THE INTRASESSION RELIABILITY OF AFFERENT INHIBITION

INVESTIGATING THE INTRASESSION RELIABILITY OF SHORT AND LONG-AFFERENT INHIBITION

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Lay Abstract

Afferent inhibition is a phenomenon where the excitability of the motor cortex, the area of the brain controlling motor actions, is reduced following stimulation of the peripheral nerves. Because afferent inhibition is a result of sensory information reducing motor output, the measure is thought to be reflective of sensorimotor integration: our ability to guide motor actions based on sensory information. Given the large number of clinical populations that have reduced levels of sensorimotor integration, as well as the reductions seen with age, afferent inhibition is a well-studied phenomenon. However, despite extensive study, there is limited information on whether this effect is reliable. This thesis focuses on understanding whether this effect can be reliably induced when tested multiple times in a single session, and also provides guidelines for improving the reliability of future procedures testing this effect. This phenomenon was found to be reliably inducible if certain testing parameters were met.

Abstract

Afferent Inhibition is the reduction in motor output when Transcranial Magnetic Stimulation (TMS) of the motor cortex is preceded by peripheral nerve stimulation. Afferent inhibition can be subdivided into two circuits of Short- (SAI) and Long-Afferent Inhibition (LAI). Reliability reflects the repeatability of a measure and can be measured in terms of both absolute and relative reliability. Relative reliability refers to the ability of a measure to identify individuals on repeated testing, measured through the Intraclass Correlation Coefficient (ICC); absolute reliability is the repeatability of scores through repeated testing, measured through Standard Error of Measurement (SEM) and Smallest Detectable Change (SDC). Current literature has highlighted only the intersession reliability of SAI and LAI, but measures of the intrasession reliability are also needed. This study aims to quantify the intrasession reliability of SAI and LAI, alongside identifying the minimum number of trials needed to obtain a reliable measure. 30 healthy individuals (21.17 ± 2.84 years) took part in one session, with SAI and LAI obtained three times at 30-minute intervals. To identify the minimum number of trials required to reliably elicit SAI and LAI, relative reliability was assessed at running intervals of every 5 trials. Results indicate that SAI had moderate–high, and LAI had high-excellent relative reliability. Both SAI and LAI had high amounts of measurement error. LAI was seen to have high relative reliability when only 5 frames of data were included, whereas for SAI, ~20-30 frames of data resulted in high relative reliability. For LAI, a minimal sample size of 19 is needed to have an $SDC_{Group} < 10$, whereas for SAI, a sample size of 22 is needed to achieve the same.

These results can be used to inform future work regarding the clinical utility of these measures, particularly in terms of their diagnostic ability.

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List of Abbreviations

APB – Abductor Pollicis Brevis

AD – Alzheimer's Disease

AP – Anterior-Posterior

ANOVA – Analysis of Variance

CS – Conditioning Stimulus

CSTS – Conditioning Stimulus-Test Stimulus

CV – Coefficient of Variation

DN – Digital Nerve

D-Wave – Direct Wave

EEG – Electroencephalography

EMG – Electromyography

FDI – First Dorsal Interosseous

GABA – gamma-aminobutyric acid

ICC – Intraclass Correlation Coefficient

ICF – Intracortical Facilitation

ISI – Interstimulus Interval

I-Wave – Indirect Wave

LAI – Long-Latency Afferent Inhibition

LICI – Long Intracortical Inhibition

M1 – Motor Cortex

MEP – Motor Evoked Potential

MCI – Mild Cognitive Impairment

MN – Median Nerve

MSE – Mean Square Error

MT – Motor Threshold

PA – Posterior-Anterior

PAS – Paired Associative Stimulation

PD – Parkinson's Disease

RMT – Resting Motor Threshold

S1 – Somatosensory Cortex

SAI – Short-Latency Afferent Inhibition

SD – Standard Deviation

SDC_{Group} – Smallest Detectable Change at the group level

$SDC_{Individual}$ – Smallest Detectable Change within an individual

SEMeas – Standard Error of Measurement

%SEMeas – Percent SEMeas

SEP – Somatosensory Evoked Potential

SICI – Short Intracortical Inhibition

TES – Transcranial Electrical Stimulation

TMS – Transcranial Magnetic Stimulation

TS – Test Stimulus

Declaration of Academic Achievement

The entirety of this thesis has been written by Ravjot Rehsi, and all experiments and statistical analyses were performed by Ravjot Rehsi. Dr. Aimee Nelson and Dr. Claudia Turco aided in the conception and design of this study. My lab mates and collaborators Karishma Ramdeo, Stevie Foglia, Stephen Toepp and Jacob Pickersgill aided in the collection of data.

CHAPTER 1: GOALS OF THE THESIS

Afferent Inhibition is a cortical phenomenon reflecting a reduction in corticospinal excitability when brain stimulation is given following a preceding nerve stimulus (Chen et al., 1999; Tokimura et al., 2000). Dependent on the time interval between the brain stimulation and the nerve stimulus, the phenomenon can be subdivided into two circuits: Short- and Long-Latency Afferent Inhibition (SAI and LAI). Both are important cortical circuits relating to sensorimotor integration, and cognitive function. However, indications of insufficient reliability for both these measures limits their translation to clinical settings.

Given their popularity and usage, the quality and reliability of afferent inhibition must be assessed. Intersession explorations of these measures have indicated moderate reliability, and high variability (Brown et al., 2017; Turco et al., 2019). A possible contributing factor to this variability is the method of collecting and processing Transcranial Magnetic Stimulation (TMS) data. Most TMS measures reflect an average of several frames of data. However, for several measures, there exists no gold standard on how many frames of data allow for a reliable assessment. Furthermore, the molecular modulation of this measure accompanied by the rapidly changing chemical environment of the brain may further lead to poor reliability (Di Lazzaro et al., 2007; Di Lazzaro, Pilato, et al., 2005; Turco, El-Sayes, Locke, et al., 2018).

The goal of this thesis is to assess and improve the intrasession reliability of SAI and LAI by exploring the reliability of Afferent Inhibition measures as a function of frame number.

The thesis begins with an in-depth review covering the history of brain stimulation, the importance and relevance of afferent inhibition, as well as the established reliability of other TMS measures. Following this, the thesis will explore the rational behind the current project and disseminate the findings of the work in relation to the larger TMS literature.

We predict that the intrasession reliability of afferent inhibition will plateau after a certain number of frames, similar to reliability metrics of other TMS measures (Biabani et al., 2018; Goldsworthy et al., 2016). Further, we predict greater reliability of LAI vs SAI, with more variability between participants in LAI, as seen in explorations of the intersession reliability of both measures (Brown et al., 2017; Turco et al., 2019).

CHAPTER 2: LITERATURE REVIEW

2.1 – An Introduction to Transcranial Magnetic Stimulation

2.1.1 – A history of Brain Stimulation

Despite its importance, investigation of the brain and its functions was limited throughout much of history. Early exploration of cranial function involved inserting electrodes into cortical tissue to administer either unipolar or bipolar current to the desired brain region (Penfield & Boldrey, 1937). Beginning with animal work, the technique was eventually applied to humans in order to study the brain (Lewes, 1876; Penfield & Boldrey, 1937). Robert Bartholow was the first to directly stimulate the human brain in 1874, doing so on a patient who had a 2 inch diameter hole in the brain where all dural layers leaving the dura mater had eroded (Bartholow, 1874). Bartholow was able to elicit bilateral muscle twitches when intracortical stimulation was given to the respective motor cortex. Further experiments continued, leading to the elicitation of both sensory and motor responses through stimulation (Bidwell, 1893; Cushing, 1909; Parker, 1893)

In perhaps some of the most influential work done using intracranial stimulation, Wilder Penfield quantified the human sensorimotor homunculus for the first time (Penfield & Boldrey, 1937). Penfield stimulated 126 patients using either unipolar or bipolar electrodes. Current levels were increased until a positive response was obtained through either a motor twitch, or the reporting of sensation by the patient. Eventually, Penfield was able to identify sites in the sensory and motor regions of the brain which controlled the body, creating the

first homunculus to depict the relative neuronal connections of these body parts (Penfield & Boldrey, 1937). However, despite the advancements pioneered by this work, intracranial stimulation was both painful and dangerous, with several patients dying following the procedure (Bartholow, 1874; Penfield & Boldrey, 1937)

Transcranial Electrical Stimulation (TES) was developed in 1980 in order to non-invasively study the brain, unlike intracranial stimulation (Merton & Morton, 1980). Utilising silver stick on electrodes, researchers were able to deliver electrical current to the cortex to elicit responses. These responses would manifest as either motor twitches, or visual phosphenes, dependent on whether stimulation was given to the motor or visual cortices (Merton & Morton, 1980). However, despite better patient outcomes when compared to intracranial stimulation, the high resistance of the scalp necessitated higher voltages, leading to the activation of sensory receptors, and as a result, participants often complained of pain.

Developed in 1985 by Barker et al. at the Sheffield University and Health Authority, TMS offered a pain free method of non-invasive brain stimulation. TMS was originally being developed for use in stimulating the peripheral nerves. However, unless nerves were located deep in the tissue, conventional methods were equivalent to TMS for stimulation of peripheral nerves; however, the resistance of the skull was 8-15 times higher than that of other soft tissues, making TMS extremely beneficial for cranial stimulation (Barker et al., 1985). Due to the lack of direct electrical stimulation, Barker et al. were able to elicit motor responses by stimulating the motor cortex (M1) without inducing pain in

participants, and recording signal using electromyography (EMG) (Barker et al., 1985). The development of TMS was revolutionary in the field of non-invasive brain stimulation, allowing for extensive study of cortical physiology.

2.1.2 – Mechanism of TMS

TMS operates through a practical application of Faraday's Law, using a capacitator to fire electrical current into a circular coil (Hallett, 2007). The electrical current produced in the coil generates a resultant magnetic field perpendicular to the plane of the electrical current. This magnetic field can be directed into the cortex, which can then generate a secondary electrical current parallel to the orientation of the coil (Hallett, 2007). This secondary electrical current is responsible for eliciting cerebral cortex activation.

TMS is technically variable, with several modulatory factors present which can influence its effectiveness. The most significant of these factors is the shape of the coil used for TMS. Traditional TMS apparatuses utilized a basic circular coil, with a diameter of 14 cm, which had the drawback of low field strength and difficulty with localization (Hallett, 2007; Terao & Ugawa, 2002). The magnetic field was strongest around the circumference of the coil, and dropped in strength linearly as it got closer to the centre (Hallett, 2007). Ultimately, this led to difficulties in localization of stimulation. The figure-of-eight coil, comprised of two individual circle coils combined to make a figure-of-eight, was able to counter this limitation. At the point of contact between these coils, the individual magnetic fields of each coil superimpose to create a stronger, singular magnetic field (Hallett, 2007; Terao &

Ugawa, 2002). This allows for higher localization of stimulation, resulting in easier targeting for brain regions located at the surface of the cortex. However, the figure-of-eight coil is not able to target areas lying deep in M1, as the magnetic field is strongest just beneath the point of contact between the coil and the skull. To stimulate such areas, a conical coil is utilised instead, which consists of two circular coils oriented at an angle of 90-100 deg relative to each other (Terao & Ugawa, 2002). This allows for deeper penetration of the signal, rendering it capable of eliciting reactions in the leg muscles for which the representation is located 3-4 cm below the scalp (Terao & Ugawa, 2002).

Despite the ability of both to elicit motor reactions, TMS and TES accomplish this through different methodologies. When stimulating muscles of the upper limb, the latency between stimulation delivery and motor reaction is ~1.5-2 ms shorter for electrical stimulation compared to magnetic (Day et al., 1989; Di Lazzaro et al., 1998; Hallett, 2007). The difference in latencies has led to the development of the D- and I-Wave hypothesis (Day et al., 1989). This hypothesis proposes that TES directly stimulates corticospinal neurons below the site of stimulation; contrastingly, TMS is proposed to stimulate those same corticospinal neurons in a transsynaptic fashion through excitatory synaptic inputs. These latency differences have been categorized into waves, with TES producing primarily Direct or D-waves, and TMS producing Indirect, or I-waves (Di Lazzaro et al., 1998). Furthermore, TMS stimulation at different coil orientations can elicit I-waves of different latencies: Lateral-Medial (LM) coil orientation can elicit D-waves; Posterior-Anterior (PA)

produces I1, I2, I3 and I4 waves; and Anterior-Posterior (AP) produces I2, I3 and I4 waves (Ni et al., 2011; Sakai et al., 1997; Ziemann & Rothwell, 2000).

2.2 – Measures of Transcranial Magnetic Stimulation

TMS, when accompanied with EMG, has been used to extensively study the cortical control of hand movement. EMG utilises pairs of skin electrodes in order to measure the size of the motor response, known as a Motor Evoked Potential (MEP) in mV. Through using EMG and TMS, we can find the optimal representation of muscles in M1, referred to as the motor hotspot (Wassermann, 2002). The hotspot is defined as the location in M1 which, when stimulated by TMS, leads to the largest MEP in the target muscle. Utilising the motor hotspot, researchers can explore a variety of TMS measures separated into two distinct sets: single-pulse measures involve the recording of output from a single TMS pulse; paired-pulse measures refer to the recording of output from sequential TMS pulses or nerve stimulation paired with TMS.

2.2.1 – Resting Motor Threshold (RMT)

The RMT is defined as the lowest TMS intensity that evokes an MEP ≥ 50 μV in peak-to-peak amplitude 50% of the time (Rossini et al., 2015). The RMT can be used as a measure of baseline motor cortex excitability (Siebner & Rothwell, 2003; Turco, El-Sayes, Locke, et al., 2018). Importantly however, RMT does not represent intracortical excitability, but instead represents overall neuronal membrane excitability, due to its inability to be

modulated by drugs incapable of modifying ion-channels (Korchounov et al., 2007; Ziemann et al., 1997).

2.2.2 – Short-Latency Afferent Inhibition (SAI)

SAI reflects a reduction in motor output when a TMS pulse to M1 is preceded by an electrical peripheral nerve stimulus at a time difference of 18-28 ms (Ni et al., 2011; Tokimura et al., 2000). Due to the reduction in motor output caused by activation of sensory afferent fibers, SAI is an indirect assessment of sensorimotor integration. Given the significant reductions in sensorimotor capabilities in the aging population, SAI remains of great interest to researchers (Brown et al., 2018; Sailer et al., 2003).

SAI has a varied pharmacological basis. When injected with muscarinic antagonists such as scopolamine, participants exhibit reduced SAI (Di Lazzaro et al., 2000). Contrastingly, the administration of various acetylcholinesterase inhibitors, which prevent the destruction of acetylcholine in the synaptic cleft, led to an enhancement of overall SAI (Di Lazzaro, Oliviero, Pilato, et al., 2005; Fujiki et al., 2006). Further, SAI is also reduced in patient populations that have altered cholinergic function such as those with Alzheimer's Disease (AD) (Benussi et al., 2018; Di Lazzaro et al., 2002; Martorana et al., 2012; Motta et al., 2018; Nardone et al., 2008) and Mild Cognitive Impairment (MCI) (Nardone et al., 2012; Peter et al., 2016; Tsutsumi et al., 2012). The evidence seems to suggest therefore, that SAI has a cholinergic root. Further modulations of SAI can also occur through pathways involving gamma-aminobutyric acid (GABA) receptors. When administered with various

drugs that modulate the activity of both $GABA_A$ and $GABA_B$ receptors, SAI was only changed through those drugs that modulated $GABA_A$ activity (Di Lazzaro et al., 2007; Di Lazzaro, Oliviero, Saturno, et al., 2005; Turco, El-Sayes, Locke, et al., 2018). Specifically, SAI is reduced when lorazepam or zolpidem are administered, but not diazepam, indicating that SAI is specifically modulated by the α-1 subunit of the $GABA_A$ receptor.

The inhibition produced by SAI predominantly reduces the later I-waves, as seen through epidural recordings (Tokimura et al., 2000). As mentioned, the orientation of the TMS coil is able to elicit differential activation of either the late I-Waves using the AP orientation (I2 – I4), or the early-late I-waves using the PA orientation (I1 – I4) (Ni et al., 2011; Sakai et al., 1997; Ziemann & Rothwell, 2000). Considering that SAI predominantly reduces the later I-waves, and given that late I-waves are generated more through AP stimulation than PA, SAI should be highest when stimulating in the AP direction. Contrastingly however, deeper SAI is observed when stimulating in the PA orientation, indicating that the neuronal populations responsible for late I-wave generation differ between PA and AP stimulation (Ni et al., 2011).

The depth of SAI can be experimentally modulated through several variables. The intensity of the conditioning nerve stimulus (CS) is indicated as one such measure, with SAI being maximal when the nerve stimulus is given at 50% of the maximum Sensory Nerve Action Potential ($SNAP_{Max}$) for mixed nerves, and at the $SNAP_{Max}$ for sensory nerves (Bailey et al., 2016). This reflects a continual increase in inhibition until all afferent fibers are

recruited. The TMS intensity administered to M1 can also modulate SAI, with higher TMS intensities resulting in decreased inhibition, as the conditioning stimulus is not strong enough to reduce the MEP (Cash et al., 2015; Ni et al., 2011; Udupa et al., 2009). The Somatosensory Evoked Potential (SEP), a recording of the sensorimotor cortex while nerve stimulation is given to the hand, also contributes to the depth of SAI. The N20 component of the SEP reflects the latency between the delivery of nerve stimulus and the arrival of corresponding sensory information to the somatosensory cortex (S1). When the time period between the delivery of nerve stimulus and TMS is normalized to the latency of the N20 component, SAI is maximal (Bailey et al., 2016; Tokimura et al., 2000). Normalization of the Interstimulus Interval (ISI) allows for TMS delivery to directly coincide with the arrival of the inhibitory signal to M1, resulting in maximal inhibition of the signal. Further evidence also exists to purport that higher amplitudes of the N20 potential correlate with stronger SAI (Bailey et al., 2016).

While the neural pathway underlying SAI is currently unknown, there exist several hypothetical models. Two primary models theorize that SAI is elicited through either an activation of thalamocortical connections to M1 interneurons that inhibit descending corticospinal volleys, or the disinhibition of connections from S1 which excite inhibitory interneurons within M1 (Di Lazzaro et al., 2000; Turco, El-Sayes, Savoie, et al., 2018). Evidence for the former model arises due to the lack of SAI in individuals with paramedian thalamic infarct who continue to exhibit SEPs (Oliviero et al., 2005). Further, a recent case report presents an individual with posterolateral thalamic infarct leading to a complete loss

of sensation on the left side of their body and a non-existent N20 component of the SEP, yet continues to show SAI (Alaydin et al., 2021). The presence of SAI in this individual without a notable N20 component in the SEP indicates that thalamocortical inhibition of M1 drives the effect with the disinhibition of excitatory S1-M1 connections acting as a modulator instead (Alaydin et al., 2021). As well, the differential effects on SAI dependent on the location of stroke (posterolateral vs paramedian) support Oliviero's initial theory that SAI is generated specifically in the paramedian thalamic nuclei (Oliviero et al., 2005).

2.2.3 – Long-Latency Afferent Inhibition (LAI)

LAI provides an indirect assessment of sensorimotor integration, reflecting a reduction in motor output when brain stimulation is preceded by a peripheral nerve stimulus at a time difference of 200-1000 ms (Chen et al., 1999). While similar to SAI, LAI has a distinct pharmacological basis and neural organization.

Considerably less is known regarding the pharmacological basis of LAI than for SAI. LAI seems to be primarily $GABA_A$ mediated, as administration of Lorazepam, a $GABA_A$ agonist, significantly inhibited LAI (Turco, El-Sayes, Locke, et al., 2018). However, unlike with SAI, it is unknown whether the $GABA_A$ receptors involved in LAI operate through cholinergic pathways. Similarly, the relationship between the depth of LAI and various disorders is less clear. LAI is reduced in patient populations with Parkinson's Disease (PD); however, the reduction in LAI may not be related to dopaminergic losses within the population, as L-Dopa therapy failed to normalize LAI (Sailer et al., 2003).

Several modulatory variables also influence LAI. When stimulation is given to a mixed nerve such as the median nerve (MN), LAI is maximal at 50% of $SNAP_{Max}$, which represents the recruitment of all sensory fibers, with only motor efferent fibers remaining (Turco et al., 2017). When a sensory nerve such as the digital nerve (DN) is stimulated, LAI appears at and is maximal at ~50% of $SNAP_{Max}$, failing to increase when more sensory fibers are recruited. Therefore, unlike SAI, which is maximally elicited by the DN when all sensory fibers are recruited at 100% $SNAP_{Max}$, LAI is less dependent on the sensory afferent volley. Also, unlike SAI, no current work has investigated the modulation of LAI through SEPs. However, LAI is influenced by the intensity of TMS, as inhibition decreases as TMS intensity increases (Kukaswadia et al., 2005).

The neural pathway underlying LAI is even less clear than that of SAI. When delivering peripheral nerve stimulation to either the DN or the MN, there is activation of several brain areas (Posterior Parietal Cortex, Secondary Somatosensory Cortex, Somatosensory Cortex) at the latency at which LAI is seen (~100-200 ms) (Turco, El-Sayes, Savoie, et al., 2018). This invokes the potential for all such areas to be involved in producing the inhibition seen with LAI. However, given the abnormal LAI seen in patients with PD, the Basal-ganglio-thalamocortical loop may also be involved, as PD is primarily a Basal-Ganglia mediated disease (Sailer et al., 2003).

2.3 – Reliability Testing

Reliability is a metric referring to the reproducibility of measurements and is an integral component to the data collection process. Establishing the reliability of a data set allows for researchers to make certain that changes in the measure are due to experimental methodology, not random error. Original assessments of reliability are based around the classical test theory, which defines three components: True score, error score, and observed score (Šerbetar, 2015). The true score represents the average score from multiple trials, and the observed score represents the individual score on each trial. The error score represents variability that cannot be experimentally explained (Bruton et al., 2000; Šerbetar, 2015). The components can be arranged in an equation: Observed Score = True Score + Error Score (Bruton et al., 2000; Šerbetar, 2015). Reliability testing reflects the attempt to isolate and assess the true variability of an observed score from the error score.

Common attempts at establishing the reliability of a measure often include Pearson's Correlation Coefficients and Paired Sample's T-Tests. Users of Pearson's R assume that a high correlation coefficient reflects high test-retest reliability. However, a high correlation coefficient only indicates that changes in all scores are consistent. If all scores increase by 5 from time 1 to time 2, the correlation coefficient would be high, but the measures would not have good test-retest reliability. In other words, Pearson's R does not account for systemic error and thus is an inappropriate tool for accurate assessments of reliability (Bruton et al., 2000; Šerbetar, 2015). Similar problems exist in the usage of paired sample T-tests in assessing reliability. While a paired sample's t-test is able to identify systematic

changes in the data from T1 to T2, the test cannot detect random fluctuations (Beaulieu, Flamand, et al., 2017). If the scores for half the dataset increased by 10 from T1 to T2, and the other half decreased by 10, the average for both time points would be the same. In this case, the paired sample's t-test would indicate that the measures did not change over time; however, there was random error in the measures that the T-test could not assess for. Rather than utilizing such metrics, researchers are now promoting the usage of more robust tests that directly assess the relative and absolute reliability of a measure (Bruton et al., 2000).

2.3.1 – Relative Reliability

Relative reliability defines the ability of a measure to identify individuals on repeated testing (Bruton et al., 2000). A measure with high relative reliability allows for an individual to retain their position relative to each other in a dataset. Relative reliability was originally assessed through Pearson's R; however, due to its bivariate nature, insensitivity to systemic error, and its lack of usability when more than two instances of measurement are concerned, Pearson's R is a non-ideal method of assessment (Bruton et al., 2000; Šerbetar, 2015; Weir, 2005). The Intraclass Correlation Coefficient (ICC) represents a better method to assess relative reliability as it is univariate in nature, and is sensitive to systemic errors alongside being able to handle more than two instances of measurement (Beckerman et al., 2001; Šerbetar, 2015). The ICC can be defined using the equation (Weir, 2005):

$$ICC = \frac{Between\ Subject\ Variability}{Beween\ Subject\ Variability + error}$$

The ICC, therefore, is inherently dependent on the sample demographics. In a sample with large between subject variability, the ICC can be large even if there is a large amount of error. Large values of the ICC indicate high relative reliability of the measure.

ICC values are calculated from the outputs of repeated measures ANOVAs (Bartlett & Frost, 2008; Weir, 2005). There exist several different forms of the ICC, namely 1-way and 2-way models. The 1-way model collapses the variability due to time and error, whereas the 2-way model explores them independently (Weir, 2005). However, for both mentioned models, the values for calculation are obtained from a one-way repeated measures ANOVA. The error term within this ANOVA reflects the interaction between participants and timepoints, such that if all participants change similarly across time the error will be minimized. The ICC 2,k model is often used for studies where one experimenter tests all participants (2 way model), when the results are intended to be generalized to other works, and when the scores represent an average of several trials (Weir, 2005).

$$ICC\ 2,k = \frac{MS_S - MS_E}{MS_S + \frac{k(MS_T - MS_E)}{n}}$$

This formula represents a computationally equivalent model of the formula presented above, with MS_S representing the subjects mean-square, MS_E representing the error mean square, MS_T representing the trials mean square, and K the number of trials.

However, the presentation of an individual ICC value is not enough to inform relative reliability. As indicated, the ICC is dependent on the Between Subject Variability, and as

such, two measures that have differing amounts of variability between subjects but similar amounts of variability from trial to trial will have different ICC values (Turco et al., 2019). Therefore, in conjunction with the ICC, the coefficient of variation (CV) for a measure is needed, which provides a metric for the between subject variability of the data (Turco et al., 2019). The formula for the CV is as follows: $CV = \frac{Standard\ Deviation}{Mean} \times 100$.

2.3.2 – Absolute Reliability

Absolute Reliability is concerned with the repeatability of individual scores through repeated testing (Bruton et al., 2000; Turco et al., 2019). Unlike relative reliability, which is dependent on the heterogeneity of the dataset, the absolute reliability is an independent assessment (Weir, 2005).

1.3.2.1 – Standard Error of Measurement

A common approach to assessing absolute reliability is calculation of the Standard Error of Measurement (SEMeas). The SEMeas represents the standard deviation of all the errors present in a measure, or in other words, the sum of random variation in a measure when an individual is repeatedly tested (Beckerman et al., 2001; Hopkins, 2000; Šerbetar, 2015). Larger values of the SEMeas indicate greater total variance of the scores around the true score, and lower precision of individual scores on a measure.

Initial attempts to calculate the SEMeas did so as a function of the ICC through the formula: $SEM_{eas} = SD\sqrt{1 - ICC}$. However, calculation of the SEMeas using this formula results in

the calculation being influenced by the value of the ICC, and can therefore be modulated dependent on the ICC model used (Šerbetar, 2015). Furthermore, the SEMeas as calculated above would be influenced by the sample demographics, as the ICC is a function of between-subject variability, a factor that should minimally influence absolute reliability measures. Rather, optimal calculation of the SEMeas utilizes the formula shown: $SEMeas = \sqrt{MSE}$ (Beaulieu, Massé-Alarie, et al., 2017; Šerbetar, 2015). The MSE term indicated in the formula represents the Mean Squared Error, which is the total error variance as indicated in the output table of a Repeated Measures ANOVA assessing changes in the measure over time. Using this formula allows SEMeas to be calculated independent of the ICC, and provides an accurate assessment of variance around the mean. Given that the SEMeas is in the units of the collected measure, it can be difficult to compare scores between measures with different units. To compare such, the SEMeas can be normalized by the following formula: $\%SEMeas = \frac{SEMeas}{Mean} * 100$. This presents the absolute error as a percentage, allowing easier comparison between studies, with a value greater than 10% indicating high measurement error (Beaulieu, Massé-Alarie, et al., 2017; Schambra et al., 2015; Turco et al., 2019).

2.3.2.2 – Smallest Detectable Change

The Smallest Detectable Change (SDC) is the quantification of baseline variation in a measure due to measurement error. The SDC reflects the smallest change necessary to be seen in order to be quantified as real change in the outcome (Šerbetar, 2015). If an intervention modulates the outcome measure by a value smaller than the SDC, that

modulation is attributed to random variations rather than to experimental change. Calculation of the SDC is accomplished using the SEMeas through the formula: $SDC = 1.96 \times \sqrt{2} \times SEMeas$ (Beckerman et al., 2001; Šerbetar, 2015; Weir, 2005). 1.96 reflects the z-score at the 95% confidence level and the value is multiplied by $\sqrt{2}$ to reflect the change across two time points (Šerbetar, 2015). The above formula leads to the calculation of the $SDC_{Individual}$, which reflects the minimum change required at an individual level to indicate experimental change. However, given the prevalence of group level statistics in the sciences, the SDC_{Group}, which indicates the minimum change needed to be seen at the group level to be considered real, is far more important. The SDC_{Group} can be calculated using the SDC through the formula: $SDC_{Group} = \frac{SDC_{Individual}}{\sqrt{n}}$, where n represents the total sample size (Šerbetar, 2015). Therefore, as the sample size increases, a smaller change is required to be seen at the group level for the change to be considered "real". This follows trends seen in other statistical tests, where a larger sample size increases the power of the test.

2.4 – Reliability of TMS Measures

TMS, despite its popularity and increasing use, is a variable method of stimulating the brain due to the multitude of biological and technical factors that govern its usage. Many studies have indicated large intraindividual variability of the MEPs elicited by TMS, with a primary explanation being fluctuations in the excitability of corticospinal and motoneurons (Kiers et al., 1993). Coil orientations also play a significant role in governing variability to TMS, with certain coil orientations leading to the induction of a stronger magnetic field

(Jung et al., 2010; Lee et al., 2018). Further, despite the usage of neuronavigation systems which use optical tracking to ensure coil placement to a degree of error within 2.5 mm, the variability of MEPs is unchanged when compared to non-neuronavigated TMS (Jung et al., 2010). This leads to the belief that while coil orientation may play a role in the variability of TMS, the fundamental source is likely neurophysiological factors that influence baseline corticospinal excitability and lead to trial-trial variations (Beaulieu, Flamand, et al., 2017; Jung et al., 2010). Therefore, given the significant variability of TMS responses, there is a need to explore the reliability of these measures.

Despite the prominent variability of these measures, there is a significant lack of research exploring their reliability. The limited research studying such effects indicate that assessments of motor threshold (MT) have generally good intersession (Cacchio et al., 2009, 2011; Fleming et al., 2012; Forster et al., 2014; Hermsen et al., 2016; Liu & Au-Yeung, 2014; Malcolm et al., 2006; Ngomo et al., 2012; Sankarasubramanian et al., 2015) and intrasession relative reliability (Cacchio et al., 2009). The reliability of MT stays similarly high regardless of whether threshold was gathered during rest or active contraction. Furthermore, patients with chronic and acute stroke had similar levels of relative reliability for motor threshold as patients without stroke (Cacchio et al., 2011; Schambra et al., 2015). The limited number of studies exploring the absolute reliability of MT, indicate that the measure has low measurement error (Samusyte et al., 2018; Schambra et al., 2015). Post-publication analysis of data in a systematic review has further

corroborated that measures of MT have low measurement error (Beaulieu, Flamand, et al., 2017).

While single-pulse measures such as motor threshold seem to have high relative reliability, the reliability of paired-pulse measures are more varied. Short- and Long-Intracortical Inhibition (SICI and LICI) are paired pulse measures consisting of two TMS pulses that, when delivered to M1, inhibit the excitability. Similarly, Intracortical Facilitation (ICF) is a paired-pulse paradigm where the first TMS pulse increases the excitatory effect of the second TMS pulse. The relative reliability of these measures ranges, with SICI and LICI generally having moderate-good inter and intrasession relative reliabilities (Biabani et al., 2018; Fleming et al., 2012; Ngomo et al., 2012; Samusyte et al., 2018; Schambra et al., 2015), with one study presenting low intersession relative reliability of SICI (Sankarasubramanian et al., 2015). The relative reliability of ICF is even further reduced, with most studies reporting poor relative reliability (Biabani et al., 2018; Fleming et al., 2012; Schambra et al., 2015). The absolute reliabilities for SICI, LICI and ICF are all lower than that for MT (Beaulieu, Flamand, et al., 2017; Beaulieu, Massé-Alarie, et al., 2017; Samusyte et al., 2018; Schambra et al., 2015).

However, despite the appreciable amount of literature exploring the reliability of single and paired pulse TMS measures, there is a dearth of studies exploring the reliability of SAI and LAI in particular. The limited literature present indicates that SAI and LAI both have moderate intersession relative reliability (Brown et al., 2017; Turco et al., 2019), and

moderate intersession absolute reliability (Turco et al., 2019). No study to date has explored the intrasession reliability of these measures, nor has any work explored the minimal number of pulses required to obtain reliable measures of the data.

CHAPTER 3: THE EXPERIMENT

3.1 – Introduction

Afferent Inhibition is a phenomenon reflecting decreases in Transcranial Magnetic Stimulation (TMS) induced motor output following peripheral nerve stimulation. The reduction in motor output due to the presence of nerve stimulation supports this phenomenon as an indirect assessment of sensorimotor integration. Afferent Inhibition can be subdivided into two separate circuits elicited at distinct Interstimulus Intervals (ISI); Short-Latency Afferent Inhibition (SAI) is elicited at ISIs of 18-28 ms (Ni et al., 2011; Tokimura et al., 2000), while Long-Latency Afferent Inhibition (LAI) is elicited at ISIs of 200-1000 ms (Chen et al., 1999).

Alongside reflecting the integrity of sensorimotor systems, afferent inhibition is also associated with cognitive well-being and neurological disorders. Particularly, SAI is reduced in various disorders impairing cognition, such as Alzheimer's Disease (AD) (Di Lazzaro et al., 2002, 2004), Parkinson's Disease (PD) with Dementia (Celebi et al., 2012; Yarnall et al., 2013), and Mild Cognitive Impairment (MCI) (Nardone et al., 2012; Peter et al., 2016) whereas LAI is reduced in disorders of the sensory-motor system such as PD (Sailer et al., 2003). The differentiation between the two may lie in their molecular basis, with SAI reflecting both cholinergic and gamma-aminobutyric acid type A (GABA) receptor activity, compared to LAI which appears to be solely GABAergic (Di Lazzaro et al., 2007; Di Lazzaro, Pilato, et al., 2005; Turco, El-Sayes, Locke, et al., 2018).

Importantly, despite their popularity in the sensorimotor literature, afferent inhibition measures show moderate to low reliability in most cases (Brown et al., 2017; Toepp et al., 2021; Turco et al., 2019). Determining the reliability of both SAI and LAI would allow experimenters to assess whether changes in the measure are due to physiological change or simply the variability of the measure itself.

Reliability can be partitioned into two forms: relative and absolute reliability. Relative reliability reflects the ability of a measure to allow for stable individuals to maintain their position relative to each other, by providing the correlation between repeated measures (Beaulieu, Flamand, et al., 2017; Šerbetar, 2015). Intraclass Correlation Coefficients (ICC) are a common form of assessing relative reliability, reflecting the between subject variability as a function of total error (Šerbetar, 2015; Weir, 2005). Higher ICC values indicate a greater ability of the measure to identify differences between individuals, an essential trait for the diagnostic usage of a measure. Absolute reliability reflects the total variability of a measure across time (Beaulieu, Flamand, et al., 2017; Šerbetar, 2015). Unlike relative reliability, absolute reliability is not dependent on the characteristics of the sample (Turco et al., 2019; Weir, 2005). Traditionally, absolute reliability can be assessed through the Standard Error of Measurement (SEMeas), reflecting the standard deviation of all errors in the measure (Beckerman et al., 2001; Hopkins, 2000; Šerbetar, 2015). Smaller SEMeas values thus result in a measure that is less likely to change with variability. The SEMeas can be further used to calculate the Smallest Detectable Change (SDC) in the measure at both the group and individual level. The $SDC_{Individual}$ reflects the smallest

amount of change required to be seen in an individual across time that can be attributed to sources other than measurement error (Beaulieu, Flamand, et al., 2017; Šerbetar, 2015). SDC_{Group}, similar to $SDC_{Individual}$, reflects the smallest amount of change needed to be seen at the group level which can be attributed to real change (Beaulieu, Flamand, et al., 2017; Šerbetar, 2015).

Previous work has only explored the intersession reliability of SAI and LAI, with the three studies reporting the relative reliability of both measures as ranging from low to strong (Brown et al., 2017; Toepp et al., 2021; Turco et al., 2019). Even more limited are quantifications of the absolute reliability of these measures, with a single study reporting low absolute reliability as indicated by high SEMeas and high SDC values, indicating large deviations in the measure are needed in order to be significantly different from measurement error (Turco et al., 2019). The literature therefore seems to suggest that while SAI and LAI are able to identify distinguishable individuals on repeated testing, the variability in true scores across time is high.

To date, no study has examined the absolute and relative reliability of SAI and LAI when collected at multiple time points within a single session. This is important in informing whether the measures are stable enough to reflect changes within a session when an intervention is administered. Furthermore, the literature exploring reliability of other TMS measures often include a frame-by-frame analysis in order to determine the smallest number of frames required to produce a reliable measure (Biabani et al., 2018;

Goldsworthy et al., 2016) and this has yet to be explored for afferent inhibition. In the present study, we explore both the absolute and relative intersession reliability of SAI and LAI evoked by stimulation of the Median Nerve (MN) at three time points separated by 30 minutes in 30 healthy, young individuals. Given that past work has indicated higher intersession relative reliability for LAI compared to SAI, we predict the same relationship in our intrasession exploration (Turco et al., 2019).

3.2 – Methods

3.2.1 – Participants

Thirty healthy, young, right-handed participants took part in this experiment (17 females; age = 21.17 ± 2.84 years). Participants were involved in one session spanning ~3 hours. All participants partook in the experiment after 12 PM in order to account for fluctuations in diurnal cortisol levels that may influence TMS measures (Milani et al., 2010). Participants were screened for contraindications to TMS prior to taking part in the study, and handedness was confirmed using a modified version of the Edinburgh Handedness Questionnaire. All individuals provided informed written consent prior to study onset, as well as verbally reconsenting prior to any measure. This research was approved by the Hamilton Integrated Research Ethics Board and conformed to the Declaration of Helsinki.

3.2.2 – Electromyography (EMG)

Surface electrodes (9 mm, Ag-AgCl) were used to record activity from the first dorsal interosseous (FDI) muscle of the right hand. The active electrode was placed over the

muscle belly, and the reference electrode was placed on the metacarpal joint of the first digit. A grounding electrode was placed at the styloid process at the wrist. EMG signals were first magnified x1000 and were then band pass filtered between 20 and 2.5 kHz (Intronix Technologies Corporation Model 2024F, Bolton, Canada). Data were digitized at 5 kHz through the usage of a digital to analog converter (Power1401; Cambridge Electronics Design, Cambridge, UK), and were then analyzed through commercial software (Signal v7.02; Cambridge Electronics Design, Cambridge, UK).

3.2.3 – Electroencephalography (EEG)

Somatosensory Evoked Potentials (SEPs) were recorded by placing EEG electrodes on the scalp at C3' over S1 and referencing activity to Fz (International 10-20 System). A ground electrode was placed on the clavicle ipsilateral to the stimulated nerve. Electrical stimulation (Digitimer DS7AH, 200 μs square wave pulses) was delivered using a bar electrode at the right MN at the wrist, at 3Hz and an intensity corresponding to the motor threshold of the participant. 500 stimuli were delivered and time-locked averaged to determine the latency of the N20 potential.

3.2.4 – Transcranial Magnetic Stimulation (TMS)

TMS was performed with a monophasic waveform using a figure of eight coil connected to a Magstim 200^2 stimulator. The coil was oriented at a 45 degree angle posterior-anterior over the motor representation of the right FDI hotspot over the left motor cortex. The motor

hotspot was registered using Brainsight Neuronavigation Software, and was identified as the location that elicited the largest Motor Evoked Potential (MEP) in the right FDI muscle.

3.2.5 – Afferent Inhibition

The intensity of the nerve stimulus, also known as the conditioning stimulus (CS) was set to the motor threshold of the MN, assessed through visual inspection of the right Abductor Pollicis Brevis (APB) muscle. The motor threshold of the MN has previously been found to correlate with 50% of the maximum sensory nerve action potential elicited by the nerve, which is the intensity at which maximum inhibition occurs for both SAI and LAI (Bailey et al., 2016; Turco et al., 2017). TMS stimulation was delivered at the lowest intensity needed to elicit a 1 mV peak-peak MEP in the target muscle. The ISI between the nerve stimulus and TMS pulse was set to the latency of the N20 component + 4 ms for SAI (Di Lazzaro, Pilato, et al., 2005; Tokimura et al., 2000; Turco et al., 2019), and 200 ms for LAI (Chen et al., 1999). SAI and LAI were collected sequentially. Unconditioned pulses contain only the TMS pulse and are referred to as Test Stimulus (TS) frames. Conditioned pulses contain both the nerve stimulus and TMS pulse and are referred to as Conditioning Stimulus-Test Stimulus (CSTS) frames. For each circuit, 40 TS frames and 40 CSTS frames were delivered pseudo-randomly such that there were never 3 of any one type delivered in succession. SAI and LAI were collected at three time-points within the session, separated by 30-minute breaks.

3.3 – Statistical Analysis

EMG trials were discarded if the peak-peak amplitude of the signal 100 ms before the TMS pulse was greater than 50 μv. Normality was assessed using Shapiro-Wilks tests, and heteroscedasticity was assessed using Bland-Altman plots. Bland-Altman plots were created comparing the respective variables at T1-T2, T1-T3, and T2-T3. Outliers were identified and removed using Grubb's Test. Afferent Inhibition was calculated as a ratio of the peak-peak amplitudes of the mean CSTS MEPs to mean TS MEPs. A repeated measures ANOVA was used to compare the across the three time points to discover if systematic error was present. Significance was set to alpha = 0.05, and Bonferroni corrections were used for multiple comparisons.

3.3.1 – Reliability Assessment

Relative reliability was assessed on running averages of the MEP amplitudes, defined as the average of all preceding trials. ICCs were evaluated for both SAI and LAI using both the overall measure, as well as at intervals of every 5 trials. As all data was collected by a single experimenter, a two-way random effects model was used (ICC 2,k). To aid in the interpretation of the measures, coefficients of variation (CV) were also calculated for each measure. ICCs were categorized based on suggested cut off points where ICC with 95% CI above 0.9 is Excellent; 0.75 < ICC < 0.9 is High; 0.5 < ICC < 0.75 is Moderate; and ICC < 0.5 is considered Low (Koo & Li, 2016; Portney & Watkins, 2009). Absolute reliability was assessed using SEMeas values obtained over the whole dataset. SEMeas was converted to be expressed as a percentage of the mean using the formula %SEMeas =

(SEMeas/mean)*100%, in order to provide a unitless assessment of the measurement error. %SEMeas < 10% was used as a cut off to indicate low measurement error (Schambra et al., 2015). These SEM values were then used to calculate both $SDC_{Individual}$ and SDC_{Group}, which provide the minimum amount of change needed to be seen at the individual and group level to be considered real change and not due to measurement error.

3.4 – Results

All participants tolerated the experimental conditions well, with no reported adverse effects. In accordance with our noise threshold, a total of 58 CS-CSTS pairs were removed for SAI and 59 CS-CSTS pairs were removed for LAI. For P12, because more than 50% of the LAI dataset at T1 was removed due to noise, this participant was excluded from the analysis. Further, LAI data could not be processed in P29 due to an inability to relax the hand. As well, Grubb's test necessitated the removal of a singular outlier from the LAI at T1 dataset. The outlier datapoint was removed from overall analysis of reliability only and was included in the frame-by-frame analysis method. Therefore, for overall assessments of reliability, SAI yielded n = 30 and LAI yielded n = 27. For the frame-by-frame analysis of relative reliability SAI yielded n = 30 and LAI yielded n = 28.

The datasets for both SAI and LAI were normally distributed. Homoscedasticity was maintained for LAI, however, Bland-Altman plots comparing T1-T3 and T2-T3 for SAI indicated heteroscedasticity in the dataset, with $R^2 > 0.1$. Corrections for heteroscedasticity normally require a log-transformation on the dataset. However, given that log-

transformations change the dataset to a ratio scale, we did not perform the log transformation, and analysed the data with an assumption of heteroscedasticity, as done previously (Liu & Au-Yeung, 2014; Ngomo et al., 2012; Sankarasubramanian et al., 2015)

Across the sample size, TMS was delivered at the lowest intensity to elicit a ~1mV peak-peak MEP, which equated to 134 ± 5.61% of RMT averaged across participants. The average latency for the N20 potential in the dataset was 18.64 ± 1.01 ms.

3.4.1 – Short-Latency Afferent Inhibition (SAI)

The mean ± SD of SAI across the three timepoints is shown in Figure 1A. A repeated measures ANOVA with factors of Time (3 Levels), and State (TS vs CSTS) indicated a main effect of State ($F_{(1, 29)} = 52.350$, $p < 0.001$, $\eta_p^2 = 0.644$) with no other significant main or interaction effects. Therefore, we can conclude a significant difference in peak-peak MEPs between states, such that CSTS was suppressed relative to TS. For SAI ratio, a repeated measures ANOVA indicated no main effect of time on SAI magnitude ($F_{(2,58)} = 0.330$, $p = 0.720$, $\eta_p^2 = 0.011$) indicating that SAI was not different across timepoints, and that there was no detectable systematic error in the dataset. Overall, 29 out of 30 participants displayed inhibition at a minimum of one time point (Figure 1C). Compared to the TS, the CSTS was reduced by an average of 27.74% at T1, 30.65% at T2, and 27.45% at T3.

Overall, high relative reliability was observed for the SAI inhibition ratio (ICC = 0.79; 95% CI [0.61 – 0.89]), and moderate reliability for the conditioned frames (ICC = 0.73; 95% CI [0.51 – 0.86]). The %SEMeas for SAI was 23.65, indicating large amounts of measurement error. For SAI, the $SDC_{Individual}$ indicates that a minimal change of 46.80 is needed to be seen over time to be considered physiological change on an individual level. Our data also indicates that for our sample size of 30 individuals, a change of 8.54 is needed to be considered physiological change in the measure at a group level (Figure 1B).

The ICCs as a function of the number of stimulus pairs are shown in Figure 1D. The running-averages are pooled into groups of 5, and the ICC values are shown individually for the 8 created groups in Table 1. For SAI, the first 5 collected datapoints indicated low reliability of the measure (ICC = 0.47). The 95% CI of the first 5 frames of data was also noticeably large, with a width of ~0.7 (See Table 1). Increasing the number of frames included in the analysis steadily increased the relative reliability of the measure, with high reliability being achieved with 20 stimulus pairs (ICC = 0.76).

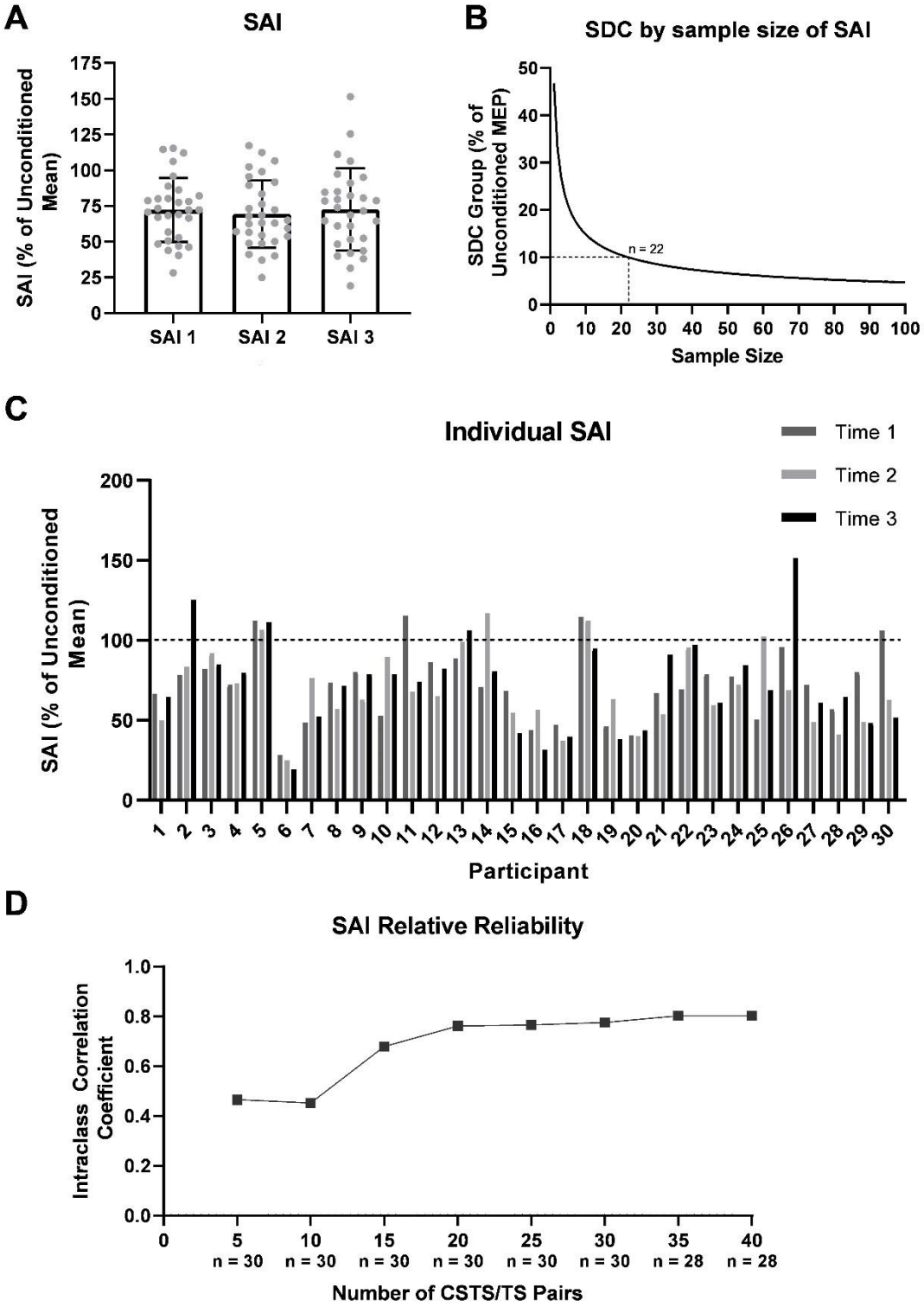
A
SAI
SAI (% of Unconditioned Mean)
175
150
125
100
75
50
25
0
SAI 1
SAI 2
SAI 3
B
SDC by sample size of SAI
SDC Group (% of Unconditioned MEP)
50
40
30
20
10
0
n = 22
0 10 20 30 40 50 60 70 80 90 100
Sample Size
C
Individual SAI
Time 1
Time 2
Time 3
SAI (% of Unconditioned Mean)
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Participant
D
SAI Relative Reliability
Intraclass Correlation Coefficient
1.0
0.8
0.6
0.4
0.2
0.0
0
5 n = 30
10 n = 30
15 n = 30
20 n = 30
25 n = 30
30 n = 30
35 n = 28
40 n = 28
Number of CSTS/TS Pairs

Figure 1: Group Averaged and Individual SAI Data. A. Average SAI, expressed as a % of the unconditioned mean, alongside Standard Deviation error bars, and individual scores. The CV was 31 at T1, 34 at T2, and 40 at T3. B. SDC_{Group} presented as a function of the sample size. C. Individual scores on SAI by timepoint for each participant. D. ICC values depicted as a function of the number of CS/CSTS pairs included to determine SAI.

Number of CSTS/TS Pairs	*SAI ICC (95% CI)*	*LAI ICC (95% CI)*
5	.47 (0.02 – 0.73)	.84 (0.69 – 0.92)
10	.45 (-0.02 – 0.72)	.86 (0.74 – 0.93)
15	.68 (0.41 – 0.84)	.84 (0.69 – 0.92)
20	.76 (0.56 – 0.88)	.86 (0.74 – 0.93)
25	.77 (0.57 – 0.88)	.87 (0.76 – 0.94)
30	.78 (0.59 – 0.89)	.89 (0.79 – 0.95)
35	.80 (0.63 – 0.90)	.89 (0.80 – 0.95)
40	.80 (0.63 – 0.90)	.90 (0.81 – 0.95)

Table 1: Frame-by-Frame analysis of relative reliability. ICC values are presented as a function of number of CSTS/TS pairs used to calculate afferent inhibition, with the 95% confidence interval presented in brackets.

3.4.2 – Long-Latency Afferent Inhibition (LAI)

The mean ± SD of LAI across the three timepoints is shown in Figure 2A. A repeated measures ANOVA with factors of Time (3 Levels) and State (CSTS vs TS) indicated a main effect of State ($F_{(1,27)} = 32.36$, $p < 0.001$, $\eta_p^2 = 0.545$) with no other significant main or interaction effects. Therefore, we can conclude that MEPs were significantly suppressed during CSTS compared to TS alone. A 3-level repeated measures ANOVA indicated no main effect of time on LAI magnitude ($F_{(2, 52)} = 0.366$, $p = 0.695$, $\eta_p^2 = 0.014$) indicating that LAI was not different across time points. All participants showed inhibition at a minimum of one time point (Figure 2C). Compared to the TS, the CSTS was reduced by an average of 34.57% at T1, 38.13% at T2, and 36.75% at T3.

Overall, excellent relative reliability was observed for both the LAI inhibition ratio (ICC = 0.90, 95% CI [0.82 – 0.95]) and the conditioned frames (ICC = 0.92, 95% CI [0.84 – 0.96]). The %SEMeas for LAI was 24.29, indicating that there was large measurement error in the dataset. The $SDC_{Individual}$ indicates that a difference of 42.77 in % of unconditioned mean across time is required for the change in LAI to be considered significant in a given individual. Our calculations also indicate that for our sample size of 27 individuals, a considerably smaller change of 8.23% is needed in order to be considered physiological change at the group level analysis (Figure 2B).

The ICCs as a function of number of stimulus pairs is shown in Figure 2D. The running-averages are pooled into groups of 5, and the ICC values are shown individually for the 8

created groups. For LAI, the first 5 collected datapoints indicated high relative reliability of the measures (ICC = 0.84), with a narrow 95% confidence interval (0.69 – 0.92) (See Table 1). Increasing the number of CS/CSTS pairs continued to increase the ICC value until all 40 pairs of data were included, indicating excellent relative reliability of the measure (ICC = 0.90). The 95% confidence interval also continued to narrow as the number of frames analysed increased.

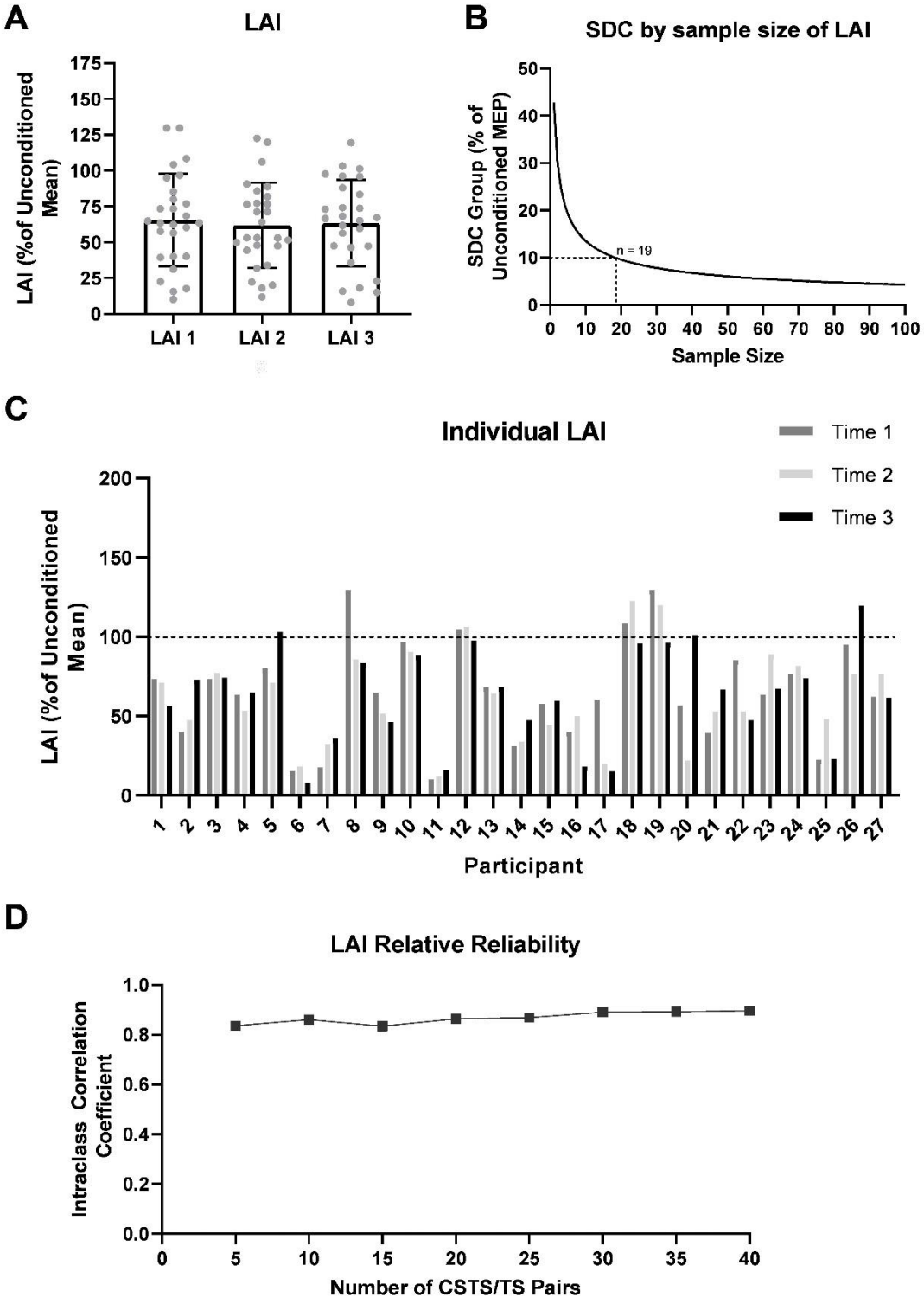
A
LAI
LAI (%of Unconditioned Mean)
175
150
125
100
75
50
25
0
LAI 1
LAI 2
LAI 3
B
SDC by sample size of LAI
SDC Group (% of Unconditioned MEP)
50
40
30
20
10
0
n = 19
0 10 20 30 40 50 60 70 80 90 100
Sample Size
C
Individual LAI
Time 1
Time 2
Time 3
LAI (%of Unconditioned Mean)
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Participant
D
LAI Relative Reliability
Intraclass Correlation Coefficient
1.0
0.8
0.6
0.4
0.2
0.0
0 5 10 15 20 25 30 35 40
Number of CSTS/TS Pairs

Figure 2: Group Averaged and Individual LAI Data. A. Average LAI, expressed as a % of the unconditioned mean, alongside Standard Deviation error bars, and individual scores. The CV was 50 at T1, 48 at T2, and 48 at T3. B. SDC_{Group} presented as a function of the sample size. C. Individual scores on LAI by timepoint for each participant. D. ICC values depicted as a function of the number of CS/CSTS pairs included to determine LAI.

3.5 – Discussion

The goal of the present study was to assess the intrasession reliability of SAI and LAI. To do so, we elicited SAI and LAI through delivering peripheral nerve stimulation to the MN, followed by TMS to M1. We assessed the relative reliability of these measures using ICCs overall and as a function of the total number of stimuli pairs included in calculating SAI and LAI. The absolute reliability of these measures was assessed through calculations of the %SEMeas and the SDC.

3.5.1 – Relative Reliability

Relative reliability reflects the ability of a measure to consistently identify individuals within a dataset during repeated testing. Within this study, we found that **SAI had moderate-high relative reliability, whereas LAI had high-excellent relative reliability**, supporting our hypothesis that LAI would have higher relative reliability than SAI and is in line with previous findings (Turco et al., 2019). The differences in relative reliability

between SAI and LAI observed both here and previously in the literature, may be due to differences in the between subject variation of these measures. For SAI, the calculated CVs in our study ranged from 31% to 40%, whereas for LAI they ranged from 48% to 50%. This same differentiation was also seen in Turco et al.'s work, with LAI having higher levels of between subject variability when compared to SAI (2019). Given that ICC values are a function of the between subject variability in a sample, with large amounts of between subject variability leading to higher estimates of relative reliability, this likely explains the trends in reliability seen across the literature and supported here (Bruton et al., 2000; Weir, 2005). We recently published a large retrospective analysis of SAI and LAI data which also showed larger amounts of between subject variability for LAI compared to SAI, with a plausible reasoning being the different neurological pathway that LAI may traverse (Toepp et al., 2021). We theorize that because of the large ISI of LAI, there is possible activation of the basal-ganglio-thalamocortical loop, the posterior parietal cortex, and the secondary somatosensory cortex (Toepp et al., 2021). The potential activation of these various brain regions introduces several avenues of variability between individuals, which is not seen in SAI. Similar trends are seen with reliability comparisons of SICI and LICI, where LICI, with its longer ISI and potential for involvement of several cortical regions and thus greater variation between individuals, has more between subject variability and higher levels of relative reliability (Schambra et al., 2015).

Previous work has quantified the reliability of these measures between sessions, allowing us the opportunity to compare intrasession to intersession reliability (Brown et al., 2017;

Turco et al., 2019). However, as Brown et al. did not include CVs in their work, it is difficult to compare our ICC values with theirs, as ICC is a function of between subject variability (Turco et al., 2019; Weir, 2005). Similar CVs between our work and Turco et al.'s allow for such comparisons and indicate that both SAI and LAI have better *intrasession* relative reliability compared to *intersession* relative reliability. Similar findings have been reported when comparing the relative reliability of other single and paired pulse TMS measures, with intrasession reliability being higher than intersession (Biabani et al., 2018; Goldsworthy et al., 2016). Given that relative reliability reflects the ability to identify individuals on repeated testing, it is likely easier to do so in an intrasession format due to less opportunity for change in the various neurochemical and biological factors that govern afferent inhibition (Bruton et al., 2000; Di Lazzaro, Oliviero, Saturno, et al., 2005; Turco, El-Sayes, Locke, et al., 2018). Therefore, for future work exploring the modulation of afferent inhibition across time, we recommend intrasession approaches if possible.

Analysing relative reliability as a function of the number of stimuli pairs led to some interesting findings. For LAI, the relative reliability is initially high and continues to stay high as more pairs are added, with the greater change being the narrowing of the 95% Confidence Intervals. This again, can likely be explained by the large amounts of between subject variation in the LAI measure leading to overall higher values for the ICC. However, for SAI we found that the relative reliability is initially low when only 5 pairs of data are included and ICCs continue to increase as more pairs are included in the average calculation, eventually reaching a "high" level once 20 pairs are included. Similar trends

have been explored with assessments of both, single and paired pulse TMS measures, indicating that 20-30 frames are needed to produce a reliable estimate (Biabani et al., 2018; Goldsworthy et al., 2016). Future studies exploring afferent inhibition may consider collecting between 20-30 stimuli pairs to ensure high relative reliability, without providing unnecessary stimulation to participants.

3.5.2 – Absolute Reliability

Absolute Reliability assesses the repeatability of scores through repeated testing (Bruton et al., 2000). We found that both SAI and LAI had high levels of measurement error, quantified by the %SEMeas being greater than 10%. This high measurement error of SAI and LAI has also been documented previously in between session explorations; however, while for SAI the error is largely the same as that reported here, the previously reported measurement error for LAI is ~20% greater than reported in our work (Turco et al., 2019). Given that SEMeas is considered to be a fixed characteristic of the measure, which is uninfluenced by the sample demographics, it stands to reason that for LAI particularly, there is more measurement error between sessions compared to within sessions. The lack of consistency may be attributed to the largely unknown biochemical underpinnings of LAI, as suggested elsewhere (Turco et al., 2019). This may be further explained by the longer ISI traversed by the LAI circuit as discussed earlier; similar to our SAI findings, previous work exploring the absolute reliability of SICI indicated no significant difference in intersession and intrasession measurement error, perhaps owing to the shorter ISI of this circuit (Samusyte et al., 2018). However, validation of this theory would require

investigations of the inter and intrasession absolute reliabilities of LICI as well. We currently recommend that future studies exploring interventional modulations of LAI consider intrasession models, due the decreased amount of measurement error present.

Calculations of SDC indicated that, at the individual level, large amounts of within session change in both SAI and LAI is required in order for the change to be considered significant. This limits the utility of SAI and LAI to detect significant change on an individual level. Previous work has cited an SDC_{Group} level <10% as a recommended threshold (Schambra et al., 2015). Given this recommended threshold, both SAI and LAI have utility at the group level, as sample sizes of ≥22 and ≥19 respectively lead to SDC_{Group} < 10%. This indicates that for adequately attainable sample sizes, SAI and LAI are able to establish whether interventions lead to changes across groups. High levels of $SDC_{Individual}$ have been reported for other paired pulse TMS measures as well; however, single pulse TMS measures such as MT generally present with much higher levels of absolute reliability (Beaulieu, Flamand, et al., 2017; Beaulieu, Massé-Alarie, et al., 2017; Samusyte et al., 2018; Schambra et al., 2015). For both SAI and LAI, there is limited diagnostic utility of the measures to identify individuals with disorders such as AD or PD through reductions in the measure owing to the large $SDC_{Individual}$. However, the measures may be useful on the group level, for example to determine whether treatments or therapies are able to modulate SAI and LAI in patient populations through group statistics.

3.6 – Limitations

In the present study, we were limited by our sample demographics. We were only primarily able to recruit undergraduate students for participation in our study, and thus our sample reflects a younger group. Given the inconsistent reports of modulations in SAI and LAI with age, our study may not be applicable to an older demographic (Bhandari et al., 2016; Degardin et al., 2011; Yarnall et al., 2016; Young-Bernier et al., 2012). Further work should be done to explore the reliability of SAI and LAI in these populations. As well, we did not track the activity of the right APB muscle during the delivery of MN stimulation for SAI and LAI acquisition. As such, there may be variances in the amount of motor activity experienced in the right APB muscle across time, possibly leading to differing amounts of afferent inhibition as seen previously (Bailey et al., 2016).

3.7 – Conclusions

This study is the first to explore the intrasession relative and absolute reliability of SAI and LAI, particularly as a function of the number of stimuli pairs. High ICC levels suggest that SAI and LAI have high levels of relative reliability when 20-30 stimuli pairs are included in calculating the inhibition ratio. We also find that while the measures have limited utility to identify differences between individuals, when appropriate sample sizes are collected the measures can be used to establish group level changes. Future investigations should look at establishing the reliability metrics assessed here in clinical populations, to improve the utility of these measures.

CHAPTER 4: GENERAL DISCUSSION

This thesis has contributed novel neuroscience to the field of sensorimotor neural control, particularly regarding the phenomenon of Afferent Inhibition. A well studied phenomena, Afferent Inhibition is a hallmark of sensorimotor integration and has been linked to various clinical disorders as well, such as AD, PD, and MCI (Nardone et al., 2008, 2012; Sailer et al., 2003). SAI has even been implicated as a potential diagnostic tool to determine whether individuals with MCI will progress to AD (Padovani et al., 2018). However, despite the interest and perceived utility of the measure, little is known about its reliability.

In this thesis, I explored the *within session* reliability of both SAI and LAI and determined the minimal number of stimuli pairs required to achieve a reliable estimate. I found appreciable relative reliability of both SAI and LAI, indicating that they were adequately able to differentiate between individuals on repeated testing. However, significant levels of measurement error were observed alongside large values of the $SDC_{Individual}$, indicating that large amounts of change are required to be considered physiological change. This precludes the usage **of SAI and LAI** as individual markers of change, and further limits the diagnostic utility of these measures as well. However, the work also provides key information to guide future explorations of afferent inhibition, suggesting the collection of 20-30 stimuli pairs to ensure reliable estimates of the measure. We also indicate that despite limited utility on an individual scale, SAI and LAI remain relevant in their ability to explore differences at the group level, such as to investigate whether interventions lead to group level change.

The applicability of SAI and LAI in various research designs is shown in Figure 3. The between session recommendations are based on data presented in Turco et al.'s 2019 paper. Figure 3 only depicts the acceptable usage for SAI and LAI collected using MN stimulus at an ISI of N20+4 ms for SAI and 200 ms for LAI. As seen previously, reliability can vary largely with stimulation of different nerves, and varying ISIs (Turco et al., 2019). Therefore, prior to expanding usage guidelines of SAI and LAI using either DN stimulation or varying ISIs, intrasession explorations of their reliability must be established. In Figure 3 we can see that both SAI and LAI are consistently poor markers of individual change but may be used for diagnostic or group level analysis if appropriate assumptions are met.

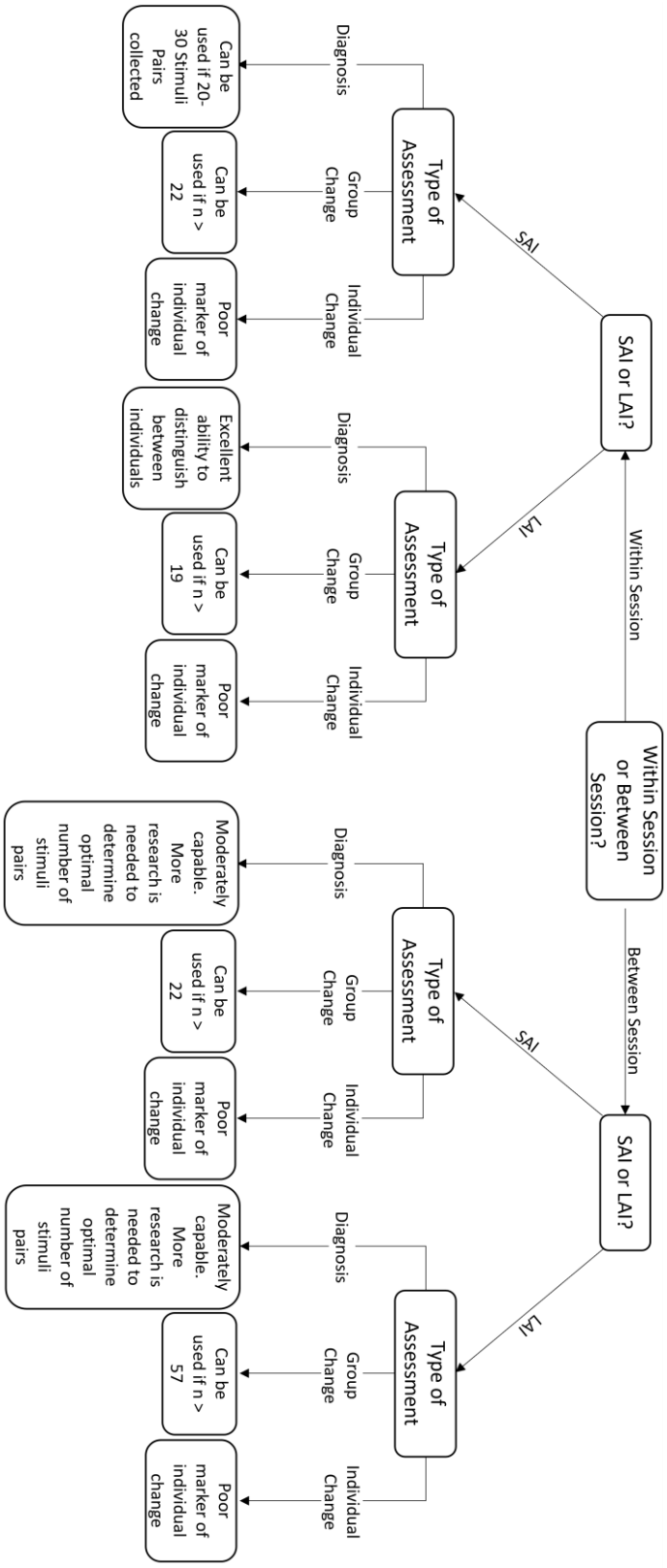
Within Session or Between Session?
Within Session
Between Session
SAI or LAI?
SAI
LAI
Type of Assessment
Diagnosis
Group Change
Individual Change
Can be used if 20-30 Stimuli Pairs collected
Can be used if n > 22
Poor marker of individual change
Excellent ability to distinguish between individuals
Can be used if n > 19
Moderately capable. More research is needed to determine optimal number of stimuli pairs
Can be used if n > 57

Figure 3: SAI and LAI Decision Making Model. This figure provides a tool for researchers to determine whether SAI and LAI are appropriate dependent measures for a given study.

4.1 – The Variability and Reliability of Afferent Inhibition

Afferent inhibition, both in the short and long form, is plagued with variability, both within and between subjects. Biological sex represents one such source of variation. Our previous work has shown no difference in the depth of LAI and SAI when comparing males and females; however, given that biological sex has been implicated in levels of inhibition with SICI (Shibuya et al., 2016), another GABAergic paired pulse measure, the lack of difference observed previously may be due to sample size limitations (Turco et al., 2021). Specifically, while SICI seems to be upregulated by increased $GABA_A$ activity, SAI is downregulated (Di Lazzaro et al., 2007); therefore, if biological sex modulates TMS circuits through GABAergic interactions, males may have increased SAI compared to females based on biological sex differences observed with SICI (Shibuya et al., 2016).

Diet may also play an important role in the variability of afferent inhibition due to its global implications on cortical function. Glucose intake has been theorized to be related to overall cortical neurophysiology, resulting in changes to the overall size of an MEP, as well as LICI (Badawy et al., 2013; Specterman et al., 2005). However, these reports are inconsistent, as other work has reported no changes to SICI, SAI or LAI following glucose intake (Toepp et al., 2019). The ketogenic diet has also been implicated in modulating

corticospinal excitability, as adherence to the diet can lead to increases in SICI (Cantello et al., 2007). Furthermore, the Gluten Free Diet has also been shown to modulate levels of cortical excitability in patients with Celiac Disease (Pennisi et al., 2017). Overwhelmingly, diet seems to play an integral role in the variability of TMS measures.

Sleep is also possibly implicated in the prominence of afferent inhibition, as when tested following a 24-hour sleep deprivation interval, participants displayed reductions in SICI and ICF (Civardi et al., 2001). Given that SICI is also $GABA_A$ modulated (Di Lazzaro et al., 2007; Di Lazzaro, Pilato, et al., 2005), lack of adequate rest could possibly lead to inadvertent changes in SAI and LAI. However, the GABAergic system may not be implicated in this case, as ICF has the opposite relationship to GABA levels as SICI (Civardi et al., 2001). Given that both are reduced by sleep deprivation, and that there are no changes in RMT, which represents changes in pre-synaptic axon membrane excitability (Ziemann et al., 1997), global post-synaptic changes in the motor cortex may be implicated. These global changes could also lead to the downregulation of both SAI and LAI. Future research should further explore this effect.

Furthermore, usage of several recreational substances may result in changes to both single and paired-pulse TMS measures. A recently published systematic review inspected the influence of several recreational substances on a multitude of TMS measures (Turco et al., 2020). Their findings indicated that both acute and chronic nicotine intake can increase levels of SAI relative to non-smokers. As well, despite no studies analysing the effects on

SAI directly, chronic cannabis use and acute alcohol use both modulate SICI, and thus may be implicated in levels of SAI as well (Turco et al., 2020). Future work should look to explore the direct influences of cannabis and alcohol usage on SAI in order to better understand their impact on variability.

For SAI in particular, attentional modulation is a large source of potential variability. Previous work has indicated that spatial attention towards the hand being stimulated increases the magnitude of SAI compared to attentional direction to the non-stimulated hand (Kotb et al., 2005). SAI also seems to be influenced by arousal and motivation, with decreases in both leading to an overall decreased strength of the inhibitory circuit (Koizume et al., 2017). Furthermore, attentional direction itself seems to modulate SAI, as internal focuses of attention reduced SAI more so than external (Suzuki & Meehan, 2020). However, to our knowledge, no yet conducted research has indicated an influence of attention on LAI. Given the multitude of methods in which attention may modulate afferent inhibition, it represents a significant source of within-subject variability.

4.2 – Gaps in SAI and LAI Research

Despite the advancements made by this work, there is a severe lack of understanding for the underpinnings of SAI and LAI. While both SAI and LAI are understood to be modulated by $GABA_A$ activity, SAI also has cholinergic roots (Di Lazzaro et al., 2000; Di Lazzaro, Pilato, et al., 2005; Turco, El-Sayes, Locke, et al., 2018). There is no current work indicating whether LAI is also similarly modulated by cholinergic circuits, further

reinforcing the lack of knowledge on this measure. The lack of investigation on cholinergic modulation of LAI may be due to limited evidence indicating that LAI is related to cognitive ability, a marker of the cholinergic system (Ballinger et al., 2016). However, a singular study has reported a positive correlation between depth of inhibition with LAI, and cognitive abilities as assessed with the Montreal Cognitive Assessment in patients with Amyotrophic Lateral Sclerosis (Cengiz et al., 2019). Unlike SAI, reduced LAI seems to be correlated with better cognitive ability; however, given that only one study has reported this thus far, future work should explore this relationship further.

There has also been speculation that dopamine may be involved in the modulation of SAI and LAI. Independently, dopamine has been shown to be capable of inducing changes to the human motor cortex, and the rat sensorimotor system (Hosp et al., 2011; Monte-Silva et al., 2009). These changes are dose dependent, as when dopamine levels are too high or low, maladaptive plasticity occurs; however, optimal levels of dopamine can allow for adaptive plasticity and motor learning to take place (Pearson-Fuhrhop et al., 2013). Given its ability to modulate both the sensory and motor systems, dopamine may be responsible for governing SAI and LAI. When administered in patient populations, Levodopa (L-DOPA) has been shown able to normalize SAI in AD patients (Martorana et al., 2009; Nardone et al., 2014), and PD patients (Sailer et al., 2003). Despite modulations in clinical populations, testing in healthy controls indicated no modulation of SAI through L-DOPA administration (Martorana et al., 2009). The lack of modulation in healthy controls may be due to the small sample size in that study. However, there is also a possibility that similar

to the dose dependent effects of dopamine on sensorimotor neuroplasticity, dose dependency may also be prevalent on the influence of dopamine on afferent inhibition (Pearson-Fuhrhop et al., 2013). Explorations of this relationship will further our current understanding of SAI and LAI, and in particular their connection to the basal-ganglio-thalamocortical loop.

4.3 – The Prevalence of Afferent Inhibition

In our study we found that ~97% of participants showed SAI at a minimum of one time point, whereas all participants showed LAI at a minimum of one time point. However, across the three time points, 30% of participants had at least one instance of collection where they did not show SAI, with ~26% of participants having at least one instance of not showing LAI. We recently published a retrospective analysis which similarly indicated that several individuals do not show afferent inhibition at all or fluctuate between showing inhibition and facilitation between sessions (Toepp et al., 2021). We found no biological differences between individuals in our study that do not show SAI (22.1 years; 5 females) or LAI at one time point (20.43 years; 4 females), and the larger sample demographic (21.16 years; 17 females). Given both our study and the present literature, these findings highlight important issues regarding the prevalence of afferent *inhibition* as a phenomenon.

It is apparent in the broader spectrum of the work, that inhibition may not be observed in all individuals. However, a lack of inhibition may prompt some researchers to exclude these individuals from certain analyses. In such cases, the nomenclature of afferent *inhibition*

guides researchers' decisions on data integrity processes, as inhibition is thought to be the expected outcome. However, nomenclature should not influence researchers' decisions on the acceptability of their data. Rather, decisions to remove or include data should be statistically founded, such as with outlier analyses. Given these discrepancies, perhaps the nomenclature needs to be changed to further best practice approaches. Redefinition of the phenomena from afferent *inhibition* to afferent *interaction* would do much to correct these preconceived biases regarding the presence of an inhibitory effect.

4.4 – Are SAI and LAI the Same or Different?

Much has been explored about the differences between SAI and LAI. SAI is evoked at ISIs of 18-28 ms (Ni et al., 2011; Tokimura et al., 2000), whereas LAI is evoked at ISIs of 200-1000 ms (Chen et al., 1999). While SAI is modulated by cholinergic activity, there is no such evidence to support the same for LAI (Di Lazzaro et al., 2000; Di Lazzaro, Oliviero, Saturno, et al., 2005). SAI is purported to be an accessory measure of cognition, with reduced levels of inhibition being correlated with poorer cognitive ability; however, the singular study exploring the relation between LAI and cognition fails to reach the same conclusion (Cengiz et al., 2019). Yet, despite the multiple differences between SAI and LAI, there are several similarities as well. SAI exerts an inhibitory effect through primarily reducing later I-waves (Ni et al., 2011; Sakai et al., 1997; Tokimura et al., 2000). While extensive studies have not been completed to assess I-wave reduction in LAI, Paired Associative Stimulation (PAS) at low TMS intensities (where PAS primarily influences I3 Waves) can modulate LAI, indicating that LAI may also reduce late I-waves (Meunier et

al., 2012; Turco, El-Sayes, Savoie, et al., 2018). Furthermore, both SAI and LAI are GABAergic in origin, being modulated through $GABA_A$ receptors. As well, both SAI and LAI are extensively reduced in patients with severe cases of PD, where dementia is also present (Celebi et al., 2012; Sailer et al., 2003; Yarnall et al., 2013).

In this thesis, there was no significant difference between the inhibition observed with SAI and LAI. Therefore, given both the multitude of similarities between the circuits as well as our finding of a lack of significant difference in the level of inhibition observed with SAI and LAI, the two may not be independent circuits as commonly believed. Rather, SAI and LAI may represent two components of a singular sensorimotor circuit that branch off into different brain areas, but synapse with M1 and present with similar outcomes (late I-wave reduction). Further work may be conducted for example to elicit SAI and LAI with a singular nerve stimulus, and two subsequent TMS pulses at the appropriate time differences required to elicit SAI (18-28 ms) and LAI (200-1000 ms). This could allow researchers to further explore the influence of a peripheral nerve stimulus on cortical activity and determine whether SAI and LAI are representative of two isolated circuits.

4.5 – Limitations

In this study, we only assessed the reliability of SAI and LAI at two consistent ISIs. However, as seen in previous work, the ISI between the nerve stimulus and delivery of TMS can significantly influence the reliability for the measure (Turco et al., 2019). Future work should look to explore the relative and absolute reliability of SAI and LAI with ISIs

that cover the range of frequently used ISIs. As well, SAI and LAI are significantly altered in various patient populations. SAI in particular is being considered as a diagnostic tool to identify individuals with MCI that may eventually progress to AD (Padovani et al., 2018). However, our reliability statistics are representative of healthy participants, and thus, are not applicable to patient populations. Reliability metrics such as those conducted in our study, should be established in clinical patient populations in order to determine whether the measures continue to maintain similar levels of reliability in those individuals.

There were several challenges to overcome in the planning and execution of this study, particularly in light of the Covid-19 pandemic. I was unsure of my ability to conduct this project considering Covid-19 guidelines until well into the first year of my Master's Degree. Once approval was given for the project to be run, we faced numerous difficulties with recruitment. Participants were initially hesitant in being involved with research due to the dangers of the pandemic, resulting in limited recruitment during the early months of this study. However, soon after recruitment reached an appreciable level, we faced closures due to another wave of Covid-19, led by the Omicron surge. Following our reopening, it was extremely difficult to actively recruit participants for a sex-balanced study, due to the large number of individuals who were contracting Covid-19. As such, we had to choose to forego our attempts to sex-balance the sample size, in lieu of being able to complete the study.

4.6 – Conclusion

In conclusion, the work presented in this thesis expands our current understanding of the reliability of both SAI and LAI. It was found that in within-session explorations of afferent inhibition, both measures were able to reach high levels of relative reliability and are capable of being used to assess group level changes. However, for both SAI and LAI, the ability to assess changes within an individual is limited. This is reflected by both large amounts of measurement error, and a large value of the $SDC_{Individual}$. However, SAI and LAI can be used to determine whether group level changes have occurred between time points. The outcomes gained from this thesis add to the growing base of knowledge regarding the reliability of afferent inhibition and contribute essential guidelines for the prescribed usage of these measures. Future work should aim to maintain the reliability standards outlined herein and contribute to the reliability of SAI and LAI in clinical populations.

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