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# Modulation of corticospinal excitability induced by paired associative stimulation combined with movement

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## ABSTRACT

## MODULATION OF CORTICOSPINAL EXCITABILITY INDUCED BY PAIRED ASSOCIATIVE STIMULATION COMBINED WITH MOVEMENT

**by**
**Ahmad O. Alokaily**

An essential feature of the brain is its capacity to undergo long-lasting morphological or functional changes in response to experiences or trauma. Advances in noninvasive brain stimulation techniques have led to increased interest in understanding neural mechanisms of neuroplasticity at the network level. Paired associative stimulation (PAS) is one of the most commonly used applications for noninvasive brain stimulation because of its clinical potential as an adjuvant rehabilitative intervention. However, the optimal method for incorporating PAS into rehabilitative activities remains unknown. This dissertation explores different approaches to combining PAS with movement and investigates the enhancement of the specificity of conventional PAS-induced effects.

A fundamental aspect in combining PAS and voluntary movement is the timing of stimuli with respect to muscle activation onset. Therefore, this dissertation first focuses on determining the effect of PAS on the primary motor cortex (M1) excitability when the stimuli are delivered during preparation or execution of a voluntary finger extension in a reaction time setup. The results of this investigation show that applying PAS during voluntary contraction or at rest increases the corticospinal excitability (CSE), while PAS delivered during movement preparation decreases CSE. This suggests that the direction of PAS-induced plasticity is dependent on the order of stimulation and the phase of the movement.

Next, combining PAS with the movement of the stimulated limb may further increase the enhancement of CSE. However, individuals with moderate to severe motor function impairment due to stroke may not be able to engage in the necessary repetitive voluntary movements of the paretic limb. The objective of this study is to investigate the feasibility of contralaterally coordinated PAS applied to the resting hand's extensors during fast extension of the contralateral hand in healthy individuals. PAS delivered during the muscle contraction of the left hand, and PAS delivered at rest both increase the CSE. Delivering PAS during the preparation phase of the left-hand movement leads to a decrease in the CSE. Thus, PAS-induced bidirectional plasticity effects that are dependent on the phase of the movement of the opposite hand.

Additionally, given the noted topographical specificity of the somatosensory cortex and reported muscle-specific PAS-induced changes, this dissertation also evaluates the feasibility of enhancing the specificity of PAS-induced effects through a simultaneous application of facilitatory PAS to hand extensor muscles and inhibitory PAS to flexor muscles while at rest in healthy individuals. The simultaneous application of PAS, targeting the hand extensor muscle with facilitatory PAS and hand flexor muscle with inhibitory PAS leads to a consistent and significant increase in CSE of the extensor muscle.

Finally, in a pilot study, two scenarios combining PAS with dynamic hand movements in a reaction time paradigm are explored in people with stroke. PAS of the ipsilesional M1 is combined with a voluntary activation of finger extensors of the paretic limb or during a nonparetic finger extension. This is done to evaluate the feasibility of combining PAS with voluntary movement in chronic stroke patients. Although there were

no notable changes in the CSE, recruited stroke patients are able to tolerate and perform a motor task with both their affected and less affected hand while PAS is applied.

# MODULATION OF CORTICOSPINAL EXCITABILITY INDUCED BY PAIRED ASSOCIATIVE STIMULATION COMBINED WITH MOVEMENT

**by**
**Ahmad O. Alokaily**

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**APPROVAL PAGE**

**MODULATION OF CORTICOSPINAL EXCITABILITY INDUCED BY PAIREDASSOCIATIVE STIMULATION COMBINED WITH MOVEMENT**

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To my friends and family

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# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Stroke Rehabilitation

As reported by the American Heart Association, stroke is among the leading causes of death in the U.S. Although 75% of stroke cases occur in people aged 65 or older, strokes can affect people of all ages (Mozaffarian et al., 2015; Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). More than three million Americans experience difficulties in their daily lives from disabilities due to stroke (Dobkin, 2005). These numbers indicate the continuous need for rehabilitative and therapeutic interventions.

A stroke can be classified as either hemorrhagic, when blood vessels in the brain rupture, or ischemic, when caused by occluded blood vessels in the brain. Both types of strokes can cause hemisyndromes associated with motor and sensory disabilities, which result from a brain injury to the motor cortices, the descending pathways, basal ganglia, and/or cerebellum (Sacco et al., 2013).

The ability of the human brain to reorganize its neural networks by learning or responding to pathological changes such as brain lesions caused by a stroke or injury is known as neuroplasticity (Dickins, Kamke, & Sale, 2017). This cortical remapping occurs in an activity-dependent manner and based on a competitive pattern (Murphy & Corbett, 2009). In the case of a small stroke, residuals of damaged networks compete with neighboring healthy tissues that share the same functions in order to recover their territory (Nudo, Wise, SiFuentes, & Milliken, 1996). On the other hand, the lost function of damaged circuits in a large stroke can take place at different structures distant from an

infarct location or contralaterally in the other hemisphere (Murphy & Corbett, 2009; Winship & Murphy, 2009). Through this compensatory process, along with the creation of new connections between intact neurons, functions can be recovered (Lin & Liebeskind, 2016). In stroke rehabilitation, motor recovery can be augmented through an emphasis on many factors such as timing and intensity of treatment and neuroplasticity. Some of the current commonly used rehabilitative approaches in clinical practice are constraint-induced movement therapy and bilateral arm training.

Constraint-induced movement therapy is a task-specific upper extremity rehabilitative method. In constraint-induced movement therapy, patients are forced to use only their paretic limb to complete tasks (Liu, Huai, Gao, Zhang, & Yue, 2017). The positive outcomes of increased motor function, dexterity, and activity of daily living have made constraint-induced movement therapy the most clinically used therapeutic intervention (Kwakkel, Veerbeek, van Wegen, & Wolf, 2015). Another known therapeutic intervention post-stroke is bilateral arm training. In bilateral arm training, stroke survivors are instructed to use both hands to perform movements at the same time. Bilateral arm training motor recovery is believed to be a result of the rebalancing of the interhemispheric inhibition between affected and less affected hemispheres along with the activation of the affected hemisphere (Pollock et al., 2014).

Although these treatments have been proven to be effective to a certain extent, moderate to severely impaired stroke patients cannot benefit from them due to their lack of motor function. Thus, their treatment options are limited. Additionally, it has been reported that currently used therapeutic interventions for stroke survivors do not deliver satisfactory outcomes (Dąbrowski et al., 2019).

## 1.2 Non-Invasive Brain Stimulation

Noninvasive brain stimulation is a term used to describe different technologies and techniques that are noninvasively, able to modulate the cortical excitability using transcranial stimulation (Boes et al., 2018). Recently, a massive interest has developed around the use of noninvasive brain stimulation techniques because of their abilities to induce corticospinal excitability changes, which might enhance the outcomes of traditional rehabilitative practices (Harris-Love & Cohen, 2006). Another important role of noninvasive brain stimulation is providing a substrate to explore brain plasticity at the system level (Carson & Kennedy, 2013; Muller-Dahlhaus, Ziemann, & Classen, 2010).

The activity-dependent modification of synaptic weight is known as synaptic plasticity (Citri & Malenka, 2008). The foundation of our understanding of neural synaptic plasticity comes from Hebb's postulate: "*When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.*" (Hebb, 2005). Thus, the synchronous activation of pre-synaptic and postsynaptic neurons leads to the strengthening of synaptic connections, inducing long-term potentiation (LTP) where asynchronous depolarization decreases synaptic connections causing long-term inhibition (LTD) (Bliss & Gardner-Medwin, 1973). Both LTP and LTD are often proposed as candidate mechanisms for learning and memory (Martin, Grimwood, & Morris, 2000; Thabit et al., 2010).

One of the most used noninvasive, painless, and safe modalities for brain stimulation is transcranial magnetic stimulation (TMS). In the TMS device, an electric current is induced by rapid magnetic field changes generated by a TMS electromagnetic

coil, which depolarizes the cerebral cortex neurons (Barker, Jalinous, & Freeston, 1985). When applied over the motor cortex, a TMS pulse depolarizes interneurons, activating the pyramidal neurons. The depolarization of pyramidal neurons arouses descending volleys that travel down the corticospinal tract. These descending volleys activate the motor-neurons, causing a contraction in the peripheral muscle. By using a surface electromyography (EMG) electrode, motor-evoked potential (MEP) can be recorded in response to TMS (Klomjai, Katz, & Lackmy-Vallee, 2015). This TMS-elicited MEP provides a quantitative measure of corticospinal excitability (Bestmann & Krakauer, 2015) (See Figure 1.1). Since it was introduced in the late 1980s, TMS has been used to investigate cortical reorganization and excitability (Cohen et al., 1998). The viability of TMS in inducing short-term changes in corticospinal excitability in different brain diseases, such as strokes (Liepert, 2003), Parkinson's (Behzad Elahi, Elahi, & Chen, 2009), and dystonia (Schneider et al., 2010) has been investigated. The TMS modality is considered a prominent neurophysiological tool for the diagnosis of the integrity of corticospinal pathways (Groppa et al., 2012) and investigation of the brain plasticity in humans as in the experimental paired associative stimulation (PAS) paradigm (Muller-Dahlhaus et al., 2010).

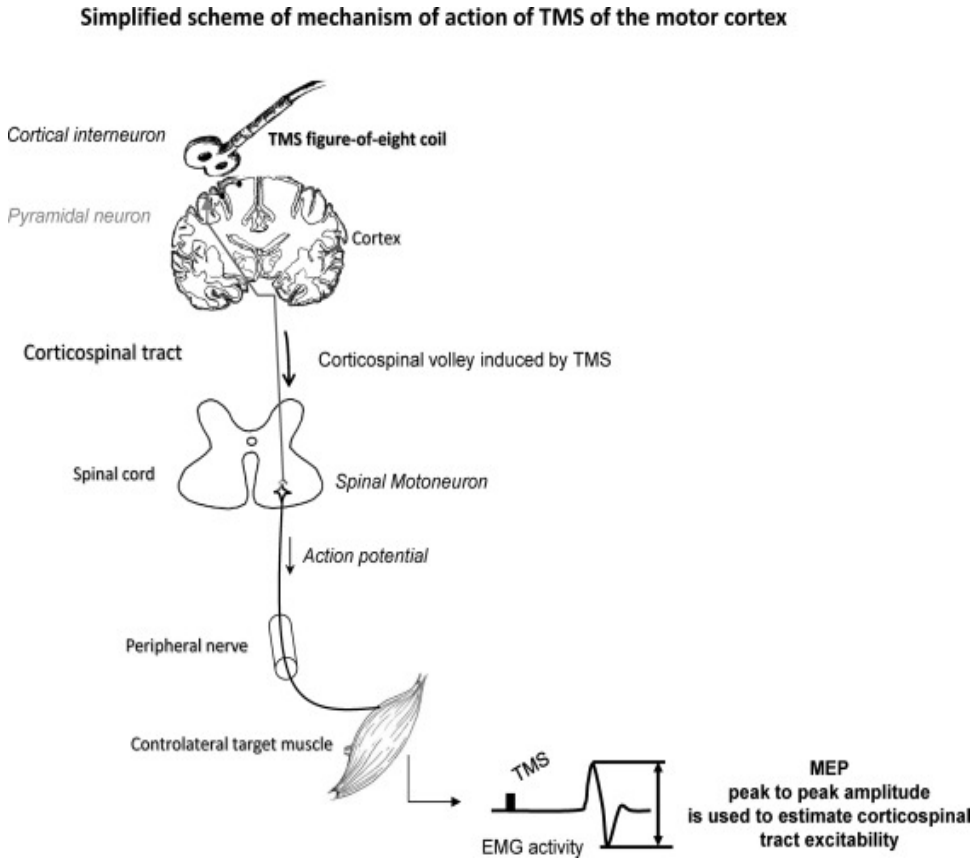


**Figure 1.1** Mechanism of action of TMS of the motor cortex.

*Source: (Klomjai et al., 2015).*

### 1.2.1 Paired Associative Stimulation

One of the most used noninvasive brain stimulation paradigms to modulate cortical excitability is PAS. This paradigm combines the electrical stimulation of the peripheral nerve and the central stimulation of the motor cortex by the TMS in order to investigate synaptic plasticity in humans at the system level. Since it was introduced by Stefan and colleagues (2000), this technique has been studied extensively for its ability to modulate corticospinal excitability, which might help in the restoration of motor functions in persons who have experienced strokes (Borich, Wolf, Tan, & Palmer, 2018; Castel-Lacanal, Gerdelat-Mas, Marque, Loubinoux, & Simonetta-Moreau, 2007).

This experimental paradigm is thought to follow a type of spike-timing dependent plasticity (Muller-Dahlhaus et al., 2010). This is because PAS induces bidirectional changes in the corticospinal excitability that are dependent on the activation order of the received stimulation. Hence, when the TMS pulse is delivered after the arrival of an afferent volley, an increase in the MEPs amplitude can be observed in the M1, leading to an LTP-like effect (Stefan et al., 2000). In the original PAS study by Stefan et al. (2000), an ISI of 25ms between the electrical stimulation and TMS was found to induce this LTP-like effect. This was established based on the assumption that the conduction time of the afferent volley in reaching the somatosensory cortex would be 20 ms and then 3 ms for the information to be conveyed to M1 (see Figure 1.2). On the other hand, if the sequence of events is reversed, so that the TMS of M1 is activated before the arrival of the afferent volley, a decrease in the corticospinal excitability is noted. This is believed to resemble LTD-like plasticity changes.

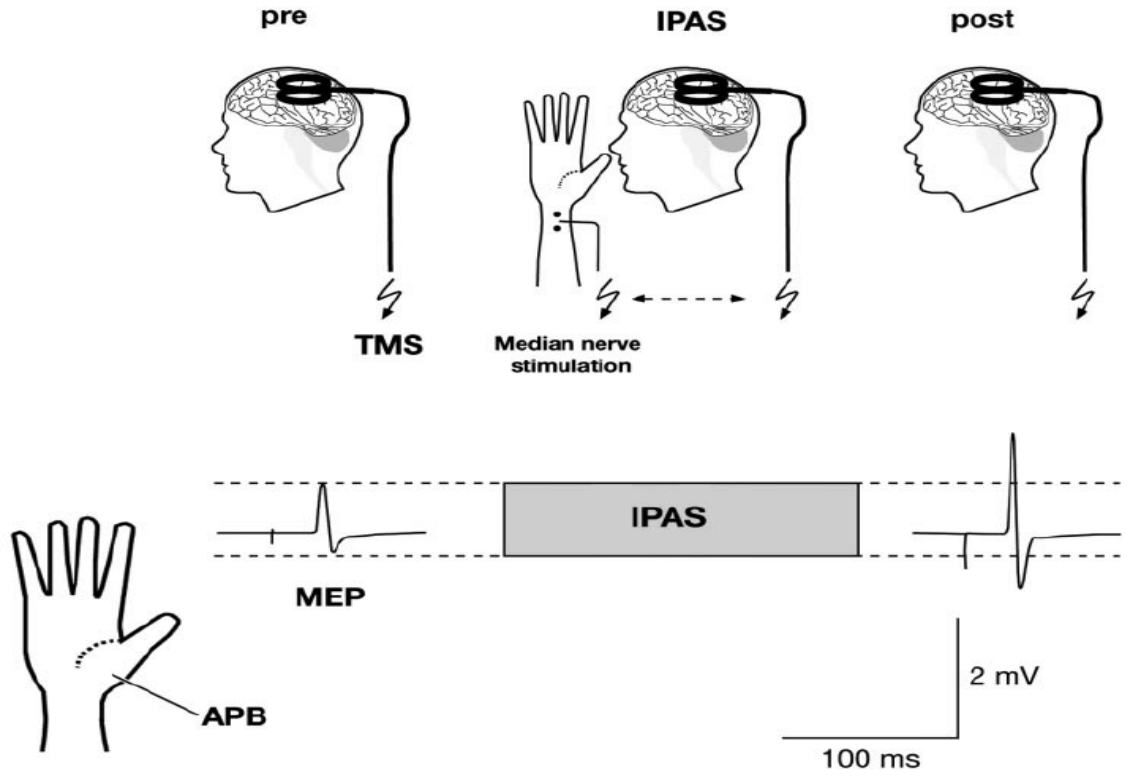


**Figure 1.2** Schematic view of the PAS protocol: PAS consists of medial nerve electrical stimulation paired with a TMS pulse with 25ms ISI. The MEPs were elicited by TMS only before (pre) and immediately after (post) the intervention (IPAS). An increase in the MEPs amplitude measured from abductor pollicis brevis (APB) muscle is noted following the PAS protocol.

*Source: (Stefan et al., 2000).*

Several studies have investigated the site at which PAS-induced excitability changes occurs. The effects induced by PAS are widely believed to occur at the level of the cortex. This is evident, as the application of PAS does not have any modulatory changes on the F-wave, which is considered an indicator of spinal motor neuron excitability (Castel-Lacanal et al., 2007; Stefan et al., 2000).

However, administrating PAS at rest is not always effective in inducing changes in corticospinal excitability (Castel-Lacanal et al., 2007; M. R. Kamke, A. S. Nydam, M. V. Sale, & J. B. Mattingley, 2016). Therefore, some researchers used modified PAS protocols

to augment PAS-induced changes. For instance, Kujirai et al. (2006) found that introducing minimal contraction (5% maximum voluntary contraction (MVC) of the PAS targeted first dorsal interosseous muscle (FDI) muscle accelerated the induced PAS modulatory effect (Kujirai, Kujirai, Sinkjaer, & Rothwell, 2006). In their PAS combined with muscle contraction protocol, only 50 stimulation pairs were sufficient to induce an LTP-like increase in the MEPs while conventional PAS at rest needed at least 90 pairs to induce the same effect. Moreover, Mrachacz-Kersting et al. (2007) compared the outcomes of applying PAS during dorsiflexion to applying PAS at rest (Mrachacz-Kersting, Fong, Murphy, & Sinkjaer, 2007). They found that PAS delivered during active dorsiflexion elicited significant increases in average MEPs following PAS, while PAS at rest did not induce notable changes.

Furthermore, based on the same time-dependency plasticity concept, others successfully induced corticospinal plasticity changes by pairing voluntary muscle contraction (instead of artificial peripheral stimulation of the targeted muscle) and TMS pulse over M1 or with peripheral electrical stimulation to the targeted muscle (Edwardson, Avery, & Fetz, 2014; Thabit et al., 2010). This type of stimulation, like PAS, was found to have inter-stimulus, time-dependent, and bidirectional induced effects. In a study by Thabit et al. (2010) the movement-related cortical stimulation (MRCS) protocol led to an increase of cortical excitability when the TMS pulse over M1 was 50 ms before the estimated abductor pollicis brevis (APB) muscle activation. On the other hand, delivering TMS 100 ms after the estimated voluntary muscle activation led to a significant decrease in the APB MEPs after the intervention (Thabit et al., 2010).

Thus, enhancing PAS-induced corticospinal excitability changes in combination with voluntary muscle activation could be the result of decreasing in the activity cortical inhibitory circuitry (Kujirai et al., 2006). Voluntary muscle activation was found to be associated with a decrease in the excitability of intra-cortical inhibitory networks when evaluated through short-interval intracortical inhibition (SICI) (Ridding, Taylor, & Rothwell, 1995). Reducing the activity of inhibitory circuits within the M1 was suggested in order to augment the development of LTP-like effects (Stefan, Kunesch, Benecke, Cohen, & Classen, 2002).

# CHAPTER 2

# OBJECTIVE

With ~800,000 cases every year in the U.S., stroke is considered a major cause of disabilities in adults (Mozaffarian et al., 2015). The ability of the brain to reorganize in response to pathological or environmental changes is important for the recovery of motor functions after a stroke (Cramer et al., 2011). Following a stroke, the corticospinal excitability of the ipsilesional cortex has been found to be suppressed when compared to contralesional or in neurologically intact individuals (Stinear, Petoe, & Byblow, 2015). The re-establishment of lesioned corticospinal excitability has been associated with improved functional outcomes of the paretic limb (Pomeroy et al., 2011).

One promising adjuvant therapeutic approach to modulate corticospinal excitability in the primary motor cortex (M1) is paired associative stimulation (PAS). PAS has been described as a peripheral nerve stimulation paired with transcranial magnetic stimulation (TMS) of the M1 in a timing-dependent manner in order to induce Hebbian-like plasticity (Stefan et al., 2000). This repetitive paired stimulation induces bidirectional changes in M1 corticospinal excitability depending on the interval between the peripheral and central stimulation (Carson & Kennedy, 2013). However, the small effect size and high variability of the PAS intervention leads one to question its therapeutic potential (Lahr et al., 2016).

Furthermore, the ideal method for incorporating PAS into rehabilitative activities remains unknown. In this dissertation, we investigate different approaches that combine PAS with movement. Unlike the overwhelming majority of PAS studies, in which stimulation is delivered to flexor muscles of the hand, we focused on applying PAS to

extensor muscles, which is more relevant for stroke rehabilitation (Castel-Lacanal et al., 2009). Rehabilitative interventions directed at enhancing wrist extensor muscles resulted in increased motor function post-stroke (Powell, Pandyan, Granat, Cameron, & Stott, 1999). The modulation of corticospinal excitability was assessed by TMS-elicited motor evoked potential (MEP) amplitude, which is a quantitative measure of corticospinal excitability (Bestmann & Krakauer, 2015).

In summary, the specific aims of this dissertation are as follows:

Aim1: To determine the optimal timing of triggering PAS over the contralateral motor cortex during unilateral hand movement in healthy people.

The majority of published PAS studies have investigated the modulation of corticospinal excitability while the targeted muscle is at rest (Castel-Lacanal et al., 2007; Fratello et al., 2006; Lahr et al., 2016; Stefan et al., 2000). Furthermore, introducing a slight isometric contraction of the targeted muscle during PAS has been shown to accelerate the induction of LTP-like plasticity and lessen the required number of stimulations compared to PAS at rest (Kujirai et al., 2006; Mrachacz-Kersting et al., 2007). However, to our knowledge, no study has investigated the optimal timing of triggering PAS when combined with isotonic finger extension, which closely mimics motor training protocols used in stroke rehabilitation. Thus, for this study, noting the importance of temporal dependency of PAS and the changes in cortical activity associated with volitional movement (Kornhuber, 1965), we investigated the effect of PAS on M1 excitability when the stimuli are delivered during the preparation or execution phase of voluntary activation of the finger extensors. This aimed to test the

hypothesis that triggering PAS during the different phases of contralateral hand movement could affect the direction of the PAS-induced effect.

Aim 2: To determine the optimal timing of triggering PAS over the ipsilateral motor cortex during unilateral hand movement in healthy people.

Combining PAS with the movement of the stimulated limb may further facilitate the corticospinal excitability. However, stroke individuals with a moderate to severe motor function may not be able to engage in the necessary repetitive voluntary movements of the paretic limb. We investigated the effects of PAS delivered during movement of the opposite hand on corticospinal excitability of the hemisphere ipsilateral to the moving hand. Critical to the effective application of contralaterally coordinated PAS (ccPAS) might be the timing of the stimuli relative to voluntary activation, as ipsilateral M1 excitability during unilateral hand movement is known to vary with the movement phase (Beaule, Tremblay, & Theoret, 2012). Therefore, we specifically explored the impact of the timing of PAS delivery relative to the onset of contralateral hand extension on M1 excitability as measured by MEP amplitude. This tested the hypothesis that triggering ccPAS during the execution phase of contralateral hand movement may further facilitate the corticospinal excitability of ipsilateral M1.

Aim 3: To investigate the feasibility of enhancing the specificity of PAS-induced effects through the simultaneous application of facilitatory PAS to hand agonist muscles and inhibitory PAS to hand antagonist muscles while at rest in healthy people.

It is widely believed that PAS-induced effects are topographically specific to the muscles innervated by the peripherally stimulated nerve. Other studies describe

PAS-induced effects spreading to surrounding muscles that are not being innervated by the stimulated peripheral nerve (see review (Carson & Kennedy, 2013)). In PAS protocols, inter-stimulus interval (ISI) of 25 ms between peripheral and central stimulation (PAS25) induces an increase in the MEP amplitudes of the targeted muscle that is associated with an increase of MEP amplitudes acquired from other untargeted muscles (Stefan et al., 2000). On the other hand, an ISI of 10 ms (PAS10) leads to a reduction in the MEP amplitudes of the PAS targeted muscle that is accompanied with an increase in MEP amplitudes of muscles that are not innervated by the targeted nerve (Wolters et al., 2003). Thus, we investigated the ability to augment the PAS-induced corticospinal excitability and/or its specificity effect by simultaneously targeting the finger extensors with PAS25 and flexors with PAS10 (dual-PAS). This tested the hypothesis that dual-PAS may increase the MEP amplitudes of finger extensors and decrease the MEP amplitudes in finger flexors.

Aim 4: To explore the feasibility of combining PAS with voluntary movement in persons with chronic stroke (a preliminary investigation).

Conventional PAS has previously been demonstrated as effective in inducing a short-term increase of corticospinal excitability in stroke patients (Castel-Lacanal et al., 2007; Fratello et al., 2006). In addition, improvement in motor functions as a result of traditional intensive task-specific training has been associated with an increase in the cortical excitability of the lesioned M1 post-stroke (Tarkka, Könönen, Pitkänen, Sivenius, & Mervaala, 2008). It has been suggested that functional recovery could be augmented through a combination of rehabilitative training and neuromodulatory techniques (Hoyer & Celnik, 2011). In this feasibility study, we examined whether

combining PAS with a contralesional or ipsilesional task-specific movement would conform to PAS-induced effects obtained from Aim1 and Aim 2. This approach was used to test the hypothesis that triggering PAS during the execution phase of the paretic or nonparetic hand movement might facilitate the PAS-stimulated ipsilesional M1 for people with stroke.

# CHAPTER 3

# THE DIRECTION OF PAS-INDUCED MODULATION OF CORTICOSPINAL EXCITABILITY DEPENDS ON TIMING BETWEEN STIMULATION AND MOVEMENT ONSET

## 3.1 Abstract

Paired associative stimulation (PAS) is a recognized technique to induce neuroplastic changes in the human motor cortex (M1). PAS modulates cortical excitability by pairing peripheral nerve stimulation with TMS of M1 in a timing-dependent manner to induce Hebbian-like plasticity. To date, PAS is most often performed with the subject at rest. A key question about how to combine PAS and voluntary movement is the timing of stimuli with respect to muscle activation onset. In this study, we investigate the effect of PAS on M1 excitability when the stimuli are delivered during the preparation or execution of a voluntary movement. Sixteen healthy right-handed subjects participated in the experiment. Prior to each session, 20 trials were conducted without stimulation in order to compute the mean reaction time (RT) for timing the stimulation during PAS. Subjects were instructed to extend their right fingers, activating the extensor digitorum communis (EDC) in response to a visual cue. Each PAS session consisted of 240 pairs applied at a rate of 0.2 Hz. All subjects completed the following three PAS sessions triggering the TMS: 1) during movement preparation (PAS RT-100ms), 2) during movement execution (PAS RT+50ms), and 3) conventional PAS at rest. Transcranial magnetic stimulation-induced motor evoked potentials (MEPs) were measured in the muscles of interest prior to and following the intervention to assess changes in corticospinal excitability. The MEP amplitudes were used for the statistical analysis. PAS triggered during voluntary contraction (RT+50) or at rest increased excitability, while PAS delivered at the same inter-stimulus interval during

movement preparation (PAS RT-100) decreased excitability. Additionally, these changes were significant only for the targeted EDC muscle and not the flexor digitorum superficialis (FDS), indicating a muscle-specific effect. Unlike most PAS studies, our focus was on hand extensors for their rehabilitative importance. The results of this investigation suggest that the direction PAS-induced plasticity was dependent on the order of stimulation and voluntary movement onset. These findings have important implications for the incorporation of PAS into neuromotor rehabilitative training. Future investigations should explore the underlying neurophysiological mechanisms and possible clinical applications.

## 3.2 Introduction

In the last two decades, a strong interest has developed around using non-invasive brain stimulation approaches as they have demonstrated promising results in modulating the brain corticospinal excitability with long-lasting effects (Kubis, 2016). One promising adjuvant rehabilitative intervention is paired associative stimulation (PAS). The repetitive paired stimulation has been found to induce long-lasting, muscle specific, bidirectional changes in M1 cortical excitability depending on the interval between the peripheral and central stimulation (Suppa et al., 2017). Previous literature suggests that PAS-induced effects are temporally dependent. Several studies of the upper extremity have demonstrated that if a single pulse of peripheral electrical stimulation precedes a TMS pulse delivered over the representative area of the contralateral M1 by 20 to 25 ms, an increase in M1 cortical excitability represented as an increase in the motor evoked potentials (MEPs) following PAS is achieved. This induced change appears to cause a type of long-term-

potentiation (LTP-like) effect (Castel-Lacanal et al., 2007; Stefan et al., 2000). On the other hand, a decrease in the cortical excitability can be induced with the use of an intra-stimulus interval (ISI) of 10 ms, during which the TMS stimulation occurs before the arrival of the afferent stimulation to M1, leading to long-term depression (LTD)-like phenomena (Wolters et al., 2003). The majority of previous PAS studies have investigated the ability of PAS to modulate M1 excitability while the targeted muscle is at rest (Fratello et al., 2006; Stefan et al., 2000).

Based on the same time-dependent plasticity concept examining PAS, other researchers have successfully induced short-term cortical plasticity by pairing voluntary muscle contraction (instead of artificial peripheral stimulation of the targeted muscle) with TMS pulse over M1 or with peripheral electrical stimulation to the targeted muscle (Jochumsen et al., 2016; Thabit et al., 2010). These alternate approaches to paired stimulation, like conventional PAS, have been found to have inter-stimulus time-dependent effects. In a study by Thabit et al. (2010), movement-related cortical stimulation (MRCS) protocol led to an increase of cortical excitability when the TMS pulse over M1 was delivered 50ms before the estimated abductor pollicis brevis (APB) muscle activation. On the other hand, delivering the TMS pulse 100 ms after the estimated voluntary muscle activation led to a significant decrease in the APB MEPs after the intervention (Thabit et al., 2010).

Few studies have investigated the effect of delivering PAS during minimal isometric voluntary activation of the targeted muscle compared with PAS at rest. Studies combining PAS with voluntary activation of the targeted muscle suggest that the addition of voluntary activation results in a greater increase in corticospinal excitability and greater

consistency of effects across subjects compared to PAS delivered at rest (Khaslavskaia & Sinkjaer, 2005; Kujirai et al., 2006; Mrachacz-Kersting et al., 2007).

That said, the optimal method for incorporating PAS into rehabilitative activities remains unknown. To our knowledge, no study has yet investigated the optimal timing of triggering PAS when combined with isotonic volitional movement. Thus, in this study, noting the importance of temporal dependency in PAS and temporal pattern of cortical activity in relation to movements, we aimed to investigate the effect of PAS on M1 corticospinal excitability when the stimuli are delivered during the preparation or execution phase of a voluntary activation of the extensor digitorum communis (EDC) muscle. Unlike the overwhelming majority of PAS studies where stimulation was delivered to flexor muscles of the hand, we focused on PAS of the extensors, which is more relevant for stroke rehabilitation (Castel-Lacanal et al., 2009). The modulation of corticospinal excitability was assessed by TMS-elicited motor evoked potential (MEP) amplitudes. We hypothesized that PAS triggered during different phases of voluntary activation would differentially effect the direction and amplitude of intervention-induced modulation of excitability and that this effect would be muscle-specific.

## 3.3 Material and Methods

### 3.3.1 Participants

Sixteen subjects participated in all study conditions (11 male and 5 female; mean age $25.37 \pm 3.10$ years; range 23–34 years). All subjects were right-handed as determined by the Edinburgh Handedness Inventory (Oldfield, 1971). Participants were neurologically

intact, provided written consents, and completed the study protocol that was approved by the Institutional Review Board of New Jersey Institute of Technology and Rutgers University. The order of the PAS sessions was randomly assigned. Subjects completed all PAS sessions, which were assigned randomly and separated by a week to avoid any residual effects (Edwardson et al., 2014).

### 3.3.2 Electromyography (EMG) recording

The muscle activity (EMG) was recorded in all study conditions from the EDC muscle, and its antagonist, the flexor digitorum superficialis (FDS) muscle with the use of a wireless surface electrode (Trigno™ electrodes, Delsys Inc.) placed over each muscle belly. EMG signals were amplified (x1000) and band-pass filtered (10 – 300Hz), then digitized at a frequency of 1000 Hz. The EMG signals were then stored for further analysis to quantify reaction times and MEP amplitudes using a custom-built MATLAB analysis software.

### 3.3.3 Stimulation

**3.3.3.1 Peripheral Electrical Stimulation.** The EDC muscle was stimulated using a constant-current square-wave pulse of 1000μs duration (DS7A stimulator, Digitimer Ltd, Welwyn Garden City, UK) delivered through bipolar surface electrodes (3 cm apart) placed over the EDC muscle belly. The stimulation intensity was set to be 300% of the subject's perceptual threshold (Stefan et al., 2000).

**3.3.3.2 Neuronavigated Transcranial Magnetic Stimulation (TMS).** To ensure TMS precision, a canonical high-resolution anatomical MRI was co-registered with the subject's head for frameless neuro-navigation (Yarossi, Manuweera, Adamovich, & Tunik, 2017). Throughout testing, the TMS coil (Magstim Rapid2, Air Film) was held tangentially

to the scalp with the handle posterior 45° off the sagittal plane. Following a rough mapping in order to determine the hotspot for the right EDC muscle in M1, the resting motor threshold (RMT) was defined as the minimum intensity required to elicit MEPs >50μV in the EDC muscle in three of six consecutive trials (Butler, Kahn, Wolf, & Weiss, 2005; Yarossi et al., 2017). The TMS intensity was then set to be 120% of RMT intensity throughout all sessions (Mrachacz-Kersting & Stevenson, 2017).

**3.3.3.3 Paired Associative Stimulation (PAS).** The PAS stimulation protocol used in all three sessions consisted of 240 pairs of peripheral electrical stimulation applied to the right EDC muscle (300% perceptual threshold) followed by TMS pulse delivered the contralateral M1 at an inter-stimulus interval of 25ms. The PAS stimulation rate was set to be at 0.2 Hz, as it was found to be the most effective frequency in inducing the LTP effect in the motor cortex (Wischnewski & Schutter, 2016).

### 3.3.4 Experimental protocol

**3.3.4.1 Setup.** Participants were seated in a comfortable reclining chair with their hands and forearms resting on the armrests. Subjects were instructed to remain relaxed and focus their attention on the targeted EDC muscle and presented visual cues on the screen in front of them. Following the determination of each subject's RMT, peripheral electrical stimulation threshold, and mean reaction time (RT), subjects were instructed to keep focused for the "Move" visual cues and to respond with an immediate extension of their right-hand fingers without activating their wrist muscles, hold their contraction, and then return to their relaxed position once "Relax" cues were presented (Figure 3.1).

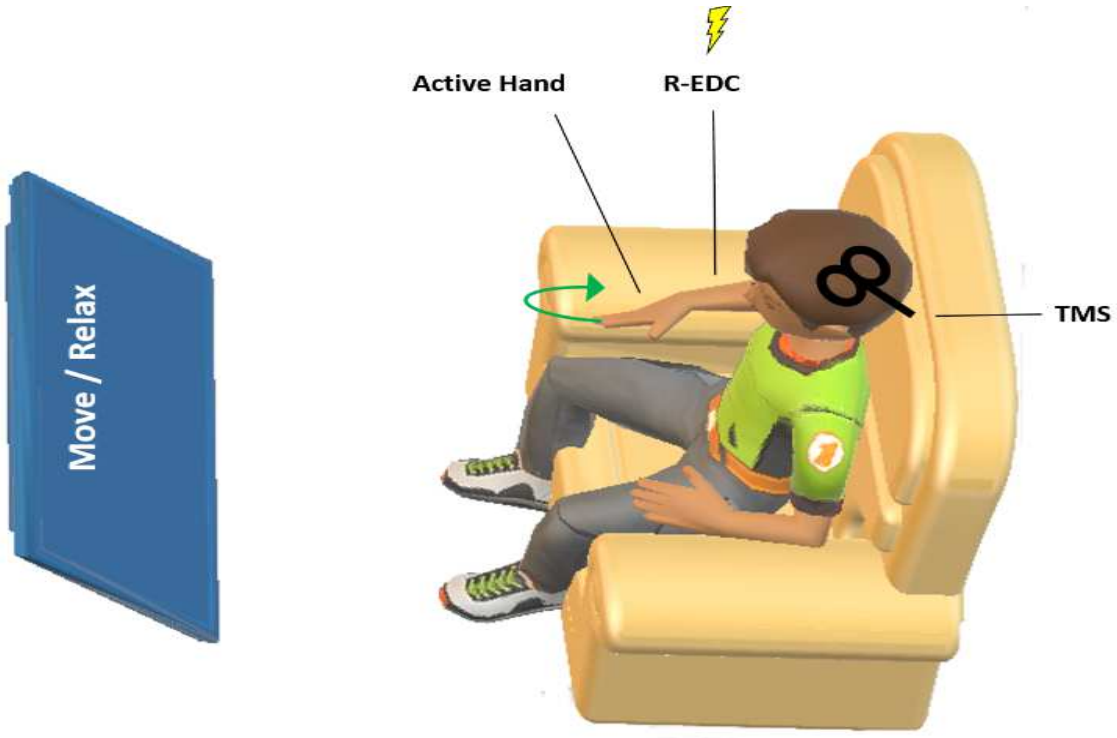


**Figure 3.1** PAS protocol setup: 240 pairs of peripheral electrical stimulation applied to the right EDC muscle followed by TMS stimulation over the identified EDC hotspot at a rate of 0.2 Hz. In PAS at rest, subjects were instructed to relax both hands at all times (no cues were presented). In conditions where PAS was combined with hand movement, subjects were engaged in an identical hand extension task to the one described for determination of the reaction time.

### 3.3.4.2 Task

***3.3.4.2.1 Behavioral task.*** In a simple reaction time paradigm, before each PAS session, subjects were instructed to rapidly extend the fingers of the right hand in response to a visual cue (Move), pause, and return (Relax) to a resting posture. Twenty trials at a rate of 0.2 Hz were conducted without stimulation to compute the mean RT for timing the stimulation during PAS.

***3.3.4.2.2 During PAS intervention task.*** In both (PAS RT-100) and (PAS RT+50) in all 240 trials, subjects were asked to follow the exact instructions that were given during

the behavioral simple reaction time task. Subjects reacted to a visual cue "Move" by extending their right-hand fingers, paused, and then relaxed while their movement was simulated simultaneously on the screen (Figure 3.1). In each trial, a single TMS pulse was delivered to the predefined EDC hotspot 25 ms following the peripheral electrical stimulation directed to the EDC muscle.

**3.3.4.3 Experimental conditions.** As this research focused on investigating the effect of the phase of the volitional movement on the induced PAS effect, the investigation consisted of three PAS conditions. Following the determination of the subject's finger extension mean RT, the TMS in the PAS intervention was set to be triggered during 1- the movement preparation phase, when the TMS pulse in each trial preceded the subject's mean RT by 100ms (PAS RT-100); 2- during the movement execution phase, when the TMS pulse was delivered 50ms after the movement onset of a unilateral fingers extension, and 3- during PAS at rest (Figure 3.2).

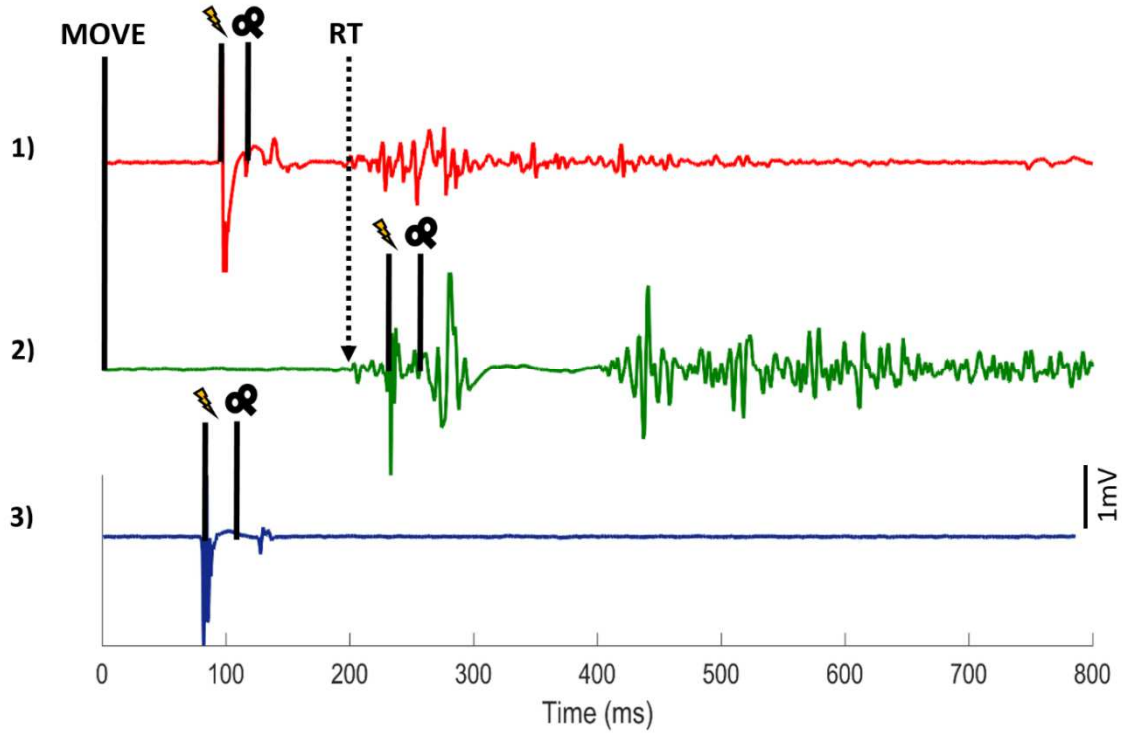


**Figure 3.2** Stimulation timing relative to subject's RT for one trial in: 1) PAS RT-100, 2) PAS RT+50, and 3) Conventional PAS with both hands at rest. The ISI was always fixed at 25ms.

### 3.3.5 Determination of reaction time

EMG signals were filtered using a band-pass filter (10-300 Hz) and then rectified off-line with the use of a custom-built Matlab software. Following baseline removal and rectifying, EMG envelopes were generated by a root mean square (RMS) filter. The RT was quantified as the EMG onset, calculated as the time point when EMG activity exceeded three standard deviations above the baseline amplitude (taken as the mean of 1000 ms window prior to the "Move" cue) (Figure 3.3).

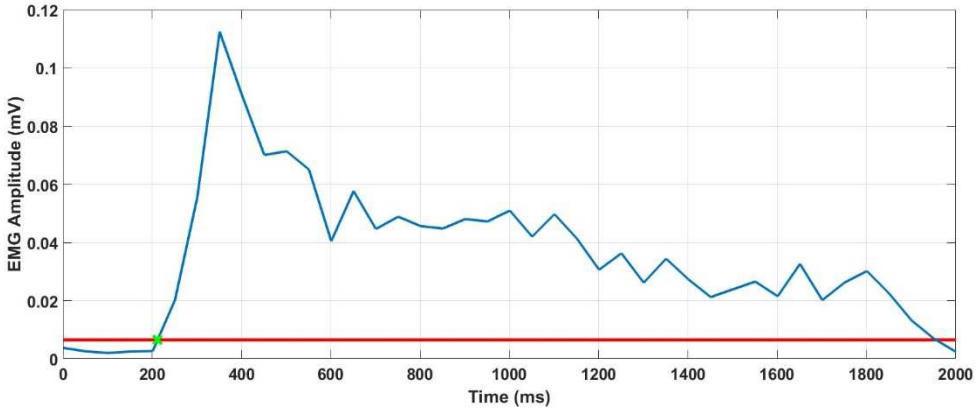


**Figure 3.3** EMG envelop of EDC muscle in response to a move cue (blue). Movement onset is marked (X) as the first time point to exceed the threshold (red line) of three standard deviations from averaged baseline ( taken as the mean of 1000 ms window prior to "Move" cue)

### 3.3.6 Data Analysis

**3.3.6.1 Cortical excitability assessment.** To evaluate cortical excitability, in a block of 20 trials, the peak-to-peak MEP amplitudes were quantified and averaged for both EDC and FDS muscles before (PRE) and immediately after each session (POST). The MEP amplitude was calculated as the peak-to-peak amplitude of the EMG signal 20 to 50ms following the TMS pulse. Individual MEPs were excluded if the MEP amplitude exceeded three standard deviations of the block average (Yarossi et al., 2017). MEPs were analyzed using rmANOVA with factors of Condition (PAS RT-100, PAS RT+50, and PAS at rest) and Time (PRE and POST). The Greenhouse–Geisser correction was used to adjust for violations of sphericity. Condition X Time interaction was considered significant at $p < 0.05$. Post-hoc pairwise comparisons (paired-samples t-tests) were conducted using Bonferonni correction with alpha adjusted to 0.0167 to protect significance.

Additionally, POST MEP values normalized to PRE were analyzed with a one-way ANOVA with a within-subjects factor Condition to determine whether PAS-induced changes in MEP amplitude were significant.

## 3.4 Results

**Table 3.1** Stimulation parameters. (Means ± SD)

| Condition | RMT (% MSO) | Test pulse (% MSO) | Electrical stimulation (300% of perceptual level) (mA) |
|---|---|---|---|
| **PAS RT-100** | 59.88 ± 8.91 | 71.85 ± 10.69 | 3.78 ± 2.81 |
| **PAS RT+50** | 57.9 ± 8.47 | 69.45 ± 10.16 | 3.60 ± 2.39 |
| **PAS at rest** | 60.75 ± 9.59 | 72.9 ± 11.50582 | 3.00 ± 2.47 |

*MSO: Maximum stimulator output*

### 3.4.1 Session to session within-subject variability

To ensure a stable and comparable cortical excitability baseline across sessions, a 1x3 rmANOVA for each individual muscle with a factor of Condition (PAS RT-100, PAS RT+50, and PAS at rest) was conducted on the amplitude of MEPs. Results of the rmANOVA confirmed no effect of Condition on the EDC muscle ($F(2,30)=1.39$, $p=0.26$) nor on the FDS muscle ($F(2,30)=1.32$, $p=0.28$).

### 3.4.2 During PAS, TMS timing relative to reaction time

The mean per-trial timing of the TMS pulse relative to the per-trial RT for the PAS sessions with volitional movements was -121.83 ms (SD: 37.36) for the PAS RT-100 condition and 42.18 ms (SD: 26.98) for the PAS RT+50 session.

### 3.4.3 Changes in MEPs amplitude following PAS

**3.4.3.1 Changes in the targeted EDC muscle.** A two-way rmANOVA revealed a significant Condition (PAS RT-100, PAS RT-50, and PAS at rest) x Time (PRE, POST) interaction for the stimulation-targeted EDC ($F(2,30)=16.36$, $p<0.001$). Post-hoc pairwise comparisons (paired-samples t-tests, with Bonferroni correction) revealed that triggering the TMS during unilateral finger extension (PAS RT+50) ($t(15)=-3.76$, $p=0.002$) and while at rest (PAS at rest) ($t(15)=-3.12$, $p=0.007$) both significantly increased the MEPs of the targeted EDC muscle. Applying the TMS during the movement preparation phase (PAS RT-100) led to a significant decrease ($t(15)=3.466$, $p=0.003$) in the MEP amplitudes of EDC (see Figure 3.4).

The repeated measures ANOVA of the POST MEPs normalized to baseline revealed a significant effect of Condition on the PAS-targeted EDC muscle ($F(2,30)= 22.16$, $p= 0.0005$). Paired t-test analyses of normalized MEP changes for PAS RT+50 and PAS RT-100 compared to PAS at rest were conducted. The corticospinal excitability decreased significantly after PAS in the PAS RT-100 condition when compared to PAS at rest ($t(15)= -5.78$, $p= 0.001$), while there were no significant differences after PAS in the PAS RT+50 condition compared to PAS delivered at rest ($t(15)= 1.06$, $p= 0.31$) (see Figure 3.5).

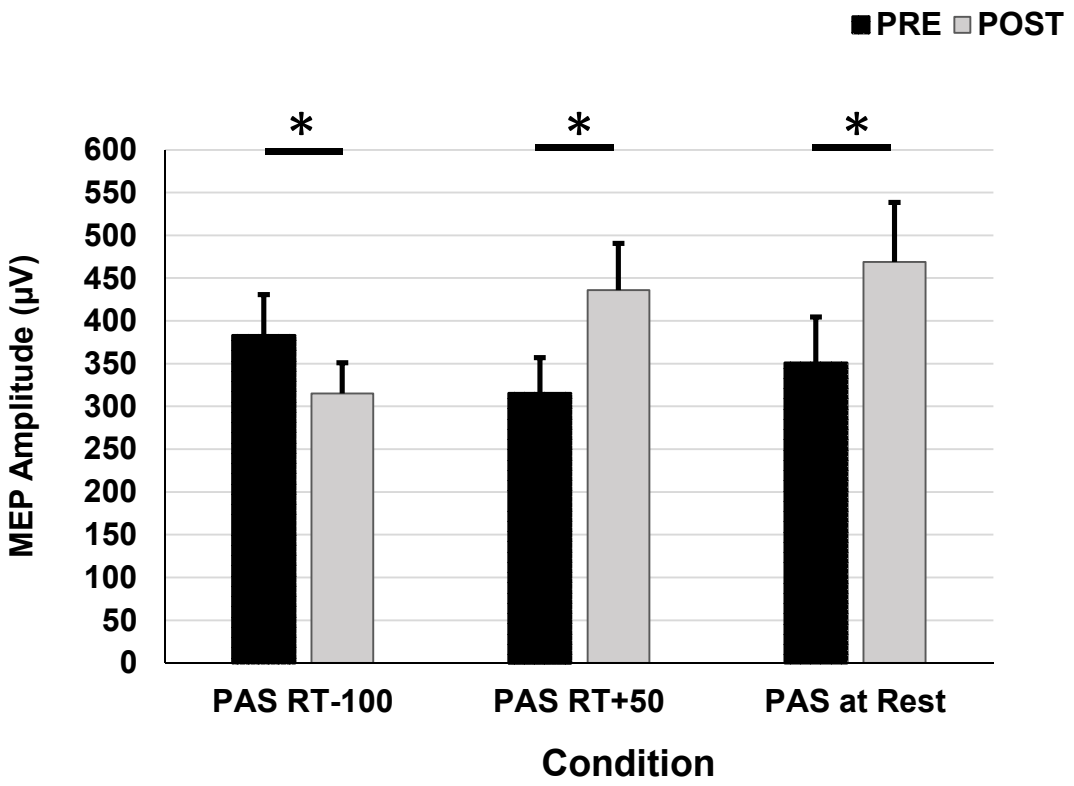


**Figure 3.4** Effect of PAS on MEP amplitudes (Mean±SEM) of the right EDC muscle before (PRE) and after (POST) PAS. A significant decrease was observed when the TMS was triggered 50ms before the estimated RT, while a significant increase was observed when TMS was delivered 50ms after the movement onset as well as in the PAS at rest condition. (Paired t-test, *$p<0.01$).

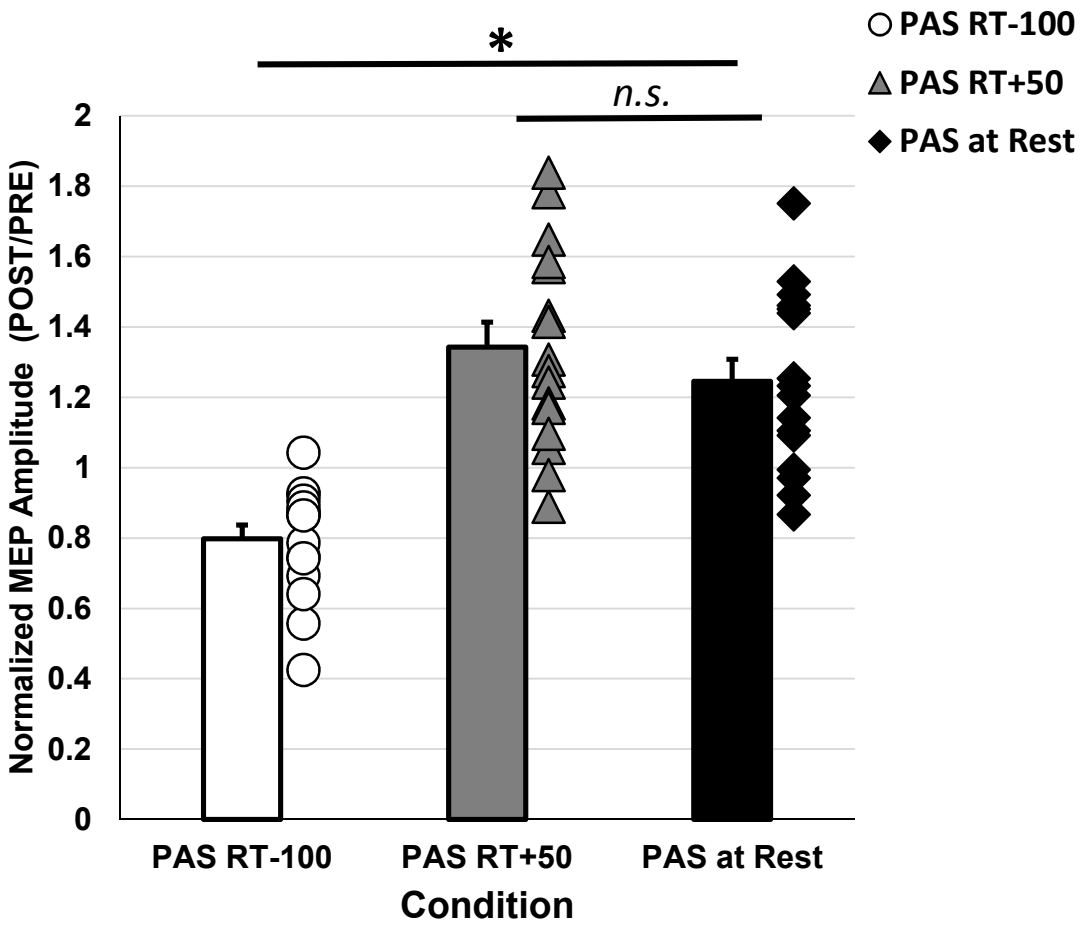


**Figure 3.5** Mean change in MEP amplitude relative to the baseline (POST/PRE) for the EDC muscle in each condition. Individual responses relative to their baseline are presented for each condition. * $p<0.05$

**3.4.3.2 Changes in the untargeted antagonist FDS muscle.** A two-way rmANOVA demonstrated no significant interaction condition with time for the untargeted FDS ($F(2,30)=0.191$, $p=0.827$) (Figure 3.6).

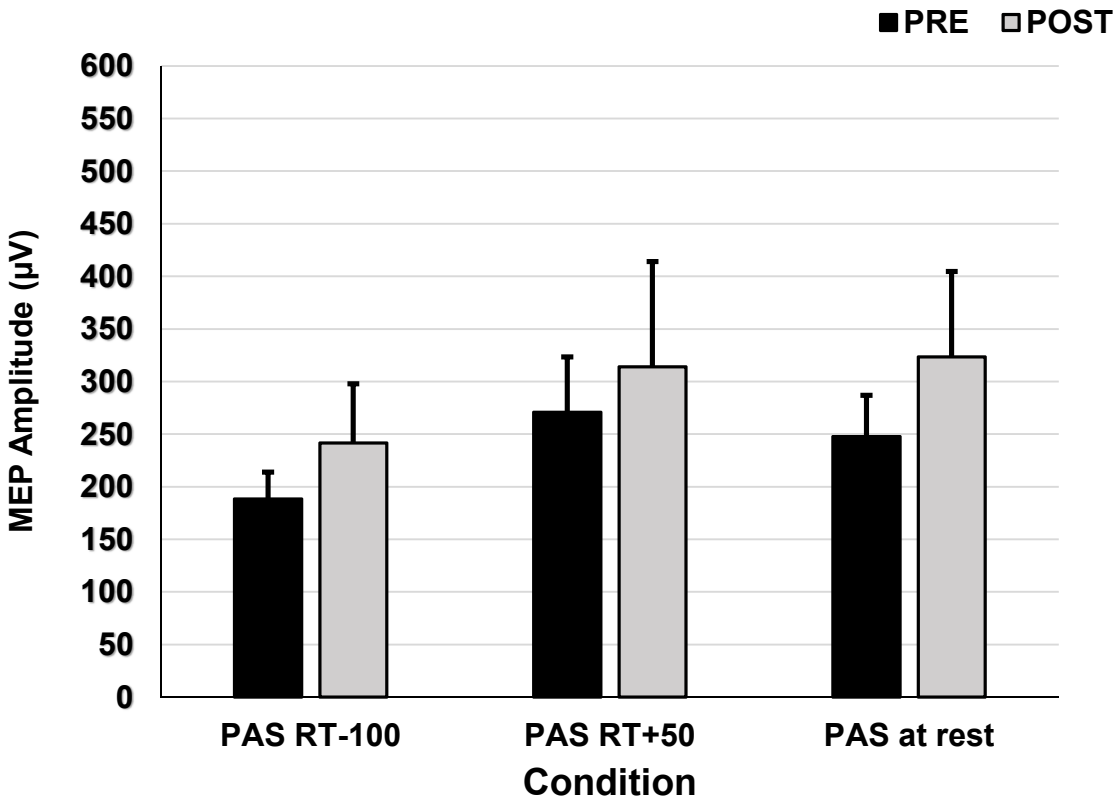


**Figure 3.6** Effect of PAS on MEP amplitudes (Mean±SEM) of the right FDS muscle before (PRE) and after (POST) PAS. No significant changes were seen.

**3.4.4 Changes in reaction time (RT).** A 2x3 rmANOVA for EDC muscle of the computed reaction time (RT) for each PAS session with factors of TIME (PRE and POST) and CONDITION (PAS RT-100, PAS RT+50 and PAS at rest) revealed no significant main effect of Condition ($F(2,30)=1.61$, $p=0.22$) nor Time ($F(1,30)=1.12$, $p=0.32$). Additionally, interaction of Condition X Time was not significant ($F(2, 30) =0.1$, $p=0.81$) (Figure 3.7)

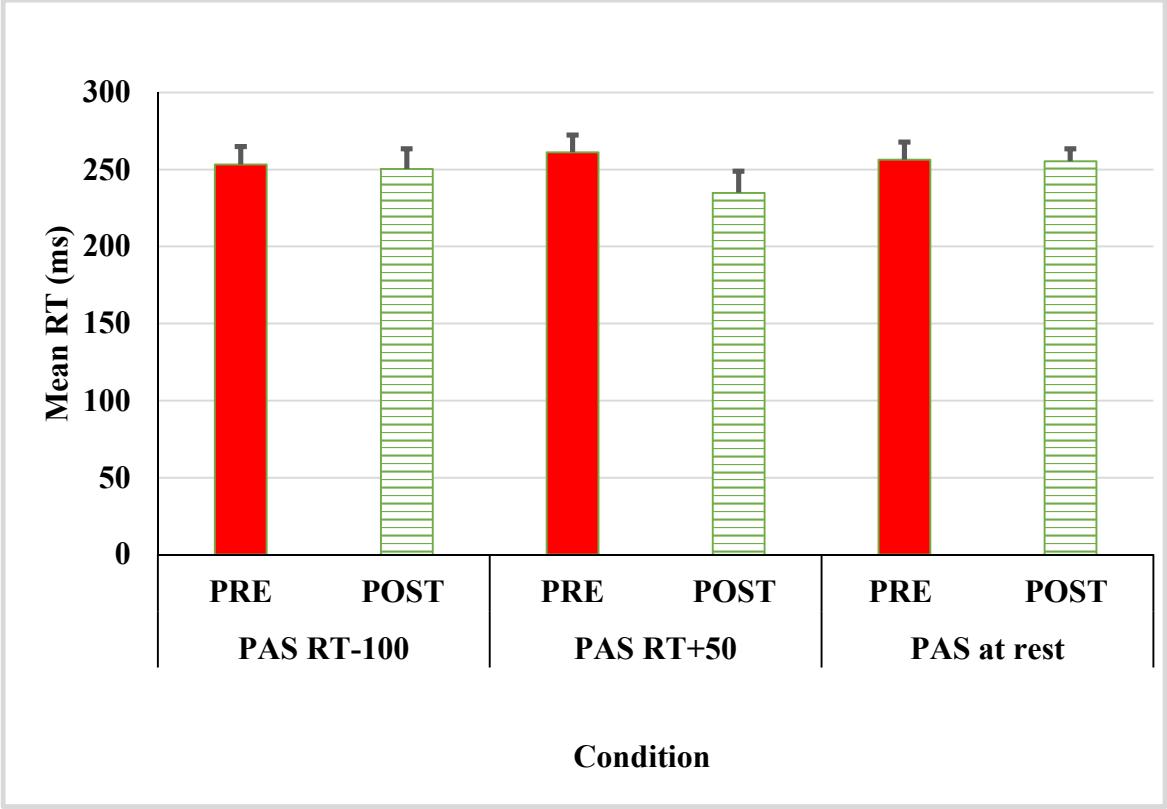


**Figure 3.7** Effect of PAS on reaction time (Mean±SEM) of the right EDC muscle before (PRE) and after (POST) each PAS session. No significant changes were seen.

### 3.5 Discussion

Using our approach to pair PAS with visually guided movement, triggering PAS during the execution phase (PAS RT+50) led to an increase in the cortical projection of the EDC muscle (Mean 34.23%± SD: 28.38). This is in line with other studies that used PAS with used PAS combined with isometric muscle contraction (Kujirai et al., 2006; Mrachacz-Kersting et al., 2007). Additionally, conducting conventional PAS while the targeted muscle is at rest also led to an increase in the cortical excitability for the targeted EDC muscle (Mean: 24.48%± SD: 25.36) (Figure 3.5).

Interestingly, despite the fact that we implemented an ISI of 25 ms between the peripheral and central stimulation, which is known to have a facilitatory effect, triggering

PAS during the movement preparation phase (PAS RT-100) led to a decrease in the cortical excitability of the targeted EDC muscle in 15 out of the 16 subjects (Mean: -20.25%± SD: 15.71) (Figure 3.5). Thus, these bidirectional induced changes in the corticospinal excitability reemphasize the importance of the presynaptic to postsynaptic activation timing and suggest that the timing of stimulation relative to the onset of muscle activity is crucial when PAS is combined with movement.

It was previously demonstrated by Kujirai and colleagues (2006) that introducing a slight tonic contraction of the targeted muscle during PAS accelerates the induction of LTP-like plasticity and lessens the required number of stimulation pairs compared to PAS at rest. Additionally, a modified PAS protocol that paired peripheral electrical stimulation to different phases of cortical potentials recorded during the motor imaginary task, was found to be effective in inducing LTP-like plasticity changes in M1 only when the peripheral stimulation was set to be triggered during the movement execution phase (Mrachacz-Kersting, Kristensen, Niazi, & Farina, 2012). Possible neural mechanisms that might be enhancing the LTP-like effect when PAS is delivered during the execution phase could be related to the increase in size and number of descending volleys as measured by I-waves during muscle contraction (V. Di Lazzaro et al., 1998; Kujirai et al., 2006). Moreover, the reduction of intracortical inhibition as measured by short intracortical inhibition (SICI) within M1 seen during voluntary contraction of the targeted muscle could enhance the development of LTP-like PAS-induced effect (Ridding et al., 1995; Stefan et al., 2002). The voluntary activation of the PAS targeted muscle provides additional afferent feedback that was found to be vital in inducing plasticity changes (Mrachacz-Kersting et al., 2007).

One neural mechanism that could explain our finding that triggering PAS during the preparation phase of a simple task leads to the LTD-like effect could be related to the temporal changes in the M1 excitability before movement onset. The M1 cortical activity is known to be changing based on the movement phase, as seen in different physiological studies (Chen & Hallett, 1999). Studies that monitored the movement-related cortical potentials (MRCPs) and more specifically, its initial slope motor potential component (MP), found an increase in the cortical excitability of M1, 50-100 ms before the EMG onset (Chen & Hallett, 1999). This was also supported by TMS and transcranial electrical stimulation (TES) in which the MEP amplitudes started to increase above baseline around 80 ms before the EMG onset (Chen, Yaseen, Cohen, & Hallett, 1998; Rossini, Zarola, Stalberg, & Caramia, 1988; Starr, Caramia, Zarola, & Rossini, 1988). In PAS, the induced excitability changes are believed to be analogous to spike-timing-dependent plasticity (STDP) in which the direction of the induced effects depends on the activation order of presynaptic and postsynaptic neural activity within a time window (Carson & Kennedy, 2013; Stefan et al., 2002; Stefan et al., 2000; Wolters et al., 2003). Thus, the LTP-like effect could be induced in the human cortex when the timing of stimulation is set so that the afferent volley arrives at the M1 exactly at or shortly before the activation of M1 by TMS. On the other hand, if the order of events is reversed, an LTD-like effect is induced. We believe that the LTD-like effect that resulted when PAS was applied before the EMG onset (PAS RT-100) might be happening because the cortical activity of M1 (postsynaptic) had already started to rise before the arrival of the ascending afferent volley of PAS.

Another candidate neural mechanism that might explain the development of LTD-like effect, seen when $PAS_{25}$ is triggered during the preparation phase of an intended

movement, could be the result of increased neural suppression of local motor circuits. It is believed that the preparation of a movement is accomplished by changes in M1 cortical activities. Numerous studies have reported the overall increase in M1 excitability during the preparation phase of a movement and that, associated with a decrease in cortical inhibition, directed towards the cortical neural population responsible of the movement (Floeter & Rothwell, 1999; Reynolds & Ashby, 1999). However, a recent animal study investigated the relationship between the modulation of cortical activities and motor performance in mice at the circuit level (Hasegawa et al., 2017). By implementing a delayed reach task and calcium-imaging technique during the preparation phase, this study was able to identify a different subpopulation of neurons whose activity was suppressed and other that maintained its increased activity during that period. This neural activity pattern correlated with improved motor performance as quantified by RT. Consequently, applying PAS during the preparatory phase of an intended movement might have interfered with this increased inhibitory mechanism.

We are the first to investigate the optimum timing window of delivering the paired artificial stimulation in PAS when combined with dynamic voluntary activation of the targeted muscle in a reaction time setup. Compared to other studies that incorporated minimal static voluntary muscle activation with PAS (Kujirai et al., 2006; Mrachacz-Kersting et al., 2007), we used a within-subject experimental design in order to better compare the induced intervention effects. This is due to the fact that PAS is found to be prone to inter-subject variability (Lahr et al., 2016). One limitation of this study that needs to be addressed in the future is monitoring the retention of the PAS-induced effects.

These findings have important implications for the incorporation of PAS into neuromotor rehabilitative training. Future investigations should explore possible clinical applications and the neurophysiological mechanisms governing the relationship between PAS-induced effects and movement-related cortical potentials by combining PAS and EEG.

# CHAPTER 4

# THE EFFECT OF THE MOVEMENT PHASE ON THE CONTRALATERALLY COORDINATED PAIRED ASSOCIATIVE STIMULATION-INDUCED EXCITABILITY IN HEALTHY INDIVISUALS

## 4.1 Abstract

Paired associative stimulation was found to increase corticospinal excitability (CSE), providing a promising adjuvant therapeutic approach for stroke. Combining PAS with the movement of the stimulated limb may further increase enhancement of CSE. However, individuals with moderate to severe stroke may not be able to engage in the necessary repetitive voluntary movements of the paretic limb. The objective of this study was to investigate the feasibility of contralaterally coordinated PAS (ccPAS) applied to the resting hand extensors during fast extension of the contralateral hand. A potential dependency of CSE modulation on the phase of the movement of the opposite hand was evaluated. Sixteen participants each completed three sessions: PAS applied to the resting right hand during the preparation phase of the extension of the contralateral (left) hand; PAS applied during the execution phase of the left-hand extension, and PAS applied with both hands at rest. Motor evoked potentials (MEPs) were evoked from the right extensor digitorum communis (EDC) and flexor digitorum superficialis (FDS) muscles prior to and immediately after each session. PAS-induced effects were monitored for one hour after each session. PAS delivered during the muscle contraction of the left hand, and PAS delivered at rest both increased the MEP amplitude in the right EDC. Delivering PAS during the preparation phase of the left-hand movement led to a decrease in the MEP amplitude measured in the right EDC muscle. We conclude that PAS-induced bidirectional changes in the amplitude of MEPs were dependent on the phase of the movement of the opposite hand.

## 4.2 Introduction

To date, there has been little success developing rehabilitative treatments designed to ameliorate moderate to severe paralysis of the hand caused by stroke. It has been hypothesized that treatments designed to enhance the excitability of the motor cortex may prove to be effective adjuvants to existing therapies. Task-oriented functional electrical stimulation (FES) has demonstrated promise in promoting functional recovery in stroke by combining FES and repetitive training of the affected limb (Dang, Chen, He, & Chen, 2017). However, combining FES with repetitive task practice of the affected limb may not be possible for those with severe impairments. Contralaterally controlled FES (ccFES), in which stroke patients use their unaffected hand to control the stimulation of the paretic limb, has been found to improve hand function and does not require functional movements of the affected hand, making it suitable for more impaired individuals. Though not explicitly tested, functional improvements due to ccFES are thought to be associated in part with the induction of increased cortical excitability via the temporal correlation between peripheral and central neural activity (Knutson, Gunzler, Wilson, & Chae, 2016; Knutson et al., 2012; Knutson, Harley, Hisel, Makowski, & Chae, 2014). In this case, central activity refers to activation generated by voluntary contraction; however, it is also possible to generate central activation through non-invasive transcranial stimulation.

Paired associative stimulation (PAS) refers to the pairing of electrical peripheral nerve stimulation with stimulation of the M1 via transcranial magnetic stimulation (TMS) (Stefan et al., 2000). Repetitive pairs of stimuli delivered in a single session have been found to result in changes in MEPs, indicating modulation of corticospinal excitability (CSE). The directionality of modulation of CSE induced by PAS has been shown to depend on the inter-stimulus interval (ISI) between the peripheral stimulation and the TMS pulse

(Carson & Kennedy, 2013). Importantly, the induction of excitability via PAS has been shown to be at least partially preserved in the lesioned hemisphere of patients post-stroke (Castel-Lacanal et al., 2009). To date, the majority of PAS studies have investigated the modulation of CSE while the targeted (for peripheral nerve stimulation) muscle is at rest (Castel-Lacanal et al., 2009; Stefan et al., 2000). Studies combining PAS with voluntary activation of the targeted muscle indicate the addition of voluntary activation results in a greater increase in CSE and greater consistency of effects across subjects compared to PAS delivered at rest (Kujirai et al., 2006; Mrachacz-Kersting et al., 2007).

The purpose of this study is to investigate the effects of PAS delivered during the movement of the opposite hand on CSE of the hemisphere ipsilateral to the moving hand. Critical to the effective application of contralaterally coordinated (ccPAS) might be the timing of the stimuli relative to voluntary activation, as ipsilateral M1 excitability during a unilateral hand movement is known to vary with the movement phase (Leocani, Cohen, Wassermann, Ikoma, & Hallett, 2000). Therefore, we specifically investigated the impact of the timing of PAS delivery relative to the onset of contralateral hand extension on M1 excitability as measured by MEP amplitude in healthy individuals. This work was partially published as a conference proceeding (Alokaily, Yarossi, Fluet, Tunik, & Adamovich, 2018).

## 4.3 Methods

### 4.3.1 Participants

Following screening for TMS contraindications (Rossi, Hallett, Rossini, & Pascual-Leone, 2011), sixteen young, right-handed, healthy adults (10M, 6F; mean age 23.56 ± SD 2.63 years; range 20–33 years) were recruited and consented in accordance with the Institutional

Review Boards of NJIT and Rutgers University. All participants completed all PAS sessions, which were assigned randomly and separated by one week to avoid any ordering or carry-over effects.

### 4.3.2 Electromyography (EMG) recording

Wireless surface electrodes (Trigno™ electrodes, Delsys Inc.) were placed over the left and right EDC and FDS muscles. EMG signals were amplified (x1000), band-pass filtered (10 – 300Hz), and digitized at a frequency of 1000 Hz. EMG signals were stored for further analysis to quantify the reaction time and MEP amplitude with the use of a custom-built MATLAB analysis software.

### 4.3.3 Stimulation

**4.3.3.1 Peripheral electrical stimulation.** The EDC muscle was stimulated with the use of a constant-current square-wave pulse of 1000μs duration (DS7A stimulator, Digitimer Ltd, Welwyn Garden City, UK) delivered through bipolar surface electrodes (3 cm apart) placed over the EDC muscle belly. The stimulation intensity was set to be 300% of the subject's perceptual threshold (Stefan et al., 2000).

**4.3.3.2 Neuronavigated TMS.** To assure TMS precision, a canonical high-resolution anatomical MRI was co-registered with the subject's head for frameless neuro-navigation. During testing, the TMS coil (Magstim Rapid 2, Air Film) was held tangentially to the scalp with the handle posterior 45° off the sagittal plane. Following a rough mapping to determine the hotspot for the right EDC muscle in M1, the resting motor threshold (RMT) was defined as the minimum intensity required to elicit MEPs >50μV in the EDC muscle on three of six consecutive trials. The TMS intensity was then set for all sessions to be 120% of RMT intensity.

**4.3.3.3 PAS.** The PAS protocol that was implemented in all the study conditions comprised 240 pairs of peripheral electrical stimulation applied to the right EDC muscle followed by TMS pulse delivered to the contralateral (left) motor cortex M1 with the inter-stimulus interval of 25 ms (Stefan et al., 2000). The PAS stimulation rate was set to be 0.2 Hz based on previous evidence that this frequency is most effective for inducing the potentiation of M1 (Wischnewski & Schutter, 2016).

### 4.3.4 Experimental protocol

Participants were seated in a comfortable reclining chair with their hands and forearms rested on the armrests. All subjects were instructed to remain relaxed and focus their attention on words on a screen placed in front of them. Following the determination of stimulation parameters, subjects performed a simple reaction time task (25 trials) with the ipsilateral left hand. Starting in a relaxed position, subjects were instructed to respond to a visual cue ("Move") presented on the screen with immediate full extension of their left fingers (while minimizing activation of wrist muscles), hold the hand fully extended for about one to two seconds, then return to a relaxed posture at the appearance of a cue to "Relax." Following baseline removal and rectifying, EMG envelopes were generated by a root mean square (RMS) filter. The reaction time (RT) was quantified as the EMG onset, calculated as the time point when EMG activity exceeded three standard deviations above baseline amplitude (taken as the mean of 1000 ms window prior to the "Move" cue).

For all conditions, PAS was administrated on the right resting hand (Figure 4.1). In PAS at rest, subjects were instructed to relax their both hands at all times (no cues were presented). In conditions where PAS was combined with contralateral hand movement, subjects were engaged in an identical hand extension task to the one described for the determination of the reaction time. Using the predetermined RT, PAS was either delivered

in 1) the contralateral movement preparation phase (the TMS pulse in each trial preceded the subject's mean RT by 100ms, ccPAS RT-100) or 2) the contralateral movement execution phase (the TMS pulse in each trial was delivered at 50ms following the subject's mean RT, ccPAS RT+50) (Figure 4.2). To assess changes in CSE, 20 MEPs were collected before (PRE) and directly following (POST) the PAS session.

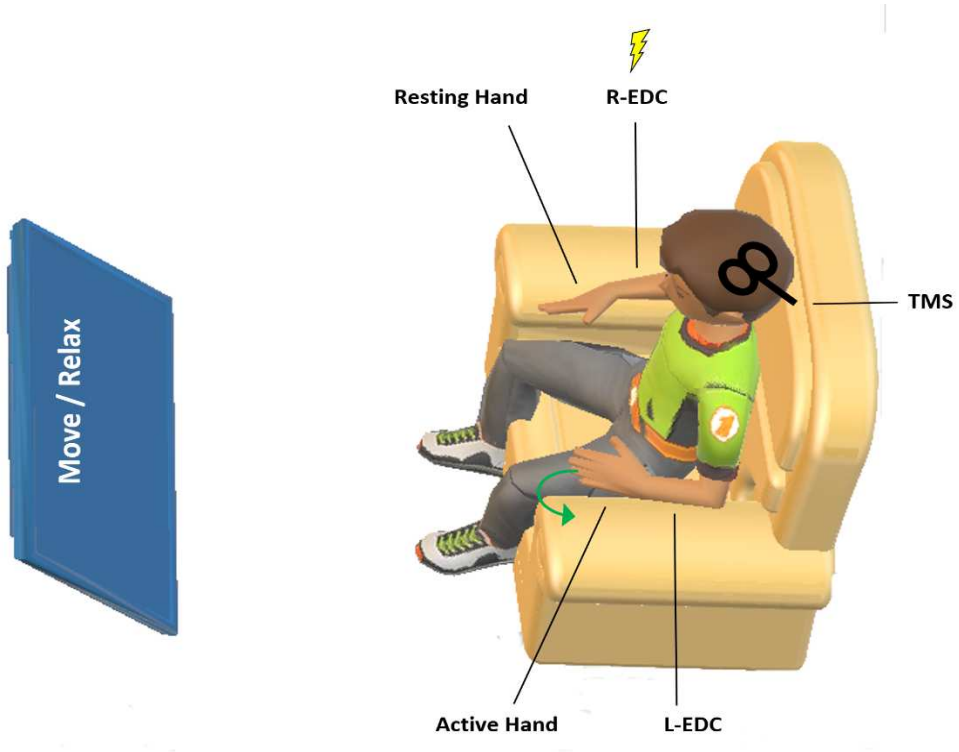


**Figure 4.1** Experimental protocol: 240 pairs of peripheral electrical stimulation applied to the right resting EDC muscle (R-EDC) followed by TMS stimulation over the identified EDC hotspot at rate of 0.2 Hz. For PAS interventions that required left EDC (L-EDC) movements, participants were instructed to extend their left-hand fingers in response to the visual cue (Move), and then relax according to the visual cue (Relax).

*Source: (Alokaily et al.,2018).*

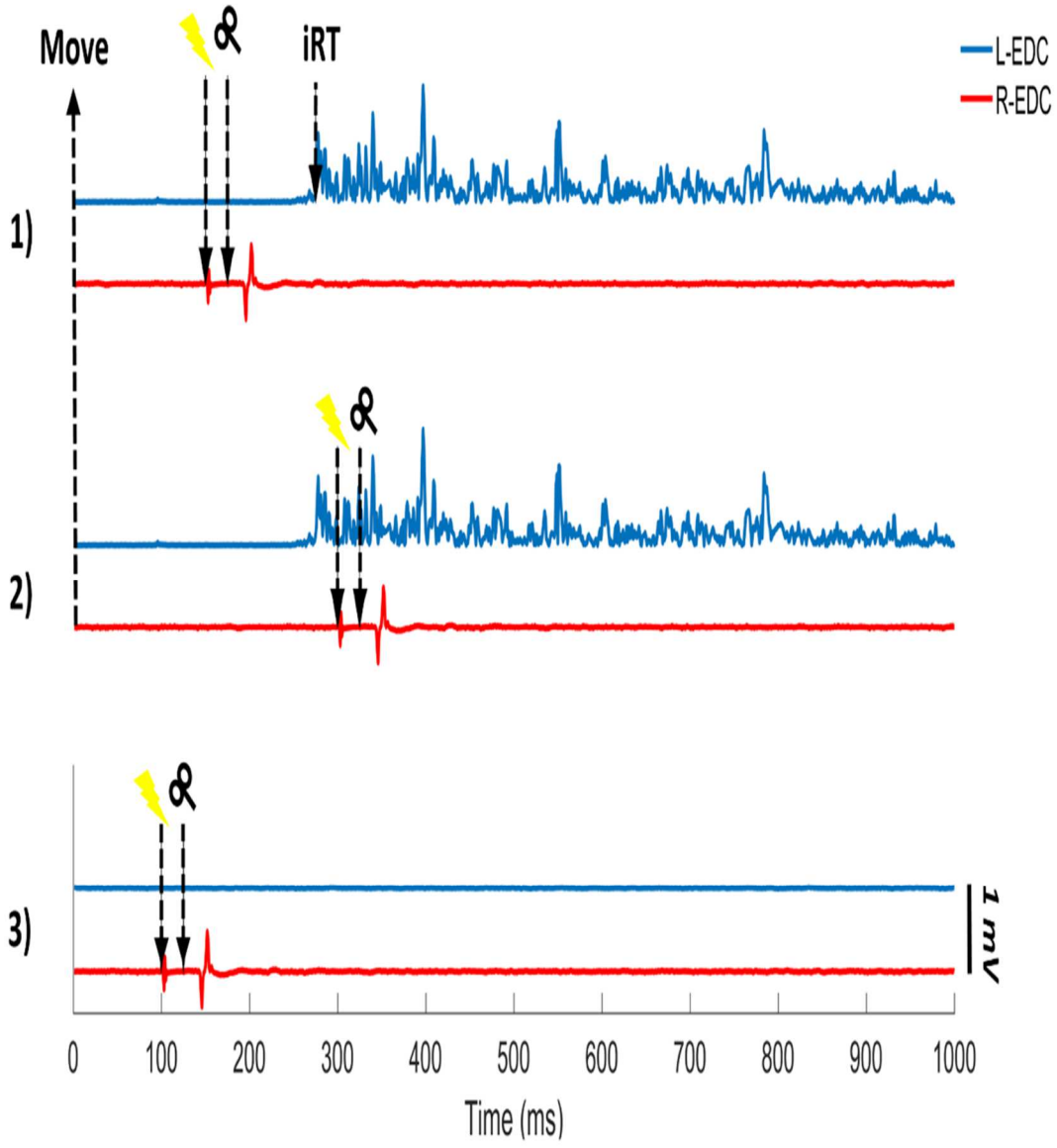


**Figure 4.2** Stimulation timing relative to subject's RT for one trial in: 1) ccPAS-100, in which TMS pulse was applied to the left ipsilateral M1 100 ms prior to the EMG onset of the left EDC muscle (L-EDC). 2) ccPAS+50, where TMS pulse was applied to the left M1 50 ms after the EMG onset of the L-EDC. 3) Conventional PAS at rest: Both hands at rest. In each of the three conditions, peripheral electrical stimulation was applied 25 ms before the TMS pulse. Electrical stimulation artifact was reduced using template subtraction method.

*Source: (Alokaily et al.,2018).*

### 4.3.5 Cortical excitability assessment

To evaluate changes in CSE in targeted EDC and untargeted FDS muscles, MEP amplitudes were calculated as the peak-to-peak amplitude of the EMG signal 20–50ms following the TMS pulse and averaged within collection blocks. Individual MEPs were excluded if the MEP amplitude exceeded three standard deviations of the block average (Yarossi et al., 2017).

### 4.3.6 Data analysis

To assess the induced PAS effect, we conducted a 3x5 rmANOVA using averaged MEP amplitudes as a dependent variable, separately for each of the two muscles. The ANOVA had within-subjects factors of Condition (PAS RT-100, PAS RT+50, Rest) and Time (PRE, POST, POST30, POST45, and POST60), with $\alpha$=0.05. If necessary, the Greenhouse-Geisser method was used to correct for non-sphericity. Additionally, POST MEPs values normalized to PRE were analyzed with a one-way ANOVA with a within-subjects factor Condition to determine whether PAS-induced changes in MEP amplitude were significant. Post-hoc analysis was done using Holm-Bonferroni correction for multiple comparisons.

## 4.4 Results

**Table 4.1** Mean Stimulation Intensities. (Means ± SD)

| Condition | RMT (% MSO) | Test TMS pulse (% MSO) | Peripheral electrical stimulation (300% of perceptual level) (mA) |
|---|---|---|---|
| **ccPAS RT-100** | 60.94 ± 9.42 | 73.13 ± 11.30 | 2.17 ± 1.27 |
| **ccPAS RT+50** | 60.69 ± 8.65 | 72.83 ± 10.39 | 2.34 ± 1.14 |
| **PAS at rest** | 59.88 ± 10.62 | 71.85 ± 12.74 | 2.41 ± 1.19 |

*MSO: Maximum stimulator output*

### 4.4.1 Session to session variability

To ensure stable and comparable cortical excitability baseline of subjects between sessions, one-way repeated measures ANOVA of the PRE MEPs with a factor of Condition (ccPAS RT-100, ccPAS RT+50 , and Rest) was conducted for each muscle individually. Results confirmed no effect of Condition in the EDC ($F(2,30) = 2.22$, $p = 0.13$) or FDS muscle ($F(2,30) = 0.95$, $p = 0.36$).

### 4.4.2 TMS pulse timing relative to the ipsilateral hand reaction time during PAS

The mean per-trial timing of the TMS pulse relative to the per-trial RT for the PAS sessions with ipsilateral hand movement was -80.30 ms (SD: 33.59) for ccPAS RT-100 condition and 48.67 ms (SD: 21.98) for the ccPAS RT+50 session. The mean RT during PAS sessions was 230.27 ms (SD: 46.81) for ccPAS RT-100 and 243.11 ms (SD: 25.78) for ccPAS RT+50.

### 4.4.3 Changes in MEPs amplitude following PAS

**4.4.3.1 Changes due to PAS in the targeted right EDC muscle.** The rmANOVA on the averaged MEP amplitudes revealed a significant Condition x Time interaction for the PAS-targeted EDC muscle ($F(8, 120) = 4.54$, $p = 0.002$) as well as a significant effect of Time ($F(4, 60) = 4.45$, $p = 0.012$) and no effect of Condition ($F(2,30) = 2.11$, $p = 0.14$). Two-tailed paired t-tests were used to compare MEPs between PRE and POST. There was a significant increase in the MEP amplitudes for ccPAS RT+50 (46.21 ± 9.34 %, $t(15) = -4.37$, $p = 0.001$). Conventional PAS at rest was also found to have a significant PRE to POST increase (21.57 ± 5.15 %), $t(15) = -3.42$, $p = 0.004$). For ccPAS RT-100, the paired t-test showed a significant PRE to POST decrease in average MEP amplitude (-18.41 ± 4.77%, $t(15) = 3.48$, $p = 0.003$).

Additionally, to assess the retention of each the PAS-induced corticospinal excitability changes, paired t-test were also conducted to compare each of after-PAS time point (POST30, POST45 and POST60) to its baseline (PRE). This revealed that ccPAS RT+50 PAS-induced effect remind significantly higher than baseline in the in POST30 ($t(15) = -4.583$, $p = 0.0001$) and POST45 ($t(15) = -3.70$, $p = 0.002$) and at POST60 ($t(15) = -3.24$, $p = 0.005$). PAS-Induced increase of excitability brought by conventional PAS at rest remained significantly higher than PRE at POST30 ($t(15) = -3.10$, $p = 0.007$), POST45 ($t(15) = -3.14$, $p = 0.007$) but not at POST60 ($t(15) = -2.83$, $p = 0.013$). Moreover, for ccPAS RT-100, there was no significant changes in the corticospinal excitablity compared to PRE at POST30 ($t(15) = 0.57$, $p = 0.58$), POST45 ($t(15)=1.06$, $p = 0.31$) nor POST60 ($t(15)=0.43$, $p =0.68$). (Figure 4.3)

The one-way rmANOVA comparing PAS-induced effects across conditions after PAS (POST) revealed a significant main effect of Condition ($F(2, 30) = 28.26$, $p = 0.001$).

Follow up paired t-test analysis of normalized MEP changes for ccPAS RT+50 and ccPAS RT-100 compared to PAS at Rest were conducted. PAS-induced effect caused by ccPAS-100 was significantly less than those induced by PAS at rest ($t(15)=6.26$, $p=0.001$). On the other hand, PAS in the ccPAS RT+50 condition induced a larger increase in the corticospinal excitability than PAS delivered at rest ($t(15)=-2.34$, $p=0.033$).(Figure 4.4).

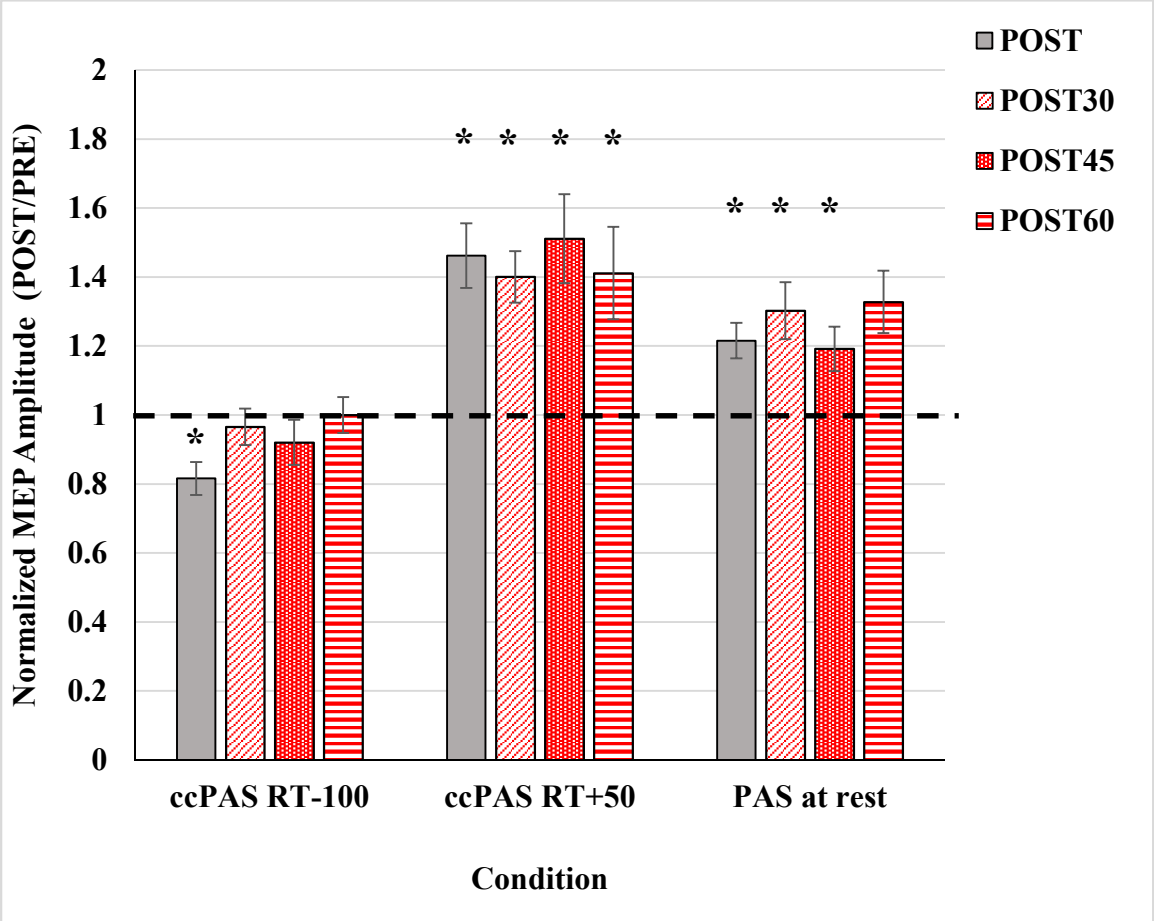


**Figure 4.3** Mean change in MEP amplitude relative to baseline (POST/PRE) for the EDC muscle in each condition (PAS25, dual-PAS, and PAS10) over time (POST, POST30, POST45 ,and POST30) in the PAS targeted right EDC muscle: MEP decrease was observed when the TMS was triggered 100 ms before the estimated movement onset in the left EDC while MEP increase was observed when TMS was delivered 50ms after the estimated movement onset in the left EDC and persisted 45 minutes after termination of the session. Conventional PAS at Rest condition induced a significant increase in MEPs amplitudes following PAS session. *$p<0.05$

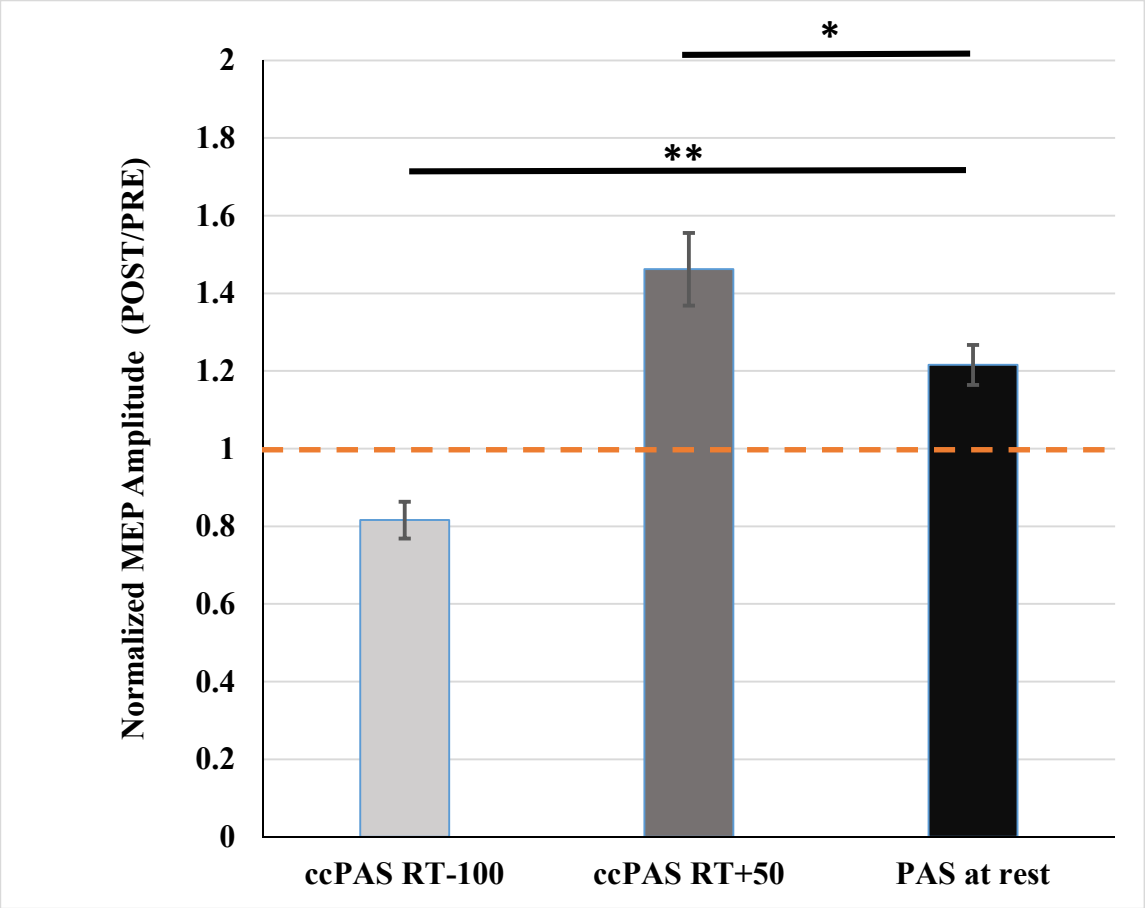


**Figure 4.4** POST PAS-induced changes in the MEPs normalized to baseline (post/pre) in the EDC muscle. Triggering PAS during contralateral hand movement induced greater increase in the corticospinal excitability compared to conventional PAS at rest. (* *p<0.05,* ** *p<0.01)*

**4.4.3.2 Changes due to PAS in the untargeted right FDS muscle.** A 3x5 rmANOVA of the averaged MEP amplitudes in the right FDS muscle untargeted by PAS revealed a significant effect of Time ($F(4, 60) = 6.07$, $p=0.008$), but no significant effect of Condition ($F(2, 30) =0.83$, $p=0.41$), nor significant Condition x Time interaction ($F(8, 120) =1.02$, $p=0.43$).

Paired t-tests to compare PRE to POST, POST30, POST45 , and POST60 changes in each condition were not significant when corrected for multiple comparisons. In ccPAS RT-100, paired t-tests to compare baseline measure (PRE) to POST ($t(15) =-1.56$, $p=0.14$),

POST30 (t(15)=-2.25, p=0.04), POST45(t(15)=-2.13, p=0.05) and POST60 (t(15) =-1.35, p=0.20) showed no significant change. Moreover, ccPAS RT+50, paired t-tests to compare baseline measure (PRE) to POST (t(15)=-1.62, p = 0.13), POST30 (t(15)=-2.17, p=0.047), POST45( t(15)=-2.32, p=0.035) and POST60 (t(15)=-2.36, p=0.032). Finally, no significant changes in the FDS were found when the paired t-test of PRE was compared to POST (t(15) =-2.61, p=0.02), POST30 (t(15)=-2.82, p=0.013), POST45(t(15)=-3.00, p =0.009) and POST60 (t(15)=-2.32, p=0.035) (Figure 4.5).

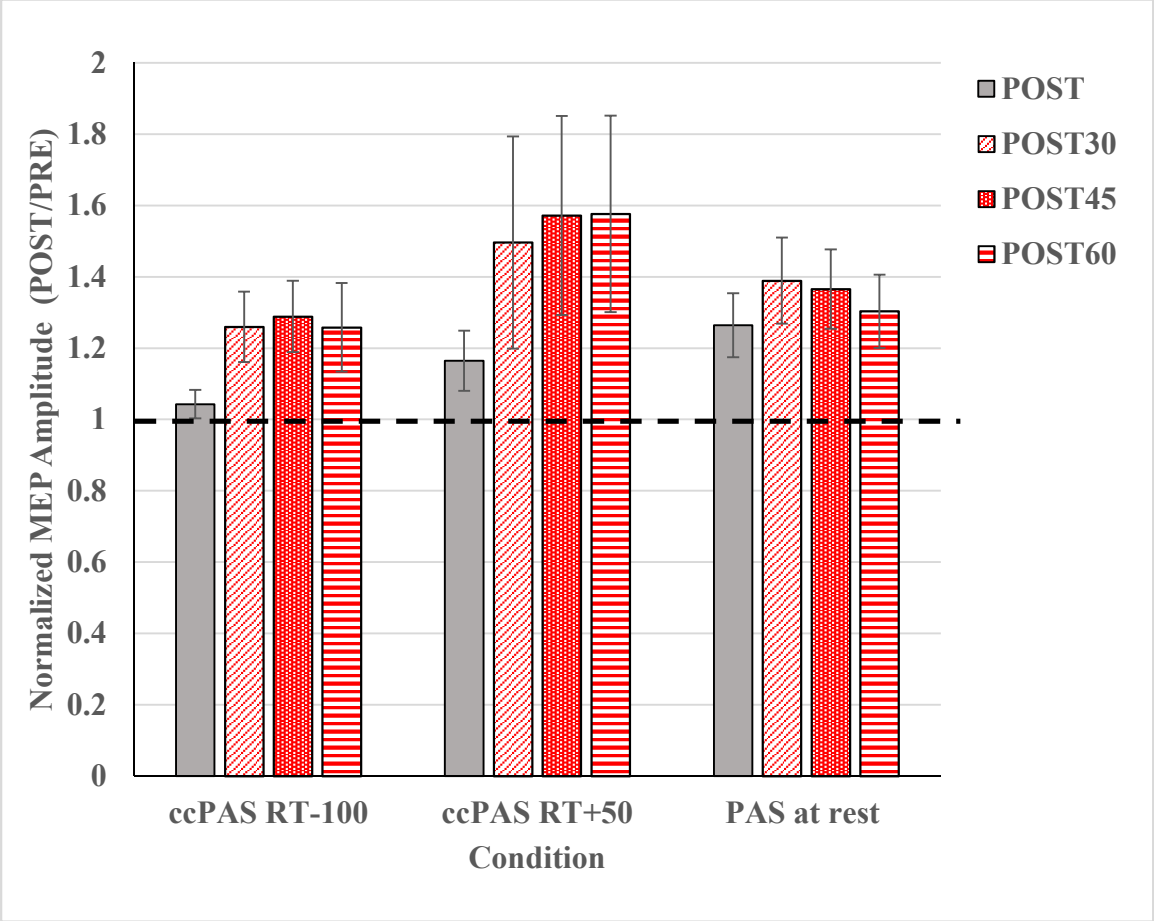


**Figure 4.5** Group effect of PAS condition (ccPAS RT-100, ccPAS RT+50 and PAS at rest) on POSTs MEP amplitude relative to baseline (mean±SEM) after (POST) each PAS session and up to one hour of follow up (POST 30, POST45, POST60) in the PAS untargeted right FDS muscle. No significant changes were found.

## 4.5 Discussion

Inducing cortical excitability changes in M1 using PAS can be a promising therapeutic intervention for stroke functional recovery (Castel-Lacanal et al., 2009). This study aimed to determine the feasibility of inducing M1 excitability changes with ccPAS. This was investigated through an examination of the effect of delivering PAS stimuli during the preparation or execution phases of the contralateral hand movement on corticospinal excitability. Our results emphasize the key role of ipsilateral M1 activity during unilateral hand movements on PAS-induced effects. Triggering PAS during the execution of a contralateral hand movement led to a robust increase in the cortical excitability in the stimulated M1 (ipsilateral to the moving hand). Compared to administrating PAS at rest, excitability changes in M1 were significantly higher. These PAS corticospinal excitability changes could resemble an LTP-like (Stefan et al., 2002; Stefan et al., 2000). Interestingly, although a well-known facilitatory PAS protocol (with a 25ms between peripheral and central stimulation) was implemented, when PAS was delivered during the contralateral hand movement preparation phase, a decrease in the M1 excitability was found. This may indicate a paradoxical induction of long-term depression-like effects (LTD-like), usually associated with PAS protocols using 10ms ISI (Wolters et al., 2003).Possible neural mechanisms underlying these PAS-induced effects in the targeted M1 might be related to the temporal changes in the interhemispheric inhibition, which is directed from right M1 towards left M1. Several TMS and fMRI studies have reported the significant role of the ipsilateral motor cortex during a contralateral hand movement. The co-activation of the ipsilateral motor cortex is believed to have a key role in processing and controlling the unilateral movement. It has been shown that the activation of the ipsilateral motor cortex during a unilateral hand movement is being affected by several factors, such as movement

phase, rhythm, and contraction level and task complexity (Beaule et al., 2012). In a simple reaction-time task, the resting ipsilateral M1 activity assessed with TMS undergoes deep inhibition 80 to 120ms before the initiation of unilateral hand movement, while the contralateral M1 activity increased (Leocani et al., 2000). As the movement is initiated, the MEPs of the ipsilateral hand were shown to increase when the contralateral homologues muscle was voluntarily contracted (Stedman, Davey, & Ellaway, 1998). Inhibition of the ipsilateral hemisphere during movement preparation followed by facilitation during execution may explain the effects seen in the current study.

Further investigations are needed to assess the feasibility of ccPAS in chronic stroke patients as they demonstrate interhemispheric imbalance (Dodd, Nair, & Prabhakaran, 2017). Resting and movement related power changes in cortical alpha and beta range oscillations have been previously linked to the modulation of MEP amplitude (Karabanov, Thielscher, & Siebner, 2016). Their role in ccPAS should be the subject of future investigations combining PAS and electroencephalography.

The findings of this feasibility study may have an important implication for the use of PAS applied during movement of the contralateral limb as an adjuvant therapy for severely impaired stroke patients. Further investigation into the underlying mechanisms and optimal parameters for administration of ccPAS should be considered.

# CHAPTER 5

# THE EFFECT OF APPLYING SIMULTANEOUS EXCITATORY AND INHIBITORY PAIRED ASSOCIATIVE STIMULATION PROTOCOLS ON CORTICOSPINAL EXCITABILITY

## 5.1 Abstract

Paired associative stimulation (PAS) is one of the most intensively investigated experimental paradigms to study the Hebbian principles of synaptic plasticity in humans. PAS consists of repetitive pairing of peripheral electrical stimulation and transcranial magnetic stimulation (TMS) of the M1. PAS induces bidirectional changes in the corticospinal excitability depending on the timing between the received stimuli. Although PAS-induced changes are often proposed as topographically specific to muscles innervated by the peripheral electrical stimulation, other PAS non-targeted muscles demonstrated an increase of their excitability with both facilitatory and inhibitory PAS. The objective of this study was to investigate the ability to augment the PAS-induced corticospinal excitability and/or its specificity effect by simultaneously targeting the finger extensors with PAS25 and flexors with PAS10 (dual-PAS). Eighteen volunteers each completed three sessions: facilitatory PAS25 targeted the extensor digitorum communis (EDC) muscle and the inhibitory PAS10 was applied to the flexor digitorum superficialis (FDS) and dual-PAS. Motor-evoked potentials (MEPs) were induced from the right EDC and FDS muscles prior to and immediately after each session. MEPs were also monitored for 30 minutes after each PAS session. Simultaneous application of facilitatory PAS to the hand extensor muscle and inhibitory PAS to the hand flexor muscle produced a consistent increase in the cortical excitability of the EDC muscle that lasted for at least 30 minitues and was only distinguishable in the EDC muscle.

## 5.2 Introduction

Recent advances in non-invasive brain stimulation tools and techniques have broadened our understanding of synaptic plasticity (Edwardson et al., 2014). At the systemic level, Stefan and others were able to induce cortical plasticity changes in the M1 using the PAS technique. Paired associative stimulation modulates cortical excitability by pairing peripheral nerve stimulation with TMS of M1 in a timing-dependent manner to induce Hebbian-like plasticity following the concept of spike-time dependent plasticity (Stefan et al., 2000). This repetitive paired stimulation has been demonstrated to induce long-lasting, muscle specific, bidirectional changes in M1 cortical excitability depending on the interval between the peripheral and central stimulation (Carson & Kennedy, 2013; Suppa et al., 2017).

In the PAS paradigm, it is well established that inter-stimulus interval (ISI) of 25 ms (PAS25) between peripheral and central stimulation leads to an increase in M1 cortical excitability. Thus, in PAS25, it is assumed that the peripheral stimulation evokes an afferent volley that reaches M1 synchronously or shortly before the trans-synaptic activation of corticospinal neurons caused by the TMS pulse (Muller-Dahlhaus et al., 2010). On the other hand, implementing a fixed ISI of 10 ms (PAS10), which causes the afferent signal to reach M1 after the TMS of corticospinal neurons, reduces M1 corticospinal excitability (Weise, Schramm, Beck, Reiners, & Classen, 2011; Wolters et al., 2003).

Paired associative stimulation-induced excitability changes are presumed to occur only at the muscles innervated by the nerve that is targeted by the peripheral electrical stimulation in PAS (Nitsche et al., 2007; Quartarone et al., 2008; M. C. Ridding & J. L. Taylor, 2001; Stefan et al., 2000; K. Stefan, M. Wycislo, & J. Classen, 2004). This is

frequently termed “topographical specificity.” However, a large volume of PAS literature reported PAS-induced excitability changes in muscle cortical representations that are not innervated by the electrical stimulation (Carson & Kennedy, 2013; Potter-Nerger et al., 2009). For instance, in the original cortical excitability enhancement study by Stefan et al. (2000), the electrical nerve stimulation in PAS was applied to the median nerve. Following the PAS session, MEPs recorded from the median nerve innervated abductor pollicis brevis (APB) muscle increased in comparison to the baseline. Although the results did not reach statistical significance, MEP amplitudes were obtained from the ulnar nerve innervated abductor digiti minimi (ADM) and the musculocutaneous nerve innervated biceps brachii (BB) muscles demonstrated a similar increasing trend. Additionally, when the PAS-induced effect in the PAS-targeted APB muscle was compared to the induced effects in untargeted ADM muscle, the changes were not distinguishable (Stefan et al., 2000). This was also reported with different muscles in several other PAS25 studies (B. Elahi, Gunraj, & Chen, 2012; Quartarone et al., 2003; Quartarone et al., 2006; Rosenkranz & Rothwell, 2006).

The PAS inhibitory paradigm, PAS10, also demonstrated changes of the corticospinal excitability for muscles that are innervated by nerves that were not targeted by electrical stimulation (Weise et al., 2013; Weise et al., 2011; Weise et al., 2006). In the study by Kamke et al. (2014), for example, the independent application of PAS25 and PAS10 led to an increase of corticospinal excitability of PAS targeted muscle following PAS25 and diminution of corticospinal excitability after PAS10. Nevertheless, PAS-induced changes in both protocols were associated with an increase in corticospinal excitability in untargeted muscles (M. R. Kamke et al., 2014).

Several studies have investigated the ability to modulate cortical excitability through the alteration of afferent inputs. These changes were found to influence the inhibitory circuits within M1 (Ridding, Pearce, & Flavel, 2005). Additionally, these changes are considered to be topographically specific (Ridding & Rothwell, 1999). Muscle-specific changes brought by changes in afferent inputs might be crucial for focusing targeted muscle activation during movement. For example, in the study by Ridding et al. (2005), when appropriately timed, electrical stimulation of digit II led to a reduction of short intracortical inhibition (SICI) for the first dorsal interosseous (FDI) muscle, where no changes were found when measured from the ADM muscle. In contrast, when digit V was stimulated, SICI reduced in the ADM muscle only. This muscle-specific decrease in SICI might lead to an increase in the synaptic connection of targeted muscles, as was found during motor learning (Muellbacher, Ziemann, Boroojerdi, Cohen, & Hallett, 2001; Ridding et al., 2005).

Based on the topographical specificity of afferent inputs to the sensorimotor cortex, this study investigated the feasibility of further enhancing PAS-induced effects and/or their specificity by simultaneously targeting hand extensors with facilitatory PAS25 and flexors with inhibitory PAS10 (dual-PAS). This might be of therapeutic benefit for the reduction of spasticity in stroke patients as they demonstrate hypertonicity in flexor muscles (Marciniak, 2011; McIntyre et al., 2018).

## 5.3 Methods

### 5.3.1 Participants

Following the screening for TMS contraindications (Rossi et al., 2011), eighteen young, right-handed, healthy adults (11M, 7F; mean age 24.06 ± SD 1.95 years; range 20–27

years) were recruited and consented in accordance with the Institutional Review Boards of NJIT and Rutgers University. All participants completed all PAS sessions, which were assigned randomly and separated by one week to avoid any ordering or carry-over effects.

### 5.3.2 Electromyography (EMG) recording

Wireless surface electrodes (Trigno™ electrodes, Delsys Inc.) were placed over the left and right EDC and FDS muscles. EMG signals were amplified (x1000), band-pass filtered (10 – 300Hz), and digitized at a frequency of 1000 Hz. signals were stored for further analysis to quantify the reaction time and MEP amplitude using a custom-built MATLAB analysis software.

### 5.3.3 Stimulation

**5.3.3.1 Peripheral Electrical Stimulation** Depending on the PAS session, an electrical stimulation pulse was applied to EDC or/and FDS muscles. Electrical stimulation pulse with the use of a constant-current square-wave pulse of 1000μs duration (DS7A stimulator, Digitimer Ltd, Welwyn Garden City, UK). It was delivered through bipolar surface electrodes placed over the EDC or/and FDS muscles. The stimulation intensity was set to be 300% of the subjects' perceptual threshold (Stefan et al., 2000).

**5.3.3.2 Neuronavigated Transcranial Magnetic Stimulation (TMS).** In order to ensure TMS precision, a canonical high-resolution anatomical MRI was co-registered with the subject's head for frameless neuro-navigation. Throughout testing, the TMS coil (Magstim Rapid 2, Air Film) was held tangentially to the scalp with the handle posterior 45° off the sagittal plane. A rough mapping was conducted to determine the optimal hotspot that produced the largest and most consistent MEP in the right EDC and FDS muscles (M. R. Kamke et al., 2016). Following the definition of the resting motor threshold (RMT) as

the minimum intensity required to elicit MEPs >50μV in the EDC muscle in three of six consecutive trials, the TMS intensity was then set to 120% of RMT intensity throughout all sessions.

**5.3.3.3 Paired Associative Stimulation (PAS).** The PAS protocol that was implemented in all the study conditions comprised 240 pairs of peripheral electrical stimulation applied to the right EDC or/and FDS muscles followed by a TMS pulse delivered to the contralateral (left) motor cortex M1. The PAS stimulation rate was set to be 0.2 Hz based on previous evidence that this frequency is most effective for inducing the potentiation of M1 (Wischnewski & Schutter, 2016).

### 5.3.4 Experimental protocol

**5.3.4.1 Setup.** Participants were seated in a comfortable reclining chair with their hands and forearms rested on the armrests (Figure 5.1). All subjects were instructed to remain relaxed and focus their attention on the targeted muscles. Following the determination of the subject's RMT and peripheral electrical stimulation threshold, a baseline reading of corticospinal excitability was taken acquiring MEPs from both EDC and FDS muscles. Then, a PAS session was randomly assigned, followed by three measurement points in time to evaluate PAS-induced changes.

**5.3.4.2 Experimental conditions.** As this study aimed to explore the ability to induce both facilitatory and inhibitory PAS effects to different cortical representations, the investigation consisted of three PAS conditions. The PAS sessions were 1- conventional facilitatory PAS25 targeting the EDC muscle; 2-inhibitory PAS10 where the electrical stimulation was applied to the FDS muscle; and 3- dual-PAS, in which, one electrical stimulation pulse was applied to the EDC muscle, and another was applied to the FDS muscle followed by a single TMS pulse. These two electrical pulses in dual-PAS were

delivered to FDS and EDC muscles, 10 ms and 25 ms before the TMS pulse respectively. (Figure 5.1)

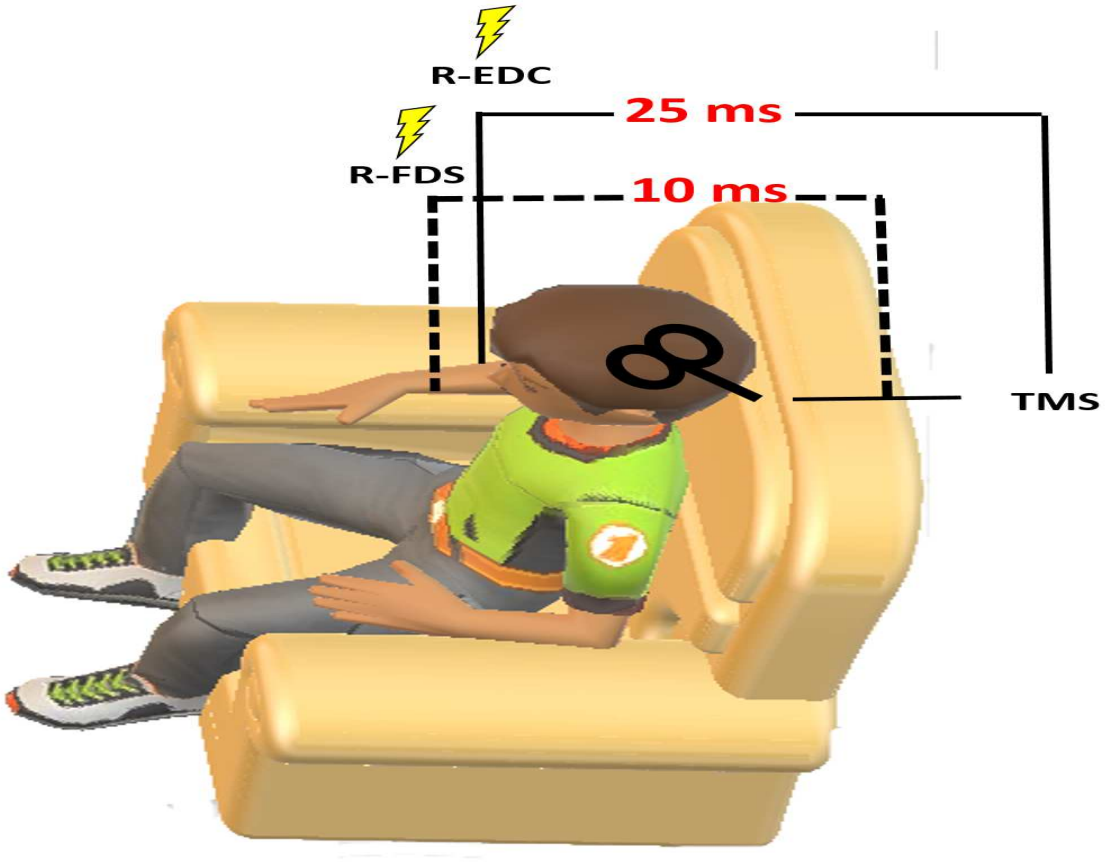


**Figure 5.1** The experimental protocol comprising 240 pairs of stimulation, the peripheral electrical stimulation was applied to 1) the right EDC muscle (R-EDC) 25 ms prior to TMS stimulation over the hotspot (PAS25), 2) right FDS muscle (R-FDS) 10 ms before TMS pulse (PAS10), and 3) two electrical pulses in dual-PAS were delivered to FDS and EDC muscles, 10 ms and 25 ms before the TMS pulse respectively. The stimulation frequency was set at 0.2 Hz.

### 5.3.5 Cortical excitability assessment.

To evaluate the corticospinal excitability changes brought by PAS in EDC and FDS muscles, MEP amplitudes were calculated as the peak-to-peak amplitude of the EMG signal 20–50ms following the TMS pulse and averaged within each collection block (Macdonald, Skinner, Shils, & Yingling, 2013). Individual MEPs were excluded if the

MEP amplitude exceeded three standard deviations of the block average (Yarossi et al., 2017). Twenty MEPs were acquired before (PRE) and immediately after (POST), and then at two other points to monitor any retention of an effect 15 min (POST15) and 30 min (POST30) after each PAS session.

### 5.3.6 Data analysis

To assess the induced PAS effects, for each of the two muscles, a 3x4 repeated measure ANOVA using averaged MEP amplitudes as a dependent variable was conducted. The ANOVA was constructed with within-subjects factors of Condition (dual-PAS, PAS25, PAS10) and Time (PRE, POST, POST15, POST30). The Greenhouse–Geisser correction was used to adjust for violations of sphericity when needed. An $\alpha$ of 0.05 was used as a criterion for statistical significance.

## 5.4 Results

**Table 5.1** Stimulation Parameters. (Means ± SD)

| Condition | RMT (% MSO) | Test TMS pulse (% MSO) | Peripheral electrical stimulation (300% of perceptual level) (mA) | |
|---|---|---|---|---|
| | | | PAS25 | PAS10 |
| **PAS25** | 59.80 ± 9.47 | 71.32 ± 11.46 | 2.45±1.04 | --- |
| **dual-PAS** | 60.50 ± 8.53 | 71.79 ± 10.05 | 2.87±1.82 | 2.09±1.02 |
| **PAS10** | 59.65 ± 9.31 | 71.05 ± 11.17 | --- | 1.58 ± 0.54 |

*MSO: Maximum stimulator output.*

### 5.4.1 Session to session within-subject variability

In order for the study to ensure a stable and comparable cortical excitability baseline across sessions, a 1x3 rmANOVA for each individual muscle with a factor of the Condition (PAS25, dual-PAS, and PAS10) was conducted on the amplitude of MEPs. Results of the rmANOVA confirmed no effect of Condition on EDC muscle MEPs ($F(2,34)=0.47$, $p=0.56$) or on FDS muscle MEPs ($F(2,34)=1.31$, $p=0.28$).

### 5.4.2 Changes in MEP amplitudes following PAS

**5.4.2.1 Changes in the targeted EDC muscle.** The rmANOVA on the averaged MEP amplitudes revealed a significant Condition X Time interaction ($F(6,102) =2.25$, $p=0.04$) and a main effect of Time for the EDC muscle ($F(3, 51)=3.84$, $p = 0.015$), but there was no significant effect of the Condition ($F(2,34)=3.19$ $p=0.082$).

Two-tailed paired t-tests were used to compare MEP averages between PRE and POST measures. There was a significant increase in the MEP amplitudes for dual-PAS ($t(17)=-3.51$, $p=0.003$) at POST. Conventional PAS at rest was not found to experience a significant POST-to-PRE increase ($t(17)=-1.05$, $p=0.031$). For PAS10, the paired t-test demonstrated no significant PRE to POST changes in average EDC muscle MEP amplitude ($t(17)=0.89$, $p=0.39$).

Follow-up post-hoc paired t-tests with corrected alpha (Bonferroni-Holm) were also used to assess the retention of each of the PAS-induced corticospinal excitability changes between after-PAS time points (POST15, POST30) and their baseline (PRE). This revealed that dualPAS corticospinal excitability-induced effects remained significantly higher than the baseline at POST15 ($t(17)=-3.31$, $p=0.004$) or POST30 ($t(17)=-3.61$, $p=0.002$). On the other hand, changes in excitability brought by conventional PAS at rest were found to be unreliable compared to PRE at POST15 ($t(17)=-1.13$, $p=0.28$) or POST30

(t(17) =-2.08, p =0.053). Finally, for PAS10, there were no significant changes in the corticospinal excitability compared to PRE at POST15 (t(17) =0.17, p=0.87) or POST30 (t(17)=-0.83, p = 0.42) (See Figure 5.2).

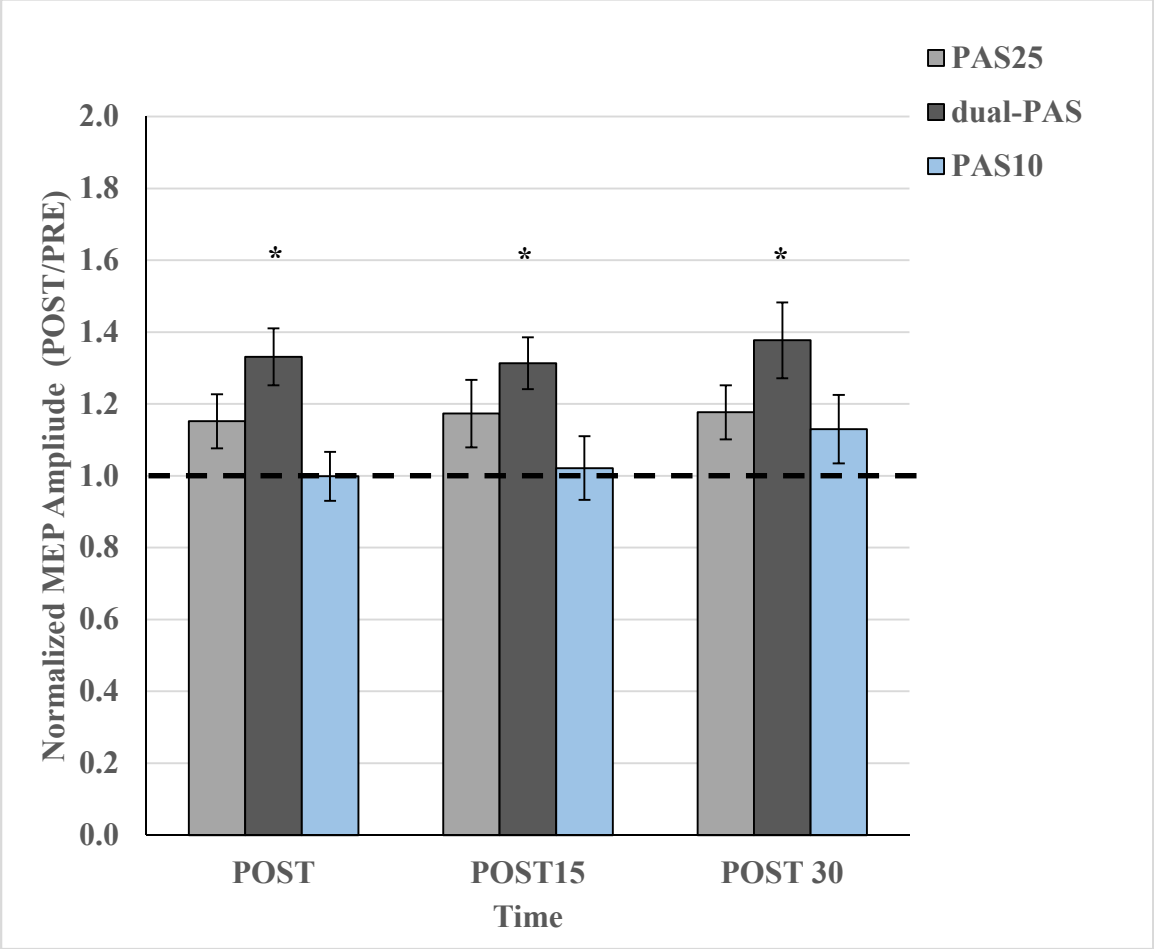


**Figure 5.2** Mean ±SEM change in MEP amplitude relative to baseline (POST/PRE) for the EDC muscle in each condition (PAS25, dual-PAS, and PAS10) over time (POST, POST15, and POST30).

**5.4.2.2 Changes in the targeted FDS muscle.** The rmANOVA on the average MEP amplitudes revealed a significant Condition x Time interaction for the FDS muscle (F(6, 102)=3.09, p=0.026), but there was no significant main effect of Condition (F(2, 34) =1.84, p=0.17) or Time (F(3,51) =0.55, p=0.54).

However, the follow-up post-hoc paired t-tests revealed no significant differences when compared to PRE for each of the conditions at the adjusted α. In the dual-PAS condition, no significant differences in the corticospinal excitability were found at POST (t(17)=1.62, p=0.12), POST15 (t(17)=0.83, p=0.42) or POST30 (t(17)=0.38, p=0.71). No differences were seen in the FDS muscle following PAS25 when POST, POST15, and POST30 were compared to PRE, (t(17)=-1.77, p=0.09), (t(17)=-1.85, p=0.08),and (t(17)=-2.47, p=0.024), respectively. Finally, as for PAS10, no differences were observed with the corrected α when we compared POST (t(19)=262, p=0.018), POST15 (t(17)=2.72, p=0.015), and POST30 (t(17)=2.08, p=0.053) with PRE (see Figure 5.3).

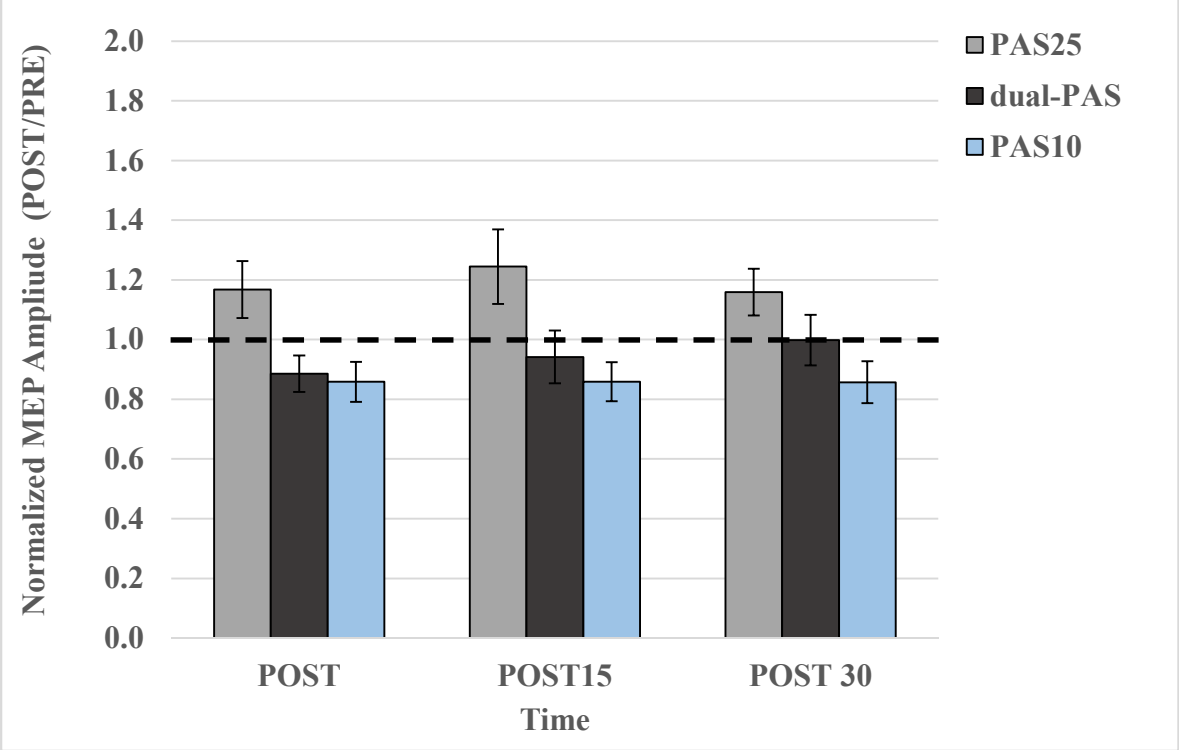


**Figure 5.3** Mean ±SEM change in MEP amplitude relative to baseline (POST/PRE) for the FDS muscle in each condition (PAS25, dual-PAS, and PAS10) over time (POST, POST15, and POST30).

**5.4.3 PAS-induced specificity effects.** The three-way rmANOVA with factors of Condition (PAS25, dual-PAS, and PAS10), Time (POST, POST15, and POST30) and muscle (EDC and FDS) of normalized MEPs (POST/PRE) revealed a significant Condition X Muscle interaction ($F(2,34)=4.78$, $p=0.015$). Additionally, there were significant main effects of Condition ($F(2,34)=6.50$, $p=0.004$) and Muscle ($F(1,17)=12.43$, $p=0.003$). There was significant effect of Time ($F(2,34)=1.15$, $p=0.33$), or significant interaction between Condition X Time X Muscle ($F(4,68)=0.51$, $p=0.73$) nor Time X Muscle ($F(2,34)=0.08$, $p=0.92$).

Follow up paired t-tests contrasts between the PAS-induced effects in EDC and FDS muscles revealed a significant difference between the effects induced in the EDC muscle versus those in the FDS muscle ($t(17)=4.36$, $p=0.0001$) (Figure 5.4). There was no significant differences between muscles following cessation PAS25 ($t(17)=-0.73$, $p=0.48$) and PAS10 ($t(17)=1.24$, $p=0.23$).

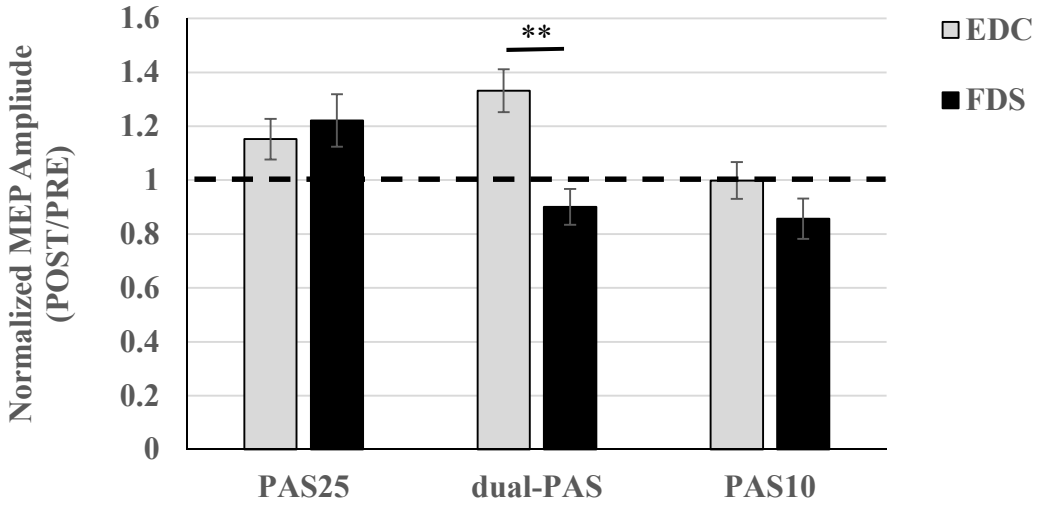


**Figure 5.4** Mean ±SEM change in MEP amplitude relative to baseline (POST/PRE) for the EDC and FDS muscle immediately following each PAScondition (PAS25, dual-PAS, and PAS10). By using dual-PAS, the PAS-induced effects were significantly limited to the EDC muscle. *(**p<0.001)*

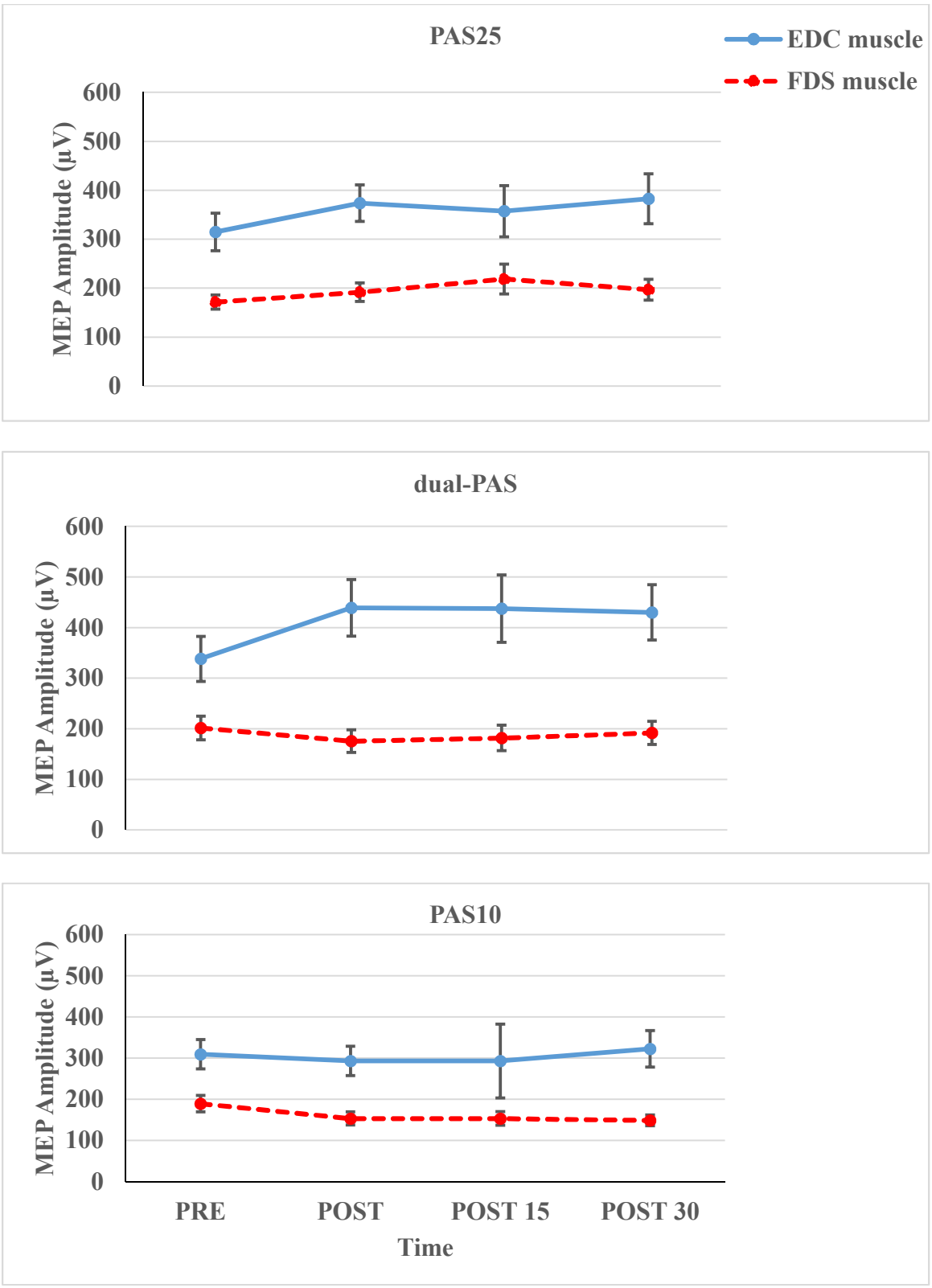


**Figure 5.5** The effect of PAS on MEP amplitudes (mean±SEM) before (PRE) and after (POST) PAS sessions up to 30 minutes follow-up (POST15, POST30) in the PAS-targeted right EDC and FDS muscles for PAS25(*top*), dual-PAS (*middle*), and PAS10 (*bottom*).

## 5.5 Discussion

Paired associative stimulation is a well-known experimental paradigm used to induce corticospinal changes in the M1. Many conventional PAS (PAS25 or PAS10) studies have identified increased MEPs in surrounding muscles, not innervated by the stimulated nerve. This makes the proposed PAS topographical specificity relative rather than absolute (Carson & Kennedy, 2013). The results of this study demonstrated that conventional application of facilitatory PAS25 to the EDC muscle increased the MEPs amplitude (average ± SEM, +15.21± 7.52%). However, this enhancement in corticospinal excitability was not found to be significant. This inconsistency of PAS-induced changes brought by PAS25 at the group level was also found in other studies in which PAS25 failed to induce reliable changes in M1 (Marc R Kamke, Abbey S Nydam, Martin V Sale, & Jason B Mattingley, 2016; López-Alonso, Cheeran, Río-Rodríguez, & Fernández-del-Olmo, 2014; Müller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008).

For the inhibitory PAS10 that was directed towards the FDS muscle, we found a decreasing trend in MEPs acquired, measured based on the targeted PAS10 (average ± SEM,-14.36±6.73%), which failed to reach significance.

However, as this study aimed to enhance the PAS-induced excitability effect and its specificity, the simultaneous application of PAS, targeting EDC muscle with PAS25 and FDS muscle with PAS10 (dual-PAS) led to a consistent and significant increase in the EDC corticospinal excitability. This was represented as an increase in the MEP amplitudes following dual-PAS (average ± SEM, +33.13±7.95). For changes in the FDS muscle following dual-PAS, a decreasing trend (average ± SEM, -10.08±6.62%) was found, but this was not found to be statistically significant (Figure 5.3). Moreover, dual-PAS induced changes were found to be specific to the facilitatory PAS-targeted finger extensors muscle

(i.e. EDC). Conventional PAS25 and PAS10 induced changes in the EDC and FDS muscles that were not distinguishable. Thus, dual-PAS showed to induce confined changes in the corticospinal excitability which provide enhanced muscle specific effects.

One possible neural mechanism that could explain the changes brought by the simultaneous application of excitatory and inhibitory PAS could be the concept of STDP. In my STDP model, the relationship of pre- and post-synaptic activation determine the direction of induced plasticity (Muller-Dahlhaus et al., 2010). Therefore, when the afferent volley arrives at M1 before the TMS pulse (as in PAS25), a long-term potentiation-like effect is demonstrated as an increase in the overall corticospinal excitability(Stefan et al., 2000). On the other hand, the late arrival of the afferent volley to M1 (as in PAS10) leads to a long-term depression-like effect (Wolters et al., 2003).

In addition, since inhibitory changes caused by dual-PAS in the FDS muscle were not reliable, another mechanism that might better explain the observed effect could be related to activity-dependent plasticity (Carson & Kennedy, 2013; Marc R Kamke et al., 2016). The activity-dependent plasticity model, the increase of overall network activity is achieved via the convergence of spatial and temporal summation of inputs. (Marc R Kamke et al., 2016; Thickbroom, 2007)

Although responses were found to be highly variable among participants, this preliminary investigation suggests that applying both facilitatory and inhibitory PAS can lead to a consistent augmentation of corticospinal excitability in the extrinsic EDC muscle and a decreasing trend in the extrinsic FDS muscle. In comparison with a recent study that concurrently targeted separate intrinsic muscle representation with excitatory and inhibitory PAS protocol, we find that our results are in line with changes found in their concurrent PAS protocol being more consistent in enhancing corticospinal excitability than

conventional PAS alone (Marc R Kamke et al., 2016). However, in their concurrent PAS protocol (PAS25 to the median nerve and PAS10 to the ulnar nerve) the corticospinal excitability showed a significant increase in the PAS25 targeted FDI muscle (median nerve innervated) as well as an increasing trend in the ADM muscle (ulnar nerve innervated). In our protocol, we adopted a muscle-located electrical stimulation instead of the commonly used nerve-located stimulation. Given that we focused on two muscle representations that are agonist "EDC" and antagonist "FDS" muscles, there is a possibility that reciprocal inhibition might have played a role in the induced changes. However, this was not tested in this study.

In conclusion, the results of this study propose more reliable PAS-induced changes in the corticospinal excitability. Further studies are required to underpin the origin of these changes as short-intracortical inhibition was found to be altered following PAS10 but not PAS25 (Carson & Kennedy, 2013).

# CHAPTER 6

# THE PAIRD ASSOCIATIVE STIMULATION INDUCED EFFECT COULD BE ENHANCED BY INTRODUCING CONTRALATERAL OR IPSILATERAL MUSCLE CONTRACTION IN CHRONIC STROKE: PILOT STUDY

## 6.1 Introduction

Stroke is considered the leading cause of adult disabilities in the U.S., where there are around seven million stroke survivors, most of whom suffer from sensorimotor disabilities (Mozaffarian et al., 2015). Functional recovery of the upper extremity is challenging with current rehabilitative interventions. It is reported that around 33% to 66% of stroke patients experience none to minor improvements in their arm function six months post-stroke, and only 50% experience substantial recovery in arm function five years after the stroke (Bolognini, Pascual-Leone, & Fregni, 2009; Kwakkel, Kollen, van der Grond, & Prevo, 2003; van Lieshout, Visser-Meily, Neggers, van der Worp, & Dijkhuizen, 2017).

Compared to neurologically intact individuals, stroke patients have significantly lower levels of corticospinal excitability at the lesioned M1 (Ward & Cohen, 2004). This atypical, reduced M1 excitability has been linked to the severity of motor dysfunction (Koski, Mernar, & Dobkin, 2004). In addition to the changes to the inhibitory-excitatory circuits within lesioned M1, stroke patients demonstrate an interhemispheric imbalance (Dodd et al., 2017). Therefore, in an attempt to enhance motor recovery in stroke patients, many studies have recommended functional improvement might be enhanced through a combination of rehabilitative training and neuro-modulation approaches such as TMS (Hoyer & Celnik, 2011; van Lieshout et al., 2017).

Paired associative stimulation modulates cortical excitability by pairing peripheral nerve stimulation with TMS of the M1 in a timing-dependent manner to induce Hebbian-like plasticity (Carson & Kennedy, 2013). This repetitive paired stimulation has

demonstrated long-lasting, muscle-specific, bidirectional changes in M1 cortical excitability depending on the interval between the peripheral and central stimulation (Suppa et al., 2017). Although PAS has been introduced as a promising adjuvant therapeutic approach in stroke rehabilitation, only a few studies have investigated PAS-induced changes in persons with stroke.

Castel-Lacanal et al. (2007) previously studied PAS-induced changes in chronic stroke patients with subcortical lesions. Their results demonstrated the ability of PAS to induce short-term changes in the M1 as quantified by an increase in the MEPs of the targeted wrist extensors. This was also associated with a decrease in the subjects resting motor threshold (RMT) (Castel-Lacanal et al., 2007). Moreover, a PAS-induced increase in corticospinal excitability was found to be more pronounced when the PAS intervention was applied earlier (five months post-stroke) rather than later (12 months post-stroke) (Castel-Lacanal et al., 2009). This suggests that PAS might be more beneficial when introduced in the acute stage rather than the chronic stage of stroke (Suppa et al., 2017). Additionally, a recent preliminary study conducted on seven chronic stroke patients demonstrated that conventional PAS induced an increase in the corticospinal excitability when applied to the paretic or nonparetic limb (Palmer, Wolf, & Borich, 2018). Though it was not significant in their study, following PAS, motor function was improved over time, which might indicate an association between corticomotor excitability-induced effects and motor function in stroke patients.

In healthy individuals, studies that explored the effect of applying PAS during minimal isometric muscle activation, have suggested that the addition of voluntary activation results in accelerated and more consistent effects across subjects compared to

PAS delivered at rest (Khaslavskaia & Sinkjaer, 2005; Kujirai et al., 2006; Mrachacz-Kersting et al., 2007). However, this has not yet been tested in persons with stroke.

In this pilot study, we aimed to explore two scenarios combining PAS with dynamic hand movement in a reaction time paradigm. In the first scenario, PAS of the ipsilesional M1 was combined with a voluntary activation of finger extensors of the paretic limb to test whether the PAS-induced effects would conform with results obtained in Chapter 3 with healthy individuals. We previously found (see Chapter 3) that delivering PAS during the movement execution phase induced an increase in corticospinal excitability. Secondly, we explored the feasibility of applying PAS to the ipsilesional M1 during a nonparetic finger extension (ccPAS) which might be of substantial benefit for moderate to severely impaired stroke patients, for whom the rehabilitative intervention is limited. In a previous chapter (Chapter 4), we reported that the PAS-induced effect caused by ccPAS when delivered during the execution phase in neurologically intact participants was higher than those induced when PAS was administrated at rest.

## 6.2 Methods

### 6.2.1 Participants

Following screening for TMS contraindications (Rossi et al., 2011), three moderately impaired persons with chronic stroke (Fugl Mayer score of 28 to 57 out of 66 points (Pang, Harris, & Eng, 2006)) (3M; mean age 59.33 ± SD 3.32 years; range 57–63 years) (See Table 6.1) were recruited and consented in accordance with the Institutional Review Boards of NJIT and Rutgers University. All participants completed all PAS sessions, which were assigned randomly and separated by one week to avoid any ordering or carry-over effects.

**Table 6.1** Stroke Patients Demographics

| Subject | Age | Gender | General location | Specific lesion location | Impaired hand | Fugl Meyer score (FM) | Type of elicited MEPs |
|---|---|---|---|---|---|---|---|
| **S1** | 57 | Male | Subcortical | L- Thalamus | R | 45/66 | Active MEPs |
| **S2** | 63 | Male | Cortical | R- Middle cerebral artery | L | 54/66 | Resting MEPs |
| **S3** | 58 | Male | N/A | N/A | L | 47/66 | Resting MEPs |

### 6.2.2 Electromyography (EMG) recording

Wireless surface electrodes (Trigno™ electrodes, Delsys Inc.) were placed over the left and right extensor digitorum communis (EDC) muscles. EMG signals were amplified (x1000), band-pass filtered (10 – 300Hz), and digitized at a frequency of 1000 Hz.

### 6.2.3 Experimental Protocol

**Setup** Patients were seated with their hands and forearms fully relaxed in front of an LCD screen, and they viewed real-time visual feedback of hand motions displayed as Virtual Reality (VR)-rendered hand models actuated by kinematic data streaming from data gloves (Cyber Glove3) (Figure 6.1) worn on the affected or unaffected hand depending on the study condition. The VR setup was developed with Virtools (Dassault Systems), and a VRPack plugin that communicates with an open source Virtual Reality Peripheral Network interfaced with an instrumented glove.

Subjects were instructed to remain relaxed and focus their attention on visual commands displayed on a screen placed in front of them. Following the determination of stimulation parameters (RMT and electrical stimulation intensity), the instrumental glove was calibrated to register the subjects' fully relaxed position, at which the represented angle is 0˚.

Three PAS conditions were conducted for each patient and applied to the paretic limb. PAS was either combined with the more affected (paretic) hand movement (PAS+Vol) or less affected (nonparetic) hand movement (ccPAS). The additional PAS at-rest session was conducted as a control. In sessions that required movements, subjects were instructed to respond to a visual cue "Move" presented on the screen with immediate full finger extension of their paretic hand for PAS+Vol or with the non-paretic hand for ccPAS sessions, and then returned to a relaxed posture at the appearance of a cue to "Relax." PAS stimulation was triggered by an angle change of five degrees measured by the CyberGlove to ensure the delivery of PAS during the execution phase of the movement. In the PAS at-rest condition, subjects were instructed to keep both hands relaxed at all times (no visual cue were given). In order to assess changes in corticospinal excitability, blocks of 20 MEPs were collected prior to (PRE) and following (POST) each PAS session and up to one hour (POST15, POST30, POST45, POST60).

**Figure 6.1** Cyber Glove was worn by patients to acquire their kinematic data during the finger extension movements and deliver PAS stimulation to the affected hand. PAS was triggered by a change in the angle of five degrees from the patient's relaxed position following the presentation of a move cue on the screen.

### 6.2.4 Stimulation

**6.2.4.1 Peripheral electrical stimulation.** The more affected (paretic) EDC muscle was stimulated using a constant-current square-wave pulse of 1000μs duration (DS7A stimulator, Digitimer Ltd, Welwyn Garden City, UK) delivered through bipolar surface electrodes placed over the EDC muscle belly. The stimulation intensity was set to be 300% of the subjects' perceptual threshold (Stefan et al., 2000).

**6.2.4.2 Neuronavigated TMS.** To ensure TMS precision, a canonical high-resolution anatomical MRI was co-registered with the subject's head for frameless neuro-navigation. Throughout the process of testing, the TMS coil (Magstim Rapid 2, Air Film) was held tangentially to the scalp with the handle posterior 45° off the sagittal plane. Following a rough mapping for determination of the hotspot for the EDC muscle in lesioned M1, the RMT was defined as the minimum intensity required to elicit MEPs

>50μV in the EDC muscle in three to six consecutive trials. The TMS intensity was then set throughout all sessions to be 120% of RMT intensity.

In case we were not able to acquire MEPs while the subject was at rest, active MEPs were acquired. This was done by asking the subject to contract their EDC muscle when the "Go" cue was represented on the screen. Transcranial magnetic stimulation was set to trigger at 10% of the subjects maximum voluntary contraction (MVC). For those who did not have resting MEPs, the active motor threshold (AMT), which was determined as the minimum TMS intensity, was used to elicit MEPs of 100μV (M. Ridding & J. Taylor, 2001).

**6.2.4.3 PAS.** The PAS protocol that was implemented in all the study conditions comprised 240 pairs of peripheral electrical stimulation applied to the more affected EDC muscle followed by the TMS pulse delivered to the contralateral (left) motor cortex M1 with the inter-stimulus interval of 25 ms (Stefan et al., 2000). The PAS stimulation rate was set to be 0.2 Hz based on previous evidence that this frequency is most effective for inducing potentiation of M1 (Wischnewski & Schutter, 2016).

### 6.2.5 Assessment of corticospinal excitability

The corticospinal excitability of the lesioned side was measured before (PRE) and at different points in time following each PAS session (POST, POST15, POST30, POST45, and POST60). A block of 20 MEPs was collected and averaged for each subject within each point.

### 6.2.6 Statistical Analysis

At each point in time, MEPs of the EDC muscle were averaged and represented as a ration of PRE (POST/PRE). The normalized MEP ratio was then submitted to a 3x5 repeated

measure ANOVA with within-subject factors of Condition (PAS25, PAS+Vol, and ccPAS) and Time (POST, POST15, POST30, POST45, and POST60) to test changes in corticospinal excitability. An $\alpha$ of 0.05 was used as a criterion for statistical significance.

## 6.3 Results

All subjects were able to complete all PAS sessions without any fatigue or adverse events. In one of the recruited patients (**S1**), we were not able to elicit resting MEPs. Thus, active motor potentials were acquired instead.

The overall changes in corticospinal excitability following each condition over time is presented in Figure 6.2. The results demonstrate that PAS+Vol induced a greater increase in the MEPs amplitude (POST: 48.35±13.44% mean±SEM) directly following the PAS session compared to PAS25 at rest (POST: 14.35±29.91% mean±SEM) and ccPAS (11.79±15.25% mean±SEM). This increase in the corticospinal excitability following PAS+Vol remained higher than baseline for 30 minutes after cessation of the PAS session (POST15: 53.84±12.41%, POST30: 30.71±11.79%, POST45: 11.79±14.14%, POST60: 11.43±20.23%)

On the other hand, for ccPAS conditions, an increase in the overall corticospinal excitability was developed later at POST30: 46.85±32.64 and remained higher than the baseline (POST45: 48.63±44.54%, POST60: 71.14±36.08%).

Additionally, as ccPAS, PAS25 demonstrated a similar trend and the MEPs peaked 60 minutes after the session (POST15: 26.21±29.41%, POST30: 2.12±8.51%, POST45: 48.35±11.81%, POST60: 58.15±51.43%).

However, due to the small sample size and high variability of the induced effects among subjects (see Figure 6.2 and 6.3), the rmANOVA did not reveal a significant effect

of the Condition (F(2, 4) =0.36, p=0.72) or Time (F(4,8) =0.90, p=0.51) or Condition X Time interaction (F(8,16) =1.13, p=0.40).

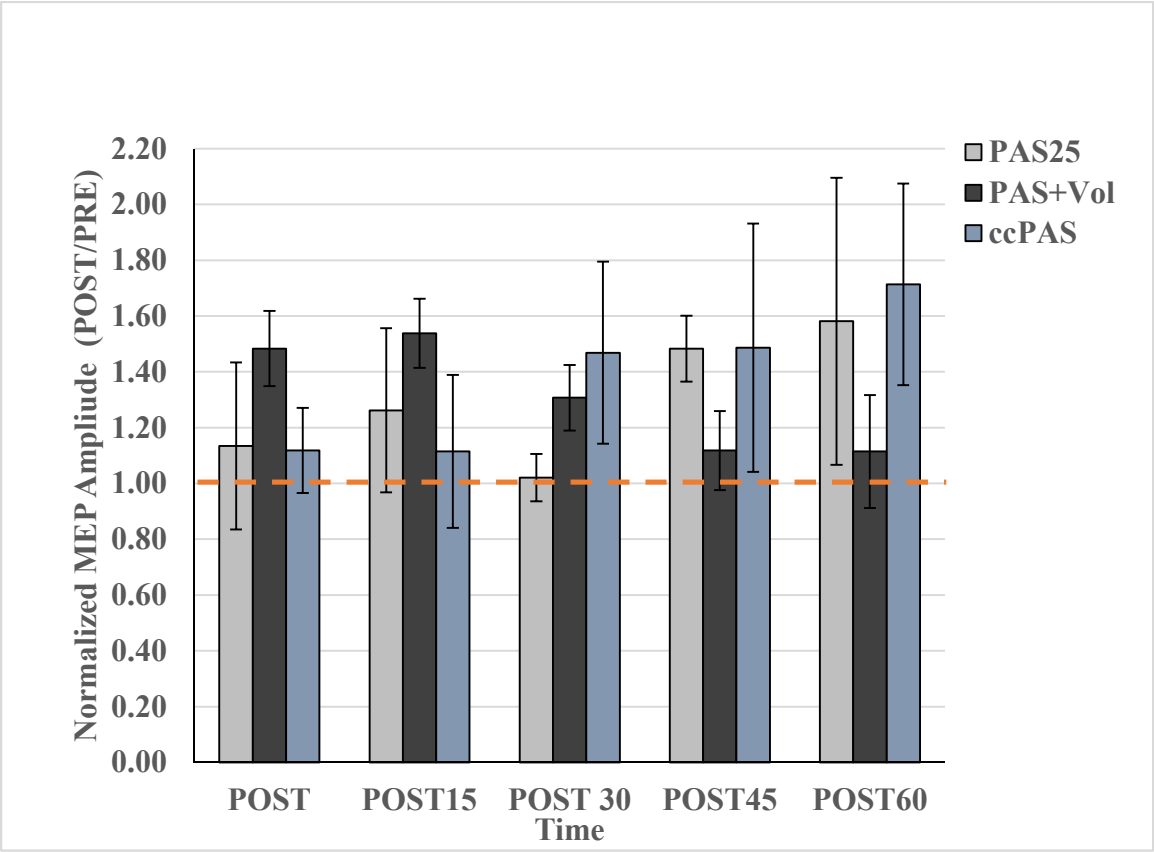


**Figure 6.2** Group (Mean±SEM) change in MEP amplitude relative to baseline (POST/PRE) for the EDC muscle in each condition (PAS25, PAS+Vol, and ccPAS) over time (POST, POST15, POST30, POST45, and POST60). No differences were observed.

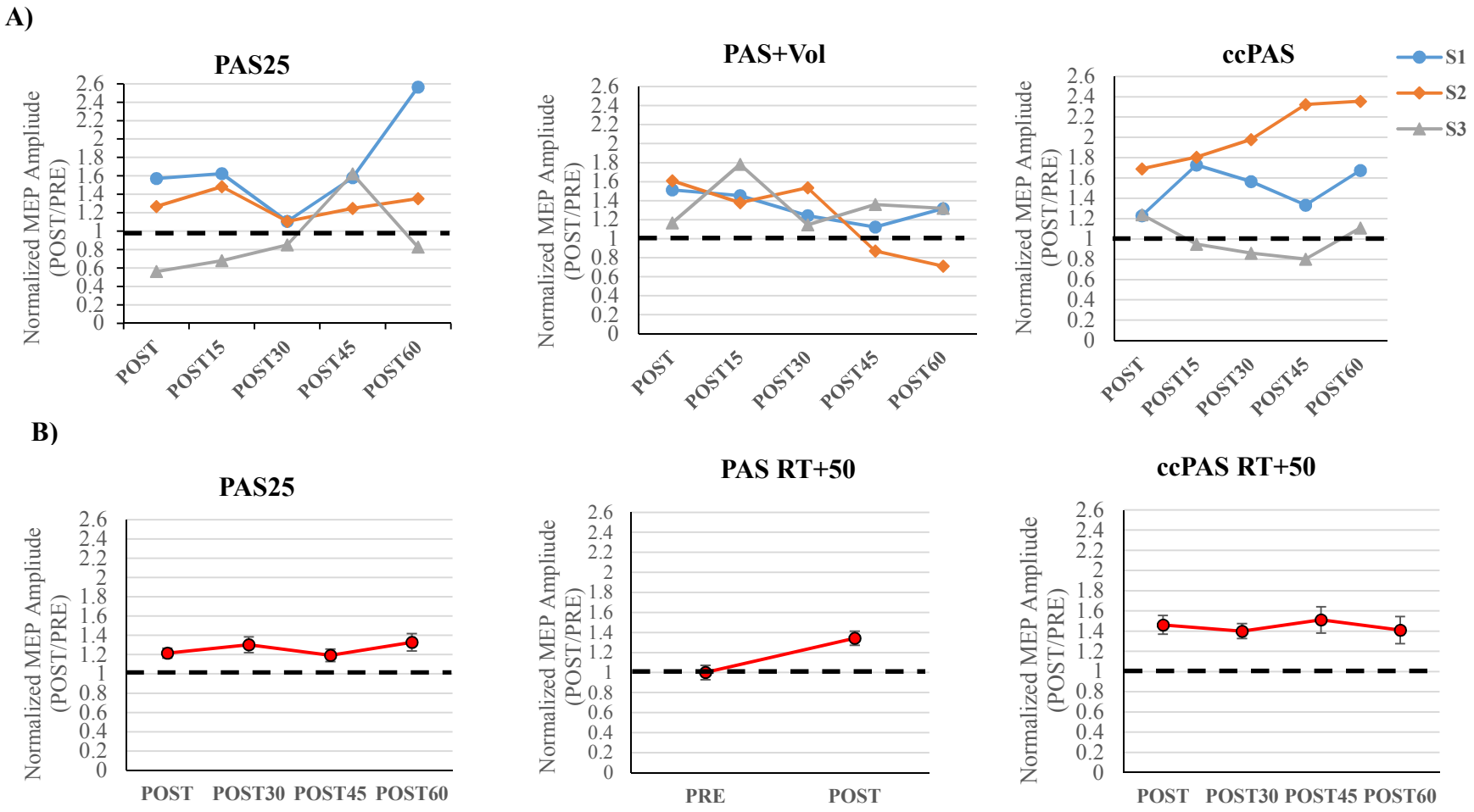


**Figure 6.3** A) Individual stroke patients normalized to PRE MEP amplitude following PAS at rest (PAS25), PAS combined with voluntary contraction of paretic limb (PAS+Vol), and PAS triggered during ccPAS B) Group average (±SEM) of normalized to PRE MEP amplitude following PAS for healthy participant obtained from Chapter 3 and 4.

## 6.4 Discussion

The results of this pilot investigation intended to test the feasibility of combining motor training with the well-known modulatory PAS protocol in stroke patients. Although the effects induced by the different PAS interventions were not statistically significant, on average, PAS combined with contralesional (paretic) hand movement demonstrated a greater increase in the corticospinal excitability when compared with conventional PAS at rest. In addition, when PAS was delivered during an ipsilesional (nonparetic) hand, an increasing trend of corticospinal excitability was noted.

In the PAS paradigm, it was specifically emphasized that attention plays a key role in inducing the associative plasticity effects (Marc R Kamke et al., 2014; Katja Stefan, Matthias Wycislo, & Joseph Classen, 2004). This may be because attention has a role in determining which neural network should endure modification (Roelfsema, van Ooyen, & Watanabe, 2010). In the study by Kamke et al. (2014), it was reported that visual spatial attention has an opposite effect on LTP-like and LTD-like PAS-induced plasticity (Marc R Kamke et al., 2014). In their investigation, they found that spatial attention directed at the stimulated limb enhanced the corticospinal excitability. In contrast, administrating a spatial attention task during inhibitory PAS reduced these induced LTD-like effects. Thus, delivering PAS with a specific task of the targeted muscle in a reaction time setup may make subjects more focused, leading to enhanced effects compared to PAS at rest.

The small sample size, differences in lesions size, and locations might be the cause of the high variability of the obtained results. Thus, we did not find any significant differences in the current study. These factors are vital in determining the stability and effect size of PAS-induced changes in these interventions. In the future, other neurophysiological markers such as short intracortical inhibition (SICI), cortical silent

period, and interhemispheric inhibition (IHI) should be investigated. Additionally, in order to monitor the changes in motor performance, using clinical tests such as the Fugel Meyer and arm mobility test, before and after PAS must be considered to evaluate the effectiveness of PAS.

In conclusion, several studies suggest that combining noninvasive brain stimulation with other traditional therapies has the potential to enhance motor recovery in stroke patients (Page, Cunningham, Plow, & Blazak, 2015; Vaz et al., 2019). As PAS is a promising adjuvant therapeutic approach, combining PAS with traditional motor training, passive robotics, or mirror therapy might boost motor recovery.

# CHAPTER 7

# SUMMARY AND CONCLUSION

Compared to other noninvasive brain stimulation protocols, paired associative stimulation (PAS) is one of the most promising paradigms to induce corticospinal excitability changes (Vincenzo Di Lazzaro et al., 2011). Motor recovery is claimed to be dependent on plastic changes in the brain. Thus, combining noninvasive brain stimulation with motor training is believed to have a potential in boosting the therapeutic outcomes in persons with neurological disorders (Bolognini et al., 2009).

This dissertation mainly investigated the effect of incorporating PAS with motor training with the objective of inducing reliable corticospinal excitability changes in the primary motor cortex (M1). One key element that we focused on is investigating the interaction between PAS and the temporal pattern of cortical activity in relation to the movement phase. Our findings show that the movement phase directly influences the direction of the PAS-induced effects. The movement phase dependency was noticeable when PAS was combined with both ipsilateral or contralateral, unilateral hand movements in neurologically intact individuals. This is of significant importance for future studies, which aim to combine PAS with a rehabilitative approach to enhance the therapeutic-induced recovery in stroke patients. This could be achieved by ensuring the application of PAS during the execution phase of movement to induce an increase in the cortical excitability of M1 (LTP-like effect). More investigations should be considered to understand the neural mechanisms responsible for the interaction between PAS and the activity of the cortical neural network of M1 or the surrounding areas such as the somatosensory cortex and premotor cortex. This might be best done by combining TMS

with electroencephalography (EEG) to assess the temporal cortical activity during PAS when combined with overt movements. Additionally, using combined TMS/EEG protocol offers an advantage of providing information on the connectivity between the stimulated areas and other cortical areas such as posterior parietal cortex (PPC) (Veniero, Ponzo, & Koch, 2013). Furthermore, the use of paired-pulse TMS modality may also be beneficial in observing the changes of the excitatory and inhibitory circuits within the stimulated M1 following the combined PAS and movement protocol. Paired-pulse TMS could also be used to evaluate the changes in the interhemispheric inhibition between the hemispheres to examine any direct their effect on the seen PAS-induced effects of contralaterally coordinated PAS (ccPAS).

Though we did not show significant changes in the corticospinal excitability, the feasibility study we conducted on moderately impaired post-stroke patients, gives us an indication of their ability to tolerate, engage and perform a motor task with both their affected and less affected hand. In the future, attempting to combine PAS with well-established clinical rehabilitative protocols such as constraint-induced movement therapy should be considered for those who are mild to moderately impaired. On the other hand, for those who are severely impaired and have challenges participating in PAS protocol that requires activating the muscles of the affected hand, combining ccPAS with mirror therapy, for instance, might be subject of future studies. Mirror therapy consists of performing a task-oriented movement with the unimpaired limb that is reflected using a mirror placed in the midsagittal plane, so the patient perceives it as a movement of the impaired limb. This makes mirror therapy suitable for severely impaired patients was shown previously shown to improve the functional recovery post-stroke (Yavuzer et al., 2008).

Finally, another goal this dissertation observed was attempting to enhance the induction and topographical specificity of PAS-induced effects. We found that simultaneous application of facilitatory PAS (PAS25) to the hand extensor muscle (EDC) and inhibitory PAS (PAS10) to the hand flexor muscle (FDS), not only produced a consistent increase in the cortical excitability of the EDC muscle but the PAS-induced effects were only distinguishable in the EDC muscle. This might be of a functional significance if applied as a therapeutic approach in stroke rehabilitation as the induced effects were manifested at the targeted EDC muscle. Thus, using dual-PAS with stroke patients to enhance the ipsilesional cortical excitability of their affected extensor muscles is the next logical step to evaluate its effectiveness in improving the motor function. Additionally, confining the PAS-induced effects to the facilitatory PAS-targeted EDC muscle and not the inhibitory PAS-targeted antagonist FDS flexor muscle could reduce the spasticity in the affected hand. A low-frequency repetitive TMS (rTMS) intervention demonstrated its usefulness in improving the spasticity in stroke patients (Etoh et al., 2013; Kakuda et al., 2012). It will be interesting to investigate the utilization of dual-PAS to reduce the spasticity of flexor muscles in persons with stroke.

## 7.1 Limitations

Although we believe that neurologically intact individuals could perform cued movements in an accurate manner, the stimulation timing of some trials might not be timed correctly. Thus, in the future, especially for individuals with a neurological disorder such as stroke, EMG driven stimulation should be considered. Another way to accurately deliver the PAS stimulation is by syncing the triggering mechanism of PAS with an endogenous cortical activity by the online recording of movement-related cortical excitability (MRCP).

Another limitation is the small sample size of recruited stroke patients and the differences in their stroke onset, infarct size and location, and side of the lesions limited our ability to investigate further the feasibility of combining PAS with motor training. For instance, it was shown that rTMS in stroke patients is more efficient in improving the motor function of stroke patients with subcortical infarcts compared with those with cortical ones (Zhang et al., 2017). Thus, increasing the number of recruited stroke patients and controlling for the lesion location (cortical versus subcortical) should be considered in the future for a better understanding of the induced effects.

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