INVESTIGATING THE EFFECTS OF ATTENTION ON AFFERENT INHIBITION VIA TRANSCRANIAL MAGNETIC STIMULATION

INVESTIGATING THE EFFECTS OF ATTENTION ON AFFERENT INHIBITION VIA TRANSCRANIAL MAGNETIC STIMULATION

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Lay Abstract

Attention can alter transcranial magnetic stimulation (TMS) evoked afferent inhibition. Measures of afferent inhibition are emerging as valuable tools for clinical assessments of sensorimotor function. However, the reliability of afferent inhibition remains relatively low, limiting its value in the clinic. Afferent inhibition is increased when the one's attention is focused on the peripheral nerve stimulation used to elicit afferent inhibition. However, it is unknown whether afferent inhibition, with attention directed to somatosensory input, will improve the *reliability* of these measures. This is important as it suggests that changes to the methodology used to acquire afferent inhibition can improve the reliability of this measure, thereby increasing the opportunity for translation to the clinic. The goal of this study was to assess the influence of attention on afferent inhibition circuits, short afferent inhibition (SAI) and long afferent inhibition (LAI) and determine whether attention modulation would increase the reliability of afferent inhibition.

Abstract

Evidence indicates attention can alter afferent inhibition, a Transcranial Magnetic Stimulation (TMS) evoked measure of cortical inhibition following somatosensory input. This measure is emerging as a valuable tool for clinical assessment of sensorimotor function. However, the reliability of the measure remains relatively low. Further, attention is capable of modifying the magnitude of afferent inhibition. Therefore, for afferent inhibition to become an assessment with translation within and beyond the research lab, the reliability of the measure must be improved. Controlling the focus of attention may be one method to improve the reliability of afferent inhibition. In the present study, two experiments were conducted. One to assess the biological effects of attention on SAI and LAI, and the other to address whether the reliability of SAI and LAI are altered in the presence of varying attentional demands. The magnitude of short- and long-latency afferent inhibition (SAI and LAI, respectively) was assessed under four conditions with varying attentional demands focused on the somatosensory input that mediates SAI and LAI circuits. Further, the reliability of SAI and LAI was assessed with and without directed attention to the relevant somatosensory input to explore whether attention to the tactile stimulation can improve intrasession and intersession reliability of these measures. Thirty individuals participated in four conditions; three conditions were identical in their physical parameters and varied only in the focus of directed attention (visual attend, tactile attend, non- directed attend) and one condition consisted of no external physical parameters (no stimulation). Reliability was measured by repeating conditions at three time points to assess intrasession and intersession reliability. Results indicate the magnitude of SAI and LAI were not modulated by varied attention. Reliability assessments demonstrated that the attention manipulations increased intrasession and intersession reliability of SAI and LAI compared to the no stimulation

condition. This research exposes the influence of attention, and its impact on the reliability of afferent inhibition. By quantifying these influences, this research has identified new information to inform the design of TMS research in sensorimotor integration.

Keywords: TMS, SAI, LAI, Visual, Peripheral Nerve Stimulation, Reliability

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Declaration of Academic Achievement

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

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LIST OF ABBREVATIONS

ACh: acetylcholine
AD: Alzheimer's disease
ANOVA: analysis of variance
APB: abductor pollicis brevis
Covid-19: Coronavirus-19
CV: coefficient of variation
D-wave: direct wave
DlPFC: dorsolateral prefrontal cortex
EEG: electroencephalography
EMG: electromyography
EPSP: excitatory post-synaptic potential
GABA: gamma-aminobutyric acid
HiREB: Hamilton Integrated Research Ethics Board
ICC: intraclass correlation coefficient
IPSP: inhibitory post-synaptic potential
IQ: intelligence quotient
ISI: interstimulus interval
I-wave: indirect wave
LAI: long-latency afferent inhibition
LTP: long-term potentiation
MEP: motor-evoked potential
$MEP_{conditioned}$: MEP evoked by nerve stimulation paired with TMS
$MEP_{unconditioned}$: MEP evoked by TMS alone
MCI: Mild cognitive impairment
MN: median nerve
MSO: maximum stimulator output
MT: motor threshold
MTAT: motor threshold assessment tool
M1: primary motor cortex
NDA: non-directed attend
NS: no stimulation
PD: Parkinson's disease
RMT: resting motor threshold
rTMS: repetitive transcranial magnetic stimulation
SAI: short-latency afferent inhibition
SD: standard deviation
$SDC_{individual}$: smallest detectable change at the individual level
SDC_{group}: smallest detectable change at the group level
SEMeas: standard error of measurement
SEMeas%: relative SEMeas
SEP: somatosensory-evoked potential
SES: somatosensory electrical stimulation
SICI: short-interval intracortical inhibition
ST: sensory threshold
S1: primary somatosensory cortex
S2: secondary somatosensory cortex
TA: tactile attend
TS: test stimuli
TMS: transcranial magnetic stimulation
TRN: thalamic reticular nuclues
VA: visual attend
VPN: ventral posterior nucleus
WRST: Wilcoxon-signed rank test

CHAPTER 1: GOALS OF THE THESIS AND HYPOTHESIS

Evidence indicates attention can alter afferent inhibition, a Transcranial magnetic stimulation evoked measure of cortical inhibition following somatosensory input. This measure is emerging as a valuable tool for clinical assessment of sensorimotor function. However, the reliability of the measure remains relatively low. It is known that focused attention is capable of modifying the magnitude of afferent inhibition. It is unknown whether afferent inhibition, in the presence of attention directed to a component of afferent inhibition, will exhibit improved reliability. This is an important question because it suggests that methodology can improve the reliability of this measure improving the translation of afferent inhibition in clinical applications.

The goal of this thesis is to assess the influence of attention on human neurophysiology, specifically on afferent inhibition circuits. These circuits can be subdivided into two types short-latency afferent inhibition (SAI) which occur at interstimulus intervals of ~20-25 ms and long-latency afferent inhibition (LAI) which occur at ISI of 200-1000 ms (Chen et al., 1999; Tokimura et al., 2000). The magnitude of afferent inhibition is directly related to the amplitude of the sensory afferent volley, such that greater inhibition is observed as a larger volume of sensory afferents are recruited (Bailey et al., 2016; Turco et al,. 2017). Both SAI and LAI are thought to reflect cortical rather than spinal inhibitory mechanisms (Chen et al., 1999; Tokimura et al., 2000, Asmussen et al., 2013).

The reliability of SAI and LAI will be assessed with and without directed attention to the relevant somatosensory input to explore whether a focused attention state can improve intrasession and intersession reliability of these measures. To achieve this goal, an experiment was conducted in thirty participants at three separate time points, to evaluate intersession and

intrasession reliability. At each time point participants were asked to undergo four conditions all varying in attention tasks (‘*visual attend’*, ‘*tactile attend’*, ‘*non-directed attend’* and ‘*no stimulation’*). During all conditions measures of SAI and LAI were recorded. It was hypothesized that when participants focus their attention towards the somatosensory stimuli this would elicit the most reliable measures of intrasession and intersession SAI and LAI.

CHAPTER 2: REVIEW OF LITERATURE

2.1. Neurophysiology

The origins of neurophysiology have aimed at exploring the intercommunication of neuronal circuits with the objective of undercovering the functioning of the nervous system (Terao & Ugawa et al., 2002). From Galen's proposal of fluid filled transmission secreted from the brain and spinal cord to nerves, to Golgi and Cajal's discovery of nerve cell anatomy, the field of neuroscience has continued to grow (Kandel et al.,2000). As a result, the need for physiological investigation gave rise to Galvani's discovery of electricity as a by-product of muscle and nerve cells, which led to experiments uncovering the speed of conduction of electrical activity along the axon and its effects on neighbouring axons (Kandel et al.,2000). With the understanding of animal-based models of the nervous system, researchers began to use invasive electrical stimulation to undercover the inner workings of the human nervous system (Terao & Ugawa et al., 2002). From 1874 to 1985, the use of electric stimulation was able to support the field of neurophysiology, providing the first representations of the homunculus (Terao & Ugawa et al., 2002). Although the findings of these studies are essential to the current understanding of the central and peripheral nervous system, the amount of electrical current required to demonstrate a response was excruciating to the participant (Terao & Ugawa et al., 2002). In 1985 the understanding of human neurophysiology transitioned to a more ethical way of investigation with the use of non- invasive brain stimulation in the form of Transcranial Magnetic Stimulation (Hallet et al., 2007, Terao & Ugawa et al., 2002).

2.1.1 Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a non-invasive brain stimulation technique used to investigate the neurophysiological mechanisms in the central and peripheral nervous system (Hallet et al., 2007; Terao & Ugawa et al., 2002). This non-invasive stimulation technique is based on the principle of Faraday's law of induction, which demonstrates that a changing magnetic field can cause current to flow through conductive material such as the neurons in the cortex of the brain (Hallet et al.,2007; Terao & Ugawa et al., 2002). TMS uses a brief, high current pulse which is delivered via a magnetic coil (Hallet et al., 2007). As result a magnetic field is created which induces an electrical field perpendicular to it, which in turn excites neurons resulting in a motor evoked potential (MEP) (Hallet et al., 2007; Terao & Ugawa et al., 2002).

2.1.2 Transcranial Magnetic Stimulation: Motor Cortex

For most neurophysiological investigations using TMS, the target site of stimulation is the motor cortex, specifically the M1 region (Hallet et al., 2007). For such studies a figure 8 coil, which is more focal in structure, is placed 45 degrees to the medial- sagittal plane of the subject's head in the posterior to anterior direction. This orientation is optimal for stimulation to M1, inducing an electrical field perpendicular to the central sulcus (Hallet et al., 2007). This orientation allows for the depolarization of cortical interneurons, represented as I-waves, (Di Lazzaro et al., 1998) which create a descending path of activation via descending volleys. This results in transsynaptic activation of pyramidal neurons (Di Lazzaro et al., 1998). This projection transcends to the corticospinal tract which results in a contraction of the muscle (Auvichayapat et al., 2009). The contraction that occurs in the muscle gives rise a MEP which is recorded via electromyography (EMG) (Hallet et al., 2007). The peak-to-peak amplitude of the MEP demonstrates the integrity

of corticospinal tract, specifically it reflects the amount of upper and lower motor neurons that are recruited via TMS (Terao & Ugawa et al., 2002). TMS is capable of both excitatory and inhibitory modulation of neurons within the region of stimulation (Hallet et al., 2007). However, TMS is unable to differentiate between excitatory and inhibitory neurons. Thus, when stimulation occurs the response can either cause excitation, acting as an agonist, or inhibition, acting as an antagonist (Tokimura et al.,2000).

2.1.3 Afferent Inhibition

Corticospinal excitability can be modulated via peripheral nerve stimulation prior to the delivery of TMS, resulting in either inhibition or facilitation depending on the interstimulus interval (ISI). When peripheral nerve stimulation is delivered prior to TMS a phenomenon known as afferent inhibition occurs (Tokimura et al.,2000). The latency between the peripheral nerve stimulation dictates the subtype of afferent inhibition evoked, either short latency afferent inhibition (SAI) or long latency afferent inhibition (LAI) (Chen et al., 1999; Hallet et al., 2007; Tokimura et al., 2000; Terao & Ugawa et al., 2002). It is thought that the amplitude of the sensory afferent volleys is directly related to the magnitude of inhibition (Bailey et al., 2016). Hence, it is thought that afferent inhibition reflects cortical inhibitory mechanisms (Chen et al., 1999; Tokimura et al., 2000; Asmussen et al., 2013; Turco et al., 2020).

SAI is described as peripheral nerve stimulation paired with TMS to the M1 region separated by an interstimulus interval (ISI) of approximately 18-25ms (Tokimura et al., 2000). The N20 latency of the somatosensory evoked potenital is recorded to determine the ISI for SAI, as it reflects the latency for the signal to travel to the S1 region of the brain (Turco et al., 2019). In

contrast, LAI is characteristic of an ISI ranging from 100-1000ms (Chen et al., 1999). The neural pathways of SAI and LAI are currently not clear. SAI has shown a reduction in the presence of positive allosteric modulators of gamma aminobutyric acid type A ($GABA_A$) receptors (Di Lazzaro et al., 2007). SAI is also reduced by muscarinic antagonist, scopolamine, which indicates the mediation of SAI via cholinergic transmission (Di Lazzaro et al., 2000). In addition, SAI is reduced in disorders of cognition, which have cholinergic deficits (Sakuma et al., 2007, Nardone et al., 2006). Currently, the molecular underpinning of LAI is inconclusive. LAI seems to be modulated by the $GABA_A$ circuits (Turco et al., 2018) but it is unknown if those circuits influence the cholinergic system.

Afferent inhibition is abnormally reduced in clinical populations. SAI is reduced in cognitively impaired populations such as AD, MCI and Lewy body dementia (Di Lazzaro et al., 2004; Di Lazzaro et al., 2008; Di Lorenzo et al., 2013). SAI has the potential to be used to monitor cognitive function, disease progression and recovery of function (Turco et al., 2018; Fujiki et al., 2006; Turco et al., 2021). Increased SAI has been correlated with better performance in executive functioning, verbal memory and visuospatial tasks (Suzuki & Meehan, 2018; Miradamadi et al., 2017). It is suggested that SAI could assess cortical cholinergic activity. Studies demonstrate disorders related to cholinergic dysfunction also demonstrate reduced SAI (Di Lazzaro et al., 2004; Turco et al.,2021).

In addition, reductions in SAI are observed in PD, stroke and spinal cord injury (Nardone et al., 2005; Di Lazzaro et al., 2012; Bailey et al.,2015). Similarly, LAI is also impaired with PD. However, the understanding of the neural mechanism underlying it is currently unknown, thus its mechanistic effect on human neurophysiology is currently poorly understood.

2.1.4 Attention

Attention is the ability to enhance the detection and response to a target stimulus (Gazzingia et al., 2014). It is thought that attentional networks can be categorized as one of the three types, **Alerting** speaks to how one maintains an alert state (Driver & Frackowiak, 2001; Petersen & Posner, 2012), **Orienting** is how selective mechanism operate on sensory input (Knudsen et al., 2007; Petersen & Posner, 2012), and **Executive** examines the regulation of thoughts feelings and behaviour (Petersen & Posner, 2012). It is thought that each attentional network is modulated by neurotransmitters. Alerting is suggested to be largely driven by norepinephrine (Witte & Marrocco, 1997). In contrast, executive attention is suggested to stem from the anterior cingulate and prefrontal cortex and is driven by dopamine and serotonin (Davidson& Marrocco.,1998; Robbins et al., 2004). Last, orienting is driven by acetylcholine in parietal and frontal cortices (Beane & Marrocco, 2004).

Acetylcholine is a key neurotransmitter responsible for orienting attention systems in humans (Petersen & Posner, 2012). Experimental studies, at the molecular level, have demonstrated that altering the cholinergic system via muscarinic or nicotinic antagonists have decreased attention (Mirza et al., 1999). Studies have highlighted the effects of nicotine, demonstrating an increase in attentional focusing and filtering, which are important to selective attention processes (Thienel et al., 2009, Davidson & Marrocco, 2002). Muscarinic receptors also seem to play a similar role in the selective attention processes but also executive functioning (Thienel et al., 2009; Furey et al., 2008; Klinkenberg et al., 2002; Herro et al., 2008). In a study conducted by Thienel et *al* (2009), twelve healthy volunteers were given 0.4mg of scopolamine and placed in a 1.5 T

magnetic resonance scanner (Thienel et al., 2009). During the study participants completed the Attention Network Task, which assessed the three main components of attention (Fan et al., 2002). Scopolamine ingestion resulted in increased reaction times and decreased brain activation in anterior cingulate cortex, when compared to the placebo group, suggesting muscarinic modulation of attention (Thienel et al., 2009).

The studies outlined above lay the groundwork for the bridge between attention and afferent inhibition. SAI and attention both seem to be influenced by cholinergic circuits within the brain (Di Lazzaro et al.,2000; Di Lazzaro et al.,2008; Everitt & Robbins, 1997; Davidson & Marrocco, 2000. This suggest that controlling for attention could influence the magnitude and reliability of SAI. It has previously been demonstrated that the generation of SAI has been linked to circuitry involved in learning, memory, attention, and cognition (Mirdamadi et al., 2017; Suzuki & Meehan 2018; Kotb et al., 2005). The effects of spatial attention on sensorimotor integration via SAI and LAI were explored by Kotb *et al* (2005). Nine- right handed individuals attended to a somatosensory stimulus being delivered to the digital nerve on either the right or left hand while receiving TMS (Kotb et al., 2005). During the task individuals instructed to count the number of shocks received on either the right or left hand (Kotb et al., 2005). The results demonstrated that attention to the right hand resulted in increased afferent inhibition. Although compelling, the methodology of the study measured LAI at an ISI of approximately 100ms, which is not frequently used in the literature. Future studies should address the effects of spatial attention on LAI using an ISI of 200ms (Chen et al., 1999). Mirdamadi *et al.* (2017) demonstrated that attentional load can alter SAI magnitude. Individuals participated in a high visual attention task and a low visual attention task (Mirdamadi et al., 2017). During the high visual attention task,

individuals were asked to report the number of upright yellow and inverted green crosses. In contrast the low visual attention task asked participants to count the number of red crosses regardless of orientation (Mirdamadi et al., 2017). The study found SAI was reduced during the high visual attention task compared to the low visual attention task. However, the sample size of this study was rather small (n=12) and should be conducted with a larger sample to determine if this finding can be replicated but also if this attention manipulation can affect the reliability of SAI and LAI.

The literature demonstrates the effects of attention on afferent inhibition, suggesting that attention could modify the magnitude of afferent inhibition. Attention focused on the somatosensory input is capable of eliciting a deeper magnitude of afferent inhibition (Kobt et. al.,2009). However, it is unknown whether afferent inhibition, with attention directed to somatosensory input, will improve the reliability of these measures. This is important as it suggests that methodology can improve the reliability of this measure thereby increasing the opportunity for translation to the clinic.

2.2. Reliability

Afferent inhibition has the ability to be used to assess sensorimotor integration in humans (Turco et al., 2021). However, SAI and LAI display low to moderate levels of reliability which limit its clinical utility. Until the reliability of SAI and LAI measures improve, it cannot be used as a clinical assessment of sensorimotor integration. Reliability, an indicator of the consistency of a measure, is essential to the validity of the assessment (Beaulieu et al., 2017). If the measurement of interest is not reliable, it provides inaccurate and inconsistent data overtime. This creates

difficulty when testing hypotheses and comparing data across groups or studies. Researchers have explored the reliability of afferent inhibition and concluded that SAI and LAI demonstrated poor to moderate reliability (Turco et al., 2019). The relative reliability of afferent inhibition measures across studies also seem to vary substantially, which demonstrates an inability to be used as a reliable measure of sensorimotor integration in clinical populations (Toepp et al., 2021). Until more reliable acquisition of SAI and LAI can be determined, this measure cannot be used with certainty. Hence, the need for reliability statistics when conceptualizing new devices, techniques and treatments are essential to understand, consistency and accuracy of the measure and researchers (Turco et al., 2019).

2.2.1 Classical Test Theory

The foundation of reliability assessments lies within *Classical Test Theory*, which states every individual has a 'true' score that would be obtained if there were no errors present in the measurement (Nunnally & Berinstein, 1994). This theory relies on the true score, observed score and error score (Šerbetar, 2015). The observed score can be defined as the sum of the true score and the error score. The errors can be attributed due to random and systematic error (Bruton et al., 2000).

Observed score= True score + Error score

Observed score= True score + Random Error + Systematic Error

Systematic error is a unidirectional error which is consistent and occurring in one direction (Bruton et al., 2000). In contrast, a random error is unpredictable and inconsistent in nature

(Bruton et al., 2000). To assess reliability of a variable or measurement both absolute and relative reliability need to be assessed.

2.2.2 Commonly used Statistics

One commonly used assessments of test- retest reliability is Pearson correlation coefficient. Pearson's R is used to describe the degree to which two measures are related. A high correlation coefficient indicates that all scores are consistent. Although informative, it does not allow for an indication of consistency in the data over time to be explored. This indicates that Pearson's R unable to account for systemic error and therefore is not useful for assessments of reliability (Bruton et al., 2000). Another common method of assessment is a Paired Sample's T-Tests, which conducts a comparison of means. This test is capable of detecting systematic error that occurs between session one and session two (Beaulieu et al., 2017). While this is important to reliability assessments it cannot detect random fluctuations in the data (Beaulieu et al., 2017). These tests are important for statistical evaluations, however, they are not suitable assessments to evaluate reliability. Currently researchers are shifting towards the use of more accurate testing to determine absolute and relative reliability (Bruton et al., 2000).

2.2.3 Absolute Reliability

Absolute reliability is an independent assessment aimed at investigating how a metric can change over time in a stable individual (Bruton et al., 2000; Weir, 2005). It allows for the amount of measurement error to be evaluated (Bruton et al., 2000). To assess the absolute reliability standard errors of measurement (SEM_{eas}) and smallest detectable changes (SDC) within the group and individuals are calculated (Bruton et al., 2000).

The standard error of measurement allows researchers to uncover how measures in a stable individual changes over time, which quantifies within-subject variability (Weir, 2005). There are two methods in which the SEM_{eas} can be calculated. The first relies upon $SEM_{eas} = Standard\ Deviation\sqrt{1 - Intraclass\ corelational\ coefficent}$. The incorporation of the intraclass correlational coefficient (ICC) introduces between subject variability, which should not be in methods of assessing absolute reliability. In contrast, the second method to calculate the SEM_{eas} is more appropriate, $SEM_{eas} = \sqrt{MeanSquared_{error}}$. This method allows for the calculation to be independent of the ICC and allows for greater consistency. The use of the mean squared error, which indicates the total error variance, allows for the variance around the mean to be considered. When comparing SEM_{eas} values across studies the SEM_{eas} must be converted to the $\%SEM_{eas}$, $\%SEM_{eas} = \frac{SEM_{eas}}{mean} * 100\%$. The $\%SEM_{eas}$ displays the absolute error as a percentage, $\%SEM_{eas}$ > 10% are considered to reflect high measurement error (Schambra et al., 2015; Turco et al., 2019).

The smallest detectable change is indicative of the minimum amount of change that is real and not due to measurement error (Turco et al., 2019; Weir, 2005). $SDC_{individual.}$ represents the minimum amount of change within an individual to be considered real. It relies upon the SEM_{eas} multiplied by 1.96, which is reflective of the 95% confidence interval, and the square root of two to reflect the change at two time points, $SDC = SEMeas$ x 1.96 × $\sqrt{2}$ (Weir, 2005). In addition, the SDC_{group} will also be calculated to demonstrate the minimum amount of change in the group mean that is considered to be a real change (Weir, 2005). To calculate the SDC_{group}, the $SDC_{individual}$ will be divided by the square root of the sample size, $SDC_{group} = \frac{SDC_{indiv}}{\sqrt{n}}$

(Schambra et al.,2015). This indicates that as sample size increases, the level of change required to see a real change will decrease.

2.2.4 Relative Reliability

Relative reliability refers to the ability of a metric to distinguish individuals from each other (Schambra et al., 2015; Weir, 2005). Methods to assess relative reliability includes the intraclass correlational coefficients and coefficients of variation. ICC's are reflective of the ability of a test or measure to accurately differentiate between individuals (Weir, 2005). The ICC can be represented by the following equation, $\mathrm{ICC} = \frac{between\ subject\ variability}{between\ subject\ varibility+error}$ (Weir, 2005). This indicates that the ICC is dependent on the sample, meaning a sample with a large amount of between subject variability will indicate high relative reliability of the measure, even if there is a large amount of error. This indicates that the ICC is context specific, and the heterogeneity of the sample collected is imperative to the calculation (Weir, 2005).

Popular models of ICC are the one-way ICC models and two-way ICC models. The one-way model breaks down the variability due to the time and error, whereas the two-way model allows for the integrity of time and error to be assessed separately (Weir, 2005). The ICC model selected is dependent upon the study design of the experiment (Weir, 2005). The (1,k) model can be used when the subject is being measured by a different rater and each rater is selected at random (Weir, 2005). In contrast, the (2,k) model is selected when the subject is measured by the same rater, who is generalizable to other raters (Weir, 2005). For the assessments occurring in TMS studies, typically 2-way random effects (2,k) model is used as TMS scores are reported as an average of many data points (Turco et al., 2019).

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

This calculation of the ICC incorporates the subjects mean square ($MS_{s)}$, error mean square (MS_E), the trials mean square (MS_T), the number of trials (k) and the sample size (n) (Weir 2005). This is an essential assessment for reliability but is unfortunately not comparable between studies. Therefore, the coefficient of variation, CV = Standard Deviation / mean *100%, which is an indication of the sample heterogeneity, is reported as well (Bruton et al., 2000; Turco et al., 2019).

The usage of reliability statistics is imperative to the evaluation of measures and metrics, such as paired pulse TMS measures. Unfortunately, the use of these statistics are not widespread in the field of neurophysiology. The literature has demonstrated moderate intersession reliability for measures of afferent inhibition (Turco et al., 2019) hence future studies should determine if the reliability can be improved by manipulating the experimental parameters, such as directing attention.

CHAPTER 3: EXPERIMENT – EFFECTS OF ATTENTION ON AFFERENT INHITION AND RELIABITY OF AFFERENT INHIBITION

3.1 Introduction

The literature highlights that the relationship between afferent inhbition and human behaviour is relatively unclear (Turco et al., 2021). Research has shown that afferent inhibition could potentially be modulated with attention (Kobt et al., 2009; Suzuki & Meehan, 2018). These findings suggest that attention could potentially influence the process of sensorimotor integration creating more reliable SAI and LAI. How might attention modulate SAI? The literature suggest that attention will cause changes in cortex activation, which could cause an enhancement in the afferent volley going to the cortex. Studies demonstrate that attention to specific features of a sensory stimulus leads to activation in the cortical region responsible for processing that stimulus feature (Nelson et al.,2004; Corbetta et al.,1991). Based on the literature, it is possible that attention directed towards sensory mechanisms involved in afferent inhibition could lead to greater inhibition.

The purpose of the present study is to investigate the effects of attention on SAI and LAI. With the growing use of afferent inhibition as a marker of sensorimotor integration the need to improve its reliability is essential. With the knowledge that attention influences the magnitude of SAI, it is proposed that focusing attention on the somatosensory input could elicit more reliable measures of SAI. By directing attention in a specific way, it could create a homogenous state amongst participants, potentially enhancing the reliability and reducing the variability of these measures. To address the biological mechanisms of attention, *we hypothesize that attention focused to the somatosensory stimuli will elicit deeper SAI.* The results from this study will aid

the design of future TMS studies and the usage of SAI and LAI in basic and clinical neuroscience settings.

As shown above, attention appears to be an important factor influencing the magnitude of inhibition assessed. This may indicate that between- and within-subject variability in afferent inhibition is partially driven by wandering and/or the focus of attention. *The exploratory hypothesis will examine whether or not the reliability of SAI and LAI can be improved if the focus of attention during the acquisition of data is controlled.* If controlling the focus of attention improves inter-session reliability of SAI and/or LAI, this would suggest that focus of attention should be incorporated into future TMS study designs.

The experiment discussed in this thesis will consist of two parts. Part A " Biological Effects" will examine the effects of attention of SAI and LAI. Part B " Statistical Effects of Attention" will explore whether or not a focused state of attention towards the somtatosensory stimuli will elicit improved intrasession and intersession reliability.

3.2 Methods

3.2.1 Participants

Thirty right-handed, healthy participants (15 females; age =21.50 ± 3.00 years), were recruited. Participants attended two separate sessions (Figure 2), both scheduled in the afternoon to control for the effects of diurnal cortisol levels (Milani et al., 2010) which may influence TMS measures. Sessions were separated by 1-3 days after the initial testing session to allow for flexible scheduling for participants (Figure 2). Participants passed a screening for TMS contraindications

and were identified as right-handed using a handedness questionnaire which required them to indicate which hand they use for several common manual tasks. This study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and conformed to the declaration of Helsinki.

3.2.2 Peripheral Nerve Stimulation

Median nerve stimulation was delivered at motor threshold. To estimate motor threshold, electrical stimulation begun at a sub-threshold intensity, and the intensity (mA) was increased until a slight twitch is visible in the APB muscle. When motor threshold was determined, this intensity was used to evoke SAI and LAI.

3.2.3 Electromyography:

Surface electrodes (9 mm Ag-Cl) were used to record activity from the abductor polliculus brevis (ABP) muscle of the right hand. In order to reduce signal noise, a dry ground was placed on the wrist. EMG signals were magnified x1000 and bandpass filtered between 20 Hz-2.5 kHz (Intronix Technologies Corporation Model 2024F, Bolton, Canada). An analog-digital converter was used to digitize data at 5 kHz (Power1401; Cambridge Electronics Design, Cambridge, UK), prior to being analyzed through commercial software (Signal v7.01; Cambridge Electronics Design, Cambridge, UK). The hotspot of the right APB muscle is defined as the location on the left motor cortex that, when stimulated with TMS, consistently led to the largest MEP in the muscle. This point was found and registered using Brainsight Neuronavigation and Transcranial magnetic stimulation (Rogue Research, Montreal, Canada).

3.2.4 Electroencephalography

Electroencephalography (EEG) electrodes were used to acquire somatosensory-evoked potentials (SEPs) from the primary somatosensory cortex (S1). Electrodes were placed at C3' (2 cm posterior to C3) with signals referenced to Fpz (international 10-20 system). A ground electrode was placed on the right clavicle. A bar electrode with the anode positioned distally was used to stimulate the median nerve at the wrist. 500 stimuli (200 µs square wave pulses; 3 Hz) were delivered at the minimum intensity needed to observe a visual contraction in the right APB muscle using a constant current stimulator (Digitimer DS7AH, Hertfordshire, UK). Resultant signals were averaged over the 500 epochs to identify the latency of the N20 component of the SEP. The N20 latency+ 4 ms was used for the measurement of SAI (Di Lazzaro et al., 2005; Tokimura et al., 2000; Turco et al., 2019).

3.2.5 Transcranial Magnetic Stimulation

TMS was performed using a Magstim 200^2 stimulator (Magstim, Whitland, UK). A 50 mm figure-of-eight branding coil was positioned over the left M1 at the optimal location to evoke MEPs from the right APB muscle. The coil was oriented at a 45-degree angle to the sagittal plane to induce a posterior-to-anterior current. This was registered using Brainsight Neuronavigation and TMS (Rogue Research, Montreal, Canada).

3.2.6 Resting Motor Threshold

Resting motor threshold (RMT) was defined as the stimulus intensity (%MSO) that evokes and MEP (i.e. peak-to-peak amplitude >50 µV) 50% of the time. This value was determined using TMS_MTAT_2.0 freeware (http://clinicalresearcher.org/software.htm). The stimulus intensity

was set to 37 %MSO, and twenty TMS pulses were distributed over M1, specifically the APB hotspot, with the stimulus intensity being adjusted after each subsequent pulse as advised by the MTAT software based on the presence or absence of an MEP on the previous trial.

3.2.7 Afferent inhibition

Afferent inhibition was acquired by delivering peripheral nerve stimulation paired with TMS. The intensity of TMS was adjusted to evoke a ~1 mV peak-to-peak amplitude MEP.

The intensity of median nerve stimulation was set to motor threshold, which reflects the intensity at which maximum afferent inhibition is observed (Bailey et al., 2016). For SAI, the ISI was set to the latency of the N20 + 4ms (Di Lazzaro et al., 2005; Tokimura et al., 2000; Turco et al., 2019) whereas for LAI the ISI was approximately 200ms (Chen et al., 1999). SAI and LAI were delivered randomly within each section approximately 6-8 seconds apart therefore ensuring that participants could not predict the onset of afferent inhibition. Within each condition, 20 SAI conditioned stimulus (CS)/test stimulus (TS), 20 LAI CS/TS and 20 TS were delivered. During all conditions SAI and LAI were collected. The conditions are described in Figure 1.

3.2.8 Experimental parameters

The experiment consisted of four conditions: *Visual Attend (VA), Tactile Attend (TA), Non-Directed Attend (NDA), No Stimulation (NS).* Figure 1 outlines the parameters of each condition.

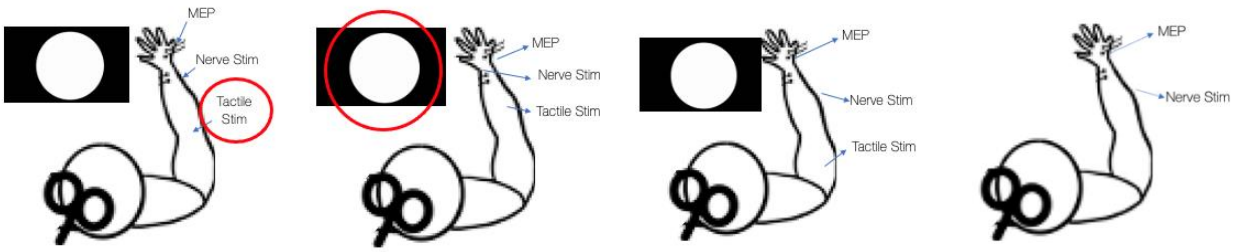


Figure 1 Experimental Parameters. Participants were seated in front of a visual stimulus while receiving nerve stimulation and TMS. A) Tactile Attend B) Visual Attend C) Non-directed Attend D) No Stimulation

Visual stimulation used in VA, TA and NDA Conditions

The visual stimulus for this experiment consisted of a white circle appearing on the black screen coded in MATLAB software (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). Throughout the condition the intensity of brightness fluctuated, and participants were probed to respond with a button press (Adapted/Modified from Hsiao, Lane, and Fitzgerald 2002). The number of intensity fluctuations for each condition was set to twenty changes and each participants onset of change was randomized for each condition.

Tactile stimulation used in VA, TA and NDA Conditions

Individuals received several electrical stimuli to the median nerve, proximal to the median nerve stimulus required for TMS. The intensity of the tactile stimulation was set to sensory threshold, which reflects the intensity required for the individual to detect the stimuli. The nerve stimulus varied as a single pulse, double pulse, or triple pulse (Adapted/Modified from Kotb et al., 2005). Each pulse was approximately 200 microseconds long. Depending on the condition, the participant was asked to respond with a button press when a triple-pulsed electrical stimuli is

received. For *Visual Attend (VA), Tactile Attend (TA) Non- Directed Attend (NDA)* twenty triple pulse nerve stimuli were delivered.

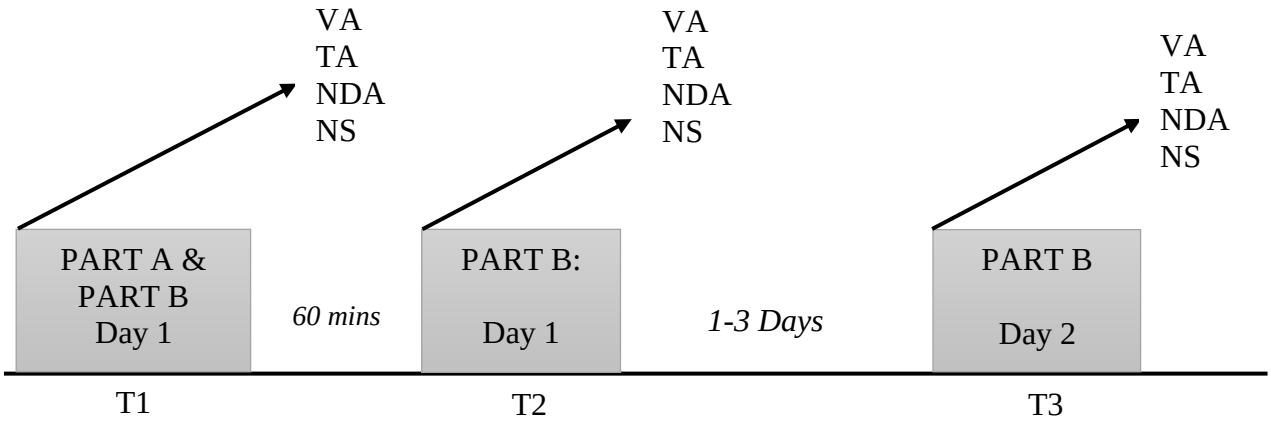


Figure 2: **Timeline for Experiment A and B**. Participants were presented with four conditions. All conditions were completed in a succession. Conditions were presented in three blocks (T1, T2, T3). The order of conditions (VA, TA, NDA, NS) were pseudorandomized across participants and preserved within participants at T1, T2 and T3.
VA; visual attend, TA: tactile attend, NDA: non-directed attend, NS: no stimulation

3.3 Data Analyses Generalized to Part A and Part B

Peak-to- peak EMG trials with activity exceeding 100 μV in a 100 ms window proceeding the TMS artefact were removed (Schambra et al., 2015; Turco et al 2019). The magnitude of afferent inhibition present was expressed as a ratio of the conditioned MEP amplitude to the unconditioned MEP amplitude.

$$SAI\ or\ LAI = \frac{MEP_{CONDITIONED}}{MEP_{UNCONDITIONED}} * 100\%$$

3.3.1 Part A. Biological effects of attention

To address the effects of attention and its influence on measures of afferent inhibition the following statistical test were completed. A one-way ANOVA within- subject design with a factor of condition was completed using data points collected at T1. Furthermore, the normality

of the residuals was determined through the Shapiro-Wilk test. If data was considered non-parametric, a Friedman's analysis of variance was conducted with a corresponding Wilcoxon Signed Rank post-hoc analysis. Post-Hoc tests were conducted using Tukey's Honest Significant Difference (HSD), which examine all relevant pairwise comparisons between groups. Significance was set to $\alpha < 0.05$. The accuracy data acquired in the *VA* and *TA* conditions was analysed using a Wilcoxon Signed Rank test, which is a non- parametric scale allowing the assessment of accuracy.

3.3.2 Part B. Statistical Effects of Attention

Normality was assessed using Shapiro-Wilks tests, and heteroscedasticity was assessed using Bland-Altman plots. Violations of normality, normally require a transformation on the dataset, but due to the reliability analysis that must take place, transformations were not implemented as it would cause a change in the ratio scale (Liu & Au-Yeung, 2014; Ngomo et al., 2012; Sankarasubramanian et al., 2015). Bland-Altman plots were created comparing the respective variables at T1-T2, and T1-T3. Outliers were identified and removed using Grubb's Test. Multiple paired t-test were used to compare each condition for both intrasession and intersession time points to discover if systematic error was present. Significance was set to alpha = 0.05. Relative reliability was quantified by averages of MEP amplitudes for all subsequent trials. ICCs were calculated for SAI and LAI using all data points within the condition. The data was recorded by one experimenter and hence ICC (2,k) model was implemented. To supplement the ICC calculation CVs were also calculated for SAI and LAI for all conditions (Appendix 5.3.1 & 5.3.2). ICCs were evaluated using recommended guidelines 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 determined for each condition using the SEM_{eas} values, which were then converted to represent $\%SEM_{eas}$ values. This assessment of absolute reliability will indicate the measurement error that is present within the data. %SEMeas < 10% was used as a cut off to indicate low measurement error (Schambra et al., 2015). Furthermore, the SEM_{eas} was then used to determine the $SDC_{individual}$ and SDC_{group}, which indicates the minimum amount of change needed to be observed at the individual and group level to be considered a real change and not a change due to measurement error.

3.4 Results

All participants underwent the experimental manipulation with no adverse effects. Table 1 displays the group-averaged dependent measures across all three time points of acquisition. These data reveal no significant differences in nerve stimulation delivered at motor threshold ($\chi^2(2) = 3.045$, $p=0.218$), tactile stimulation delivered at sensory threshold ($\chi^2(2) = 1.19$, $p=0.551$), SAI N20+4 latency (Wilcoxon signed-ranks; $Z=-0.378$, $p=0.705$), RMT (Wilcoxon signed-ranks, $Z=-0.332$, $p=0.74$) and 1mV ($\chi^2(2) = 2.53$, $p=0.282$).

	T1(n=30)	**T2(n=30)**	**T3(n=30)**
Nerve Stimulation Motor Threshold (mAmp)	5.98 ± 1.51	6.24 ±1.84	6.23 ±1.84
Tactile Stimulation Sensory Threshold (mAmp)	0.42 ±0.13	0.43 ±0.14	0.43 ±0.15
SAI N20+4 latency (ms)	21.9 ±0.92	-	21.9 ±0.87
TMS Resting Motor Threshold (%MSO)	46.57 ±6.16	-	46.37 ±6.50
1mV as a %RMT	125.73± 11.25	128.02 ±12.64	125.34 ± 10.46

Table 1: Group averaged measures (with standard deviations). SAI acquired with an ISI of N20+4ms, evoked by median nerve stimulation.

%RMT: resting motor threshold, SAI: short latency afferent inhibition, %MSO= maximum stimulator output

Grubbs test was performed and allowed for the removal of two outliers: a SAI data point at T2 and a LAI data point from T3. The biological question utilized a sample of n=30 to demonstrate the effects of attention on afferent inhibition. The statistical question was addressed with an n=30, expect for the *VA* SAI T1-T2 and *TA* - LAI at T1- T3 which utilized a sample size of n=29 for the following intersession analysis.

3.4.1 Part A. Biological Effects of Attention

The data from T1 was used to address the effects of attention on afferent inhibition. The repeated measures ANOVA with factors Condition (*VA, TA, NDA, NS*) and State (TS vs CSTS) confirmed a main effect of State ($F_{(1,29)}$=38.326, p<0.001, η_p^2 = 0.970) such that CSTS is significantly supressed relative to TS indicating that significant inhibition was observed in the group. All individuals displayed inhibition in at least one condition (see Appendix A.1, A.2, A.3). Figure 3A plots the group-averaged mean with whiskers spanning 1.5 X interquartile range of SAI across all conditions. One-way ANOVA revealed no effect of condition ($F_{(3,87)}$ =1.082, p=0.361, η_p^2 = 0.036). At the individual level 43% of participants demonstrated deeper inhibition during TA versus VA as hypothesized while 57% revealed the opposite response or no notable difference between these conditions.

For LAI, the data from T1 was used to assess the effects of attention on afferent inhibition. A repeated measures ANOVA with factors of Condition (*VA, TA, NDA, NS*), and State (TS vs CSTS) indicated a main effect of State ($F_{(1,29)}$=102.90, p<0.001, η_p^2 = 0.780) with no other significant main or interaction effect. This concludes that the peak-to-peak MEPs between states

is significant and CSTS is supressed relative to TS. All individuals displayed inhibition in at least one condition (see Appendix A.4, A.5, A.6). Normality of the LAI data was violated, and a Friedman's two-way analysis of variance was implemented to examine the effects of attention on LAI. The results shown in Figure 3B demonstrate non-significant difference, $\chi^2(3) = 1.080$, $p=0.782$, indicating no statistical difference in LAI across conditions. Upon individual analysis, 26% of participants demonstrated deeper inhibition during *TA* versus *VA* as hypothesized while 74% revealed the opposite response or no notable difference between these conditions.

Figure 3C plots the performance data for *VA* and *TA* indicates that individuals displayed higher % correct on the *TA* (Wilcoxon signed-ranks; mean rank=15.84) when compared to *VA* (Wilcoxon signed-ranks; mean rank=5.50), $Z=-4.681$, $p<0.001$). Further, to examine relationships between performance and depth of SAI and LAI, Spearman correlations were conducted for SAI (refer to appendix A.5.2.1) and LAI (refer to appendix A.5.2.2) to demonstrate whether performance was correlated to levels of inhibition for SAI and LAI. Accuracy of *VA* was not significantly correlated with SAI ($r_s(28)= -0.087, p>0.05$) or LAI ($r_s(28)= 0.096, p>0.05$). The same analysis was repeated for *TA* for SAI ($r_s(28)= 0.106, p>0.05$) and LAI ($r_s(28)= 0.239, p>0.05$) and similarly there was no significant correlation.

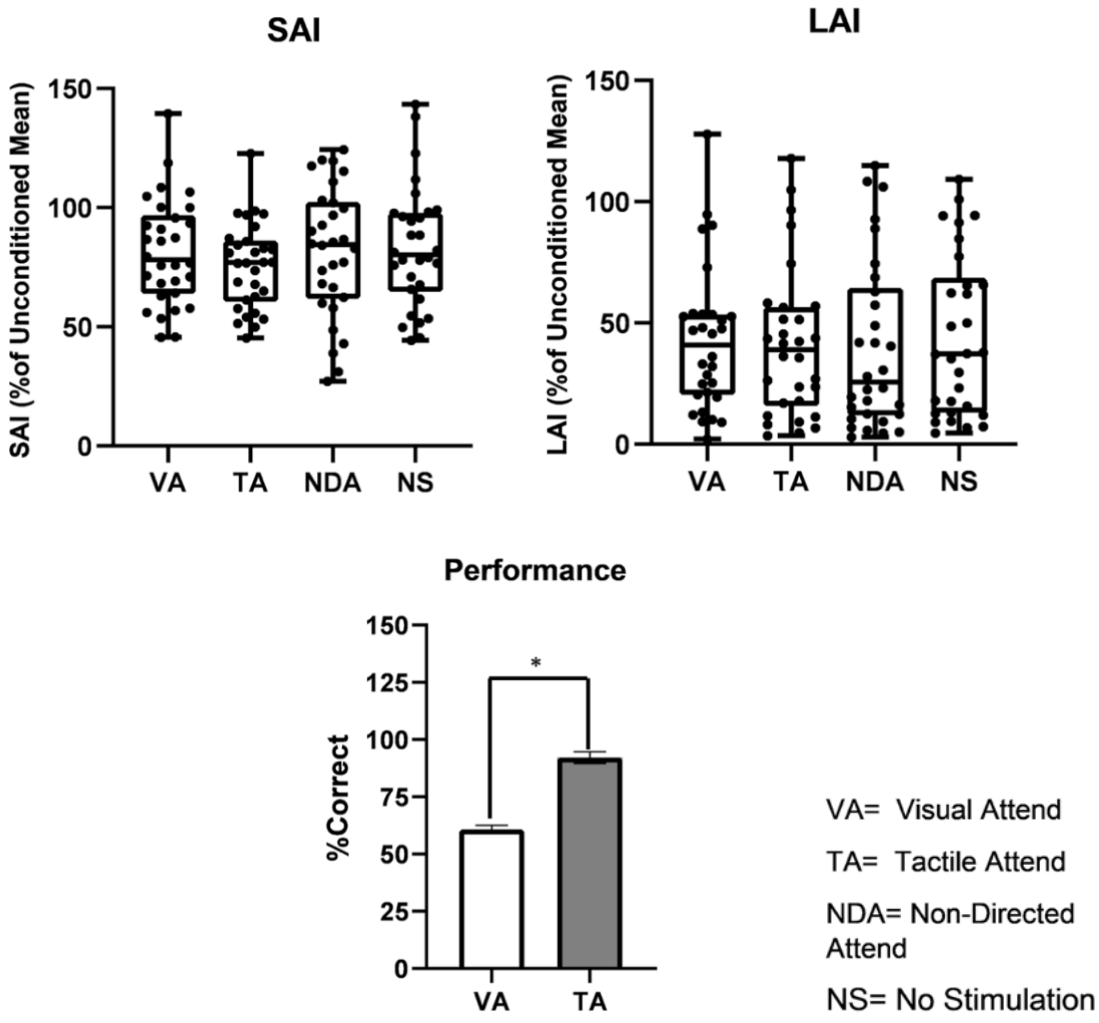


Fig3: Biological effects of Attention A. Average SAI, expressed as a % of the unconditioned mean at. T1. Shown is the mean with whiskers spanning 1.5X the interquartile range. B. Average LAI, expressed as a % of the unconditioned mean. Shown is the mean with whiskers spanning 1.5X the interquartile range C. Performance data plotting % Correct for *VA* and *TA* at T1, alongside standard error of measurement bars. *indicates significant difference between performance on *VA* compared to *TA.*
LAI: long latency afferent inhibition, NDA: non-directed attend, NS: no stimulation, SAI: short latency afferent inhibition, TA: tactile attend, VA: visual attend

3.4.2 Part B. Statistical Effects of Attention

The SAI dataset was normally distributed across all time points and conditions, in contrast the LAI dataset was normally distributed for all LAI datapoints except at T3. Paired t-tests were completed for each condition at its corresponding timepoint, which indicated that no systematic error was present within comparisons of conditions at T1 vs T2 and T1 vs T3 for SAI and LAI ($p>0.05$). Homoscedasticity, determined via Bland Altman plots, was preserved for SAI at T1-T2 and T1-T3, however for LAI it was upheld at T1-T3 but was heteroscedastic for T1-T2 specifically for the *NS* condition, with an $R^2>0.1$. Due to the heteroscedastic nature of LAI at T1-T2, usually a correction via log transformation can be implemented on the dataset, however given that the transformations lead to a change to a ratio scale the transformation was not performed and hence the data was analyzed with an assumption of heteroscedacity as done in previous studies (Liu & Au-Yeung, 2014; Ngomo et al., 2012; Sankarasubramanian et al., 2015).

SAI - Intrasession Analysis

As shown in Table 2, SAI demonstrates poor reliability for the *NS* condition (ICC= 0.10) compared to the moderate reliability obtained in all attention manipulation conditions (*VA*, *TA* and *NDA*). For all conditions, the % SEM_{eas} demonstrate large amounts of measurement error (%SEM >10%) (Table 2). The $SDC_{individual}$ indicates that a minimum change of 52, 45, 59 and 70 is needed to be considered physiological change at the individual level for the *VA*, *TA*, *NDA* and *NS*, respectively (Table 2). In summary, each condition requires a large physiological change to occur to be considered a real change at the level of an individual. Furthermore, in order to see a real physiological change at the group level a sample size of 24 is needed for *VA* (Fig 4A), 19 is needed for *TA* (Fig 4B), 32 is needed for *NDA* (Fig 4C) and 45 is needed for *NS* (Fig 4D).

SAI - Intersession Analysis

As shown in Table 3, SAI demonstrates poor reliability for the *NS* condition (ICC= 0.25) compared to the moderate reliability obtained in all attention manipulation conditions (*VA, TA* and *NDA*). For all conditions, the % SEM_{eas} demonstrates large amounts of measurement error (%SEM_{eas} >10%) (refer to Table 3). The $SDC_{individual}$ indicates that a minimum change of 65, 47, 61 and 64 is needed to be considered physiological change at an individual level for the *VA, TA, NDA* and *NS*, respectively (Table 3). This indicates each condition requires a large physiological change to occur to be considered a real change at the level of the individual. Furthermore, to see a real physiological change at the group level a sample size of 39 is needed for *VA* (Fig 5A), 21 is needed for *TA* (Fig 5B), 34 is needed for *NDA* (Fig 5C) and 37 is needed for *NS* (Fig 5D).

Intrasession SAI	ICC (95% CI)	SEM_{eas}	SEM_{eas}%	SDC_{group}	$SDC_{Individual}$
VA	0.45 (-0.161 to 0.741)	18.89%	24.01%	9.72%	52.36%
TA	0.56 (0.06 to 0.791)	16.27%	21.55%	8.24%	45.12%
NDA	0.44 (-0.186 to 0.732)	21.40%	26.97%	10.83%	59.31%
NS	0.101 (-0.88 to 0.57)	25.31%	31.62%	12.81%	70.15%

Table 2: Intrasession Reliability Statistics SAI. SEM_{eas}, $SDC_{individual,}$ SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, %SEM_{eas} is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed attend, NS: no stimulation, SDC: smallest detectable change, SEM_{eas}: standard error of measurement, %SEM_{eas}: relative $SEM_{eas.}$ TA: tactile attend, VA: visual attend.

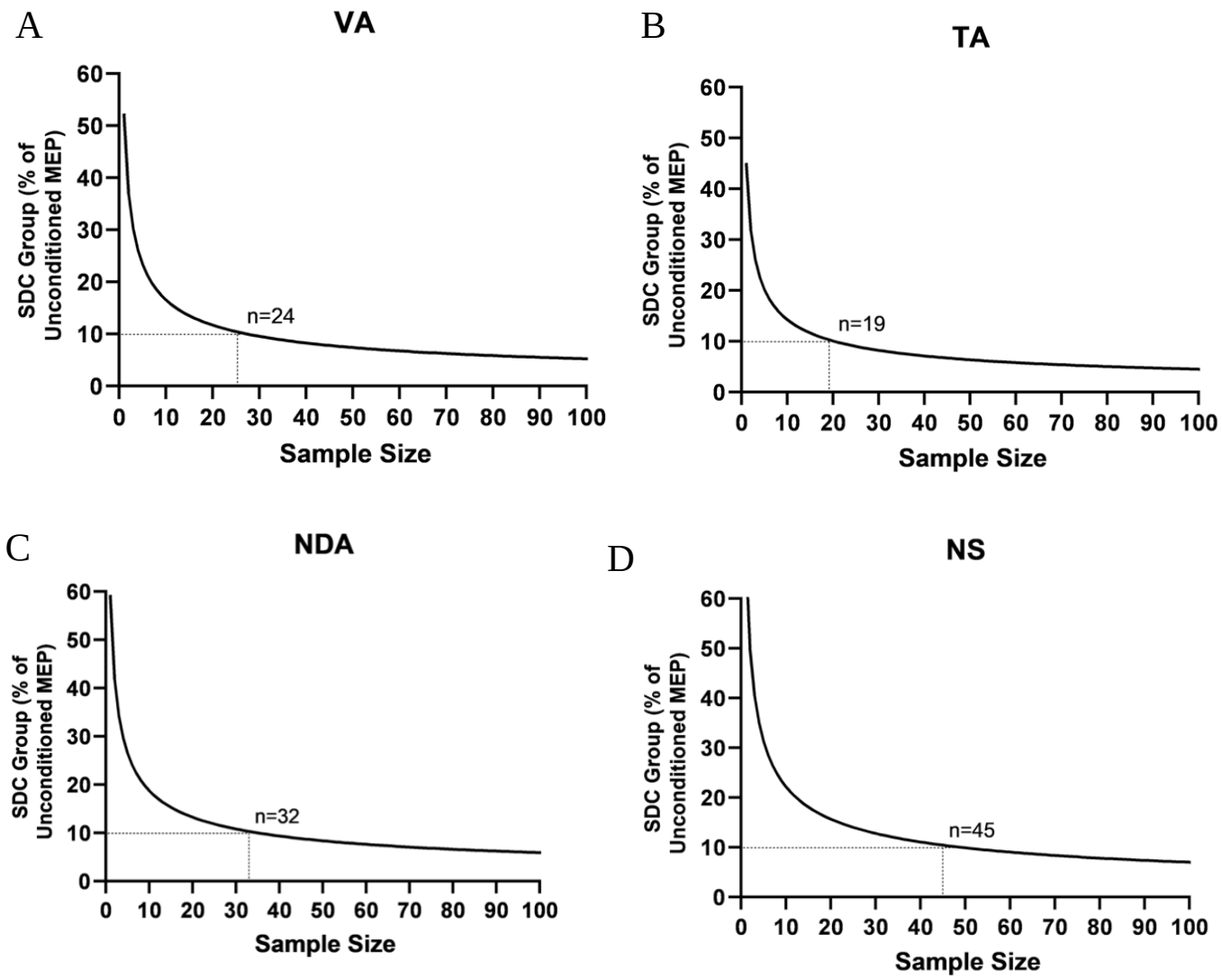


Fig4. SDC_{group} for Intrasession SAI. SDC_{Group} presented are as a function of the sample size (n) for intrasession SAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).
SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

Intersession SAI	ICC (95% CI)	SEM_{eas}	SEM_{eas}%	SDC_{group}	$SDC_{indivdual}$
VA	0.42 (-0.22 to 0.72)	23.57%	27.94%	11.93%	65.34%
TA	0.41(-0.27 to 0.72)	16.98%	22.34%	8.59%	47.06%
NDA	0.54 (0.01 to 0.78)	22.18%	26.86%	11.22%	61.48%
NS	0.25 (-0.53 to 0.64)	23.06%	29.19%	11.67%	63.92%

Table 3: Intersession Reliability Statistics SAI SEM_{eas}**,** $SDC_{individual,}$ SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.

CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed attend, NS: no stimulation, SDC: smallest detectable change, SEM_{eas}*: standard error of measurement,* $\%SEM_{eas}$*: relative* $SEM_{eas.}$ *TA: tactile attend, VA: visual attend.*

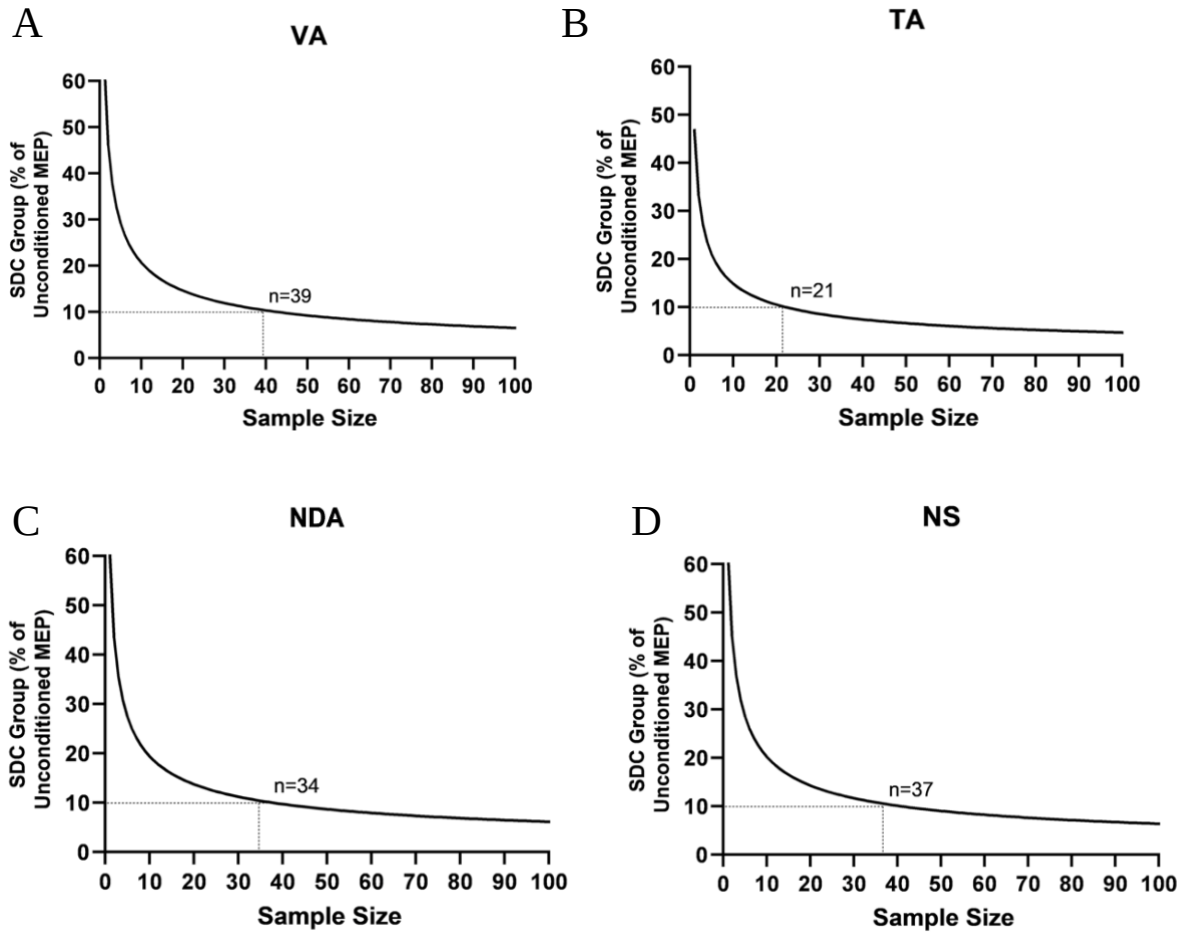


Fig5. $\mathbf{SDC_{group}}$ **for Intersession SAI**. SDC_{Group} presented are as a function of the sample size (n) for intrasession SAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).

SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

Intrasession analysis of LAI

As shown in Table 4, LAI demonstrates high relative reliability (0.75 <ICC< 0.9) for all attention manipulation conditions (*VA, TA, NDA*) and moderate reliability (0.5<ICC<0.75) for the *NS* condition. For all conditions, the % SEM_{eas} demonstrates large amounts of measurement error (%SEM_{eas} >10%) (Table 4). The $SDC_{individual}$ indicates that a minimum change of 41, 50, 47 and 59 is needed to be considered physiological change at an individual level for the *VA, TA, NDA* and *NS*, respectively (Table 4). In summary, each condition requires a large physiological change to occur to be considered a real change at the level of an individual. In order to see a real physiological change at the group level a sample size of 16 is needed for *VA* (Fig 6A), 23 is needed for *TA* (Fig 6B), 21 is needed for *NDA* (Fig 6C) and 32 is needed for *NS* (Fig 6D).

Intersession Analysis of LAI

As shown in Table 5, LAI demonstrates high relative reliability (0.75 <ICC< 0.9) for all conditions (*VA, TA, NDA*) and moderate reliability (0.5<ICC<0.75) for the *NS* condition. For all conditions, the %SEM_{eas} demonstrate large amounts of measurement error (%SEM_{eas} >10%) (Table 5). The $SDC_{individual}$ indicates that a minimum change of 55, 48, 48 and 54 is needed to be considered physiological change at an individual level for the *VA, TA, NDA* and *NS*, respectively (Table 5). In conclusion, each condition requires a large physiological change to occur to be considered a real change at the level of an individual. Furthermore, in order to see a real physiological change at the group level a sample size of 29 is needed for *VA* (Fig 7A), 21 is needed for *TA* (Fig 7B), 21 is needed for *NDA* (Fig 7C) and 28 is needed for *NS* (Fig 7D).

Intrasession LAI	ICC (95% CI)	SEM_{eas}	SEM_{eas} %	SDC_{group}	$SDC_{individual}$
VA	0.86 (0.71 to 0.93)	14.97%	32.97%	7.58%	41.52%
TA	0.82 (0.63 to 0.92)	18.15%	41.09%	9.19%	50.32%
NDA	0.85 (0.69 to 0.93)	17.15%	40.42%	8.68%	47.54%
NS	0.75 (0.47 to 0.89)	21.51%	47.13%	10.88%	59.64%

Table 4: Intrasession Reliability Statistics LAI SEM_{eas}, $SDC_{individual}$, SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed Attend, NS: no Stimulation, SDC: smallest detectable change, SEM_{eas}: standard error of measurement, $\%SEM_{eas}$: relative $SEM_{eas.}$ TA: tactile attend, VA: visual attend.

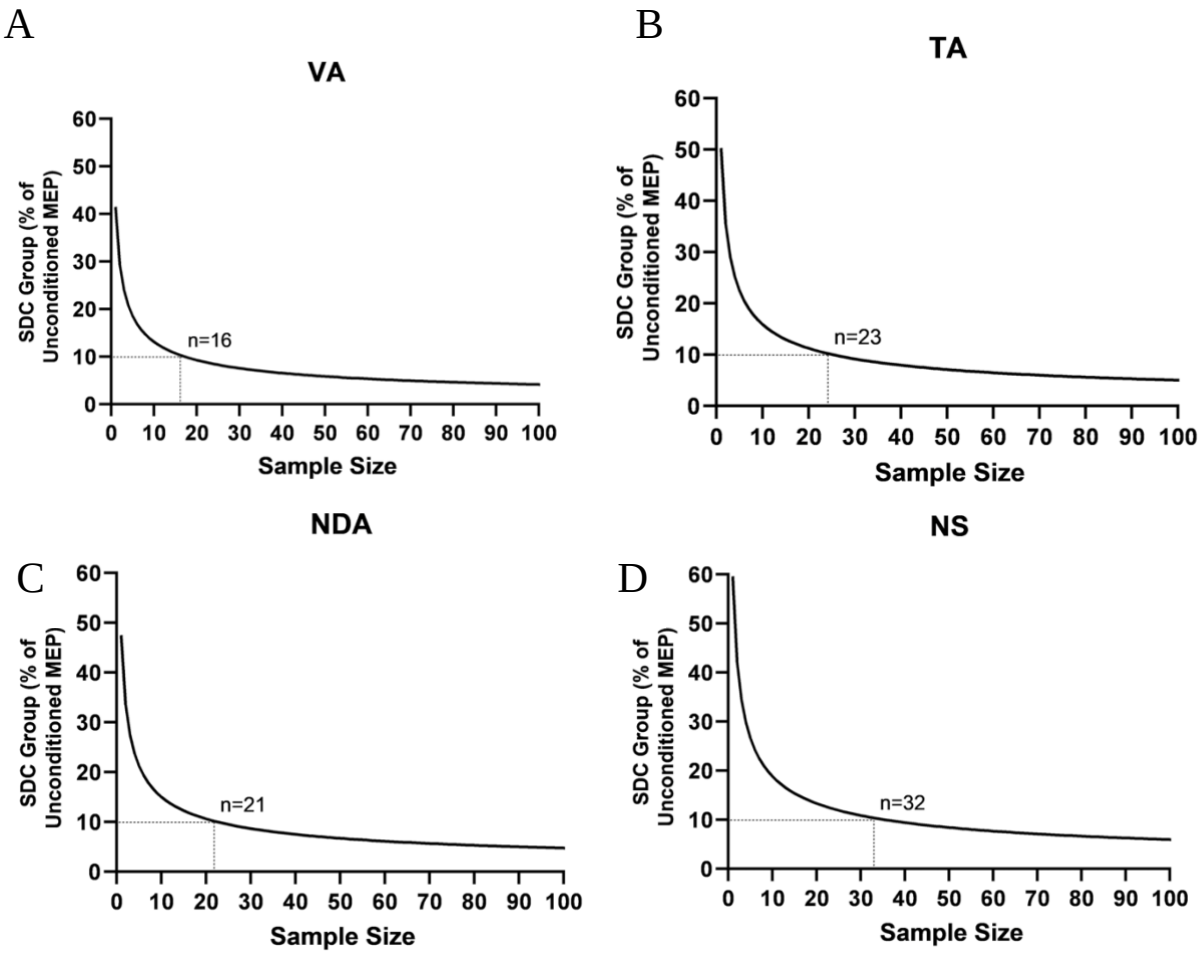


Fig6. SDC_{group} for Intrasession LAI. SDC_{Group} presented are as a function of the sample size (n) for intrasession LAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).

SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

Intersession LAI	ICC (95% CI)	SEMeas	SEMeas%	SDC_{group}	$SDC_{individual}$
VA	0.76 (0.49 to 0.88)	20.00%	44.02%	10.12%	55.45%
TA	0.80 (0.56 to 0.90)	17.44%	43.64%	8.98%	48.34%
NDA	0.86 (0.70 to 0.93)	17.49%	41.55%	8.85%	48.48%
NS	0.73 (0.43 to 0.87)	19.68%	47.00%	9.96%	54.55%

Table 5: Intersession Reliability Statistics LAI. SEM_{eas}, $SDC_{individual,}$ SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed Attend, NS: no Stimulation, SDC: smallest detectable change, SEM_{eas}: standard error of measurement, $\%SEM_{eas}$: relative $SEM_{eas.}$ TA: tactile attend, VA: visual attend.

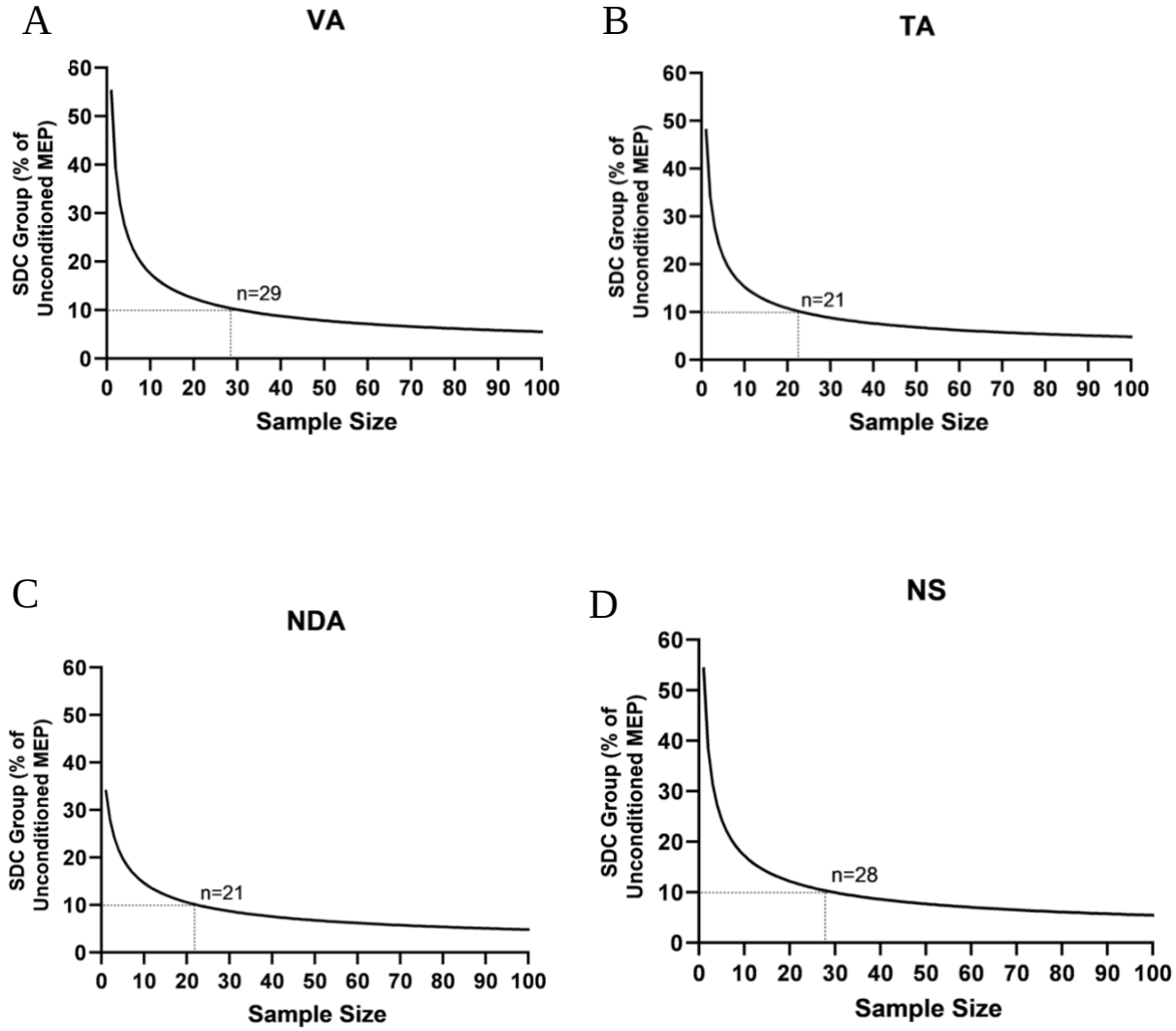


Fig 7. SDC_{group} for Intersession LAI. SDC_{Group} presented are as a function of the sample size (n) for intrasession SAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).
SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

3.5 Discussion

The goals of the present study were to examine the effect of attention on both the magnitude and reliability of afferent inhibition. SAI and LAI were elicited through the delivery of peripheral nerve stimulation to the right MN followed by TMS to the left M1. While inducing afferent

inhibition the participant performed various conditions, each with a different attention manipulation (*VA, TA, NDA, NS*). Attention did not modulate the depth of afferent inhibition, seen by the magnitude of both SAI and LAI being consistent across the four conditions. The intersession and intrasession relative and absolute reliabilities were assessed through measurements of ICC, the $\%SEM_{eas}$ and the SDC_{Group} and $SDC_{Individual}$ Overall, LAI had higher levels of relative reliability compared to SAI across all four conditions. However, while attention did not modulate the depth of afferent inhibition, it seems to influence the reliability of the measure. For both SAI and LAI, relative reliability was higher for conditions where attention was manipulated in comparison to no stimulation.

3.5.1 Biological effects of Attention

This study collected SAI and LAI under various attention manipulations to determine whether the focus of attention influences the depth of inhibition. The data indicate that attention does not have a significant effect on depth of inhibition for SAI and LAI. However, previous research has demonstrated deeper SAI and LAI when attention was directed towards the stimulated versus non-stimulated hand (Kotb et al., 2009). One explanation for the discrepancy between the present study and previous work may be the visual environment. In the present work, all attention manipulations were performed in the presence of identical visual stimulation–the computer monitor with the changing contrast– regardless of whether attention was directed to the visual stimulation. In Kotb *et al.*'s (2009) experiment, no visual stimulation existed within the experiment design; therefore, attentional resources were focused solely on the sensory stimulation, rather than being allocated towards visual features in the external environment as

well. Therefore, visual stimulation might be distracting attention from the tactile stimulation, irrespective of the intended focus of attention, leading to no observable change in SAI or LAI.

One mechanism to explain the idea that visual stimulation competes with SAI and LAI pathways comes from the work of Suzuki and Meehan (2018). Their work evaluated the effect of working memory demands on sensory motor function. Participants were presented with a low working memory load (two digits) or a high working memory load (six digits), followed by a two second delay. Participants then had to indicate whether a presented number was a part of the previous set. SAI was reduced during the high working memory load condition compared to the low load condition, suggesting that working memory modulates the SAI pathway (Suzuki and Meehan, 2018). The reduction in SAI may be due to the suppression of sensory afferent input, which is irrelevant to the numeric working memory task. Another study conducted by Miradamadi *et al* (2017) measured SAI during the performance of high versus low visual attention demand tasks (Miradamadi et al., 2017). Participants were provided with an array of crosses which varied in both colour and orientation. In the low demand condition participants were asked to count the number of red crosses regardless of orientation (Miradamadi et al., 2017). In the high demand task participants were asked to identify the number of upright yellow or inverted green crosses (Miradamadi et al., 2017). SAI was reduced during periods of high visual attention demands in comparison to periods of low visual attention demands (Miradamadi et al., 2017). In addition, studies demonstrate that attention can suppress the N20-P25 somatosensory evoked potential (Meehan et al., 2009), which is the same thalamocortical afferent projection that modulates SAI (Baliey et al., 2016). In the present study, the presence of visual stimulation combined with tactile stimulation showed no difference in SAI or LAI magnitude across conditions, regardless

of the direction of attention. Future work should consider sensory deprivation to selectively modulate specific sensory modalities such as visual, tactile, or auditory. This will explore whether or not individual modalities exert different effects on SAI and LAI. In addition, the exploration of low and high load demands should also be explored within each modality to determine whether or not a specific load combined with a specific sensory type can create deeper inhibition of SAI and LAI.

3.5.2 Effects of Attention on Reliability

Relative reliability aims to quantify the ability of a measure to consistently identify individuals or groups from one another with repeated testing (Bruton et al., 2000; Schambra et al., 2015). The present study found that for both inter- and intra-session assessments, SAI had moderate reliability for all attention manipulation conditions and poor reliability during the no stimulation condition. Similarly, LAI demonstrated high relative reliability for all attention manipulation conditions, and moderate reliability during the no stimulation condition for the intrasession and intersession analyses.

The findings demonstrate that attention manipulations led to the most reliable measures of SAI and LAI. All attention manipulation conditions were identical in the presence of external stimuli–tactile and visual–and hence it is inconclusive whether the observed effect is due to attention or the presence of external stimuli. The *NS* condition had poor reliability of SAI, with the only difference between *NS* and the attention manipulation conditions (*VA, TA, NDA*) being the lack of external stimuli present during the condition. The reliability of SAI during all attention manipulation conditions was moderate, suggesting that directed attention towards a

particular stimulus (*TA* vs *VA*) does not act to improve reliability beyond the *NDA* condition. These data suggest it is the mere presence of the external stimuli which increases the reliability of afferent inhibition.

An unexpected finding was that *NDA* displayed different levels of reliability compared to *NS.* I anticipated that these two conditions would show no difference in SAI and LAI, as individuals were not asked to direct their attention in a particular way in the *NDA* condition. Yet the difference in reliability seen between the two conditions suggests that it is not the focus of attention that may be driving the changes observed, but simply the presence of external stimulation. One neural mechanism that could possibly explain this finding is the contribution of cholinergic, muscarinic and nicotinic receptors. During *VA, TA* and *NDA* the individual is experiencing stimuli associated with attention demands. The presence of the visual and tactile stimuli across the three conditions may be causing a constant state of receptor activation within the cholinergic system. The upregulation of cholinergic receptors in these three conditions and the downregulation during the *NS* condition, could be a possible explanation for the difference in SAI reliability, thus supporting the known finding that SAI is cholinergic in nature (Di Lazzaro et al., 2000).

Furthermore, the poor reliability of the no stimulation condition within this experiment contradicts previous work demonstrating moderate reliability for SAI (Turco et al., 2019). It is possible that the introduction of attention manipulations effects the perception of the no stimulation condition. A possible explanation for this discrepancy could lie in arousal. Decreases in arousal and motivation have been correlated with reductions in SAI (Koizume et

al., 2017). It is possible that when individuals have no cue suggesting an attention task, such as in the *NS* condition, motivation and arousal states decrease, which may contribute to the reduced SAI reliability during the *NS* condition in this experiment.

The reliability of LAI was not heavily influenced by attention manipulations. The results of the reliability assessments for the intersession measures were similar to that of previous studies (Turco et al., 2019). The molecular underpinnings and circuity of LAI have yet to be explored. While much of the mechanism underlying LAI is currently unknown, studies have demonstrated that $GABA_A$ agonists lead to significant reductions in LAI (Turco et al., 2018). However, unlike SAI, no work to date has attempted to establish a connection between LAI and the cholinergic system. In this study, the relationship between attention and LAI is not apparent. Given that attention is governed by the cholinergic system (Petersen & Posner, 2012), this effect may indicate that LAI is not cholinergic in nature, supporting pharmacological studies which have suggested this (Teo et al.,2009).

3.6 Limitations

The present study was limited by sample demographics. The participants in this study consist of only undergraduate and graduate students. Hence, the findings are not easily generalizable to an older demographic (Bhandari et al., 2016; Degardin et al., 2011; Young-Bernier et al., 2012). Future work should aim to replicate this study in older individuals. In addition, during the *NDA* and *NS* condition, it was not possible to determine exactly where the individual's attention was directed to. A solution to this limitation would be implementing eye tracker technology or resting state EEG during conditions to determine cortical activity with specific attention to the alpha and

beta waves, as they are representative of a relaxed state and alertness (da Silva, 1991). In addition, the impact of directed attention vs the presence of environmental stimulation could not be differentiated. Hence it is difficult to conclude if attention or the environmental stimulation is responsible for the differences in reliability observed in SAI and LAI.

3.7 Conclusion

This study investigated the effects of attention on SAI and LAI. The goal of the experiment was subdivided into two objectives; the first was to assess the biological effects of attention and second aimed to uncover the effects of attention on reliability. The biological component uncovered that attention had no significant effect on the magnitude of SAI and LAI. The second objective of this study revealed that attention can modulate the intrasession and intersession reliability of SAI and LAI. The results indicated that, for both SAI and LAI, the attention manipulations conditions (*VA, TA, NDA*) all led to higher levels of relative reliability when compared to the *NS* condition at both the intra and intersession levels. Future studies should aim at uncovering the impact of external stimulation on the magnitude of SAI and LAI.

CHAPTER 4: GENERAL DICUSSION

This thesis has made significant contributions to the field of sensorimotor neural control, in the area of somatosensory afferent inhibition. Afferent inhibition is speculated to reflect sensorimotor integration and is reduced in clinical populations such as AD, PD, MCI (Nardone et al 2006, 2012; Di Lorenzo et al 2013; Terranova et al., 2013; Sakuma et al., 2007; Tsutsumi et al., 2012). Afferent inhibition is influenced by attention which may alter the reliability of this measure. Low or moderate reliability of this measure can indicate whether it has utility as a clinical diagnostic tool, index of rehabilitation or aid in the pursuit of novel human neuroscience.

In this thesis, I explored the impact of attention on the magnitude and reliability of SAI and LAI. I found that attention manipulations did not alter the depth of SAI or LAI. Further, I assessed whether attention can increase intrasession and intersession reliability of SAI or LAI. The findings indicated moderate reliability of SAI during the attention manipulation conditions, and poor reliability during the no stimulation condition. Similarly, LAI demonstrated high relative reliability for attention manipulation conditions and moderate reliability for the no stimulation condition.

Interestingly, ‘non-direct attend’ demonstrated greater reliability in comparison to the ‘no stimulation’ condition for both SAI and LAI. This suggests the mere presence of external stimuli may be responsible for modulating reliability, rather than the focus of attention. This thesis provides key information regarding the design of future afferent inhibition studies, suggesting that the experimental environment could impact the reliability of SAI or LAI within and between

sessions. The following section will discuss the biological basis of afferent inhibition in humans, the neural mechanisms by which attention may influence afferent inhibition and the factors that may contribute to the reliability of afferent inhibition.

4.1 Biological Basis of Afferent Inhibition

Afferent inhibition has been used as a tool to test sensorimotor integration (Turco et al., 2021). SAI and LAI are a result of the sensory afferent volley inhibiting the motor response in a target muscle when peripheral nerve stimulation precedes TMS to M1 (Turco et al., 2018). The magnetic pulse delivered to M1, trans synaptically depolarizes corticospinal neurons which synapse on upper motor neurons to elicit MEPs. Shown Figure 8 is a series of excitatory neurons that contribute I-waves to the descending volley. Also shown is the effect of acetylcholine (ACh) which is suggested to reduces the amplitude of glutamatergic EPSPs originating in layer III and layer VI (Di Lazzaro et al., 2000; Metherate &Ashe, 1995). Another plausible mechanism by which acetylcholine acts is the muscarinic stimulation on ion channels in pyramidal cells. A muscarinic antagonist might increase inward Na^+ current, potentially allowing for rapid depolarization to occur (Mittman & Alzheimer, 1998). Di Lazzaro *et al.* (2000) demonstrated that scopolamine, which acts as a muscarinic antagonist, reduces SAI (Di Lazzaro et al., 2000). Further, acetylcholinesterase inhibitors, which stop acetylcholine breakdown in the synaptic cleft, increased the depth of SAI (Fujiki et al., 2006; Di Lazzaro et al.,2004). Further, populations characteristic of cholinergic dysfunction such as AD (Nardone et al 2006; Di Lorenzo et al 2013; Terranova et al., 2013), MCI (Sakuma et al., 2007; Tsutsumi et al., 2012) have reduced SAI.

Evidence suggest that GABA could modulate SAI as well. The binding of GABA to $GABA_A$ receptors enhance chloride channel mediated hyperpolarization of the cell membrane (Di Lazzaro et al.,2005). Similarly, when GABA binds to $GABA_B$ receptors, inhibitory signals occur via G Proteins and second messengers (Terunuma, 2018). Previous research has indicated that SAI is indeed contributed by GABA neurotransmission acting at $GABA_A$ receptors (Di Lazzaro et al., 2005; Turco et al., 2018). Last, the combination of both cholinergic and GABAergic transmission is thought to interact to modulate SAI. Nicotine is also a known modulator of GABA release, causing a reduction in GABA release to pyramidal neurons. Therefore, it is possible that GABA controls the release of acetylcholine which is responsible for the cholinergic pathways involved in SAI (Figure 8) (Yamakazi et al., 2005). Studies have explored the effects of nicotine intake on intracortical excitability in healthy smokers and non-smokers (Grundey et al., 2013). Results indicate that following the ingestion of nicotine, in non – smokers, increased SAI and SICI compared to baseline. This suggests that cholinergic pathways may modulate SAI depth.

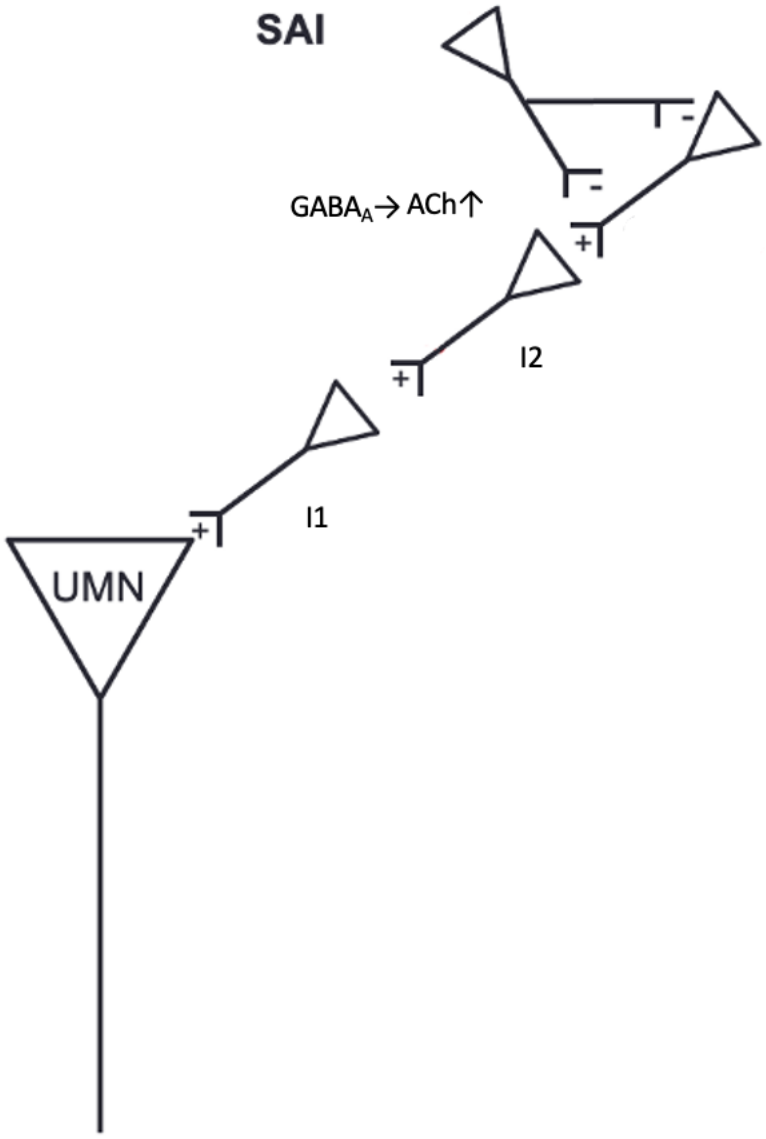


Figure 8: Demonstrates the cortical pathway of SAI. The activation of interneurons will descend and synapse upon the upper motor neurons. SAI is dependent upon acetylcholine and $GABA_A$ receptors. It is found that acetylcholine antagonist will lead to the reduction in SAI, in contrast the upregulation of $GABA_A$ receptors will lead to an increase in the depth of SAI. It is though that $GABA_A$ may modulate acetylcholine release.
UMN: upper motor neuron, ACh: acetylcholine

4.2 Pathways in which Attention may modulate Afferent Inhibition

Attention was hypothesized to increases measures of afferent inhibition via increased processing of the afferent volley at any of the synapses on the ascending axis, including the dorsal column nuclei, thalamus and the primary somatosensory cortex. It is possible that orienting attention would enhance sensory processing in this pathway, thereby yielding a stronger sensory signal. The greater sensory afferent projection would act on inhibitory interneurons in the motor cortex enhancing afferent inhibition (Nelson et al., 2004; Noppeney et al.,1999).

4.2.1 What brain areas can mediate changes in afferent inhibition?

The prefrontal cortex, which contributes to the phenomena of sensory gating, may modulate afferent inhibition. Sensory gating is the ability to filter irrelevant from relevant sensory information (Wiseman et al.,2020). Enhanced sensory gating has been linked with reduced distractibility and faster reaction times in the continuous performance of sustained attention tasks (Jones et al., 2016; Karper et al., 1996). Studies demonstrate that when attention is directed towards a relevant somatosensory stimulus, enhanced sensory gating of neural somatosensory responses, related to 'bottom up' stimulus processing, is observed (Hari and Forss, 1999). Wiseman *et al* (2020) examined the relationship between directed attention and somatosensory gating. Participants were instructed to direct their attention either towards or away from a somato-visual -paired pulse oddball paradigm (Wiseman et al., 2020). The results of the study demonstrated attention to the somatosensory stimuli increased sensory gating. The alpha coherence between the prefrontal cortex and somatosensory cortices was higher when attention was directed towards the somatosensory stimulation (Wiseman et al., 2020). This suggests that the prefrontal cortex is fundamental to somatosensory processing (Staines et al., 2002). In

addition, studies also demonstrate the effects of sensory gating on S1 activation. In this study individuals received vibrotactile stimulation to either the right hand or both hands and were asked to detect frequency changes that occurred in the right hand (Staines et al.,2002). When stimulation was delivered to both hands and the individual was instructed to focus on the frequency delivered to the right-hand, S1 activation increased. This was correlated with the recruitment of the right prefrontal cortex, suggesting that S1 is modulated by task relevancy and that this modulation stems from the prefrontal cortex (Staines et al., 2002). In contrast, damage to prefrontal cortex of the brain is correlated with deficits in sensory gating and sustained attention (Knight et al., 1999). Individuals with lesions to the dorsolateral prefrontal cortex were asked to experience a task irrelevant auditory and somatosensory stimulation paradigm. Results showed disinhibition of both the primary auditory and primary somatosensory evoked responses in individuals with prefrontal damage (Knight et al., 1999). This supports the role of the prefrontal cortex in inhibitory control. These studies suggest that sensory gating, an important phenomenon in attentional filtering, could modulate afferent inhibition. It is possible that attention to the somatosensory stimulation, relevant to afferent inhibition, could be modulated by projections from the prefrontal cortex to S1.

Another plausible mechanism, by which attention could modulate afferent inhibition involves the thalamic reticular nucleus (TRN). This nucleus can be subdivided and related to different sensory modalities (Visual, Somatosensory, Auditory) (Guillery et al.,1998). The TRN functions as a gateway between the thalamus and the cerebral cortex via thalamic relay cells. The fibres which transverse the TRN allow for excitatory, glutamatergic synapses onto the cells of the TRN which in turn send inhibitory GABAergic fibres back to the thalamus (Jones, 1985, Guillery et

al.,1998). Projections from the thalamus to the cortex and vice versa must transverse the TRN, which controls the firing of thalamocortical relay cells, which fire either tonically or in bursts. "Tonic" firing allows thalamic relay from ascending pathways to the cortex, whereas "burst" firing prevents the relay of information to the cortex. TRN cells have the ability to respond to two sensory modalities, which propose that during attention, reticular cells may selectively excite thalamic regions through inhibitory/disinhibitory mechanisms (Pinault 2004). In addition, each sensory modality has more than one related thalamic nuclei connected to the TRN. 'First Order' circuits are driven by ascending afferents from various anatomical areas (legs, arm, face) to the TRN whereas "Higher Order" projections, driven by descending afferents from the cortex, are established with the S2 region (Guillery et al., 1998). In addition to the cortical glutamatergic afferents and thalamic GABAergic inputs, the TRN also receives inputs from cholinergic areas (Hallenger et al.,1987). Due to the location of the TRN between the cortex and the thalamus it controls thalamocortical circuits activities, through inhibitory or disinhibitory mechanisms (Weese et al., 1999; Guillery et al., 1998; Crick 1984). Studies highlight that damage to the TRN leads to behavioural neglect. Particularly, rats with TRN lesions demonstrate impaired attentional orienting (Weese et al., 1999). This suggests that the TRN could be a possible mechanism driving attention and thus could modulate SAI and LAI pathways.

4.2.2 Potential factors responsible for the present outcome

Although the PFC and TRN mechanisms can alter SAI and LAI these substrates may not have contributed to the results of the study. Conditions including attention modulation had increased reliability, however the fact that *NDA* also had similar reliability to *'tactile attend'* and *'visual attend'* indicates rather than attention, arousal mechanisms may be influencing the reliability.

Within this study the reliability of conditions which contained the presence of external stimuli elicited the greatest levels of reliability. A potential mechanism to explain this finding could be explained by arousal, described as a state of alertness (Gazzinga et al., 2014). The presence of the external stimuli (*VA, TA* and *NDA*) could have increased levels of arousal potentially leading to the differences in reliability. Evidence to suggest the link between arousal and afferent inhibition can be seen with the usage of benzodiazepines (Turco et al.,2018). In this study, the effects of lorazepam ($GABA_A$ agonist) and baclofen ($GABA_B$ agonist) were explored, with lorazepam reducing SAI and LAI, but baclofen leading to no changes (Turco et al.,2018). Although lorazepam reduced SAI and LAI, it also increased sedation compared to baclofen, which demonstrated no change. Therefore, perhaps a reduction in arousal could explain the effects of lorazepam and not the drug-action pathway.

4.2.3 Factors that may influence the reliability and amplitude of afferent inhibition

Sources of inter- subject variability play an important role in reliability of afferent inhibition. Biological sex may be particularly important (Figure 9), with research having demonstrated that healthy young males have elevated levels of GABA in the dorsolateral prefrontal cortex (O'Gorman et al.,2011) while healthy young females have increased GABA within the sensorimotor cortex (Grachev et al.,2000). Given the relevance of GABA to afferent inhibition, there may be a sex-based difference in SAI and LAI.

The influence of diet on afferent inhibition is also an unknown factor (Figure 9). In previous studies the implementation of diets has demonstrated effects on the central and peripheral

nervous system. A TMS study examining the effects of the ketogenic diet found that while adhering to the diet individuals demonstrated deeper SICI, which is reflective of $GABA_A$ receptor functioning (Cantello et al., 2007). Human trials studying the effects of various ketogenic compounds such as medium chain triglycerides and the ketogenic compound AC-1202, found that subjects with Alzheimer's disease on the ketogenic diet exhibited improvements in cognitive function (Henderson et al., 2009; Ota et al., 2019). SAI, which is reduced in disorders of cognition such as AD, could hence potentially be increased. With this knowledge, it is possible that the ketogenic diet or supplement could also modulate SAI and LAI, as the underpinning of SAI is thought to be partly contributed by GABA.

4.3 Future Directions

4.3.1 Considerations for the use of Reliability Statistics

Reliability assessments rely heavily upon the interpretation of the ICC. ICCs are generally 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). Although scales such as this one provide guidelines to interpretation, they also create very rigid categories for interpreting an ICC value. For example, and ICC value of 0.76, would reflect high relative reliability, whereas a 0.74 would be interpreted as moderate reliability. Hence, the use of categories to identify ICC values should be used with caution.

4.3.2 Considerations for Biological underpinnings of Afferent inhibition: Arousal

Sleep is also an important factor when considering brain excitability (Figure 9) (Kreuzer et al.,2011). The influence of sleep deprivation has been found to decrease measures of inhibition, such as SICI and CSP, when compared to a normal night of sleep (Scalise et al., 2006; Kreuzer et al., 2011; Placidi et al., 2013). Another study demonstrated that, following sleep deprivation, measures of CSP did not change but the depth of intracortical inhibition and facilitation were reduced (Civardi et al 2001), which are GABA modulated. Sleep disturbances such as REM sleep behaviour disorder (RBD) have also demonstrated decreases in SAI compared to controls (Nardone et al., 2012). This reduction in SAI is thought to reflect cholinergic dysfunction causing RBD, demonstrating the impact of sleep related disorders on levels of afferent inhibition. With the notion that sleep can alter measures of inhibition, the effect of sleep deprivation in healthy controls needs to be explored for SAI and LAI to truly understand its impact on the variability of the phenomena.

In addition, states of arousal need to be explored to determine whether or not they can influence the magnitude and reliability of afferent inhibition. Future studies should look at the effects *donepezil, galantamine* or *mematine*, which are all alertness enhancers (Mehlman,2004). Measures pre- and post-intake should be quantified to determine whether or not arousal can influence the depth of inhibition. In addition, studies evaluating the effects of acetylcholine on afferent inhibition should be explored. The neural mechanisms of both attention and afferent inhibition suggest cholinergic underpinnings, hence the introduction of an acetylcholine agonist could expose the neural mechanism underlying both of these systems.

4.4 Limitations

Afferent inhibition can be elicited at a variety of ISIs. In the present study SAI was only determined using an ISI of N20+4ms, while LAI was evoked using 200ms. Research indicates that SAI can be evoked at 18- 28ms (Tomikura et al., 2000) and LAI at 100-1000ms (Chen et al.,1999), a future study should investigate the effect of various latencies on SAI and LAI reliability in the context of attention manipulations. In addition, future studies should be adequately powered investigate sex-based differences to determine whether males and females display different magnitudes of afferent inhibition.

4.5 Conclusion

This study examined the effects of attention on SAI and LAI. The experiment assessed the biological effects of attention and effect of attention on reliability. The biological component uncovered that attention had no significant effect on the magnitude of SAI and LAI, while the statistical component uncovered the effects of attention on intrasession and intersession reliability of SAI and LAI. The results concluded the attention manipulations conditions (*VA, TA, NDA*) all demonstrated higher levels of relative reliability when compared to the *NS* condition for SAI and LAI. Future studies should aim at uncovering the impact of different states of arousal on the magnitude of SAI and LAI.

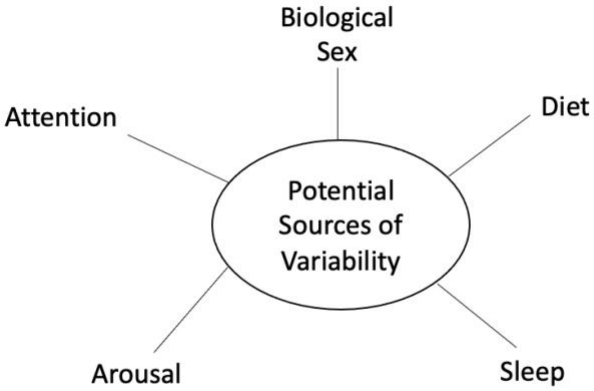
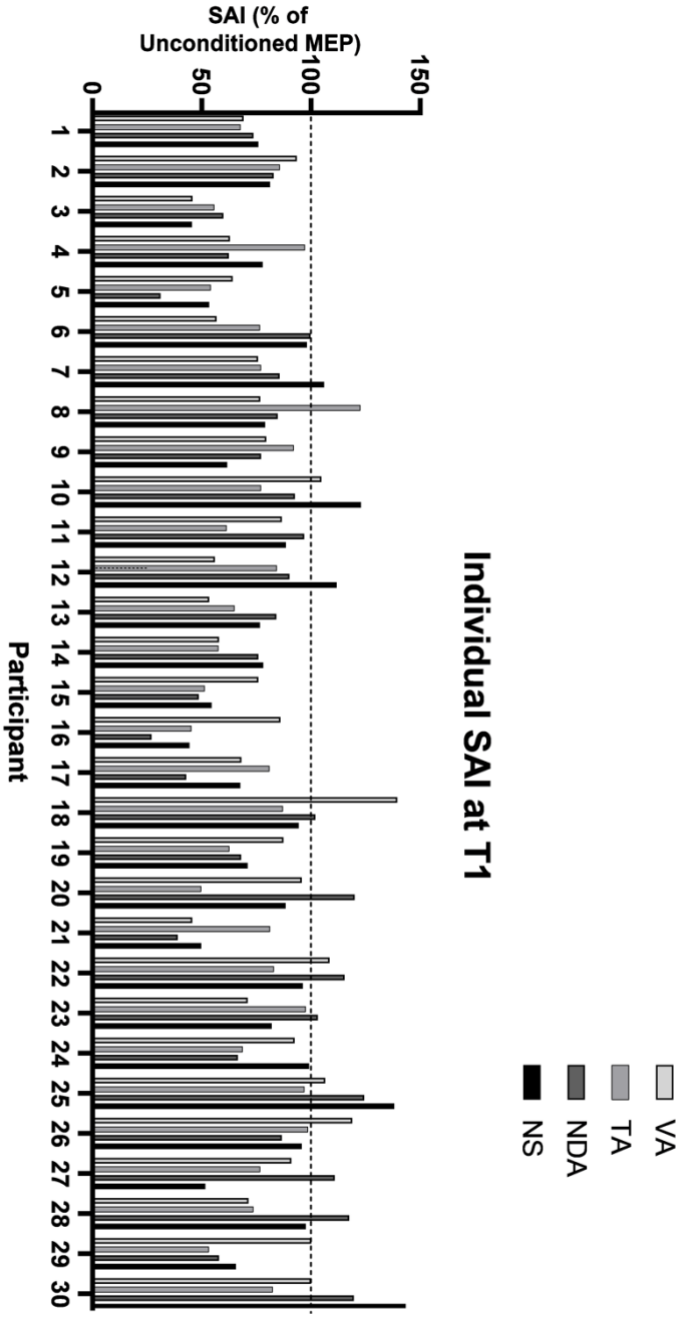
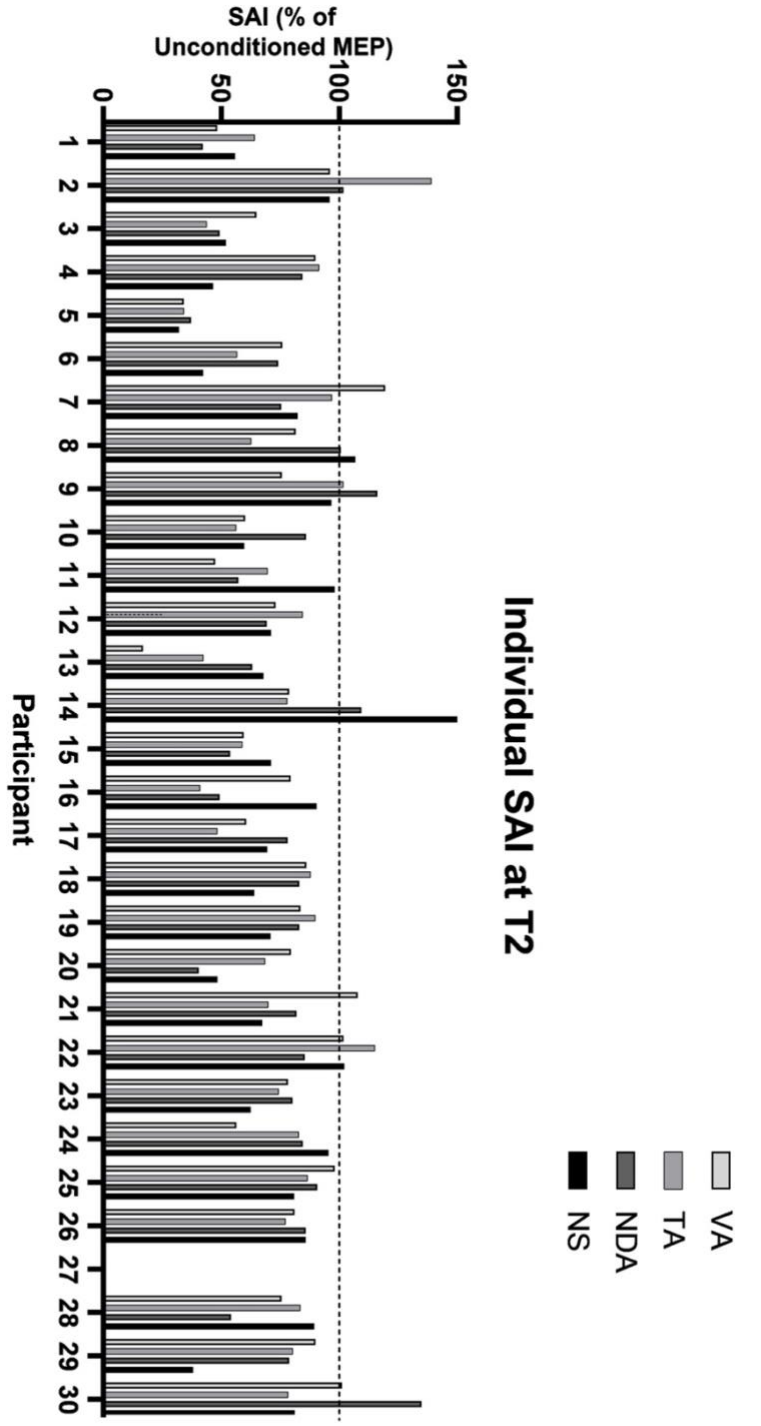


Figure 9: Potential sources of variability This diagram displays sources of variability that may affect afferent inhibition. It includes environmental factors such as attention, biological sex, diet, and sleep. Currently the effect of an arousal state is unknown. Future directions need to explore whether or not manipulations of arousal (alert vs sedated) will influence SAI.

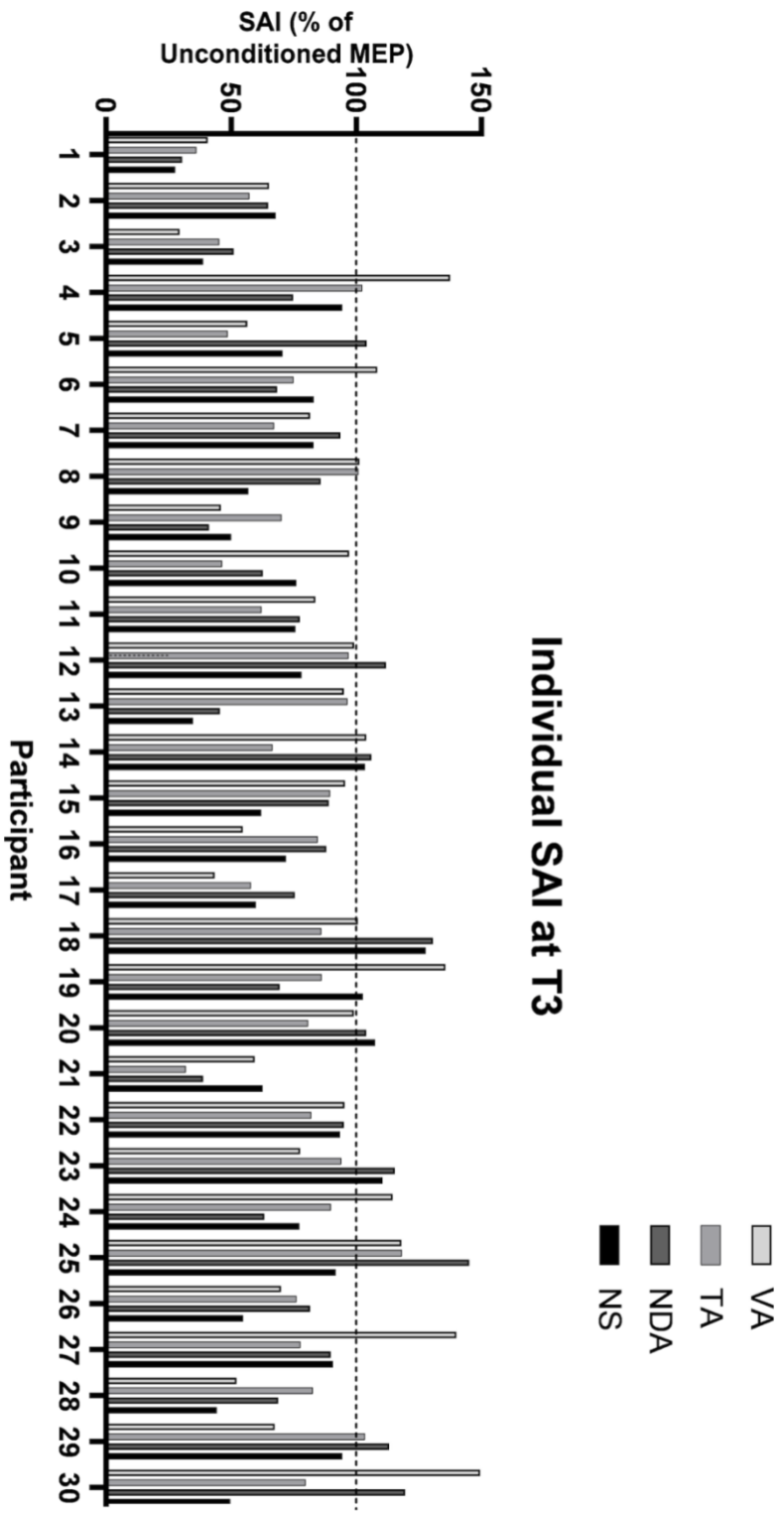
CHAPTER 5: APPENDIX



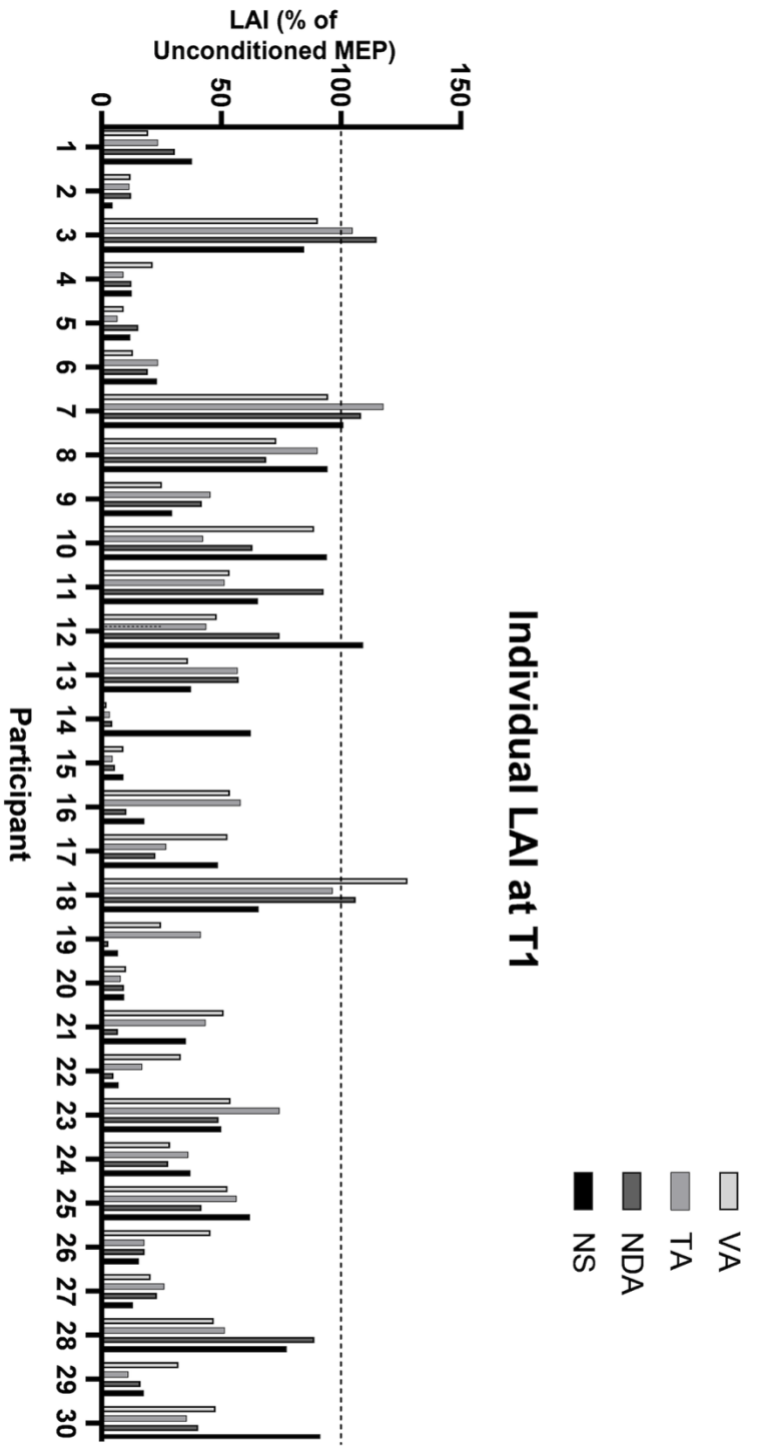
Appendix Figure 5.1.1: Individual SAI at T1 Graph depicts SAI for each individual at all four conditions



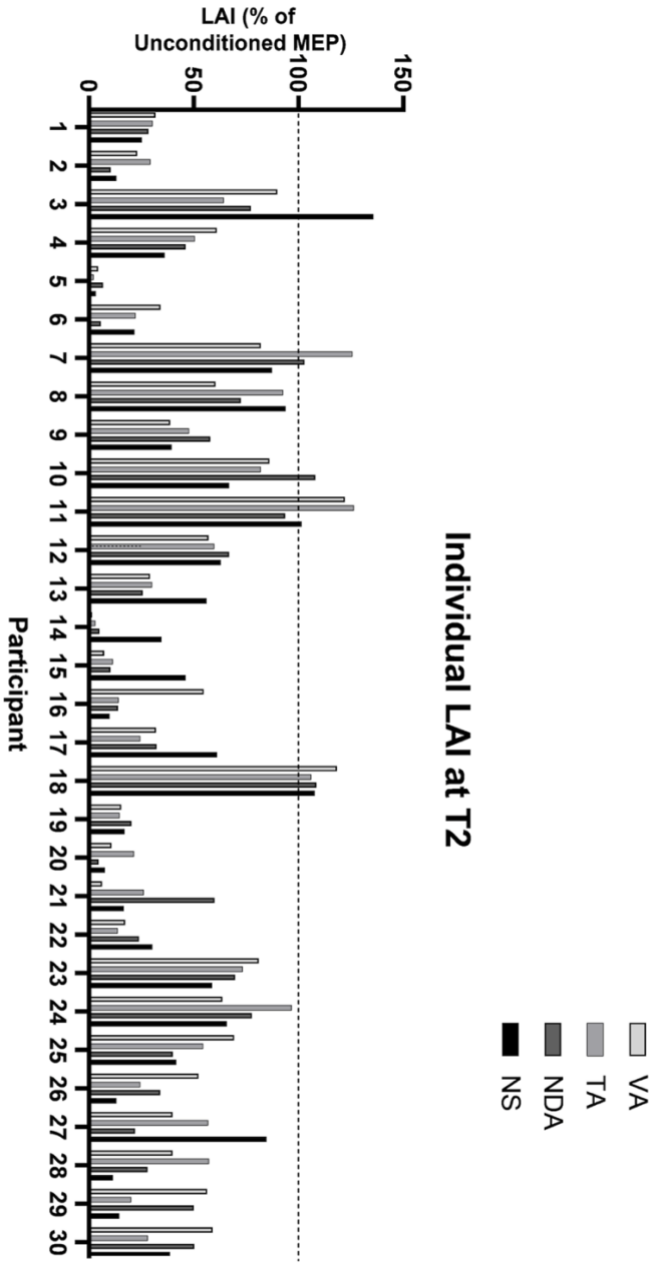
Appendix Figure 5.1.2: Individual SAI at T2 Graph depicts SAI for each individual at all four conditions



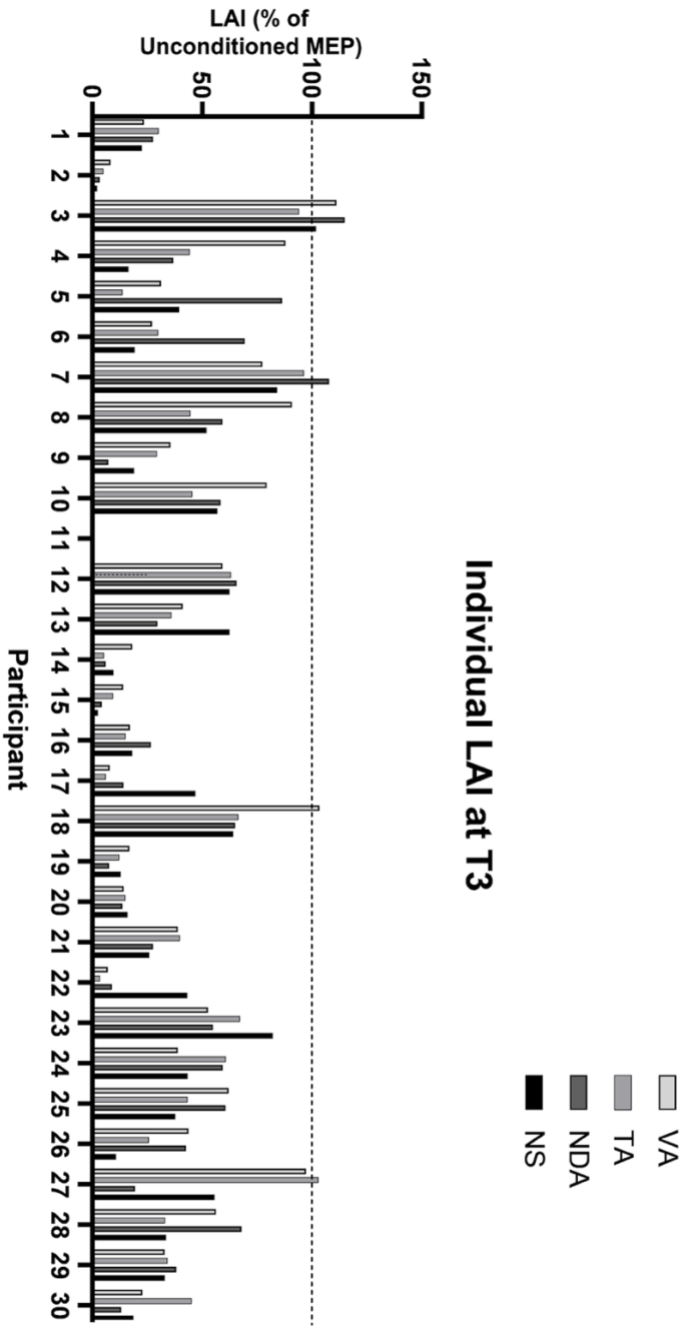
Appendix Figure 5.1.3: Individual SAI at T3 Graph depicts SAI for each individual at all four conditions



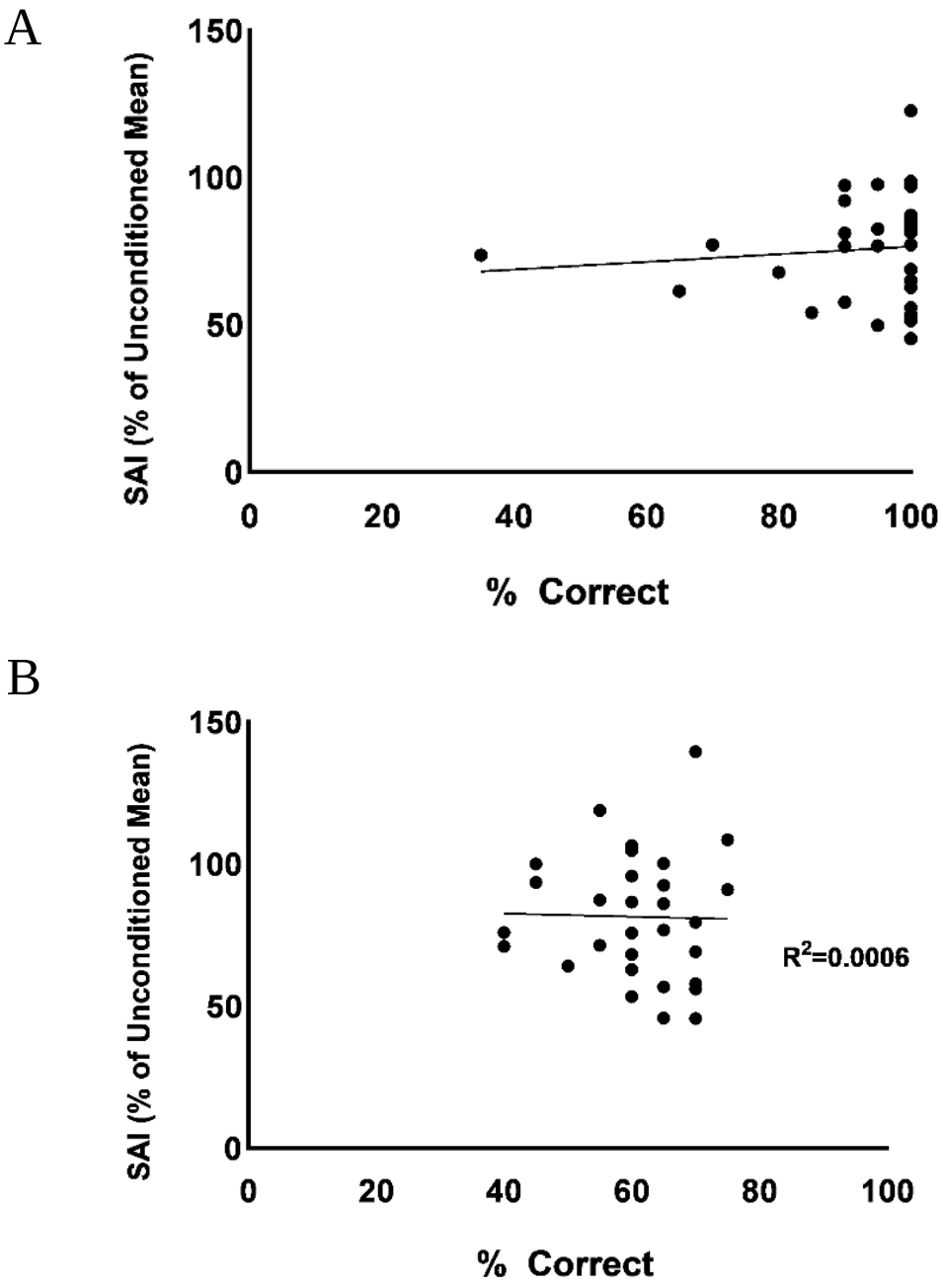
Appendix Figure 5.1.4: Individual LAI at T1 Graph depicts LAI for each individual at all four conditions



Appendix Figure 5.1.5: Individual LAI at T2 Graph depicts LAI for each individual at all four conditions

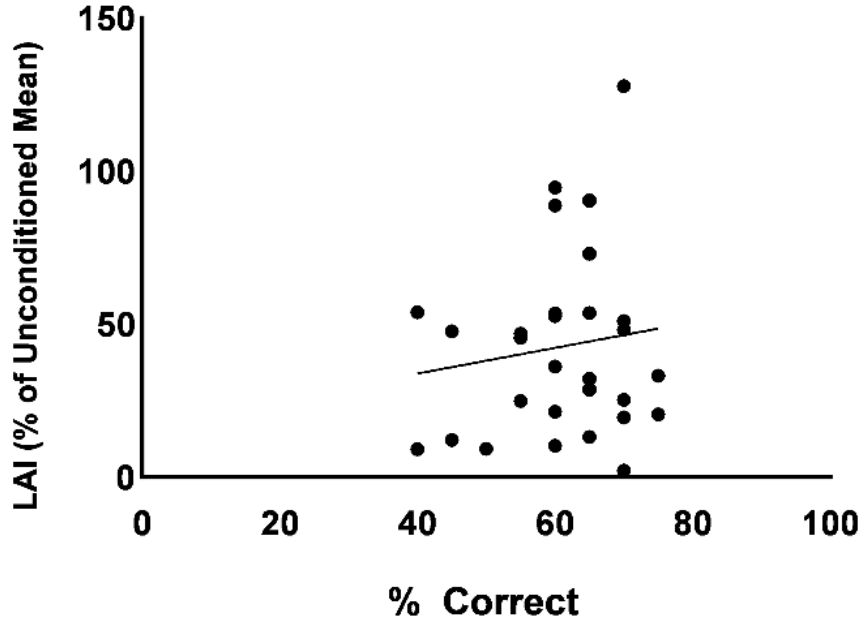


Appendix Figure 5.1.6: Individual LAI at T3 Graph depicts LAI for each individual at all four conditions

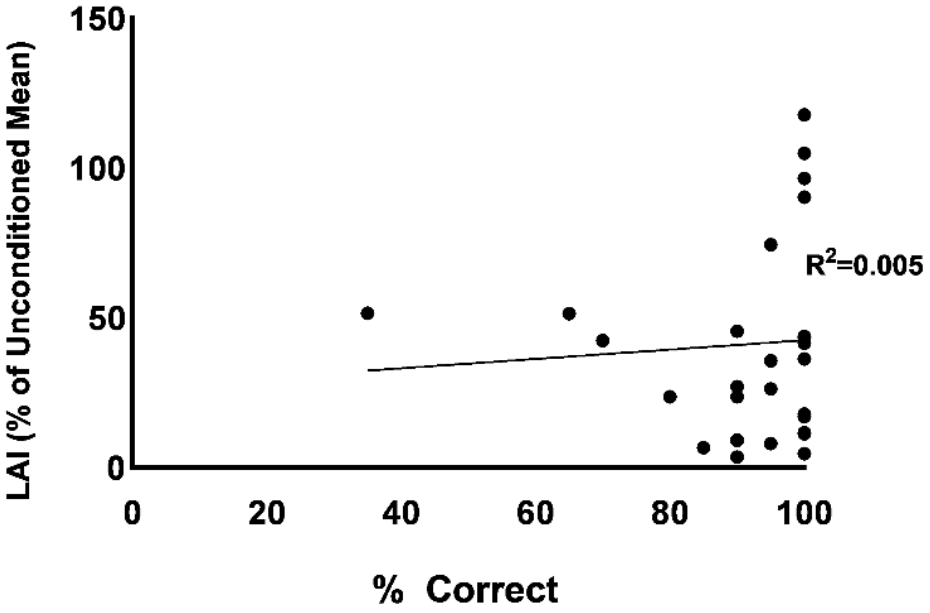


Appendix Figure 5.2.1: SAI Accuracy A) Represents visual attend accuracy plotted against SAI values. B) Represents tactile attend accuracy plotted against SAI values

A



B



Appendix Figure 5.2.2: LAI Accuracy A) Represents visual attend accuracy plotted against LAI values. B) Represents tactile attend accuracy plotted against LAI values

	VA	TA	NDA	NS
T1	27.39	23.77	33.44	30.60
T2	29.30	30.52	29.65	34.53
T3	36.48	27.69	33.60	35.59

Appendix Table 5.3.1: Coefficient of Variation (CV) of SAI for each condition at three different timepoints.

	VA	TA	NDA	NS
T1	69.22	74.67	87.61	74.56
T2	66.77	75.23	71.95	73.45
T3	71.23	72.03	78.79	67.97

Appendix Table 5.3.2: Coefficient of Variation (CV) of LAI for each condition at three different timepoints.

Appendix Table 5.4: Intersession analysis including Outliers for SAI and LAI across conditions

Intrasession SAI	ICC (95% CI)	SEM_{eas}	SEM_{eas}%	SDC_{group}	$SDC_{Individual}$
VA	0.39 (-0.321 to 0.711)	24.84%	30.68%	12.78%	68.84%
TA	0.56 (0.06 to 0.791)	16.27%	21.55%	8.24%	45.12%
NDA	0.44 (-0.186 to 0.732)	21.40%	26.97%	10.83%	59.31%
NS	0.101 (-0.88 to 0.57)	25.31%	31.62%	12.81%	70.15%

Table 5.4.1: Intrasession Reliability Statistics SAI including outliers. SEM_{eas}**,** $SDC_{individual,}$ SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed attend, NS: no stimulation, SDC: smallest detectable change, SEM_{eas}*: standard error of measurement,* $\%SEM_{eas}$*: relative* $SEM_{eas.}$ *TA: tactile attend, VA: visual attend.*

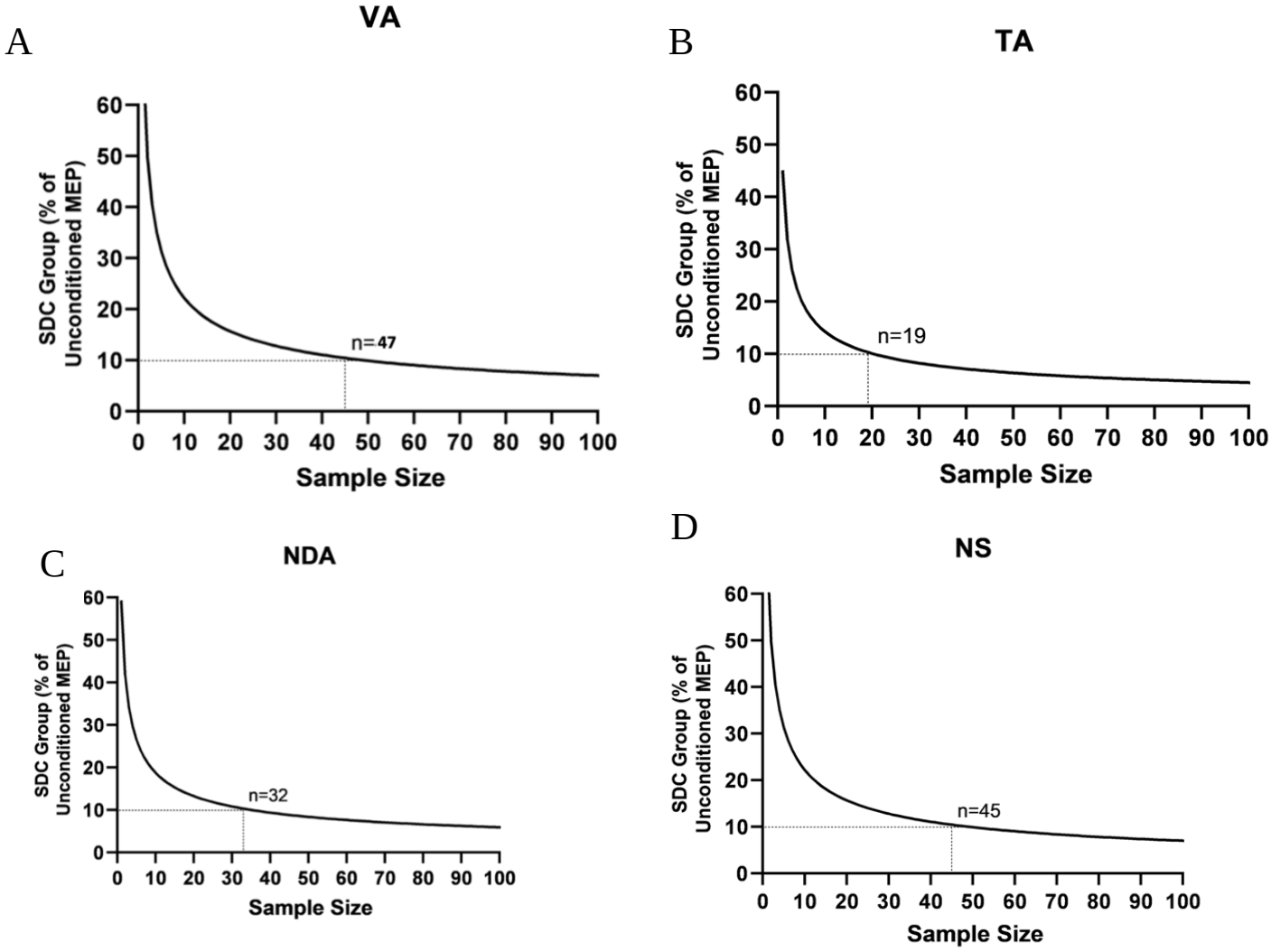


Fig 5.4.2. SDC_{group} for Intrasession SAI including outliers. SDC_{Group} presented are as a function of the sample size (n) for intrasession SAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).
SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

Intersession SAI	ICC (95% CI)	SEM_{eas}	SEM_{eas}%	SDC_{group}	$SDC_{indivdual}$
VA	0.42 (-0.22 to 0.72)	23.57%	27.94%	11.93%	65.34%
TA	0.41(-0.27 to 0.72)	16.98%	22.34%	8.59%	47.06%
NDA	0.54 (0.01 to 0.78)	22.18%	26.86%	11.22%	61.48%
NS	0.25 (-0.53 to 0.64)	23.06%	29.19%	11.67%	63.92%

Table 5.4.3: Intersession Reliability Statistics SAI including outliers SEM_{eas}, $SDC_{individual}$, SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed attend, NS: no stimulation, SDC: smallest detectable change, SEM_{eas}: standard error of measurement, $\%SEM_{eas}$: relative $SEM_{eas.}$ TA: tactile attend, VA: visual attend.

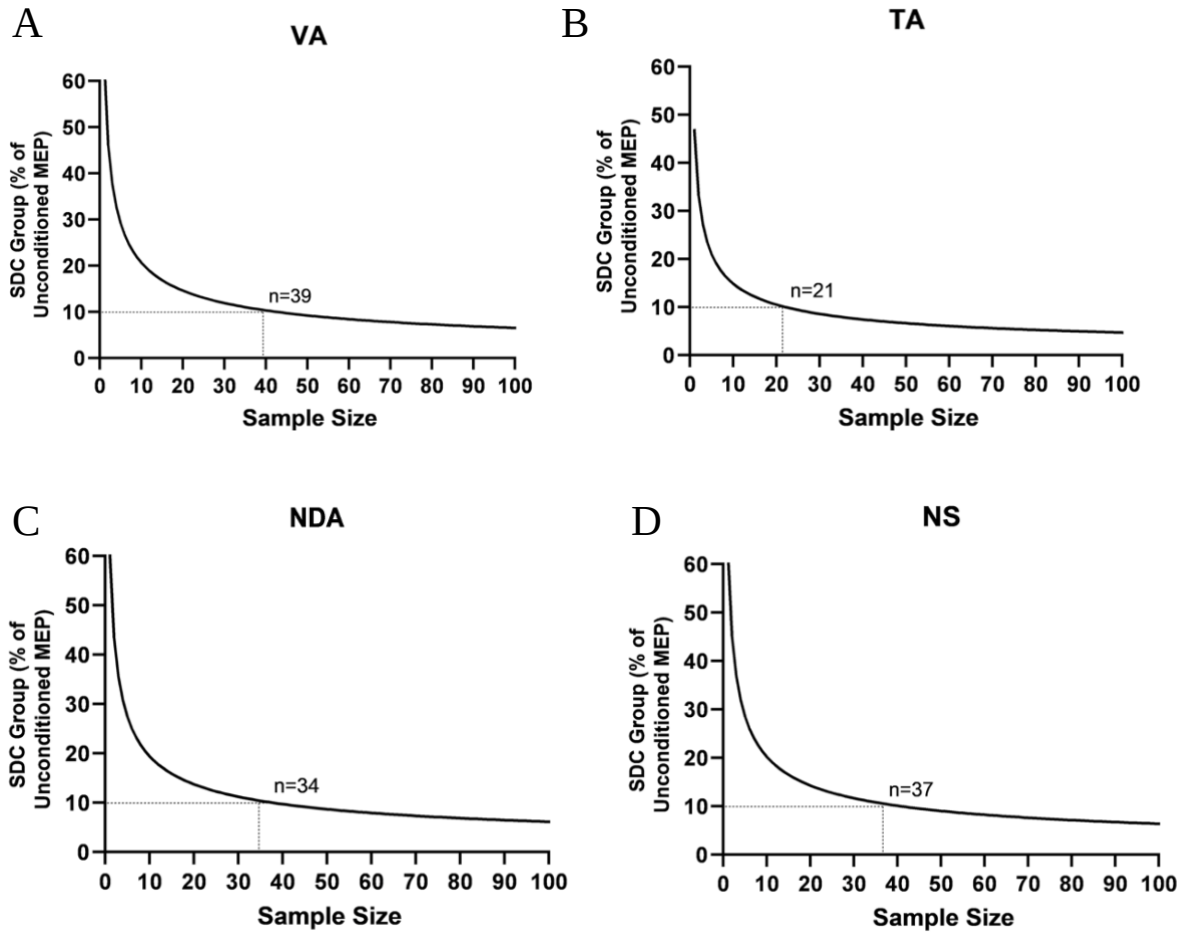


Fig5.4.4. SDC_{group} for Intersession SAI including outliers. SDC_{Group} presented are as a function of the sample size (n) for intrasession SAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS*

(D).
SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

Intrasession LAI	ICC (95% CI)	SEM_{eas}	SEM_{eas} %	SDC_{group}	$SDC_{individual}$
VA	0.86 (0.71 to 0.93)	14.97%	32.97%	7.58%	41.52%
TA	0.82 (0.63 to 0.92)	18.15%	41.09%	9.19%	50.32%
NDA	0.85 (0.69 to 0.93)	17.15%	40.42%	8.68%	47.54%
NS	0.75 (0.47 to 0.89)	21.51%	47.13%	10.88%	59.64%

Table 5.4.5: Intrasession Reliability Statistics LAI including outliers. SEM_{eas}, $SDC_{individual}$, SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed Attend, NS: no Stimulation, SDC: smallest detectable change, SEM_{eas}: standard error of measurement, $\%SEM_{eas}$: relative $SEM_{eas.}$ TA: tactile attend, VA: visual attend.

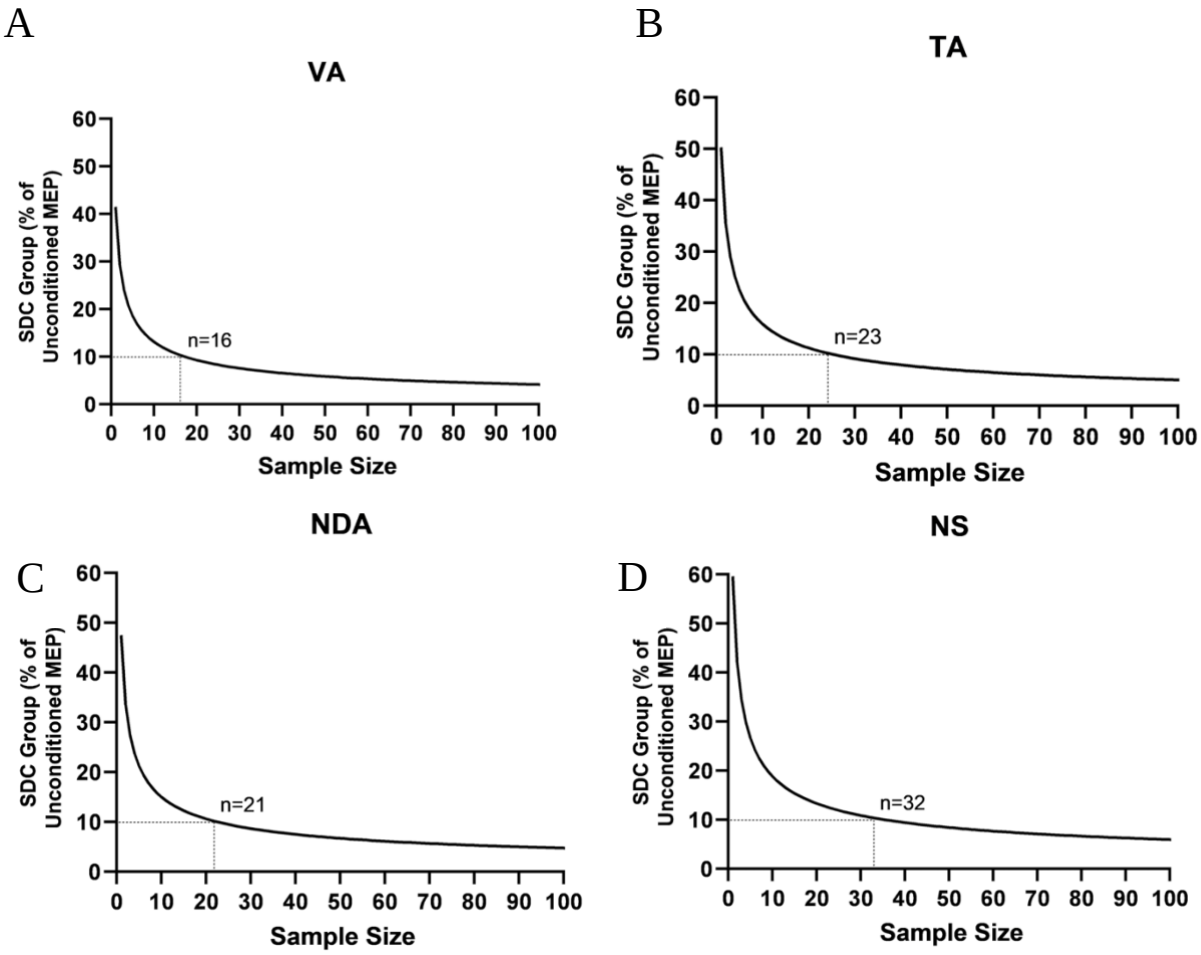


Fig5.4.6. SDC_{group} for Intrasession LAI including outliers. SDC_{Group} presented are as a function of the sample size (n) for intrasession LAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).
SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

Intersession LAI	ICC (95% CI)	SEMeas	SEMeas%	SDC_{group}	$SDC_{individual}$
VA	0.76 (0.49 to 0.88)	20.00%	44.02%	10.12%	55.45%
TA	0.64 (0.24 to 0.83)	26.54%	58.38%	13.43%	73.55%
NDA	0.86 (0.70 to 0.93)	17.49%	41.55%	8.85%	48.48%
NS	0.73 (0.43 to 0.87)	19.68%	47.00%	9.96%	54.55%

Table 5.4.7: Intersession Reliability Statistics LAI including outliers. SEM_{eas}, $SDC_{individual}$, SDC_{group} are expressed as a percentage of the unconditioned MEP. In contrast, $\%SEM_{eas}$ is expresses the SEM_{eas} as a percentage of the mean.
CI: confidence Interval, ICC: intraclass correlational coefficient, NDA: non-directed Attend, NS: no

Stimulation, SDC: smallest detectable change, SEM_{eas}: standard error of measurement, $\%SEM_{eas}$: relative $SEM_{eas.}$ TA: tactile attend, VA: visual attend.

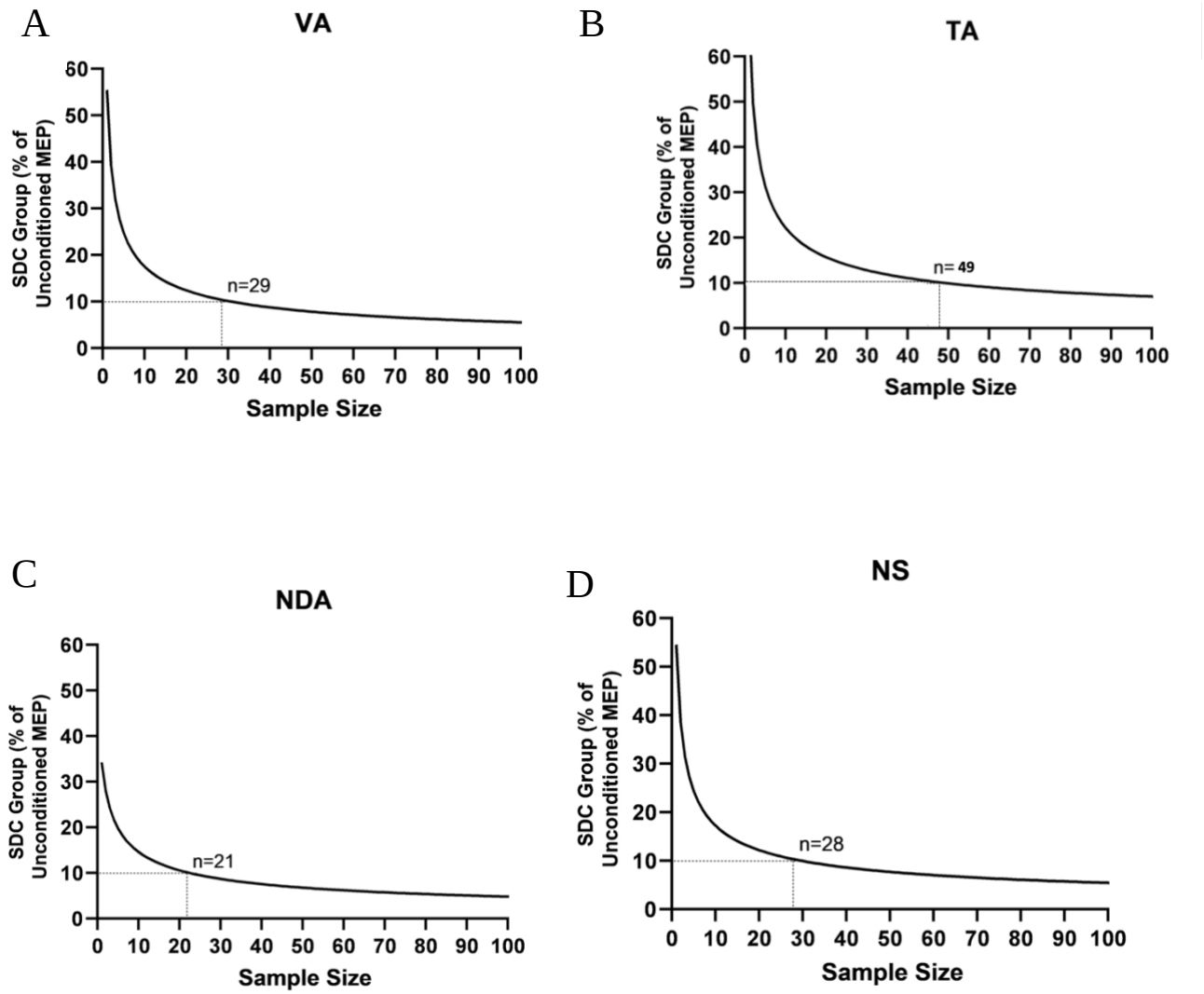


Fig 5.4.8. SDC_{group} for Intersession LAI including outliers. SDC_{Group} presented are as a function of the sample size (n) for intrasession SAI are shown for *VA* (A) *TA* (B) *NDA*(C) and *NS* (D).
SDC_{group}: smallest detectable change at the group level. NDA: non-directed attend, NS: no stimulation, TA: tactile attend, VA: visual attend.

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