental protocol, namely the resting motor threshold (RMT) and the 1mV intensity (Ziemann et al., 1998). RMT is defined as the minimum intensity as a percentage of maximum stimulator output to elicit a MEP amplitude of 0.05 millivolts (mV) in at least five out of ten consecutive trials in the relaxed FDI (Rossini et al., 2015). The 1 mV intensity was defined as the intensity required to elicit an average peak-to-peak MEP amplitude of ~1 mV in the resting FDI (Opie et al., 2018).

**SICF.** SICF was measured by delivering single-pulse and paired-pulse stimuli. Single-pulse stimuli were delivered using the TMS intensity set to elicit the average 1 mV MEP. The 1 mV MEP provides a baseline measure of corticospinal activity. Paired-pulse stimuli involved the delivery of the 1 mV intensity (S1) followed by a stimulus intensity of 90% of RMT (S2). The selection of S1 at 1 mV and S2 at 90% RMT is consistent with previous TMS literature (Clark et al., 2011; Opie et al., 2018; Ziemann et al., 1998). Paired pulse stimulation was delivered at 20 distinct ISIs to measure SICF as a function of ISI (1.1, 1.3, 1.5, 1.7, 1.9, 2.1, 2.3, 2.5, 2.7, 2.9, 3.1, 3.3, 3.5, 3.7, 3.9, 4.1, 4.3, 4.5, 4.7 and 4.9 ms). TMS delivery was divided into 15 blocks of 48 trials. Each block consisted of 2 paired-pulse trials for each of the 20 ISIs (40 in total) and 8 single-pulse trials, delivered in pseudo-random order. The experimental procedure consisted of 30 paired pulse trials at each of the 20 ISIs and 120 single pulse trials. The larger number of single-pulse trials compared to paired-pulse trials was to ensure a stable measure of baseline cortical excitability throughout the experiment. The interval between each trial was on average 5 seconds, with +/- 20% jitter. There is reasonable evidence to suggest that time between pulses can affect MEP amplitudes, with intervals longer than 4-6 seconds producing larger MEP amplitudes (Vaseghii, Zoghi, & Jaberzadeh, 2015). Thus, the present study opted to use a 5-second interval between trials,
which aligned with other studies of test-retest reliability of intracortical activity (Brown et al., 2017). The total time for each block to be completed was between 4.5 to 5 minutes. Each block was followed by a short break of ~2 minutes. Total experimental time varied between 2 and 2.5 hours.

**Manual Dexterity**

The Purdue pegboard test was used to assess manual dexterity (Lafayette Instrumental model). The Purdue pegboard involved four subtests: right-handed; left-handed; bimanual; and bimanual assembly. For the right-handed subtest, participants were asked to pick up pegs from the right side of the board and insert them in a vertical line of holes using only their right hand. For the left-handed subtest, participants were asked to pick up pegs from the left side of the board and insert them in a vertical line of holes using only their left hand. The bimanual subtest involved participants picking up pegs from both sides of the board using both hands (i.e. right hand picking up pegs on the right side and left hand picking up pegs from the left side) and placing them both in the vertical line of holes at the same time. Participants were given 30 seconds to complete each of these tasks, and the number of pegs placed in the hole correctly was then scored. The bimanual assembly task involved participants using both hands to assemble and place 4 objects in the vertical line of holes. The assembly involved placing a pin, a washer, a collar, and another washer in that order. Items were to be placed by both hands sequentially and not at the same time as in the simple bimanual subtest. Each item corresponded to one point and participants were given 60 seconds to complete this task. The one trial administration of the Purdue Pegboard has shown moderate to good test-retest reliability (Buddenberg & Davis, 2000).

**Sleep, Caffeine and Alcohol Questionnaire**

A sleep, caffeine and alcohol questionnaire was administered to all participants. (Appendix D). In order to establish reliability, it was important to determine whether sleep
quality, length of sleep, caffeine intake and alcohol intake was significantly different across sessions.

**Experimental Procedure**

The experiment consisted of 2 identical experimental sessions separated by at least one week (\textit{Mdn} days between sessions = 8, range: 7-14). Participants were tested at the same time of day for both sessions. There is evidence to suggest that time of day can affect measures of corticospinal activity, thus the current study sought to eliminate the time of day as a confounding variable (Hermsen et al., 2017). Testing was conducted in the same laboratory for all sessions. In the first session, participants were first asked to complete the TMS safety screening questionnaire (Appendix B). Participants were then to carefully read the consent form (Appendix C) and provide informed consent if they felt comfortable. The setup of the EMG was then conducted, followed by the TMS SICF protocol. The second session was identical to the first, except that participants did not complete the handedness inventory or consent form, and participants completed the Purdue pegboard test after the completion of SICF measures.

**Data Analysis**

EMG activity was monitored via visual inspection throughout the session. Some participants were unable to sustain relaxed muscle activity, which contaminated numerous trials during the SICF protocol. To compensate for this, additional blocks of SICF were delivered which lengthened the time of the sessions. EMG data was saved for offline analysis. Each individual trial of EMG activity was inspected to determine if the activity 50 ms preceding the pulse exceeded .02 mV. EMG activity higher than .02 mV indicated voluntary muscle activity which may have contaminated the amplitude of the subsequent MEP. Trials that met this exclusion criterion were discarded (8.34\% of all trials).
Peak-to-peak amplitude was obtained from EMG activity occurring from 10 ms to 50 ms after the delivery of the pulse. Mean MEP amplitude for all single-pulse trials and paired-pulse trials was calculated. SICF is quantified by expressing the MEP elicited by single-pulse TMS as a ratio of the MEP elicited by paired-pulse TMS. Thus, a ratio greater than 1.0 indicates facilitation, a ratio equal to 1.0 indicates no facilitation, and a ratio lower than 1.0 indicates inhibition.

**Statistical Analysis**

IBM SPSS Statistics version 24 was used to conduct the statistical analyses for this study (IBM Corp, Armonk, NY). All graphs were generated using Prism GraphPad 8 (GraphPad Software, La Jolla, CA). Assumptions were tested. Shapiro-Wilk statistics showed some moderate violations, however, t-tests and analysis of variances (ANOVA) are robust against violations of normality (Blanca, Alarcón, Arnau, Bono & Bendayan, 2017). Mauchly’s Test of Sphericity was not violated. A one-way repeated measures ANOVA was conducted to determine whether SICF ratios differed across ISIs and testing sessions. Session delivery and ISI were the 2 factors, with each having 2 and 20 levels, respectively. The reliability of SICF between sessions was calculated using intraclass correlation coefficients (ICC). Individual means for both sessions were correlated with each other at the three ISIs in which peak facilitation occurred. Thus, three separate ICC measures were conducted with corresponding 95% confidence intervals.

The ISI in which the SICF peaks were maximal was also examined. This was conducted by subtracting the individual ISIs in which SICF was maximal in session 2 from the individual ISIs in which SICF was maximal in session 1. A difference score of 0 would indicate that maximal SICF occurred at the same ISI in both sessions; a positive score would indicate that SICF peaked later in session 1 as compared to session 2; a negative score would indicate that SICF peaked earlier in session 1 as compared to session 2.
Associations with SICF and manual dexterity were examined using Pearson’s bivariate correlations. The four subtests of the Purdue pegboard test were individually correlated with each SICF peak across both sessions. With three SICF peaks across two sessions and four subtests of the Purdue, a total of 24 correlational analyses were conducted. Due to the associations between SICF and manual dexterity being only exploratory in nature, the current study accepted \( p \) values below .05.

**Results**

**Neurophysiological Measures**

In general, RMT was similar across sessions (session 1 \( M \pm SD \): 50.1 \( \pm \) 9.0\% of maximal stimulator output; session 2 \( M \pm SD \): 50.6 \( \pm \) 8.9\% of maximal stimulator output). A paired-samples \( t \)-test indicated that the difference in TMS intensity between sessions was not significant, \( t(15) = -0.83, p = .42, d = .06 \).

The 1mV single pulse paradigm was very consistent between sessions (session 1 \( M \pm SD \): 62.9 \( \pm \) 11.4\% of maximal stimulator output; session 2 \( M \pm SD \): 62.8 \( \pm \) 11.3\% of maximal stimulator output). A paired-samples \( t \)-test indicated that the difference in TMS intensity between sessions was not significant, \( t(15) = 0.15, p = .88, d = .01 \). MEP amplitude was also similar across sessions (session 1 \( M \pm SD \): 0.99 \( \pm \) 0.25; session 2 \( M \pm SD \): 0.94 \( \pm \) 0.32). A paired-samples \( t \)-test indicated that the difference in MEP amplitude between sessions was not significant, \( t(15) = 0.95, p = .36, d = .17 \).

**SICF**

Figure 3 shows group SICF as a function of ISI with the mean and standard error of the mean plotted at each ISI for session 1 and session 2. It is clear from the figure that mean facilitation across both sessions was similar in the two sessions, both in terms of the magnitude of facilitation and the ISIs at which the peaks of facilitation occurred. Each of the three SICF peaks were separated by troughs in which limited facilitation occurred. For both
sessions peak 1 showed the greatest facilitation of the three peaks, with session 1 peaking at the 1.5 ms ISI and session 2 peaking at the 1.3 ms ISI. Peak 2 occurred at the 2.5 ms ISI for session 1 and at the 2.7 ms ISI for session 2. Peak 2 showed lower facilitation than peak 1, but higher facilitation than peak 3. Peak 3 occurred at the 4.1 ms ISI for session 1 and at the 4.3 ms ISI for session 2. The third peak showed the lowest facilitation of the three peaks. A repeated-measures ANOVA was conducted to determine whether SICF differed across ISIs and sessions. The ANOVA results indicated that there was a main effect of ISI, $F(19, 285) = 10.65, p < .001, \eta^2 = .42$. As expected, these results indicate that SICF occurs as a function of ISI, which is reflected in the three peaks of facilitation occurring at three distinct ISIs (peak 1: 1.3-1.5ms; peak 2: 2.5-2.7 ms; and peak 3: 4.1-4.3 ms). The ANOVA showed no main effect for session, ($F(1, 15) = 0.003, p = .96, \eta^2 = <.001$) and no interaction of session and ISI ($F(19, 285) = 0.82, p = .69, \eta^2 = .05$), indicating that SICF as a function of ISI was similar across the two sessions.

![Graph](image)

*Figure 3.* SICF as a function of interstimulus interval (ISI) for both sessions are shown. Each data point reflects the mean SICF ratio at each ISI, with error bars representing standard error
of the mean. Session 1 data is represented by a filled circular point with solid lines while session 2 data is represented by an unfilled circular point with dotted lines. ISIs in milliseconds are presented on the $x$-axis. SICF ratio is presented on the $y$-axis. Ratios greater than 1.0 indicate facilitation; ratios equal to 1.0 indicate no facilitation. Data points are offset to allow for a clearer presentation of data.

**Test-Retest Reliability**

RMT and the 1mV intensity were tested for their test-retest reliability in order to establish the stability of these measures, which were used to set the protocol to assess SICF. The ICC for RMT was found to be .98 with a 95% confidence interval from .94 to .99, indicating excellent test-retest reliability of RMT. A high degree of reliability was found also between the 1mV amplitude in both session 1 and session 2. The ICC for the 1mV amplitude was .87 with a 95% confidence interval from .64 to .95.

Figure 4 displays scatterplots showing the relationship between SICF magnitude at each peak in session 1 and session 2. It is clear from the scatterplots that there are strong positive associations between SICF in both sessions at all peaks. The ICC of SICF magnitude at the first peak was .91 with a 95% confidence interval from .76 to .97, indicating excellent test-retest reliability between session 1 and session 2. An ICC of .88 was found for the second peak with a 95% confidence interval from .67 to .96, indicating excellent test-retest reliability at the second peak between sessions. The ICC for the third peak was .86 with a 95% confidence interval from .58 to .95, indicating excellent test-retest reliability at the third peak between sessions.
Figure 4. Scatterplots of SICF ratios in session 1 and session 2 at all three peaks are shown. Trendlines are applied to all graphs.
ISIs at which SICF was Maximal

Figure 5 shows frequency plots of the difference score in SICF peak between each session at each ISI. For each of the three SICF peaks, difference scores were calculated by subtracting the ISI in which the peak occurred in session 2 from the ISI in which the peak occurred in session 1. Thus, a difference score of 0 indicates no difference in ISI between sessions, a positive score would indicate that the peak occurred earlier in session 2, and a negative score would indicate that the peak occurred earlier in session 1. The first peak indicated that there was a normal distribution of SICF latency. A paired-samples $t$-test comparing the ISI of the first peak between both sessions found that there was no statistical difference between the ISIs in which peak 1 occurred, $t(15) = -0.24, p = .82, d = .05$. The second peak saw no meaningful difference between the ISI at which the peak occurred in session 1 or 2. A paired-samples $t$-test comparing the ISI of the second peak between both sessions found that there was no statistical difference between the ISIs in which peak 2 occurred, $t(15) < .001, p = 1, d < .001$. The difference score of the third peak between sessions was the most variable ($M = .05, SD = .32$) and had the largest spread of all three peaks (range: -.4-.8). However, a paired-samples $t$-test comparing the ISI of the second peak between both sessions found that there was no statistical difference between the ISIs in which peak 3 occurred, $t(15) = 0.61, p = .55, d = .03$. 
Figure 5. Frequency bar graphs showing the distribution of ISI difference score in milliseconds between sessions at each peak. Peak 1 (A) and peak 2 (B) show small spreads of data that centres on an ISI difference score of 0. Peak 3 (C) shows a larger spread of average difference scores.
Association Between SICF and Manual Dexterity

Bivariate correlations were performed in order to examine the relationship between SICF and manual dexterity: separate correlations were performed at each peak for each session for performance in all four subtests of the Purdue pegboard task. Due to the associations between SICF and manual dexterity being only exploratory in nature, $p$ values below .05 were accepted as significant correlations. SICF and right-hand performance were the only variables to show any significant correlations. Figure 6 shows the scatterplots of the relationship between SICF at each peak and right-hand performance in the Purdue Pegboard task. The figure shows no association between right-hand performance in the Purdue test and SICF at either peak 1 or peak 2. The figure does show a positive association between right-hand performance in the Purdue test and SICF peak 3 in both sessions. The third peak of session 1 showed a significant strong positive relationship with the right-handed subtest of the Purdue Pegboard task, $r = .59$, $p = .02$; 95% CIs [.13, .84]. The third peak of session 2 also showed a significant strong positive relationship with the right-handed subtest of the Purdue Pegboard task, $r = .53$, $p = .04$, 95% CIs [.05, .81].

Regarding the left-handed subtest of the Purdue, no significant correlation was found between SICF across both sessions at any of the 3 peaks (all $r < .4$, all $p > .12$). No significant correlation was found between SICF across both sessions at any of the 3 peaks and the bimanual subtest (all $r < .37$, all $p > .17$). Regarding the bimanual assembly subtest of the Purdue, only the third peak of session 1 showed a significant strong positive relationship, $r = .56$, $p = .03$, 95% CIs [.09, .83]. A non-significant moderate positive relationship was found between peak 3 of session 2 and the assembly subtest of the Purdue, $r = .33$, $p = .22$, 95% CIs [-.21, .70]. No association was found between peak 1 and peak 2 and bimanual performance in the Purdue test.
Figure 6. Correlations between right-hand subtest of Purdue pegboard task and SICF ratios at each peak for both sessions. Top panels (A, B, C) shows correlations at each peak for session 1. Bottom panels (D, E, F) shows correlations at each peak for session 2. *p < .05.
Questionnaire Results

Results from the sleep, caffeine and alcohol questionnaire showed that participants had similar patterns of sleep, caffeine and alcohol intake in both sessions. Across the two sessions, there was no difference in quality of sleep (rated on a scale of 1 to 10; session 1 $M \pm SD$: 7.31 ± 1.78; session 2: 7.40 ± 1.02; $t(15) = -0.15, p = .88, d = .06$) or length of sleep (session 1 $M \pm SD$: 7.66 ± 1.18; session 2 $M \pm SD$: 7.28 ± 0.95; $t(15) = 1.03, p = .32, d = .35$). Average caffeine intake was similar across both sessions (session 1 $M \pm SD$: 0.88 ± 1.15; session 2 $M \pm SD$: 0.81 ± 0.83; $t(15) = 0.25, p = .81, d = .07$). Average alcohol consumption was 0 across both sessions.

Discussion

The present study assessed the test-retest reliability of paired-pulse TMS to measure SICF. Establishing the test-retest reliability of SICF is important in determining whether TMS can be used to reliably assess the neural mechanisms involved in motor control. There were three main findings in the current study. First, excellent test-retest reliability of SICF magnitude at all three peaks across sessions. Second, the ISIs in which SICF peaked were consistent across sessions at all three SICF peaks. Third, SICF magnitude at the third peak was positively associated with the right-handed subtest of the Purdue pegboard test.

SICF Function

Consistent with the literature, the present study demonstrated three main peaks of facilitation at ISIs of ~1.5, 2.5, and 4.1 ms, separated by troughs in which there was no facilitation (Clark et al., 2011; Opie et al., 2018; Ziemann et al., 1998). This is supported by the analysis that showed a main effect of ISI on SICF magnitude. Also consistent with the previous literature, the magnitude of SICF declined from peak 1 to peak 2 to peak 3 (Clark et al., 2011; Opie et al., 2018; Peurala et al., 2008; Ziemann et al., 1998). Adding to this literature, the current study shows, for the first time, that SICF as a function of ISI was
similar across sessions. Analyses showed no effect of session and no interaction of session and ISI on the magnitude of SICF. These results eliminate session delivery as a confounding variable on SICF.

Whilst the physiological mechanisms underlying SICF are yet to be fully elucidated, a broad range of research, including direct measures of brain activity in animals and epidural recordings in human beings, have suggested a cortical origin of SICF (Ziemann et al., 1998). Suprathreshold stimulation of the hand area of M1 has repeatedly been shown to produce a wave of activity within corticospinal neurons that descend along the corticospinal tract. This phenomenon is referred to as the descending volley. The descending volley was first observed in early animal studies involving direct electrical stimulation of M1 on nonhuman primates (Patton & Amassian, 1954). Patton and Amassian (1954) delivered single pulses of electrical stimulation through electrodes placed on the exposed brains of monkeys and recorded responses through electrodes in the corticospinal tract. Their results found that M1 stimulation led to an initial volley in the corticospinal tract followed by later volleys occurring at a periodicity of 1.5 ms. The authors found that the first volley recruited following M1 stimulation is likely due to direct activation of the corticospinal neurons and that later volleys are likely due to the indirect activation of the corticospinal neurons through intracortical circuits.

Invasive recordings within conscious human subjects have confirmed the observation of two different volleys following stimulation of M1. Di Lazzaro and colleagues (1998) recorded and compared the descending volleys from electrodes implanted on the spinal cord of conscious human subjects following electrical stimulation and TMS of the motor cortex. Figure 7, from Di Lazzaro et al. (1998), shows the descending volleys evoked following different intensities of electrical stimulation and TMS. First, they showed that electrical stimulation (with an intensity sufficient to evoke a MEP recorded in a hand muscle) elicited
an initial descending volley but did not produce later volleys unless stimulus intensity was increased (Figure 7, panel A); the authors suggested that the initial volley was due to direct activation of the corticospinal cells, consistent with the non-human primate study described above. Second, they showed that TMS (with an intensity sufficient to evoke a MEP recorded in the hand muscle) did not elicit the initial volley that was observed following electrical stimulation but did elicit a later volley, occurring approximately 1.5 ms later than the initial volley elicited by electrical stimulation. When the TMS intensity was increased, additional later volleys were elicited, separated by approximately 1.5 ms. The predominant theory regarding this activity is that following stimulation there are two waves of activity: (1) an initial direct-wave (D-wave), which reflects the direct activation of axons descending along the corticospinal tract; and (2) subsequent indirect-waves (I-waves), which are hypothesised to be a result of transsynaptic activation of corticospinal neurons from non-invasive brain stimulation. Figure 7, panel B illustrates the descending volleys recruited following TMS as well as EMG responses at varying intensities. The number and size of I-waves increase as the intensity increases. With the recruitment of additional I-waves, the resulting MEP also increases, suggesting that I-wave recruitment plays a role in MEP facilitation. The initial I-wave following stimulation is referred to as the first I-wave, which is present during low-intensity stimulation. The subsequent I-waves are referred to as late I-waves and are distinct from the first I-wave due to their recruitment only at higher TMS intensities.
Figure 7. Descending volleys elicited using electrical stimulation (Panel A) and TMS (Panel B) with increasing intensities. The left shows electrical activity as recorded from the spinal cord following stimulation; the right of the figure shows the corresponding MEP. The left-most dotted line indicates the initial D-wave while the dotted line to the right indicates the
first I-wave. For TMS, the I-wave occurs around 1.5 ms following the expected time the D-wave occurs following electrical stimulation. As TMS intensity increases, the number of I-waves increases as well as the size of each I-wave. With each additional I-wave recruited, the size of the MEP increases. Figure adapted from “Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans”, by V. D Lazzaro et al., 1998, *Electroencephalography and Clinical Neurophysiology, 190*, 399. Copyright 1998 by Elsevier Science Ireland Ltd. Used with permission.

**SICF Peak 1**

The magnitude of SICF peak 1. As expected, the magnitude of facilitation at the first SICF peak showed excellent test-retest reliability. As the first SICF peak occurs at ~1.5 ms interval, which is consistent with the occurrence of the first indirect wave, it is thought that SICF reflects the facilitatory interaction of the early indirect waves that occur following paired-pulse TMS (Clark et al., 2011; Opie et al., 2018; Ziemann et al., 1998). It is hypothesised that the first stimulus, which is at an intensity sufficient to activate corticospinal neurons, also leads to partial depolarisation of a subpopulation of cortical neurons; the second stimulus delivered ~1.5 ms later can then cause the depolarised neurons to reach the threshold required to elicit an action potential, which results in facilitation of the MEP (Ziemann et al., 1998). As such, the first peak of SICF likely reflects the interaction of the second I-wave from stimulus 1 being facilitated by the first I-wave from stimulus 2. In the current study, the ICC performed on SICF magnitude at the first peak (1.3-1.5 ms) was the highest of all three peaks. This is the first TMS study to demonstrate consistent facilitation at the first SICF peak (1.3-1.5 ms) across two experimental sessions. Taken together with the previous literature, the current findings suggest that paired-pulse TMS is a reliable method to measure the recruitment of early I-waves.
**ISI at which SICF peak 1 was maximal.** For both sessions, peak 1 occurred within the expected ISI range for early I-wave generation (~1.3-1.5 ms). When calculating the difference between the ISIs in which SICF peak 1 occurred, there was a normal distribution of scores, suggesting no systemic difference in the ISI in which peak 1 occurred in the two sessions. This was further supported by analyses finding no statistical significance in the difference between the ISI in which SICF peak 1 occurred between sessions. This is the first report showing consistent ISIs in which SICF was maximal. This finding suggests that the temporal properties of the I-waves generated by TMS are stable across sessions. As peak 1 likely reflects the interaction of early I-waves, the results of the current study suggest a remarkably consistent interaction of early I-waves following TMS.

**SICF Peak 2 and 3**

**The magnitude of SICF peak 2 and 3.** SICF reliably peaked at two later ISIs, 2.3-2.5 ms and 4.1-4.3 ms. This aligns with previous TMS studies that have consistently found distinct SICF peaks between 2.3-2.7 ms and 4.1-4.5 ms ISIs (Clark et al., 2011; Opie et al., 2018; Peurala et al., 2008; Ziemann et al., 1998). The second and third SICF peak is hypothesised to reflect the recruitment of late I-waves (i.e. those occurring after the first I-wave; Ziemann et al., 1998). This is supported by the fact that the second and third peaks occur at multiples of the ~1.5 ms intervals that I-waves are expected to occur. The later SICF peaks are thought to reflect the summation of excitatory synaptic activity following paired-pulse stimulation (Di Lazzaro et al., 1998). The excitatory synapses alone are unable to produce an action potential, however, when activated in close succession they are summed together to result in an action potential. As mentioned earlier, Figure 3B shows later I-waves following TMS: it is clear from Figure 3B that the magnitude of the I-waves decreases, with the first I-wave being the largest in magnitude and the subsequent I-waves decreasing in magnitude systematically (Di Lazzaro et al., 1998). As SICF at the second and third peak is
hypothesised to result from the summation of the later and smaller I-waves, the magnitude of MEP facilitation at these points is likely to be smaller than the interaction of the earlier and larger I-waves (i.e. SICF at peak 1). This hypothesis explains the lower magnitude of the second and third SICF peaks in comparison to the first, consistent with previous research (Clark et al., 2011; Opie et al., 2018). This is the first TMS study to demonstrate consistent SICF at the 2.3-2.5 ms and 4.1-4.3 ms ISIs across sessions. The reliability of SICF magnitude at the expected ISI range indicates that TMS is a reliable method to measure the recruitment of late I-waves.

The reduced magnitude of SICF at the second and third peak compared to the first peak may be explained by the interaction of inhibition and facilitation in M1, which both contribute to the MEP recorded in the hand muscle. Figure 8, from Reis et al. (2008), shows the interaction of SICI on the neural pathway of SICF. This suggests that the TMS protocol used to assess SICF may be measuring the net effect of the interaction of facilitation and inhibition. Even though it is possible to set TMS protocols to preferentially activate inhibitory or facilitatory circuits, the MEP still reflects the net effect of both inhibitory and facilitatory processes. Peurala et al. (2008) explored the effects of SICF on a form of intracortical inhibition called short-interval intracortical inhibition (SICI), which can be explored through paired-pulse TMS at short ISIs of 1-6 ms. The authors measured SICF at 1.5 ms to 4.5 ms ISIs and measured SICI troughs based on the ISIs in which SICF peak 1, peak 2 and peak 3 occurred. Their results found a significant positive relationship between SICF peak 2 and SICI, suggesting that strong SICF is associated with reduced SICI. Taken with the hypothesis that peak 2 and peak 3 reflect the interaction of late I-waves, these results suggest that SICI likely has a significant contribution to the recruitment of late I-waves. Future research may be best served to measure the contribution of SICI on SICF and across at least two sessions of delivery.
Figure 8. Schematic showing the pathway of SICF and the effect of short-interval intracortical inhibition (SICI) on output from the primary motor cortex (M1) is shown. The early I-wave (I1) and late I-waves are a response following TMS, and reflect the earliest and later I-waves, respectively. SICF is shown enhancing both the earliest and late I-waves. SICI is shown to modulate the later I-waves and affect output from M1. Figure adapted from “Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control”, by J. Reis et al., 2008, The Journal of Physiology, 586(2), 327. Copyright 2008 by the Journal of Physiology. Adapted with permission.

**ISI at which SICF peak 2 was maximal.** The difference in ISI between sessions where SICF was maximal showed a consistently small spread for peak 2. For both sessions, peak 2 occurred within the expected time course expected for late I-wave generation - between 2.5 and 2.7 ms (Clark et al., 2011; Opie et al., 2018; Ziemann et al., 1998). There was very little difference in the sample in the latency of peak 2 between sessions. This is the first study to report consistent SICF latency at the second peak. This finding suggests that the temporal properties of the late I-waves generated by TMS are stable across sessions.

**ISI at which SICF peak 3 was maximal.** At the group level, the third SICF peak occurred between the 4.1-4.3 ms ISI found within the literature (Clark et al., 2011; Opie et al., 2018; Ziemann et al. 1998). At the individual level, there was a moderate spread of ISIs at
which peak 3 occurred across the two sessions. Given that peak 3 is also thought to reflect late I-waves, the ISIs at which peak 3 is maximal might be more reliably measured using a coil orientation that induces anterior-posterior flow (Di Lazzaro et al., 2001). The current study used a coil orientation that induced posterior-anterior current flow, which is known to preferentially elicit early I-waves. Anterior-posterior induced current flow is optimal in preferentially recruiting later indirect waves (Di Lazzaro et al., 2001). In a recent study (accepted for publishing in August 2019), Opie, Hand and Semmler (in press) assessed the differences in the ISI in which SICF was maximal when inducing either posterior-anterior current flow or anterior-posterior current flow in healthy young adults (N = 23). Opie et al. (2019) found that the ISI in which SICF peak 3 occurs is both broadened and delayed when inducing posterior-anterior flow when compared to anterior-posterior flow. The current study only used posterior-anterior current flow, thus the larger spread of peak 3 in the present study may be explained by the finding that MEP facilitation at the third peak is broadened and delayed in young adults when using posterior-anterior current flow. However, as the second SICF peak is thought to reflect the interaction of late I-waves, the effect of coil orientation on peak 3 should have been evident in peak 2. As this was not the case, the increased spread of within-individual variability in peak 3 compared to peak 2 may suggest that the late I-waves reflected in peak 3 interact in a different manner than the late I-waves reflected in peak 2. Further examination of the effect of coil orientation on the properties of peak 2 and peak 3 SICF is required.

**Associations between SICF and manual dexterity**

**SICF and manual dexterity of the right-hand.** The present study examined the association between SICF magnitude at each peak and manual dexterity as measured by the Purdue pegboard test. These analyses were only exploratory in nature. No significant associations were found between peak 1 or peak 2 and performance in the right-hand subtest
of the Purdue pegboard task. The lack of association between peak 1 and peak 2 with the right-hand performance subtest of the Purdue is consistent with the previous literature (Clark et al., 2011). The current study found a significant and strong positive relationship with SICF peak 3 and right-hand Purdue performance. These results suggest that increased SICF in the left hemisphere is indicative of greater manual dexterity in the right hand. This the first study to suggest a positive association between manual dexterity and SICF peak 3. The only studies to have investigated peak 3 and manual dexterity have been conducted by Clark et al. (2011) and Opie et al. (in press). Clark et al. (2011) found no association between SICF peak 3 and manual dexterity of the right hand in a sample of younger and older adults. In younger adults, Opie et al. (in press) found no association between the third SICF peak and manual dexterity. However, the authors found that increased SICF in peak 3 was associated with reduced performance in the right-hand subtest of the Purdue in older adults. The variation in results between the current study and Clark et al. (2011) and Opie et al. (in press) may be explained by the inclusion of older adults within their samples. Previous literature has shown differences in SICF function between young and older adults (Clark et al., 2011; Opie et al., 2018). Indeed Opie et al. (in press) found significant age-related differences in both the magnitude of SICF peak 3 and in the ISIs in which SICF peak 3 was maximal. As the current study only included healthy young adults, it is still unknown whether SICF magnitude and the ISIs in which SICF is maximal are reliable in all populations. Further, the low sample size of the study makes interpreting the associations between SICF and manual dexterity difficult. Prel, Hommel, Röhrig & Blettner (2009) note that high confidence intervals caused by low sample sizes indicate that knowledge of the true effect size is limited and may exaggerate the relationship between two variables. Thus, caution is required when interpreting the correlational data in both the present study and within the literature.
**SICF and bimanual dexterity.** The present study did not find any significant associations between SICF and bimanual performance in the Purdue pegboard task. This is consistent with studies finding no significant associations between SICF peak 1 and bimanual dexterity (Catteneo et al., 2005; Clark et al., 2011). However, the results are inconsistent with previous studies showing an association between SICF and bimanual performance (Cattaneo et al., 2005; Clark et al., 2011). Clark et al., (2011) observed that SICF peak 2 was significantly and positively associated with the object assembly subtest of the Purdue in both younger and older adults. Further, Cattaneo et al. (2005) found that the differences in facilitation at the second SICF peak was associated with differences in the way both hand muscles prepared to grasp two objects that were presented to individuals. The inconsistency between the current results and the previous literature may be explained in two ways. Firstly, that associations between SICF and manual dexterity found in Clark et al. (2011) included both young and older adults, whilst the present study only included young adults. As observed by the authors, SICF at the second peak is affected by age, with older adults showing reduced magnitude at peak 2 compared to younger adults. Secondly, the associations made by Cattaneo et al. (2005) were done using measurements of SICF during task performance, as opposed to resting SICF. The current study only assessed SICF during a resting state and did not examine SICF during task performance. The lack of association between SICF peak 3 and bimanual dexterity is consistent with current literature which has examined no association between SICF peak 3 and manual dexterity (Clark et al., 2011).

**Future research**

As described above, the early I-waves are optimally recruited by posterior-anterior current flow while late I-waves are optimally recruited by anterior-posterior flow (Delvendahl et al., 2014; Di Lazzaro et al., 2001). One study recently investigated the third peak of SICF using anterior-posterior flow (Opie et al., in press). Opie et al. (in press) found
that the ISI in which SICF peak 3 occurs is both broadened and delayed when inducing posterior-anterior flow when compared to anterior-posterior flow. However, the reliability of anterior-posterior coil orientation was not assessed, thus the reliability of SICF using anterior-posterior flow is unknown. To date, there is no TMS research that has utilised anterior-posterior flow to capture the full SICF function across all three peaks. Future research assessing the effect of alternative coil orientation on the reliability of SICF magnitude and latency may increase our understanding of the characteristics of SICF, particularly at the later peaks.

The present study established the test-retest reliability of SICF in the left M1 of right-handed individuals. It is unclear as to the extent of differences between SICF in dominant M1 and non-dominant M1. A possible avenue for research may be to assess the test-retest reliability of SICF in non-dominant M1 to determine whether there are asymmetries in the properties of SICF that might underlie the asymmetries in the way people use their hands. Measuring SICF as a function of ISI (as in the present study) in both left and right M1 in right-handed individuals may increase our understanding of asymmetrical differences in motor control. Only one study assessing the hemispheric asymmetry of intracortical facilitation was identified, which found no differences in intracortical facilitation (Illic, Jung & Ziemann, 2004). However, this study did not test for the full function of SICF and no test of reliability was conducted.

Whilst the present study established the test-retest reliability of SICF in young adults, the reliability of SICF measures in older adults has yet to be assessed. This is particularly important when we consider that motor control is known to decline with age, which has consequences for functional independence (Seidler et al., 2010). Thus, if SICF is known to be important for motor control, having reliable measures of SICF in both younger and older populations is critical in understanding the decline in motor control with age. Studies have
suggested age-related differences in SICF magnitude and the ISIs in which SICF is maximal, with older adults showing the reduced magnitude and significant delays in SICF at the third peak compared to young adults (Clark et al., 2011; Opie et al., 2018; Opie et al., in press). However, neither study assessed the magnitude and temporal differences in SICF peak 3 across sessions, thus it is unclear whether temporal differences in SICF can be attributed to age or whether there are underlying mechanisms that may explain shifts in the temporal dimensions of SICF peaks. Future studies may be best served to compare the test-retest reliability of SICF in older adults compared to younger adults to further the understanding of age-related declines in manual dexterity.

With the reliability of SICF now established, it provides the platform for exploring the reliability of the interaction between SICF and inhibitory processes in M1 (such as SICI). As discussed previously, the MEP recorded in the hand muscle reflects the net effect of facilitatory and inhibitory circuits (Reis et al., 2008). Thus, the role of intracortical facilitation in motor control cannot be understood in isolation from inhibitory processes. Peurala et al. (2008) used TMS to assess the interaction between SICF and SICI by measuring the three SICF peaks and the corresponding SICI troughs. Specifically, SICF was found to interact with SICI, with increased SICF resulting in reduced SICI responses (Peurala et al., 2008). However, the test-retest reliability of the SICF and SICI interaction has yet to be determined. Future research assessing the reliability of the interaction between SICF and SICI may provide a more comprehensive understanding of the intracortical mechanisms underlying motor control.

**Clinical implications**

Previous studies of neurological disorders such as Parkinson’s disease and multiple sclerosis have shown altered SICF as measured by TMS (Ho et al., 1999; Mori et al., 2013; Ni et al., 2013). Establishing TMS as a reliable measure of SICF in younger adults provides a
basis for investigating SICF as a potential biomarker for early diagnosis of movement disorders (such as Parkinson’s disease and multiple sclerosis). Research has suggested increased SICF in individuals with Parkinson’s disease compared to healthy controls (Ni et al., 2013). Measures of SICF could be included in clinical screenings and diagnostic sessions for people with suspected Parkinson’s disease. Further, a reliable measure of SICF provides a basis for investigating whether tracking changes in SICF over time could be a useful biomarker for disease progression. Measures of SICF could be included in routine follow-up clinical sessions to more comprehensively examine potential disease progression trajectory and SICF changes overtime.

The reliability of SICF measures is particularly important for emerging TMS treatments targeting SICF, such as I-wave periodicity TMS (iTMS). The iTMS technique is a repetitive TMS technique involving suprathreshold stimulation of M1 at 1.5 ms intervals, which has been shown to increase corticospinal activity (Kidgell, Mason, Frazer & Pearce, 2016). The length of iTMS sessions ranges from 10 to 30 minutes and can lead to increases in corticospinal excitability for up to 30 minutes following the protocol (Murray, Nosaka & Thickbroom, 2011). Opie et al. (2018) observed increased SICF following iTMS targeting ISIs in which SICF was maximal in both young and older adults. Thus, the reliability of SICF across sessions is important in establishing the efficacy of iTMS as a potential therapeutic target to restore altered SICF and potentially restoring altered motor control. By having a reliable measure of SICF, iTMS is likely to be effective in inducing cortical plasticity in populations with defective SICF, such as individuals with multiple sclerosis.

Limitations

The present study utilised a disproportionate number of females compared to males. Future studies may be best served utilising an equal distribution of sex. There is research to suggest that the menstrual cycle can affect cortical excitability (Zoghi, Vaseghi, Bastani,
Jaberzadeh & Galea, 2015). Whilst there are no TMS studies suggesting sex differences in SICF, accounting for this potential confound should be considered when utilising SICF protocols. Gender balanced samples are generally a prerequisite for funding towards clinical trials as outlined by institutions such as the National Institutes of Health ("NIH Policy and Guidelines on The Inclusion of Women and Minorities as Subjects in Clinical Research", 2017). In addition, the sample consisted of mostly undergraduate university students, suggesting that they were of a similar educational level. Thus, the study may be subject to sampling bias as it is not representative of the population.

**Conclusion**

The present study established the test-retest reliability of paired-pulse TMS to measure SICF. The magnitude of SICF at all three peaks was reliable across sessions. The ISIs in which SICF was maximal was also reliable across sessions. Additionally, the third SICF peak was found to be positively associated with manual dexterity of the right-hand. This is the first study to establish the test-retest reliability of TMS to measure SICF, a form of intracortical facilitation important in motor control. The peaks of SICF are thought to reflect the recruitment and interaction of early and late I-waves following stimulation of M1. As such, the present study has shown that TMS is a reliable measure of I-wave activity. The current finding of excellent test re-test reliability of SICF provides a platform for future research to investigate the role of SICF in manual dexterity, as well as investigate the role of SICF in the age-related decline in motor control and in populations with movement disorders, such as Parkinson’s disease and multiple sclerosis.
References


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