Investigating if Motor Preparation Enhances Visuomotor Associative Neuroplasticity

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The human brain is known to be highly adaptive which has important implications for motor learning and recovery from neurologic injury. It has been shown that long term potentiation-like neuroplasticity can be experimentally induced via a protocol termed visual paired-associative stimulation (V-PAS) (Suppa et al, 2015). V-PAS can be used to investigate neuroplastic mechanisms and understand how additional variables interact to alter plasticity induction. Preparation of a reaching movement was used in this study as an additional variable to influence neuroplasticity induction. Research has shown the superior parietal occipital cortex (SPOC) is critically involved in orchestrating a reaching movement (Vesia et al, 2013). The present study combined these two findings to observe how reach preparation paired with V-PAS influenced the resultant plasticity induced. We hypothesized that: (1) V-PAS without reach preparation would exhibit comparable excitability changes to Suppa et al, (2) incorporation of reach planning during V-PAS would significantly increase excitability beyond that of V-PAS alone, (3) projections from SPOC to the primary motor cortex (M1) would be enhanced following V-PAS combined with reach preparation. Ten participants completed two experimental sessions in a repeated measures study design with observations of cortical excitability recorded prior to and following V-PAS. Session 1 implemented V-PAS alone (resting V-PAS) while session 2 incorporated reach preparation with V-PAS (active V-PAS).

Results showed approximately half of the sample experienced facilitation of motor evoked potential amplitude (MEP) following V-PAS while the other half exhibited suppressed MEP sizes. The sample collectively did not show significant excitability change following V-PAS. However, when parsed out by V-PAS response (increased or decreased excitability), both groups observe dramatic effects of V-PAS in their respective direction. No effect of V-PAS condition was observed in either of these groups which suggests reach preparation as performed in this study does not enhance cortical excitability changes to a detectable degree. SPOC to M1 projections were found to increase MEP amplitudes during

all timepoints and conditions. While not preparing a reach, MEP amplitudes were further enhanced to a similar degree following both V-PAS interventions. However, when participants prepared a reach during post-testing, MEP size was only increased following active V-PAS. These results may point to a region of premotor cortex whose projections to M1 are altered selectively by V-PAS incorporating reaching preparation. Our research serves to further develop early literature describing the V-PAS technique and guide future research in the field. In time, we hope this work will help elucidate currently unknown neuroplastic mechanisms which can then be translated into improving clinical outcomes.

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Introduction

A hallmark of the human brain is its ability to adapt to and learn from the environment. With some exceptions, the brain cannot accomplish these adaptions through the creation of new cells; a luxury which many of the body's other physiologic systems retain. The nervous system circumvents this limitation via exploitation of a high degree of interconnectivity between neurons. As will be discussed below, neurons can change dramatically in response to stimuli with much of these alterations being localized to synapses between neurons[1]. Numerous techniques have been discovered to experimentally induce neuroplasticity in vivo. This accessibility has helped uncover mechanisms critical to neuroplasticity allowing deeper research into the underlying mechanisms and bolstering evidence-based treatment approaches in clinics.

This document presents a thesis project aimed to further understand neuroplasticity and methods to enhance induction through use of the visual paired-associative stimulation (V-PAS) technique[2]. V-PAS is a technique recently published in the literature and remains a relatively unexplored protocol. We first provide the reader with a review of critical concepts and relevant literature, moving into the objectives, rationale, and hypotheses for the study. A comprehensive methodology is then reported followed by observed results and related discussion.

Background

Dorsal and Ventral Visual Streams

Beyond the primary visual cortex, information proceeds through the secondary visual area (V2) towards extrastriate areas and then to either the parietal or temporal lobe for increasingly specialized processing. Signals which travel to the parietal cortex make up the dorsal stream. The dorsal stream generally processes information related to spatial localization and action[3,4]. Visual area 5 (V5) and 6 (V6) are important early relays for the dorsal stream[3,5]; consistent with function, it has been shown areas V5[6] and V6[7] are sensitive to motion direction of visual stimuli. Conversely, object recognition information is transmitted by the ventral stream which passes to the temporal lobe. Ganglion cell types are thought to preferentially sort into one of the visual streams with M-cells entering the dorsal stream and P-cells the ventral stream[3,8,9]. However, evidence shows while this claim largely remains accurate, a small portion of each cell type will contribute to the other visual stream[10].

Much of the current understanding of visual stream roles stems from visual disorders involving damage to one stream while sparing the other. Damage to the ventral pathway can result in visual agnosia, a condition characterized by an impaired ability to recognize objects in the environment[8]. Individuals with visual agnosia can present with unremarkable acuity indicating visual information is unimpeded on its way to the brain. Given deficits in recognition of objects, faces, pictures, and/or other designs, those with visual agnosia remain able to navigate and interact with the world; a process which requires strong interpretation of visuospatial information from the environment[8]. A Case study of patient DF has supported the claim that visual agnosia spares dorsal vision while impairing the ventral visual pathway[11]. When instructed to match hand aperture to an object based solely on visual perception of the target, DF was unable to accurately gauge aperture size. However, DF matched aperture size correctly when instructed to reach to grasp the object[11,12]. The critical difference between

these conditions was the instructions guiding execution. In the first, DF had to match aperture using only visual cues which selectively recruited ventral stream visual processing to garner perception of the object. When instructed to reach to grasp the object however, this task activates the dorsal stream to guide the reach to grasp movement. Given that DF has impaired ventral stream processing, it is logical that tasks requiring ventral pathway processing will be impaired while performance on tasks utilizing the dorsal stream will remain largely unaffected. On the contrary, damage to the posterior parietal cortex may impair the dorsal visual stream. Damage to this stream may result in optic ataxia, a condition which presents with difficulty coordinating visually guided movements. More specifically, research has shown that damage of this nature negatively affects online control of reaching. In these studies, participants would initially reach to an incorrect location and adjust to the target after terminating the initial movement[13,14]. These findings highlight the separate application of the ventral and dorsal streams: during the reach, the dorsal stream is likely prioritized to help guide the movement; following the movement, the ventral stream is likely utilized to assess task success. Since the ventral stream is unaffected, perception of objects/targets is generally unaffected while online control via the dorsal stream is impacted. This observation reinforces the dichotomy between the dorsal and ventral visual streams[8,15].

Mechanism of Long-term Potentiation Induction

The nervous system possesses the ability to adapt itself in response to the various stimuli it is presented with. One well studied mechanism through which this is accomplished is termed long-term potentiation (LTP). LTP is often discussed at the cellular level in conjunction with the Hebbian theory of plasticity which follows the saying: neurons that fire together, wire together[16]. That is, when neuron A fires onto neuron B repeatedly over time, eventually the synapse between these neurons will adapt to strengthen that connection. The resultant enhancement is generally considered LTP when the changes

last for a relatively long period of time beyond the induction[1]. Artificially-induced LTP (such as with TMS) implemented in one session often expires within 60min with commonly applied protocols[17]. Conversely, endogenously-produced LTP or artificially-induced LTP provided over repeated, adjacent sessions has been documented to persist for weeks to months at a time[18]. Arguably the most important factor in maintaining LTP within a synapse is through the creation of an environment that encourages LTP retention such as regular activation of the synapse. In this way, LTP plasticity retention closely follows a "use it or lose it" paradigm.

The early cellular mechanism underlying LTP induction has been well described in previous research[1,19]. In an example synapse which releases the excitatory transmitter glutamate, glutamate will cross the synaptic cleft and bind to both N-methyl-D-aspartate (NMDA) and non-NMDA receptor channels. Non-NMDA receptors, being simply ligand gated, will immediately open allowing Na^+ to enter the post-synaptic cell causing a slight cellular depolarization. NMDA receptors in addition to being ligand gated have a Mg^{2+} ion blocking passage through the channel. Upon local depolarization via non-NMDA channels, the Mg^{2+} leaves the NMDA receptor thereby allowing influx of Na^+ and Ca^{2+}. Upon influx of Ca^{2+}, a complex cellular cascade begins facilitated by several proteins including calmodulin[20]. This chemical cascade acts to improve the synapse through various short-term and long-term effects. Some short-term effects may be increased receptor trafficking to the post-synaptic membrane or greater neurotransmitter release from the pre-synaptic cell via retrograde signalling[1]. Long-term effects require at least several hours to a day or longer to be implemented and will include alterations such as synaptogenesis and/or additional receptor synthesis.

Transcranial Magnetic Stimulation

TMS Background

TMS is an effective method to stimulate and probe the brain in a non-invasive manner. At its core, TMS consists of a coil of wire encased in plastic with which a current is passed through[1]. The passage of current through the wire produces a magnetic field orthogonal to the coil. This magnetic field can be directed into a participant's scalp and influence underlying neuronal activity. Interneurons are largely targeted due to a population effect but other neurons such as motor neurons can be activated by TMS directly or indirectly through interneurons[1]. Because of the shape of the magnetic field, stimulation of the cortex is not pinpoint with the use of just one coil which can cause a spread of the excitation. Alternative coil designs have been implemented to address this concern. One such design termed a figure-eight or butterfly coil, positions two coils adjacent to one another and are activated simultaneously. When activated in combination, the epicentre of the magnetic field is observed below the junction of the two coils with progressively weaker fields observed farther away[21]. With this configuration, stimulator precision is enhanced but it should be noted that the immediate surrounding area can still be influenced to a degree.

Single-Pulse TMS

There are several ways to use TMS which can probe or impart temporary changes to cortical excitability. The most straightforward application is through single pulses which are oftentimes employed as an indicator of cortical excitability[22]. Single-pulse TMS has also been used to prepare targeted cortex for immediately incoming information to enhance perception[23]. Motor neurons are often targeted when assessing cortical excitability as quantification of cortical changes is available through measurement of evoked muscle activity. For example, if one records muscle twitch amplitude evoked by single pulse TMS prior to an intervention and repeats the procedure afterwards any muscle

twitch amplitude changes can be indirectly associated with motor neuron excitability in theory (for more information, see Methods below).

Paired-Pulse TMS

Beyond single-pulse, paired-pulse TMS may be incorporated to assess cortico-cortical connections' influence on cortical outputs. In this protocol, a sub-motor threshold conditioning stimulus (CS) is delivered to the participant followed by a supra-threshold test stimulus (TS)[24]. Depending on the duration of the interstimulus interval (ISI) between pulses, different effects can be observed on the resultant neural output. An ISI between 1-6 ms will result in short-interval intracortical inhibition (SICI) which will act to reduce a muscle twitch relative to a TS alone. Current research suggests SICI is mediated by ionotropic $GABA_A$ receptors[25]. Conversely, a facilitation effect termed intracortical facilitation (ICF) is observed at an ISI of 6-20 ms which is thought to be mediated through excitatory glutamatergic interneurons within M1 and NMDA receptors[26,27]. Lastly, long-interval intracortical inhibition (LICI) can be evoked with a suprathreshold CS followed by a TS 50-200 ms later[28]. Mechanistically, it is thought LICI is mediated via metabotropic $GABA_B$ receptors[29]. Paired-pulse TMS can also be implemented between two distinct cortical regions; for example, to examine intracortical connections between the premotor and primary motor cortex[2]. This technique necessitates two distinct coils and requires various ISI timings due to the distance between stimulation sites.

Paired Associative Stimulation

TMS can also be joined with a peripheral stimulus in a protocol termed paired associative stimulation (PAS)[27,30]. Commonly, the peripheral stimulus takes the form of a median nerve stimulus which then ascends to the primary somatosensory cortex (S1) via somatosensory pathways[3]. Following the median nerve stimulus, a single, suprathreshold TMS pulse is delivered to the primary motor cortex contralateral to the median nerve stimulus. The timing between these two events is set such that the

median nerve stimulus signal arrives at the primary motor cortex as the TMS pulse is executed[31]. In the case of a median nerve stimulus, an interstimulus interval of 25 ms is often set before TMS onset[32]. If this pattern of stimulation is applied repeatedly (e.g. 180 trials[32]), LTP-like plasticity can be induced in the primary motor cortex within the region targeted by TMS[27,31]. The important underlying mechanism supporting PAS is the coincident timing between peripheral and TMS stimuli over the desired target. However, if these stimuli do not occur at the correct timing, specifically if TMS occurs before the peripheral stimulus arrives, the effects can be reversed[31]. Research with protocols using a 10 ms ISI between a median nerve stimulus and contralateral primary motor cortex have shown long-term depression like plasticity induced in the cortex[31]. The authors suggest these findings suggest that orthodromic activity (sensory then TMS) must be demonstrated in the synapse to foster LTP-like plasticity since the 10 ms ISI likely evoked antidromic activity (TMS then sensory) within the synapse.

Review of Guiding Literature

Suppa et al, 2015

This group set out to investigate a novel PAS protocol through substitution of a visual stimulus in place of the traditional median nerve stimulation (V-PAS)[2]. Six small experiments were included in this study, each following a pre-post study design with an intervention delivered in the middle. Fourteen participants with normal or corrected to normal visual acuity and no history of neurological disorder were enrolled in the study and completed each small experiment with at least 7 days between sessions. Once seated, participants were provided an eye patch to cover their right eye and instructed to look at a fixation point in the centre of a screen in front of them during the experiment. The visual stimulus consisted of a square black and white checkerboard pattern whose checks reversed once every second. Participants were seated 70cm away from the screen resulting in each check subtending a visual angle of 24° (80% luminance contrast) contained in an 8x8 configuration. While the checkboard extended both to the left and right of the fixation cross, only the right half of the screen reversed during the experiment thereby limiting stimulation to the right visual field. The group collected electroencephalography (EEG) from participants specifically to quantify visual evoked potentials (VEPs) observed at the primary visual cortex in response to the checkerboard reversal. This data allowed a precise calculation of when information reached the visual cortex to better estimate an effective ISI for use during V-PAS as discussed below. To quantify LTP induction, motor evoked potentials (MEPs) were recorded as a peripheral indicator of cortical excitability. These were evoked with single pulse TMS of sufficient intensity to generate MEPs of approximately 1mV at baseline and measured prior to and following V-PAS. By seeking out 1mV twitch intensity first, this helped bring down the study's intersubject variability since everyone was now starting at a similar baseline. MEPs were recorded at 5, 15, 25, 35, 45, 55 min post cessation of V-PAS to track excitability over time. The intensity used at baseline was applied for all MEP measures and during V-PAS. One session collected VEPs shortly after V-PAS to examine any

differences in cortical potentials. Lastly, paired-pulse TMS between the posterior parietal cortex (PPc) and the dorsal (PMd) and ventral premotor cortex (PMv) to M1 were collected before and after V-PAS to examine the connectivity between these regions.

A critical portion of the study was dedicated to uncovering the optimal ISI to use for V-PAS. Adapted from previous PAS protocols, the authors included a VEP generated by checkerboard reversal as a peripheral stimulus met with a single suprathreshold TMS pulse delivered over contralateral M1. Due in part to the new travel distance, increased number of synapses, and previous research in animals, it was hypothesized that the optimal ISI for V-PAS would not be the same as other PAS protocols. In order to address some inter-subject variability, the researchers recorded the VEP P100 potential and treated this time as 0 for each participant for the purposes of calculating an ISI. On top of the observed P100 time, intervals of 40 to 140 ms incremented by 20 ms were included during separate sessions of V-PAS; this meant that ISIs ranged in total 140 to 240 ms from the onset of the VEP to TMS onset on average but individuals could vary slightly based on P100 timing (Avg. 101 ± 5.4 ms). Only one ISI was ever used during a given V-PAS protocol with multiple sessions being required to collect data on each time interval. In general, V-PAS consisted of 600 pulses delivered at 1 Hz. Two conditions were included with V-PAS at 0.25 Hz, the first executed 600 pulses while the second tested a dose effect of 150 pulses. Lastly, an investigation was included in which single pulse TMS alone over M1 was delivered at 1 Hz to investigate any cortical excitability induced which may confound observed V-PAS effects.

Results of the study showed that an ISI of 100 to 120 ms following P100 latency was effective at inducing strong LTP-like plasticity in the contralateral M1 observed as approximately 140% of baseline MEP amplitude. This effect remained for approximately 1 hour. Most other ISIs did not significantly alter excitability, however the 40 ms condition elicited significant decline in M1 excitability. This LTD-like plasticity is likely caused by the consistent timing of TMS prior to visual stimuli arrival. In this paradigm the motor cortical neurons and interneurons would systematically be exposed to stimuli in this

unnatural order which could lead to the observed LTD-like plasticity. Examining 1 Hz TMS alone, a significant depression in excitability indicated by reduced MEP amplitudes was observed. It is important to note that this effect should be present in the 1 Hz V-PAS data which showed an excitation of M1 neurons. This concept is further elucidated by the 0.25 Hz TMS data which presents even greater excitation of approximately 170% baseline MEP amplitudes following 600 pulses of V-PAS at this frequency. The authors also tested V-PAS at a lower "dosage" of only 150 trials, they found no significant change to cortical excitability implying there is a minimum number of trials needed to induce a measurable effect. Lastly, paired-pulse data provided a glimpse into underlying mechanisms facilitating the induced plasticity. PPc to M1 and PMd and PMv to M1 *facilitatory* connections did not present significant changes following V-PAS. However, PMd and PMv to M1 *inhibitory* projections were observed to be significantly reduced following V-PAS presenting larger MEP amplitudes compared to baseline. This increase in MEP amplitude suggests a disinhibition effect underlies the plasticity induced by V-PAS but the observed MEPs were still not as large as when induced through facilitatory projections suggesting that some inhibition yet remained after V-PAS.

Vesia et al, 2013

This group endeavoured to investigate the role of two regions within PPc as they pertain to the control of reaching and grasping[33]. Although on the surface a reach to grasp may appear trivial, successful execution requires strong visuomotor integration to coordinate an effective reach as well as aperture formation for grasping. In general, the PPc is critical for high-level action plans, intention, and decision making[34]. Imaging research has identified two specific regions within PPc with apparent functions related to reaching and grasping movements[35]. In response to extension of the arm, a region known as the superior parietal-occipital cortex (SPOC) exhibited increased BOLD signal suggestive of its involvement in the action. Similarly, in response to forming a grasp, BOLD signals were elevated in the

anterior intraparietal sulcus (aIPS). Vesia et al, aimed to examine this relationship through behavioural tests and through the use of paired-pulse TMS[33].

Seven right-handed participants were recruited to complete the study. A 5 cm wide, 2 cm deep, 1.5 cm high object was placed 30 cm away from seated participants. Four conditions guided participants' engagement with the object: the first and second condition started participants hands near their body requiring a reach to contact the object. Within these two conditions, one instructed participants to grasp the object while the other simply instructed participants to touch the object with a finger without forming a grasping posture. The third and fourth conditions positioned participants' hands adjacent to the object. From this position, participants were instructed to grasp the object or simply touch the object which did not require reaching. Paired-pulse TMS was positioned at SPOC to M1 or aIPS to M1 during the four conditions. The CS was delivered at 90% resting motor threshold (RMT) over SPOC or aIPS matched with a 120% rMT TS over M1. ISIs of 4, 6, 8 and 10 ms were used for both SPOC and aIPS to M1 connections. During a given trial, participants would be presented the object in their field of view. While presented, an auditory cue would sound signalling participants to begin planning the instructed movement to the object. This planning period lasted 500 ms at which time paired-pulse TMS would be initiated signalling participants to execute the motor response. Importantly, this meant the brain was stimulated by TMS while actively planning the motor response but prior to any motor output being initiated.

In both aIPS and SPOC projections, it was shown that an ISI of 4 ms produced the largest modulation on MEP size. Projections between aIPS and M1 appear to be net inhibitory in their influence, suppressing MEP amplitudes by approximately 30% while participants remain at rest. These projections are net facilitatory however when probed during the planning of a grasping movement. During conditions which had participants preparing to grasp the object regardless of starting location, aIPS to M1 TMS produced a MEP on average 25% larger than the TS alone. Conversely, SPOC to M1 projections

at rest did not significantly alter MEP amplitude compared to TS alone. This trend was also observed during both conditions where participants began adjacent to the object. Once participants were required to incorporate a reaching movement however, MEP amplitudes following SPOC to M1 TMS were significantly elevated by approximately 30%. The data suggest that SPOC is critically involved when planning a motor action which involves a reaching component. On the other hand, aIPS appears to assist in the formation of hand aperture to facilitate the grasping component.

Research suggests SPOC communicates with M1 through several intermediary steps prior to reaching its terminal destination[36]. Visual cortex will project onto SPOC which in turn will influence both the angular gyrus (AG) and midposterior intraparietal sulcus (mIPS). Area AG is thought to communicate relevant somatosensory information while mIPS encodes visual information related to hand positioning[36]. Projections from mIPS directly to premotor (dorsal) cortex (PMd) are then proposed to connect this parietal association activity with frontal regions to aid in reach movement production[36]. Lastly, projections from PMd to M1 then influence motor cortical neurons to aid in the generation of a reaching movement.

Objectives, Hypotheses, and Rationale

The aim of this study was to investigate plasticity induction through the V-PAS protocol. Specifically, we endeavoured to enhance the degree to which plasticity was induced in the primary motor cortex. Both PAS and V-PAS rely on coincident timing of two stimuli over many trials to encourage cortical plasticity. This study was conceived out of curiosity towards how plasticity induction is altered when more than two stimuli are coincident during each V-PAS trial. To accomplish this, we combined the methodology applied in the two studies reviewed above[2,33].

Firstly, we expect to replicate published results[2] which show increased cortical excitability when V-PAS is implemented without a reaching goal. Furthermore, we hypothesize when V-PAS is executed in conjunction with planning a reaching movement that the resultant cortical excitability will be enhanced compared to V-PAS at rest. The inclusion of SPOC to M1 activity during *active* V-PAS should increase the amount of coincident signalling present relative to during *resting* V-PAS and potentially translate to greater neuroplastic effect. This network likely relies heavily on layer II & III neurons and may relay through the premotor cortex before influencing neurons within the primary motor cortex. Hypotheses 1 and 2 will assess cortical excitability change utilizing single pulse MEP measures (see below). Lastly, we anticipate SPOC to M1 projections to promote greater cortical excitability relative to baseline specifically following V-PAS incorporating planning of a reaching movement during the intervention. These circuits should not be significantly activated during *resting* V-PAS; conversely, we expect these projections to be facilitated following *active* V-PAS due to their coincident timing with the visual stimulus and TMS pulse. SPOC to M1 projections will be assessed via 2-coil paired pulse measures (see below); change is hypothesized to occur selectively while participants are preparing a reaching movement during TMS onset.

This study will further the literature related to V-PAS and general neuroplasticity. Limited research is available regarding V-PAS due to its recency. Our work will help elucidate neurologic mechanisms facilitating V-PAS's effects and applications for future investigation. We are also in a broad sense examining techniques to foster greater neuroplastic change in the cortex. Although not a primary goal of the study, our findings may provide direction towards practices which will enhance the plastic nature of the brain. This may extend to the facilitation of recovery from stroke in which the understanding of neuroplastic mechanisms is paramount[37,38]. Therefore, if our findings suggest methods to enhance neuroplasticity induction, this project may become a small piece of the puzzle informing rehabilitative practice one day.

Methodology

Subjects

A total of 10 participants were enrolled into the study. A sample size analysis was completed following the study using G*power based on collected data related to the paired-pulse TMS outcome measure as this was initially anticipated to be most difficult measure to obtain significant effects. A sample size estimate (mean paired difference = 1.2, standard deviation = 0.46, power = 80%, level of significance $\alpha = 0.05$) suggested a sample size of 5 was sufficient for this study[39]. Inclusion criteria were set as follows: all participants will be free of neurologic illness such as stroke, epilepsy, or peripheral neuropathy, fluent in English, have known allergies to alcohols or conductive gels, have no metal or magnetized objects in the body, and be at least 18 years of age. In addition, all participants had their visual acuity and stereoacuity assessed upon arrival to the lab. Visual acuity was measured using a Snellen chart; participants were required to score 20/20 or superior to be eligible. Likewise, stereoacuity was assessed using the Randot Stereo test with participants who scored ≥ 20" being eligible for the study. Lastly, participants completed a TMS screening form (Appendix A) to minimize the risk of encountering adverse reactions to the TMS procedures. We only accepted right hand dominant individuals into this study, assessed and recorded using the Waterloo Handedness Questionnaire (Appendix B). Although unclear how left hand dominant individuals would respond compared to right handers, it is possible that the effect size may be dramatically unequal between these two groups contributing to variability within the dataset. Subjects were recruited primarily from the University of Waterloo via posters placed on campus and personal contact.

Experimental Design

A repeated measures study design was implemented to examine the effects of a V-PAS intervention on cortical excitability (See Figure 1). One group was used in which each participant completed each of the two intervention conditions. To accommodate each condition, two sessions were completed by each participant with sessions separated by an average of 8 days. Subjects always underwent the Resting V-PAS intervention during session 1 to ensure any learning effects were consistent among participants during Active V-PAS during session 2.

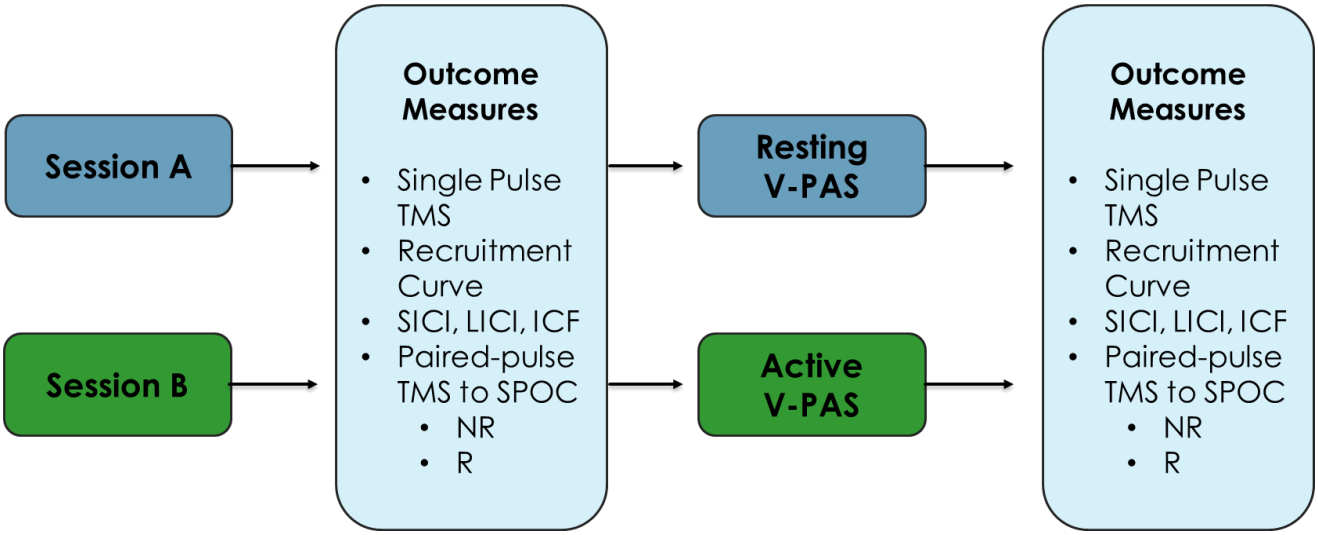


Figure 1: Example session schedule. During the first session a participant would follow the blue line. Upon return for the second session, the green line would be followed, leading to the alternate V-PAS intervention.

Apparatus

For the duration of the study, participants were seated comfortably in front of a 19” computer monitor at a 1280x1024 resolution located 40 cm away when their head rested on a chin rest. Affixed to the monitor was a touch sensitive transparent screen which participants could interact with. The computer monitor display was available to the participant throughout the session to provide a fixation target and to facilitate outcome measures and the intervention. As described below, Single-Pulse MEPs, Recruitment Curves, and SICI, ICF, and LICI displayed a simple fixation target for participants to access (visual angle = 0.72°). Visual stimuli were presented for both Paired-Pulse TMS and the V-PAS intervention in each session. These two protocols use the same stimulus presentation pattern except for the addition of a brief VEP-inducing stimulus during V-PAS. Each screen features a red dot which participants are instructed to focus on; this helps to prevent participants from moving their eyes excessively and is critical for selectively presenting stimuli to one visual hemifield during the intervention. Eye movements were monitored utilizing electrooculography to ensure participants did not deviate from the fixation point. If a participant exhibited significant eye movements leading up to or during the VEP presentation for more than 10% of trials, that subject was dropped from further analysis. Our protocol was designed to minimize the intervention trials required with participant comfort in mind; it is therefore possible that subjects who did not optimally receive over 90% of trials would exhibit a reduced effect from the intervention. The stimulus presentation pattern always began with display of a cue screen for 500 ms (see Appendix C). The cue screen contained a red square (visual angle = 1.15°) in one of 8 possible locations on the right side of the screen in addition to the fixation dot. Eight locations on the right side were selected to require participants to maintain attention throughout the protocol. The right side of the screen subtended 50.58° with half of the cue/target squares presented 9.29° and the other half 14.25° from the centre fixation point. Trials were organized into blocks of 8 trials throughout the study where each target location was randomized to occur once per block. In this way,

we could partially-randomize target presentation to participants while both ensuring each target location was presented equally and effortlessly control the duration of the protocol through manipulating the number of blocks delivered. The cue screen prepared participants for the location where the target would be displayed later in the trial. During the V-PAS intervention only, the cue screen was followed by a checkerboard pattern presented selectively on the right side of the screen. The pattern was 9 checks tall and 5 checks wide with each check subtending 5.44° vertically and 4.58° horizontally. During the study, the top and bottom row of the pattern became cut off resulting in an actual height of 1.58° for each of these rows. The checkerboard screen would induce a VEP upon onset and was displayed for 200 ms. Both Paired-pulse TMS protocol and V-PAS protocols then presented a target screen following the cue or VEP screen respectively. The target screen contained a green square in the exact same location as the cue was located. In the active condition of Paired-pulse TMS and active V-PAS, onset of the target stimulus signaled the participant to execute their prepared reach to touch movement. During resting conditions, participants remained at rest while the target screen was presented and awaited the next trial to begin. The target was presented for 2 seconds which provided ample time to execute the motor response without extending the protocol unnecessarily. Once touched, the overlaid touch screen moved the mouse cursor to the location touched. At the termination of each trial, LabView wrote the current location of the cursor into an Excel file before beginning the next trial. This process allowed us to track participant precision since each reach to touch performed during the active conditions moved the cursor to the location touched. During analysis, these recorded locations were compared to the known locations of each target to observe participant precision for each reach. In total, each trial subtended 2.5s during Paired-pulse TMS and 2.7s during V-PAS with the 200 ms difference being attributed to the addition of the VEP stimulus during V-PAS.

Electro-oculography (EOG) was collected during both sessions to ensure participants were maintaining fixation when required during V-PAS. To capture horizontal eye movement, 2 Ag-AgCl

electrodes were placed lateral to the outer canthi (lateral corner of the eye where the eyelids meet) of both eyes[40]. A reference electrode was attached over the ulnar styloid process of the right forearm. EOG signals were amplified 2500x and band-pass filtered at 0.05 Hz and 35 Hz. A sampling rate of 176 Hz has been shown to be sufficient[40] and we sampled EOG at 200 Hz as this is still sufficient to avoid significant aliasing and presented a round number to work with.

Outcome Measures

Each outcome measure was collected prior to and following the V-PAS intervention in both conditions (i.e. each participant had these collected four times over the course of the study). All outcome measures as well as the intervention involved the use of TMS. A Magventure stimulator (Model: MagPro R30, Magventure, www.magventure.com) attached to a figure-of-eight butterfly coil was used for the V-PAS intervention within each session. BrainSight (Rogue Research, Canada) aided placement of the coil over the target region using a template MRI scan calibrated to each participant. Calibration was completed at the beginning of each session and involved linking participant physical landmarks (nasion, inion, and R/L tragus) with the Brainsight camera. The representation created can then be overlaid with a template MRI which allows the use of coordinates to estimate the location of specific structures in the cortex. The coil was placed 45° to the mid-sagittal line and tangential to the scalp which induced a current from posterior to anterior in the underlying neural tissue. All outcome measures utilized a Magstim TMS stimulator (Model: Magstim^2, Magstim, www.magstim.com) to impart single and paired-pulses coordinated between two TMS coils. The TS coil was positioned 45° to the mid-sagittal line and tangential to the scalp as with the Magventure stimulator. The CS coil was placed along a parasagittal line and oriented approximately 15° medial above the SPOC region (see Paired-pulse TMS). Both coils were held tangentially to the scalp and guided by BrainSight neuronavigation software.

Single Pulse Motor Evoked Potentials

The first outcome measure in this study were MEPs evoked by a single pulse of TMS. Twenty-four individual MEPs were averaged at each timepoint. This number was selected to be consistent with later measures which must be obtained in blocks of 8. Each MEP was visually inspected following evocation to ensure a distinct result is obtained before proceeding to the next trial. If a clear MEP was not evoked that trial was discarded and repeated until a MEP of sufficient quality was observed. MEPs

were triggered manually at an intensity sufficient to evoke a 1mV MEP at the baseline timepoint within each session. Following collection, peak to peak amplitudes were extracted from each MEP and averaged across a given block of 24 trials.

Electromyography (EMG) was utilized to quantify MEP amplitude within the right FDI. Surface electrodes placed over the right FDI were applied preceded by a thorough cleaning of the skin. Raw EMG data was amplified 1000x and band-pass filtered between 2 Hz and 2.5 kHz. Data was digitized at 5 kHz and recorded with SIGNAL software and stored offline for later analysis.

Paired-pulse TMS

Paired-pulse TMS was utilized to investigate the interaction between SPOC and M1. By stimulating SPOC prior to M1, we allowed the SPOC neurons to exert any influence they have on M1 to alter the neural environment of FDI motor neurons when we subsequently evoked a MEP from M1. Two TMS coils were used simultaneously (Model: Magstim^2, Magstim, www.magstim.com) with one being positioned over the previously-determined FDI motor hotspot and the other over SPOC guided by Brainsight (Talairach coordinates x = -9, y = -74, z = 41)[33]. These coordinates represent the average location of SPOC determined from MRI scans of 8 individuals in a previous study[33]. During each trial, the CS was applied over SPOC at 90% RMT followed by a TS of 120% RMT over M1 with a 4 ms ISI. RMT was determined at the beginning of each session defined as the lowest stimulator intensity capable of evoking a MEP of at least 50 μV peak-to-peak in at least 5 out of 10 trials. The amplitude of the resultant MEP was recorded following collection and compared to other MEP measures. Within a given block, 24 MEPs were collected. Analysis of these MEPs consisted of averaging over each condition alike single pulse MEPs.

To isolate the influence of SPOC's projections with confidence, we used two distinct conditions aimed at manipulating SPOC activity. These conditions were *Reaching (R)* and *No Reaching (NR)* paired-

pulse TMS, relating the degree of involvement the participant experiences. Both conditions used a modified version of the computer interface provided during the V-PAS intervention (See below) where the presentation of the checkboard pattern was omitted (i.e. Fixation screen, followed by a cue screen, followed by a target screen, then returning to the fixation screen). In both NR and R conditions, paired-pulse TMS was initiated following a cue screen presented for 200 ms. During the NR condition, participants remained fixated on the central dot present throughout the entirety of each of the 24 trials. Conversely, during the R condition, participants remained fixated at the centre of the screen during the cue screen but used this time to prepare a reach to touch to the target with their right index finger. TMS onset in this outcome measure acted to signal participants to execute the reach as did the presentation of the target screen.

Recruitment Curve

Single pulse TMS over the FDI motor hotspot will be used to create recruitment curves across a range of stimulator intensities. Ten MEPs were evoked and averaged at intensities of 110, 120, and 130% RMT. All 10 MEPs within a stimulator intensity bracket were collected in succession with at least a 5s ISI. The order with which intensity levels are presented were collected sequentially from lowest to highest intensity. Intensity were set by the experimenter manually between blocks while MEPs were executed and recorded using SIGNAL software. The advantage of this technique lies in uncovering the pattern of excitability over a range of intensities which may help capture change from the intervention but also intersubject differences as participant response to TMS can be variable.

SICI, LICI, and ICF

Lastly, paired-pulse TMS localized over the FDI motor hotspot was applied using a single TMS coil. Firstly, SICI was evoked with a CS of amplitude 80% RMT followed 2.5 ms later by a 120% RMT TS. ICF was evoked using an 80% RMT CS followed 10 ms later by a 120% RMT TS. Lastly, LICI used a 120%

RMT CS followed by a 120% RMT TS with an ISI of 100 ms[17,32]. In each condition, 10 MEPs were evoked and recorded using SIGNAL software to be averaged together during analysis.

Visual Paired-Associative Stimulation (V-PAS)

V-PAS protocol was adapted from previous work[2]. As presented above, this study consisted of two sessions differentiated by the nature of the V-PAS intervention participants received. The rest condition was designed to closely reflect guiding literature to allow comparison with this work. Unfortunately, we were unable to recreate this protocol exactly as published as a time window for participant response during each trial must be incorporated. This resulted in our protocol executing at 0.37 Hz compared to 1 Hz in the literature[2]. However, 1 Hz repeated, single-pulse TMS (rTMS) is well known for its induction of LTD-like plasticity in the underlying cortex. This was observed following 1 Hz V-PAS as well resulting in an approximately 20% decrease in cortical excitability[2]. Fortunately, performing V-PAS at a slower frequency of 0.37 Hz should have circumvented much of the LTD-like plasticity induced at 1 Hz. We proposed a V-PAS intervention with 304 trials to ensure the intervention did not last a long duration or caused participants discomfort while still imparting measurable effects in the cortex. Previous work[2] observed consistent induction of LTP-like plasticity following 600 trials of V-PAS. The same group found non-significant results following only 150 trials of V-PAS indicating a minimum dose requirement of between 150-600 trials at 1 Hz to observe significant changes to cortical excitability. We found 304 trials completed at 0.37 Hz was sufficient to induce measurable change in the motor cortex.

Participants underwent V-PAS in the same seated position as during the baseline outcome measure assessment. Within session 1, participants always experienced resting V-PAS while session 2 exposed participants to active V-PAS. V-PAS was executed with SIGNAL software coordinated with a LabView program displayed on a monitor in front of the participant. Each trial began with the presentation of a cue screen for 500 ms, followed by a checkerboard screen for 200 ms, and concluded by display of the target screen for 2000 ms. Onset of the checkerboard evoked a VEP within the participant's left primary visual cortex and was followed up with a TMS pulse of intensity 120% RMT

over the FDI motor hotspot in the left hemisphere at the termination of the checkerboard screen. As such, TMS onset also signified the appearance of the target screen. During both V-PAS conditions, this sequence of events was repeated 304 times over an approximately 15min timeframe.

The sole difference between sessions related to the participant's involvement during the V-PAS intervention. In the Resting condition, participants sat quietly while fixating on a dot displayed on the monitor in front of them for the duration of the intervention. The Active condition required participants to prepare and execute a reach-to-touch during each trial. While the cue screen was displayed, participants in the active condition were instructed to mentally plan and visualize reaching to touch the target with their right index finger. These participants should have retained the target location in memory during the checkerboard screen presentation and maintained preparation for the upcoming motor response. Following the TMS pulse, participants were presented with the target screen which they will then reach and touch with their right index finger. Each contact was recorded using a touch sensitive screen layered over the display monitor.

Statistical Analysis

For single pulse MEP, recruitment curve, SICI, ICF, and LICI data, separate 2x2 repeated measures ANOVAs with factors of Time (Pre/Post) within session and V-PAS (Rest/Active) between session were conducted. SPOC-M1 paired-pulse TMS data was first tested using a 3-way repeated measures ANOVA with factors of Time (Pre/Post), V-PAS (Rest/Active), and Activity (NR/R). This was followed with 2, 2-way repeated measures ANOVAs across the V-PAS condition (Rest/Active) such that each ANOVA had factors of Time (Pre/Post) and Activity (NR/R). Hypotheses 1 and 3 were tested using pre-planned contrasts while hypothesis 2 tested for a significant interaction effect within the 2-way repeated measures ANOVA conducted as stated above.

Results

Sample Characteristics

A total of 16 individuals were invited to participate in this study. Of this number, 4 individuals presented with TMS thresholds greater than 70% of maximal stimulator output thus precluding them from the study due to device limitations. One individual observed an abnormal response to TMS resulting in deviations from expected MEP latency and amplitudes and as such was not enrolled in the study. The remaining 11 individuals participated in the full study. Of these 11 participants, one individual did not appear to fixate properly during the *active* V-PAS procedure and behavioural performance exhibited poor accuracy and response time. Due to these factors, this participant was removed from further analysis resulting in a sample size of 10 (5 male) being carried forward in the study.

Participant responses to both V-PAS interventions represented as MEP size following V-PAS relative to before V-PAS are shown in Figure 2. Cortical excitability changes following V-PAS were observed to be variable both in amplitude and polarity (enhanced or suppressed) between participants. While variation existed with participant responses to V-PAS, only 1 individual experienced opposite effects between sessions (participant 8 in Fig. 2). The remaining 9 participants either observed minimal change in excitability following V-PAS in 1 session or both sessions influenced them in the same direction. For statistical analysis, all 10 participants were analyzed together, regardless of individual response to V-PAS. Due to this approach and the variable response from V-PAS, some results presented may have blunted or absent effects. Statistical testing was completed using SAS University Edition; for all ANOVA tests, assumptions of normality, common variance, and sphericity were assessed and passed before reporting results.

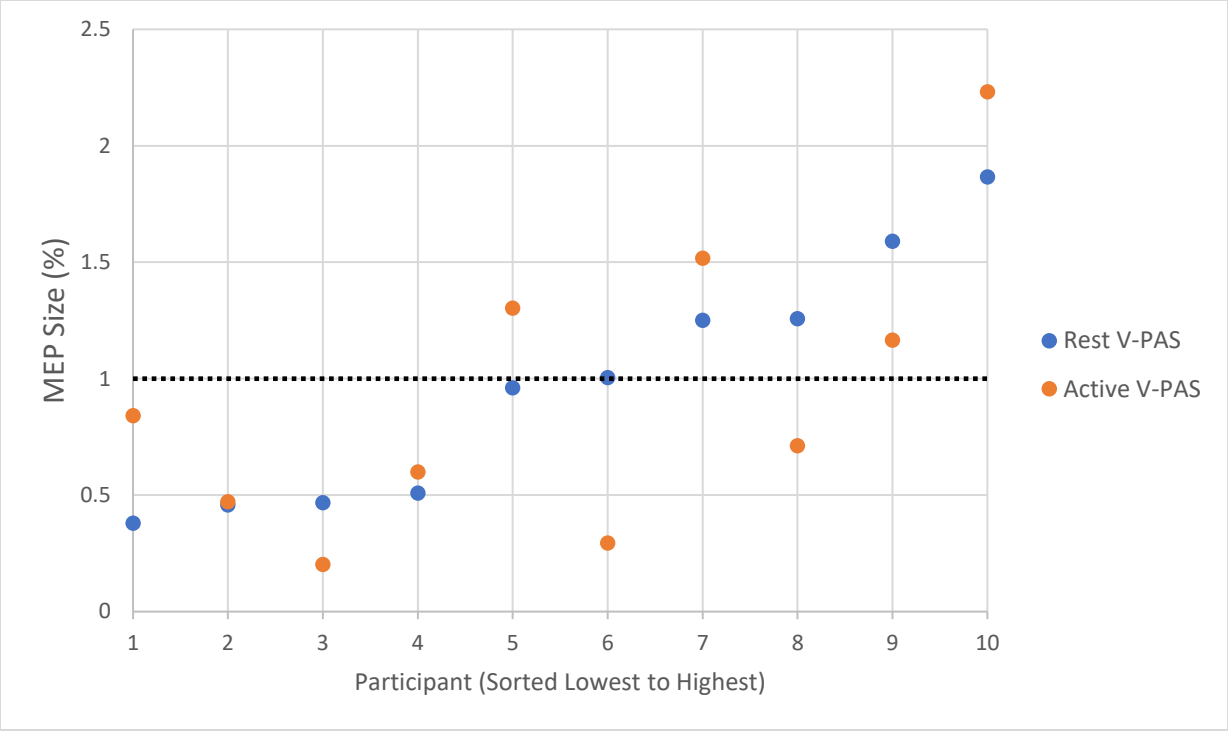


Figure 2: Participant MEP size following V-PAS. Data are represented as ratios of Post V-PAS/Pre V-PAS within a given intervention. Values of 1 visualized by the black dotted line represent no change from baseline following V-PAS.

Hypothesis Testing

Our first hypothesis stated that participants would exhibit greater MEP sizes following Resting V-PAS specifically. To test this, a paired T-Test was utilized comparing within session 1 across time before and after Resting V-PAS. Results of this test present a non-significant effect of Resting V-PAS on MEP sizes ($p > 0.05$) (see Fig 3). Therefore, resting V-PAS did not significantly increase MEP size on average which does not support our first hypothesis.

The second hypothesis for this study looked to build upon the first and postulated that MEP size would be increased significantly more by active V-PAS compared to resting V-PAS. A 2-way repeated measures ANOVA with factors Time (Pre/Post) and V-PAS (Rest/Active) was selected to assess for an expected significant interaction effect. The statistic showed no significant interaction between V-PAS and Time on MEP size ($F_{1,9} = 0.00$, $p = 0.9481$) (see Fig. 3) which does not suggest that Active V-PAS

facilitated MEP sizes to a greater extent than Resting V-PAS. Therefore, we do not support our second hypothesis.

Lastly, our third hypothesis proposed SPOC to M1 projections would be significantly elevated specifically following Active V-PAS and while preparing a reaching movement at the time of TMS onset. A 3-way repeated measures ANOVA was conducted with factors Time (Pre/Post), V-PAS (Rest/Active), and Activity (NR/R) was conducted to examine the data. A pre-planned contrast showed MEP size was significantly greater following Active V-PAS specifically and while preparing a reach ($p < 0.05$) (see Fig. 6). This finding guides us to supporting our third hypothesis.

Single Pulse TMS

MEP sizes observed prior to and following both V-PAS interventions are displayed in Figure 3. Presented are average participant raw MEP values. A two-way repeated measures ANOVA with factors Time (Pre/Post) and VPAS (Rest/Active) was run to investigate any differences between these timepoints. No main effect of time was found ($F_{1,9} = 0.25$, $p = 0.6315$) nor a significant interaction ($F_{1,9} = 0.00$, $p = 0.9481$); however, a main effect of VPAS was evident in the dataset ($F_{1,9} = 14.83$, $p = 0.0039$). These data suggest no differences within a session as a result of the intervention, though there is an apparent difference between sessions.

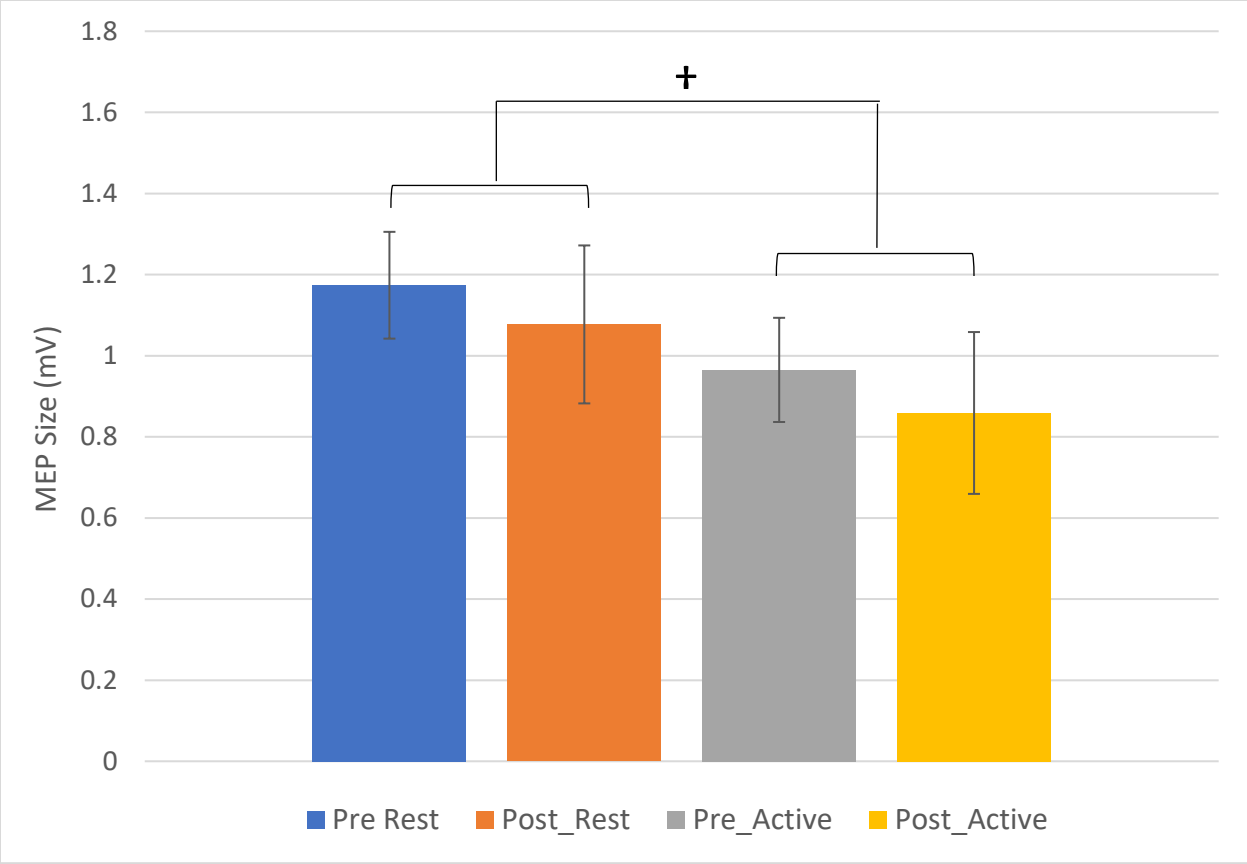


Figure 3: Average MEP sizes before and after resting and active V-PAS. Error bars represent standard error of the mean. † = $p < 0.01$.

Recruitment Curve

For statistical analysis, recruitment curve data was averaged across the 3 intensities evoked during the study. Data was grouped by time before and after V-PAS and by each intervention. Prior to averaging data across intensity, patterns of activity remained similar across time and VPAS condition via visual inspection (Figure 4a). Further highlighted through the adjacency of each line is the small degree to which conditions differ in this dataset. A two-way repeated measures ANOVA was performed with factors Time (Pre/Post) and VPAS (Rest/Active) to assess the averaged recruitment curve data (Figure 4b). No main effect of time ($F_{1,9} = 0.39$, $p = 0.5464$),V-PAS ($F_{1,9} = 0.45$, $p = 0.5316$), nor an interaction ($F_{1,9} = 0.00$, $p = 0.9864$); was revealed.

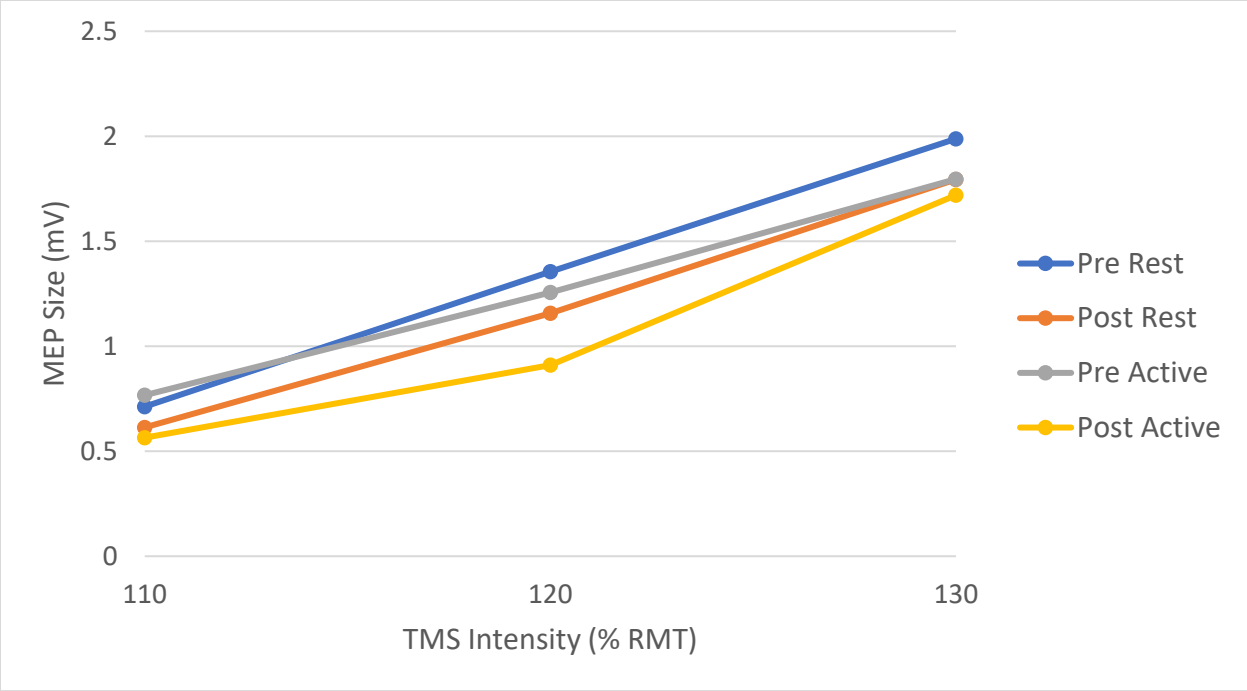


Figure 4a: Recruitment curve across 3 intensities. Intensity is a percentage of resting motor threshold (RMT). Data is raw, averaged across subjects within condition.

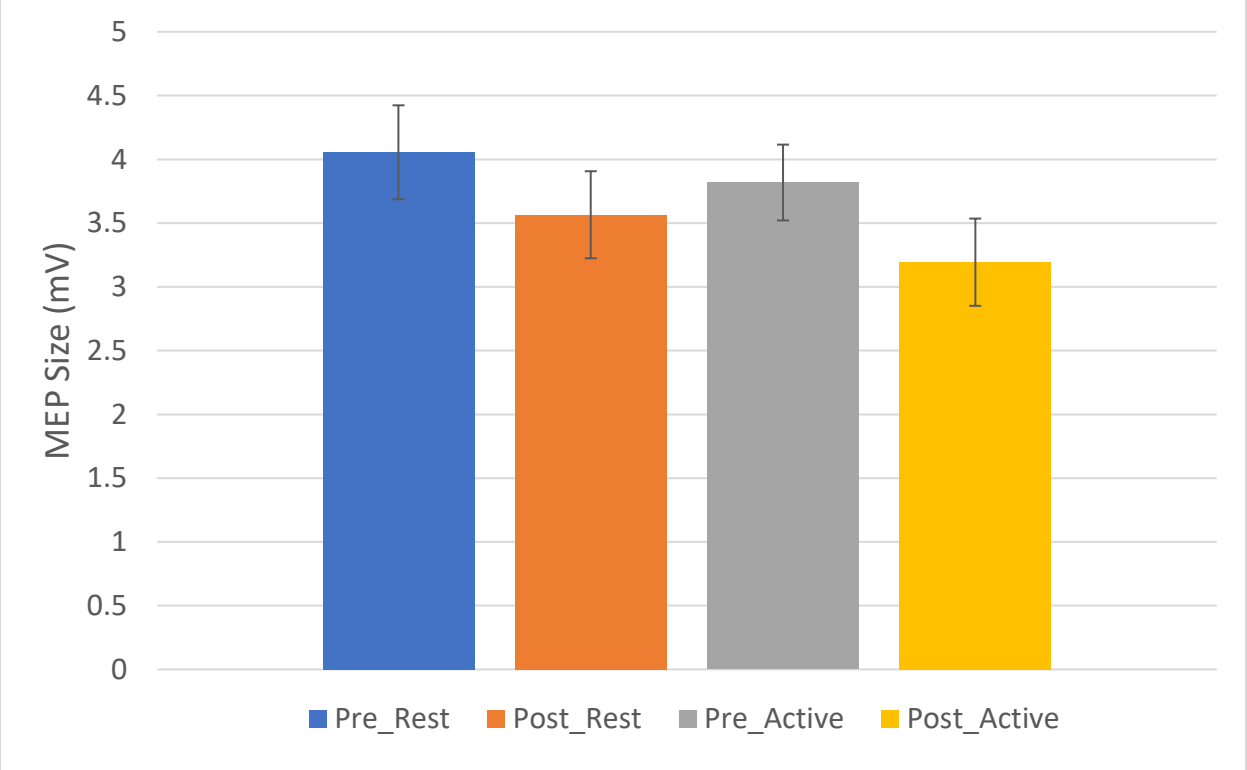


Figure 4b: Recruitment curve summed over 3 intensities (110, 120, 130% RMT). Error bars represent the standard error of the mean.

One-Coil Paired-Pulse (SICI, ICF, LICI)

For each of SICI, ICF, and LICI, all 10 trials were first averaged within each subject. Data were then normalized to the MEP amplitude found via the 120% RMT single pulse measure at each respective time point and session (i.e. Pre-Resting V-PAS SICI compared to Pre-Resting V-PAS 120% RMT and Post Resting-V-PAS SICI compared to Post Resting V-PAS 120% RMT). In this way, the general excitability changes induced from V-PAS are controlled for while sparing any specific changes related to receptor signalling. At this time, any observations outside of 3 standard deviations from the mean and the matching data point(s) within a session were removed from further analysis. For instance, a participant exhibited an abnormally large SICI value during the Pre-Resting V-PAS condition. This value in addition to the Post-Resting V-PAS value was removed from the analysis procedure to maintain equal observation counts within a session. During the Active V-PAS session, the participant presented values within 3 standard deviations from the mean at both time points and was included in the analysis for the Active V-PAS session. Lastly, data was averaged across participants at each timepoint for both sessions leading to a total of 4 observation groups.

A two-way repeated measures ANOVA on SICI data (Figure 5a) with factors: Time (Pre/Post) and V-PAS (Rest, Active) revealed no main effect of Time ($F_{1,9}$ = 1.50, P= 0.2320), V-PAS ($F_{1,9}$ = 0.03, p = 0.8645), nor an interaction ($F_{1,9}$ = 0.07, p = 0.7938). While no significant effects are observed, a slight disinhibition may be present across time points within each V-PAS condition. Similarly, ICF data (Figure 5b) showed no significant main effect of Time ($F_{1,9}$ = 2.26, p = 0.1450), V-PAS ($F_{1,9}$ = 0.20, p = 0.6607), nor interaction ($F_{1,9}$ = 0.07, p = 0.7938). Lastly, LICI data shown in Figure 5c observe no significant main effect of Time ($F_{1,8}$ = 0.04, p = 0.8498),V-PAS ($F_{1,8}$ = 1.73, p = 0.2015), nor interaction ($F_{1,8}$ = 0.07, p = 0.7960).

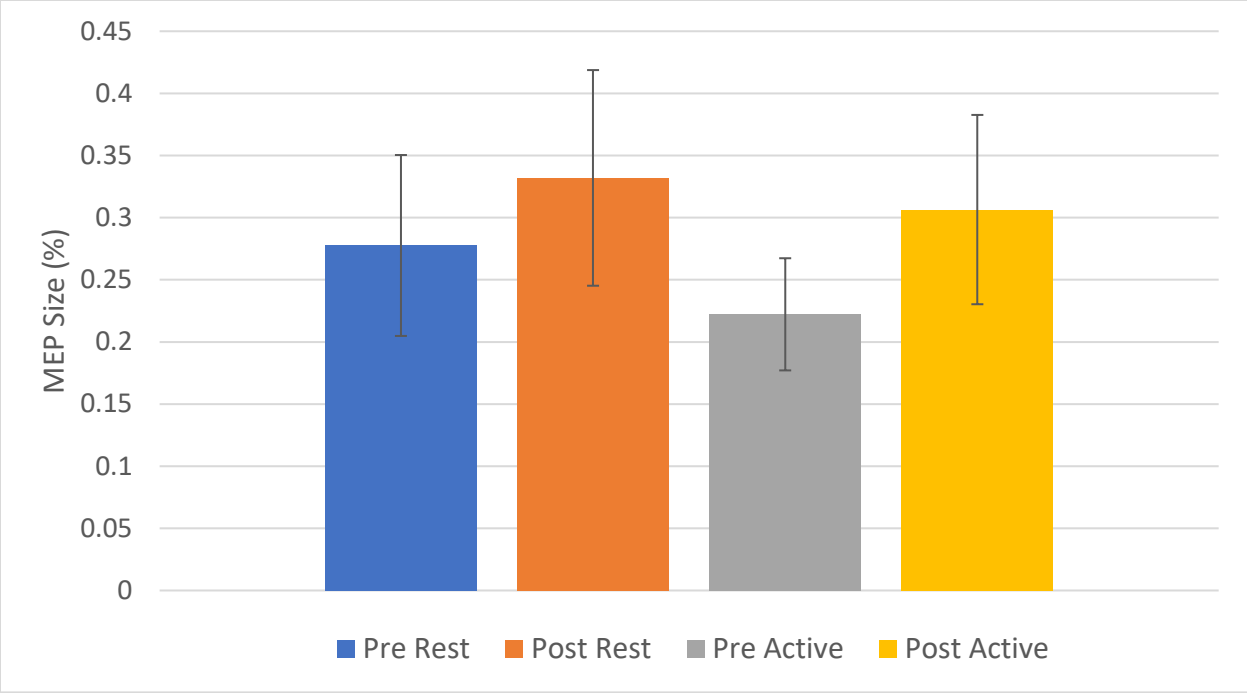


Figure 5a: Short-interval inhibition data. Data are displayed as a percentage of MEP intensity observed following a single TMS pulse at 120% RMT at the matching time point. Error bars represent standard error of the mean.

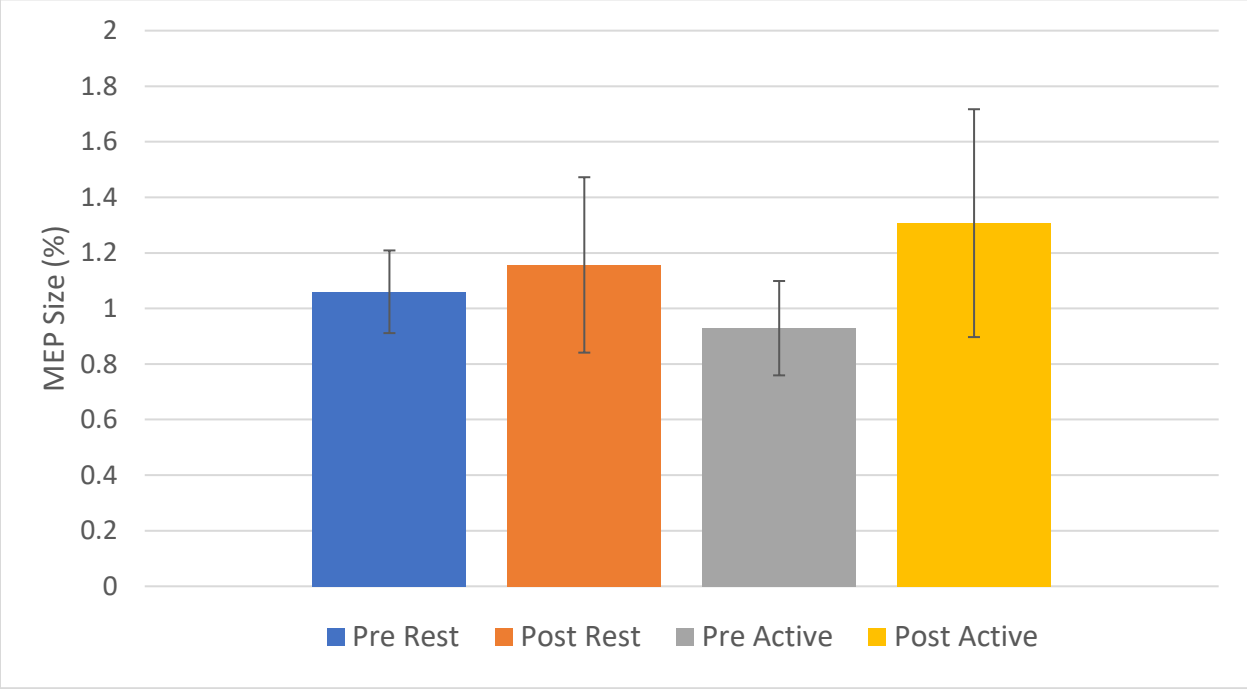


Figure 5b: Intracortical facilitation data. Data are displayed as a percentage of MEP intensity observed following a single TMS pulse at 120% RMT at the matching time point. Error bars represent standard error of the mean.

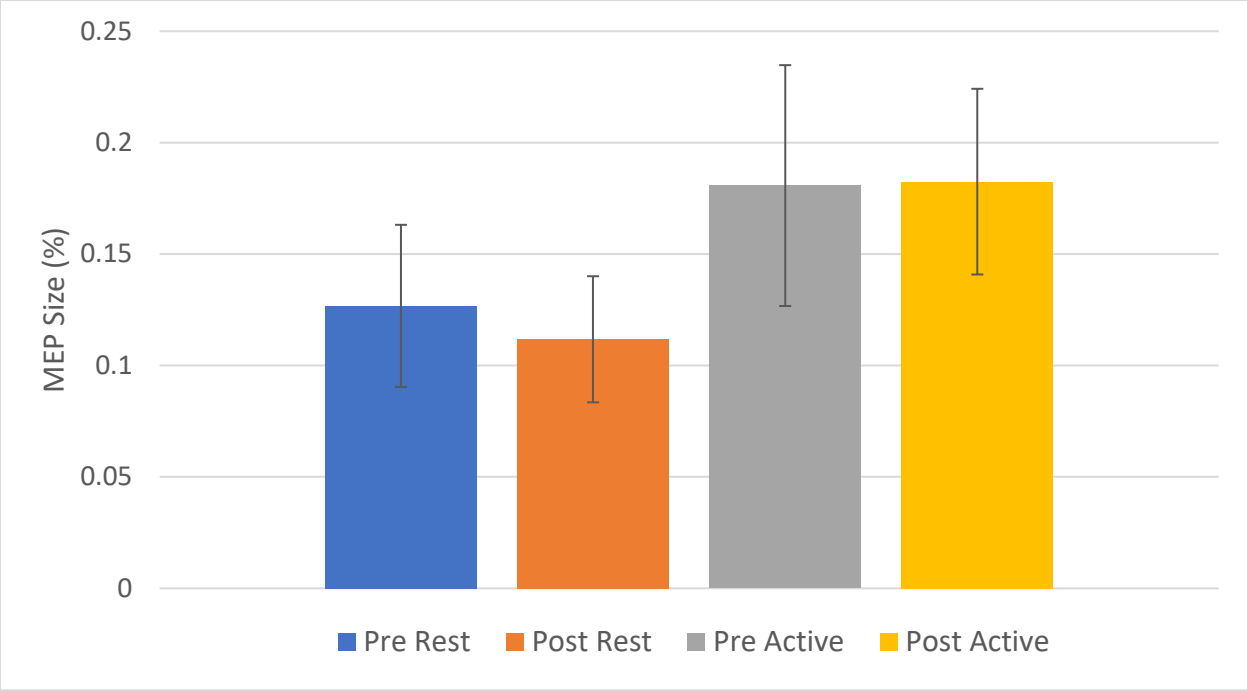


Figure 5c: Long-interval inhibition data. Data are displayed as a percentage of MEP intensity observed following a single TMS pulse at 120% RMT at the matching time point. Error bars represent standard error of the mean.

2-Coil Paired Pulse (SPOC to M1)

Within each participant, 24 trials were averaged to yield an average response during 8 different conditions generated by Time (Pre/post), Activity (Not reaching vs. reaching), and V-PAS (Rest/active). This average data was then normalized to each participant's MEP size following single pulse TMS at 120% RMT intensity; these values corresponded in time and V-PAS condition. At this time, one participant's session 1 data was removed from analysis due to values that extended beyond 3 standard deviations from the mean. As displayed in Figure 6, each condition appears proportionally larger on average than a 120% RMT stimulus alone. This observation was confirmed by paired 8 T-tests which compared MEP sizes following ppTMS between SPOC and M1 and single pulse TMS at 120% RMT intensity. These tests confirmed that all 8 conditions were significantly different from the 120% RMT baseline indicated by the black dotted line in Fig. 6 (all $p < 0.05$).

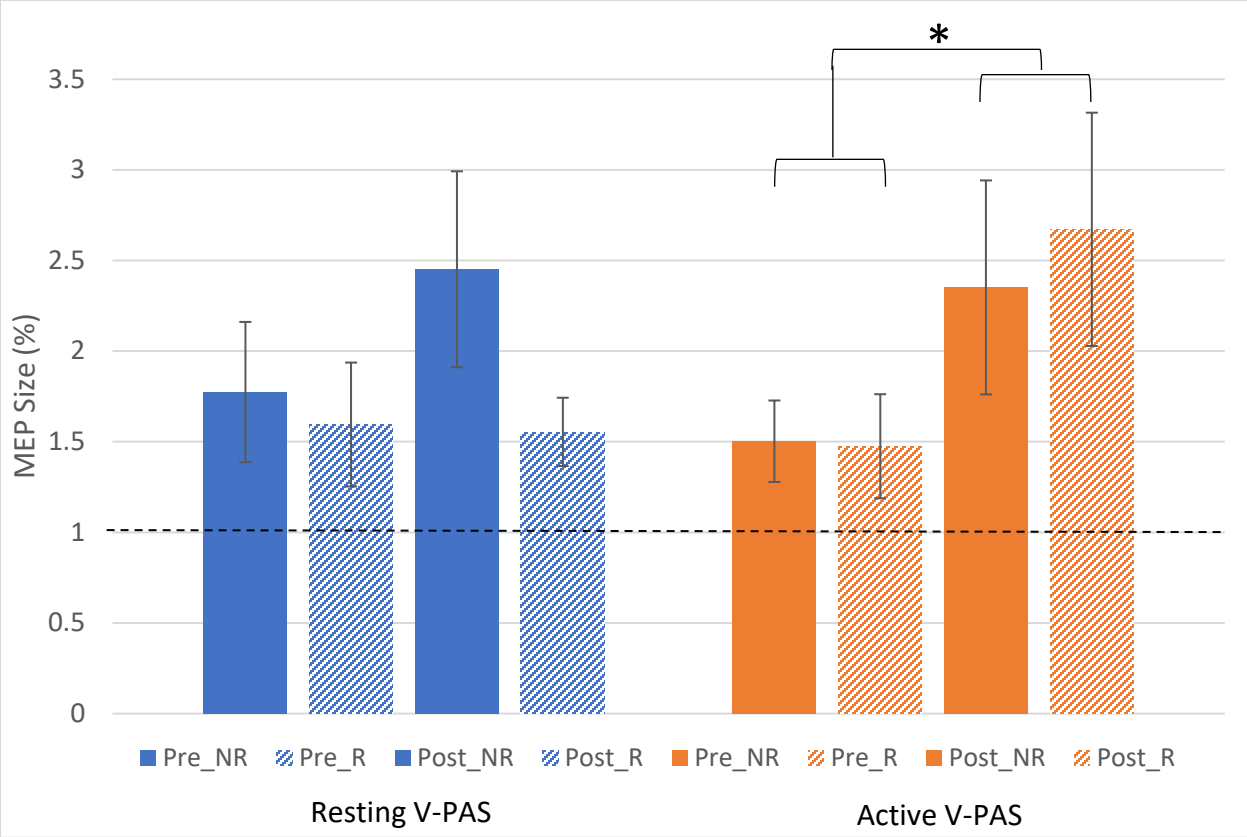


*Figure 6: 2-coil paired-pulse data represented as a percentage of MEP intensity observed following a single pulse of 120% RMT intensity. Pre/Post refers to time relative to V-PAS intervention. NR/R indicates the action during the trial, either not reaching (NR), or reaching (R). Error bars represent the standard error of the mean. * indicates $p < 0.05$*

To follow up on the ppTMS data, we executed a 3 way, repeated measures ANOVA with factors of V-PAS (Rest/Active), Time (Pre/Post), and Activity (NR/R). A natural log transformation was applied to obtain normality in the data. A significant 3-way interaction was discovered between the factors ($F_{1,9}$ = 8.82, p = 0.0208) in addition to a significant main effect of Time ($F_{1,9}$ = 6.47, p = 0.0315) but non significant main effects of V-PAS ($F_{1,9}$ = 0.00, p = 0.9868) or Activity ($F_{1,9}$ = 0.44, p = 0.5215). To investigate the interaction effect, 2 separate 2-way repeated measures ANOVAs were completed with Time (Pre/Post) and Activity (NR/R) as factors. The first 2-way repeated measures ANOVA utilized Resting V-PAS data specifically and found no significant main effect of Time ($F_{1,8}$ = 1.15, p = 0.3147) nor Activity ($F_{1,8}$ = 2.59, p = 0.1463) and no significant interaction effect ($F_{1,8}$ = 1.06, p = 0.3327). The second 2-way repeated measures ANOVA on Active V-PAS data revealed a significant main effect of Time ($F_{1,9}$ =

8.55, p = 0.0169) but no significant main effect of Activity ($F_{1,9}$ = 0.01, p = 0.9304) or significant interaction ($F_{1,9}$ = 1.69, p = 0.2257). $p = p = p = p =$

Split-Sample Exploratory Analysis

Due to the varied responses from our sample, we elected to examine trends in the data when the sample was split based upon V-PAS response. To divide the dataset, response to V-PAS was calculated as a ratio of single pulse MEP amplitude before and after V-PAS within a session. This generated 2 values for each participant: 1 for each V-PAS intervention which were then averaged together to provide an overall response score. Participants with a value greater than 1 were sorted as "Up" responders while participants with a score less than 1 were sorted as "Down" responders. One participant appeared to exhibit opposite reactions to V-PAS between sessions and was not sorted into either group (See participant 8 in figure 7). After sorting, 4 participants were selected for the "Up" group, while 5 were deemed "Down" responders as shown in Fig. 7. Due to the small sample sizes, statistical tests were not run on these groups. Similarly, only outcome measures which showed interesting trends will be reported as several measures presented too much variability to warrant interpretation.

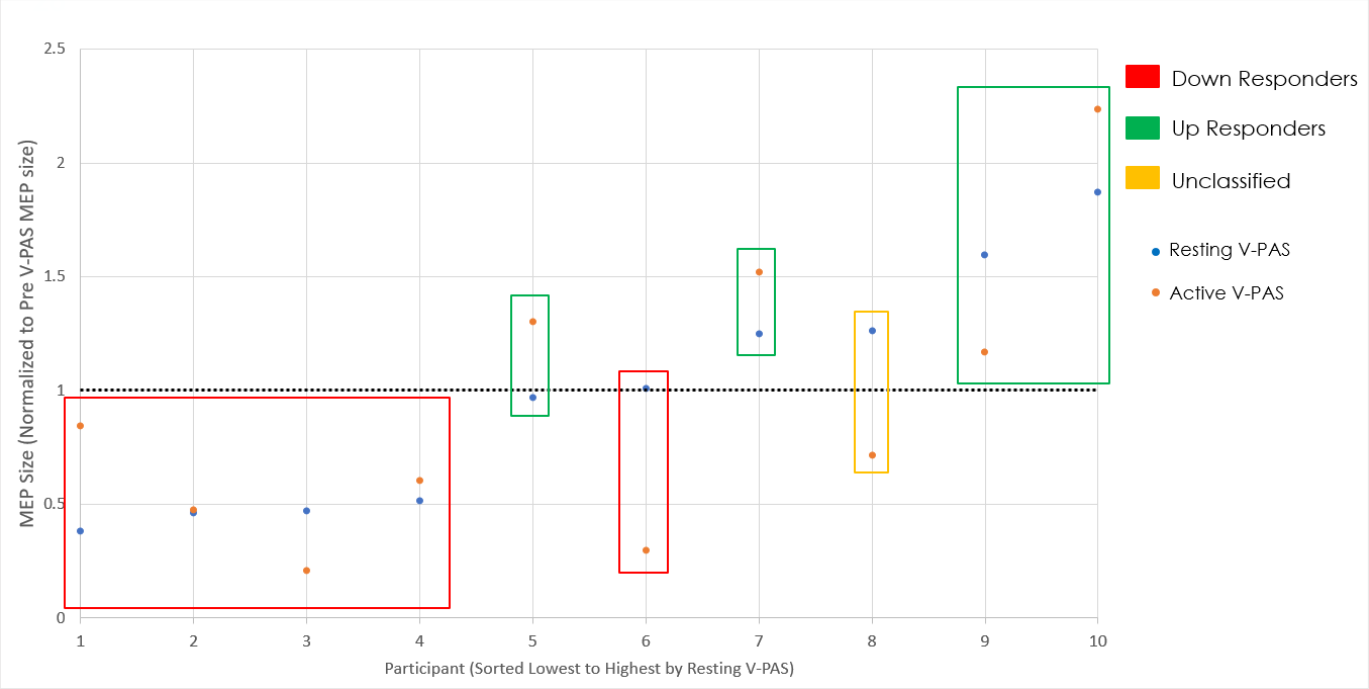


Figure 7: Participant responses to Resting and Active V-PAS. Evident is the variable influence on excitability changes following V-PAS. Highlighted are groupings applied to the sample based upon average participant response to V-PAS.

Examination of single pulse MEP data in Figure 8a and 8b presents trends closer to what was hypothesized during this study's design. Between the groups, the pattern of excitability change appears mirrored. At the post timepoint following each intervention, a sizeable change in excitability is observed in each group's respective direction. Furthermore, within an intervention, it appears active V-PAS may evoke slightly larger effects compared to resting V-PAS in both responder groups. However, it is important to note that much of the excitability change we see across all conditions appears to be driven by a time effect, irrespective of the form of V-PAS experienced.

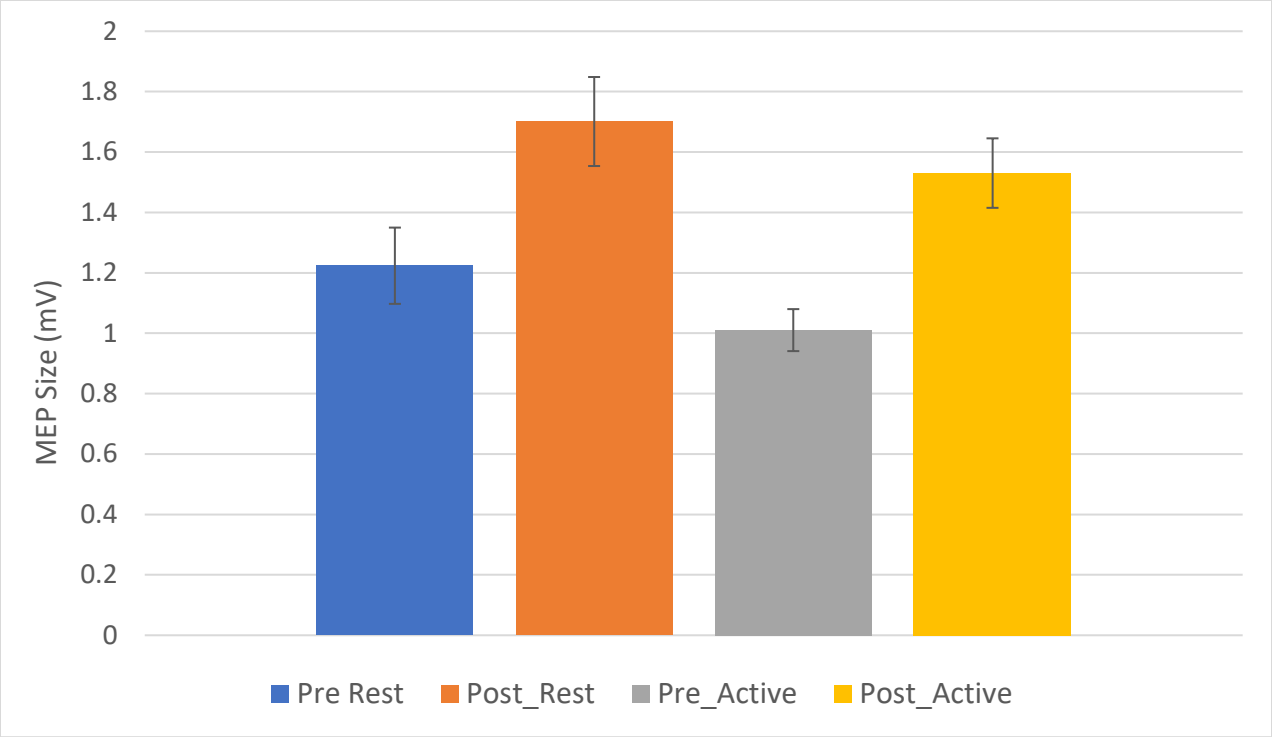


Figure 8a: Single pulse MEP data from participants sorted as "Up" responders. Error bars represent standard error of the mean.

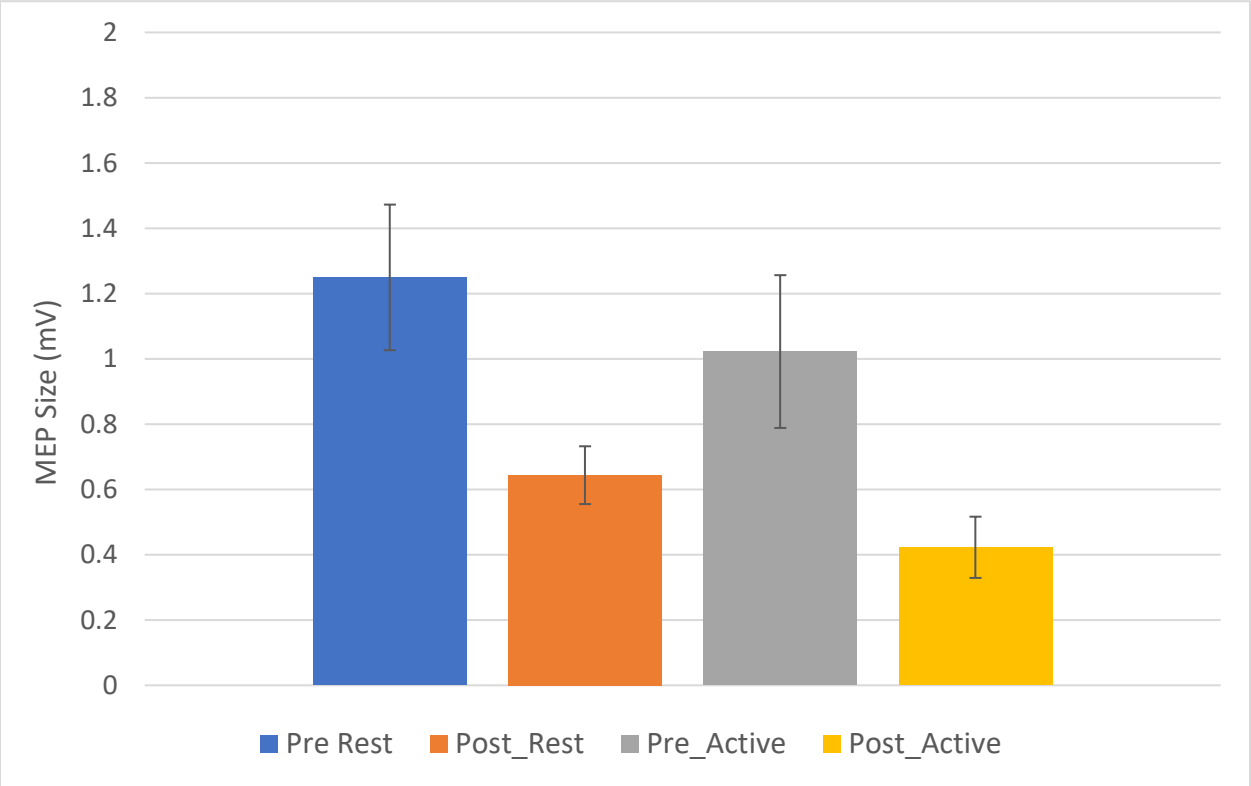


Figure 8b: Single pulse MEP data for participants sorted "Down" responders. Error bars represent standard error of the mean.

An interesting contrast also emerged between groups within the SICI measure as displayed in Figure 9a and 9b. While the data is variable in places, we do see similar trends between V-PAS conditions within each responder group. The *Up*-responder group appeared to experience decreased MEP sizes following both resting and active V-PAS. Interestingly, the *Down* responders tended to see larger MEP amplitudes after both V-PAS conditions. In contrast to the single pulse MEP data in Fig 8a and 8b, there is little indication of a main effect of V-PAS condition.

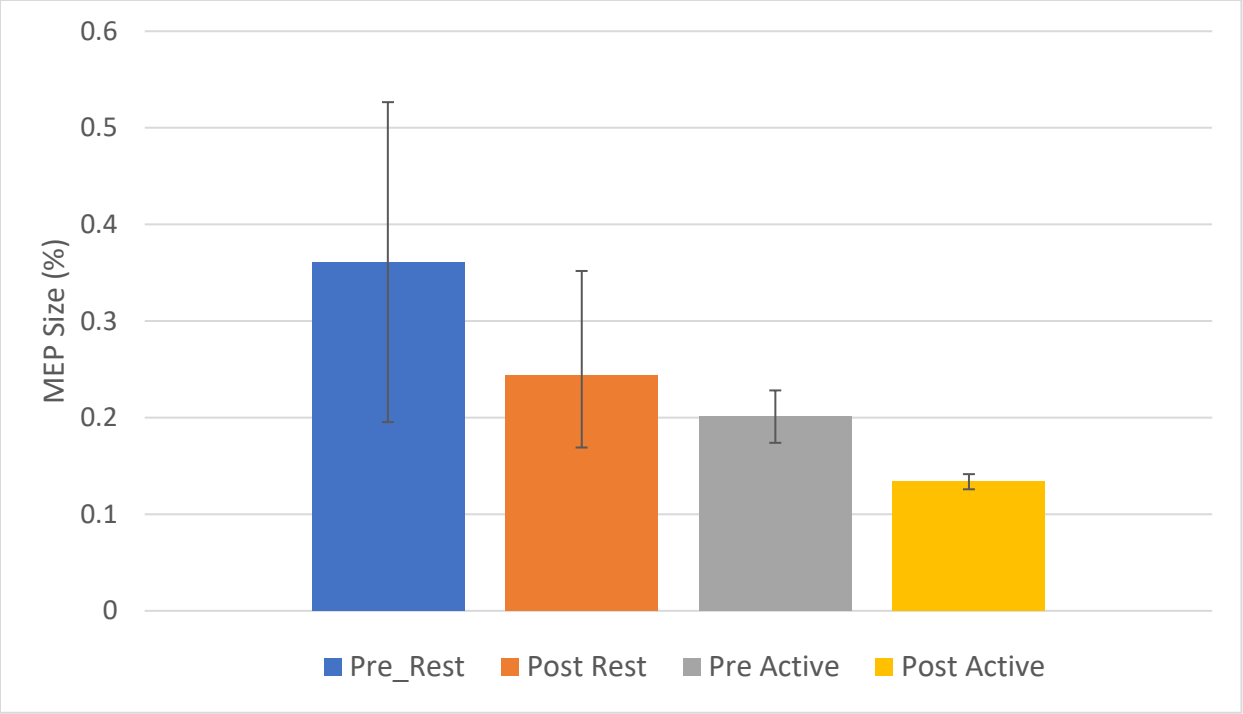


Figure 9a: Short-interval intracortical inhibition data for participants sorted "Up" responders. Error bars represent standard error of the mean.

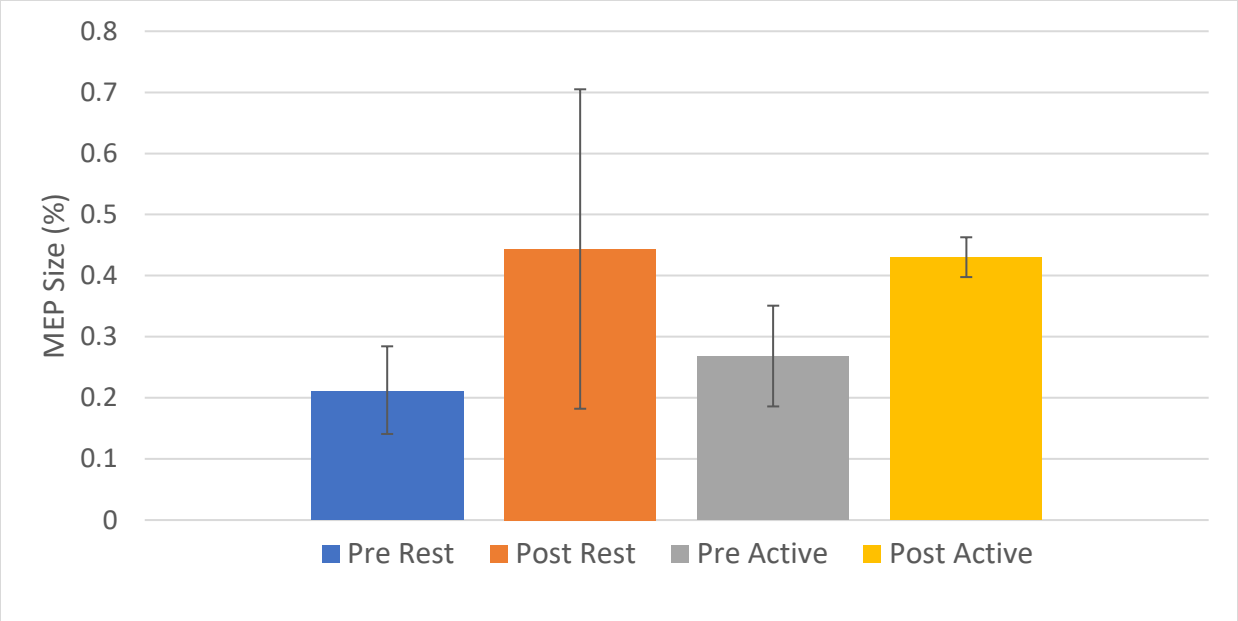


Figure 9b: Short-interval intracortical inhibition data for participants sorted "Down" responders. Error bars represent standard error of the mean.

Discussion

Sample Characteristics

Given our inclusion criteria, the data appear to be a fair sample of the population we were targeting. We enrolled an equal number of men and women and several potentially confounding factors were controlled for with our inclusion criteria (e.g. handedness). This was further represented in our raw data which consistently passed tests of ANOVA assumptions during many of our statistical tests for our outcome measures. Although unforeseen, our sample also was approximately equally split in how they responded to the V-PAS intervention. However, while this finding may lend support to the diversity of the sample, it is yet unclear the response characteristics following V-PAS observed in the population. In general, participants responded in the same direction (upward/downward excitability changes) across both V-PAS interventions. However, participants were influenced to different degrees with some individuals experiencing no effect following session 1 with a sizeable effect in session 2. It is unclear exactly why these individuals responded only to one session. Although, it is attractive to point to participant arousal as a source of inter- and intrasubject variability. An effort was made to observe these effects with oculography and behavioural responses; although we found no cause for concern, we do not have the sensitivity with these measures to interpret each subject's attentional foci or general arousal state. During active V-PAS, participants were much more involved in the intervention which may have maintained attention to a larger degree thereby contributing to some of the differential effects between sessions.

Single Pulse MEP

With the combined sample, there was no observed effect of V-PAS within session (Fig. 3). However, there was a significant difference between sessions such that session 2 on average yielded significantly lower MEP sizes. It is unlikely this observation is an experimental effect. Each participant

waited several days between sessions for a washout period to ensure no effects carried over between sessions. V-PAS effects have been shown to endure for approximately 1 hour[2] before excitability within the primary motor cortex returns to baseline levels. Furthermore, interventions which achieve long-term effects generally consist of 1000 or more pulses at higher frequencies than experienced in this study[41,42]. Lastly, rTMS of < 1 Hz has been shown to not significantly alter markers of neuroplasticity (BDNF and GluR1 subunit of AMPA receptors)[43]. As it is very unlikely carryover effects were present, this effect could be attributed to a learning effect on the side of the experimenter. We see a reduction in "Pre" timepoint MEP sizes down closer to 1mV across sessions which is the target intensity to obtain. It may be the experimenter gained experience in this process over time. This could have been accounted for by randomizing session order between participants, however we chose to order resting V-PAS before active V-PAS to ensure participants had equal exposure to the intervention apparatus at each timepoint throughout the study.

We do not observe a significant effect of time within either session which suggests our V-PAS intervention was ineffective at altering cortical excitability. Given the split nature of the sample in their responses to V-PAS, it is possible any true effect of V-PAS is being masked by the conflicting effects between participants. Data presented in Figures 8a and 8b suggests the possibility of a significant effect on excitability driven by the V-PAS interventions. Within the "Up" responders, those who experienced elevated MEP amplitudes following V-PAS, we observe an approximate 40% and 50% increase in MEP size following resting and active V-PAS respectively. This was met with a similar effect for the "Down" responders who observed a decrease in MEP amplitude of 50% and 60% following resting and active V-PAS respectively. Assuming these values to be accurate, we can compare effect sizes with that of Suppa, et al (2015). This group observed an approximate 25% increase[2] in cortical excitability utilizing what is comparable to resting V-PAS in this study. Our potential effects of approximately 40 – 50% following resting V-PAS are dramatically larger which brings attention to the differences between our

interventions. We presented a spatially-larger visual stimulus which may contribute to this difference with a potentially stronger visual stimulus being propagated to motor areas as a result. However, the primary difference is likely due to the frequency with which we executed V-PAS compared to Suppa, et al (2015) (0.37 Hz vs. 1 Hz). When V-PAS was executed at 0.25 Hz by Suppa et al (2015), an approximately 50-60% increase in MEP size was observedwhich is much closer to the effect our study finds. It is known that 1 Hz rTMS induces transient LTD-like plasticity in the targeted region[44] which likely explains the differences between the frequencies tested by Suppa, et al.

When comparing effects within session in the split analysis data, we see a possible effect of V-PAS intervention such that active V-PAS appears to influence cortical excitability more than resting V-PAS. Certainly, with the small sample sizes, it is difficult to interpret this finding. However, we do notice this trend in both the Up and Down responders which may add credence to this observation. It is possible with a larger sample size this effect becomes more pronounced and achieves significance. In this event, our hypothesis stating MEP amplitudes will be significantly enhanced following active V-PAS compared to resting V-PAS could be confirmed. However, if there is indeed a true effect related to reaching action during active V-PAS specifically, the current data suggests that its effect is overshadowed by the changes induced by resting V-PAS alone.

Recruitment Curve

Given that recruitment curve slopes remained relatively equal, it is not surprising to see a similar trend to what is observed from the single pulse MEP data (Fig. 2). The advantage and intent in including a recruitment curve outcome measure was to track excitability over a range of intensities compared to just a single intensity. Since we found no dramatic changes in the progression along the curve between intensities, this measure becomes very comparable to our single pulse MEP data which explains the similar findings between these measures.

Since each condition follows a similar pattern with excitability changes affecting a given condition relatively uniformly, this implies that the population of neurons being influenced by V-PAS in both rest and active can be activated by both low intensity and high intensity TMS. More specifically, this information suggests that any mechanism of change was not likely related to the introduction of a group of neurons with thresholds that are significantly elevated compared to the neurons targeted prior to V-PAS. However, this does not indicate if changes are facilitated through rapid synaptic efficacy alterations within previously active networks, and/or if V-PAS encourages the activation of silent synapses and/or the uncovering of latent ones[1].

One-Coil Paired-Pulse

The full dataset does not exhibit any significant differences within the SICI outcome measure largely due to the variability within the data (Fig. 5a). Although, we do see a potential disinhibition effect upon MEP sizes following V-PAS in both sessions. Further speculation also points to a slightly larger effect following active V-PAS compared to resting V-PAS. Neither of these observations achieved significance; however, when the data was grouped according to the nature of V-PAS response we see evidence suggesting that effects in the whole dataset are once again masked due to dichotomous effects within the sample. The split data presents a disinhibition effect (Fig. 9b) specifically within the Down responder group similar to observed in the whole dataset. While it is difficult to compare across sessions due to the variability, it appears that SICI was enhanced following V-PAS selectively in the Up responders. This is consistent with the single pulse MEP data in two ways. First, the whole sample generally presented net characteristics associated with the Down responder group. Second, we observe opposite effects of V-PAS between responder groups for both single pulse MEP and SICI measures. However, we have no indication in this instance to propose that active V-PAS produced a greater effect than resting V-PAS.

Officially, there is no significant effect of time or V-PAS condition to discuss. Given that there is a strong trend which may become a significant effect provided larger sample sizes (include estimate here) and uniform participant responses to V-PAS, the implications warrant a brief discussion. A change in the amount of SICI observed implies that $GABA_A$ receptors are implicated in facilitating some, or all of that change mechanistically[25]. Given that GABA is associated with inhibitory influence upon the post-synaptic membrane, it is interesting to find that Up responders appear to exhibit greater inhibition from SICI and thereby greater influence from $GABA_A$-related signalling (Fig. 9a). Furthermore, Down responders observed less inhibition from SICI corresponding with less $GABA_A$-related signalling (Fig. 9b). On the surface this appears counterintuitive, one would expect Up responders to present with less $GABA_A$ activity and Down responders more. However, these observations may indicate that the neurons activated by TMS pass through an additional inhibitory projection prior to influencing motor output. In this way, increased GABAergic activity would lead to a disinhibition effect upon a consecutive MEP and vice-versa following decreased GABAergic activity. This additional projection may reside in motor preparatory areas such as the supplementary motor area or premotor cortex as this would prevent these neurons from directly being influenced by the TMS pulse.

Similar to SICI, there is no significant effect observed within ICF data (Fig. 5b) which does not confidently implicate glutamate as a mediator of neuroplastic change following V-PAS. Although small, there is a potential enhancement of the MEP following V-PAS. This is more noticeable following active V-PAS specifically which may point to a glutamatergic mechanism[26,27] underlying greater induced effects of V-PAS when a reaching movement is incorporated.

Conversely, we do not observe a clear effect of V-PAS on $GABA_B$[28] activity as assessed by LICI. This does make sense as short-term plasticity is associated in part with trafficking of non-NMDA receptors specifically to and from the post-synaptic membrane[1]. Being that the $GABA_B$ receptor is

metabotropic in nature, it is possible that the influence of this receptor will change some time beyond the early stage.

Two-Coil Paired Pulse

In all paired-pulse conditions between SPOC and M1, a significant increase in MEP size was observed compared to single pulse TMS alone (Fig. 6). This finding suggests SPOC exhibits a net excitatory effect upon the primary motor cortex which is consistent with previous literature[33]. Each of the 4 baseline conditions appear relatively equal which adds confidence that there were no carryover effects within our dataset and that subject variability remained relatively constant. Connections between SPOC and M1 are thought to be mediated through areas mIPS and AG which then track to premotor cortex prior to reaching the primary motor cortex[36]. Interestingly, we found significant facilitatory effects of these projections during all conditions including rest whereas Vesia et al (2013) only see facilitatory effects when a participant actively prepares a reaching movement. We observe a MEPs sizes of approximately 150% of the test stimulus alone when participants remained at rest and approximately 250% of the test stimulus alone while participants were reaching. This compares to Vesia et al (2013) who observed no significant increase in MEP sizes at rest and approximately 130% of the test stimulus while participants prepared a reaching movement. Given the small sample sizes in our study (10) and Vesia et al (2013) (7), it is possible these results are born of intersubject variability. Alternatively, our protocol did present participants with the required visual stimuli (i.e. a target) which may have permitted subconscious movement planning accompanied with suppression of that motor response even though participants were instructed to remain at rest.

Data from this outcome measure begin to reveal how cortical networks may be differentially modulated by the two V-PAS interventions. The following observations have been organized into Figure 10 which depicts a possible model explaining the apparent differential effects from the V-PAS

intervention. The model presents simplified connections between V1 and premotor cortex which is then connected to M1. This portion highlights projections which are thought to be influenced by V-PAS as described in previous literature[2]. Specifically, resting V-PAS appears to modulate premotor to M1 projections[2]. Examining ppTMS from our study (Fig. 6) displays a couple interesting findings which help build our proposed model. The first is that SPOC to M1 projections tested at rest (NR condition) appear to be influenced by both resting and active V-PAS. This modulation is a significant effect following the active V-PAS condition but fails to reach significance following resting V-PAS likely due to baseline variability. Given that both interventions see a similar effect size within the "NR" condition, we will treat this as a true effect in both resting and active V-PAS conditions. What this implies is that SPOC to M1 projections appear facilitated following both resting and active V-PAS. That is to say, these projections are influenced in some way by V-PAS independently of whether the participant prepares reaching responses during the intervention. Our proposed model reconciles this finding by presenting SPOC to M1 projections sharing a common or similar projection to M1 from premotor cortex. In this way, resting V-PAS will influence premotor cortex to M1 which will modulate visuomotor networks as thought[2] but also target terminal projections from the SPOC to M1 network. This effect would likely be a result of premotor cortex to M1 projections between the two networks being physically shared or having spatially close projections; either of which option may expose both networks to effects from resting V-PAS. However, this does not explain the clearly selective influence on SPOC to M1 projections when participants are reaching (R condition) following active V-PAS selectively. The primary difference to consider in this case is the presence of motor preparatory networks which will be utilized during the active V-PAS intervention. These networks encompass many regions within the brain, some of which likely include the supplementary motor area, premotor cortex, cingulate cortex, and parietal association regions[45] as well as basal ganglia[46] and cerebellum[47] to name a few. We contest the important difference is that these motor preparatory projections influence M1 in a way which exposes them to V-PAS effects

selectively when they are recruited during the intervention (i.e. active V-PAS). In Figure 10, we propose there are spatially distinct projections between premotor cortex and M1 which are not exposed to excitability modulation from resting V-PAS. Therefore, when motor preparation networks are engaged during ppTMS in the reaching condition, we see modulation upon MEP sizes following resting V-PAS. This modulation appears to be significantly altered following active V-PAS however.

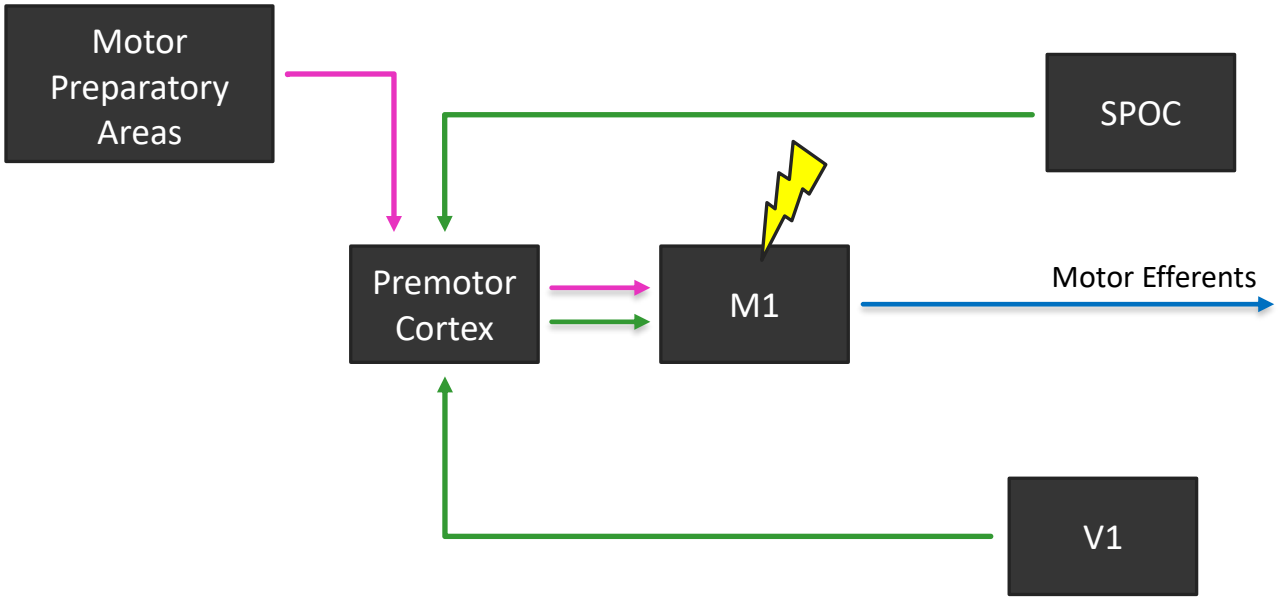


Figure 10: Schematic depicting simplified, relevant possible connectivity being influenced by V-PAS.

Limitations

Our ability to directly compare with work published by Suppa, et al (2015) was hampered in this study since we did not replicate the exact same conditions between studies. Most notably, our visual stimulus differed in its characteristics. We presented participants a visual stimulus that subtended approximately twice the visual angle cited by Suppa, et al (50° vs. 24°) and resultantly had larger checks within the checkerboard (5° vs. 0.5°). However, we did present a similar number of checks to participants (9x5 vs. 8x4). The differences between our setups evolved in large part from the viewing distance which Suppa, et al included of 70cm while we required a shorter distance, settling on 40cm. We chose the viewing distance of 40cm because participants were required to be able to comfortably reach to touch the screen from where they were seated. We considered adjusting the size of the visual stimuli to match the visual angles provided by Suppa, et al but decided against this to ensure our cue and target stimuli remained salient and spatially disparate from each other.

Following in the footsteps of Suppa et al, we did not feel the need to assess P100 latency in each participant prior to their first experimental session. While it does not appear that timing of stimuli was a significant issue, given the dichotomy observed in the sample following V-PAS, having visual waveform latencies to refer to would lend confidence to these findings. Results from Suppa et al show LTD-like plasticity only at 140 ms with ISI ranges of 160 ms and 180 ms inducing no significant change in cortical excitability. Therefore, even if our ISI of 200 ms was too brief for a participant, we would expect to see no change rather than a significant reduction in cortical excitability.

A third limitation to the study relates to the reaching component within our study. During the intervention, we anticipated reaching would augment the V-PAS protocol through facilitating neuroplastic change related to V-PAS specifically. However, we did not include a control to assess the effect of repetitive reaching itself on the motor cortex. As such, it is unclear what effects observed from

active V-PAS are related to V-PAS mechanisms compared to effects one would see after simply reaching to point for 304 trials. It has been shown that repetitive bimanual training will facilitate an expansion of the trained muscle representation within M1 but does not significantly increase the MEP size localized to the motor hotspot[48]. While participants in this study completed unimanual rather than bimanual training, these results approximate the physiologic changes we may have observed independent of the V-PAS intervention. The ideal method to control for a purely reaching effect would be to include a third session where the same participants come in and do a full session without the presence of TMS during the intervention. Including a third session would still permit a repeated measures approach to the study.

During the intervention, electrooculography and behavioural performance were recorded to assess if the participant was engaged with the intervention. This included the detection of any eye movements leading up to the onset of the VEP and observing where participants touched on the computer screen. Eye movements were a valuable tool to ensure the visual stimulus was being delivered properly and our behavioural measure helped assess how accurately our participants were performing. Unfortunately, our behavioural measure did not help us during resting V-PAS as no reaching was performed. However, neither of these measures allowed us to fully verify a participant's attention and focus. One concern in this regard is the difference in attentional demand between sessions. During active V-PAS, participants are focused on executing a reach to touch movement every few seconds. This task will draw attention at least during each reach likely but still leaves room for mind wandering following each reach. Resting V-PAS however has no attention-grabbing aspects to it, participants were simply instructed to maintain fixation at the central red dot for the duration of the intervention. Even though the cue, checkboard, and target stimuli were presented to participants, they were not required to attend to their precise location and integrate a movement to that target. Therefore, resting V-PAS contained arguably an approximately 15min block of uninterrupted time which welcomed mind wandering through an admittedly uninteresting task. This limitation could also mean some of the effect

we see from active V-PAS may have attentional effects contributing as well. However, currently effect sizes between resting and active V-PAS interventions are not significantly different which could indicate an attentional effect or reaching effect as described previously did not differentially influence the interventions. One method to engage participants more during both V-PAS interventions would be to include symbols throughout the intervention presented between a select number of trials for participants to count and report after the intervention. Alternatively, the protocol could be organized such that periodic gaps are spaced randomly throughout the intervention protocol and participants are instructed to count those gaps and report at the end.

Future Directions

Beyond the study's limitations, many important observations were found from this work which will help guide future research efforts. A remarkable finding in the present study was the approximately equal split response across participants following V-PAS. This phenomenon should be further investigated to uncover potential predictors regarding an individual's response to V-PAS. Additional research will allow us to better understand what percentage of individuals on average experience elevated or depressed cortical excitability following V-PAS. It would be wise for these future projects to include an initial screening session to confirm eligibility and to classify each participant ahead of time based on their response to V-PAS. This early session would only need a subset of outcome measures with a V-PAS intervention delivered in the middle. Screening of participants will be critical to ensure consistent effects are observed within each group which will in turn facilitate observable effects in the dataset. Genetic testing may also be wise for future studies which could correlate specific gene(s) (see if there are any to look into) with an individual's response to V-PAS. If a correlation is found, the correlated gene(s) may suggest subtle differences in connectivity for example which lead to the observed differences in V-PAS response.

V-PAS proved to be an effective technique capable of augmenting cortical excitability. However, the effects of this intervention were not consistent across participants. It is unclear with the current dataset if adding a reaching movement during V-PAS increases the resultant effects beyond that of V-PAS alone. For V-PAS to become a common intervention protocol in future research and/or clinical practice, it will be important to understand the proportion of the population that will experience MEP facilitation or depression. Once a movement response is involved in this protocol, there is the capacity for additional cortical networks to become involved related to that reach. Each of these networks adds

potential variables that can alter a participants' response to the intervention. In future, care must be taken to control for as many of these variables as possible to yield a more consistent effect.

References

1. Butler, A. J. & Wolf, S. L. Putting the brain on the map: use of transcranial magnetic stimulation to assess and induce cortical plasticity of upper-extremity movement. *Phys. Ther.* **87,** 719–736 (2007).

2. Suppa, A., Li Voti, P., Rocchi, L., Papazachariadis, O. & Berardelli, A. Early visuomotor integration processes induce LTP/LTD-like plasticity in the human motor cortex. *Cereb. Cortex* **25,** 703–712 (2015).

3. Martin, J. *Neuroanatomy Text and Atlas*. (McGraw-Hill, 2012).

4. Chapman, C. S., Gallivan, J. P., Culham, J. C. & Goodale, M. A. Mental blocks: fMRI reveals top-down modulation of early visual cortex when obstacles interfere with grasp planning. *Neuropsychologia* **49,** 1703–1717 (2011).

5. Tosoni, A. *et al.* Resting-state connectivity and functional specialization in human medial parieto-occipital cortex. *Brain Struct. Funct.* **220,** 3307–3321 (2015).

6. Born, R. T. & Bradley, D. C. Structure and Function of Visual Area Mt. *Annu. Rev. Neurosci.* **28,** 157–189 (2005).

7. Galletti, C., Fattori, P., Gamberini, M. & Kutz, D. F. The cortical visual area V6: Brain location and visual topography. *Eur. J. Neurosci.* **11,** 3922–3936 (1999).

8. Goodale, M. A. & Milner, A. D. Separate visual pathways tor perception and action. *Trends Neurosci.* **15,** 20–25 (1992).

9. Livingstone, M. . & Hubel, D. Segregation of Form, Color, Movement, and Depth: Anatomy, Physiology, and Perception. *Science (80-.).* **240,** 740–749 (1988).

10. Maunsell, J., Nealey, T. & DePriest, D. Magnocellular and parvocellular contributions to responses in the middle temporal visual area (MT) of the macaque monkey. *J. Neurosci.* **10,** 3323–3334 (1990).

11. Milner, A. *et al.* Perception and action in'visual form agnosia'. *Brain* **114,** 405 (1991).

12. Goodale, M. A., Milner, A. D., Jakobson, L. S. & Carey, D. P. A neurological dissociation between perceiving objects and grasping them. *Nature* **349,** 154–156 (1991).

13. Gréa, H. *et al.* A lesion of the posterior parietal cortex disrupts on-line adjustments during aiming movements. *Neuropsychologia* **40,** 2471–2480 (2002).

14. Rossetti, Y. *et al.* An automatic pilot for the hand in human posterior parietal cortex: toward reinterpreting optic ataxia. *Nat. Neurosci.* **3,** 729–736 (2000).

15. Perenin, M. T. & Vighetto, A. Optic ataxia: A specific disruption in visuomotor mechanisms: I. Different aspects of the deficit in reaching for objects. *Brain* **111,** 643–674 (1988).

16. Hebb, D. O. *Organization of Behaviour: A Neuropsychological Theory*. (New York: Wiley, 1949).

17. Huang, Y.-Z., Edwards, M. J. ., Rounis, E., Bhatia, K. P. . & Rothwell, J. C. Theta burst stimulation of human motor cortex. *Neuron* **45,** 201–206 (2005).

18. Abraham, W. C. How long will long-term potentiation last? *Philos. Trans. R. Soc. B Biol. Sci.* **358,**

735–744 (2003).

19. Nicoll, R. A. & Roche, K. W. Long-term potentiation: Peeling the onion. *Neuropharmacology* **74,** 18–22 (2013).

20. Matsuzaki, M.; Honkura, N.; Ellis-Davies, G.; Kasal, H. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429,** 761–766 (2004).

21. Deng, Z. De, Lisanby, S. H. & Peterchev, A. V. Electric field depth-focality tradeoff in transcranial magnetic stimulation: Simulation comparison of 50 coil designs. *Brain Stimul.* **6,** 1–13 (2013).

22. Liew, S.-L., Santarnecchi, E., Buch, E. R. & Cohen, L. G. Non-invasive brain stimulation in neurorehabilitation: local and distant effects for motor recovery. *Front. Hum. Neurosci.* **8,** 378 (2014).

23. Abrahamyan, A., Clifford, C. W. G., Arabzadeh, E. & Harris, J. A. Low intensity TMS enhances perception of visual stimuli. *Brain Stimul.* **8,** 1175–1182 (2015).

24. Kujirai, T. *et al.* Corticocortical inhibition in human motor cortex. *J. Physiol.* **471,** 501–519 (1993).

25. Ilić, T. V. *et al.* Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. *J. Physiol.* **545,** 153–167 (2002).

26. Ziemann, U., Rothwell, J. C. & Ridding, M. C. Interaction between intracortical inhibition and facilitation in human motor cortex. *J. Physiol.* **496,** 873–881 (1996).

27. Reis, J. *et al.* Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. *J. Physiol.* **586,** 325–351 (2008).

28. Wassermann, E. M. *et al.* Responses to paired transcranial magnetic stimuli in resting, active, and recently activated muscles. *Exp. Brain Res.* **109,** 158–63 (1996).

29. McDonnell, M. N., Orekhov, Y. & Ziemann, U. The role of GABAB receptors in intracortical inhibition in the human motor cortex. *Exp. Brain Res.* **173,** 86–93 (2006).

30. Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R. & Classen, J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* **123 Pt 3,** 572–584 (2000).

31. Wolters, A. *et al.* A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J. Neurophysiol.* **89,** 2339–45 (2003).

32. Singh, A. M., Neva, J. L. & Staines, W. R. Acute exercise enhances the response to paired associative stimulation-induced plasticity in the primary motor cortex. *Exp. Brain Res.* **232,** 3675–3685 (2014).

33. Vesia, M., Bolton, D. A., Mochizuki, G. & Staines, W. R. Human parietal and primary motor cortical interactions are selectively modulated during the transport and grip formation of goal-directed hand actions. *Neuropsychologia* **51,** 410–417 (2013).

34. Andersen, R. A. & Cui, H. Intention, Action Planning, and Decision Making in Parietal-Frontal Circuits. *Neuron* **63,** 568–583 (2009).

35. Cavina-Pratesi, C. *et al.* Functional Magnetic Resonance Imaging Reveals the Neural Substrates of Arm Transport and Grip Formation in Reach-to-Grasp Actions in Humans. *J. Neurosci.* **30,** 10306–10323 (2010).

36. Vesia, M. & Crawford, J. D. Specialization of reach function in human posterior parietal cortex. *Exp. Brain Res.* **221,** 1–18 (2012).

37. Lefebvre, S. *et al.* Increased functional connectivity one week after motor learning and tDCS in stroke patients. *Neuroscience* **340,** 424–435 (2017).

38. Okabe, N. *et al.* Neural network remodeling underlying motor map reorganization induced by rehabilitative training after ischemic stroke. *Neuroscience* **339,** 338–362 (2016).

39. Dhand, N. K. & Khatkar, M. S. Statulator: An online statistical calculator. Sample Size Calculator for Comparing Two Paired Means. (2014). Available at: http://statulator.com/SampleSize/ss2PM.html. (Accessed: 24th August 2018)

40. Singh, H. & Singh, J. Human Eye Tracking and Related Issues: A Review. *Int. J. Sci. Res. Publ.* **2,** 2250–3153 (2012).

41. Kleinjung, T. *et al.* Long-term effects of repetitive transcranial magnetic stimulation (rTMS) in patients with chronic tinnitus. *Otolaryngol. - Head Neck Surg.* **132,** 566–569 (2005).

42. Kim, Y. *et al.* Long-term effects of rTMS on motor recovery in patients after subacute stroke. *J. Rehabil. Med.* **42,** 758–764 (2010).

43. Gersner, R., Kravetz, E., Feil, J., Pell, G. & Zangen, A. Long-Term Effects of Repetitive Transcranial Magnetic Stimulation on Markers for Neuroplasticity: Differential Outcomes in Anesthetized and Awake Animals. *J. Neurosci.* **31,** 7521–7526 (2011).

44. Muellbacher, W., Ziemann, U., Boroojerdi, B. & Hallett, M. Effects of low frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. *Clin. Neurophysiol.* **111,** 1002–1007 (2000).

45. Deiber, M. P., Ibanez, V., Sadato, N. & Hallett, M. Cerebral structures participating in motor preparation in humans: a positron emission tomography study. *J. Neurophysiol.* **75,** 233–247 (1996).

46. McNeill, T. H., Brown, S. a, Rafols, J. a & Shoulson, I. Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. *Brain Res.* **455,** 148–152 (1988).

47. Galea, J. M., Vazquez, A., Pasricha, N., Orban De Xivry, J. J. & Celnik, P. Dissociating the roles of the cerebellum and motor cortex during adaptive learning: The motor cortex retains what the cerebellum learns. *Cereb. Cortex* **21,** 1761–1770 (2011).

48. Neva, J. L., Legon, W. & Staines, W. R. Primary motor cortex excitability is modulated with bimanual training. *Neurosci. Lett.* **514,** 147–151 (2012).

Appendix A

TRANSCRANIAL MAGNETIC STIMULATION (TMS) SCREENING FORM

Below is a questionnaire used to exclude participants considered not suitable for transcranial magnetic stimulation (TMS). This information, as well as your identity, will be kept confidential in all future publications.

PLEASE COMPLETE FORM BELOW:

Participant's Code: _____ **Age:** _____

Please **CIRCLE ONE**:

Neurological or Psychiatric Disorder	YES	NO	Multiple Sclerosis	YES	NO
Head Trauma	YES	NO	Depression	YES	NO
Stroke	YES	NO	treatment with amitryptiline and haloperidol	YES	NO
Brain surgery	YES	NO	Implanted medication pump	YES	NO
Metal in cranium	YES	NO	Intracranial Pathology	YES	NO
Brain Lesion	YES	NO	Albinism	YES	NO
Pacemaker	YES	NO	Intractable anxiety	YES	NO
History of seizure	YES	NO	Pregnant	YES	NO
Family history of epilepsy	YES	NO	Headaches or Hearing problems	YES	NO
History of epilepsy	YES	NO	Family History of Hearing Loss	YES	NO
Intracorporal electronic devices	YES	NO	Other medical conditions (please specify)	YES	NO
Intracardiac lines	YES	NO			

I hereby declare that all information given on this TMS screening form is true and complete in every respect.

_____ _____

Signature of Participant Date

Signature of Witness Date

Appendix B

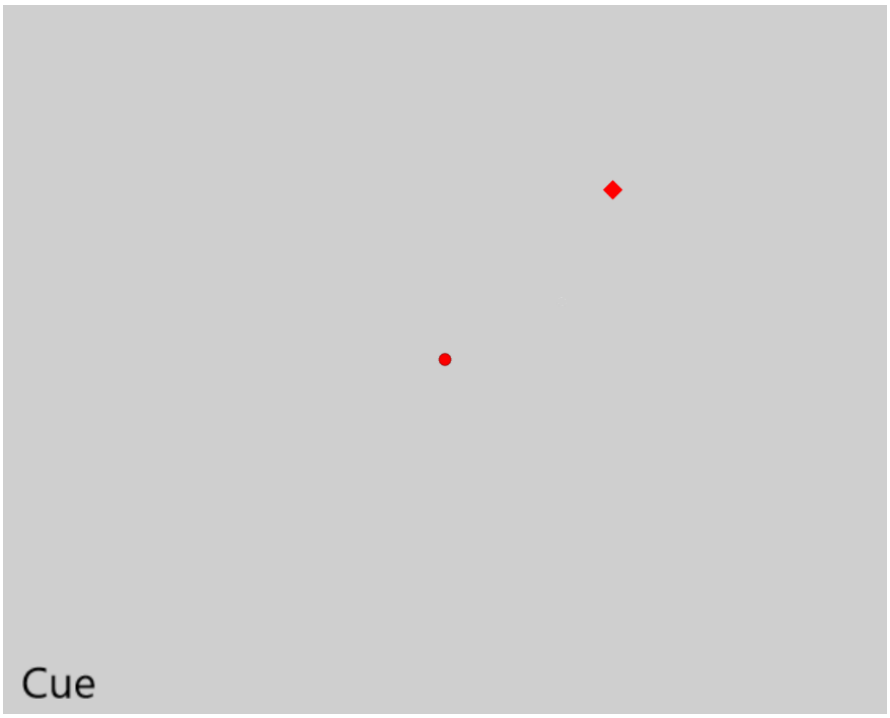
Waterloo Handedness Questionnaire—Revised

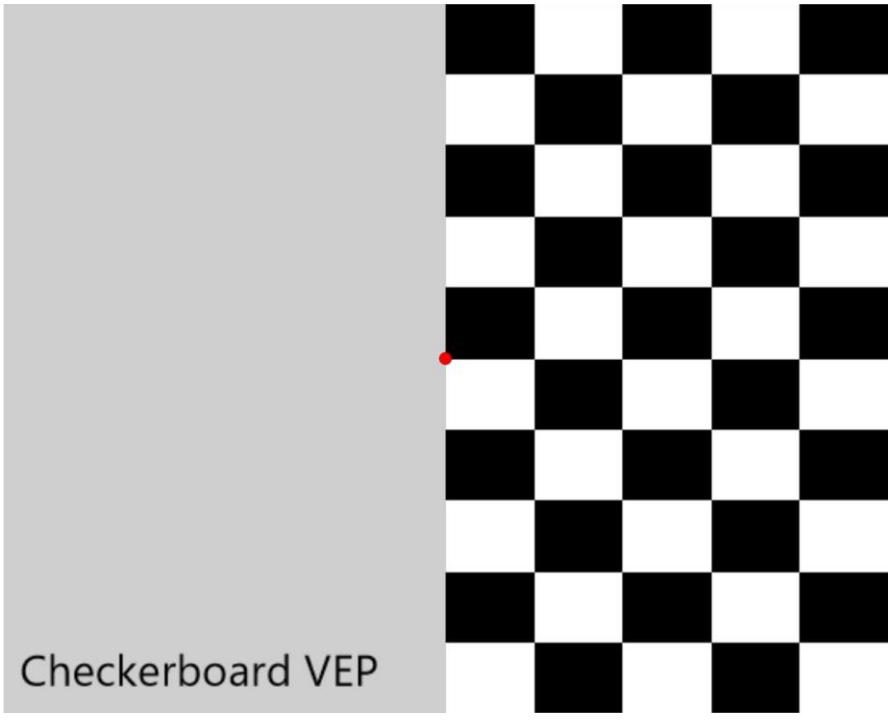
Instructions: Please indicate your hand preference for the following activities by circling the appropriate response. If you **always** (i.e. 95% or more of the time) use one hand to perform the described activity, circle **Ra** or **La** (for **right always** or **left always**). If you **usually** (i.e. about 75% of the time) use one hand circle **Ru** or **Lu** as appropriate. If you use both hands **equally often** (i.e. you use each hand about 50% of the time), circle **Eq**.

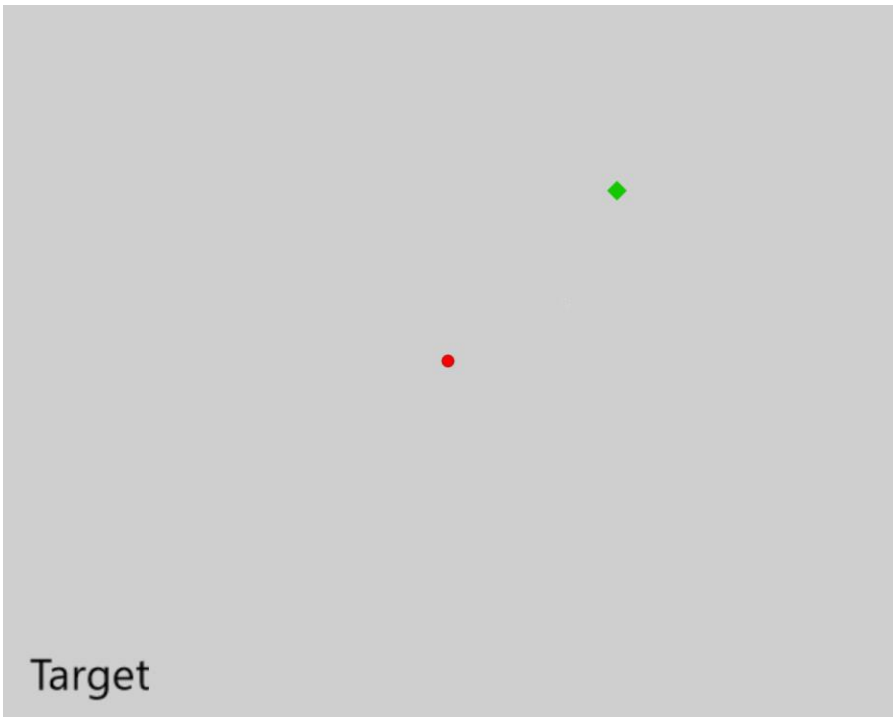
1.	Which hand would you use to adjust the volume knob on a radio?	La	Lu	Eq	Ru	Ra
2.	With which hand would you use a paintbrush to paint a wall?	La	Lu	Eq	Ru	Ra
3.	With which hand would you use a spoon to eat soup?	La	Lu	Eq	Ru	Ra
4.	Which hand would you use to point to something in the distance?	La	Lu	Eq	Ru	Ra
5.	Which hand would you use to throw a dart?	La	Lu	Eq	Ru	Ra
6.	With which hand would you use the eraser on the end of a pencil?	La	Lu	Eq	Ru	Ra
7.	In which hand would you hold a walking stick?	La	Lu	Eq	Ru	Ra
8.	With which hand would you use an iron to iron a shirt?	La	Lu	Eq	Ru	Ra
9.	Which hand would you use to draw a picture?	La	Lu	Eq	Ru	Ra
10.	In which hand would you hold a mug full of coffee?	La	Lu	Eq	Ru	Ra
11.	Which hand would you use to hammer a nail?	La	Lu	Eq	Ru	Ra
12.	With which hand would you use the remote control for a TV?	La	Lu	Eq	Ru	Ra
13.	With which hand would you use a knife to cut bread?	La	Lu	Eq	Ru	Ra
14.	With which hand would you use to turn the pages of a book?	La	Lu	Eq	Ru	Ra
15.	With which hand would you use a pair of scissors to cut paper?	La	Lu	Eq	Ru	Ra
16.	Which hand would you use to erase a blackboard?	La	Lu	Eq	Ru	Ra
17.	With which hand would you use a pair of tweezers?	La	Lu	Eq	Ru	Ra
18.	Which hand would you use to pick up a book?	La	Lu	Eq	Ru	Ra
19.	Which hand would you use to carry a suitcase?	La	Lu	Eq	Ru	Ra
20.	Which hand would you use to pour a cup of coffee?	La	Lu	Eq	Ru	Ra
21.	With which hand would you use a computer mouse?	La	Lu	Eq	Ru	Ra
22.	Which hand would you use to insert a plug into an outlet?	La	Lu	Eq	Ru	Ra
23.	Which hand would you use to flip a coin?	La	Lu	Eq	Ru	Ra
24.	With which hand would you use a toothbrush to brush your teeth?	La	Lu	Eq	Ru	Ra
25.	Which hand would you use to throw a baseball?	La	Lu	Eq	Ru	Ra
26.	Which hand would you use to turn a doorknob?	La	Lu	Eq	Ru	Ra
27.	Which hand would you use for writing?	La	Lu	Eq	Ru	Ra
28.	Which hand would you use to pick up a piece of paper?	La	Lu	Eq	Ru	Ra
29.	Which hand would you use a hand saw?	La	Lu	Eq	Ru	Ra
30.	Which hand would you use to stir a liquid with a spoon?	La	Lu	Eq	Ru	Ra
31.	In which hand would you hold an open umbrella?	La	Lu	Eq	Ru	Ra
32.	In which hand would you hold a needle while sewing?	La	Lu	Eq	Ru	Ra
33.	Which hand would you use to strike a match?	La	Lu	Eq	Ru	Ra
34.	Which hand would you use to turn on a light switch?	La	Lu	Eq	Ru	Ra
35.	Which hand would you use to open a drawer?	La	Lu	Eq	Ru	Ra
36.	Which hand would you use to press buttons on a calculator?	La	Lu	Eq	Ru	Ra
37.	Is there any reason (i.e. injury) why you have changed your hand preference for any of the above activities?	**YES/NO**	(circle one)			
38.	Have you been given special training or encouragement to use a particular hand for certain activities?	**YES/NO**	(circle one)			
39.	If you have answered YES for either Questions 37 or 38, please explain:					

Appendix C

Example of 1 of 8 possible visual stimulus patterns presented to participants during V-PAS (presented to participants without labels). During Paired-pulse TMS, the same images are presented with the omission of the Checkerboard VEP image. The Cue screen is presented for a total of 500ms, followed by the Checkerboard VEP for 200ms, concluded by the Target screen for 2000ms. This leads to a total trial time of 2.7s during V-PAS and 2.5s during Paired-pulse TMS.






Target

Appendix D

Display of the 8 possible locations for Cues and the matching Targets that will be presented to participants 1 at a time. To ensure equal frequency of each target, trials are grouped into blocks of 8 with each location being presented once during a block. Within a block, the exact order of locations displayed is randomized.