USING PRIMING TO PROMOTE NEUROPLASTICITY AND MOTOR LEARNING POST-STROKE

by

Xin Li

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biomechanics and Movement Science

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ABSTRACT

The majority of stroke survivors experience persistent motor impairments even with rehabilitation treatments. An underlying mechanism for this is the decreased motor cortical excitability in the lesioned hemisphere after stroke. Priming techniques, such as acute exercise and transcranial direct current stimulation (tDCS), can increase motor cortical excitability and enhance motor learning in healthy individuals. But whether they have the same effects in people with stroke is unclear. Selective serotonin-reuptake inhibitors, a type of antidepressant medication, can change motor cortical excitability in healthy individuals and in acute stroke survivors. Moreover, they can interact with tDCS, changing the effects of tDCS in healthy individuals. Given that up to 30% of stroke survivors take antidepressant medications, this is an important factor to consider when evaluating the effects of tDCS in stroke. The overall purpose of this dissertation was to investigate the neurophysiological effects of exercise priming and tDCS (with chronic antidepressant intake as a factor), and to investigate the effects of tDCS on locomotor learning in people with chronic stroke.

In Aim 1, we showed that exercise priming, in the form of 5 minutes of high-intensity walking, induced increased motor cortical excitability in the lesioned hemisphere, as measured in a resting upper extremity muscle. This finding is significant because it provides evidence on the effectiveness of a clinically feasible exercise priming paradigm to induce broad excitability changes in the brain.

In Aim 2, we showed that stroke survivors taking antidepressant medications had higher motor cortical excitability in the non-lesioned hemisphere compared to

those not on antidepressants. We also found that application of anodal tDCS as a primer over the lesioned hemisphere produced differential effects on excitability in the unstimulated, non-lesioned hemisphere, depending on antidepressant-taking status. In antidepressant-takers, motor cortical excitability in the non-lesioned hemisphere increased, while it decreased compared to sham in those not taking antidepressants. These findings draw attention to the fact that stroke survivors may not respond in the same way to tDCS as healthy individuals, and that antidepressants, and potentially other medications and stroke-related factors, must be considered and their effects investigated before providing tDCS as a clinical treatment.

Finally, in Aim 3, we showed that anodal tDCS over the lesioned hemisphere did not have any effect on split-belt treadmill locomotor learning and retention in chronic stroke survivors. We speculate that split-belt adaptation may not be sensitive to modulation by tDCS. Future studies should investigate whether tDCS affects other types of locomotor learning.

Overall, this work demonstrates the potential of exercise priming for stroke recovery, and highlights the complexity of tDCS usage in people with chronic stroke. Future studies should focus on how individual differences affect priming in stroke.

Chapter 1

INTRODUCTION

Nearly 800,000 people in the United States (US) experience a new or recurrent stroke each year, and stroke remains the leading cause of long-term disability in the US (Benjamin et al., 2017). The majority of stroke survivors have persistent motor function deficits in both the upper and lower extremities (Dickstein, 2008; Hatem et al., 2016; Jørgensen, Nakayama, Raaschou, & Olsen, 1995; Kelly-Hayes et al., 2003; Preston, Ada, Dean, Stanton, & Waddington, 2011) despite receiving rehabilitation treatments. These deficits contribute to higher risks of falls and complications, decreased quality of life, increased need of care, and higher costs (Campbell & Matthews, 2010; Joo, George, Fang, & Wang, 2014; Pulman & Buckley, 2013). Understanding the underlying neurological mechanisms that contribute to these motor impairments is critical for developing new interventions that promote improved motor recovery.

One important neurophysiological factor that has received considerable attention recently is the excitability level within the primary motor cortex (M1), measured through the safe and noninvasive use of transcranial magnetic stimulation (TMS). Motor cortical excitability within the lesioned hemisphere is decreased after stroke, and this decrease is related to poor motor functional outcomes (Butler & Wolf, 2007; Dimyan & Cohen, 2010; Palmer, Hsiao, Awad, & Binder-Macleod, 2016). Thus, interventions that can increase motor cortical excitability in people post-stroke are of interest because of their potential impact on motor function improvements.

Modern training-based neurorehabilitation techniques have a positive effect on motor cortical excitability through use-dependent plasticity (Butefisch et al., 2000; Classen, Liepert, Wise, Hallett, & Cohen, 1998; J. W. Stinear & Byblow, 2004; Yen, Wang, Liao, Huang, & Yang, 2008), however, training and practicing alone does not provide full recovery for many stroke survivors. Therefore, finding and testing other techniques that increase motor cortical excitability is important to promoting better stroke recovery.

Priming techniques have gained interest in past years for their abilities to modulate cortical excitability. The term "priming" originates from the psychology literature and describes a type of cognitive implicit learning where behavior is changed based on previous stimuli, usually tested in word recall/memory tasks (Friederici, Steinhauer, & Frisch, 1999; Maxfield, 1997; Meyer & Schvaneveldt, 1971; Sherman & Jordan, 2011; Weingarten et al., 2016). For example, responses to pairs of associated words, such as "nurse" and "doctor", are faster than responses to pairs of non-associated words (Meyer & Schvaneveldt, 1971). The term "motor priming" was coined to refer to interventions that facilitate brain activation changes that improve subsequent motor learning or performance (C. M. Stinear, Barber, Coxon, Fleming, & Byblow, 2008; Stoykov & Madhavan, 2015; Stoykov & Stinear, 2010). Exercise and transcranial direct current stimulation (tDCS) are such priming techniques that can increase motor cortical excitability (Neva, Brown, Mang, Francisco, & Boyd, 2017; Nitsche & Paulus, 2000; Smith, Goldsworthy, Garside, Wood, & Ridding, 2014), and more importantly, enhance motor learning and retention (Reis et al., 2009; Roig, Skriver, Lundbye-Jensen, Kiens, & Nielsen, 2012) in healthy individuals. As a result, they have gained traction in recent years as potential adjunct tools to enhance motor

learning and recovery in stroke. However, whether they have the same effects on motor cortical excitability and/or motor learning in stroke survivors is unclear.

Motor Cortical Excitability Post-Stroke

A series of neurophysiological changes happen in the brain post-stroke that can be measured with TMS. TMS works by electrical current flowing rapidly through a TMS coil placed on the head, which creates a rapidly changing magnetic field, which in turn induces current in the brain (Rothwell, 1997). With an adequate stimulus intensity, the current is strong enough to depolarize neurons to create action potentials. When the M1 is stimulated, the action potentials propagate down the corticospinal tract, finally arriving at the target muscle and creating a muscle contraction, which can be measured with surface electromyography (EMG) from the muscle. This response is called a motor evoked potential (MEP), and its peak-to-peak amplitude is an indicator of the strength of the connection between the M1 and the target muscle.

After stroke affecting the corticospinal pathway, upper extremity MEPs from the lesioned hemisphere are decreased compared to the non-lesioned hemisphere (Di Lazzaro et al., 2010; C. M. Stinear, Petoe, & Byblow, 2015; Traversa, Cicinelli, Pasqualetti, Filippi, & Rossini, 1998) and compared to healthy controls (Traversa et al., 1998). Furthermore, increases in lesioned hemisphere MEP amplitudes are associated with motor function improvement (C. M. Stinear et al., 2015; Traversa et al., 1998; Trompetto, Assini, Buccolieri, Marchese, & Abbruzzese, 2000). MEPs from the non-lesioned hemisphere are larger than normal acutely after stroke, but decrease if MEPs from the lesioned hemisphere increase over time. However, they continue to increase if MEPs from the lesioned hemisphere cannot be elicited (Traversa et al., 1998). Therefore, it seems that a between-hemisphere asymmetry (under-excitability

in the lesioned side with over-excitability in the non-lesioned side) in motor cortical excitability post-stroke is associated with poor motor recovery in the upper extremity (Catano, Houa, Caroyer, Ducarne, & Noël, 1996; Harris-Love, Chan, Dromerick, & Cohen, 2016; Hummel & Cohen, 2006; Murase, Duque, Mazzocchio, & Cohen, 2004; Rossini, Calautti, Pauri, & Baron, 2003). The same asymmetry of motor cortical excitability is also found in lower extremity muscles and is correlated with poor walking recovery (Hendricks, Pasman, van Limbeek, & Zwarts, 2003; Palmer, Hsiao, et al., 2016; Steube, Wietholter, & Correll, 2001). Therefore, the evidence suggests that an imbalance of motor cortical excitability between the lesioned and non-lesioned hemispheres is associated with poor functional outcomes post-stroke. Many investigators have suggested that interventions aiming to restore interhemispheric balance should be pursued (Boddington & Reynolds, 2017; Hummel & Cohen, 2006; Takeuchi & Izumi, 2012), however today, the efficacy of such interventions is uncertain, with the literature providing mixed results (Cunningham et al., 2015; Di Pino et al., 2014; Harris-Love & Harrington, 2017). Although the interhemispheric imbalance theory is widely accepted, it is worth noting that not all studies have found a change in asymmetry over time (C. M. Stinear et al., 2015). Instead, decreased excitability of the lesioned hemisphere M1 seems to be the most consistent and reliable finding (McDonnell & Stinear, 2017).

Aside from the measurement of corticospinal output through MEPs, the strength of intracortical circuits can also be measured with TMS. Several measures of the excitability of intracortical inhibitory circuits suggest that after stroke affecting the corticospinal tract, the lesioned hemisphere M1 has altered activity patterns within these inhibitory circuits, sometimes reflecting increased inhibition within the M1

(Classen et al., 1997) and sometimes reflecting decreased inhibition within the M1 (Manganotti et al., 2002). Changes in intracortical inhibitory circuits are also reported in the non-lesioned hemisphere M1 (Honaga et al., 2013; Manganotti et al., 2002; Shimizu et al., 2002).

Together, the evidence suggests that motor cortical excitability changes bilaterally post-stroke, and improvements of motor cortical excitability in the lesioned hemisphere, along with a return to normal excitability in the non-lesioned hemisphere, is associated with better motor recovery. Therefore, interventions that change motor cortical excitability, i.e. priming techniques, may be helpful tools to facilitate motor recovery post-stroke. In the next two sections, we will describe the evidence for the use of two different priming techniques that could increase the excitability of the lesioned hemisphere motor cortex post-stroke.

Exercise as a Primer

Exercise can induce brain excitability changes broadly, including in areas not directly involved in the exercise, but that could be engaged during another motor learning task (Taubert, Villringer, & Lehmann, 2015). As such, exercise may have facilitating effects on motor learning through enhancing neuroplasticity in these common areas. Based on findings indicating a positive effect of exercise on cognitive learning (Y. K. Chang, Labban, Gapin, & Etnier, 2012; Chin, Keyser, Dsurney, & Chan, 2015; Hindin & Zelinski, 2012), there has been growing interest over the past few years in investigating the effects of exercise on motor learning. To date, most studies have been done in young healthy individuals, and results show that a single bout of high-intensity exercise can indeed increase retention of a motor task (Ferrer-Uris, Busquets, Lopez-Alonso, Fernandez-del-Olmo, & Angulo-Barroso, 2017; Roig

et al., 2012; Stavrinos & Coxon, 2017; Thomas, Beck, et al., 2016; Thomas, Flindtgaard, et al., 2016; Thomas, Johnsen, et al., 2016). Using a visuomotor accuracy tracking task, Roig et al. (2012) showed that 20 minutes of high-intensity cycling improved retention of the task at 24 hours and 7 days after initial learning, compared to controls that did not exercise. Moreover, by varying the timing of exercise, they found that exercising after initial learning produced better retention at 7 days compared to exercising before learning. This indicates that exercise may enhance retention of learning through an action on the consolidation process. In a further series of experiments, factors that may contribute to retention were systematically explored. Retention was best at both 24 hours and 7 days after initial learning when the exercise takes place 20 minutes after learning, compared to 1 hour or 2 hours after learning (Thomas, Beck, et al., 2016). In terms of exercise intensity, high-intensity exercise has a positive effect on retention, whereas moderate and low intensities do not have consistent effects (Snow et al., 2016; Thomas, Johnsen, et al., 2016). Lastly, the muscle groups used during exercise do not appear to have a differential effect on retention (Thomas, Flindtgaard, et al., 2016).

Concurrently, investigations on motor cortical excitability changes after exercise show results in line with the theory that exercise induces global neuroplasticity. A single bout of low-moderate or moderate-high intensity cycling may induce decreased intracortical inhibition in a non-exercised hand muscle, however, MEP amplitudes may not change after either exercise intensity (Smith et al., 2014). Similarly, Singh et al. (2014) also showed decreased intracortical inhibition in a non-exercised wrist muscle after a bout of moderate-intensity cycling exercise. In addition, they found significant increases in intracortical facilitation, but, like the other study,

no changes in MEP amplitudes were observed. In another study utilizing moderate intensity cycling, intracortical facilitation in the hand increased, intracortical inhibition decreased, and, curiously, measures of interhemispheric inhibition decreased bilaterally after exercise (Neva et al., 2017). They also did not see changes in MEP amplitudes or in spinal circuitry excitability. Together, these results suggest that in the healthy brain, acute exercise can increase motor cortical excitability through modulation of a variety of intracortical circuits, but may not have an effect on corticospinal output directly. Intracortical inhibition is thought to be mediated by GABA receptors (Di Lazzaro et al., 2005; Ziemann, Lonnecker, Steinhoff, & Paulus, 1996), and intracortical facilitation may be mediated via NMDA receptors (Liepert, Schwenkreis, Tegenthoff, & Malin, 1997; Ziemann, Chen, Cohen, & Hallett, 1998). Acute exercise is thought to influence brain excitability by modulating the balance between these intracortical circuits, preparing the brain for optimal plasticity (Neva et al., 2017; Singh, Duncan, et al., 2014; Smith et al., 2014). This idea is supported by evidence showing that acute exercise increases the amount of plasticity induced by neuromodulation protocols (McDonnell, Buckley, Opie, Ridding, & Semmler, 2013; Singh, Neva, & Staines, 2014). MEPs, which reflect the combined corticospinal output in response to stimulation, may not change because the overall excitability has not been modified. These neurophysiological effects of exercise may explain how exercise enhances retention of motor learning.

Combining behavioral testing with neurophysiological testing, Ostadan et al. (2016) sought to investigate whether there is a relationship between exercise-induced motor cortical excitability changes and retention of motor learning. Interestingly and contrary to previous findings, they found increases in MEP amplitudes after high-

intensity cycling, but increases in retention of the motor skill did not reach statistical significance. The differences in the behavioral results may be explained by the difference in the motor task. Ostadan et al. (2016) used a key-press serial reaction time task, while all the aforementioned studies used a visuomotor tracking task. Their most significant finding was that the increases in MEP amplitudes were positively correlated with retention of motor learning, thus providing a link between behavioral gains from exercise with neurophysiological changes. So far, evidence suggests that acute exercise can enhance retention of motor learning through neuromodulation during motor memory consolidation in healthy individuals. If the same holds true in people with stroke, exercise could be an easy, convenient method to promote motor learning and retention in rehabilitation. Unfortunately, to date, this has not been well-studied in the stroke population.

To our knowledge, only two studies have examined the effects of a single bout of acute exercise for priming in stroke. Murdoch et al. (2016) found that a single bout of low-moderate intensity cycling failed to induce motor cortical excitability changes in a hand muscle in chronic stroke survivors. Since high-intensity exercise is best at enhancing retention in healthy individuals (Thomas, Johnsen, et al., 2016), it may be possible that the exercise intensity used in this study was not enough to induce changes in the stroke-affected brain. Another study utilizing high-intensity exercise found that exercise increased retention of a time-on-target hand motor task, but motor cortical excitability measurements in a hand muscle were inconclusive (Nepveu et al., 2017). Limitations to this study may have contributed to these results. The exercise protocol used to induce changes in motor learning was high-intensity interval training, whereas the exercise protocol to induce motor cortical excitability changes was a

graded exercise test. The difference in the exercise may have contributed to the differential results in motor learning and motor cortical excitability changes. Lactate change, which is a typical indicator of exercise intensity, was also not reported. Moreover, most participants in the study had very high-levels of hand function, so the results are likely not generalizable to individuals with greater impairments. In addition, the exercise protocols in the study may be fatiguing and take up to 15 minutes to complete, therefore they may not be feasible for use in the clinic in addition to regular physical therapy. In **Aim 1**, we will determine the effects of a short bout of high-intensity exercise on motor cortical excitability in people with chronic stroke, including participants with a broader range of impairments, using what we think is a more feasible exercise protocol. *The contribution that **Aim 1** will make is to thoroughly investigate motor cortical excitability changes after acute exercise in stroke, providing mechanistic evidence for utilizing exercise as a primer in stroke rehabilitation.*

tDCS as a Primer

tDCS delivers weak current to the brain through electrodes applied non-invasively over the scalp. The weak current alters resting membrane potentials of the neurons underneath the electrodes, and depending on the polarity, either depolarizes (under the anodal electrode) or hyperpolarizes (under the cathodal electrode) the neurons, thus making them able to fire more easily or harder to fire (Stagg & Nitsche, 2011). The weak current itself is not enough to create action potentials directly (unlike TMS); instead, tDCS only changes the resting state of the neurons. The overall effect of tDCS is that the brain area underneath the anode becomes more excitable, and the brain area underneath the cathode becomes less excitable. The effects of tDCS on

motor cortical excitability can be measured with TMS by applying the same intensity TMS pulse to the same brain location before and after applying tDCS to that same spot. In healthy individuals, following anodal tDCS over the M1, MEP amplitudes of the stimulated hemisphere increase (Furubayashi et al., 2008; Jeffery, Norton, Roy, & Gorassini, 2007; Nitsche & Paulus, 2000). The reverse is also true; cathodal tDCS over the M1 decreases MEP amplitudes from the stimulated hemisphere (Ardolino, Bossi, Barbieri, & Priori, 2005; Furubayashi et al., 2008; Nitsche & Paulus, 2000). The effects of tDCS on motor cortical excitability are dependent on current intensity and the duration of stimulation (Nitsche et al., 2003; Nitsche & Paulus, 2000, 2001), and can last up to 60 - 90 minutes after stimulation has ended (Nitsche et al., 2003; Nitsche & Paulus, 2001). Aside from effects on corticospinal output, tDCS can also affect the excitability of intracortical and interhemispheric connections (N. Lang, Nitsche, Paulus, Rothwell, & Lemon, 2004; Nitsche et al., 2005). These changes likely contribute to the MEP amplitude modulations caused by tDCS. While evidence of the effects of tDCS on motor cortical excitability in healthy individuals is abundant, it is important to note that responses to tDCS can be variable between individuals (Davidson, Bolic, & Tremblay, 2016; Wiethoff, Hamada, & Rothwell, 2014), which need to be considered when utilizing tDCS in stroke rehabilitation.

Effects of tDCS on Motor Performance Post-Stroke

As previously discussed, motor cortical excitability is altered after stroke (McDonnell & Stinear, 2017), thus, tDCS may facilitate stroke recovery through its effects on motor cortical excitability. Based on the interhemispheric imbalance theory, where the lesioned hemisphere has decreased excitability, and the non-lesioned hemisphere has increased excitability, various tDCS montages have been used in order

to restore interhemispheric balance. The most straightforward way is to apply anodal tDCS over the lesioned hemisphere M1 to increase cortical excitability. In this setup, the anode is placed over the lesioned hemisphere M1, and the cathode is placed over the contralateral supraorbital area. A single session of anodal tDCS over the lesioned hemisphere M1 during motor practice can increase performance of a paretic hand motor function task during and following stimulation (Fregni et al., 2005). tDCS-induced improvements in motor performance are correlated with increased motor cortical excitability in the lesioned hemisphere (Hummel et al., 2005), and with increased activation of the lesioned hemisphere M1 (Lindenberg, Renga, Zhu, Nair, & Schlaug, 2010; Stagg et al., 2012). Conversely, a single session of excitability-decreasing cathodal tDCS applied over the non-lesioned hemisphere M1 (anode over contralateral supraorbital area) during motor practice can also improve motor performance of the paretic hand (Fregni et al., 2005). Multiple sessions of either ipsilesional anodal or contralesional cathodal tDCS in conjunction with motor practice or rehabilitation can increase paretic upper extremity motor function, and the effects can outlast the duration of treatment up to 6 months (Allman et al., 2016; Boggio et al., 2007; Hesse et al., 2007; Kim et al., 2010; Nair, Renga, Lindenberg, Zhu, & Schlaug, 2011). Bihemispheric tDCS is applied with the anode over the lesioned hemisphere M1 to upregulate cortical excitability, with simultaneous cathodal stimulation over the non-lesioned hemisphere M1 to downregulate cortical excitability. Similar to ipsilesional anodal and contralesional cathodal tDCS, this method in conjunction with rehabilitation can also improve paretic upper extremity motor function (Bolognini et al., 2011; Goodwill, Teo, Morgan, Daly, & Kidgell, 2016; Lindenberg et al., 2010; Naros et al., 2016). The effects of tDCS on lower-limb

motor function are less studied, but the general effects seem to hold true. A single session of anodal tDCS over the lesioned hemisphere M1 can improve paretic ankle motor control (Madhavan, Weber, & Stinear, 2011) and paretic quadriceps force (S. Tanaka et al., 2011). A single session of bihemispheric tDCS can improve performance of the timed up and go test in stroke (Tahtis, Kaski, & Seemungal, 2014). However, recent reviews show that responses to tDCS in stroke are highly variable, and not all studies show positive effects (Cunningham et al., 2015; Elsner, Kugler, Pohl, & Mehrholz, 2013; Hesse et al., 2011). The heterogeneity of stroke survivors may be the reason for these inconsistencies (Cunningham et al., 2015; Elsner et al., 2013; Horvath, Carter, & Forte, 2014). For example, in a large clinical trial investigating the effects of tDCS with combined robotic arm training, no effect of tDCS on upper limb motor function is observed when the analysis includes all participants (Hesse et al., 2011). However, secondary analysis of a small subgroup of participants with purely subcortical lesions reveals that contralesional cathodal tDCS is effective in improving paretic arm function in these participants. Besides lesion location, many other factors, such as severity of stroke, motor function level, and medications may all affect behavioral responses to tDCS in stroke. From a mechanistic perspective, these differences are likely related to differences in the neurophysiological responses to tDCS. Thus thorough understanding of the neurophysiological responses to tDCS in stroke is needed in order to understand the differences in behavior. In summary, tDCS may be an effective method to at least temporarily improve motor performance post-stroke, but the underlying sources of the variability of responses need to be investigated.

Effects of tDCS on Motor Cortical Excitability Post-Stroke

While there is good evidence that tDCS can improve motor performance post-stroke, the underlying neurophysiological mechanisms are less well understood. When testing the effects of tDCS on motor performance, tDCS is almost always applied during motor practice or training. When this is the case, tDCS increases motor cortical excitability of the lesioned hemisphere in both the upper (Bolognini et al., 2011; Hummel et al., 2005) and the lower extremities (M. C. Chang, Kim, & Park, 2015). However, the majority of evidence on the neurophysiological effects of tDCS in healthy individuals has been collected when tDCS is applied without performing a motor task. Surprisingly, studies of the same nature in stroke are very limited. The effects of tDCS in people post-stroke may be different from that of healthy individuals because of neurophysiological and neurochemical changes to the brain after stroke. To our knowledge, only two studies have examined the effects of tDCS on motor cortical excitability in individuals with stroke not performing a motor task. Edwards et al. (2009) showed in 6 ischemic stroke survivors that after 20 minutes of anodal tDCS over the lesioned hemisphere M1, active MEP amplitudes from the lesioned hemisphere increase in a wrist muscle, and intracortical inhibition from the same hemisphere decreases. In another study, Suzuki et al. (2012) found that 10 minutes of anodal tDCS over the lesioned hemisphere M1 increases MEP amplitudes in a hand muscle, compared to sham tDCS. Interestingly, 10 minutes of cathodal tDCS over the lesioned hemisphere M1 also increased MEPs, whereas in healthy controls, cathodal tDCS decreases MEP amplitudes. While these studies show that stroke survivors may respond to tDCS similarly to healthy controls with regard to some motor cortical excitability measures, the study by Suzuki et al. (2012) also reveals some major inconsistencies that need to be explored. In addition, in the study by Suzuki et al., the

participants had mild impairments, and only 2 out of the 7 stroke survivors were in the chronic stage (> 6 months). Motor cortical excitability from the non-lesioned hemisphere, which could provide information on interhemispheric interactions, has not been reported. *Thorough investigation of the effects of tDCS on motor cortical excitability in people with stroke is needed to further understand neurophysiological changes after stroke, and for better utilization of tDCS in motor recovery post-stroke.*

Antidepressant Medications Modify the Neuroplastic Effects of tDCS

Up to 30% of individuals take antidepressant medications post-stroke (El Husseini et al., 2012; Eriksson et al., 2004; Paolucci, 2008; Ried, Tueth, & Jia, 2006). Reasons for this are likely two-fold. First, there is growing recognition that post-stroke depression is a common and serious consequence of stroke that is associated with increased disability (Ayerbe, Ayis, Rudd, Heuschmann, & Wolfe, 2011; Espárrago Llorca, Castilla-Guerra, Fernández Moreno, Ruiz Doblado, & Jiménez Hernández, 2015; Paolucci, 2008; Robinson & Spalletta, 2010). Second, even without a diagnosis of depression, antidepressant medications have been shown to improve functional outcomes when prescribed immediately after stroke (Chollet et al., 2011). The use of antidepressants may be another factor that contributes to the heterogeneity of responses to tDCS in stroke. The trend for antidepressant prescription to become the standard of care for acute stroke recovery is likely to continue. The same is true for rehabilitation interventions focused on utilizing tDCS to trigger enhanced neuroplasticity. Therefore, it is critical to try to understand how antidepressant medications may affect brain excitability and interact with mechanisms of tDCS post-stroke. Yet, to date, we are unaware of any such investigations in individuals with chronic stroke (and only one in acute stroke).

In healthy adults, a single dose of sertraline (a selective serotonin-reuptake inhibitor, SSRI) increases MEP amplitudes but decreases intracortical facilitation (Ilic, Korchounov, & Ziemann, 2002). However, when SSRIs are ingested chronically, as one would when treating depression, the effects of SSRIs on motor cortical excitability are reversed. For example, ingesting 20 mg of paroxetine (another SSRI) daily for 30 days induces an increase in intracortical facilitation compared to placebo, which is correlated with increased speed in a finger tapping task (Gerdelat-Mas et al., 2005). The chronic paroxetine ingestion also decreases MEP amplitudes. In patients with acute stroke, one month of citalopram (another SSRI) 10 mg daily increases intracortical inhibition and the threshold for excitability in the non-lesioned hemisphere compared to placebo medication, but does not appear to affect MEP amplitudes (Acler, Robol, Fiaschi, & Manganotti, 2009). The lesioned hemisphere was not differentially modulated by the medication. Thus, the existing evidence suggests that SSRIs may have significant effects on motor cortical excitability in stroke survivors. These effects may contribute to the heterogeneity of behavioral responses to tDCS in stroke, yet to date, antidepressant medication use has been widely ignored in post-stroke rehabilitation studies involving tDCS (Allman et al., 2016; Boggio et al., 2007; Fleming, Rothwell, Sztriha, Teo, & Newham, 2017; Fregni et al., 2005; Hesse et al., 2011; McCambridge, Stinear, & Byblow, 2018; van der Vliet, Ribbers, Vandermeeren, Frens, & Selles, 2017).

SSRIs have been shown to interact with tDCS in healthy individuals. Both an acute dose and chronic application (35 days) of citalopram in healthy individuals facilitate the effects of anodal tDCS by increasing MEP amplitudes and reverse the inhibitory effects of cathodal tDCS into facilitation (Kuo et al., 2016; Nitsche et al.,

2009). These effects are thought to be NMDA-receptor dependent because dextromethorphan, an NMDA-antagonist, abolished the effects produced by chronic SSRI (Kuo et al., 2016). Modulation of membrane conductance (Andrade & Chaput, 1991; Panicker, Parker, & Miledi, 1991) and increases in calcium influx (Gu, 2002) are proposed mechanisms of action. To date, there have been no studies examining the effects of antidepressant medication intake on responsiveness to tDCS post-stroke. Therefore, in **Aim 2**, we will determine the effects of anodal tDCS on motor cortical excitability in chronic stroke survivors on vs. off antidepressants. *The contributions that **Aim 2** will make is to thoroughly examine the effects of chronic antidepressant taking on motor cortical excitability in a chronic stroke population, and to thoroughly examine the effects of tDCS on motor cortical excitability in a broader stroke population, with chronic antidepressants as a factor. This will provide mechanistic evidence on how tDCS affects motor outcomes post-stroke, and help make more informed decisions in utilizing tDCS in stroke survivors taking antidepressants.*

Motor Learning through Neuroplasticity

Motor learning is a relatively permanent change in the capability to perform movements, through practice or experience, that persist beyond the practice session, where the capabilities can be thought of as processes that permits acquisition of new skilled behaviors (Salmoni, Schmidt, & Walter, 1984). Neuroplastic changes underlie the processes of motor learning (Dayan & Cohen, 2011), thus priming techniques that promote neuroplasticity may be beneficial for motor learning. Although true skill learning is a long-term process, short-term changes in behavior are the focus of many laboratory studies of motor learning, because this allows for better experimental control. Early stages of motor learning can be observed within a single practice

session, called online learning. This initial motor memory is fragile at first, but then goes through a process known as consolidation, where the motor memory becomes stable and resistant to interference over a few hours (Brashers-Krug, Shadmehr, & Bizzi, 1996; Shadmehr & Brashers-Krug, 1997; Walker et al., 2003). Consolidation is therefore considered to be essential for retention of recently learned movements. Modern motor rehabilitation post-stroke is based on principles of motor learning and neuroplasticity (Krakauer, 2006). Consolidation is a key component of this for people post-stroke because motor learning is only useful if the newly learned information can be retained. Interestingly, during the consolidation period the brain engages areas not necessarily required for the initial learning (Shadmehr & Holcomb, 1997), and converging evidence suggest that M1 may be crucial for the consolidation of motor memory. In healthy individuals, disruption of M1 activity via inhibitory repetitive TMS immediately following learning a ballistic finger opposition task prevents retention of the task (Muellbacher et al., 2002). Likewise, enhancing M1 activity with tDCS is known to enhance consolidation and retention across a variety of motor tasks (Galea, Vazquez, Pasricha, de Xivry, & Celnik, 2011; Reis et al., 2009; Rumpf et al., 2017).

Effects of tDCS on Motor Learning

Given the positive effects of tDCS on motor cortical excitability (N. Lang et al., 2004; Nitsche & Paulus, 2000), it is plausible to consider using tDCS to enhance motor learning. The location of tDCS needs to be carefully considered. Previous studies suggest that M1 may be the neural substrate for consolidation of motor memory (Muellbacher et al., 2002; Tunovic, Press, & Robertson, 2014). Thus, if the goal is to improve consolidation, tDCS over the M1 may be the best choice. Indeed,

anodal tDCS applied to the M1 during initial learning can enhance retention of a learned motor skill in healthy individuals (Reis et al., 2009).

The critical role of the M1 in motor consolidation can be shown through studies of one type of motor learning called motor adaptation. Motor adaptation is the short-term adjustment of motor commands through trial and error practice in response to changes in the environment (Martin, Keating, Goodkin, Bastian, & Thach, 1996b). The cerebellum is thought to be crucially involved in the initial adaptation process because patients with cerebellar damage cannot adapt (C. E. Lang & Bastian, 1999; Martin, Keating, Goodkin, Bastian, & Thach, 1996a; Morton & Bastian, 2006). Anodal tDCS applied to the cerebellum can accelerate the rate of initial adaptation in upper extremity (Galea et al., 2011) and locomotor adaptation tasks (Jayaram et al., 2012) in young healthy individuals, with no effect on storage of learning (measured as deadaptation in these studies). However, when anodal tDCS is applied over the M1, it can enhance storage of an upper extremity visuomotor rotation task (Galea et al., 2011). Similar results are found in healthy older adults, where it has been shown that they have impaired motor adaptation compared to young healthy adults (Bock, 2005; Seidler, 2006). Anodal tDCS over the cerebellum in healthy older adults enhances the rate of adaptation in a visuomotor rotation task to a rate comparable with young healthy individuals (Hardwick & Celnik, 2014). In another study, anodal tDCS over the M1 during initial learning in healthy older adults was found to enhance relearning (learned based on prior retention) of the visuomotor rotation 50 minutes later, so that their performance at the end of relearning was comparable with young healthy adults (Panouillères, Joundi, Brittain, & Jenkinson, 2015). Anodal tDCS over the cerebellum did not produce the same effect.

In people with stroke, tDCS can enhance motor performance (Butler et al., 2013; M. C. Chang et al., 2015; Fregni et al., 2005; Hummel et al., 2005; Lindenberg et al., 2010). However, studies regarding tDCS and motor learning in stroke are limited and have been focused on the upper extremity. Bihemispheric tDCS can increase online learning of a circuit tracing task and lead to markedly increased retention of the task one week later (Lefebvre et al., 2013). Cathodal tDCS over the non-lesioned hemisphere M1 can improve retention of a finger motor sequence learning task in a group of well-recovered stroke survivors (upper extremity Fugl-Meyer Assessment, FMA ≥ 61) (Zimerman et al., 2012). In a more recent study, different tDCS montages were compared in their efficacy to improve an upper extremity motor sequence learning task in a group of stroke survivors with a wider range of impairments. Contrary to previous findings, they showed that neither anodal tDCS over the lesioned hemisphere M1, cathodal tDCS over the non-lesioned hemisphere M1, or bihemispheric tDCS had an effect on motor sequence learning (Fleming et al., 2017). Therefore, to date, studies examining the effects of tDCS on motor learning post-stroke are limited and show mixed results, which may be affected by a number of factors, including but not limited to, stroke severity, lesion location, type of learning, tDCS montage and current density, duration of tDCS, and timing of tDCS relative to learning. Moreover, locomotor learning is very different from the upper extremity learning that have been studied, as it requires bilateral movements involving interlimb coordination and postural control. Therefore, the effects of tDCS on locomotor learning post-stroke warrants separate examination.

Locomotor Adaptation in Stroke

The split-belt treadmill is commonly used in the laboratory setting to study locomotor adaptation. The treadmill has two belts that can be controlled independently, and participants walk with one leg on each belt. By setting the belts at two different speeds, we introduce a perturbation in the walking environment which causes the participant to walk asymmetrically. However, participants can quickly adapt to this perturbation and regain symmetrical or near symmetrical walking, even though the belts are still running at two different speeds. When the belts are set at the same speed again after walking symmetry has adapted, participants produce an asymmetry in the opposite direction. This so-called "negative after effect" indicates that the adaptation is stored in the nervous system, and not simply a reactive response to the perturbation. After stroke not involving the cerebellum, the ability to adapt is preserved (Reisman, Wityk, Silver, & Bastian, 2007), however, the rate of adaptation is slowed and retention is poor (Savin, Tseng, Whitall, & Morton, 2013; Tyrell, Helm, & Reisman, 2014). Given that tDCS may improve retention of upper extremity motor learning post-stroke, perhaps it can also be used with locomotor learning to maximize its retention. In **Aim 3**, we will determine the effects of anodal tDCS on locomotor adaptation and retention in people with chronic stroke. *The contributions that **Aim 3** will make is to expand our current knowledge by providing evidence on the efficacy and feasibility of utilizing tDCS to improve retention of locomotor learning post-stroke, and provide some mechanistic evidence for the neural substrate of consolidation of locomotor adaptation. This will pave the path for future studies that investigate the utilization of tDCS to enhance locomotor learning in clinical settings, and with more clinically-relevant tasks.*

Summary, Aims and Hypotheses

Despite current rehabilitation efforts, motor function deficits of both the upper (Hatem et al., 2016) and lower extremities (Dickstein, 2008) persist post-stroke. This is problematic in that persistent motor impairment is associated with increased disability and decreased activity and participation (English, Manns, Tucak, & Bernhardt, 2014; Singam, Ytterberg, Tham, & von Koch, 2015). Therefore, it is important to understand the sources of this persistent impairment in order to identify new methods to reduce impairment. One important neurophysiological factor is the excitability changes within the M1 post-stroke. Motor cortical excitability of the lesioned hemisphere is decreased post-stroke, and is related to poor functional performance (Butler & Wolf, 2007; Dimyan & Cohen, 2010; Mang et al., 2015; Palmer, Needle, Pohlig, & Binder-Macleod, 2016; Palmer, Zarzycki, Morton, Kesar, & Binder-Macleod, 2017; Trompetto et al., 2000). Priming techniques can alter cortical excitability, and therefore have gained interest as adjunct tools to rehabilitation interventions through their benefits on neuroplasticity (Cunningham et al., 2015; Di Pino et al., 2014; Robertson & Takacs, 2017). One technique, exercise priming, can increase motor cortical excitability (Neva et al., 2017; Singh, Duncan, et al., 2014; Smith et al., 2014) and motor learning (Roig et al., 2012; Stavrinos & Coxon, 2017; Thomas, Beck, et al., 2016; Thomas, Flindtgaard, et al., 2016; Thomas, Johnsen, et al., 2016) in healthy individuals, but its effects in people post-stroke are inconclusive (Murdoch et al., 2016; Nepveu et al., 2017). Another technique, tDCS, can change motor cortical excitability (Nitsche & Paulus, 2000, 2011), enhance motor performance (Boggio et al., 2006), and promote motor learning and retention (Reis et al., 2009) in healthy individuals. Post-stroke, tDCS can improve motor performance (Hummel et al., 2005; Lindenberg et al., 2010), but there is surprisingly limited

reporting on the cortical neurophysiological changes induced by tDCS in the absence of a motor task (Edwards et al., 2009; Suzuki et al., 2012). Moreover, responses to tDCS is highly variable post-stroke (Cunningham et al., 2015; Elsner et al., 2013), and medications may contribute to the heterogeneity. Antidepressant medications, which are often prescribed to stroke survivors, can affect motor cortical excitability (Ilic et al., 2002) and interact with tDCS (Kuo et al., 2016; Nitsche et al., 2009) in healthy individuals, but understanding of how they may affect stroke survivors chronically taking antidepressants is poor. Furthermore, it is not clear whether tDCS may affect motor learning and retention post-stroke.

Our long-term goals are to fully understand the neurophysiological and behavioral effects of different priming techniques in people post-stroke, so that interventions utilizing these techniques can be adequately applied in stroke survivors with different characteristics. The objective of this work was to investigate the neurophysiological effects of exercise priming and anodal tDCS (with chronic antidepressant intake as a factor) in people post-stroke, and to investigate the effects of tDCS on locomotor adaptation and retention post-stroke. Accordingly, we pursued the following three specific aims with hypotheses:

Aim 1: To determine the effects of a short bout of high-intensity exercise on motor cortical excitability in people with chronic stroke.

H1: Motor cortical excitability of non-exercised upper extremity muscles will increase bilaterally after a short bout of high-intensity lower extremity exercise post-stroke.

Aim 2: To determine the effects of anodal tDCS on motor cortical excitability in chronic stroke survivors on vs. off antidepressants.

H2.1: Baseline motor cortical excitability will be increased bilaterally in stroke survivors taking antidepressants, compared to those not taking antidepressants.

H2.2: When applied during rest, anodal tDCS over the lesioned hemisphere M1 will alter motor cortical excitability in chronic stroke survivors, producing increases in excitability of the lesioned hemisphere and decreases in excitability of the non-lesioned hemisphere.

H2.3: When applied during rest, the effects of anodal tDCS over the lesioned hemisphere M1 on motor cortical excitability will be enhanced in stroke survivors taking antidepressants, compared to those not taking antidepressants.

Aim 3: To determine the effects of anodal tDCS on locomotor adaptation and retention in people with chronic stroke.

H3: Anodal tDCS over the lesioned hemisphere M1 lower extremity representation applied during split-belt treadmill adaptation will increase 24-hour retention of the novel walking symmetry pattern.

Together, Aims 1 – 3 will thoroughly examine the effects of two priming techniques that are known to induce neuroplasticity in healthy adults, high-intensity exercise (Aim 1) and tDCS (Aim 2), on motor cortical excitability in people with chronic stroke, and examine the effects of tDCS on a behavior, locomotor learning and its retention, post-stroke (Aim 3). The significance is that this will provide a deeper understanding of the neurophysiological and behavioral effects of exercise and tDCS in people with chronic stroke, and help investigators and clinicians make more targeted, evidence-based decisions on utilizing these techniques in stroke rehabilitation.

Chapter 2

A SHORT BOUT OF HIGH-INTENSITY EXERCISE ALTERS IPSILESIONAL MOTOR CORTICAL EXCITABILITY POST-STROKE

Abstract

Objectives: To investigate whether a short bout of high-intensity exercise increases motor cortical excitability in non-exercised muscles in chronic stroke survivors.

Methods: Thirteen participants with chronic, unilateral stroke participated in two sessions, at least one week apart. In each session, they underwent either high-intensity lower extremity (walking) exercise or quiet rest. Motor cortical excitability of the extensor carpi radialis muscles was measured bilaterally with transcranial magnetic stimulation (TMS) before and immediately after either exercise or rest. Post-exercise or rest measures were normalized to pre-test measures. Paired t-tests or Wilcoxon signed-ranks tests were used to compare the changes in motor cortical excitability following exercise vs. rest conditions.

Results: All participants were able to reach the target exercise intensity level. Blood lactate levels increased significantly after exercise ($p < 0.001$, $d = 2.85$). Resting MEPs from the lesioned hemisphere increased after exercise compared to the rest condition ($p = 0.046$, $d = 2.76$). All other TMS measurements were not different between exercise and rest sessions.

Conclusions: Our results indicate that a short bout of high-intensity exercise can increase lesioned hemisphere motor cortical excitability in a non-exercised muscle post-stroke.

Significance: Our short, non-fatiguing, easily-administered exercise protocol shows promise as a potential priming method in stroke rehabilitation.

Introduction

Many stroke survivors have persistent motor function deficits despite receiving intensive rehabilitation (Dickstein, 2008; Hatem et al., 2016). These deficits lead to increased disability and decreased activity and participation (English et al., 2014; Singam et al., 2015). Motor impairments post-stroke are associated with motor cortical excitability changes within the primary motor cortex (M1), which can be measured with transcranial magnetic stimulation (TMS). After stroke affecting the corticospinal pathway, motor evoked potentials (MEPs), which are a measure of the strength of connection from the M1 to contralateral muscles, from the lesioned hemisphere to the paretic upper extremity are decreased compared to the non-lesioned hemisphere (C. M. Stinear et al., 2015; Traversa et al., 1998) and compared to healthy controls (Traversa et al., 1998). Furthermore, increases in lesioned hemisphere MEP amplitudes are associated with motor function improvement (C. M. Stinear et al., 2015; Traversa et al., 1998; Trompetto et al., 2000). Therefore, interventions that increase motor cortical excitability within the lesioned hemisphere may be beneficial for motor recovery post-stroke.

Exercise can increase excitability broadly within the brain. That is, brain areas not directly involved in the exercise show increased excitability following exercise (Taubert et al., 2015), an effect known as exercise 'priming'. This is potentially

beneficial for stroke rehabilitation because the brain regions controlling more impaired muscle groups or limbs can capitalize on the broad effects of exercising intact limbs via improved excitability, without fatiguing impaired limbs with exercise or being limited by an inability to exercise very severely impaired limbs. However, most work showing the effects of exercise on motor cortical excitability has been done in young, healthy adults. In this population, a single bout of low-moderate or moderate-high intensity cycling can decrease short-interval intracortical inhibition (SICI) to a non-exercised upper extremity muscle, however, MEP amplitudes do not seem to change after either exercise intensity (Neva et al., 2017; Singh, Duncan, et al., 2014; Smith et al., 2014). In addition, significant increases in intracortical facilitation (Neva et al., 2017; Singh, Duncan, et al., 2014) and decreases in interhemispheric inhibition (Neva et al., 2017) in upper extremity muscles have been found after cycling exercise. Together, these results suggest that in the healthy brain, acute exercise can increase motor cortical excitability through modulation of a variety of intracortical circuits, but may not have an effect on corticospinal output directly.

Behaviorally, acute exercise has been shown to increase retention of learning a motor task in healthy individuals (Roig et al., 2012; Thomas, Beck, et al., 2016). Twenty minutes of high-intensity cycling can improve retention of an upper extremity visuomotor tracking task at 24 hours and 7 days after initial learning, compared to controls that do not exercise (Roig et al., 2012). Interestingly, retention at 7 days is better if the exercise occurs after initial learning, compared to before. This indicates that exercise may enhance retention of learning through an action on the motor memory consolidation process. Further studies have corroborated the results and systematically examined factors that may contribute to this effect. Retention is best if

the exercise occurs 20 minutes after learning, rather than 1 hour or 2 hours after learning (Thomas, Beck, et al., 2016). High-intensity exercise has a positive effect on retention, whereas moderate and low intensities do not have consistent effects (Snow et al., 2016; Thomas, Johnsen, et al., 2016). Lastly, the muscle groups used during exercise do not appear to have a differential effect on retention (Thomas, Flindtgaard, et al., 2016).

Interestingly, Ostadan et al. (2016) investigated whether there was a relationship between exercise-induced motor cortical excitability changes and retention of motor learning. Indeed, not only did they report a significant effect of exercise on increasing MEP amplitudes in a non-exercised muscle for the first time, they also showed that the increases in MEP amplitudes after high-intensity cycling were positively correlated with the degree of retention of a motor sequence learning task, thus providing a potential mechanism of behavioral gains from exercise through neurophysiological increases in cortical excitability.

To our knowledge, only two studies have examined the effects of a single bout of acute exercise in stroke, and the results remain inconclusive. Murdoch et al. (2016) found that a single bout of low-to-moderate intensity cycling failed to induce motor cortical excitability changes in a hand muscle in chronic stroke survivors. Since high-intensity exercise is best at enhancing retention in healthy individuals (Thomas, Johnsen, et al., 2016), it may be possible that the exercise intensity used in this study was not enough to induce changes in the stroke-affected brain. Another study utilizing high-intensity exercise found that exercise increased retention of a time-on-target hand motor task, but motor cortical excitability measurements in a hand muscle were inconclusive (Nepveu et al., 2017). The fact that the exercise protocol used to induce

changes in motor learning was high-intensity interval training, whereas the exercise protocol used to induce motor cortical excitability changes was a graded exercise test may have contributed to the differential results in motor learning and motor cortical excitability changes. Lactate change, which is a typical indicator of exercise intensity, was also not reported.

Thus it remains unclear whether or not exercise priming is an effective method to enhance motor cortical excitability in individuals with brain damage caused by stroke. The purpose of this study was to determine whether or not a short bout of high-intensity exercise (exercise priming) alters motor cortical excitability in people with chronic stroke. Unlike previous work, we included participants with moderate-severe paresis and we used a shorter priming protocol that we believe is more feasible for potential future clinical application in this population (Charalambous, Helm, Lau, Morton, & Reisman, 2018). We hypothesized that motor cortical excitability of non-exercised upper extremity muscles would increase bilaterally after a short bout of high-intensity lower extremity exercise post-stroke.

Materials and Methods

Participants

Thirteen participants (2 female, mean age 65.77 ± 7.20 years) with chronic (> 6 months), unilateral stroke affecting the corticospinal tract participated in the study. They all went through a sensorimotor evaluation to determine their functional status, and brain MRI or CT scan reports were obtained to verify stroke type and lesion location. All participants were able to walk on a treadmill continuously for 5 minutes without assistance. Exclusion criteria included multiple strokes affecting both

hemispheres, any cerebellar lesion, or any other significant neurological, cardiovascular or musculoskeletal condition besides stroke. Individuals with contraindications to TMS were also excluded. Participants taking or withdrawing from (< 8 weeks) medications affecting the central nervous system or heavy alcohol users were also excluded to rule out their potential effects on the central nervous system. The experimental protocol was approved by the University of Delaware Institutional Review Board and all participants gave written informed consent. Participant demographic information is provided in Table 1.

General Paradigm

Participants completed two testing sessions separated by at least one week. They performed a short bout of high-intensity exercise priming (i.e. fast treadmill walking) in one session and sat quietly in the other session. Before and immediately after exercise or rest, motor cortical excitability of the extensor carpi radialis (ECR) muscles was measured bilaterally with TMS. The ECR muscle was selected because (a) we wanted to select a muscle not directly involved in the high-intensity exercise (i.e., an upper extremity muscle) and (b) in more impaired stroke survivors, the chances of eliciting MEPs from the paretic ECR is typically better than from an intrinsic hand muscle.

Transcranial Magnetic Stimulation

At each TMS session, participants sat in a comfortable chair and were instructed to completely relax their arms with the elbow flexed over a pillow or armrests. A 70 mm diameter figure-of-eight coil was used in conjunction with Magstim 200^2 and BiStim^2 electromagnetic stimulation units (Magstim, Ltd., Wales,

UK) for all single-pulse and paired-pulse TMS measures, respectively. Signal 6.03 software (Cambridge Electronic Design, Ltd., Cambridge, UK) was used to control and trigger the magnetic stimulator through a 16-bit data acquisition unit (Micro 1401-3, Cambridge Electronic Design, Ltd., Cambridge, UK), and to collect and store electromyography (EMG) data for offline analysis.

Table 1 Participant demographic information[1].

PID	Gender	Age (yrs)	Time Since Stroke (m)	Side of Stroke	Type of Stroke	Stroke Location	UE-FMA	Beta-Blockers
001	M	65	26	R	I	SC	61	N
002	F	63	39	R	I	SC	60	Y
003	M	68	14	R	I	SC	58	N
004	M	75	20	R	I	SC	32	Y
005	M	55	51	R	I	C	10	Y
006	M	77	28	R	I	SC	27	Y
007	F	62	36	R	I+H	SC	59	Y
008	M	70	8	L	I	SC	61	Y
009	M	73	31	R	I	SC+C	59	N
010	M	64	73	R	I	SC	21	Y
011	M	67	42	R	I	SC	33	N
012	M	52	135	L	I	SC+C	12	N
013	M	64	11	R	I	SC+C	60	N
Mean ± SD		**65.77 ± 7.20**	**39.54 ± 33.68**				**42.54 ± 20.35**	

[1] Abbreviations: PID = participant ID number, M = male, F = female, yrs = years, m = months, R = right, L = left, I = ischemic, H = hemorrhagic, SC = subcortical, C = cortical, UE-FMA = upper extremity Fugl-Meyer Assessment, Y = yes, N = no.

The vertex of the skull was identified and marked on the scalp with a washable marker. The coil was held tangential to the scalp at a 45° angle to the mid-sagittal line to induce a posterior-to-anterior current in the upper extremity motor strip (Brasil-Neto et al., 1992; Kammer, Beck, Thielscher, Laubis-Herrmann, & Topka, 2001; Mills, Boniface, & Schubert, 1992). The "hot spot" for the ECR muscle was defined as the spot that produced the biggest and most consistent peak-to-peak MEP amplitudes at a given stimulus intensity, and marked carefully on the scalp for use throughout the session. In cases where no MEPs could be elicited from the lesioned hemisphere at 100% of the maximum stimulator output (MSO), we used a symmetrical "hot spot" to the non-lesioned hemisphere. Resting motor threshold (RMT) was defined as the lowest stimulus intensity that produced at least 5 out of 10 MEPs in the contralateral ECR muscle with a peak-to-peak amplitude over 50 μV (Groppa et al., 2012). Ten MEPs were collected from each hemisphere at 120% RMT before and after exercise or rest.

Active motor thresholds (AMTs) were determined while participants were holding a light, isometric contraction of the ECR muscle. The contraction level for each subject and arm was either 3%, 5%, or 10% of their individual maximal voluntary isometric contraction (MVIC). We were unable to use the same percentage MVIC for all participants (e.g., 5% for all) because the EMG produced by the same %MVIC differed for different people and therefore could make discerning the presence of an MEP over and above background EMG difficult. Importantly, however, the contraction level for each subject and arm was held consistent for both testing bouts (pre and post) and sessions (exercise and rest). The AMT was defined as the minimum stimulus intensity that could elicit at least 5 out of 10 MEPs in the

contralateral contracting ECR muscle, where the MEP exceeded 200 μV (Groppa et al., 2012), i.e., clearly over and above the background contraction level, and was followed by a silent period. Fifteen active MEPs were collected from each hemisphere at 120% AMT before and after exercise or rest.

During the exercise session only, we also assessed SICI. With the subject relaxed, a conditioning stimulus (CS) was delivered at an intensity of 90% RMT, followed by a test stimulus (TS) delivered at 130% RMT, with the inter-stimulus interval (ISI) set at 2.5 ms (Roshan, Paradiso, & Chen, 2003). Ten trials each of the TS alone and of the paired CS-TS, presented in random order, were delivered to each hemisphere.

All TMS single- or paired-pulses were delivered with a random ISI varying between 5.50 – 7.00 s to ensure no inhibitory effects from prolonged stimulation and to prevent anticipation of the stimulus timing.

Electromyography

EMG was collected using a 10-channel system with double differential surface electrodes with an integrated ground (Motion Lab Systems, Inc., Baton Rouge, LA). The skin over the ECR muscles were cleaned with alcohol to remove any dirt, dead skin cells and oil. EMG electrodes were placed over the ECR muscle bellies bilaterally, longitudinally following the direction of the muscle fibers. Electrodes were secured with tape and self-adhesive wrap. A ground electrode was placed over the lateral epicondyle of the humerus. EMG data were collected with a sampling rate of 5000 Hz and online low-pass filtered at 2000 Hz.

Exercise / Rest Protocol

The exercise protocol (exercise priming session only) used was similar to that described in our previous work (Charalambous, Helm, et al., 2018). Briefly, participants completed 5 minutes of high-intensity walking on an instrumented treadmill (Bertec Corp., Columbus, OH). We defined high-intensity as exercise reaching 70% - 85% of the age-adjusted maximum heart rate (HR) (220 – age) (Fletcher, 1997), or, for participants taking beta-blocker medications, reaching a rate of perceived exertion (RPE) of 13 - 15 on the Borg scale (scale of 6 - 20) (Borg, 1970). HR was monitored during walking with a wireless HR monitor (Polar Electro Inc., Lake Success, NY). To reach and maintain the target HR or RPE throughout exercise, treadmill speed was adjusted around the participant's fastest comfortable speed. The fastest comfortable speed was determined at the beginning of data collection, i.e., before any TMS measurements. This exercise protocol has previously shown to be effective and tolerable for participants with stroke (Charalambous, Helm, et al., 2018). A harness and a front handrail were used during all walking procedures to ensure safety. Finger-tip blood lactate was measured using a portable Lactate Plus Meter (Nova Biomedical, Waltham, MA) before and immediately after exercise as an additional measure of exercise intensity. EMG of the ECRs was collected throughout the exercise and analyzed offline to ensure that participants were not exercising the wrist muscles during walking. The rest protocol consisted of 10 minutes of quiet sitting.

Data Analysis

Our main outcome measures were normalized (post-to-pre ratio) resting MEP and active MEP amplitudes, normalized cortical silent period (CSP) durations, and

SICI measured with TMS. EMG data from TMS were analyzed in Signal (Cambridge Electronic Design, Ltd., Cambridge, UK) and custom written software in MATLAB (MathWorks, Inc., Natick, MA). All raw EMG data were demeaned, and any gain removed. For resting MEP and SICI trials, EMG was notch-filtered at 60 Hz with a 2nd order Butterworth filter to remove electrical noise, and trials with baseline (10 - 60 ms before stimulus artifact) peak-to-peak EMG exceeding 30 μV were discarded, as this could indicate the participant was not at rest. Peak-to-peak resting MEP amplitudes were calculated for each trial and averaged over all trials. For active MEP and CSP trials (i.e., trials with an active light contraction being held), average rectified EMG during baseline (50 - 150 ms before stimulus artifact) of each trial was evaluated to ensure the correct contraction level. We used the following criteria as acceptable contraction levels for the various target contraction levels: within ± 5% for 10% MVIC contraction, within ± 3% for 5% MVIC contraction, within ± 1% for 3% MVIC contraction. Frames that did not meet the criteria were discarded. Peak-to-peak active MEP amplitudes were calculated for each trial and averaged over all trials. CSP onset was defined as the first time point when the rectified EMG fell below, and stayed below, the average rectified baseline EMG of that trial for at least 10 ms; CSP offset was defined as the first time point after CSP onset when the rectified EMG was at or above the average rectified baseline EMG, and the average rectified EMG during the following 25 ms was also at or above the averaged rectified baseline EMG. CSP duration was defined as the time from the stimulus artifact to CSP offset. CSP duration was calculated for each trial and averaged over all trials. SICI was calculated as the ratio of the mean peak-to-peak MEP amplitude of CS-TS trials to TS-only trials. Thus, a ratio value of 1 would indicate no intracortical inhibition, whereas values of <

1 would indicate inhibition. For all other TMS outcomes, measures were expressed as a ratio of that obtained during the post-test (for exercise or rest) to that obtained during the pre-test. This allowed us to compare changes of motor cortical excitability in individuals with widely varying degrees of impairment.

Exercise intensity was assessed as the ability to reach 70% - 85% of the age-adjusted maximum HR, or for participants taking beta-blockers, the ability to reach an RPE of 13 - 15. Time at target intensity was our outcome measure for exercise intensity and was averaged across all participants (Charalambous, Helm, et al., 2018). Blood lactate levels before and after exercise were also averaged across all participants during the exercise priming session only. HR, RPE and lactate levels were used as verification of adequate exercise intensity being reached.

To determine whether the ECR muscles were used extensively during exercise priming, we analyzed the last 100 s (1/3 of total exercise time) of ECR EMG during exercise because we thought participants were more fatigued towards the end of exercise, and would have tended to use their arms more then, if ever. EMG during exercise was demeaned, rectified, and the average of the rectified EMG was normalized to the individual EMG during MVIC.

Statistical Analysis

Statistical analyses were performed in IBM SPSS Statistics 24 (IBM Corp., Armonk, NY). Normality was checked with Kolmogorov-Smirnov tests. Depending on the normality or valid sample size for each measurement, paired t-tests or Wilcoxon signed rank tests were applied to compare differences in motor cortical excitability changes between exercise vs. rest sessions. Paired t-tests or Wilcoxon signed-rank tests were also applied to assess SICI changes before vs. after exercise.

Paired t-test were used to compared lactate levels before vs. after exercise. To be sure the post-to-pre normalization procedure did not mask any real differences in raw values, we performed additional analyses using the non-normalized TMS measures. For variables that were normally distributed, repeated-measures ANOVA were performed with within-subject factors session (exercise, rest) and time (pre, post). For not normally distributed variables, Wilcoxon signed-rank tests were used to compare motor cortical excitability levels before and after exercise or rest, and between after exercise and rest.

Results

All subjects completed the experiment with no adverse events. All data are reported in mean ± SEM unless otherwise stated.

Exercise Intensity

All subjects were able to reach the target exercise intensity level, and the average time spent at the target level was 66.15% ± 4.35% (range 35% - 85%) of the total exercise time. Our other measure of exercise intensity, blood lactate levels, increased significantly post-exercise (4.28 ± 0.36 mmol/L) compared to pre-exercise (1.68 ± 0.11 mmol/L), $t(12) = -7.53$, $p < 0.001$, $d = 2.85$.

EMG During Exercise

The averaged rectified ECR EMG magnitude during the last 100 s of exercise priming was 2.90% ± 0.77% MVIC for the non-paretic ECR, and 5.14% ± 1.11% MVIC for the paretic ECR. Importantly, all individuals' paretic ECR contraction level during exercise were less than their contraction level during active TMS testing,

suggesting that the light handrail holding during lower extremity exercise priming was unlikely to affect TMS results.

Baseline Motor Cortical Excitability Between Sessions

In the lesioned hemisphere, we were able to obtain RMTs from 8 participants (61.54%) in the exercise session. Because RMTs were very high (82 %MSO and 90 %MSO) from 2 participants in this session, we were only able to obtain resting MEPs and SICI from 6 participants (46.15%). We were able to obtain RMTs, resting MEPs, and SICI from 6 participants (46.15%) in the rest session, and we were able to obtain AMTs, active MEPs, and CSP durations from 8 participants (61.54%) from the exercise session and from 9 participants (69.23%) in the rest session. We were able to obtain complete datasets from all participants in the non-lesioned hemisphere. Baseline TMS measures were similar across the two sessions, with the exception of active MEP amplitudes from the non-lesioned hemisphere, which were higher in the rest session (0.87 ± 0.11 mV) than in the exercise session (0.70 ± 0.09 mV), $t(12) = -2.52$, $p = 0.027$. All other measurements, including active MEPs from the lesioned hemisphere, and bilateral RMTs, AMTs, resting MEP amplitudes and CSP durations, were not significantly different between sessions at baseline (all $p > 0.09$). Table 2 shows the mean baseline RMTs and AMTs from both sessions.

Resting MEP Amplitude Changes After Exercise / Rest

In Figure 1, we show traces of resting MEPs from one exemplar individual. Note that the MEP from the lesioned hemisphere had a larger peak-to-peak amplitude following exercise (compare thick black trace to thin gray trace, bottom panel, right), while it remained approximately the same size after rest (upper panel, right). This

observation was confirmed in the statistical comparisons of the entire group using MEPs expressed as a ratio (post/pre), which showed that excitability within the lesioned hemisphere increased more after exercise (1.66 ± 0.24) than rest (1.23 ± 0.30), $Z = 1.99$, $p = 0.046$, $d = 2.76$ (see Figure 2).

Table 2 Baseline group means ± SEM for RMTs and AMTs in rest and exercise sessions[2].

	RMT (%MSO)		AMT (%MSO)	
	NL	**L**	**NL**	**L**
Rest	43.92 ± 2.48	56.33 ± 6.83	34.54 ± 1.67	44.78 ± 5.52
Exercise	46.23 ± 3.41	61.38 ± 6.68	36.23 ± 2.08	40.00 ± 3.64
	$p = 0.19$	$p = 0.79^{\dagger}$	$p = 0.09$	$p = 0.40$

Active MEP Amplitude and CSP Duration Changes After Exercise / Rest

Figure 3 shows active MEP and CSP traces from an exemplar subject. Neither MEP amplitudes nor CSP durations changed much after exercise or rest in both hemispheres. The group post-to-pre ratios of active MEP amplitudes and CSP durations for the exercise and rest sessions are shown in Figure 4. Neither of these measures changed after either exercise or rest. Between-session comparisons for active MEPs and CSPs were all non-significant (all $p > 0.05$).

[2] †Wilcoxon signed-rank test; all others paired t-tests. RMT = resting motor threshold, AMT = active motor threshold, %MSO = percentage of maximum stimulator output, NL = non-lesioned hemisphere, L = lesioned hemisphere.

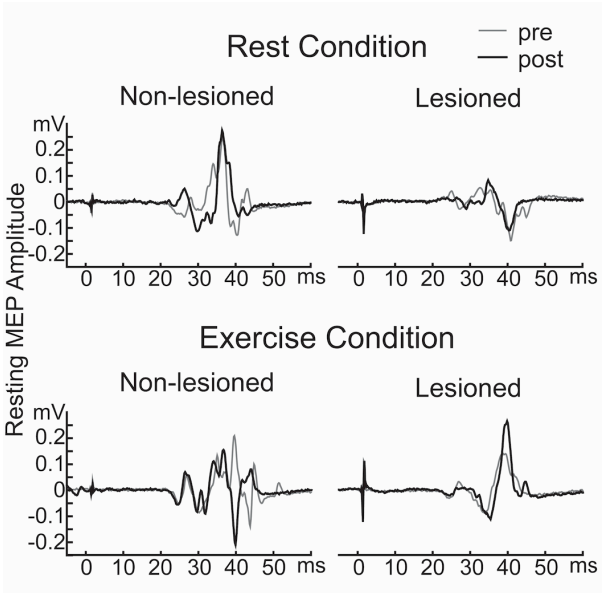

Figure 1 Raw EMG showing resting MEP traces (TMS intensity, 120% RMT) from an exemplar participant. Thin gray traces show pre-exercise or rest MEPs; thick black traces show post-exercise or rest MEPs. MEPs from the lesioned hemisphere increased after exercise, while they did not change much after rest. MEPs from the non-lesioned hemisphere did not change after either exercise or rest. X-axis show time from TMS stimulus (ms).

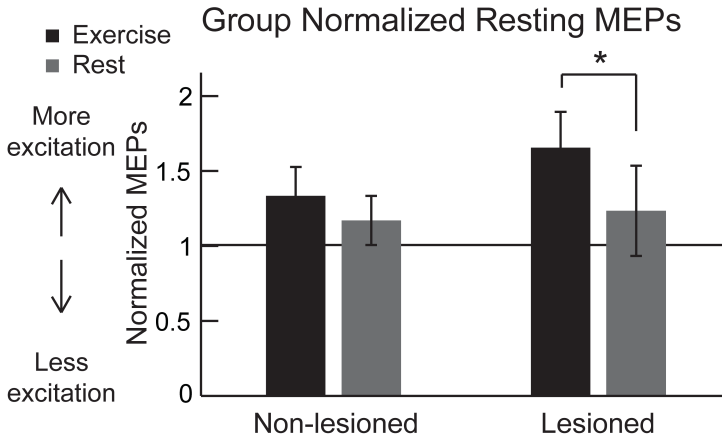

Figure 2 Group normalized post-to-pre resting MEP ratios. Data from the exercise session are in black bars; data from the rest session are in gray bars. Value of 1 means no changes in MEP amplitude after exercise or rest. *Indicates significance between exercise vs. rest sessions at $p < 0.05$.

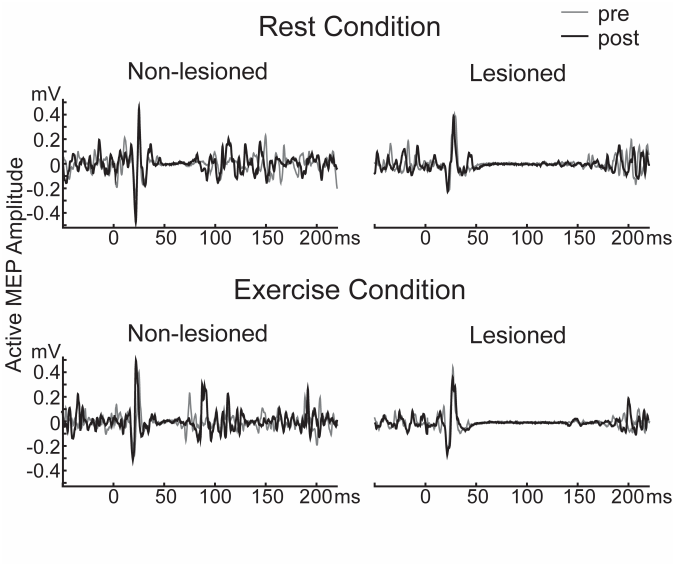

Figure 3 Raw EMG showing active MEP and CSP traces (TMS intensity, 120% AMT) from an exemplar participant. Thin gray traces show pre-exercise or rest data; thick black traces show post-exercise or rest data. In both hemispheres, active MEP amplitudes and CSP durations did not change much after either exercise or rest. X-axis show time from TMS stimulus (ms).

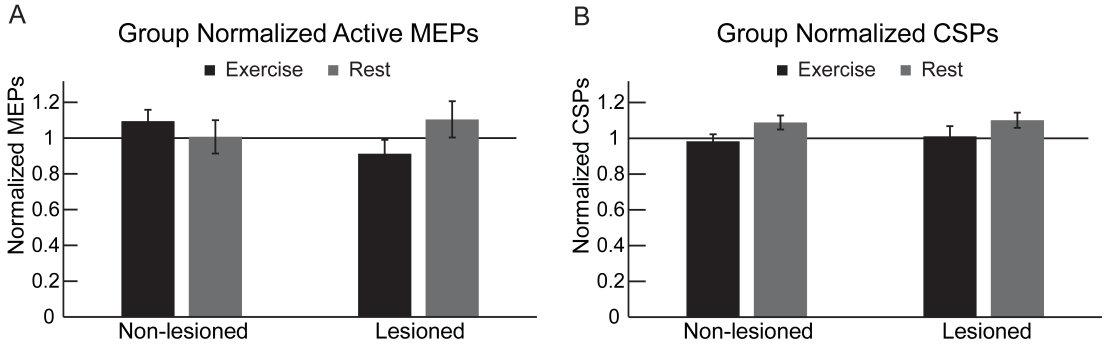

Figure 4 A. Group normalized post-to-pre active MEP ratios. There were no differences in normalized active MEP amplitudes between exercise vs. rest sessions in either hemisphere. B. Group normalized post-to-pre CSP ratios. There were no differences in normalized CSP durations between exercise vs. rest sessions in either hemisphere. For both panels, data from the exercise session are in black bars; data from the rest session are in gray bars. Value of 1 means no changes in MEP amplitude or CSP duration after exercise or rest.

SICI Changes After Exercise

SICI was also not significantly different before and after exercise in both the non-lesioned (pre, 0.47 ± 0.13, post, 0.55 ± 0.19) and the lesioned hemispheres (pre, 0.55 ± 0.19, post, 0.50 ± 0.12), all $p > 0.60$.

Analyses of Raw Motor Cortical Excitability Measures

Resting MEPs from the lesioned hemisphere before rest, and CSP durations from the non-lesioned hemisphere after exercise were not normally distributed, so non-parametric tests were performed on these variables. Consistent with the results from the normalized data, Wilcoxon signed-rank tests revealed that resting MEPs from the lesioned hemisphere increased significantly after exercise (0.49 ± 0.14 mV) compared to before exercise (0.28 ± 0.06 mV), $Z = 2.20$, $p = 0.028$, while they were not different before (0.21 ± 0.06 mV) and after rest (0.23 ± 0.05 mV), $Z = 0.11$, $p = 0.92$. Comparing the same measure between sessions, we found that resting MEPs from the lesioned hemisphere were also significantly higher after exercise compared to after rest ($Z = 2.20$, $p = 0.028$), and they were not significantly different before exercise and rest ($Z = 1.36$, $p = 0.17$). CSP durations from the non-lesioned hemisphere were significantly longer after rest (106.28 ± 6.76 ms) compared to before (99.02 ± 6.83 ms), $Z = 2.06$, $p = 0.039$. They were not significantly different before and after exercise, or between after exercise and after rest (both $p = 0.25$). For the remaining variables, the ANOVAs did not reveal significant main effects of time (all $p > 0.17$) or session (all $p > 0.06$), nor any significant interaction (all $p > 0.15$). Thus, with the exception of the results of CSP durations from the non-lesioned hemisphere, analyses of raw motor cortical excitability measures showed the same results as

analyses of the normalized measures, that exercise induced increased resting MEPs from the lesioned hemisphere.

Discussion

In summary, our results provide evidence to partially support our hypothesis that a short bout of high-intensity lower extremity exercise can increase motor cortical excitability in non-exercised muscles in people post-stroke. Specifically, peak-to-peak resting MEP amplitudes from the lesioned hemisphere were increased in this population following exercise priming. Notably, this is the first study to report positive effects of exercise priming on the lesioned hemisphere in people with stroke. Interestingly, the same measure from the non-lesioned hemisphere failed to show any changes. This result may seem surprising because one might presume that the lesioned hemisphere would be less responsive to changes induced by interventions, especially in the chronic stage of stroke. However, the changes in resting MEPs from the lesioned hemisphere in our study were highly robust (Cohen's $d = 2.76$), whereas in the non-lesioned hemisphere this was not the case ($d = 0.25$). This distinction leads us to speculate that the lesioned hemisphere post-stroke may be more susceptible to acute exercise priming than the non-lesioned hemisphere. This would be an exciting finding, because the ability to increase MEP amplitudes in the lesioned hemisphere M1 is associated with better motor recovery (C. M. Stinear et al., 2015; Traversa et al., 1998; Trompetto et al., 2000). Thus, acute exercise may be a potential method to promote better motor recovery post-stroke.

Our other measurements, including active MEPs, CSPs and SICI, were all unaffected by acute exercise in either hemisphere. CSPs reflect $GABA_B$-mediated intracortical inhibition (Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999),

while SICI is a measure of $GABA_A$-mediated intracortical inhibition (Di Lazzaro et al., 2005; Ziemann, Lonnecker, et al., 1996). Based on our results, intracortical inhibitory circuits may be less susceptible to changes induced by exercise in people with stroke. The interpretation of our active MEP results is more challenging because one would expect to see the same changes in active MEPs as in resting MEPs. Given the increased motor cortical excitability in the lesioned hemisphere M1, less cortical activation may have been required to achieve the same muscle contraction after exercise, which could have potentially confounded the measurement of active MEPs.

Most studies in healthy adults have failed to detect changes in resting MEPs after exercise (Neva et al., 2017; Singh, Duncan, et al., 2014; Smith et al., 2014). Instead, increases in SICI were found in most studies (Neva et al., 2017; Singh, Duncan, et al., 2014; Smith et al., 2014). Several factors may have contributed to the difference in our results and these healthy cohort studies. Most obviously, the presence of a brain lesion in our stroke subjects would be expected to potentially influence how the brain responds to acute exercise. Notably, resting MEPs from the "healthier" non-lesioned hemisphere were not affected by exercise, similar to results from most healthy cohort studies. Second, aging can decrease motor cortical excitability (Clark & Taylor, 2011), and reduce responses to other neuroplasticity-inducing protocols (Bashir et al., 2014; Fathi et al., 2010). Thus it is likely that aging may affect plastic changes within the motor cortex in response to acute exercise as well. Last, differences in exercise paradigm may also have affected the results. We purposefully chose our exercise paradigm because it is non-fatiguing and can be easily administered in the clinic for people with stroke (Charalambous, Helm, et al., 2018). The fact that just 5 minutes of high-intensity exercise was able to induce resting MEP changes in the

lesioned hemisphere is evidence for the potential of this paradigm to be used as an adjunct to physical therapy in the clinic, as a means to induce increased cortical excitability in patients with stroke.

To our knowledge, our study is the first and only study that has found positive effects of exercise on motor cortical excitability in stroke. Using low-intensity exercise, Murdoch et al. (2016) did not find any effect of exercise on motor cortical excitability in their study. It is possible that intensity is an important factor in inducing motor cortical excitability changes in people with stroke, where high-intensity may be the most effective. In another study, Nepveu et al. (2017) found that a graded exercise test did not induce conclusive changes in motor cortical excitability in stroke. However, in that study, neither exercise duration under each intensity nor lactate changes were reported. Thus, it could be that different individuals may have exercised at different intensities, increasing the variability of the results. In our study, subjects experienced a significant increase in lactate levels after exercise, suggesting that 5 minutes of fast walking was sufficient to induce exercise priming effects in this population.

Exercise has widespread positive influences on multiple levels of brain function (Taubert et al., 2015). Some of these changes induced by exercise may explain potential mechanisms underlying our results. Exercise can enhance long-term potentiation (LTP) in mice (van Praag, Christie, Sejnowski, & Gage, 1999), as well as up-regulate genes associated with the excitatory glutamatergic system, while down-regulating genes associated with the inhibitory GABA system in rats (Molteni, Ying, & Gómez-Pinilla, 2002). In healthy humans, acute exercise has been shown to increase neuroplasticity through facilitation of the effects of neuromodulation

protocols (McDonnell et al., 2013; Singh, Neva, et al., 2014). While mechanisms underlying the cortical effects of exercise remain unclear, it is likely that many individual factors contribute to the overall result. Lactate uptake in the brain increases with high-intensity exercise (Ide, Schmalbruch, Quistorff, Horn, & Secher, 2000; Kemppainen et al., 2005), and blood lactate increases, with or without exercise, have been associated with increased motor cortical excitability in healthy individuals (Coco et al., 2010). This could explain why the high-intensity exercise used in our study may be more efficient in inducing motor cortical excitability changes. A neurochemical model has also been proposed to explain the effects of exercise on motor cortical excitability through modulation of arousal-related neurotransmitters, including dopamine, serotonin, and norepinephrine (Singh & Staines, 2015). Brain-derived neurotrophic factor (BDNF) is also a potential candidate for increasing motor cortical excitability after exercise. BDNF levels increase after exercise (Rasmussen et al., 2009; Rojas Vega et al., 2006; Skriver et al., 2014; Winter et al., 2007) and are associated with LTP in both the hippocampus (Korte et al., 1995) and the motor system (He, Zhang, Yung, Zhu, & Wang, 2013). However, interpretation of peripheral values of BDNF calls for caution (Knaepen, Goekint, Heyman, & Meeusen, 2010), and a recent study did not find increases of BDNF after exercise, nor was there a correlation between BDNF levels and acute exercise-induced neuroplasticity (McDonnell et al., 2013). In summary, the mechanisms with which exercise induces neuroplasticity are unclear and need to be studied more systematically in the future.

Some limitations to this work should be mentioned. First, because of concerns over session length, we opted not to measure intracortical facilitation or interhemispheric inhibition, both of which have been shown to change after acute

exercise in healthy individuals (Neva et al., 2017; Singh, Duncan, et al., 2014). Whether or not our priming paradigm affects these measures should be investigated. Also, it is important to note that we do not know whether the positive motor cortical excitability changes we found would be associated with gains in motor performance or learning. Nepveu et al. (2017) did find better retention of a time-on-target upper extremity motor task after high-intensity interval training in people with stroke, but other work using a different learning paradigm suggests this effect may be very task-specific (Charalambous, Alcantara, et al., 2018). It remains to be investigated whether our exercise priming protocol can enhance motor performance or learning in people with stroke.

In conclusion, we found that just 5 minutes of high-intensity lower extremity exercise in people with stroke significantly increases motor cortical excitability within the lesioned hemisphere as measured by resting MEP amplitudes. Future studies should investigate whether this priming effect in the brain can be utilized in therapeutic interventions to improve motor recovery post- stroke.

Chapter 3

EFFECTS OF CHRONIC ANTIDEPRESSANT USE ON NEUROPHYSIOLOGICAL RESPONSES TO TDCS POST-STROKE

Abstract

Background: Transcranial direct current stimulation (tDCS) induces neuroplastic changes in the motor cortex of healthy individuals and has become a candidate intervention to promote recovery post-stroke. However, neurophysiological effects of tDCS in stroke are poorly understood. Antidepressants change cortical excitability in both healthy and stroke-affected individuals, and alter the effects of tDCS in healthy individuals. Whether antidepressants alter effects of tDCS in stroke is unknown.

Objective/Hypotheses: To examine the effects of chronic antidepressant use, tDCS, and their interaction on motor cortical excitability in chronic stroke. Based on literature from healthy adults, we hypothesized: (a) Baseline motor cortical excitability would be higher in antidepressant-takers; (b) Anodal tDCS over the lesioned hemisphere primary motor cortex (M1) would increase excitability in the lesioned hemisphere M1 and decrease excitability in the non-lesioned hemisphere M1; (c) Effects of anodal tDCS would be enhanced in antidepressant-takers.

Methods: Twenty-six participants with chronic stroke (17 control, 9 antidepressant) received real and sham anodal tDCS during separate sessions at least a week apart. Motor cortical excitability was measured before and after tDCS. We

compared baseline cortical excitability and neurophysiological responses to tDCS between groups.

Results: Non-lesioned hemisphere baseline active motor thresholds were lower in antidepressant-takers than controls. Following anodal tDCS over the lesioned hemisphere M1, non-lesioned hemisphere excitability decreased in controls, but increased in antidepressant-takers.

Conclusions: Chronic antidepressant use affects motor cortical excitability in people with stroke and, importantly, reverses some effects of tDCS. Future utilization of tDCS in post-stroke neurorehabilitation should take medication status into account.

Introduction

Despite receiving standard rehabilitation treatments, the majority of stroke survivors have persistent motor function deficits in the upper extremity (Hatem et al., 2016; Kelly-Hayes et al., 2003), which lead to poorer quality of life (Joo et al., 2014; Pulman & Buckley, 2013). One underlying mechanism that contributes to these deficits is the motor cortical excitability changes within the primary motor cortex (M1) after stroke, which can be measured with transcranial magnetic stimulation (TMS). After stroke affecting the corticospinal tract, motor evoked potential (MEP) amplitudes from the lesioned hemisphere are decreased compared to the non-lesioned hemisphere (C. M. Stinear et al., 2015; Traversa et al., 1998), and to healthy controls (Traversa et al., 1998). This decrease is associated with poor motor function recovery (Butler & Wolf, 2007; Dimyan & Cohen, 2010). Furthermore, persistent abnormal increases of MEP amplitudes in the non-lesioned hemisphere are associated with persistent decreases of MEP amplitudes in the lesioned hemisphere (Traversa et al., 1998), suggesting an imbalance of interhemispheric excitability, which may be

associated with poor motor recovery (Harris-Love et al., 2016; Takeuchi & Izumi, 2012). Indeed, interventions that increase excitability within the lesioned hemisphere M1, and those that decrease excitability within the non-lesioned hemisphere M1 can both improve motor performance of the paretic arm (Fregni et al., 2005).

Transcranial direct current stimulation (tDCS) is an intervention that can change motor cortical excitability in healthy individuals (N. Lang et al., 2004; Nitsche & Paulus, 2000, 2001). Specifically, anodal stimulation over the M1 increases MEP amplitudes of the stimulated hemisphere (Furubayashi et al., 2008; Jeffery et al., 2007; Nitsche & Paulus, 2000), and cathodal stimulation over the M1 decreases MEP amplitudes (Ardolino et al., 2005; Furubayashi et al., 2008; Nitsche & Paulus, 2000). Presumably through transcallosal pathways, tDCS can also affect the excitability of the unstimulated hemisphere (Davidson et al., 2016). Thus, use of tDCS to enhance motor recovery post-stroke has become an area of interest, but the effects are not consistent (Cunningham et al., 2015; Di Lazzaro et al., 2014; Elsner et al., 2013; Fleming et al., 2017; Horvath et al., 2014; Hummel et al., 2005; Lindenberg et al., 2010). In stroke studies, tDCS is often applied during a motor task (Boggio et al., 2007; Fleming et al., 2017; Fregni et al., 2005; Goodwill et al., 2016; Hummel et al., 2005; Lefebvre et al., 2013; Lindenberg et al., 2010; Tahtis et al., 2014; S. Tanaka et al., 2011; Zimerman et al., 2012). When applied this way, anodal tDCS over the lesioned hemisphere M1 can improve motor performance of the paretic hand (Fregni et al., 2005; Hummel et al., 2005), and the performance improvement is positively correlated with increases of motor cortical excitability in the lesioned hemisphere M1 (Hummel et al., 2005). However, responses to tDCS in stroke are highly variable, and not all studies show positive effects (Cunningham et al., 2015; Elsner et al., 2013;

Hesse et al., 2011). The heterogeneity of stroke survivors may be the reason for these inconsistencies (Cunningham et al., 2015; Elsner et al., 2013; Horvath et al., 2014). It is possible that stroke survivors may have different neurophysiological responses to tDCS because of neurophysiological and neurochemical changes in the brain.

A major factor that may contribute to the heterogeneity of results of tDCS in stroke is the use of medications. The use of antidepressant medications is of particular interest because these drugs are prescribed in up to 30% of stroke survivors (El Husseini et al., 2012; Eriksson et al., 2004; Paolucci, 2008; Ried et al., 2006). Likewise, recent investigations in healthy adults indicate that selective serotonin-reuptake inhibitors (SSRIs), the most common class of antidepressant medication used (IMS Health (Firm), n.d.), affects motor cortical excitability and responsiveness to tDCS (Gerdelat-Mas et al., 2005; Ilic et al., 2002; Kuo et al., 2016; Nitsche et al., 2009; Robol, Fiaschi, & Manganotti, 2004). Therefore, understanding how these medications affect motor cortical excitability, and how they may interact with tDCS are likely important considerations for using tDCS in post-stroke neurorehabilitation. Yet to date, antidepressant medication use has been widely ignored in post-stroke rehabilitation studies involving tDCS (Allman et al., 2016; Boggio et al., 2007; Fleming et al., 2017; Fregni et al., 2005; Hesse et al., 2011; McCambridge et al., 2018; van der Vliet et al., 2017).

In healthy adults, a single dose of an SSRI increases MEP amplitudes but decreases intracortical facilitation (ICF) (Ilic et al., 2002). However, when SSRIs are ingested chronically, as the way it would be used clinically, the effects are reversed (Gerdelat-Mas et al., 2005). In patients with acute stroke, one month of SSRI ingestion increases intracortical inhibition (ICI) and motor thresholds in the non-lesioned

hemisphere compared to placebo medication, but does not appear to affect MEP amplitudes (Acler et al., 2009). These studies suggest that SSRIs may have significant effects on motor cortical excitability in chronic stroke survivors, but this has not been tested. Moreover, SSRIs have been shown to interact with tDCS in healthy individuals. Both acute and chronic application of SSRIs in healthy individuals facilitate the effects of anodal tDCS and reverse the inhibitory effects of cathodal tDCS into facilitation (Kuo et al., 2016; Nitsche et al., 2009). Whether the same effects exist in stroke survivors is unknown.

The purpose of this study was to examine the effects of chronic SSRI or selective serotonin-norepinephrine reuptake inhibitor (SNRI) use on motor cortical excitability and to examine its effects on responsiveness to anodal tDCS in chronic stroke. First, based on the prior work in healthy individuals (Gerdelat-Mas et al., 2005; Ilic et al., 2002), we hypothesized that baseline motor cortical excitability would be increased bilaterally in stroke survivors taking antidepressants, compared to those not taking antidepressants (H2.1). Second, we hypothesized that when applied during rest, anodal tDCS over the lesioned hemisphere M1 would increase excitability of the lesioned hemisphere M1 and decrease excitability of the non-lesioned hemisphere M1 in chronic stroke survivors (H2.2). Last, based on the results from healthy subjects (Kuo et al., 2016; Nitsche et al., 2009), we hypothesized that when applied during rest, the effects of anodal tDCS over the lesioned hemisphere M1 on motor cortical excitability would be enhanced in stroke survivors taking antidepressants, compared to those not taking antidepressants (H2.3).

Materials and Methods

Participants

Twenty-six (4 female, mean ± SD, 65.46 ± 8.06 years old) participants with chronic (> 6 months), unilateral stroke affecting the upper extremity (Fugl-Meyer Assessment (FMA) < 66) participated in this study. They all underwent a sensorimotor evaluation to determine their functional status, and brain MRI or CT scan reports were obtained to verify stroke type, lesion location and damage to the corticospinal tract. Participants were assigned to either the control group (n = 17) or the antidepressant group (n = 9) according to their regularly taken medications. Individuals in the antidepressant group must have been taking a single SSRI or SNRI, and must have been on the same drug and dosage for at least 3 months at the time of testing. Exclusion criteria included multiple strokes affecting both hemispheres, any cerebellar lesion, or any other significant neurological, cardiovascular or musculoskeletal condition besides stroke. Individuals with contraindications to TMS or tDCS were also excluded, as well as participants taking or recently withdrawing from (< 12 weeks) any medications affecting the central nervous system (except SSRIs or SNRIs). Individuals taking or withdrawing from any antidepressant medications were excluded from the control group. The experimental protocol was approved by the University of Delaware Institutional Review Board and all participants gave written informed consent. Participant demographic information and clinical information are provided in Table 3 and Table 4, respectively.

Table 3 Participant demographic information[3].

Control Group						
PID	**Gender**	**Age (yrs)**	**Time Since Stroke (m)**	**Side of Stroke**	**Stroke Type**	**Stroke Location**
01	M	74	9	R	I	S
02	M	66	29	R	I	S
03	F	63	31	R	I	S
04	F	68	45	R	I	S+C
05	M	76	34	R	I	S
06	M	51	125	L	I	S+C
07	M	73	10	L	I	S
08	M	55	45	R	I	C
09	M	68	9	R	I	S
10	M	77	24	R	I	S
11	M	65	25	R	I	S
12	M	70	7	L	I	S
13	M	73	30	R	I	S+C
14	M	64	72	R	I	S
15	M	47	25	R	I	S
16	M	57	50	L	I	S
17	F	69	17	L	I	S
mean ± SD		**65.65 ± 8.72**	**34.53 ± 28.91**			
Antidepressant Group						
PID	**Gender**	**Age (yrs)**	**Time Since Stroke (m)**	**Side of Stroke**	**Stroke Type**	**Stroke Location**
18	M	79	64	L	I	S
19	M	63	31	R	H	S
20	M	58	22	R	I	S+C
21	F	61	29	R	I+H	S
22	M	69	66	L	H	S
23	M	69	133	R	I+H	S
24	M	55	32	R	I	S

[3] Abbreviations: PID = participant identifier; SD = standard deviation; $p = p$ value comparing control vs. antidepressant groups; M = male; F = female; yrs = years; m = months; R = right; L = left; I = ischemic; H = hemorrhagic; S = subcortical; C = cortical. #Fisher's exact test; all others Mann-Whitney U tests. *Statistically significant between control and antidepressant groups at $p < 0.05$.

Table 3 continued

25	M	64	11	R	I	S+C
26	M	68	10	R	H	S
mean ± SD		**65.11 ± 7.13**	**44.22 ± 38.85**			
p	1.000#	0.634	0.491	1.000#	0.002#*	1.000#

Table 4 Participant clinical information[4].

Control Group					
PID	**UE-FMA**	**GDS**	**HADS-A**	**HADS-D**	**AS**
01	32	1	1	2	2
02	33	0	0	0	2
03	60	0	0	1	4
04	60	0	0	0	7
05	59	1	2	2	7
06	12	5	7	3	5
07	63	6	6	5	24
08	10	0	7	5	5
09	58	1	0	3	7
10	27	2	4	3	5
11	61	1	3	1	9
12	61	0	2	1	8
13	59	1	3	1	7
14	21	1	0	2	6
15	49	6	5	7	8
16	52	2	1	2	7
17	60	3	2	4	3
mean±SD	**45.71 ± 18.80**	**1.76 ± 2.05**	**2.53 ± 2.48**	**2.47 ± 1.91**	**6.82 ± 4.89**
Antidepressant Group					
PID	**UE-FMA**	**GDS**	**HADS-A**	**HADS-D**	**AS**
18	8	3	0	4	5
19	9	2	3	5	12
20	15	9	5	9	13
21	59	3	3	5	19
22	47	2	1	4	11
23	14	2	1	2	7
24	60	3	6	2	4

[4] Abbreviations: PID = participant identifier; SD = standard deviation; $p = p$ value comparing control vs. antidepressant groups; UE-FMA = upper extremity Fugl-Meyer Assessment; GDS = geriatric depression score; HADS-A = anxiety score of the Hospital Anxiety and Depression Scale; HADS-D = depression score of the Hospital Anxiety and Depression Scale; AS = apathy score. *Statistically significant between control and antidepressant groups at $p < 0.05$, Mann-Whitney U tests for all variables.

Table 4 continued

25	60	6	9	10	28
26	64	5	7	9	18
mean ± SD	**37.33 ± 25.02**	**3.89 ± 2.37**	**3.89 ± 3.06**	**5.56 ± 3.05**	**13.00 ± 7.68**
p	0.458	0.009*	0.263	0.009*	0.029*

General Paradigm

Participants completed three testing sessions over two weeks. During the first session, the upper extremity FMA was assessed for all participants and medication information was collected to verify eligibility and group assignment. To better understand our participants, the Geriatric Depression Survey (GDS) and the Hospital Anxiety and Depression Scale (HADS) were used to assess depression and anxiety levels, and the Apathy Scale (AS) was used to assess apathy. Session 1 was also used to familiarize subjects with the TMS and electromyography (EMG) setup for future sessions. Sessions 2 and 3 were scheduled at least a week apart to avoid carry-over effects. Participants received real tDCS in one session and sham tDCS in the other session. The order of real and sham tDCS sessions were counterbalanced, and participants were blinded to the treatment. Motor cortical excitability of the extensor carpi radialis (ECR) muscles was measured bilaterally with TMS before and immediately after real or sham tDCS.

Data Collection

Transcranial Magnetic Stimulation

The TMS-related procedures were described in Chapter 2. Participants sat in a comfortable chair with arms relaxed. A 70 mm diameter figure-of-eight coil was used

in conjunction with a Magstim 200^2 electromagnetic stimulation unit (Magstim, Ltd., Wales, UK) for all TMS measures. Signal 6.03 software (Cambridge Electronic Design, Ltd., Cambridge, UK) was used to control and trigger the magnetic stimulator through a 16-bit data acquisition unit (Micro 1401-3, Cambridge Electronic Design, Ltd., Cambridge, UK), and to record and store EMG data for offline analysis.

The vertex of the skull was identified and marked on the scalp. With the coil held tangential to the scalp and the handle pointing backwards at a 45° angle to the mid-sagittal line (Brasil-Neto et al., 1992; Kammer et al., 2001; Mills et al., 1992), the "hot spot" for the ECR muscle was found and carefully marked directly on the scalp. If no MEPs could be elicited from the lesioned hemisphere at 100% of the maximum stimulator output (MSO), we used a symmetrical "hot spot" to the non-lesioned hemisphere. Resting motor threshold (RMT) was defined as the lowest stimulus intensity that produced at least 5 out of 10 MEPs in the contralateral ECR muscle with a peak-to-peak amplitude over 50 μV (Groppa et al., 2012). Ten MEPs were collected from each hemisphere at 120% RMT before and after real or sham tDCS.

Active motor thresholds (AMTs) were determined while participants were holding a light, isometric contraction of the ECR muscle. The contraction level for each subject and arm was either 3%, 5%, or 10% of their individual maximal voluntary isometric contraction (MVIC) to ensure MEPs could be discerned over the background EMG. Importantly, however, the contraction level for each subject and arm was held consistent throughout the entire study. Likewise, across groups, average MVIC EMG (means ± SDs, non-paretic, control, 2.35 ± 0.94 mV, antidepressant, 2.36 ± 0.95 mV; paretic, control, 1.08 ± 0.67 mV, antidepressant, 1.59 ± 1.05 mV) and percent MVIC (non-paretic, control, 5.35 ± 1.87%, antidepressant, 6.22 ± 2.95%;

paretic, control, 9.33 ± 1.76%, antidepressant, 7.60 ± 3.36%) used were not different (all $p > 0.34$). AMT was defined as the minimum stimulus intensity that could elicit at least 5 out of 10 MEPs in the contralateral contracting ECR muscle, where the MEP exceeded 200 μV (Groppa et al., 2012), and was followed by a silent period. Fifteen active MEPs were collected from each hemisphere at 120% AMT before and after real or sham tDCS.

Electromyography

EMG was collected using a 10-channel system with double differential surface electrodes with an integrated ground (Motion Lab Systems, Inc., Baton Rouge, LA). The skin over the ECR muscles were cleaned with alcohol and electrodes were placed over the ECR muscle bellies bilaterally, longitudinally following the direction of the muscle fibers. A ground electrode was placed over the lateral epicondyle of the humerus. EMG data were collected with a sampling rate of 5000 Hz and online low-pass filtered at 2000 Hz.

Transcranial Direct Current Stimulation

tDCS was applied during quiet sitting using a battery-powered direct current stimulator (Chattanooga by DJO Global, Inc., Vista, CA) with two square saline-soaked sponge electrodes (effective area 25 cm^2) enclosed in rubber casings (AMREX Electrotherapy, Paramount, CA). The anode was centered over the lesioned hemisphere "hot spot" for the ECR, determined by TMS, and the cathode was placed over the contralateral supraorbital area. For real tDCS, the current was set at 1 mA and delivered for 10 minutes. The current ramped up and ramped down over 30 s at the beginning and the end of stimulation, respectively. For sham tDCS, the current was set

at 1 mA for 1 minute, with the same ramping schedule. This method creates an effective form of subject blinding, as there are no differences in sensation or level of comfort between real and sham tDCS groups (Gandiga, Hummel, & Cohen, 2006) and yet no effective stimulation is delivered during the sham condition.

Data Analysis

The data analysis procedures were described in Chapter 2. EMG data were recorded in Signal (Cambridge Electronic Design, Ltd., Cambridge, UK) and analyzed with custom written software in MATLAB (MathWorks, Inc., Natick, MA). All raw EMG data were demeaned, and any gain removed. For resting MEP trials, EMG was notch-filtered at 60 Hz with a 2nd order Butterworth filter to remove electrical noise, and trials with baseline (10 - 60 ms before stimulus artifact) peak-to-peak EMG exceeding 30 μV were discarded, as this could indicate the participant was not at rest. Peak-to-peak MEP amplitudes were calculated for each trial and averaged over all trials. For active MEP trials, average rectified EMG during baseline (50 - 150 ms before stimulus artifact) of each trial was evaluated to ensure the correct contraction level (within ± 5% for 10% MVIC contraction, within ± 3% for 5% MVIC contraction, within ± 1% for 3% MVIC contraction). Trials that did not meet the criteria were discarded. Peak-to-peak MEP amplitudes were calculated for each valid trial and averaged over all trials. In these active trials, we also measured CSP durations. CSP onset was defined as the first time point when the rectified EMG fell below, and stayed below, the average rectified baseline EMG of that trial for at least 10 ms; CSP offset was defined as the first time point after CSP onset when the rectified EMG was at or above the average rectified baseline EMG, and the average rectified EMG during the following 25 ms was also at or above the averaged rectified

baseline EMG. CSP duration was defined as the time from the stimulus artifact to CSP offset. CSP duration was calculated for each trial and averaged over all trials. For all TMS outcomes, post-tDCS measures were normalized to pre-tDCS (post-to-pre ratio) to more fairly compare participants with varying degrees of impairment.

Statistical Analysis

Statistical analyses were performed in IBM SPSS Statistics 24 (IBM Corp., Armonk, NY). Because of the small sample size of the antidepressant group, and many variables were not normally distributed, non-parametric tests were used for all statistical comparisons. Mann-Whitney U tests were used to compare baseline motor cortical excitability (RMTs, AMTs, resting MEP and active MEP amplitudes, CSP durations) between control and antidepressant groups (H2.1). For testing of H2.2 and H2.3, normalized (post/pre) TMS measures (resting and active MEP amplitudes, CSP durations) were used. Because we used non-parametric statistics, we completed separate analyses to test for between groups (control vs. antidepressant) versus between conditions (real vs. sham tDCS) effects. To test the effects of tDCS on motor cortical excitability (H2.2), Wilcoxon signed-rank tests were used separately in control and antidepressant groups to compare normalized motor cortical excitability between real and sham tDCS sessions. To test whether real tDCS had differential effects on chronic antidepressant takers (H2.3), Mann-Whitney U tests were used to compare normalized motor cortical excitability measurements in the real tDCS session between control and antidepressant groups. Finally, to make sure the two groups weren't different in sham tDCS sessions, Mann-Whitney U tests were used to compare normalized motor cortical excitability measurements in the sham tDCS session

between control and antidepressant groups. Demographic and clinical characteristics between groups were compared using Fisher's exact tests or Mann-Whitney U tests.

Results

All subjects completed the experiment with no adverse events. All data are reported in mean ± SEM unless otherwise stated. More participants in the antidepressant group had hemorrhagic strokes compared to the control group (p = 0.002). All other demographic characteristics were not significantly different between groups (all $p > 0.49$), see Table 3. Participants were not different between groups in upper extremity motor function as measured by the FMA ($p = 0.46$). However, participants in the antidepressant group showed significantly higher scores on depression scales than the control group based on both the GDS ($p = 0.009$) and the depression score of the HADS ($p = 0.009$), even though they were taking antidepressants. They were also significantly more apathetic compared to the control group ($p = 0.029$). Anxiety levels were not different between groups based on the anxiety score of the HADS ($p = 0.26$), see Table 4.

In the lesioned hemisphere, we were able to obtain RMTs and resting MEPs in 8 participants (47.1%) in the control and 4 participants (44.4%) in the antidepressant group. The remainder had no MEPs at 100 %MSO. For AMTs, active MEPs and CSPs, we were able to obtain data from 11 participants (64.7%) in the control group and 5 participants (55.6%) in the antidepressant group. In the non-lesioned hemisphere, we excluded resting MEPs after real tDCS in one participant because baseline EMG activity was too high (> 30 μV) in all trials. We were able to obtain complete data sets for all other measures from the non-lesioned hemisphere.

Baseline Motor Cortical Excitability in Control vs. Antidepressant Groups (H2.1)

Baseline excitability was obtained from the pre-tDCS TMS measures of all participants during session 3. AMTs in the non-lesioned hemisphere of the antidepressant group (32.33 ± 1.47 %MSO) were significantly lower than that of the control group (37.18 ± 1.45 %MSO), $U = 38.00$, $p = 0.039$, suggesting that participants taking antidepressants may have greater baseline cortical excitability within the non-lesioned hemisphere compared to those not taking antidepressants (see Figure 5). However, all other motor cortical excitability measurements, including AMT of the lesioned hemisphere (Figure 5), bilateral RMTs, resting and active MEP amplitudes, and CSP durations were not different at baseline between groups (all, $p > 0.07$).

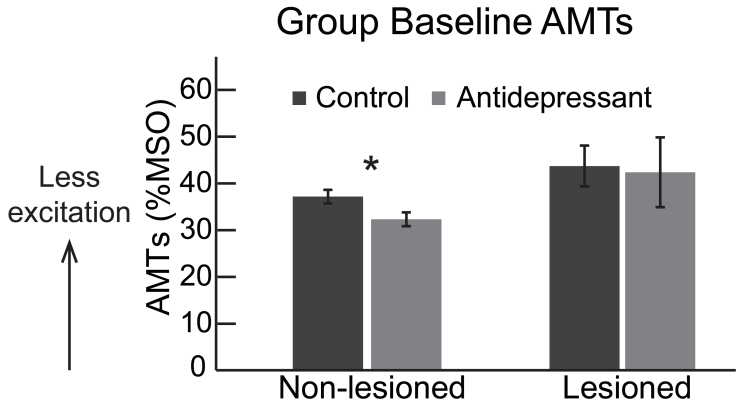

Figure 5 Group mean baseline AMTs in the non-lesioned hemisphere M1 (left) and in the lesioned hemisphere M1 (right). The antidepressant group is shown in light gray bars; the control group is in dark gray bars. Error bars, ± 1 SEM. *Significantly different between control and antidepressant groups, $p < 0.05$.

Effects of tDCS on Motor Cortical Excitability in Stroke (H2.2)

In the control group, normalized resting MEPs from the lesioned hemisphere were not different between real (1.03 ± 0.18) and sham tDCS (1.16 ± 0.18) sessions, $Z = 0.338$, $p = 0.735$. This was somewhat unexpected because we had predicted real anodal tDCS to the lesioned hemisphere M1 to increase resting MEPs from the stimulated, lesioned hemisphere. However, as expected, normalized resting MEPs from the unstimulated, non-lesioned hemisphere were relatively smaller in the real tDCS session (1.00 ± 0.10), compared to sham tDCS (1.27 ± 0.16), $Z = 2.06$, $p = 0.039$ (see Figure 6). Normalized active MEPs and CSP durations were not significantly different between real and sham tDCS sessions (all $p > 0.074$).

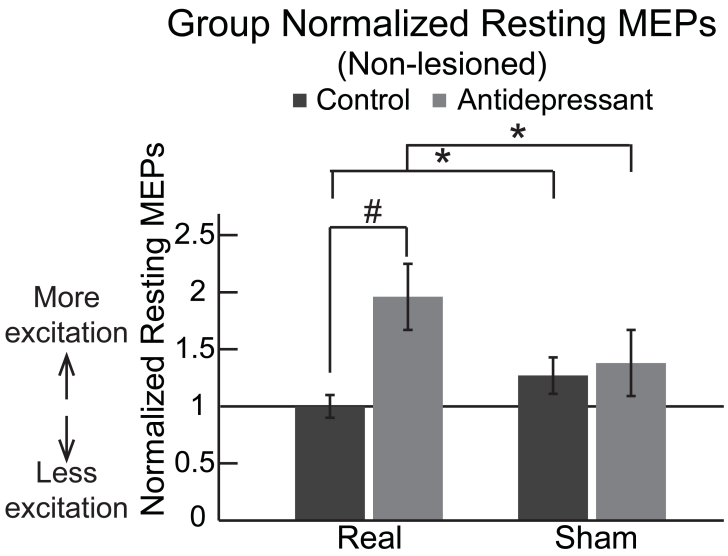

Figure 6 Group mean normalized (ratio, post-to-pre tDCS values) resting MEP amplitudes from the non-lesioned hemisphere M1. Responses to real (left) and sham (right) tDCS are shown. The antidepressant group is shown in light gray bars; the control group is in dark gray bars. Error bars, ± 1 SEM. *Significantly different between real and sham tDCS in either control or antidepressant group, $p < 0.05$. #Significantly different between control and antidepressant groups after real tDCS.

In the antidepressant group, again, normalized resting MEPs from the lesioned hemisphere were not significantly different between real (1.08 ± 0.40) and sham tDCS (1.39 ± 0.40) sessions, $Z = 0.73$, $p = 0.47$. However, normalized resting MEPs from the non-lesioned hemisphere were significantly larger after real tDCS (1.96 ± 0.30) compared to sham tDCS (1.38 ± 0.28), $Z = -2.38$, $p = 0.017$ (see Figure 6). All other motor cortical excitability measurements were not significantly different between real and sham tDCS sessions (all $p > 0.13$).

Differential Effects of Real tDCS in Control and Antidepressant Groups (H2.3)

In Figure 7, we show individual data of normalized resting MEPs from the non-lesioned hemisphere in the real tDCS session in control (Figure 7A) and antidepressant (Figure 7B) groups. Most participants in the control group, but no participants in the antidepressant group, had decreased MEPs from the non-lesioned hemisphere after real anodal tDCS was applied over the lesioned hemisphere. This observation was reflected in the group data, showing that normalized resting MEPs from the non-lesioned hemisphere were significantly higher in the antidepressant group (1.96 ± 0.30) compared to the control group (1.00 ± 0.10), $U = 122.00$, $p = 0.001$ (Figure 6). The fact that non-lesioned hemisphere MEPs were elevated after tDCS in antidepressant-takers post-stroke is surprising and suggests a reversal of the typical effects of anodal tDCS that may be caused by the medication. In terms of raw MEP values, control group resting MEP amplitudes from the non-lesioned hemisphere were 0.40 ± 0.07 mV before real tDCS and 0.37 ± 0.06 mV after tDCS; in the antidepressant group, they were 0.23 ± 0.04 mV before real tDCS and 0.38 ± 0.03 mV after tDCS. All other measures in the real tDCS session, including normalized resting MEPs from the lesioned hemisphere, bilateral normalized active MEPs and CSP

durations, were not significantly different between groups (all $p > 0.11$). All measurements in the sham tDCS session were also not significantly different between groups (all $p > 0.19$).

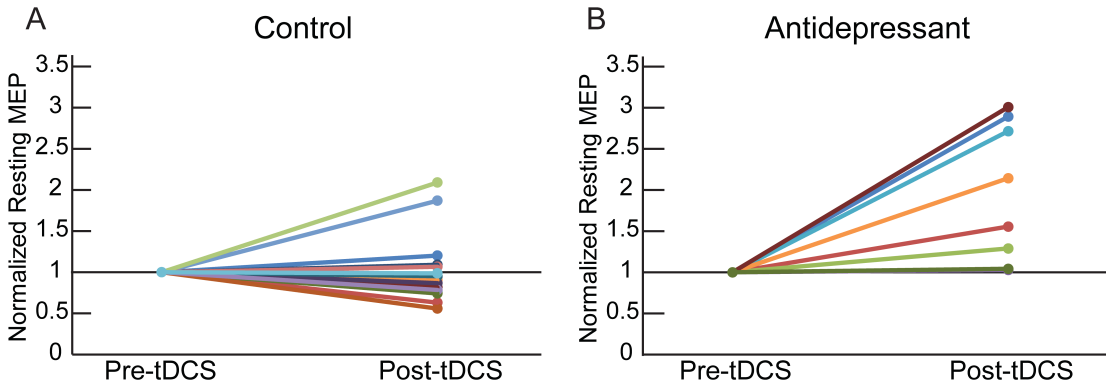

Figure 7 Individual normalized resting MEP amplitudes from the non-lesioned hemisphere M1 before and after real tDCS; each line represents data from one individual. In the control group (A), most participants had decreased resting MEPs (values < 1) after real tDCS. In the antidepressant group (B), most participants had increased MEPs (values > 1) after tDCS, and no participant had decreased MEPs.

Discussion

In our study of chronic stroke participants, we found that baseline AMT of the non-lesioned hemisphere was lower in participants chronically taking antidepressants, compared to those not taking antidepressants, suggesting greater baseline motor cortical excitability in the antidepressant group. When anodal tDCS was applied to the lesioned hemisphere M1, motor cortical excitability of the lesioned hemisphere M1 did not change in either control or antidepressant group. However, resting MEPs from the unstimulated, non-lesioned hemisphere M1 changed in both groups, but in opposite directions. In the control group, normalized resting MEPs (non-lesioned)

after real tDCS were smaller than that after sham tDCS; in the antidepressant group, normalized resting MEPs after real tDCS were much larger compared to sham tDCS. Moreover, normalized resting MEPs from the non-lesioned hemisphere after real tDCS were much larger in the antidepressant group than the control group, while the same measure after sham tDCS was not different between groups, suggesting antidepressants affected responses to tDCS.

Among all motor cortical excitability measures at baseline, chronic intake of antidepressants in stroke survivors only affected AMT in the non-lesioned hemisphere. This is in contrast with some studies in healthy individuals. An acute dose of sertraline (an SSRI) increased MEPs, and decreased ICF in healthy individuals, but motor thresholds were unchanged (Ilic et al., 2002). The same results were replicated in a small sample of healthy participants taking paroxetine (another SSRI) (Gerdelat-Mas et al., 2005). However, acute intake of another SSRI, citalopram, increased RMT in healthy individuals, along with increases of CSP durations and ICI, but with no effects on MEPs or ICF (Robol et al., 2004). The differences in these results may be explained by the fact that each specific SSRI may work on unique pathways specific to it (Sanchez, Reines, & Montgomery, 2014). We did not control which antidepressants our participants were taking, except that it must be a single SSRI or SNRI. Therefore, the heterogeneity of the medications may have limited our ability to detect subtle differences in motor cortical excitability caused by a specific medication. However, as the first study of its kind in chronic stroke, our finding at least indicates that antidepressants can indeed affect motor cortical excitability in stroke. Besides stroke, chronic vs. acute intake of antidepressants is another factor to consider when understanding these results. In healthy individuals, chronic paroxetine induced

opposite effects on motor cortical excitability than an acute dose (Gerdelat-Mas et al., 2005). This is thought to stem from additional changes in the brain due to chronic medication, such as desensitization and downregulation of receptors (Maj et al., 1996; Pineyro, Blier, Dennis, & de Montigny, 1994). In a study of acute stroke, chronic administration of citalopram increased ICI and RMT in the non-lesioned hemisphere only (Acler et al., 2009). Although the measures affected by antidepressants are different, it is interesting that the differences we found were also restricted to the non-lesioned hemisphere. Perhaps the relatively undamaged non-lesioned hemisphere is more sensitive to changes induced by chronic antidepressants than the lesioned hemisphere. AMTs are thought to reflect pyramidal axonal excitability, which is dependent on voltage-gated sodium channels (Paulus et al., 2008; Ziemann, Lönnecker, Steinhoff, & Paulus, 1996). Serotonin can affect brain areas through multiple mechanisms and pathways (Ciranna, 2006; Lesch & Waider, 2012), including an enhancement of inward sodium current in the neocortex (Aghajanian & Marek, 1997), which may explain the lower AMT in our antidepressant group.

We found that in our cohort of participants with chronic stroke, anodal tDCS over the lesioned hemisphere M1 did not induce any changes in motor cortical excitability in the stimulated, lesioned hemisphere. This is in contrast to studies in healthy individuals where upregulation of the stimulated motor cortex after anodal stimulation has been found relatively consistently (Furubayashi et al., 2008; Jeffery et al., 2007; Nitsche et al., 2008; Nitsche & Paulus, 2000, 2011). In stroke, although only two other studies have examined the effects of tDCS on motor cortical excitability *when participants were not performing a motor task*, both found increased motor cortical excitability in the lesioned hemisphere after anodal tDCS over the lesioned

hemisphere M1 (Edwards et al., 2009; Suzuki et al., 2012). In our study, we had a larger number of participants, and we did not limit the functional levels of our participants (compare Suzuki et al.), which may have increased the heterogeneity of responses to tDCS in our study. It would not be surprising to find that individuals with more severe deficits and larger brain lesions due to stroke would show somewhat less responsiveness to cortical stimulation via tDCS. Surprisingly, changes in the non-lesioned hemisphere, which were not tested in previous studies, showed changes after tDCS was applied to the lesioned hemisphere. In the control group, normalized resting MEPs from the non-lesioned hemisphere were smaller after real tDCS compared to sham tDCS. The direction of this change is consistent with our hypothesis, which stated that the non-lesioned hemisphere would show decreased excitability after anodal tDCS to the lesioned hemisphere, due to increased interhemispheric inhibition from the lesioned to the non-lesioned hemisphere. However, the normalized resting MEPs (non-lesioned) after real tDCS were close to 1, which indicates that no actual change in MEP amplitudes occurred. The statistical difference emerged because resting MEPs increased after sham tDCS (normalized 1.27 ± 0.68). Because the same changes occurred after sham tDCS in the antidepressant group, we postulate that this change may be related to a generalized effect of light exercise from muscle contractions during active TMS or an effect of time. Thus the relative decrease of motor cortical excitability in the non-lesioned hemisphere may reflect tDCS induced changes through transcallosal connections.

In the antidepressant group, normalized resting MEPs from the non-lesioned hemisphere after real tDCS were significantly larger compared to the control group and to sham tDCS, while sham tDCS did not induce differential effects between

groups. This indicates that the increased MEPs after real tDCS were likely a result of the antidepressant factor. This is an intriguing result because we had expected antidepressants to enhance the effects of tDCS, as reported in healthy individuals (Kuo et al., 2016; Nitsche et al., 2009). Instead, the direction of the change is opposite of the control group. In the only previous study examining motor cortical excitability changes due to chronic antidepressant intake in stroke, Acler et al. (2009) reported that chronic antidepressants increased ICI in the non-lesioned hemisphere in acute stroke, but MEP amplitudes did not change. Unfortunately, due to concerns for time, we did not test ICI in our study. However, if we assume ICI in the non-lesioned hemisphere was increased in our antidepressant group as well, it is possible that anodal tDCS over the lesioned hemisphere increases excitability of the transcallosal fibers within the lesioned hemisphere (Davidson et al., 2016) that have a net inhibitory effect, and this may have reduced ICI in the contralateral, non-lesioned hemisphere (Daskalakis, Christensen, Fitzgerald, Roshan, & Chen, 2002). The overall effect would thus be an increase in corticospinal output due to decreased inhibitory activity, which would be reflected through increased MEP amplitudes from the non-lesioned hemisphere. Of course, this possibility is highly speculative at this point and would require extensive neurophysiological and neuropharmacological testing. Regardless of mechanism, the overall finding of reversed effects of lesioned hemisphere M1 anodal tDCS in the non-lesioned hemisphere M1 of chronic antidepressant-takers is highly relevant. Given that antidepressant intake is rarely considered in clinical intervention studies of anodal tDCS for post-stroke recovery, this could explain some of the mixed findings and negative results in these studies.

Limitations to our study must be considered. Although we limited inclusion of the antidepressant group to those only taking one SSRI or SNRI, individual medications can have unique mechanisms of action (Sanchez et al., 2014), which may affect motor cortical excitability and/or responses to tDCS differently. Future studies should examine and compare each medication individually. Second, due to time restraints, we did not test all TMS measures available. Certain other measures, such as ICI, ICF and interhemispheric inhibition, reflect changes in other cortical pathways, and may provide mechanistic evidence for our results. Third, due to increased depression and apathy in our antidepressant group, we cannot rule out that the effects seen in our study were an effect of depression and/or apathy rather than the medication per se. However, in a preliminary study of depressed patients without stroke, responses to anodal tDCS were decreased compared to healthy controls (Palm et al., 2013), which was not found in our study of stroke survivors with depression. Finally, we do not know whether or how the neurophysiological changes are related to changes in motor performance or motor learning, which need to be investigated.

Conclusions

Our results show unexpected and complex changes of SSRI/SNRI usage on motor cortical excitability and responses to tDCS in chronic stroke survivors, which are different from those experienced by healthy individuals, and different from those experienced by stroke survivors not taking antidepressants. Thus the combination of a brain lesion due to stroke and the chronic ingestion of certain antidepressant drugs produces complex interactions in the brain that impact cortical excitability and responses to tDCS. Importantly, these interactions appear to be significant, with the potential to reverse some of the physiological effects of anodal tDCS. Therefore,

careful attention should be paid to antidepressant medication status in future studies utilizing tDCS post-stroke. Further, this and additional factors that can contribute to differential responses to tDCS in stroke need to be thoroughly tested before tDCS can be effectively used in the clinic.

Chapter 4

ANODAL TDCS OVER THE LESIONED MOTOR CORTEX DOES NOT AFFECT SPLIT-BELT TREADMILL LOCOMOTOR LEARNING POST-STROKE

Abstract

Locomotor adaptation enables human beings to adjust gait patterns in a changing environment. Stroke can negatively affect the rate of adaptation and retention, which limits the ability to relearn gait patterns in this population. Transcranial direct current stimulation (tDCS) can enhance motor adaptation and retention in healthy individuals, and enhance retention of some upper extremity learning tasks in people with stroke. The purpose of this study was to investigate whether anodal tDCS over the lesioned hemisphere primary motor cortex (M1) can enhance learning and retention of locomotor adaptation in people with chronic stroke. Because the M1 may be preferentially involved in retention of adaptation, we hypothesized that anodal tDCS over the lesioned hemisphere M1 would enhance retention. We tested our hypothesis by having participants with chronic stroke learn a new gait pattern on a split-belt treadmill, then retesting them on the same task 24 hours later. One group received anodal tDCS over the lesioned hemisphere M1 during learning on day 1, while the other group received sham tDCS. Changes in step length symmetry were used to assess learning and retention. We found that anodal tDCS did not affect the magnitude of learning on either day, nor did it affect retention compared to sham. We speculate that the effects of tDCS post-stroke may be task-specific.

Cognitive demand of the task, and the neural structures subserving learning and retention of the task may be associated with the efficacy of tDCS.

Introduction

Human beings can readily adjust motor behavior in accordance with predictable changes in the environment, for example, walking in high-heels or through deep snow. This happens through a process known as motor adaptation, which is a type of motor learning, defined as the short-term adjustment of motor commands through trial and error practice in response to changes in the environment (Martin et al., 1996b). The cerebellum is thought to play a major role in the adaptation process itself (C. E. Lang & Bastian, 1999; Martin et al., 1996a; Morton & Bastian, 2006), but the ability to retain what was learned is thought to rely more heavily on the primary motor cortex (M1) (Galea et al., 2011). After stroke affecting the corticospinal tract, the ability to adapt is preserved (Patton, Stoykov, Kovic, & Mussa-Ivaldi, 2006; Reisman et al., 2007), but, during locomotor adaptation, the rate is slowed and retention is poor (Savin et al., 2013; Tyrell et al., 2014). This is problematic because, as part of the rehabilitation and recovery process, stroke survivors have to relearn lost motor skills and be able to adapt them to different environments. Without proper retention, they would be unable to use newly learned walking patterns or make necessary adjustments of gait in a new environment.

Transcranial direct current stimulation (tDCS) can induce plasticity in the brain in a polarity-dependent fashion, where the anode increases excitability in the stimulated area (Furubayashi et al., 2008; Jeffery et al., 2007; Nitsche & Paulus, 2000), and the cathode decreases excitability in the stimulated area (Ardolino et al., 2005; Furubayashi et al., 2008; Nitsche & Paulus, 2000). More importantly, the effects

of tDCS can last up to one hour after stimulation (Nitsche et al., 2003; Nitsche & Paulus, 2001), which is important for potentially affecting consolidation of learning (Brashers-Krug et al., 1996). Because neuroplasticity is one of the processes underlying motor learning (Dayan & Cohen, 2011), tDCS may enhance motor learning by readily engaging these processes.

In healthy individuals, tDCS can enhance motor adaptation (Galea et al., 2011; Hardwick & Celnik, 2014; Jayaram et al., 2012). Anodal tDCS applied to the cerebellum accelerates the rate of initial adaptation in upper extremity (Galea et al., 2011) and locomotor adaptation tasks (Jayaram et al., 2012) in young healthy individuals, but does not affect retention of learning. However, when anodal tDCS is applied over the M1, it enhances retention of an upper extremity visuomotor rotation task (Galea et al., 2011). Healthy older adults have impaired motor adaptation compared to young healthy adults (Bock, 2005; Seidler, 2006). Anodal tDCS over the cerebellum in healthy older adults enhances the rate of adaptation in a visuomotor rotation task, so that it is comparable with young healthy individuals (Hardwick & Celnik, 2014). In another study, it was shown that anodal tDCS over the M1 during initial learning in healthy older adults enhances relearning (learning based on prior retention) of the visuomotor rotation 50 minutes later, so that performance at the end of relearning is comparable with young healthy adults (Panouillères et al., 2015). Anodal tDCS over the cerebellum does not produce the same effect. Overall, these results support the potential differential roles of the cerebellum and M1 in motor adaptation and retention, respectively, and the idea that tDCS directed to the appropriate brain region can improve adaptation or retention.

Although several studies have reported potential beneficial effects of tDCS on paretic arm or leg movements (Hesse et al., 2007; Hummel et al., 2005; Lindenberg et al., 2010; Madhavan et al., 2011; Tahtis et al., 2014; S. Tanaka et al., 2011), studies evaluating tDCS to improve motor *learning* specifically in people with stroke are limited and have been focused on the upper extremity. Bihemispheric tDCS (anode over the lesioned hemisphere M1, cathode over the non-lesioned hemisphere M1) increases online learning of a circuit tracing task and leads to markedly increased retention of the task one week later (Lefebvre et al., 2013). In well-recovered stroke survivors, cathodal tDCS over the non-lesioned hemisphere M1 improves retention of a finger motor sequence learning task in the paretic hand (Zimerman et al., 2012). The positive effects are thought to stem from inhibition of the "overactive" non-lesioned hemisphere. However, in a group of stroke survivors with a wider range of impairments, neither anodal tDCS over the lesioned hemisphere M1, nor cathodal tDCS over the non-lesioned hemisphere M1, nor bihemispheric tDCS had an effect on upper extremity motor sequence learning (Fleming et al., 2017). The evidence above indicates that the effects of tDCS on motor learning post-stroke are mixed and it remains unclear what forms of learning can be best impacted by application of tDCS. To our knowledge, the effects of tDCS on locomotor learning have not been tested in stroke. Locomotor learning is very different from the upper extremity learning that has been studied, as it requires bilateral movements involving interlimb coordination and postural control. Therefore, a separate examination of the effects of tDCS on locomotor learning post-stroke is warranted.

The current study aimed to examine the effects of anodal tDCS on locomotor adaptation and retention post-stroke with a split-belt treadmill paradigm. The split-belt

treadmill is a well-studied locomotor adaptation paradigm (Morton & Bastian, 2006; Reisman, Block, & Bastian, 2005; Reisman et al., 2007) where a perturbation is introduced by differentially changing the belt speeds, making one leg move faster than the other. Participants initially present with asymmetrical step lengths, but gradually adapt back to baseline symmetry. Stroke survivors have been shown to adapt on the split-belt treadmill (Reisman et al., 2007; Tyrell et al., 2014), but with less retention than age-matched controls (Tyrell et al., 2014). Because the M1 is thought to be involved in retention (Galea et al., 2011), we hypothesized that anodal tDCS over the lesioned hemisphere M1 would enhance retention of split-belt treadmill adaptation compared to sham tDCS.

Materials and Methods

Participants

Twenty-one people (5 female, mean ± SD, 65.0 ± 9.79 years old) with chronic (> 6 months), unilateral, ischemic stroke participated in the study. They all could walk unassisted on a treadmill moving at ≥ 0.4 m/s continuously for 5 minutes. Brain MRI or CT scan reports were obtained to verify stroke type and lesion location. Exclusion criteria included multiple strokes affecting both hemispheres, any cerebellar lesion, or any other significant neurological, cardiovascular or musculoskeletal condition besides stroke. Individuals with contraindications to tDCS were excluded, as well as individuals taking any medications that affect the central nervous system. Participants were randomly assigned to either the real tDCS group (n = 11) or the sham tDCS group (n = 10). The experimental protocol was approved by the University of

Delaware Institutional Review Board and all participants gave written informed consent. Participant demographic information is provided in Table 5.

Table 5 Participant demographic information[5].

Real tDCS Group							
PID	**Gender**	**Age (yrs)**	**Time Since Stroke (m)**	**Side of Stroke**	**Stroke Location**	**LE-FMA**	**FWS (m/s)**
01	F	67	27	R	S+C	23	1.2
02	M	60	144	R	S	26	0.9
03	M	85	13	R	S	28	0.72
04	M	62	53	R	S	16	0.67
05	M	54	35	R	C	12	0.67
06	M	74	6	R	S	25	0.5
07	M	66	29	R	S	20	1.2
08	M	65	25	R	S	29	0.9
09	M	63	14	R	S+C	25	1.3
10	F	69	17	L	S	20	0.7
11	M	77	16	L	S+C	27	0.92
Mean ± SD		**67.45 ± 8.59**	**34.45 ± 38.54**			**22.82 ± 5.31**	**0.88 ± 0.26**
Sham tDCS Group							
PID	**Gender**	**Age (yrs)**	**Time Since Stroke (m)**	**Side of Stroke**	**Stroke Location**	**LE-FMA**	**FWS (m/s)**
12	F	49	51	R	S+C	20	0.52
13	F	73	53	L	C	29	0.85
14	F	62	24	R	S	23	0.8
15	M	50	119	L	S+C	14	1
16	M	69	11	L	S	19	0.9
17	M	73	10	L	S	34	1
18	M	70	7	L	S	22	1.1
19	M	73	30	R	S+C	29	1.1
20	M	57	50	L	S	23	1.1
21	M	47	28	R	S	16	0.6
Mean ± SD		**62.3 ± 10.74**	**38.3 ± 33.22**			**22.9 ± 6.23**	**0.9 ± 0.21**
p	0.635#	0.237	0.557+	0.08#	1.00#	0.974	0.871

[5] Abbreviations: PID = participant identifier; SD = standard deviation; *p* = *p* value comparing real vs. sham tDCS groups; M = male; F = female; yrs = years; m = months; R = right; L = left; S = subcortical; C = cortical; LE-FMA = lower extremity Fugl-Meyer Assessment score; FWS = fast walking speed. #Fisher's exact test; +Mann-Whitney U test, all others independent t-tests.

General Paradigm

Participants completed two sessions approximately 24 hours apart. They walked on the treadmill with the two belts set at the same speed (tied) and different speeds (split) on each day, using a standard split-belt treadmill adaptation paradigm (Reisman et al., 2005, 2007), and received either real or sham tDCS during split-belt walking on day 1, see Figure 8.

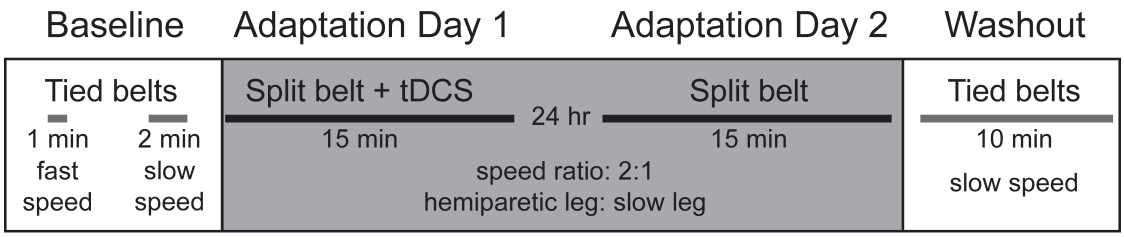

Figure 8 General experimental paradigm.

Split-belt Treadmill Adaptation

Resting heart rate (HR), rate of perceived exertion (RPE) (Borg, 1970), and blood pressure were collected at the beginning of data collection on each day to ensure safety (HR monitoring via a wireless monitor, Polar Electro Inc., Lake Success, NY). All treadmill walking was performed on an instrumented split-belt treadmill with imbedded force plates (Bertec Corp., Columbus, OH). On day 1, we determined the participants' fast walking speed (FWS) by gradually increasing the treadmill speed until it was the fastest speed at which they believed they could walk for 5 minutes without stopping. The slow walking speed (SWS) was defined as 1/2 of the FWS. They walked at their FWS for one minute (fast baseline period), followed by two minutes of walking at their SWS (slow baseline period). For the next 15 minutes, the

treadmill was changed to the split-belt configuration, in which the speeds of the two belts were controlled independently. The belt that the paretic leg was on was set to the SWS (from here on out, we will refer to this leg as the slow leg), and the belt that the non-paretic leg was on was set to the FWS (the fast leg), i.e., there was a 2:1 speed ratio. This period of split-belt walking was termed adaptation day 1. Participants went home after adaptation day 1 and were instructed to not use any treadmill while they were away. On day 2, participants started by walking on the treadmill with the belts split at the same speeds as day 1 (adaptation day 2 period) for 15 minutes, followed by 10 minutes of tied belts walking at the SWS (washout), see Figure 8. Participants wore a safety harness and held on to a front handrail during walking to ensure safety, and were instructed to look straight ahead during all walking and not look down at their feet. HR and RPE were checked every 3 minutes during walking to ensure safety. Participants were allowed to take rest breaks during walking if needed, but were not allowed to step off the treadmill.

Transcranial Direct Current Stimulation

tDCS was applied using a battery-powered direct current stimulator (Chattanooga by DJO Global, Inc., Vista, CA) with one saline-soaked oblong sponge electrode (size 5 x 1.6 cm^2) as the anode (AMREX Electrotherapy, Paramount, CA), and either a self-adhesive carbonized electrode (DUPEL B.L.U.E., Empi, St. Paul, MN, size 6.2 x 6.2 cm^2) or a saline-soaked sponge electrode (AMREX Electrotherapy, Paramount, CA; size 6.6 x 5.8 cm^2) as the cathode. The anode was placed over the lesioned hemisphere M1 lower extremity representation, with the long edge aligned with the mid-sagittal line of the skull, and the mid-point of the edge aligned with the vertex. The cathode was placed over the contralateral supraorbital area. This electrode

montage is known to successfully induce focal and bilateral (increasing excitability in the anodal-stimulated M1 and decreasing excitability in the contralateral M1) changes in motor cortical excitability of the lower extremities (Madhavan & Stinear, 2010). For the real tDCS group, the current was set at 1.5 mA and delivered throughout the entire time of the adaptation day 1 period. The current was ramped up and ramped down over 30 s at the beginning and the end of stimulation, respectively. The actual time of stimulation for each subject was recorded. For the sham group, the current was set at 1.5 mA for 2 minutes, with the same ramping schedule. This method creates an effective sham, as there are no differences in sensation or level of comfort between real and sham tDCS groups (Gandiga et al., 2006). For both groups, the tDCS was turned on after the slow baseline ended and 2 minutes before the start of adaptation day 1. This allowed a fixed time to set up data collection procedures for every participant. Thus for the sham group, no stimulation was given during walking at all. No tDCS was given on day 2 for either group of participants.

Data Collection

Kinematic data were collected using an eight-camera Vicon MX motion capture system with Nexus 1.8.5 software (Vicon Motion Systems, Ltd., Oxford, UK). Reflective markers were placed bilaterally over the first and fifth metatarsophalangeal joints, lateral and medial malleoli, heels, lateral and medial epicondyles of the femur, greater trochanters and iliac crests. Rigid-body clusters were placed over the shanks, thighs, and the sacrum. Ground reaction forces (GRFs) during walking were collected through force plates embedded in each treadmill belt separately. Marker position and force plate data were time synchronized and collected at a sampling rate of 100 Hz and 1000 Hz, respectively.

Data Analysis

Marker position data were initially processed in Vicon Nexus 1.8.5 (Vicon Motion Systems, Ltd., Oxford, UK) using custom pipelines to fill gaps and delete additional trajectories. Processed marker position data and GRFs were exported, and further processed with custom written software in MATLAB (MathWorks, Inc., Natick, MA). GRFs were low-pass filtered at 10 Hz with a 4th-order Butterworth filter, and down-sampled to 100 Hz to match the sampling rate of marker position data. Gait events were detected with GRFs using the following criteria: (a) Initial contact was defined as the first frame when the vertical GRF reached > 20 N for 8 continuous frames (80 ms); (b) Lift off was defined as the first frame when the vertical GRF reached < 20 N for 8 continuous frames (Tyrell et al., 2014). Marker position data were low-pass filtered at 10 Hz with a 4th-order Butterworth filter, and marker positions at initial contact and lift off were found.

The major kinematic variable of interest was step length asymmetry (SLA), since it is well-known to show a highly robust adaptation in response to split-belt treadmill walking (Morton & Bastian, 2006; Reisman et al., 2005, 2007; Tyrell et al., 2014). Step length (SL) was defined as the distance along the anterior-posterior axis between the lateral malleoli markers at initial contact. SLA was calculated using the formula:

$$SLA = (SL_{fast} - SL_{slow}) / (SL_{fast} + SL_{slow})$$

where SL_{fast} represents SL when the fast leg was the leading leg, and SL_{slow} represents SL when the slow leg was the leading leg. Therefore, a SLA of zero would indicate perfect interlimb symmetry.

We focused on analyzing SLA during several key walking periods:

1. Slow baseline (SB): last 40 strides of slow baseline walking period;

2. Early adaptation day 1 (EA1) and early adaptation day 2 (EA2): first 10 strides of adaptation day 1 and adaptation day 2 periods, respectively;

3. Late adaptation day 1 (LA1) and late adaptation day 2 (LA2): last 30 strides of adaptation day 1 and adaptation day 2 periods, respectively;

4. Early washout (EW): first 10 strides of washout;

5. Late washout (LW): last 30 strides of washout.

In all cases, we normalized SLA to the mean SLA during slow baseline in order to eliminate detecting differences solely due to different levels of baseline asymmetry in participants with stroke.

Learning and retention measures were calculated using mean SLA during the defined key walking periods above, with the following formulas:

1. Learning magnitude day 1 = LA1 – EA1;

2. Learning magnitude day 2 = LA2 – EA2;

3. Retention magnitude = EA2 – EA1;

4. After effect magnitude = EW.

Rate of adaptation and rate of washout were calculated using a method adapted from Malone & Bastian (2010). For each subject, each adaptation or washout curve was smoothed with a moving average of 3 strides. The rate of adaptation or washout was defined as the number of strides to reach plateau. Plateau range was defined as the mean ± SD of the last 30 strides. The beginning of the plateau, i.e., rate, was defined as the first stride at which 5 consecutive strides fell within the plateau range. We also looked at differences in rates between days 1 and 2. Change of rate was calculated as the difference between rate of adaptation on day 1 and day 2.

Statistical Analysis

Statistical analyses were performed in IBM SPSS Statistics 24 (IBM Corp., Armonk, NY). Normality of data were checked with Kolmogorov-Smirnov tests, and Levene's tests were used to assess homogeneity of variances where appropriate. Depending on normality, independent t-tests or Mann-Whitney U tests were performed to assess differences in learning magnitudes on each day, retention magnitude, after effect magnitude, rates of adaptation and washout, and change of rate between real vs. sham tDCS groups. A mixed-design analysis of variance (ANOVA) with group (real, sham) as between-group factor and walking period (SB, EA1, LA1, EA2, LA2, EW, LW) as within-group factor was performed to assess differences in SLA change across time in the two groups. Greenhouse-Geisser corrections were applied if the sphericity assumption was violated. Demographic characteristics between groups were compared with independent t-tests, Mann-Whitney U tests, or Fisher's exact tests.

Results

All participants completed the study with no adverse events. All data are presented as mean ± SEM, unless otherwise stated. Demographic characteristics and functional levels were not significantly different between real and sham tDCS groups (all $p > 0.07$), see Table 5.

Figure 9A shows stride-by-stride plots of SLA, averaged over all subjects in each group. At the beginning of adaptation day 1, both groups produced a negative SLA due to the perturbation from the different belt speeds, but were able to adapt their SLA back up toward baseline levels by the end of the adaptation day 1 period. On day 2, they again initially produced a negative SLA, but this SLA was a smaller magnitude

than during the analogous period of day 1, indicating a degree of retention over days. Again, both groups adapted back close to baseline levels by the end of day 2 adaptation. At the beginning of washout, both groups had similar negative after effects shown by the positive SLA. The negative after effects indicate storage of the newly learned walking pattern. After effects shifted back toward baseline levels by the end of the washout period. Summary data of the SLA values during each of the key walking periods, averaged by group, are shown in Figure 9B. The group x walking period ANOVA showed a significant main effect of walking period ($F(3.12, 59.21) = 59.57$, $p < 0.001$), but no main effect of group and no interaction effect (both $p > 0.69$).

In addition, learning magnitude on both days, retention magnitude, and after effect magnitude were all not significantly different between real and sham tDCS groups (all $p > 0.22$, Figure 10A). Similarly, rates of adaptation on both days, rate of washout, and change of rate were also not different between groups (all $p > 0.24$, Figure 10B). A power analysis showed that the primary outcome measure, retention magnitude, had a small effect size ($d = 0.13$), and a sample size of $n = 1860$ would be needed to reach a power of 80% with the α set at 0.05.

Discussion

In our group of chronic stroke survivors, we did not find any effect of anodal tDCS applied to the lesioned hemisphere M1 on the magnitudes of learning or retention, magnitude of after effect, rates of adaptation and washout, or change of rate in split-belt treadmill learning.

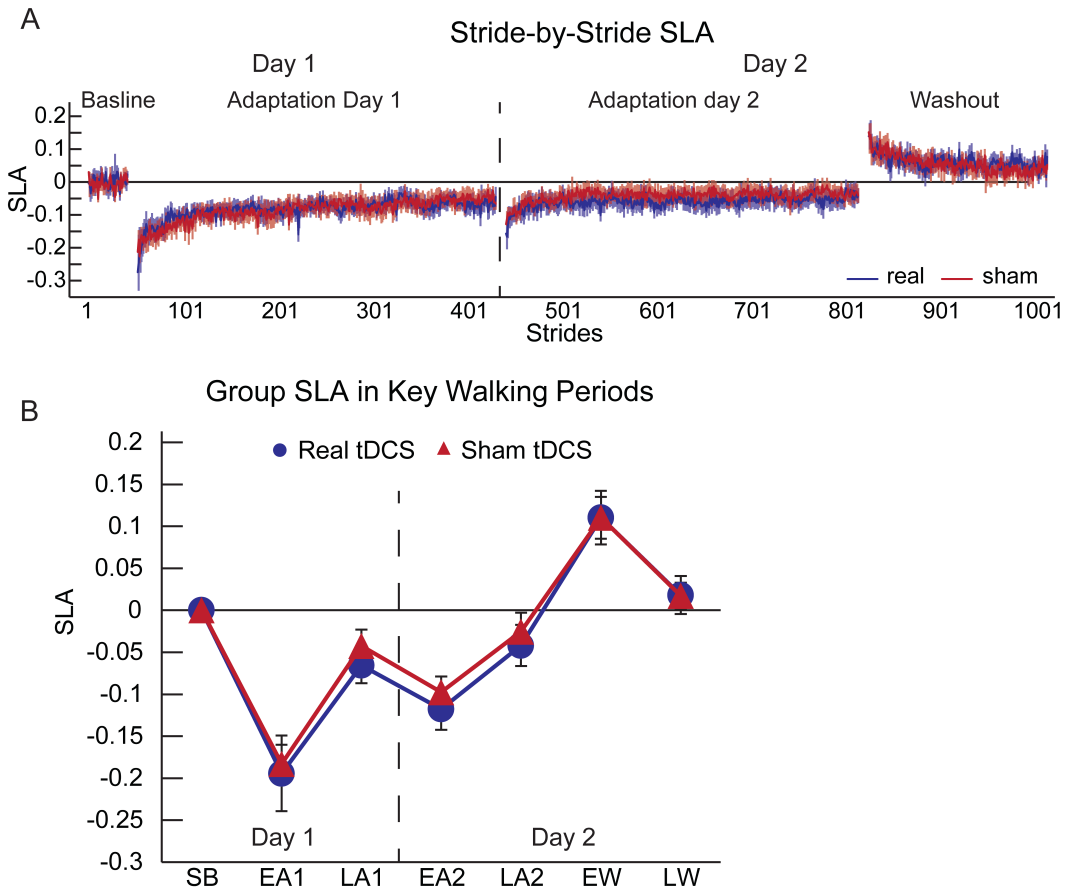

Figure 9 A. Stride-by-stride plot of averaged step length asymmetry (SLA) for real (blue) and sham (red) tDCS groups. Data are truncated to the participant with the least number of strides. Light shaded areas indicate ± 1 SEM. B. Group average SLA during key walking periods. The sham tDCS group is depicted as red triangles; the real group as blue circles. The ANOVA revealed no main effect of group or any group x time interaction. SB = slow baseline; EA1 = early adaptation day 1; LA1 = late adaptation day 1; EA2 = early adaptation day 2; LA2 = late adaptation day 2; EW = early washout; LW = late washout. Error bars, ± 1 SEM.

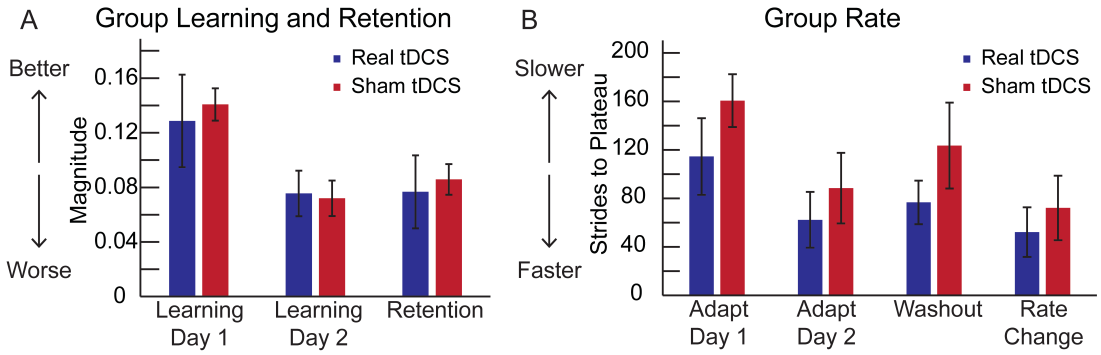

Figure 10 A. Group averaged learning magnitudes on both days and retention magnitude are not different between real (blue) and sham (red) tDCS groups. B. Group averaged rates of adaptation on both days, rate of washout, and change of rate are not different between real (blue) and sham (red) tDCS groups. Error bars, ± 1 SEM.

First, anodal tDCS over the lesioned hemisphere M1 did not have an effect on learning magnitude or rate of adaptation on day 1. This was expected, because the M1 is thought to be involved in the retention of adaptation (Galea et al., 2011; Panouillères et al., 2015), not the initial adaptation process. This was also consistent with studies in healthy individuals, where anodal tDCS over the M1 did not have any effect on initial online learning (Galea et al., 2011; Panouillères et al., 2015).

However, contrary to our hypothesis, retention was not affected by anodal tDCS. Other relevant measures, including the learning magnitude and rate of adaptation on day 2, and change of rate, were also unaffected by anodal tDCS. It is important to note that the stroke survivors from both real and sham tDCS groups in our study showed levels of 24-hour retention that were comparable to that reported in previous studies in chronic stroke and have been shown to be impaired compared to age-matched healthy controls (Tyrell et al., 2014). Therefore, it was not the case that our subjects were too mildly impaired or had reached a ceiling effect on retention

capabilities such that tDCS could not improve retention any further. Instead, it is more likely that the anodal tDCS was simply not effective for enhancing retention of split-belt adaptation in people with stroke. The fact that change of rate was also not different between groups indicates that not only the amount of retention, but also the speed of learning between days was unaffected by anodal tDCS.

Our overall result of anodal tDCS not having any effects on split-belt adaptation in stroke is inconsistent with some other studies of tDCS and motor learning in stroke. Differences between other studies and ours must be addressed in order to fully understand the results. Other studies used upper extremity learning, either a circuit tracing task (Lefebvre et al., 2013), or a finger sequence learning task (Zimerman et al., 2012). Our task (i.e., walking) is a lower extremity, bilateral motor task that involves postural control and interlimb coordination, which is, arguably, more complex than unilateral arm or finger movements. It is possible that tDCS may not be capable of modulating such complex behavior. Walking is also thought to have less cortical control than upper extremity movements, where spinal circuitry may play a larger role (Ahn & Hogan, 2012; Dimitrijevic, Gerasimenko, & Pinter, 1998; Yang et al., 2004). Thus using tDCS to target M1 may not be sufficient to affect locomotor behavior. However, we think this is not likely because M1 tDCS can modulate coordinated motor output during walking (van Asseldonk & Boonstra, 2016) and enhance lower extremity motor control (Foerster, Dutta, Kuo, Paulus, & Nitsche, 2018; Sriraman, Oishi, & Madhavan, 2014) in healthy individuals. In stroke, tDCS over the lower extremity M1 modulates paretic force output (S. Tanaka et al., 2011), paretic ankle motor control (Madhavan et al., 2011), and enhances performance of functional gait activities (Tahtis et al., 2014). This evidence suggests that tDCS over

the M1 is capable of modulating complex behavior of the lower extremities, including locomotion. Another difference between our study and previous studies is the cognitive demand of the task. The above mentioned upper extremity tasks all require a substantial amount of cognitive resources (e.g., attention, strategy, memory) in order to be successful. Similarly, previous studies in healthy individuals investigating the effects of tDCS on motor adaptation mostly used visuomotor rotation tasks. Recent evidence suggests that visuomotor rotation tasks have a large strategy-based explicit component driven by target error, and a smaller implicit component driven by sensory prediction errors (Taylor, Krakauer, & Ivry, 2014). The explicit learning component demonstrates savings of learning (faster relearning after washout – a form of retention), whereas the implicit learning component is not well-saved (Morehead, Qasim, Crossley, & Ivry, 2015). The explicit component of learning is also associated with working memory capacity (Christou, Miall, McNab, & Galea, 2016), indicating it requires more cognitive resources. On the other hand, our task, split-belt treadmill learning, is a primarily implicit type of sensorimotor adaptation driven by the mechanical perturbation from the different belt speeds (Bastian, 2008). These different types of learning rely on different neural substrates. While sensorimotor adaptation mostly relies on the cerebellum and M1 (Galea et al., 2011; Martin et al., 1996a; Morton & Bastian, 2006; Panouillères et al., 2015), these other types of learning requiring more cognitive resources also depend on the basal ganglia, premotor cortex, prefrontal and parietal cortices (Doya, 2000; Galea, Albert, Ditye, & Miall, 2010; Kantak, Mummidisetty, & Stinear, 2012; Sami, Robertson, & Miall, 2014; H. Tanaka, Sejnowski, & Krakauer, 2009). The differences in these brain areas involved, as well as the dynamic interactions within these areas during learning and consolidation may

explain the different effects of tDCS observed in our study compared to previous studies. Similarly, a recent study from our lab (Charalambous, Alcantara, et al., 2018) showed that an acute bout of high-intensity exercise also does not have any effect on split-belt adaptation in stroke, whereas others have shown that acute high-intensity exercise enhances retention of newly learned upper extremity visuomotor tracing or target matching tasks (Nepveu et al., 2017; Roig et al., 2012). In this comparison, the same difference in the cognitive demand of the tasks exists. Split-belt adaptation requires less cognitive strategy compared to the visuomotor tasks. Combining these results, we speculate that the effects of tDCS are task relevant, and cognitive demand of the motor learning task may be an important factor in determining its efficacy.

Limitations of the study must be considered. First, we only tested anodal tDCS in our study, so we do not know if cathodal tDCS or bihemispheric tDCS, which were used in previous studies (Lefebvre et al., 2013; Zimerman et al., 2012), would enhance split-belt learning in stroke. We chose anodal tDCS because it was effective in enhancing retention of motor adaptation in healthy individuals, but it is possible that other types of tDCS may work better in individuals with stroke. It is important to note, however, that neither type of tDCS was effective to enhance an upper extremity sequence learning task in stroke (Fleming et al., 2017). Second, we did not control for stroke characteristics in our study. It is possible that tDCS may be more effective in individuals with less damage to the brain due to higher availability of intact pathways. Notably, we did not find any association between stroke severity (as assessed by either lower extremity Fugl-Meyer Assessment score or FWS) and our major outcome variable, retention magnitude (both $r < 0.14$, $p > 0.16$) in the real tDCS group. Nevertheless, future studies should incorporate detailed imaging to assess structural

predictors of responses to tDCS. Third, we did not measure neurophysiological changes before and after tDCS, so we do not know if our tDCS produced the expected excitability changes in the brain, which could provide some mechanistic insight for the results.

In conclusion, we found that anodal tDCS over the lesioned hemisphere M1 does not have any effect on split-belt treadmill learning and retention in people with chronic stroke. Future studies are needed to determine whether tDCS can enhance other types of locomotor learning in stroke.

Chapter 5

DISCUSSION

Overall, this work examined the neurophysiological and behavioral effects of two types of priming techniques in people with chronic stroke. Specifically, neurophysiological responses to exercise priming and transcranial direct current stimulation (tDCS) were studied, and additionally, the effects of chronic antidepressant intake on neurophysiological responses to tDCS were investigated. Finally, the effects of tDCS on split-belt treadmill adaptation, a type of locomotor learning, were tested. Exercise priming had a positive effect on motor cortical excitability in people with chronic stroke in the form of increasing resting MEP amplitudes from the lesioned hemisphere. Anodal tDCS, however, did not induce the expected increase of motor cortical excitability in the lesioned hemisphere. Interestingly, in chronic antidepressant-takers, but not others, anodal tDCS over the lesioned hemisphere primary motor cortex (M1) induced increased resting MEP amplitudes from the non-lesioned hemisphere. Behaviorally, anodal tDCS over the lesioned hemisphere M1 did not have any effect on split-belt treadmill learning in stroke.

The results together paint a complex picture of how priming affects people with chronic stroke in the motor domain. We showed the feasibility and effectiveness of a short, 5-minute bout of high-intensity walking to broadly increase motor cortical excitability in people with stroke. This paves the way for future studies to investigate whether this type of exercise can improve motor learning, and whether the changes in

motor cortical excitability are correlated with learning. Recent work from our lab using similar exercise protocols showed that acute exercise did not affect split-belt treadmill learning in stroke (Charalambous, Alcantara, et al., 2018). However, in our view, the results should not be taken as evidence that our exercise priming protocol does not promote motor learning in stroke. Rather, the type of motor task needs to be considered, as exercise may be more effective in enhancing learning of certain types of tasks. The same consideration is also true regarding tDCS. We did not find any effect of tDCS on split-belt adaptation in Aim 3, but that does not lead to the conclusion that tDCS is ineffective in enhancing motor learning in stroke. Given that there is evidence of exercise (Nepveu et al., 2017) and tDCS (Lefebvre et al., 2013; Zimerman et al., 2012) being effective in enhancing other types of motor learning in stroke, it is likely that split-belt treadmill adaptation is not sensitive to these types of priming. Future studies should investigate the effects of exercise and tDCS priming in other tasks, such as visuomotor locomotor adaptation. Systematic collection of evidence and analyses are also needed to determine which type of tasks are sensitive to different types of priming in order to effectively utilize them in stroke rehabilitation.

The neurophysiological responses to tDCS in stroke could be considered surprising, as anodal tDCS over the lesioned hemisphere M1 did not induce the expected changes in motor cortical excitability in the lesioned hemisphere. Furthermore, there was a significant increase of excitability in the unstimulated, non-lesioned hemisphere in antidepressant takers. This is evidence to show that tDCS is not a one-size-for-all when it comes to people with chronic stroke. Many factors, such as antidepressants, as studied here, can affect how people respond to tDCS. Other

potential factors may include other medications (e.g., anti-anxiety, anticonvulsants, other types of antidepressants), stroke severity, stroke location, and comorbidities such as depression and anxiety. The specifics of tDCS parameters may also affect the responses. Future studies should investigate how each of these factors may affect neurophysiological responses to tDCS. Behaviorally, future studies should investigate whether these factors can affect motor functional changes from tDCS, and whether they are correlated with neurophysiological changes. With enough evidence, perhaps a complex model can be built to predict individuals' responses to tDCS, and the specific parameters needed to achieve maximum benefits. In the meantime, however, we should be cautious when using tDCS in stroke recovery, as it could have detrimental effects on some individuals.

Taking the results from Aims 2 and 3 together, it is possible to think that the reason tDCS did not affect split-belt adaptation was that tDCS simply was not effective in inducing neurophysiological responses in the participants. We cannot prove or disprove this line of reasoning because we did not collect motor cortical excitability measures in Aim 3. However, differences between the conditions of Aims 2 and 3 need to be considered. First, tDCS was applied when participants were not performing a motor task in Aim 2, while it was applied during split-belt walking in Aim 3. Previous work have shown that tDCS is effective in inducing motor cortical excitability changes post-stroke when applied concurrently with a motor task (Bolognini et al., 2011; M. C. Chang et al., 2015; Hummel et al., 2005), so it is possible that motor cortical excitability changes were induced by tDCS in Aim 3. Second, tDCS parameters were different in the two Aims. We selected different tDCS parameters based on previous literature and the scientific questions in each Aim to

maximize the expected effects, but it is possible that the parameters in one Aim are more effective than the other. Future studies should incorporate motor cortical excitability measures before and after motor learning with tDCS, and test neurophysiological responses to the same tDCS during rest in a separate session to fully understand the effects.

In summary, this work demonstrates the potential of exercise priming for stroke recovery, and highlights the complexity of tDCS usage in people with chronic stroke. Individual differences and priming parameters must be considered to maximize the effects of priming in stroke. A one-size-fits-all approach is not only inefficient, but could also be damaging to individual recovery.

In light of our findings, new questions arise. It is of significant importance that we start systematically investigating factors that may affect responses to tDCS in stroke. Studies should start with investigation of more common factors such as widely-used medications, depression status, and stroke location, then move on to less common or more fine-tuned factors such as less widely-used medications, dose of medication, and time on medication. Besides testing neurophysiological responses to tDCS when participants are at rest, direct comparison of the neurophysiological responses to tDCS between rest and during motor practice should also be made. Building on these mechanistic findings, the next step would be to investigate how tDCS may be applied to best enhance motor performance or motor learning in stroke, with the consideration of these influential factors. The mechanisms of exercise priming also need to be studied. Current studies suggest that exercise affects the brain by inducing multiple-level neurochemical changes (Taubert et al., 2015). However, another interesting possibility is that exercise may be indirectly inducing changes in the brain through

another mechanism, such as enhanced engagement or arousal. If this is true, other therapies that enhance engagement, such as music therapy, may also be potential priming techniques. This line of thought presents an exciting possibility of priming for individuals who may have difficulty performing exercise. Finally, the types of motor tasks that are sensitive to different priming techniques must be investigated.

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Appendix

HUMAN SUBJECTS RESEARCH APPROVAL LETTERS

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: September 27, 2017

TO: Darcy Reisman
FROM: University of Delaware IRB

STUDY TITLE: [819000-8] Behavioral and neurophysiologic processes of locomotor learning after stroke

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: September 27, 2017
EXPIRATION DATE: October 20, 2018
REVIEW TYPE: Full Committee Review

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: February 22, 2018

TO: Susanne Morton, PT, PhD
FROM: University of Delaware IRB

STUDY TITLE: [729526-6] Effects of Chronic Antidepressant Use on tDCS Responses in Stroke

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: February 21, 2018
EXPIRATION DATE: March 17, 2019
REVIEW TYPE: Full Committee Review

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

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Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE:	August 16, 2017
TO:	Susanne Morton, PT, PhD
FROM:	University of Delaware IRB
STUDY TITLE:	[509868-11] Augmenting Motor Learning Post-stroke with Non-invasive Brain Stimulation
SUBMISSION TYPE:	Continuing Review/Progress Report
ACTION:	APPROVED
APPROVAL DATE:	August 16, 2017
EXPIRATION DATE:	September 17, 2018
REVIEW TYPE:	Full Committee Review

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

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If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.