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Shiyu Lin   March 28, 2019

Neuromodulation of Interhemispheric Connectivity by Paired Associative Stimulation after Stroke

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Abstract

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Atypical interhemispheric connectivity has been observed in stroke survivors. Specifically, the presence of excessive interhemispheric inhibition (IHI) from the contralesional motor cortex (cM1) to ipsilesional motor cortex (iM1) (cM1-to-iM1 IHI) created a model of interhemispheric competition after stroke which may contribute to motor recovery. Paired associative stimulation (PAS) was found to be able to induce spike-timing-dependent plasticity, which is essential for motor relearning during rehabilitation. However, it is unknown how modulation of IHI through transcranial magnetic stimulation (TMS) might influence post-stroke motor function. The purpose of this study was to 1) determine the effect of a session of interhemispheric paired associative stimulation (ihPAS) on neuromodulation of cortical excitability, IHI, and motor performance in stroke survivors and 2) investigate the relationship between IHI and post-stroke motor function.

Thirteen participants (7 male) with ischemic chronic (>6 mo.) stroke were tested during two separate sessions with two ihPAS conditions: $ihPAS_{8ms}$ (interstimulus interval [ISI] of 8ms) and $ihPAS_{1ms}$ (ISI of 1ms). ihPAS consisted of 100 paired TMS pulses to cM1 followed by iM1 delivered at 0.25Hz. Electromyography (EMG) were placed on first dorsal interosseous muscles to measure the motor evoked potential upon TMS stimulations. IHI and cortical excitability were assessed by TMS before and after ihPAS. Motor function and skill learning were assessed by both 3-item Wolf Motor Function Test and serial reaction time task. ihPAS did not significantly modulate iM1 cortical excitability and cM1-to-iM1 IHI, but paretic motor function was improved following $ihPAS_{8ms}$. cM1-to-iM1 IHI was not strongly correlated with paretic arm motor function. Contrary our hypothesis, there was greater iM1-to-cM1 IHI than cM1-to-iM1 IHI prior to ihPAS. Secondary analyses revealed that those demonstrating exaggerated cM1-to-iM1 IHI before ihPAS had decreased cM1-to-iM1 IHI compared to those who showed diminished IHI. Our results suggest that the effect of ihPAS is variable across individuals. The absence of exaggerated cM1-to-iM1 IHI does not support the interhemispheric competition model. Future studies are necessary to further characterize the role of cM1-to-iM1 IHI in the motor recovery process on an individual level to develop effective therapies.

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ILLUSTRATIONS

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ABBREVIATIONS

TMS   transcranial magnetic stimulation

rTMS   repetitive transcranial magnetic stimulation

fMRI   functional magnetic resonance imaging

iM1   ipsilesional primary motor cortex

cM1   contralesional primary motor cortex

FDI   first dorsal interosseous

IHI   interhemispheric inhibition

ISI   interstimulus interval

MEP   motor evoked potential

EEG   electroencephalography

EMG   electromyography

PAS   paired associative stimulation

ihPAS   interhemispheric paired associative stimulation

STPD   spike-timing-dependent plasticity

LTP   long-term potentiation

LTD   long-term depression

FM-UE Fugl-Meyer Upper Extremity score

WMFT Wolf Motor Function Test

SRTT   serial reaction time task

NHPT   Nigh hole peg test

Introduction

In the United States, 2.5% of the population reported having a history of stroke. Although the age-standardized mortality rate of stroke has decreased in the past twenty-five years, the absolute number of people having a stroke each year increased to approximately 800,000. As a result, the number of survivors suffering from stroke-induced life-long disability increased (Benjamin et al., 2019). Among disabilities resulting from stroke, upper limb weakness was found in 77% of the survivors (Lawrence et al. 2001). Currently, post-stroke rehabilitative training is the most common and essential approach to help patients regain some paretic limb motor functions and promote life independence. Despite the neurorehabilitation, 65% of survivors still experience difficulty in performing daily activities using paretic upper extremity six months after stroke (Dobkin, 2005). Given the increasing prevalence of stroke-induced disability and the limitations in recovery outcome by the conventional rehabilitative training (Okabe and Miyamoto, 2018), there is a need to incorporate new technologies to boost the efficiency and efficacy of the post-stroke rehabilitative training.

Transcranial magnetic stimulation (TMS) is one of the emerging techniques that can be used to assess and modulate neural activities in the brain. When TMS coils are placed over the head, a brief electrical current runs within the coil of wires, which creates a magnetic field. The magnetic field induces currents below the scalp to allow excitation of a small area of brain without causing a painful sensation (Hallett, 2007). Repetitive TMS (rTMS) has been shown to be capable of modulating excitability depending on the stimulating frequency, intensity, and duration (Maeda et al., 2000). When stimulating at a higher frequency (>5Hz), rTMS enhanced cortical excitability (Pascual-Leone et al., 1994) while a lower frequency of 1Hz reduced excitability (Chen et al., 1997). Stroke can cause neuron loss in brain which directly impairs the

excitability and corticospinal output of ipsilesional primary motor cortex (iM1) (Blesneag et al., 2015; McDonnell and Stinear, 2017). As a result, many rTMS studies aimed to promote iM1 excitability (Di Lazzaro et al., 2008; Massie et al., 2013). Moreover, primary motor cortex has extensive connections with both its adjacent and distant areas through fiber projections. Stroke itself and the secondary adaptation process following stroke can cause atypical connectivity in those neural networks.

One of the important network is the connectivity between the two hemispheres through the transcallosal pathway. In a healthy brain, the communication between two primary motor cortices is regulated by the inhibitory circuits: the excitation of one hemisphere suppresses the activity of the other hemisphere when performing a unilateral movement (Figure 1A). Ferbert et al. (1992) first investigated the communication between the two hemispheres and defined interhemispheric inhibition (IHI) by using the non-invasive brain stimulation on the healthy human motor cortex. The researchers found that a conditioning stimulus to one hemisphere could reduce the muscle response evoked by a test stimulus to the other hemisphere when the conditioning pulse preceded test pulse at certain interstimulus intervals (ISI). Later studies found similar results that the excitation of motor cortex on one side suppressed the excitability of the contralateral motor cortex (Di Lazzaro et. al, 1999; Gerloff et al., 1998). This inhibitory regulation creates a balance of activity between the two hemispheres.

After stroke, the remaining neural circuits undergo rewiring process to compensate for the damage and thus might disrupt the balance between two hemispheres. Some of these compensatory strategies in the brain allow patients to rely more on the non-paretic limb to perform daily activities. The functional ability of paretic limb, however, may be further worsened by the lack of movement in addition to the neuronal loss (Jones, 2017). This

phenomenon supported the model of interhemispheric competition (Ward and Cohen, 2004). The interhemispheric competition model proposed that after stroke, the contralesional hemisphere is less inhibited by the affected hemisphere due to the lesion, resulting in more inhibition on the affected hemisphere from the contralesional hemisphere (Figure 1B). The over-inhibition of iM1 further reduces its corticospinal output, which impairs the motor function of the paretic limb. Functional studies have shown that when patients were trying to generate a voluntary movement of the paretic hand, there was a lack of normal release of inhibition from the contralesional primary motor cortex (cM1) to iM1, which was correlated with slower movements of the paretic hand (Murase et al., 2004; Duque et al., 2005). Imaging techniques such as functional magnetic resonance imaging (fMRI) characterized the abnormal increase in activation of both hemispheres during paretic hand movements. Greater motor deficits were found to be associated with higher level of bilateral activation (Volz et al., 2014). Another study by our lab using simultaneous electroencephalography (EEG) recording on TMS-evoked cortical response showed direct physiological evidence of abnormal interhemispheric interactions in stroke participants (Borich et al., 2016). This abnormality of increased interhemispheric connectivity during motor movement was believed to contribute to the motor deficits.

Based on those studies, much research has been focused on rebalancing IHI as the post-stroke rehabilitation strategy. Down-regulation of unaffected hemisphere by low frequency rTMS showed better motor outcomes (Mansur et al., 2005; Takeuchi et al., 2009; Du et al., 2016). Overall, many researchers are optimistic about the results of rTMS studies and consider it a promising tool to promote beneficial plasticity and to enhance motor recovery after stroke, even though there were ineffective protocols (Rose et al., 2014; Seniow et al., 2012). Many studies suffer from the small sample sizes that are unable to fully represent stroke population in
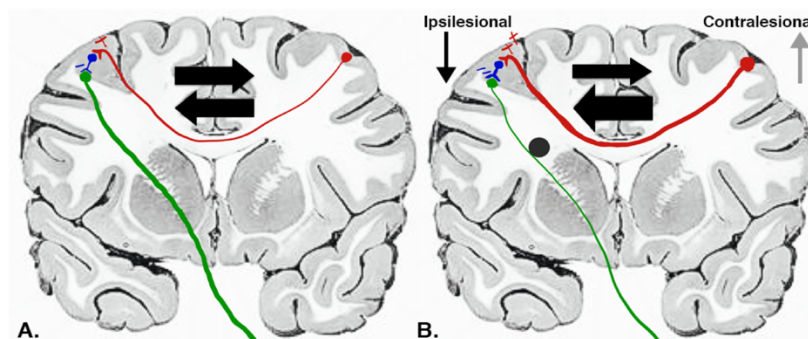
Figure 1. A) In a healthy brain, there is a balance of activity between the two cerebral hemispheres (black arrows). Interhemispheric inhibition is mediated by transcallosal excitatory projections (red) that synapse onto inhibitory interneurons (blue) to regulate the corticospinal motor output (green) of the contralateral hemisphere. B) After stroke, the interhemispheric model predicts increased activation in contralesional hemisphere that results greater excitation in transcallosal pathway, greater activation of inhibitory interneurons, and reduced output of ipsilesional hemisphere. The balance shifts to the direction with more inhibition onto and less excitability within the ipsilesional hemisphere.

different stages of recovery, and there is a lack of agreement on the most effective paradigm for

rTMS stimulation.

In addition to rTMS, paired associative stimulation (PAS) is another TMS paradigm.

Traditional PAS pairs the TMS of the brain with electrical stimulations of a peripheral nerve.

Studies have showed that PAS can induce spike-timing-dependent plasticity (STDP) in the brain

depending on the ISI (Stefan et al., 2000; Wolters et al., 2003). When the ISI precisely

synchronizes the output signal from TMS activation and the afferent signal from the periphery,

the synaptic strength is enhanced. This type of plasticity was first described by Donald Hebb in

1949 as a neural mechanism underlying associative learning. The increased efficiency at the

synapse resembles a long-term potentiation (LTP) effect. More importantly, studies on both the

animal model and human motor cortex demonstrated that afferent signals from the

somatosensory cortex is critical for inducing STDP and acquiring new motor skills (Pavlides et

al., 1993; Brodie et al., 2014), so scientists proposed that the enhanced synaptic efficiency by STDP in the primary motor cortex is critical for motor learning and memory.

Based on the STDP model for motor learning and the interhemispheric competition model explaining post-stroke neurophysiological maladaptation, we designed a TMS protocol that targets the interhemispheric interactions in the transcallosal pathway. Interhemispheric PAS (ihPAS) pairs the TMS pulses delivered to bilateral M1. The synchrony of two TMS pulses can induce STDP and modify the synaptic efficiency of the interhemispheric connection. An earlier study investigating transcallosal afferent projection to M1 by Rizzo et al. (2009) showed that repeated left-to-right ihPAS decreased the left-to-right IHI and increases the cortical excitability of the conditioned right M1 on healthy right-handed individuals. Left-to-right IHI remained reduced at 30 and 60 minutes after the intervention. Such single-session ihPAS also influenced motor performance and motor strategies during the movement tasks (Rizzo et al., 2011). However, no study has been done to investigate if this form of paired stimulation is effective for stroke patients who may present with unbalanced IHI. ihPAS might be able to diminish the excessive inhibition onto and increase the excitability of iM1. The plasticity induced by ihPAS might benefit paretic motor function and motor learning in stroke survivors.

In our study, we aimed to determine the effect of a single-session ihPAS on neuromodulation of IHI and motor performance in a group of stroke survivors in their chronic stage of recovery. We hypothesized that ihPAS would increase the cortical excitability of the lesioned hemisphere and reduce inhibition from contralesional to ipsilesional hemisphere compared to a control stimulation condition. In addition to the neurophysiological changes measured by TMS, we investigated the effect of ihPAS on motor performance and motor skill

learning. We hypothesized that ihPAS would improve participants' paretic hand motor function and motor learning.

Methods

Participants

Participants were recruited from the Emory Rehabilitation Hospital. Thirteen participants (7 male; mean age: 65±11) have been tested in this study (Table 1). Inclusion criteria to the study include 1) aged between 40-85; 2) clinically diagnosed ischemic stroke in the chronic stage of recovery (> 6 mo.) with 3) mild-moderate impairment of the paretic upper extremity (Fugl-Meyer Upper Extremity score ≥35/66); 4) no signs of dementia (Montreal Cognitive Assessment >20/30); 5) no history of head trauma, major psychiatric diagnosis, neurodegenerative disorder, or substance abuse; 6) MRI and TMS tolerable and 7) having detectable muscle response upon TMS. All participants have given written informed consent prior to the study, and the study procedure was approved by the Emory Institutional Review Board.

Experiential Paradigm

There were four assessment time points: PRE, POST 0 min, POST 30 min and POST 24 hours of ihPAS intervention (Figure 2). The PRE assessment was performed immediately before the ihPAS intervention, and the POST 0' was immediately after ihPAS. The POST time points were chosen to compare the instant and the long-term effect of ihPAS on both the physiological and functional characteristics. ihPAS intervention had two conditions, and each participants was expected to complete both conditions in separate visits.

Table 1. Demographic data of the 13 participants in the study.

ID	Age(y)/Gender	CVA location	PSD(mo)	FM-UE Score
S01	74/M	R IC/BG	11	45
S02	49/F	R IC/BG	13	60
S03	74/F	R IC/BG	13	40
S04	45/F	R PLIC	23	55
S05	65/F	L IC	185	61
S06	83/M	R MCA	68	35
S07	67/F	L ACA	75	58
S08	69/M	L BG	167	66
S09	53/M	L MCA	67	60
S10	74/M	L Brainstem(Pons)	24	51
S11	72/M	L Brainstem(Pons)	35	56
S12	56/M	L Frontal, Parietal, Cortical/Subcortical WM	70	53
S13(S16)	63/F	L PLIC/ Striatum	57	35
MEAN ± SD	65±11		62±56	52±10

Note: CVA- cerebrovascular accident; PSD- post-stroke duration; mo-month; FU-UE ((Fugl-Meyer Upper Extremity); M-male; F-female; L-left hemisphere; R- right hemisphere; IC-internal capsule; BG-basal ganglia; PLIC-posterior limb of the internal capsule; MCA-middle cerebral artery; ACA-anterior cerebral artery; WM-white matter; SD-standard deviation.

TMS Setup

The target areas of stimulation were positions on the scalp of primary motor cortex areas bilaterally that control the movement of contralateral first dorsal interosseous (FDI) muscle of the hand. Electromyography (EMG) electrodes were placed bilaterally over the FDI muscles to record the muscle activities. There were two hand-held figure-of-eight coils connected to two Magstim-200 magnetic stimulators. The large coil (70mm) was used over the lesioned hemisphere, and the small one (50mm) was used over the non-lesioned hemisphere. At the beginning of the experiment, a coil was moved around the target stimulation area to find the optimal spots (hotspots) on each hemisphere that could constantly generate the largest peak-to-
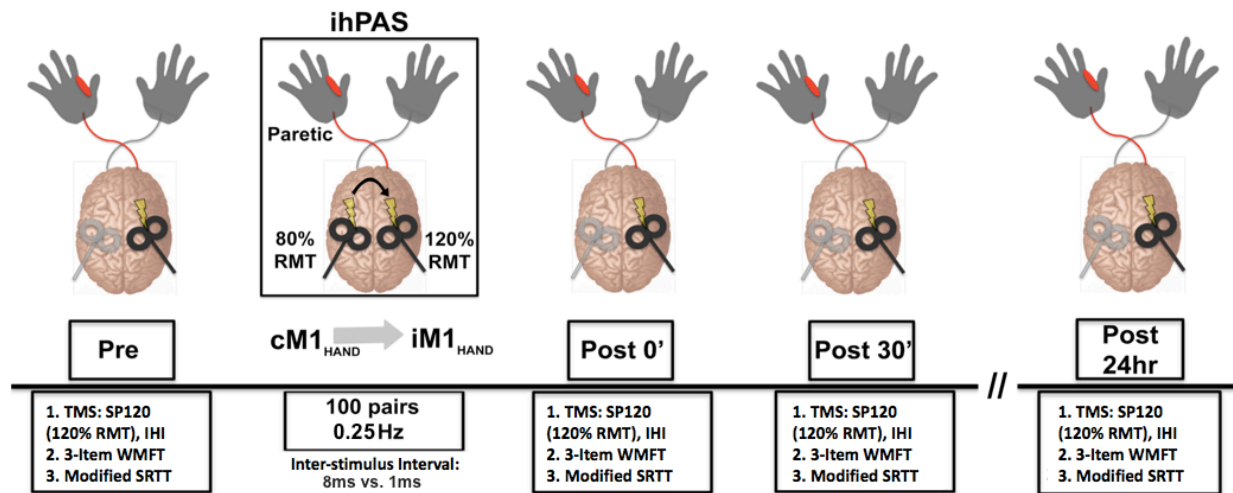
Figure 2. Experimental design for ihPAS visits. Cortical excitability (SP120), inhibition (IHI), and motor function (3-Item WMFT and modified SRTT) were assessed at four time points: PRE, POST 0', POST 30', POST 24h. Data from POST 24h was collected on the second day as a separate visit. During the ihPAS intervention, contralesional-to-ipsilesional PAS was delivered at 0.25Hz with 100 stimulus pairs each separated by either 1ms or 8ms. Each participant completed both 8ms and 1ms ISI conditions in separate visits. The order of conditions was random.

peak motor evoked potential (MEP) in the FDI (Rossini et al., 1994). Brainsight[TM] software was used to help locate optimal simulation loci. The handles of coils were placed posterolaterally at 45 ° angle to induce a posterior-anterior current (Brasil-Neto et al., 1992). Resting motor thresholds (RMTs) for both hemispheres were found after the optimal stimulation spots were determined. RMT is defined as the minimal intensity of stimulus required to evoke a motor response of 50uV peak-to-peak MEP in at least 5 out of 10 trials in the target muscle (Rossini et al., 1994). Participants were asked to sit in a comfortable chair with arms supported by pillows to maintain a resting muscle state during the entire TMS sessions. To keep participants awake and alert, they were asked to count laser dots which were randomly sent to a blank computer screen located in front of them.

ihPAS protocol

A hundred paired TMS pulses in total were delivered at a frequency of 0.25Hz. Each pulse paired the conditioning pulse applied to contralesional M1 (cM1) with a test pulse over ipsilesional M1 (iM1). Brainsight^{TM} was used to guide coils to stimulate the hotspots determined during the TMS setup. The intensity of conditioning pulses was 80% RMT and that of test pulses was 120% RMT. Each participant was expected to complete two separate visits that were at least a week apart for two conditions: ISI of 8ms (ihPAS$_{8ms}$) and 1ms (ihPAS$_{1ms}$). According to the first study investigating conditioning-test paired TMS on evaluating IHI by Ferbert et al. (1992), the onset of inhibition from one hemisphere to the other was when ISI lied in 5 to 6ms or slightly longer. ISI of 8ms can synchronize the TMS output to M1 with the input signals conducted through the transcallosal pathway, and thus repetitively applying paired TMS may induce STDP. ISI of 1ms is too fast for inhibitory pulses to reach M1 of the target hemisphere, and thus we predicted that ihPAS$_{1ms}$ would not affect cortical excitability of the lesioned hemisphere.

TMS Physiological assessments

During each time point, we investigated the cortical excitability (SP120) and IHI. The muscle responses were measured as peak-to-peak MEP by EMG electrodes on FDI muscles. SP120 was assessed first with 20 suprathreshold single-pulse stimulations sending to the M1 hotspot determined during TMS setup. The pulses were sent at the frequency randomizing from 0.125Hz to 0.25Hz with a intensity of 120% RMT (Figure 3A). IHI was then assessed by using the double-pulse paradigm: each test pulse was paired with a preceding conditioning pulse at an ISI of 10ms. Twenty paired pulses were sent at random frequencies of 0.125Hz to 0.25Hz with the intensity of 120% RMT of both pulses (Figure 3B). IHI was calculated as the ratio of MEP elicited by the double pulse (blue trace in Figure 3B) over MEP elicited by the single pulse (red trace in Figure 3B) of the testing hemisphere. The procedure was repeated for assessments on

both the lesioned and non-lesioned hemispheres to examine changes in excitability of both
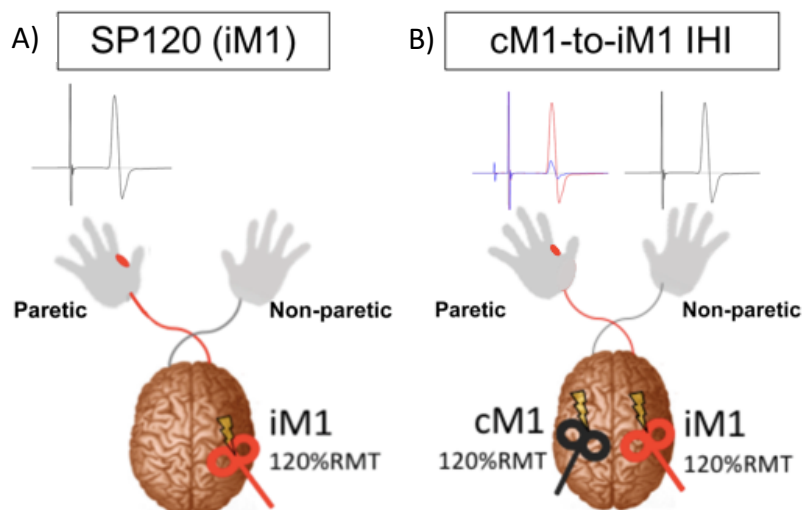
hemispheres and in IHI of both directions.

Figure 3. An illustration of unconditioned stimulation (SP120) and IHI assessments. (A) Following 120%RMT TMS stimulation over iM1, MEPs were recorded in an intrinsic muscle of the paretic hand. (B) The double-pulse TMS for measuring cM1-to-iM1 consisted of the 120%RMT conditioning pulses delivered to the cM110ms prior to the 120%RMT test pulse delivering to the iM1. The MEP response following the double-pulse recorded at the paretic hand (in blue) was smaller compared to single pulse MEP response (in red) due to trancallosally-mediated IHI.

Behavioral Assessments

3-Item WMFT and modified SRTT tasks were used to examine the motor function and

motor learning. 3-Item WMFT is an abbreviated version of WMFT (Wolf et al., 2005). It

consisted of three tasks: hand to table, lift can, and stack checkers. Each task had different

control demands and required participants to perform number of actions in order to complete the

task. Performance for each task was timed for both paretic and non-paretic limb at each

assessment time point.

During the modified SRTT, four equally sized and spaced squares appeared on a touch

screen in front of the participant (Figure 4). There was a large or small option of the size of the

square depending on the initial motor function level of participants' paretic hands. Participants

with worse initial paretic function used the modified version of SRTT with larger squares. Participants were instructed to touch the square which illuminated in sequence. Each target presented 400ms after the correct touch of the preceding target. All mistakes were recorded and an accuracy percentage was calculated. Response time (RT) was calculated as the time difference between the target highlight and the correct response. Each SRTT block consisted of trials of both repeated and random sequences of order presentation. Within each block, repeated trials occurred in between random trials: first 50 trials were presented in a random order followed by fifteen repeats of a 12-trial sequence (180 trials) and afterwards another 50 random trials. There were 280 touches in total in each block. Skill was measured by the difference in RT between last 48 repeated trials (4 groups of the 12-trial sequence) and the final random trial to examine the level of motor learning of repeated trials (Cohen et al., 2005). Performance was measured separately at each time point as the mean RT of the first 50 random trials within a block except that the mean RT of last 50 random trials was used for the PRE assessment.

Before any TMS application at PRE, the upper extremity portion of the Fugl-Meyer score was obtained to index paretic arm impairment. The Nine-hole Peg Test (NHPT) and a full WMFT were also performed to assess hand dexterity (Mathiowetz, 1990) and upper extremity motor ability (Wolf et al., 2005).

Data Acquisition and Screening

A customized MATLAB script was used to help identify peak-to-peak MEP values. All trials were then manually screened to eliminate problematic trials. The exclusion criteria of trials included background muscle activity within the 100ms window prior to the stimulations (peak-to-peak MEP > 50 uV), no detectable MEPs within 20ms-100ms window post stimulation, and peak-to-peak MEP values outside two standard deviation of the assessment mean. For SP120
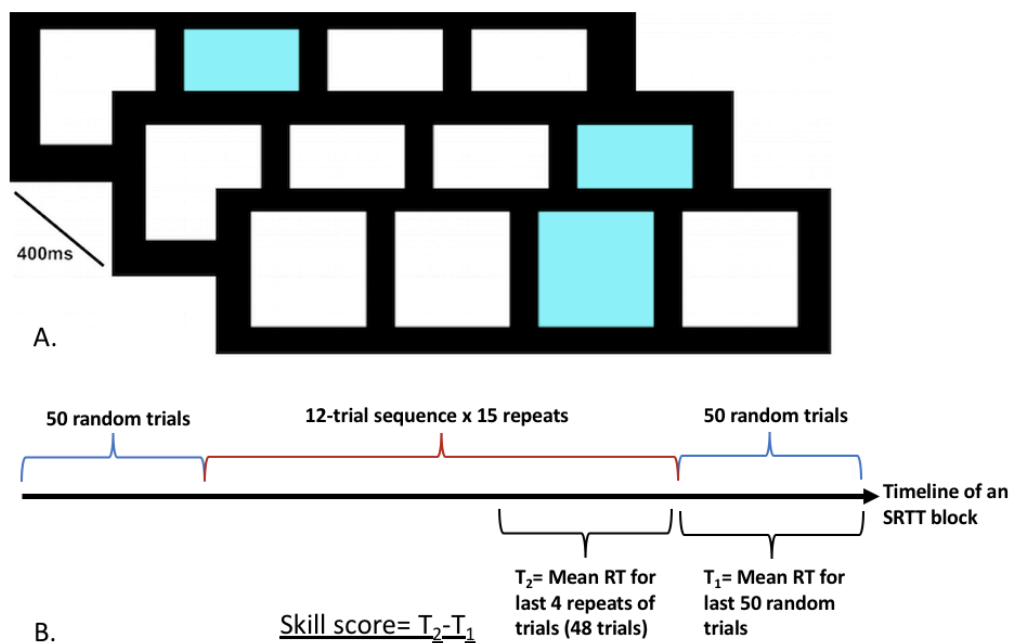
Figure 4. An illustration of the SRTT. A) One of the four squares was sequentially illuminated on the screen (in blue), and participants were asked to touch the lighted square as fast and accurately as possible. The interval between a correct touch and the next lighted square was 400ms. B) Each block of SRTT consisted of three parts in order: 50 random trials, 15 repeats of a 12-trial sequence, and another 50 random trials. The Skill score was calculated as the difference of mean response time (RT) between the last 50 random trials and the last 48 repeated trials. The Performance was defined as the mean RT for the first 50 random trials within a block except that at PRE mean RT for the last 50 random trials was used instead.

assessments, trials with peak-to-peak MEP < 50 uV responses were also eliminated because we expected muscle activity following the suprathreshold stimulation. The remaining MEP values for SP120 at each time for each hemisphere were averaged as the excitability measurements. The MEP values under double-pulse paradigm were normalized to the average SP120 MEP of the testing hemisphere to measure IHI of the testing hemisphere. A ratio smaller than 1 indicated IHI. Our primary outcomes of interest were changes of peak-to-peak MEPs of iM1 and cM1-to-iM1 IHI after applying ihPAS. SRTT results were also manually screened. Single accurate trials with RT over 3 standard deviation were eliminated.

Subject S03 did not complete the Post 24h visit for 8ms condition due to a personal reason. Subject S04 and S05 did not complete 3-Item WMFT during all visits, and S06 was not able to perform the checker task of 3-Item WMFT due to the severity of paretic hand impairment. S03, S06 and S13(S16) were unable to complete all trials within an SRTT block, and these participants were instructed to terminate after 5 minutes of pressing.

Statistical Analysis

We evaluated M1 excitability, interhemispheric connection, motor function, and motor learning by two-way (ihPAS condition x Time points) repeated-measures analysis of variance (RM ANOVA). Specifically, SP120 MEP, IHI, 3-Item WMFT, and modified SRTT were tested. Post-hoc pairwise testing with Bonferroni correction for multiple comparisons was performed for significant results. Mixed-effect analysis was applied when there was a missing value during RM ANOVA. Linear regression was used to evaluate association between neurophysiological and behavioral measures. The significant value α was 0.05. A P-value smaller than 0.05 was considered significant.

Results

Neither ihPAS$_{8ms}$ or ihPAS$_{1ms}$ significantly modulated cortical excitability and IHI

IHI was calculated as a ratio of the paired-pulse MEP over the SP120 MEP on the target hemisphere, with a smaller value indicating greater inhibition. There was no significant modulatory effect of ihPAS on bilateral cortical excitability and bidirectional IHI, and no significant difference between conditions (Figure 5). There was a trend of decreasing cM1-to-iM1 IHI in the 8ms condition (Figure 5c) at POST30, potentially indicating the LTP-like effect on the iM1 interneurons after ihPAS$_{8ms}$. On the contrary, ihPAS$_{1ms}$ increased the cM1-to-iM1 IHI, which might be explained by the long-term depression (LTD)-like plasticity at iM1

interneurons. Secondary analyses did not indicate any significant association between cM1

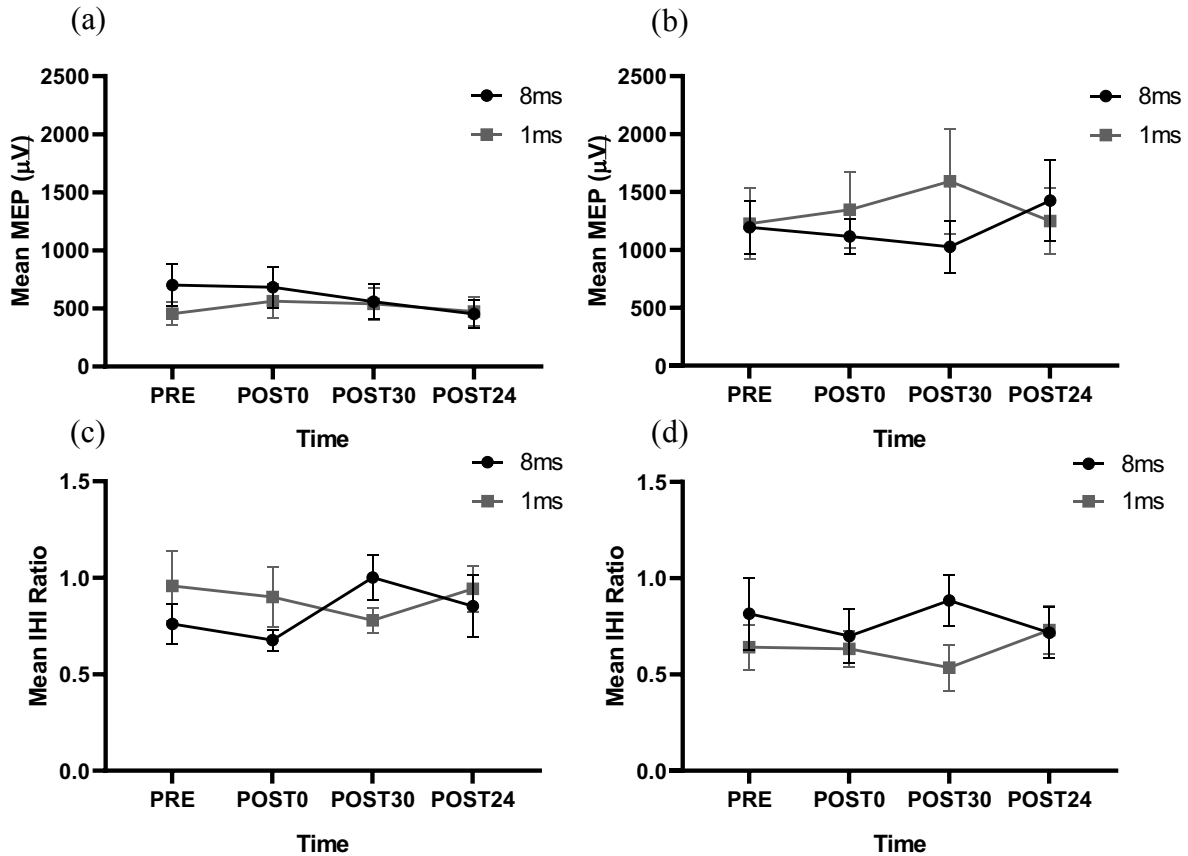excitability (b) and cM1-to-iM1 IHI (c) within a condition.

Figure 5. Bilateral iM1 excitability and bidiretional IHI at four time points in both ihPAS$_{8ms}$ and ihPAS$_{1ms}$ conditions. MEP amplitudes (mean ± SE) for cortical excitability of iM1 (a) and cM1 (b) which recorded from the contralateral FDI muscles were not significantly modulated upon ihPAS and between conditions. **(c)** cM1-to-iM1 IHI, and **(d)** iM1-to-cM1 IHI ratios (paired pulse mean MEP/ SP120 unconditioned mean MEP) were also not significantly changed or different in conditions as indicated by two-factor RM ANOVA.

Large variability was observed among individuals in response to ihPAS$_{8ms}$

Large inter-individual variability contributed to the lack of statistical significance in this

part of the study. Data with drastic changes was double-checked to ensure validity. Even prior to

ihPAS intervention, individuals demonstrated various levels of cortical excitability and IHI.

Within an individual, cortical excitability and IHI also varied between visits. Interestingly, the

trend of decreasing cM1-to-iM1 IHI after ihPAS$_{8ms}$ was showed in ten (out of twelve) of our

participants (Figure 6c), which suggested that ihPAS has the potential to modulate IHI and

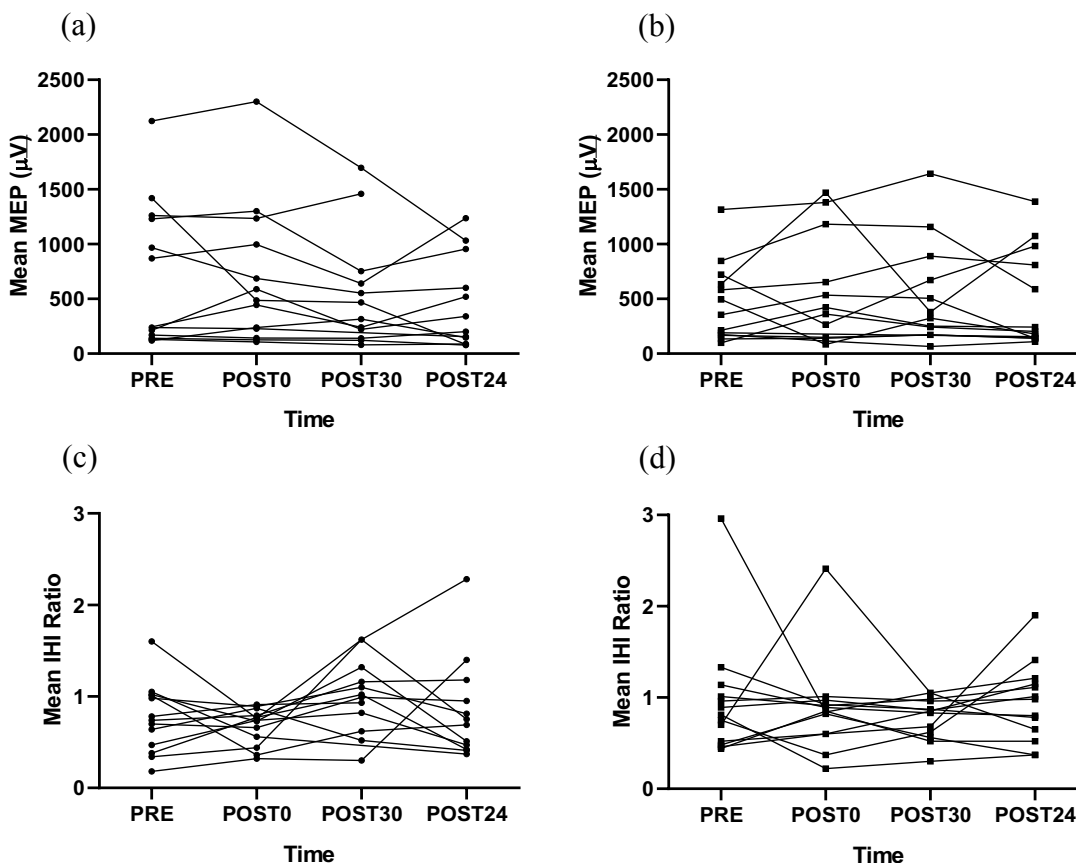enhance synaptic strength through LTP-like plasticity.

Figure 6. The individual response of iM1 SP120 and cM1-to-iM1 IHI at four time points in both
ihPAS$_{8ms}$ and ihPAS$_{1ms}$ conditions. Each point represents mean MEP or IHI ratio of all valid
trials during that assessment. The individual iM1 SP120 MEP at four time points of ihPAS$_{8ms}$ (a)
and ihPAS$_{1ms}$ (b) demonstrate large variability among individuals. cM1-to-iM1 IHI of ihPAS$_{8ms}$
(c) and ihPAS$_{1ms}$ (d) demonstrated similar variability. There is also a lack of consistency in
baseline cortical excitability and IHI between conditions.

Paretic motor function measured by 3-item WMFT was improved following ihPAS$_{8ms}$

The performance of the 3-item WMFT was measured by the response time (RT) that the

participants took to complete the task after the verbal "go" signal. A shorter RT indicated better

motor function. WMFT was assessed at each time point: PRE, POST0,POST30, and POST24h.

Paretic motor function was significantly improved over time in the "hand to table" task regardless of condition (p=0.04) (Figure 7a). The task required the functional ability of the shoulder of the involved upper extremity. Post-hoc comparison identified the significant improvement between both PRE and POST0 (p=0.03), and PRE and POST30 (p<0.01). However, the lack of difference between conditions indicated that the improvement could result from both conditions of ihPAS as well as other confounders such as practicing the task over time. In addition, ihPAS$_{8ms}$ significantly improved the performance of "stack checker" task compared to the control condition (Figure 7c) (p=0.048). Post-hoc comparison reveled that at POST0, there was a significant difference between conditions (p=0.01). The average RT reduced after ihPAS$_{8ms}$ while increased after ihPAS$_{1ms}$. The opposite direction of change following two conditions supported our hypothesis that ihPAS$_{8ms}$ could potentially induce LTP-like plasticity in iM1 interneurons and enhance paretic motor function. On the contrary, LTD-like plasticity after ihPAS$_{1ms}$ could diminish the motor function. The performance of "lift can" (Figure 7b) and "stack checkers" (Figure 7c) had larger individual variance compared to "hand to table". The increased difficulty of the tasks and more requirement on the hand function compared to "hand to table" might contribute to the larger variability. Specifically, those two tasks needed more precise motor control over the object, and the "stack checker" task needed a high level of remaining fine dexterity in order to complete the task. No other significance was found by two-factors RM ANOVA. Two participants did not complete the WMFT during all visits due to undocumented reasons, and one participant failed to do the "stack checker" task which required greater dexterity function.
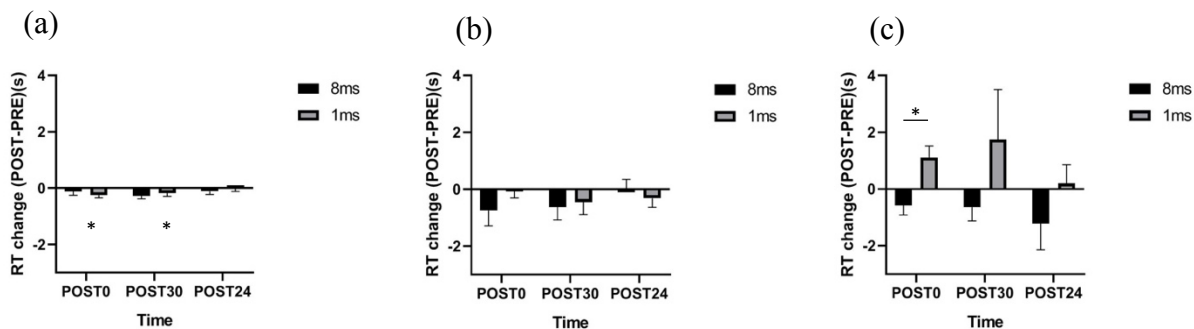
Figure 7. RT change at POST time points of 3-item WMFT relative to PRE. Each bar on the graph represents the change of RT after ihPAS (POST-PRE). The RT for 3-item WMFT-"hand to table" task (a) had a significant difference (RM-ANOVA time effect p=0.04) between PRE and POST0 (p=0.03); PRE and POST30 (p<0.01), suggesting improved paretic arm motor function immediately and 30 minutes after stimulation regardless of stimulation condition. There was a significant condition effect (p=0.048) in "stack checker" task (c). Post-hoc analysis identified the difference between conditions at POST0 (P=0.01). The performances for "lift can" (b), however, did not show significant change or difference in time, condition, or interaction of both factors after two-factor RM ANOVA. Each bar represents the average change of RT measured as POST-PRE in all participants. A negative change indicates improved performance while a positive change represents poorer performance with respect to pre-ihPAS performance. Error bars represent SE.

Paretic skill learning and motor function measured by SRTT were not improved after ihPAS

Two-factor RM ANOVA did not identify any significance in SRTT skill score (Figure 8a) and the performance (Figure 8b). The results indicated that ihPAS$_{8ms}$ did not significantly modulate motor learning and motor function compared to the control condition and to the baseline levels prior to ihPAS. Three participants in our study were not able to complete the SRTT at each time point due to the severity of their motor impairment. They reported feeling fatigue in their paretic arm. They were excluded from the analysis of skill but were included for the performance analysis which utilized the average RT of random trials which they had completed. The accuracy was not taken into consideration as some participants had longer

duration of pressing, which the program counted as mistakes. For those with milder impairment, the accuracy values were above 95%.
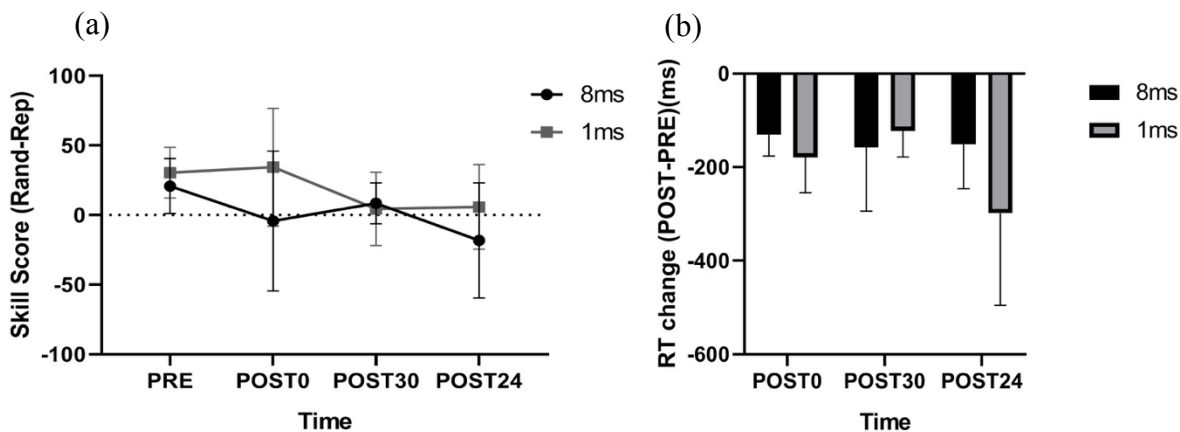

Figure 8. Skill score and performance change of SRTT. ihPAS$_{8ms}$ intervention did not significantly modulate SRTT (a) sequence learning or (b) general performance. Skill score was measured as the difference between the average RT for the last 50 random trials and last 4 groups of repeated trials (48 repeated trials). A positive skill score indicates motor learning. General SRTT performance was measured using mean RT for the first 50 random trials except the at PRE, for which the last 50 random trials were used for calculating the mean performance. The immediate effect of ihPAS$_{8ms}$, in this case, could be evaluated by the difference between performance at PRE and POST0. In (b), each bar represents the change of performance compared to baseline (POST-PRE), with a negative value meaning shorter RT and better motor function. Both ihPAS$_{8ms}$ and ihPAS$_{1ms}$ seem to decrease the RT without significant difference between conditions. Error bars represent SE.

Modification of cortical excitability and IHI might not contribute to the change of motor function

To examine the contribution of cortical excitability and IHI to the motor recovery process, we investigated if there were associations between the effect of ihPAS on neurophysiology and the motor behavior. However, after looking at the correlations between two of our neurophysiological measurements: cortical excitability and IHI, and five of our behavioral measurement: SRTT skill score, SRTT performance, WMFT-table, WMFT-can, and WMFT-checker at both POST0 and POST30, we did not find solid evidence of association between neurophysiology and motor behavior after ihPAS. In general, cM1-to-iM1 IHI change was not

strongly correlated with motor function change. Only at P0ST30 after ihPAS$_{1ms}$ was cM1-to-iM1 IHI ratio change significantly associated with SRTT performance change (Figure 9a. r=0.65, p=0.03). Moreover, there was a significant association between the greater iM1 excitability and the better paretic motor function measured by SRTT performance at ihPAS$_{8ms}$ POST30 (r=-0.94, p<0.01) (Figure 9b). However, the significance of the association might be largely driven by one value. In addition, there was a trend toward association between greater iM1 SP120 change and improved motor function measured by 3-item WMFT "stack checker" task at POST0 after ihPAS$_{8ms}$ (Figure 9c. r=-0.57, p=0.08), and all the other associations between neurophysiology and motor function measurements were not significant.
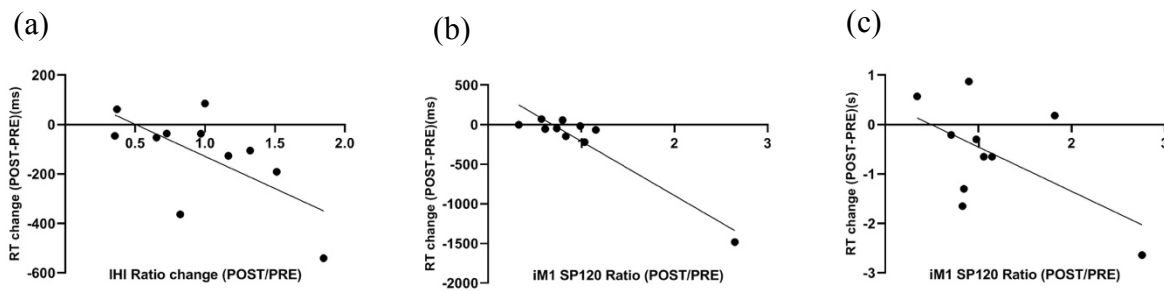

Figure 9. Associations between the effect of ihPAS on neurophysiology and motor behavior. Decreasing cM1-to-iM1 IHI was significantly associated with better performance in SRTT 30 minutes after ihPAS$_{1ms}$ (a. r=0.65, p=0.03). Better performance in SRTT had a significant association with increased iM1 excitability 30 minutes after ihPAS$_{8ms}$ (b. r=-0.94, p<0.01). Shorter RT in WMFT-"stack checkers" had a trend to be associated with increased iM1 excitability (c. r=-0.57, p=0.08) immediately after ihPAS$_{8ms}$. Changes of cortical excitability and IHI were normalized to PRE values. Behavioral measurements were showed as the difference in RT (POST-PRE). Changes of cortical excitability and IHI following ihPAS were only found to be associated with motor function change during certain task and time.

IHI was not associated with paretic hand motor function prior to ihPAS

Previously we expected an association between cM1-to-iM1 IHI and paretic hand motor function prior to ihPAS. However, we did not find any significant association between the bilateral IHI and paretic hand function (Figure 10) at baseline. The severity of motor impairment

after stroke may not be a direct consequence of abnormal IHI. Furthermore, we investigated if the baseline level of IHI could predict the change of paretic hand motor function. We evaluated the relationship between baseline cM1-to-iM1 IHI and change in motor function measurements at both POST0 and POST30. Nevertheless, no significant correlation was observed. The insignificance of IHI with both baseline motor function and modulation of motor function indicated that IHI might not have a direct contribution to the motor impairment after stroke.

There was greater iM1-to-cM1 IHI and reduced iM1 excitability prior to ihPAS

Since ihPAS did not significantly modulate SP120 and IHI, we took a step back to look at some neurophysiological characteristics of our participants. One assumption in our hypothesis was that after stroke, cM1-to-iM1 IHI would increase according to the interhemispheric competition model supported by results from previous studies (Murase et al., 2004; Duque et al., 2005; Volz et al., 2014). On the contrary to what we assumed, our data suggested the lack of inhibition from cM1-to-iM1 at baseline (Figure 11a). There was instead greater inhibition from iM1-to-cM1, which was demonstrated in the majority of our participants. There was also significantly reduced excitability in iM1 compared to cM1 (Figure 11b), which was as expected due to the direct damage and the subsequent maladaptation of ipsilesional hemisphere.

ihPAS$_{8ms}$ reduced cM1-to-iM1 IHI in participants with exaggerated baseline IHI

Since our participants as a group demonstrated the lack of inhibition from cM1-to-iM1, we were curious if ihPAS had a different modulatory effect on those with exaggerated baseline cM1-to-iM1 IHI (IHI<.6) compared to those with diminished IHI (IHI >.6). MEP amplitudes and IHI at POST0 and POST30 were normalized to PRE values respectively. Between-subjects one-way ANOVAs on normalized MEP and IHI for each condition were performed. The ANOVA showed significant between-subject difference on cM1-to-iM1 IHI (Figure 12b), indicating that
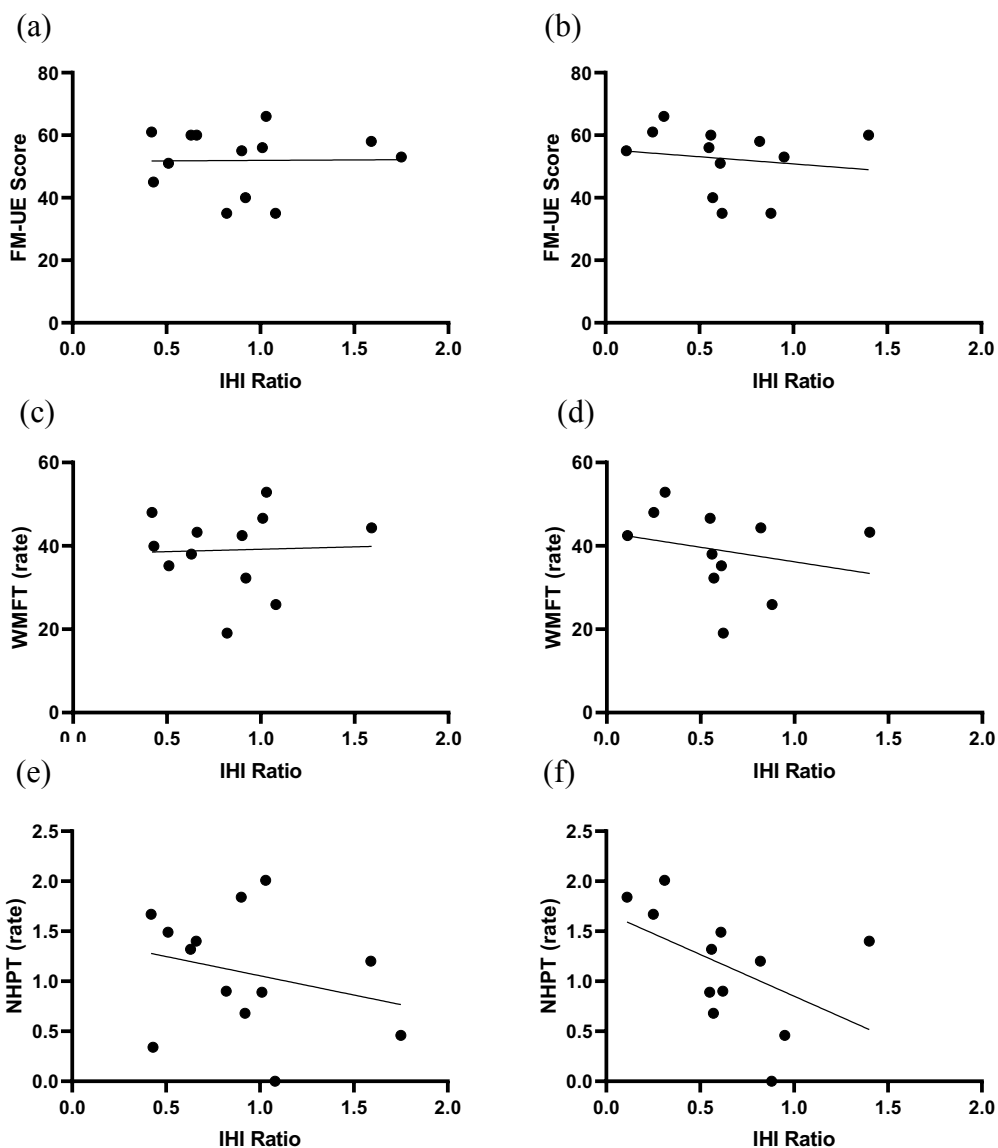
Figure 10. Baseline relationships between bilateral IHI and paretic motor function. There was no association between bilateral IHI and levels of impairment demonstrating in our participants before ihPAS intervention. Each dot represents a IHI ratio and a functional measurement for a single participant. Prior to TMS assessment, motor impairment was evaluated in FM-UE test (a, b), full version WMFT (c, d) and NHPT (e, f). The RTs were measured for WMFT and NHPT, and were converted into rates (60/RT). Neither cM1-to-iM1 IHI (a, c, e) or iM1-to-cM1 IHI (b, d, f) was significantly associated with the motor behavior.
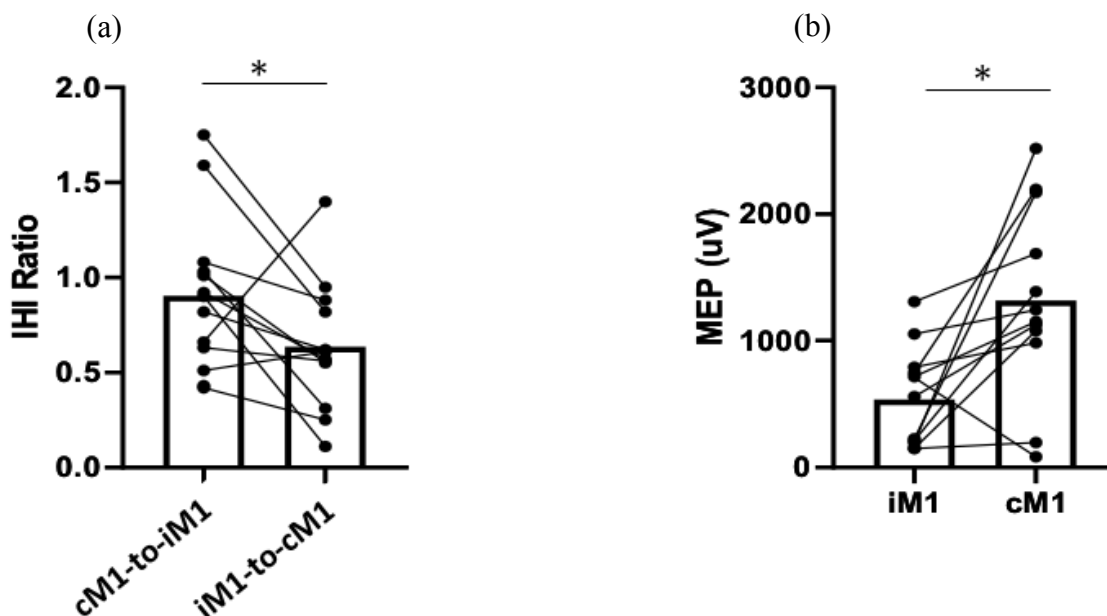
Figure 11. Baseline IHI and cortical excitability collected bilaterally at the PRE time point. Each dot represents the mean IHI (a) or mean SP120 MEP (b) of all participants. (a) iM1-to-cM1 IHI was significantly greater than cM1-to-iM1 ($p=0.04$). (b) cM1 MEP amplitude was significantly greater than iM1 MEP ($p=0.02$).

participants with exaggerated and diminished IHI responded differently to ihPAS$_{8ms}$. The difference was identified at POST0 by post-hoc test ($p<0.01$). Specifically, ihPAS$_{8ms}$ decreased cM1-to-iM1 IHI in participants with exaggerated baseline cM1-to-iM1 IHI. ihPAS$_{1ms}$, however, did not have any different modulation on SP120 (Figure 12c) and cM1-to-iM1 IHI (Figure 12d) between two groups.
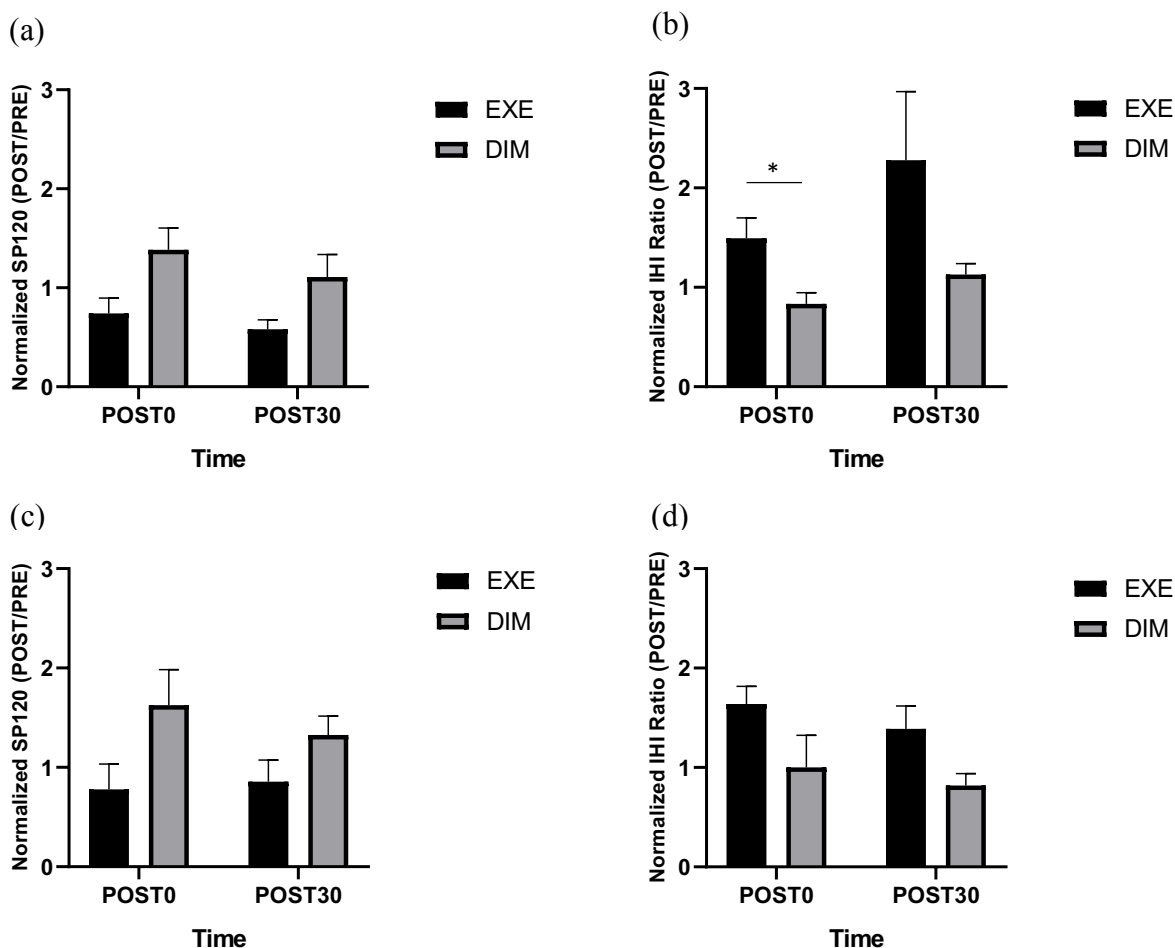
Figure 12. Effect of ihPAS on iM1 SP120 and cM1-to-iM1 IHI in participants with exaggerated or diminished baseline cM1-to-iM1 IHI. Participants were divided into two groups (EXE and DIM) to compare based on level of cM1-to-iM1 IHI prior to ihPAS. Participants with baseline cM1-to-iM1 IHI <0.6 were in the EXE group while the others were in the DIM. $ihPAS_{8ms}$ did not significantly modulate SP120 MEP (mean ± SD) in both groups (a). However, there was a significant difference of cM1-to-iM1 IHI after $ihPAS_{8ms}$ between the two groups (p=.002) at POST0 (p<0.01) (b). $ihPAS_{1ms}$ did not significantly affect both SP120 MEP (c) and cM1-to-iM1 IHI (d) in both groups.

Participants with different baseline cM1-to-iM1 IHI showed similar motor improvement after

$ihPAS_{8ms}$ and $ihPAS_{1ms}$

In the previous section of result, we identified that the baseline level of IHI could affect

the modulatory effect of $ihPAS_{8ms}$. However, we did not find the similar opposition effect of

ihPAS on motor function and motor learning between the two groups which were differentiated

by baseline cM1-to-iM1 IHI. In general, $ihPAS_{8ms}$ decreased RT in SRTT performance (Figure

13c) and WMFT tasks (Figure 14a, c, f) while ihPAS$_{1ms}$ did not consistently modify task performances.
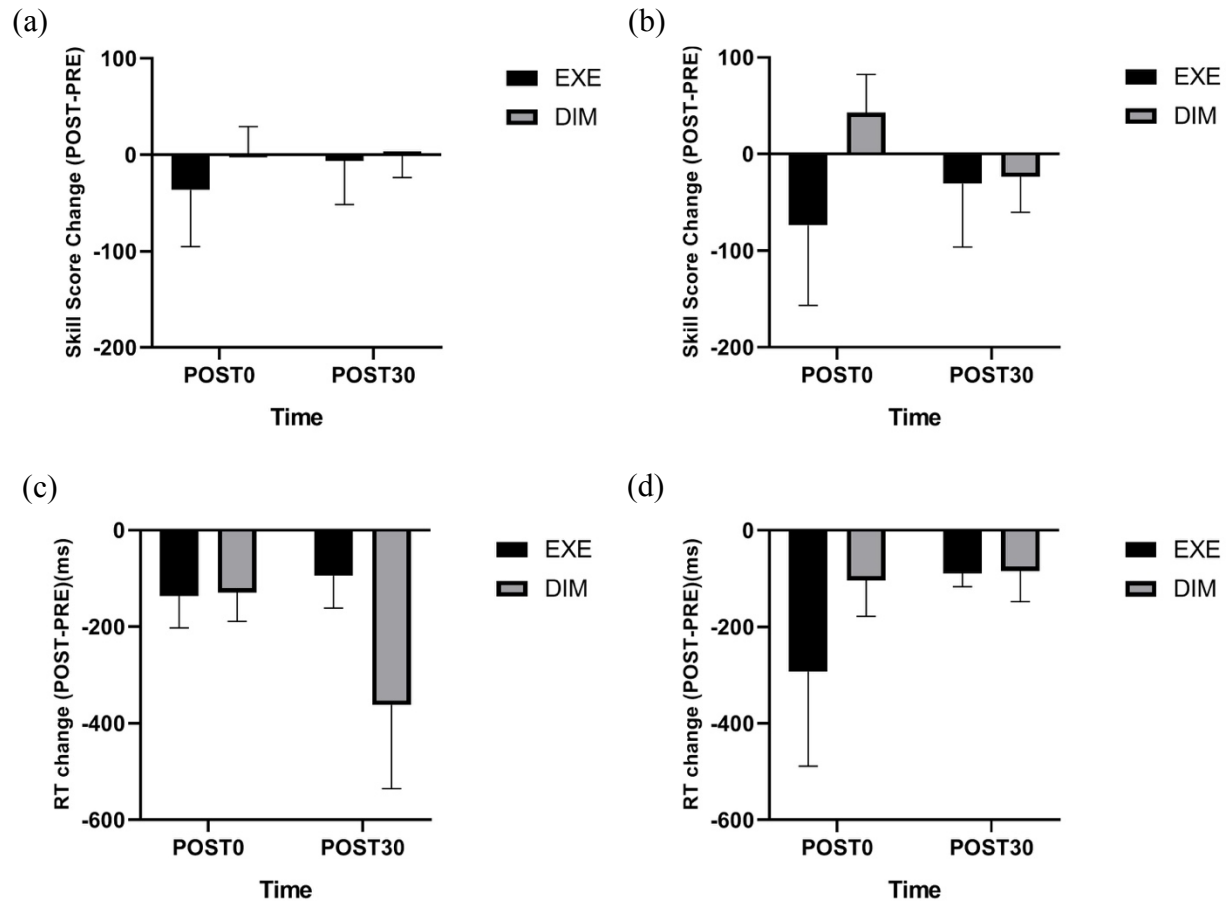
Figure 13. Effect of ihPAS on the SRTT in participants with exaggerated or diminished baseline cM1-to-iM1 IHI. ihPAS did not modulate motor learning and performance differently in participants with exaggerated (EXE) or diminished (DIM) baseline. Large variability was observed in the change of skill score (mean ± SE) after both ihPAS$_{8ms}$ (a) and ihPAS$_{1ms}$ (b) conditions. The RTs for SRTT performance were shortened after both ihPAS$_{8ms}$ (c) and ihPAS$_{1ms}$ (d) for all participants, with no significant difference between EXE and DIM group.
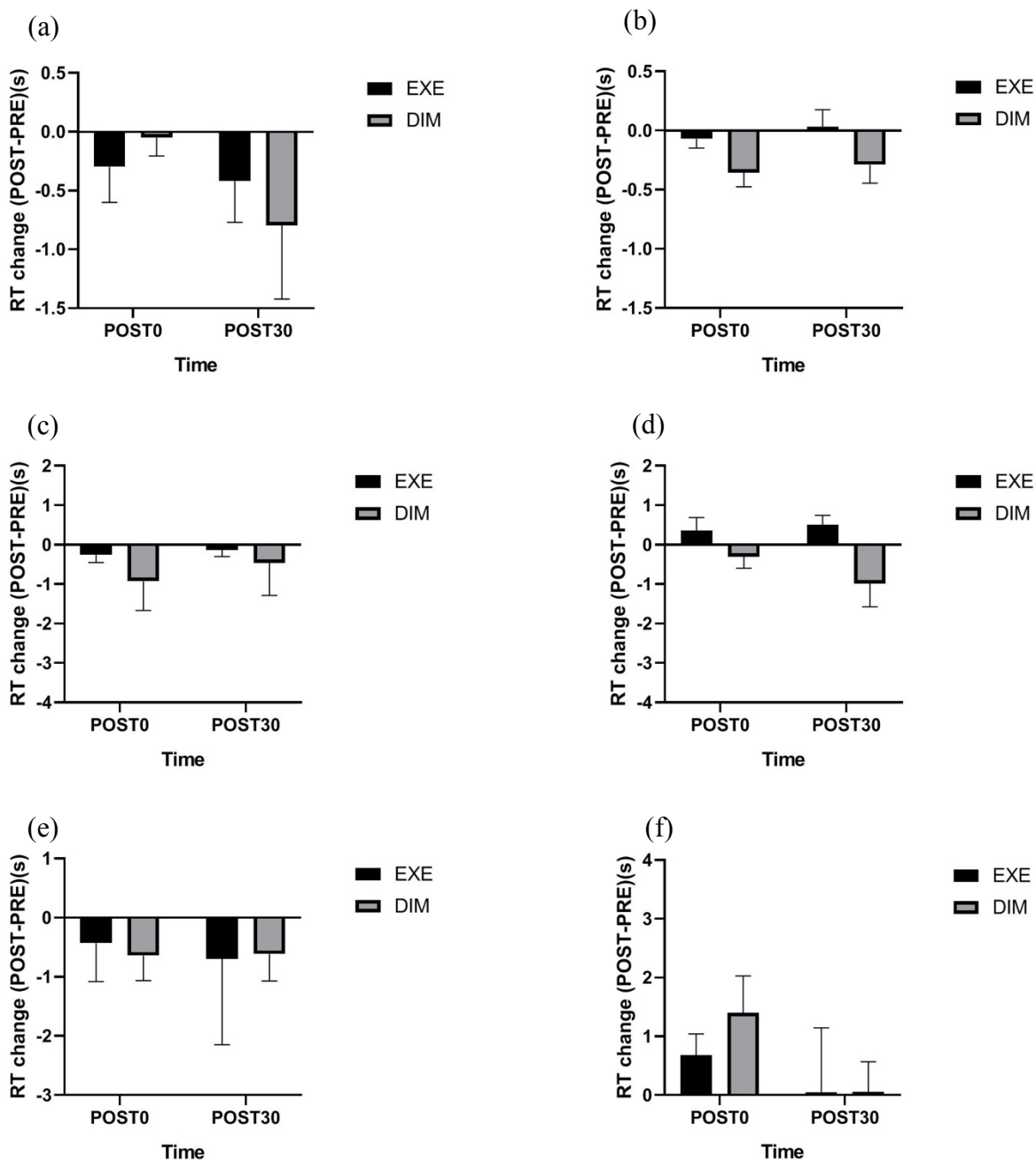
Figure 14. Effect of ihPAS on the 3-item WMFT in differentiated participants with exaggerated or diminished baseline cM1-to-iM1 IHI. Participants with exaggerated (EXE) or diminished (DIM) baseline IHI had no difference in 3-item WMFT responses. After ihPAS$_{8ms}$, the change of RT (POST-PRE) (mean ± SD) were negative in "hand to table" (a), "lift can"(c), and "stack checkers" (e) for both groups of participants. ihPAS$_{1ms}$ did not indicate consistent direction in motor function modification, and no clear difference in motor function modification between groups (b-"hand to table", d-"lift can", f-"stack checkers").

Discussion

This is the first study on stroke patients of the non-traditional PAS protocol which targets the transcallosal interhemispheric connections. Previously, ihPAS had only been applied to young healthy individuals in three studies (Rizzo et al., 2009, 2011; Koganemaru et al.2009). Each found positive results of the modulatory effect on IHI and cortical excitability after ihPAS. Moreover, the motor strategy was modified along with the neurophysiological changes. In our study, however, we did not find ihPAS to be significantly effective in modulating either brain plasticity within neural circuits or motor learning. ihPAS is still in its very early stage of clinical trial given the amount of studies on this protocol. On the contrary, according to a review by Dioniso et al. (2018), there were more than six hundred studies that have investigated the effect of rTMS on stroke rehabilitation. Although there were negative results, rTMS was still considered a promising tool given the number of randomized control trials with positive results. Another review on PAS protocol summarized the studies in the past two decades on the physiology, pharmacology, pathology, and motor effects in both healthy individuals and patients with various neurological conditions. The researchers believed that PAS is able to induce LTP/LTD -like plasticity in the human motor cortex (Suppa et al., 2017). Compared to traditional PAS and rTMS, ihPAS is relatively understudied, and it is still unclear whether it is capable of facilitating post stroke rehabilitation. It is very early for us to reach a solid conclusion about the effect of ihPAS given that our study had a small sample size and cannot fully represent stroke survivors who are in different stages in stroke recovery.

There are several explanations behind large variability which contributed to the insignificant effect of ihPAS on excitability and IHI. One possibility is the ongoing controversary about the post-stroke recovery model. The interhemispheric competition model

which was used to predict the of abnormal interhemispheric connectivity after stroke might be oversimplified. Our study was designed to reduce the abnormally high level of cM1-to-iM1 IHI and promote a balance of inhibition between hemispheres. One of the most important assumptions that was made in our hypothesis according to the competition model was that after stroke, the cM1-to-iM1 IHI would increase. However, after reexamining the baseline level of IHI before ihPAS intervention, we found a lack of resting cM1-to-iM1 IHI in our participants. This lack of IHI can be explained by reduced excitability in iM1 compared to cM1 observed at baseline. Since we normalized IHI MEP to single-pulse SP120 MEP, a smaller iM1 single-pulse MEP compared to cM1 could contribute to a greater value in normalized cM1-to-iM1 ratio. To take a step further, the diminished IHI after stroke could be a result of the lesion or the secondary degradation occurred at the transcallosal pathway (Wang et al., 2012), which might reduce the signal conductance of the cM1-to-iM1 fiber projections. Conversely, in a study by Boroojerdi et al., (1996), researchers found comparable levels of IHI in subcortical stroke patients had no direct damage in transcallosal pathway compared to healthy individuals. Another study also disagrees with the interhemispheric imbalance after stroke. The researchers found balanced IHI between hemispheres in the first 6 months of stroke in 46 participants, and the symmetric IHI remained stable over time (Stinear et al., 2015). Interhemispheric interaction needs to be characterized in more stroke survivors in different locations of lesion and different stages of rehabilitation to investigate the reason that the imbalance develops.

Because our group result did not indicate a significant effect of ihPAS, we reexamined our assumption that excessive cM1-to-iM1 IHI developed after stroke. We did not find increased level of cM1-to-iM1 IHI in all of our participants. As a result, our subsequent analysis focused on whether ihPAS affected differently those with exaggerated or diminished baseline cM1-to-

iM1 IHI. We found that for those with exaggerated baseline IHI, who fitted the expected competition model, had decreased cM1-to-iM1 IHI after ihPAS$_{8ms}$ as we hypothesized. This modulatory effect was significantly different from those with diminished baseline IHI, who did not show remarkable change in cM1-to-iM1 IHI afterwards. This divergent result indicates that the level of pre-stimulation baseline IHI is essential in determining whether the ihPAS will be effective in inducing the LTP-like plasticity in iM1. After recognizing that there was an intrinsic factor, baseline cM1-to-iM1 IHI, which could affect participants' physiological response to ihPAS, we extended the comparison between two groups to motor function results. However, participants with exaggerated cM1-to-iM1 IHI and diminished cM1-to-iM1 IHI did not always respond differently in the behavioral assessments after ihPAS$_{8ms}$. In addition, we performed analysis on the associations between changes in IHI and motor performance in participants with exaggerated cM1-to-iM1 IHI who fit our hypothesis. Nevertheless, our small number of participants who demonstrated exaggerated baseline cM1-to-iM1 IHI adds difficulty to finding significant and meaningful association. We are also unsure what contributes to the difference in baseline cM1-to-iM1 IHI in our participants. Neither physiological characteristics such as RMT or demographic characteristics such as lesion locations was revealing. Results from imaging studies on fiber tract integrity can potentially provide anatomical explanations to the strength of interhemispheric connectivity.

Furthermore, ihPAS did not show beneficiary motor outcome in all of our participants. Using the interhemispheric competition model as the "one-type-suits-all" standard to design the intervention that enhances brain plasticity might not be the most effective strategy for every survivor. Large variability was observed in our cohort of participants in respond to ihPAS. There are other models that explain the interhemispheric connectivity after stroke. The vicariation

model suggests the opposite to the rebalancing of hemispheric interactions. The activity in the intact hemisphere is believed to be compensatory to the lost function of the affected hemisphere. Activation of the intact hemisphere may facilitate the movement control and subsequent recovery of the affected hemisphere. Di Pino et al. (2014) proposed a bimodal balance-recovery model that combines the competition and the vicariation model. The researchers introduced a new independent variable that are different for each stroke patient: the level of structural reserve which indicates the spared neural pathways after the lesion. They argued that if the structural reserve after stroke is high, the interhemispheric competition model will predict the motor recovery. On the contrary, if the structural reserve is low at baseline, the functional recovery will incline to the vicariation model. The survivors in our study had mild to moderate motor impairment. They also had muscle response upon TMS simulation, which inferred high corticospinal fiber integrity. Our participants would fall in the category of high structure reserve, and thus the interhemispheric competition model would apply to this group of participants according to the bimodal-recovery model proposed by Di Pino. However, our results did not suggest that reducing cM1-to-iM1 IHI was the most effective strategy to promote motor recovery in our group of participants and thus cannot support the bimodal balance-recovery model. It is also uncertain if our indirect measures of structural reserve is effective in determining participants' recovery strategy, and if some of participants would benefited more from facilitating the cM1-to-iM1 IHI and cM1 excitability.

Furthermore, we did not find an association between baseline measures of IHI and motor impairment levels. Changes in IHI also cannot always predict changes in motor function. After stroke, the role of IHI for recovery remains unclear and might be different from the role of IHI in healthy brains. Although studies have shown that suppressing contralesional cortical excitability

improved motor outcome (Mansur et. al., 2005; Takeuchi et al., 2009; Du et al., 2016), it is possible that the modulation was mediated by different sets of fibers and networks. Inhibitory transcallosal pathways which were targeted in our study might not be the major contribution to stroke recovery in our participants. The lack of association between IHI and motor function and the large variability among individuals in response to ihPAS indicated that IHI might have even complicated levels and directions of effect in each person's recovery. Future studies on models of post-stroke recovery are necessary to investigate the relationship among structural integrity, IHI levels, and motor function on survivors with different levels of impairment. These relationships will help verify if newly proposed theory such as the bimodal-recovery model is more thorough in explaining post-stroke recovering mechanisms. If so, the further step will be finding a biomarker in guiding us design the intervention strategy which can effectively modulate interhemispheric connectivity and predict the therapeutic outcome on the level of individual.

Paretic hand motor function was improved following ihPAS intervention in the "stack checker task" which requires finger dexterity. Since we stimulated the M1 area that is responsible for FDI muscle activity, it is reasonable that we see the functional change in finger dexterity which has a strong involvement of the FDI muscle. The effect of ihPAS on brain plasticity may be local to the target areas rather than dispersive to other areas that are responsible for the grasp and shoulder function. Despite that ihPAS promoted dexterity function in the "stack checker" task, overall our motor measures showed large variability between and within individuals. For the SRTT, participants used different strategies to complete the task. Some used different fingers to tap the lightened squares; and some only used one finger throughout. It was difficult for us to examine the muscle involvement that was reflected by our measurement. The

SRTT with a keyboard to press down may work better than a touchpad since we can distinguish each finger's movement to each key. In addition, due to the length of each SRTT block, some participants with greater impairment reported fatigue and could not complete the task. And some participants had decreased attention although they were informed to complete the task as quickly as possible. Other simple motor tasks similar to the 3-Item WMFT which requires specific functionality of the paretic limb might be used in the future to increase the reliability of behavioral measurements.

Lastly, the stimulation protocol that we adopted in our study is mostly similar to Rizzo et al., 2009; however, their participants were young healthy individuals. Participants with stroke may have degeneration in the certain areas of brain which might affect the interhemispheric signal conductance rate (Wang et al., 2012; Radlinska, 2012). Aging is known be another factor of myelin coverage degradation within the transcallosal fibers (Peters, 2002), which can affect the integrity and conductance rate of the signal. The ISI of 8ms in our intervention protocol might be shorter than the optimal for an afferent signal from cM1 to arrive iM1, and 100 pairs of stimulation might not be enough to modify the synaptic strength. In the future, studies may focus more on individualizing the intervention rather than using a standard protocol. Since the synchrony of the input and output signal is critical in inducing STDP, ISI may be customized to each induvial. The optimal ISI which can elicit the largest IHI can be used for the intervention. The challenge that comes with implementing this idea is to find a reliable IHI, which might be varied by visits according to the observation on our data.

In conclusion, in the current study, ihPAS$_{8ms}$ did not significantly modulate the interhemispheric connectivity in our cohort of participants with chronic ischemic stroke. Motor function of the paretic hand was improved following stimulation. Future studies may further

characterize levels of IHI after stroke and its relativity with motor recovery. With clear biomarkers to predict the effectiveness of intervention strategy and the motor outcome, we can customize rehabilitation plan for each survivor and minimize the life-long motor function impairment resulting from stroke.

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