The Effects of Anodal Transcranial Direct Current Stimulation on Neural Excitability and
Function in Individuals with Chronic Ankle Instability: A Preliminary Investigation

A Thesis
by
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Abstract

The Effects of Anodal Transcranial Direct Current Stimulation on Neural Excitability and
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Chronic ankle instability (CAI) leads to persistent levels of disablement among physically active

individuals, potentially generating health risks. Rolling and giving-way at the ankle among this

population are associated with changes to the central nervous system that alter movement

patterns, predisposing these individuals for subsequent re-injury. This study attempted to modify

these injury-induced brain changes by using anodal transcranial direct current stimulation

(aTDCS) in conjunction with ankle eccentric exercise among individuals with CAI. Twenty-six

participants (n=26) were recruited who were 22.38 ± 2.99 years old and experienced CAI. The

participant underwent 4 testing sessions at baseline, week-2, week-4, and week-6, where an

assessment of neural excitability, dynamic balance, side-hop test and ankle strength, were

observed. Between baseline and week-2 the participants underwent 5 training sessions and the

training sessions were repeated between week-2 and week-4 (n=10). Participants partook in

eccentric exercises on an isokinetic dynamometer in conjunction with either aTDCS intervention

or sham intervention. It was observed that the aTDCS group increased their neural excitability, dynamic balance, and muscle activation over the course of this study. Eccentric exercise alone was observed to elicit improvement in functional performance, and to some extent neural excitability. However, the improvements in neural excitability with eccentric training alone were seemingly short-term adaptations from baseline to week-2 (p=0.007) where after week-2 participants became less excitable (week-4: p=0.022; week-6: p=0.006); whereas adding aTDCS in conjunction with eccentric exercise, created long-term adaptations, improving neural excitability measures from baseline to week-6 (p=0.024). Surprisingly, there were no improvement in strength measurements in both the aTDCS and sham group (p>0.05). The design of this study allowed us to examine if the use of aTDCS may positively modify the changes to cortical excitability and movement patterns that subsequently improve functional outcomes. Although caution should be exercised due to the lack of a true control, these results suggest non-invasive brain stimulation may have some efficacy in the treatment of joint injuries.

Acknowledgments

I would like to thank my thesis committee Dr. Alan Needle, Dr. Jeffrey McBride, Dr. Jennifer Howard and Dr. Herman van Werkhoven for the support and guidance they have provided me during this major project and learning process. You all have been astounding mentors and I have absolutely loved getting to know you all and getting to work with you for the past two years. I would like to give a special thanks to Dr. Alan Needle for his patience to always answer my list of never-ending questions and willingness to help anytime I ask. Thank you so much for pushing me during the past year and a half and holding me to a higher standard which has helped me realize my true potential. I would also like to thank Elisa Godoy and Zack Ripic for helping during this long data collection process and always being willing to lend a helping hand. Lastly, I would like to thank all my peers in the Biomechanics lab for all the laughs, rant sessions, and coffee runs. I could not have accomplished any of this this without the help of you all!

Dedication

I would like to dedicate this project to my Nanny and Pawpaw. Nanny was my biggest fan and always had faith in me and believed that I could do anything I set my mind to. She has given me all the love, support, encouragement, and inspiration to follow my passions wherever they may take me. I'm sorry for all the times I came home off the school bus and made you both help me with my 4th and 5th grade math and science homework for hours. Turns out I am pretty good at math and science, and ironically am making a career out of both of those subjects. You both make me strive to work hard and be a better person. I miss you both dearly and I hope I will continuously make you proud as you are watching over me.

I would also like to dedicate this project to my Mom and Dad. Thank you for being my biggest supporters and giving me all the opportunities to help me grow and become who I am today. I could not have done this without you! I love you both.

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Chapter 1: Introduction

Ankle sprains are injuries experienced by approximately 60% of the general population (Hiller et al., 2012) that despite a relatively mild rehabilitation protocol process, is estimated to generate an annual economic burden of 4 to 7.5 billion dollars on the United States healthcare system (Shah, Thomas, Noone, Blanchette, & Wikstrom, 2016; Waterman, Owens, Davey, Zacchilli, & Belmont, 2010). Approximately 70% of individuals who experience a lateral ankle ligament injury, are at an increased risk of re-injury along with continual instability and symptoms that compound this large economic demand (Anandacoomarasamy & Barnsley, 2005; Hiller et al., 2012; McKeon & Wikstrom, 2016). Chronic ankle instability (CAI) is the most common result of an ankle injury and is characterized by repeated sensations of rolling and giving-way in the ankle that contribute to the long-term degeneration of the joint within ten years of the initial ligament damage (Golditz et al., 2014). There are both neural and mechanical changes that happen when an ankle sprains occurs. A change in movement patterns during gait is a result of the reorganization of the motor cortex and alterations to the individual's sensory feedback (Needle, Lepley, & Grooms, 2017). Furthermore, individuals with CAI experience functional and mechanical deficits that result in decreased health-related quality of life (HRQOL) and decreased physical activity levels that could restrict their overall functional abilities, and further predispose them to cardiovascular disease, osteoarthritis and shorten life expectancy (Golditz et al., 2014; Houston, Van Lunen, & Hoch, 2014; Hubbard-Turner & Turner, 2015; McKeon & Wikstrom, 2016). An ankle sprain might seem insignificant at first; however, it's economic and long-term health impact make it imperative that rehabilitation techniques are re-

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evaluated and optimized in order for the individual to regain their pre-injury ankle function, limit

health related quality of life deficits, and reduce re-injury rates.

The etiology of this injury is crucial to understanding how certain interventions

contribute to the healing and rehabilitation process. Rehabilitation procedures in patients with

musculoskeletal injury try to change behavioral characteristics that potentially affect

performance, such as balance training to restore postural control, isotonic strengthening to

improve muscle function, or perturbation-based exercises to improve neuromuscular control

(Donovan & Hertel, 2012; Wright & Linens, 2017). After a ligamentous injury there is evidence

of altered cortical function; therefore, further investigation of potential rehabilitation methods are

needed to determine if they are capable of modifying neuroplasticity in individuals with CAI

(Needle et al., 2017). Ideally, a rehabilitation focused on modifying neuroplasticity in the brain

would prompt long-term potentiation (LTP) within the primary motor cortex, such that

corticospinal connections to stabilizing muscles are restored, motor cortex excitability is

enhanced, and activation of extraneous cortical areas are decreased (e.g., premotor, planning)

(Needle et al., 2017; Stagg & Nitsche, 2011).

Transcranial direct current stimulation (TDCS) is an intervention that is noninvasive and

applies a small electrical current (0.6 to 2.0 mA) for 10 to 20 minutes over the motor cortex

(M1). Anodal TDCS (aTDCS) modifies the resting membrane potentials of intracortical neurons

increasing cortical excitability via depolarization, facilitating LTP-like changes which enhances

the motor learning process, even after a single training session (Stagg & Nitsche, 2011).

Eccentric training allows for heavier loads to be placed on the muscle inducing greater

hypertrophy as well as an increase in neural recovery due to a decrease in cortical muscle

inhibition and an increase in spinal excitability via increased descending neural drive compared

to concentric and isometric exercises (Duclay, Martin, Robbe, & Pousson, 2008; Hedayatpour & Falla, 2015; Lepley & Butterfield, 2017). After ligamentous injuries, such as injuries experienced by individuals with CAI, there is a decrease in the motor neuron recruitment and neural drive (or a sum of multiple motor neuron action potentials) due to decreased cortical inhibition leading to subsequent muscle weakness; by participating in eccentric training post-injury these deficits can be recovered more quickly (Farina, Negro, & Dideriksen, 2014; Lepley & Butterfield, 2017). This intervention of aTDCS, in conjunction with an eccentric training exercises, potentially could improve task acquisition, in addition to enhancing cortical excitability to stabilizing musculature, and providing a potential correction for injury-induced maladaptive plasticity (Bikson et al., 2016; López-alonso, Cheeran, & Fernández-del-olmo, 2015; Needle, Palmer, Kesar, Binder-macleod, & Swanik, 2013).

In previous aTDCS investigations, the focus of the intervention was directed towards either individuals who were healthy or had a neurological impairment (e.g., stroke, traumatic brain injury, Parkinson's disease) (Madhavan & Stinear, 2010; Tahtis, Kaski, & Seemungal, 2014). There have been mixed conclusions to these studies potentially due to the populations that were being observed. In individuals with a neurological impairment, it is common that their brains go through a structural change that may hinder the amount of improvements achievable (Marquez, van Vliet, Mcelduff, Lagopoulos, & Parsons, 2015). In recent studies, healthy individuals were not as affected by the aTDCS intervention they received compared to the neurologically impaired individuals; this is thought to be due to their lack of deficits compared to neurologically impaired individuals (Bikson et al., 2016; Devanathan & Madhavan, 2016; Maeda et al., 2017). There have been no known studies observing the use of aTDCS in individuals with a musculoskeletal injury. Individuals with musculoskeletal injuries have previously exhibited

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altered movement patterns that are theorized to be caused by a *functional* reorganization of their motor cortex (Kapreli et al., 2009; Needle et al., 2017). These individuals with musculoskeletal injuries might display greater improvements in their performance and neurophysiological function due to the functional maladaptive neuroplasticity of their motor cortex and not a physical structural change like what is seen in individuals with neurological impairments (Needle et al., 2017).

The findings of this study could lend support towards an adjunctive therapy for joint injury rehabilitation and potentially illuminate a treatment protocol for CAI individuals that specifically addresses the maladaptive neuroplasticity that is currently an obstacle for functional recovery. Eccentric training as a current rehabilitation method may have beneficial effects in improving functional performance; however, it has not been proven sufficient in preventing future injuries or in addressing the neural deficits experienced by individuals with CAI. The current study may contribute to further rehabilitation efforts of ligamentous injuries and potentially the prevention of a decline in overall HRQOL of individuals with these injuries. By using the aTDCS intervention, stimulating the propagation of motor learning through long-term potentiation and for the disinhibition of motor pathways, along with eccentric training further may expand on basic science knowledge for the development of subsequent interventions for individuals with joint injuries such as CAI.

Problem Statement:

The purpose of this study was to examine how an anodal transcranial direct current stimulation (aTDCS) intervention compared to a sham stimulation, in conjunction with eccentric training of the ankle stabilizers, affects neuroplasticity, functional performance, and strength in individuals with CAI.

Specific Aims and Hypothesis:

This study aimed to determine the effects of an aTDCS intervention in conjunction with eccentric exercise on neural excitability and ankle joint function following a 4-week intervention in individuals with CAI. Our overall working hypothesis was that the proposed intervention would improve neural, functional performance, and strength outcome measures beyond eccentric exercise alone.

Hypothesis 1: After the aTDCS intervention, neural excitability will be enhanced.

a) The aTDCS group was expected to increase their neural excitability between each time point (baseline, week-2, week-4, and week-6).

b) The sham group was expected to increase their neural excitability between each time point (baseline, week-2, week-4, and week-6) but to a lesser extent than that of the aTDCS group.

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Hypothesis 2: Dynamic balance and associated muscle activation was expected to improve with a quicker hop to stabilization time and side-hop performance was expected to improve with a decrease in the time to complete 10 side hops.

a) The aTDCS group was expected to improve their dynamic balance with a quicker hop to stabilization time and a decrease in the time to complete 10 side hops between each testing time points.

b) The sham group was expected to improve their dynamic balance with a quicker hop to stabilization time and a decrease in the time to complete 10 side hops between each testing time points but not to the extent of the aTDCS group.

Hypothesis 3: The ankle strength after the eccentric exercise training, was expected to increase.

a) The aTDCS group was expected to increase their ankle strength between baseline and 2-weeks, and further increase it between the week-2 time point and the week-4 time point. It was not expected to observe an increase in their ankle strength between week-4 and week-6.

b) The sham group was expected to increase their ankle strength between baseline and week-2, and further increase it between the week-2 time point and the week-4 time point. It was not expected for there to be an increase in their ankle strength between week-4 and week-6.

Chapter 2: Review of Literature

Introduction:

Lateral ankle sprains are the most prevalent category of ankle sprain and musculoskeletal injury occurring in approximately 30% of active individuals and athletes. Current rehabilitation methods are seemingly ineffective and insufficient for the prevention of ankle sprains. (Donovan & Hertel, 2012; Tanen, Docherty, Van Der Pol, Simon, & Schrader, 2014). In athletic populations, it has been recorded by the National Collegiate Athletic Association injury surveillance system that 11,000 ankle sprains happen each year, with a multitude of ankle sprain injuries that go unreported due to injured people not seeking medical attention (Donovan & Hertel, 2012; Hiller et al., 2012). CAI characterized as a "giving way" sensation in the ankle with repeated ankle sprains and ankle instability occurring after the initial ankle sprain (Wright & Linens, 2017). If CAI is not effectively treated it can lead to further pathologies later in life, such as early-onset osteoarthritis (Golditz et al., 2014; McKeon & Wikstrom, 2016). The maladaptive neuroplasticity of the brain also plays a vital role in the contribution to recurrence of ankle sprains in individuals with CAI, due to the occurrence of arthrogenic muscle inhibition after an injury (Klykken, Pietrosimone, Kim, Ingersoll, & Hertel, 2011). Due to the frequency of this injury in the general population, especially in athletic or active individuals, it is necessary for further research to be conducted investigating the underlying contributing factors behind CAI and the potentially novel rehabilitation methods. The use of eccentric training on the effected ankle in conjunction with the addition of an aTDCS intervention, addressing the neural aspects of the injury as well as functional performance and strength deficits, may illuminate the need for augmented rehabilitation strategies in order to decrease the recurrence of ankle sprains and

enhance long-term functional outcomes in CAI individuals (Golditz et al., 2014; Houston, Hoch, & Hoch, 2015; Houston et al., 2014).

Chronic Ankle Instability (CAI) Barriers:

The etiological understanding of CAI and similar ligamentous injuries at the knee joint such as an anterior cruciate ligament (ACL) injury has recently been under investigation to detect potential inhibitors to the rehabilitation process that contribute to persistent poor outcomes (Needle et al., 2013; Rosen, Needle, & Ko, 2017). Previously, it was believed that damage to the physical joint contributed to these functional deficits, as joint laxity and deafferentation of the joint were believed to decrease the static and dynamic stability of the joint (Freeman, 1965; Konradsen, 2002). Current rehabilitation techniques have been implemented based on those findings and were directed towards restoring stability in the affected joint through exercises that emphasized strengthening of the stabilizing muscles and improving proprioceptive acuity in response to minute joint motions.

These strengthening exercise techniques have shown to acutely improve joint function, however they may lack the resilience required to improve long-term functional outcomes (McKeon & Wikstrom, 2016; Wright & Linens, 2017). Individuals with CAI are showing a deficiency in their ability to transfer their learning from rehabilitation to their everyday activities when new constraints are presented and affordances are required to be made in their motor planning process (Seidler, 2010). It is evident that individuals with joint instability have altered the way they process their movement planning in a potentially negative way (Needle et al., 2017). This alteration of places the joint in unstable, hazardous or unpredictable positions, where

muscle activation does not occur quickly enough to stress-shield the joint (Hass et al., 2010; Needle et al., 2017). Additionally, recent studies have suggested that the neuromechanics in unstable ankle joints possibly characterize a potential predisposition to adverse events or compensation strategies (Needle et al., 2013). A physiologic explanation for these observations is that, following ligament injury, changes within the central nervous system affect motor systems, specifically peripheral deafferentation of the sensory receptors located in the ligaments themselves; therefore, impeding the ability to shield the joint from injurious forces (Needle et al., 2017).

Mechanism of Injury:

Chronic ankle instability (CAI) is defined as the rolling or giving way of the ankle joint typically in an inversion direction causing stress to the lateral ligaments, which is recurrent post-initial sprain (Donovan & Hertel, 2012; Wright & Linens, 2017). This can result in functional instability limiting activities of daily living (ADL) and physical activity (Golditz et al., 2014; Wright & Linens, 2017). Individuals with CAI might not only incur ligamentous injury after an ankle sprain, depending on the severity, but they might also have disruptions in their proprioception and postural control, as well as experience neuromuscular and sensorimotor impairments (Munn, Sullivan, & Schneiders, 2010; Tanen et al., 2014). The brain and central nervous system are thought to be the structures eliciting these deficits (i.e., feedforward and altered motor output) during ligamentous injuries (Figure 1) (Needle et al., 2017). Impairments to the sensorimotor system have been found in CAI individuals; this is observed through a decrease of postural control and joint position sense (Munn et al., 2010). In addition, the laxity of

the ligaments in the ankle joint can lead to a loss in mechanical restraint due to arthrogenic

muscle inhibition reflex contributing to the apparent weakness of the ankle with an additional

decrease in function (Klykken, Pietrosimone, Kim, Ingersoll, & Hertel, 2011; Lepley, Lepley,

Onate, & Grooms, 2017; Needle et al., 2017). There has been evidence presented that individuals

with CAI need a greater internal stimulus or cortical drive to excite the descending cortical

neurons, and they experience a diminished ability of the corticomotor system to stimulate

neurons (Klykken, Pietrosimone, Kim, Ingersoll, & Hertel, 2011; Lepley et al., 2017; Needle et

al., 2017). Therefore, when an injury (i.e., ankle sprain) occurs, the individual is not able to

generate a motor response fast enough to prevent the injury from happening due to the

individual's muscle weakness, abnormal movement patterns, and activation failure (Needle et al.,
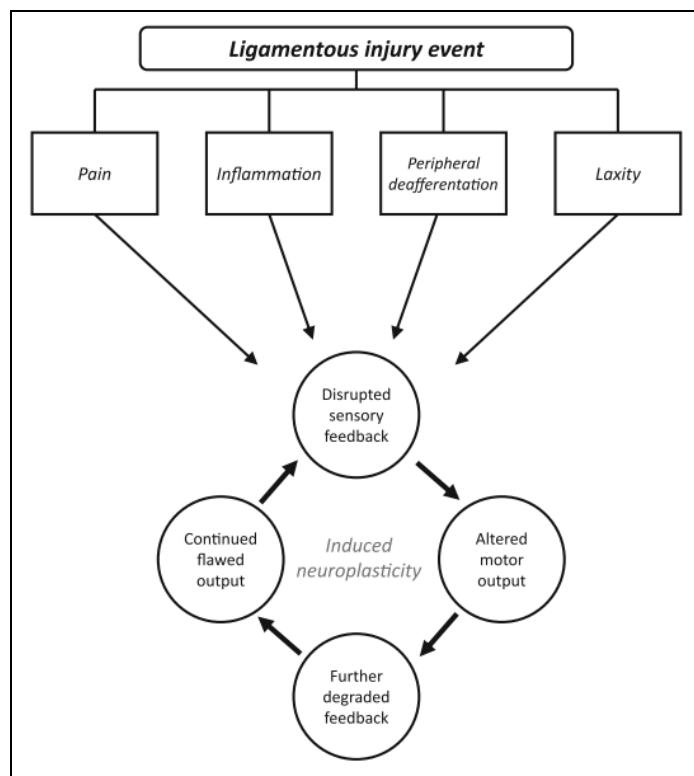
2017).

Figure 1. Theorized role of neuroplasticity on ligamentous injuries and the effect on
sensorimotor function.

Impact of Ankle Sprains on Health-Related Quality of Life (HRQOL):

After an initial ankle sprain it is probable that the individual will experience further repercussions from the injury. In recent studies of individuals who experienced a lateral ankle sprain via an inversion motion incurred residual symptoms including instability, 32% experienced recurrent sprains in addition to pain and swelling, and 15% to 64% of individuals had not recovered after three years (Anandacoomarasamy & Barnsley, 2005; Hiller et al., 2012). Prevalence of CAI is somewhat difficult to determine due to the fact that there is no "gold standard" to classify CAIs. However, there are several self-reporting questionnaires that have been created as a more objective way to measure and classify individuals with CAI: Ankle Instability Instrument (AII), Identification of Functional Ankle Instability (IdFAI), Ankle Joint Functional Assessment Tool (AJFAT), Foot Ankle Instability Questionnaire (FAIQ), etc. (Tanen et al., 2014). In this study the Identification of Functional Ankle Instability (IdFAI) will be used. Additionally, there are a plethora of undocumented cases of ankle sprains and CAI due to individuals electing not to seek medical attention after an injury, meaning there are likely more incidence of ankle sprains and CAI than what is currently reported in medical records (Hiller et al., 2012).

There are, in addition to questionnaires, functional performance tests (FPTs) that are given to identify people with CAI. Questionnaires are a good tool to utilize; however, they can be called into question due to their subjectivity (Rosen et al., 2017). By having FPTs, this gives an objective result that can be compared to established cut-point values where if an individual has a score above or below a certain point then they are classified as having CAI or not. The FTPs designed to classify an individual having CAI or not are practical due to their simplicity, ease of administration in a clinical or field setting, low-cost, and ability to be tracked over time

(Rosen et al., 2017). Side-hop, timed-hopping, multiple-hop tests and foot-lift tests have been observed as the best FPTs to determine whether or not someone has CAI (Rosen et al., 2017).

People with CAI, particularly older individuals, tend to have a decrease in their HRQOL due to, not only physiological factors, but psychological factors such as "kinesiophobia, fear-avoidance beliefs, or reinjury anxiety" (Houston et al., 2014). Individuals who are afraid of being reinjured will tend to limit their physical activity and ultimately their overall health and balance will subsequently over time decline as a result increasing their risk for cardiovascular disease, additionally with an increase in weakness they will have the potential to develop osteoarthritis. (Arnold, De La Motte, Linens, & Ross, 2009; Golditz et al., 2014; Houston et al., 2014). Overall, with an ankle sprain and the onset of CAI the HRQOL as individuals become older, is likely to decrease due to physical as well as psychological factors (Arnold et al., 2009; Golditz et al., 2014; Houston et al., 2014).

Neuroplasticity after Ligamentous Injury:

The central nervous system has the ability to adapt to changes, or become plastic, from the peripheral input stimulus that the body sends up to the brain (Ives, 2014; Raskin, 2011). More specifically the brain has the ability to have long-term or "enduring" adaptations that can change cortical properties either morphologically or functionally, which is defined as neuroplasticity (Ives, 2014; Raskin, 2011). Nervous system assessment techniques have been developed to further the understanding of the mechanism behind neuroplastic changes; these techniques include electroencephalography (EEG), transcranial magnetic stimulation (TMS), and functional magnetic resonance imaging (fMRI) (Kapreli et al., 2009; Needle et al., 2017).

Previous studies suggest that maladaptive neuroplasticity possibly influences the functional reorganization of the motor cortex post-ligamentous injury (Needle et al., 2017). The decrease and alteration of activation in the somatosensory cortex in response to external stimuli, lowered excitability in the corticospinal tract measured via TMS, and further increases in activation in the premotor cortex, planning via the anterior cingulate gyrus, and visual areas during basic movement tasks in individuals with ligamentous injury are all evidence of neuroplasticity occurring in the central nervous system (Grooms et al., 2017; Kapreli et al., 2009; Needle et al., 2014; Needle et al., 2013; Pietrosimone & Gribble, 2012; Valeriani et al., 1996).

During the acute stages of injury there are alterations to sensory feedback and long-term chronic changes to proprioception in the join itself (i.e. joint laxity and peripheral deafferentation) that contribute to down-regulation of excitability in the motor cortex; that therefore, leads to an increase on the demands of extraneous cortical areas during motor tasks (Needle et al., 2017). The deficits to the sensory receptors and somatosensory cortex are negative neuroplastic changes that potentially add to the alteration of an individual's joint proprioception, which could put them at risk for letting their ankle joint reach angles that would make them susceptible to injury or reinjury (Needle et al., 2017). This allows for a prominent disadvantage in the lower extremities as typical leg functions, such as gait and overall balance, require some small amount of cortical input; following injury however, there increased cortical activation is necessary for simple mundane tasks. This takes away from more complex tasks, such as dual-tasking (Taube et al., 2007). These cortical specific changes may therefore elucidate the reasoning as to why traditional rehabilitation, is often effective acutely, however, frequently ineffective in maintaining functional outcomes. This leads to potential future instability, altered arthrokinematics, and subsequent re-injury of the ankle joint. This risk for potential re-injury of

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the ankle could be possibly prevented with the appropriate rehabilitation techniques, such as the use of eccentric training as well as electromagnetic techniques to improve sensorimotor function through stimulation of capsuloligamentous, musculotendinous, and cutaneous receptors (Klykken et al., 2011; Needle et al., 2014, 2017; Pietrosimone & Gribble, 2012). With these different modalities the theory is that they would allow for somatosensational changes, or positive neuroplastic changes, to occur correcting proprioception, load, and movement allowing for correct positioning of the ankle joint as to avoid injury (Needle et al., 2017).

Eccentric Training:

During eccentric contractions the muscle is put under greater loads than the force that it is producing causing a stretch or lengthening contraction under tension (Hedayatpour & Falla, 2015). It is thought that eccentric contractions may result in an increase in hypertrophy of the muscle relative to isometric or concentric contractions due to the stimulation of protein synthesis and an inhibition of protein degradation (Hedayatpour & Falla, 2015; Lepley & Butterfield, 2017). The ability to stress the muscle by putting it under large forces with low metabolic costs and increased neural adaptations further enforces the decision to use an eccentric exercise protocol versus a concentric or isometric exercise protocol (Hedayatpour & Falla, 2015; Lepley & Butterfield, 2017). Both the stretch and overload that is present during an eccentric exercise induces cellular damage to the contractile and structural elements, particularly the elastic protein titin, which signals a physiological response. There is an elongation of the sarcomere via mechanical force, which then leads to the phosphorylation of titin-kinase that initiates tissue growth and an upregulation of various effector cells (e.g., receptors for cell proliferation and

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metabolism) (Hedayatpour & Falla, 2015; Lepley & Butterfield, 2017). Another advantage that mechanical stress causes, through eccentric training, is the proliferation of satellite cells aiding in the muscle recovery process repairing damaged cells or helping to generate new cells (Lepley & Butterfield, 2017).

Additionally, after an ankle injury there is a reduction in alpha motor neuron recruitment and firing rates to those muscle groups in the ankle, causing a decrease in volitional activation of those muscles that leads to subsequent muscle weakness (Lepley & Butterfield, 2017). By starting the recovery process through eccentric training post-injury, injured individuals can recover their neural activity quicker through the decreased amount of cortical muscle inhibition present which requires less volitional muscle activation for a particular eccentric exercise making it less difficult for the individual to elicit a contraction (Lepley & Butterfield, 2017). It has been observed in previous studies that the combination of eccentric contractions with a concurrent neuromuscular stimulus (e.g. neuromuscular electrical stimulation) further enhanced muscle strength via the increase in spinal excitability (Lepley, Wojtys, & Palmieri-Smith, 2015). An increase in the descending neural drive during eccentric training has been seen to increase motor neuron excitability and limit the amount of presynaptic inhibition that is occurring after arthrogenic muscle inhibition takes place (Duclay, Pasquet, Martin, & Duchateau, 2011). The results of a study investigating knee symmetries after an anterior cruciate ligament reconstruction suggested that it is beneficial to add a neuromuscular component during the rehabilitation process, not just strictly a strength protocol, in order to enhance the recovery process (Lepley et al., 2015).

Transcranial Direct Current Stimulation (TDCS):

TDCS is a type of stimulation via an electrode typically focused on an individual's primary motor cortex (M1) and a reference electrode placed over the contralateral supraorbital ridge of the skull (Stagg & Nitsche, 2011). The modern version of transcranial direct current stimulation (TDCS) has been used since the early 2000s as a tool to modulate synaptic plasticity typically in individuals with neurological conditions such as refractory epilepsy, stroke, chronic depression, etc. (Stagg & Nitsche, 2011). Recent research uses TDCS as a method to either increase or decrease cortical excitability (Chang, Kim, & Park, 2015). There are two different techniques of TDCS that can be delivered depending of the desired outcome of the stimulation. Anodal TDCS enhances cortical excitability, and its counterpart, cathodal TDCS, will do the reverse and decrease cortical excitability (Bikson et al., 2016; Chang et al., 2015; Madhavan & Stinear, 2010). For individuals with CAI, the desired outcome of TDCS could be an enhancement of cortical excitability so that long-term potentiation might occur; therefore, anodal TDCS is the ideal method of delivery of stimulation of neuroplasticity (Madhavan & Stinear, 2010; Stagg & Nitsche, 2011).

The best effects from the stimulation are observed when TDCS is given over repeated, spaced out sessions, as opposed to a single TDCS session, due to the fact that a single session's behavioral effects will dissipate after several minutes (Stagg & Nitsche, 2011). The timing of the TDCS is important to note. It was found that when applying TDCS during a motor task the rate of learning was increased compared to applying the TDCS before a motor task (Sriraman, Oishi, & Madhavan, 2014). Generally, stimulation during TDCS intervention last from 10 to 20

minutes at an intensity of 1 mA to 2 mA (Stagg & Nitsche, 2011). Recent investigations have demonstrated that TDCS stimulation over the M1 is able to target the lower extremities in order to correct potential asymmetries and enhance neuroplasticity (Madhavan & Stinear, 2010). Using similar protocols, TDCS has proven effective by increasing the cortical excitability in the lower extremities (Chang et al., 2015), balance (Kaminski et al., 2016), reaction times (Devanathan & Madhavan, 2016), and gait velocity (Tahtis et al., 2014) among healthy and neurologically-impaired populations.

The effects of TDCS on corticospinal excitability and motor learning has been extensively studied in both animal and human models, with results illustrating consistent improvements following short-term interventions, and mixed results were seen following a long-term training intervention (Cooney, Forte, & Carter, 2015; Madhavan & Shah, 2012; Stagg & Nitsche, 2011). During the anodal TDCS intervention the voltage being elicited creates a sustained direct current stimulating the motor cortex (Bikson et al., 2016). This results in a change in the membrane potential in a positive or depolarized state with a decrease in calcium and sodium channel blockers and an opening of calcium voltage gated channels. (Bikson et al., 2016; Schabrun, Chipchase, Zipf, Thickbroom, & Hodges, 2013; Stagg & Nitsche, 2011). The intervention increases cortical excitability and modulating LTP plasticity, allowing for heightened motor learning (Stagg & Nitsche, 2011). In a previous study, healthy populations have shown to produce a ceiling effect in their results, despite if the individuals received a TDCS intervention or a sham intervention, potentially due to the absence of an initial deficit compared to individuals who are neurologically impaired (Maeda et al., 2017). For individuals with CAI, TDCS is an ideal method of potential treatment due to the effect it has had on enhanced cortical excitability in the lower extremities and enhanced balance as well as several other factors in

conjunction with the intervention being short-term (Chang et al., 2015; Kaminski et al., 2016; Stagg & Nitsche, 2011).

Safety:

Considering the location placement of the electrodes on individual's scalp and the presence of a fixed constant electrical stimulation on an individual's head targeting part of their brain, there is valid need for extensive research into the safety of the TDCS intervention technique (Bikson et al., 2016). TDCS is a non-invasive intervention that an individual can be easily trained to operate. The voltage used during TDCS is typically under 20V and the stimulation typically lasts less than 40 minutes at an intensity of 1.5mA (Bikson et al., 2016).

Thus far, there have been no reported serious adverse effects from a TDCS intervention (Bikson et al., 2016). There have been cases of disruption to the skin during stimulation such as erythema; however, this subsides when the stimulation has ceased (Bikson et al., 2016). There have also been no reports of skin lesions at the stimulation site, edema, or alterations to the blood-brain barrier viewed via magnetic resonance imaging (MRI); suggesting that there is no damage conducted to the brain during a TDCS intervention (Bikson et al., 2016). A recent review of the safety of TDCS observed that in 1,000 participants after 33,000 sessions of TDCS stimulation there was no evidence of any irreversible injury to the participants when testing in the following parameters ($\leq$ 40 min, $\leq$ 4 mA, $\leq$ 7.2 C) (Bikson et al., 2016).

Summary:

There is a large demand for more research regarding individuals with CAI given that approximately a third of the population suffer from this impairment (Donovan & Hertel, 2012). Current rehabilitation methods appear to be insufficient, and this may be due to the lack of focus on neuroplasticity that occurs after an injury (Needle et al., 2017). After an ankle sprain, the neuroplasticity occurring behind the injury forms a barrier for individuals experiencing CAI to recover and restore normal movement patterns (Duclay et al., 2011; Klykken et al., 2011). Eccentric training has potential neuromodulatory effects due to its role in decreasing presynaptic inhibition. Conversely, aTDCS has potential neuromodulatory effects through an increase in cortical excitability (Chang et al., 2015; Duclay et al., 2011). Eccentric training in conjunction with aTDCS may improve strength, neural excitability and self-reported measures beyond eccentric exercise alone in patients with CAI.

Chapter 3: Methods

Introduction:

This study incorporated a longitudinal randomized, blinded, and controlled trial. Dependent variables included: resting motor threshold (RMT), stimulus-response curve slope and mid-point intensity (I_{50}), H:M ratio from Hoffmann reflex testing, cortical silent period, dynamic balance via a hop-to-stabilization test, electromyography (EMG), time on a side-hop test, and eversion and inversion ankle strength. Independent variables included: groups (TDCS intervention versus a sham intervention) and time (Testing at baseline, week-2, week-4, and week-6). Participation in this study was comprised of the completion of four testing sessions occurring in 2-week increments, in addition to 10 total training sessions (five training sessions in the first two weeks; five training sessions in the second two weeks, Table 1).

Testing Session	Neurological Variables	Performance and Strength Variables
Baseline	Cortical excitability, reflexive excitability	Ankle strength, dynamic balance; side hop test (SHT)
Participant randomization into intervention groups (aTDCS intervention or sham group)		
2 weeks training (5 total visits with the completion of a progress report the morning after each training visit)		
Week 2	Cortical excitability, reflexive excitability	Ankle strength, dynamic balance; side-hop test (SHT)
2 weeks training (5 total visits with the completion of a progress report the morning after each training visit)		
Week 4	Cortical excitability, reflexive excitability	Ankle strength, dynamic balance; side-hop test (SHT)
Week 6	Cortical excitability, reflexive excitability	Ankle strength, dynamic balance; side-hop test (SHT)

Table 1. A timeline of testing and training sessions that each participant completed with neurological, performance, and strength variables.

Participants:

Twenty-two participants between the ages of 18 to 40 with a history of CAI and recurrent ankle sprains scoring greater than or equal to 11 on the Identification of Functional Ankle Instability (IdFAI) questionnaire (classifying them as having CAI) were recruited from Appalachian State University student body, and the surrounding community in Boone North Carolina through flyers, emails, class talks, posting on the Injury Lab website (injurylab.appstate.edu), and talking to surrounding community gyms (Gribble et al., 2014) (Table 2 & Figure 2). All participants had a history of multiple ankle sprains, with the first being more than one year ago, and continue to experience recurrent sensations of ankle instability. They could have recurrent sensations of ankle instability at any time during or before the study, but if they experienced an instance of instability and sprained or rolled their ankle that limited them from physical activity within the past three months, they were not allowed to continue or

enroll in this study. All participants were willing and able to participate in an ankle training program and had no previous injuries that had inhibited their physical activity in the past 3 months. Participants had no previous history of problems during transcranial magnetic stimulation (TMS) or magnetic resonance imaging (MRI).

	aTDCS	Sham
N	13	13
Age (yrs.)	22.23 (2.81)	22.54 (3.15)
Sex (Male/Female)	3M/10F	6M/7F
Height (cm)	170.08 (9.97)	174.22 (7.53)
Mass (kg)	71.11 (14.70)	81.67 (12.80)
IdFAI Score at Baseline of Involved Limb	21.62 (5.23)	22.77 (6.19)

Table 2. Characteristics of participants who completed this study.
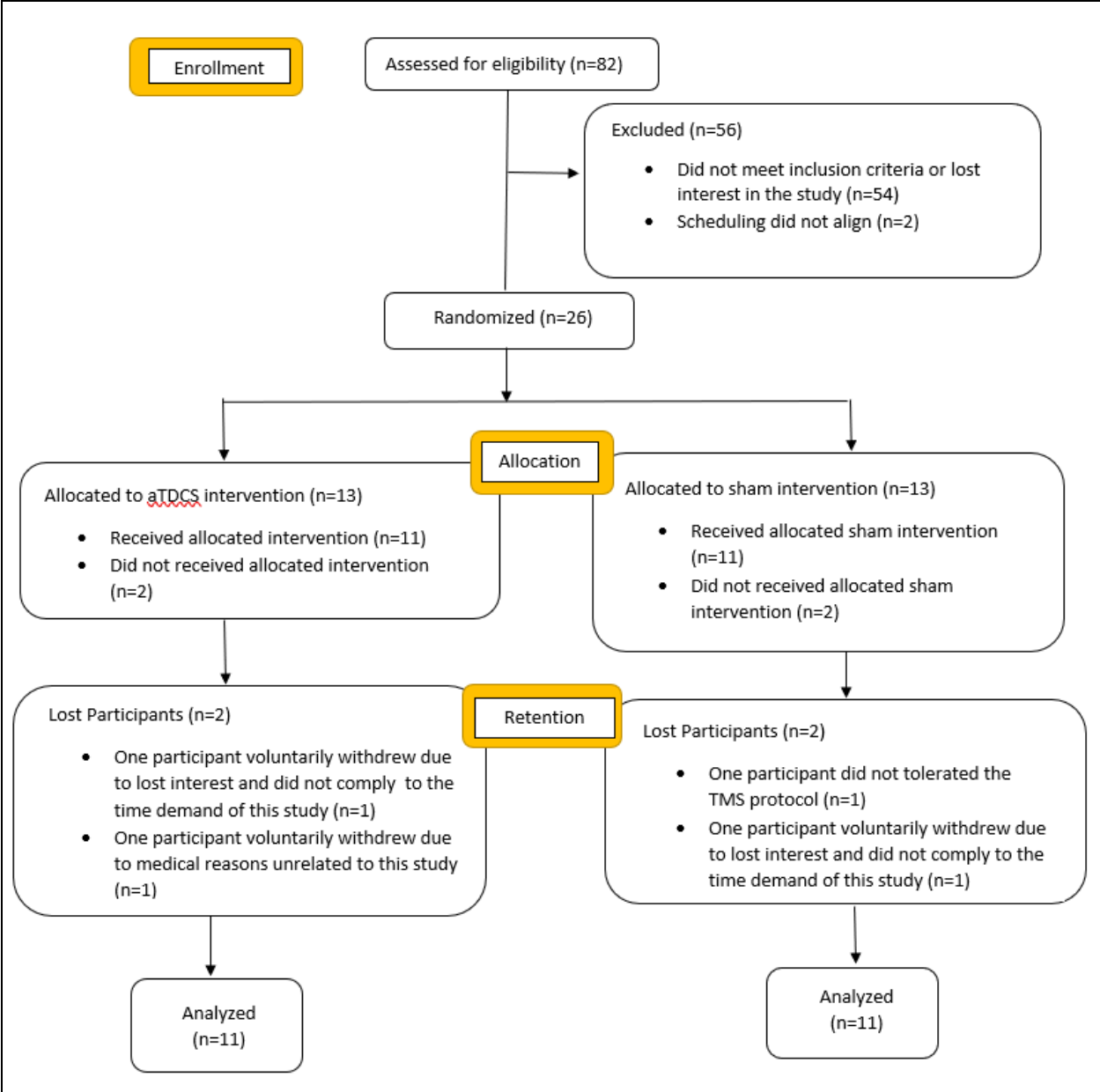
Figure 2. Consort flow diagram depicting enrollment, allocation, and retention of participants.

Participants were excluded if they had never had an ankle injury or experienced CAI, had a recent injury restricting their physical activity within the past 3 months, and/or were currently enrolled in a formal rehabilitation program for ankle instability. They were excluded if they had surgery to their lower extremities, heart, or brain, had a personal or family history of epilepsy or

seizures, experience recurrent syncope or fainting episodes, were taking medications associated with risk of seizure, or had a recent concussion within the last 6 months. They were also excluded if they had any implanted metal (including splinters, fragments, clips, etc.), pacemaker, neurostimulator, or other medical devices, had any hearing problems or cochlear implants, or experience frequent migraines headaches (recurrent or four or more in a year). Female participants were excluded if they were currently pregnant or planning to become pregnant. Lastly, participants were excluded if they had a history of skull fracture or abnormalities (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of TMS Consensus Group, 2009). Participants were asked to refrain from ingesting caffeine, alcohol, and tobacco for the 12 hours prior to reporting to the lab for testing and training sessions. At the initial testing session, group allocation was determined using a size four block randomization into one of two groups: aTDCS intervention group and a sham group. All participants (regardless of group) completed the following procedures during the testing sessions (Table 1). Each testing session took approximately two and a half hours.

Testing Sessions (Baseline, Week-2, Week-4, and Week-6):

Participants came into the Biomechanics and Electrophysiology lab for a total of four testing sessions: baseline, week-2 (training mid-point), week-4 (training completion), and week-6 (retention). The head investigator reviewed a Transcranial Magnetic Stimulation (TMS) safety questionnaire that participants completed at each testing session, ensuring that there were no changes to TMS risk factors since their previous testing session.

Assessment of Neural Excitability:

Prior to assessment of neural excitability, electromyography (EMG) sensors were placed on the muscles of the lower leg (tibialis anterior, TA; peroneus longus, PL; and soleus, SOL). The TA muscle electrode location was determined by instructing the participant to dorsiflex their involved ankle and the area was palpated. Sensor were then placed over the muscle belly lateral to the tibia. The PL muscle electrode location was determined by instructing the participant to evert their ankle and the area was palpated. Once the PL location was identified the sensor was placed over the muscle belly 25% of the distance between the tip of the head of the fibula and the tip of the lateral malleolus. The SOL muscle electrode location was determined by the participant laying prone and plantarflexing their involved ankle. The sensor for the SOL muscle was placed distal from the gastrocnemii heads and slightly lateral. The skin over the aforementioned muscles was palpated, shaved (if necessary), cleaned with an alcohol swab, and lightly abraded before placement of an electrode sensor. The sensor was placed on the leg and secured by an elastic wrap to minimize movement. Participants were seated in an arm-chair and an elastic cap was placed over their head. Participants were familiarized with TMS procedures and the coil was placed over the vertex of the skull while gradually increasing pulses, starting at 10% stimulator output, were applied over the vertex. Once a motor response was observed in the legs, that intensity was recorded and pulses of that intensity was applied every 5s while moving the coil in an approximately 5cm radius to locate the lower extremity hotspot, identified as the location on the skull that leads to the largest response in the lower extremity muscles of the involved limb. The hotspot was measured as deviation from lines measured from the pre-auricular points on each ear, and from the nasion to inion of the skull. These measurements were used to ensure proper location of aTDCS electrodes for subsequent training sessions. Once identified, the coil

was positioned at the hotspot at a range of randomized intensities, from below the point a motor response was observed to above the point a maximal leg response was observed, was applied in a randomized order every 5-7s for 50 pulses. The peak-to-peak amplitudes of the motor evoked potentials (MEP) were obtained and then plotted against the stimulus intensity, forming the stimulus-response curve using a Levenberg-Marquardt nonlinear fit with a modified Boltzmann equation (Equation 1) (Devanne, Lavoie, & Capaday, 1997; Needle et al., 2013). This data was used to identify the resting motor threshold (RMT) – the intensity that a motor response is first observed. Ten pulses were administered at 90, 110, and 130% of the RMT (n=30) as participants maintain a light contraction of their PL muscle to extract the dependent variables of interest (Stirling, McBride, Merritt, & Needle, 2018). The peak-to-peak amplitudes, 20-100ms post-stimulus, were extracted and normalized to M_{max} from electrical stimulation. MEPs from 90, 110, and 130% stimulation pulses were assessed for the cortical silent period (CSP), which is a measure of GABA-related intracortical inhibition. EMG activity was logarithmically-transformed and normalized to pre-stimulus activity to 4ms post-stimulus when EMG activity would return to pre-stimulus levels. Cursors were positioned on a graph in a Labview program (National Instruments, Austin, TX) by a trained investigator confirming locations were accurate, extracting the time between the start of the MEP and the resumption of normal EMG activity, defining that as the CSP (Stirling et al., 2018).

Equation 1.

$$y = \text{MEP}_{min} + \frac{(\text{MEP}_{max} - \text{MEP}_{min})}{1 + e^{m(I_{50}-x)}}$$

Participants were then instructed to lay prone on a padded table and their reflexive excitability was assessed using the Hoffmann reflex (H-reflex). The location of the sciatic nerve was identified in the popliteal fossa, prior to its bifurcation into tibial and common peroneal divisions, using brief (<1ms) electrical pulses at a suprathreshold intensity using a simulator (DS7AH Constant Current Stimulator, Digitimer LTD, Hertfordshire, UK). At this location, the pulse intensity was turned down to a sub-threshold intensity then gradually increasing pulses (square wave, < 1ms duration, 10s interpulse interval, increasing by 2mA) was applied at this location until maximal motor responses were observed across all three muscles (Hoffman, Palmieri, & Ingersoll, 2003). The peak-to-peak amplitudes of the H waves and M waves were measured and the maximum values for H waves and M waves were used to form the $H_{max}:M_{max}$ ratio which was then used for analysis (Hoffman et al., 2003).

Assessment of Dynamic Balance:

Dynamic balance was assessed using a hop to stabilization test on the involved limb. Participants performed 3 maximal jumps on a Vertec device (Sports Imports, Hillard, OH) to determine their maximal jump height. Participants were then instrumented with three electromyography (EMG) sensors on three muscles of the lower leg (TA, PL, and SOL) in the same manner as mentioned above in the neural excitability section. Participants were instructed to stand 70cm from the force plate and hop to a height of 50% of their maximum height average (with the Vertec device set to this height to provide a target). They landed forward on a force plate with their affected ankle limb and stabilize as quickly as possible with their hands on their hips and maintained balance for 15s (Figure 3). EMG data were extracted and used to create a

complete linear envelope by conducting a bandpass filter (20-400 Hz), rectification, and lowpass filter (10 Hz). The data were then normalized to ensemble peak activity of each muscle, and a extraction 250ms prior to and following initial ground contact with the force plate was used to obtain average EMG values used to determine a pre- and post-phase (Rosen et al., 2013). Participants performed this maneuver five successful times, without touchdown of the uninvolved limb or stabilizing by grabbing the Vertec with their hands, dynamic postural stability indices (DPSIs) were then calculated from ground reaction forces (Wikstrom, Tillman, Smith, & Borsa, 2005). Additionally, anteroposterior postural stability indices (APSIs), medio-lateral stability indices (MLSIs), and vertical stability indices (VSIs) were also calculated (Wikstrom et al., 2005) (Equations 2-5). The variable y refers to anterior or posterior movement, x refers to medial or lateral movement, and z refers to vertical movement.

Equation 2:

$$DPSI = \sqrt{\frac{\Sigma(0-x)^2 + \Sigma(0-y)^2 + \Sigma(\text{body weight} - z)^2}{\text{number of data points}}}$$

Equation 3:

$$APSI = \sqrt{\frac{\Sigma(0-y)^2}{\text{number of data points}}}$$

Equation 4:

$$MLSI = \sqrt{\frac{\Sigma(0-x)^2}{\text{number of data points}}}$$

Equation 5:

$$VSI = \sqrt{\frac{\Sigma(\text{body weight} - z)^2}{\text{number of data points}}}$$

Figure 3. Dynamic balance task for postural stability, kinematic, and EMG analysis.

Assessment of Side-Hop:

A double leg side-hop test was conducted with two parallel lines, oriented from front-to-back, were placed 30cm apart on the ground. Participants was asked to perform 10 lateral hops in each direction as quickly as possible, clearing the lines on the ground each time. Time to completion was extracted for analysis.

Assessment of Ankle Strength:

Ankle strength was assessed on an isokinetic dynamometer (HUMAC NORM, Computer Sports Medicine Inc., Stoughton, MA) to quantify direct strength gains from the eccentric training intervention. Participants were seated with the hip at approximately 60 degrees, the knee flexed at about 90 degrees, and the foot secured in a footplate adapter (Figure 4). Participants were tested for peak concentric and eccentric ankle inversion and eversion torques at 30 and 90 degrees per second through 30 degrees range of motion. The participant had multiple trial attempts, allowing them to become familiarized with the procedure. Participants underwent concentric then eccentric contractions where during test trials, they were instructed to resist the motion of the dynamometer as hard and as fast as possible by turning their ankle outward (in the direction of eversion). Five test trials for each concentric and eccentric torque in inversion and eversion were performed at 30 and 90 degrees per second and were used for analysis.

Figure 4. A picture of the body and dynamometer positioning during the strength portion of the testing sessions.

Training Sessions (n=10):

Participants completed a total of ten training sessions. Five were completed in the first two weeks and five in the following two weeks (2-3 sessions per week). The training sessions were briefer than the testing sessions. All participants partook in the following procedures once they arrived to the lab (Table 3):

Time (mins)	aTDCS	Sham
	5-minute bicycle warm-up	*5-minute bicycle warm-up*
0-2	*Instrumentation* aTDCS ramp on	*Instrumentation* aTDCS ramp on
		aTDCS gradually ramp down and completely turned off by 2 minutes
2-3	Set of 10 eccentric contractions	Set of 10 eccentric contractions
3-6	Rest	Rest
6-7	Set of 10 eccentric contractions	Set of 10 eccentric contractions
7-10	Rest	Rest
10-11	Set of 10 eccentric contractions	Set of 10 eccentric contractions
11-14	Rest	Rest
14-15	Set of 10 eccentric contractions	Set of 10 eccentric contractions
15-18	Rest	Rest

Table 3. A timeline of the procedures conducted during training sessions.

The participant underwent concentric contractions at 10 degrees per second moving in an everted direction and then eccentric contractions moving at 30 degrees per second moving in an inverted direction. In order to complete each repetition, participants must elicit at least 60% of their peak torque value that was recorded on their previous testing day (Lepley et al., 2018). If that torque requirement was not met, the dynamometer attachment would not move and they were given assistance by the researcher until they completed that set. If there were sequential sets they must complete after they failed to reach the torque requirement, then the researcher lowered their upcoming set's torque requirement by 10%. If the participant completes each of the four sets without assistance then the researcher increased their torque requirement for the following training session by 10%. Each training session lasted approximately 20-25 minutes. The difference between aTDCS and sham groups is that – while all participants were instrumented with aTDCS electrodes and turned on for a 2-minute ramp-up – the sham group had their aTDCS

turned off after the 2-minute ramp-up. This was completed by flipping the "sham" switch on the aTDCS stimulator (1x1, Soterix, New York, NY). However, the aTDCS group received aTDCS over the primary motor cortex at an intensity of 1.5mA for 18 minutes (duration of eccentric testing) (Bikson et al., 2016).

aTDCS Procedures:

Prior to participant arrival, all materials for collection (sponge electrodes, saline, stimulator, cables, measurement tape, and elastic bands) were gathered and inspected for any signs of damage. The investigator asked the participant to complete the adverse event report to track any skin irritation that occurred in the prior session and presence of exclusion criteria were reconfirmed as any changes from the initial questionnaire. The scalp was inspected for any lesions or irritation. The vertex was marked on the skin using measurements from the previous hotspot assessment (at baseline and week-2). The area of the skin was cleaned with an alcohol pad. A plastic head set was placed around the head circumference, inferior to the inion and above the eyebrows, and across the top of the head over the motor cortex. The 5x3 sponge electrodes were soaked with 4-6mL of saline solution until saturated, but not dripping. The cables were connected to the aTDCS device and the rubber electrode connected to the unit was placed in the sponge electrode, in the pocket opening. The cathode sponge was placed over the forehead on the ipsilateral side of the treated leg (contralateral to hotspot). The anode sponge was placed over the hotspot location (Figure 5 & 6). The aTDCS stimulator then elicited a pre-stimulus tickle to familiarize the participant to the sensation of stimulation, and the stimulator was set for the preset settings of 1.5 mA for 20 minutes, and the start button was engaged. A separate timer kept

a running time, so that the researcher would know when 18 minutes passed. At the completion of

18 minutes the aTDCS stimulator was off. The participant was informed of tickling or itching

sensations that are associated with the electrical stimulus period, and the participant was
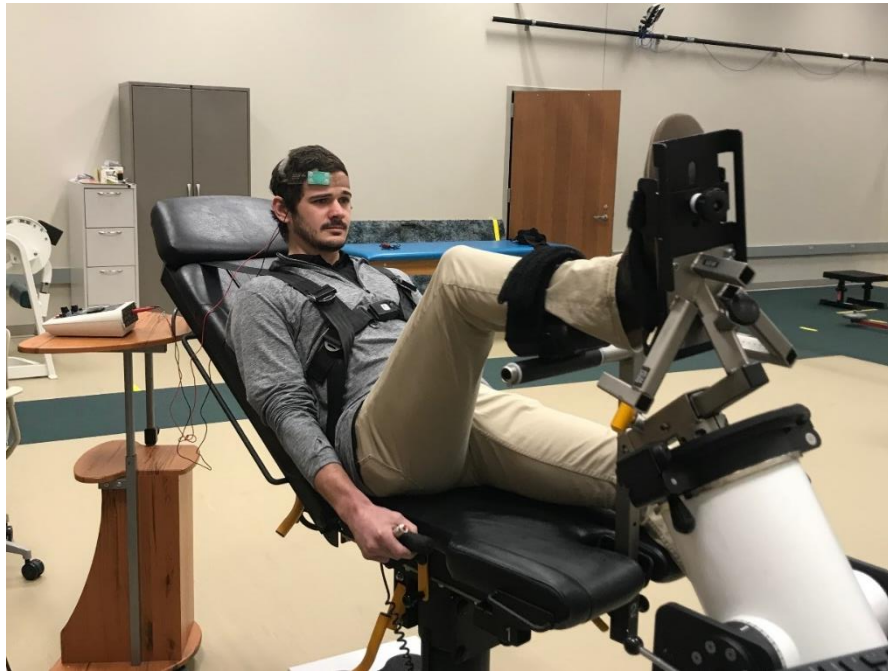
continuously monitored during treatment.

Figure 5. A front view of the set up on the dynamometer and the aTDCS electrodes inside of the
green sponge on the contralateral supraorbital side of the hotspot.

Figure 6. A side view of the set up on the dynamometer and the aTDCS electrodes inside of the green sponge above the hotspot.

Analysis:

TMS, H-reflex, balance, and EMG data were analyzed in a custom LabVIEW program (National Instruments, Austin, TX). The data that was analyzed by utilizing factorial analyses of variance, with the primary independent variables of interest being the interaction of time (4-levels, baseline, week-2, week-4, and week-6) by group (2-levels; TDCS vs Sham). For H-reflex, muscle (3 levels) was additionally considered as an independent variable to explore differences in modulation across ankle muscle. For CSP, intensity (3-levels, 90, 110, or 130) was considered an additional independent variable to account for changes in modulation of inhibition. For hop-to-stabilization, direction (3-levels; APSI, MLSI, VSI) were considered as an additionally independent variable. Finally, for strength measurements, speed (2-levels, 30 versus 90 degrees per second) were considered an additional independent variable. Cases were assessed with a per protocol analysis. Fisher's least significant difference (LSD) was used *post hoc* to determine

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locations of specific differences. Partial eta squared was used as a measure of effect size with 0.01, 0.06, and 0.14 being considered small, medium, and large, respectively (Cohen, 1988). An a priori significance level was set as 0.05.

TMS variable RMT was calculated from the stimulus-response curve obtained through the Levenberg-Marquardt nonlinear fit with a modified Boltzmann equation (Equation 1.). The I_{50} and slope (m) were estimated through this equation as well (Needle et al., 2013). The CSP was calculated manually through the EMG data pre stimulus and post stimulus. Cursors were positioned on a graph by a trained investigator confirming locations of pre stimulus and post stimulus were accurate, extracting the time between the start of the MEP and the resumption of normal EMG activity, defining that as the CSP (Stirling et al., 2018). H-reflex data were computed through peak-to-peak amplitudes of the H waves and M waves were measured and the maximum values for H waves and M waves were used to form the H_{max}:M_{max} ratio which was then used for analysis (Hoffman et al., 2003). Dynamic balance was calculated through the combination of equations for DPSI, APSI, MLSI, and VSI (Equation 2-5) (Wikstrom et al., 2005). Side-hop time to completion was record and used for analysis. Lastly, peak torque strength measures were collected from the dynamometer and used for analysis.

Chapter 4: Results

Neural Measurements:

TMS:

Resting Motor Threshold:

There was a non-significant time-by-group interaction effect for the tibialis anterior ($F_{[3,48]}$=1.460; p=0.237; η_p^2=0.084). There were no significant main effects of time or group from the tibialis anterior (p>0.05) (Figure 7).

There was a significant time-by-group interaction effect for the peroneus longus ($F_{[3,51]}$=3.401; p=0.025; η_p^2=0.167). Fisher's LSD comparisons revealed significant differences in the sham group at week-2 compared to other weeks (p≤0.022). Week-2 values in the sham group were lower, indicating more excitability, compared to baseline (p=0.007), week-4 (p=0.022), and week-6 (p=0.006). Significant differences were also observed in the aTDCS group where week-6 values were lower than week-2 indicating increased excitability at week-6 relative to week-2 (p=0.024) (Table 4 & Figure 7).

Resting Motor Threshold					
		Baseline	Week 2	Week 4	Week 6
TA	Sham	30.75 (10.20)	29.41 (13.90)	36.57 (13.68)	37.31 (15.76)
	aTDCS	38.54 (13.91)	34.83 (13.63)	36.55 (6.02)	32.90 (7.97)
PL	Sham	36.67 (12.74)	27.86 (14.69)[a]	35.63 (13.10)[b]	35.99 (13.52)[b]
	aTDCS	36.92 (11.53)	39.02 (9.30)	37.46 (9.22)	32.91 (12.33)[a]

Table 4. Means (Standard Deviations) for Resting Motor Threshold across times and groups.

[a] Significant difference from baseline (p<0.05).

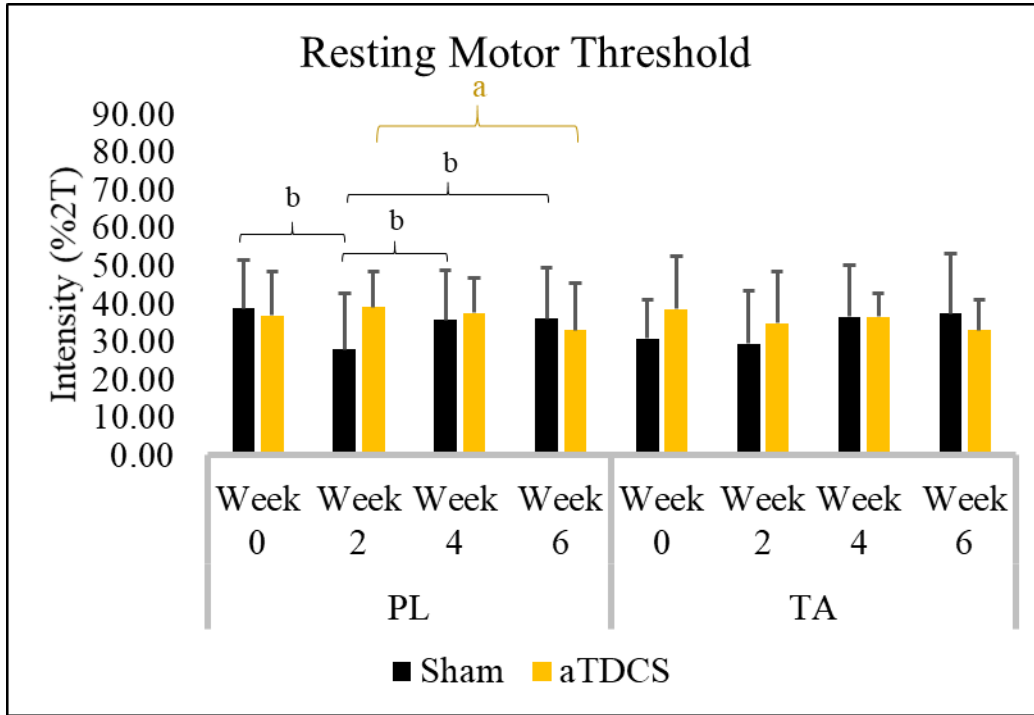[b] Significant difference from week-2 (p<0.05).

Figure 7. PL and TA resting motor threshold values across group and time.

[a] Significant differences in aTDCS group (p<0.05)

[b] Significant differences in Sham group (p<0.05)

I_{50}:

There was a significant time-by-group interaction effect for the tibialis anterior

($F_{[3,48]}$=4.538; p=0.007; η_p^2=0.221). Fisher's LSD comparisons revealed significant differences in

the sham group at week-2 compared to week-4 and week-6 (p≤0.044) and from baseline to week-

6 (p=0.016). Significant differences were observed in the sham group where values increased to

week-2 through the end of the intervention, indicating less excitability (week-4, p=0.044; week-

6, p=0.016). In the aTDCS group, significant differences were also observed where I_{50} decreased indicating more excitability between baseline and week-6, (p=0.047) (Table 5 & Figure 8).

There was a significant time-by-group interaction effect for the peroneus longus ($F_{[3,51]}$=5.290; p=0.003; η_p^2=0.237). Fisher's LSD comparisons revealed significant differences in the sham group at week-2 (p≤0.026). Significant differences were observed in the sham group, where week-2 values were lower than all other time points, indicating more excitability from baseline, and less excitability at week-4 and week-6 (baseline, p=0.026; week-4, p=0.019 week-6, p=0.001). Significant differences were also observed in the aTDCS group, where week-6 values were lower, indicating more excitability, than baseline, (p=0.025) and week-4 (p=0.001) (Table 5 & Figure 8).

		I_{50}			
		Baseline	Week 2	Week 4	Week 6
TA	Sham	49.06 (10.40)	44.62 (12.96)	53.08 (8.01)[b]	54.14 (11.42)[b]
	aTDCS	53.42 (6.19)	54.67 (11.92)	52.05 (733)	49.26 (5.93)[a]
PL	Sham	51.11 (11.27)	45.47 (10.62)[a]	52.31 (11.30)[b]	53.91 (12.04)[b]
	aTDCS	51.97 (6.47)	51.35 (9.38)	55.89 (7.63)	47.42 (5.633)[ac]

Table 5. Mean (Standard Deviation) of I_{50} across muscle, group, and time.

[a] Significant differences from baseline (p<0.05)

[b] Significant differences from week-2 (p<0.05)

[c] Significant differences from week-4 (p<0.05)
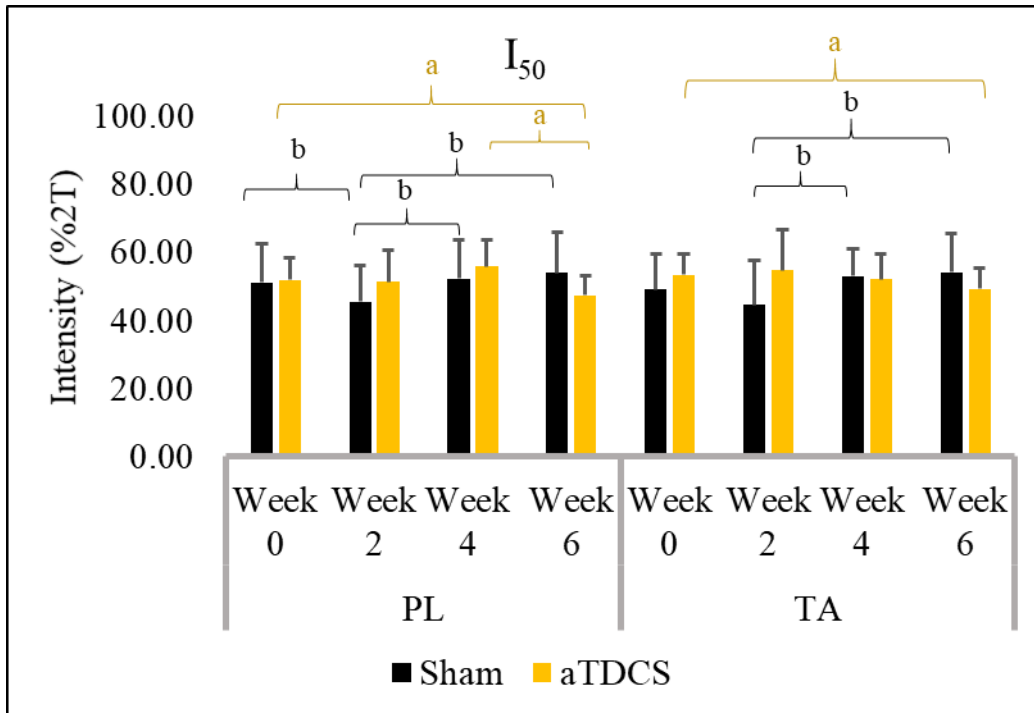
Figure 8. PL and TA of I_{50} values across group and time.
[a] Significant differences in aTDCS group (p<0.05)

[b] Significant differences in Sham group (p<0.05)

Slope:

There was a non-significant time-by-group interaction effect for the tibialis anterior ($F_{[3,48]}$=0.333; p=0.802; η_p^2=0.020). There were no main effects of time or group (p>0.05) (Table 6).

There was a non-significant time-by-group interaction effect for the peroneus longus ($F_{[3,51]}$=1.200; p=0.319; η_p^2=0.319). There were no main effects of time or group (p>0.05) (Table 6).

Slope (m)		Baseline	Week 2	Week 4	Week 6
TA	Sham	0.23 (0.13)	0.85 (1.94)	0.33 (0.22)	0.92 (2.13)
	aTDCS	0.38 (0.30)	0.85 (1.86)	0.28 (0.14)	0.43 (0.60)
PL	Sham	0.30 (0.10)	0.24 (0.14)	0.25 (0.11)	0.20 (0.05)
	aTDCS	0.31 (0.22)	0.36 (0.21)	0.21 (0.08)	0.33 (0.20)

Table 6. Mean (Standard Deviation) of slope (m) across muscle, group, and time.

Silent Period:

There were non-significant time-by-intensity-by-group ($F_{[6,24]}=1.433$; p=0.243; $\eta_p^2=0.264$), time-by-intensity ($F_{[6,24]}=1.810$; p=0.140; $\eta_p^2=0.311$), intensity-by-group ($F_{[2,8]}=1.242$; p=0.339; $\eta_p^2=0.237$), and time-by-group ($F_{[3,12]}=0.260$; p=0.853; $\eta_p^2=0.061$) interaction effects for cortical silent period for the peroneus longus. There was a significant main effect of intensity ($F_{[2,8]}=13.636$; p=0.003; $\eta_p^2=0.773$). As intensities increased so did the length of the silent period (p<0.01). There were no significant main effects of time or group (p>0.05) (Table 7).

Cortical Silent Period (ms)					
Intensity		Baseline	Week 2	Week 4	Week 6
90	Sham	155.50 (75.66)	127.00 (14.14)	117.50 (68.59)	99.50 (48.79)
	aTDCS	164.50 (57.45)	136.50 (47.29)	133.50 (30.69)	143.00 (81.13)
110	Sham	266.00 (154.15)	202.50 (33.23)	232.00 (80.61)	244.50 (167.58)
	aTDCS	249.00 (84.34)	226.25 (72.57)	274.50 (68.01)	245.75 (109.61)
130	Sham	269.50 (102.53)	165.50 (16.26)	327.00 (14.14)	179.00 (67.88)
	aTDCS	345.25 (72.53)	327.00 (105.67)	342.25 (96.07)	311.25 (89.84)

Table 7. Mean (Standard Deviation) of the Cortical Silent Period across intensity, group, and time.

H-Reflex:

For reflexive excitability, there were non-significant time-by-muscle-by-group $(F_{[6,20]}=0.457; p=0.839; \eta_p^2=0.022)$, time-by-muscle $(F_{[6,120]}=0.396; p=0.222; \eta_p^2=0.65)$, muscle-by-group $(F_{[2,40]}=1.645; p=0.206; \eta_p^2=0.076)$ and time-by-group $(F_{[3,60]}=1.165; p=0.331; \eta_p^2=0.055)$ interaction effects. There were no significant main effects of time or group ($p>0.05$) (Table 8).

		H:M Ratio ($\%M_{max}$)			
		Baseline	Week 2	Week 4	Week 6
TA	Sham	0.25 (0.18)	0.35 (0.22)	0.32 (0.13)	0.23 (0.13)
	aTDCS	0.21 (0.17)	0.24 (0.13)	0.19 (0.19)	0.18 (0.11)
PL	Sham	0.29 (0.18)	0.31 (0.17)	0.31 (0.13)	0.20 (0.11)
	aTDCS	0.19 (0.08)	0.22 (0.14)	0.19 (0.14)	0.21 (0.14)
SOL	Sham	0.26 (0.21)	0.24 (0.23)	0.21 (0.15)	0.19 (0.09)
	aTDCS	0.28 (0.20)	0.27 (0.20)	0.19 (0.16)	0.24 (0.17)

Table 8. Mean (Standard Deviation) of H:M ratio across muscle, group, and time.

Performance Measures:

Postural Stability Indices:

There was a significant time-by-group interaction effect for DPSI ($F_{[3,60]}=2.952; p=0.040; \eta_p^2=0.129$). Fisher's LSD post hoc comparisons did not reveal significant differences between groups or times (Table 9). In order to further investigate this DPSI was divided into its individual components anterior-posterior stability indices (APSI), medial-lateral stability indices (MLSI), and vertical stability indices (VSI).

There were non-significant time-by-direction-by-group ($F_{[6,120]}=1.237$; p=0.292; $\eta_p^2=0.058$), time-by-direction ($F_{[6,120]}=0.623$; p=0.711; $\eta_p^2=0.030$), and direction-by-group ($F_{[2,40]}=0.755$; p=0.477; $\eta_p^2=0.036$) interaction effects for PSI components (APSI, MLSI, VSI). There was a significant time-by-group interaction effect ($F_{[3,60]}=3.087$; p=0.034; $\eta_p^2=0.134$) (Figure 9). There was also a significant main effect of direction ($F_{[2,40]}=1000.077$; p<0.001; $\eta_p^2=0.980$). Fisher's LSD comparisons revealed significant differences in the aTDCS group, where PSI values decreased from baseline to week-6 (p=0.010) and week-4 to week-6 (p=0.026), indicating better postural stability. Directional effects revealed VSI values higher than MLSI & APSI (p<0.001), and APSI values greater than MLSI (p<0.001) (Table 9).

Postural Stability Indices					
		Baseline	Week 2	Week 4	Week 6[ab]
DPSI	Sham	0.50 (0.05)	0.52 (0.07)	0.51 (0.05)	0.51 (0.06)
	aTDCS	0.50 (0.07)	0.49 (0.06)	0.49 (0.04)	0.47 (0.05)
APSI	Sham	0.12 (0.03)	0.10 (0.05)	0.10 (0.05)	0.11 (0.04)
	aTDCS	0.12 (0.04)	0.11 (0.04)	0.13 (0.02)	0.10 (0.05)
MLSI	Sham	0.04 (0.004)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)
	aTDCS	0.04 (0.02)	0.04 (0.02)	0.03 (0.01)	0.04 (0.01)
VSI	Sham	0.47 (0.05)	0.50 (0.07)	0.49 (0.06)	0.50 (0.07)
	aTDCS	0.48 (0.07)	0.47 (0.06)	0.47 (0.04)	0.46 (0.06)

Table 9. Mean (Standard Deviation) of Postural Stability Indices across group and time.

[a] Significant difference in aTDCS group from baseline (p<0.05).

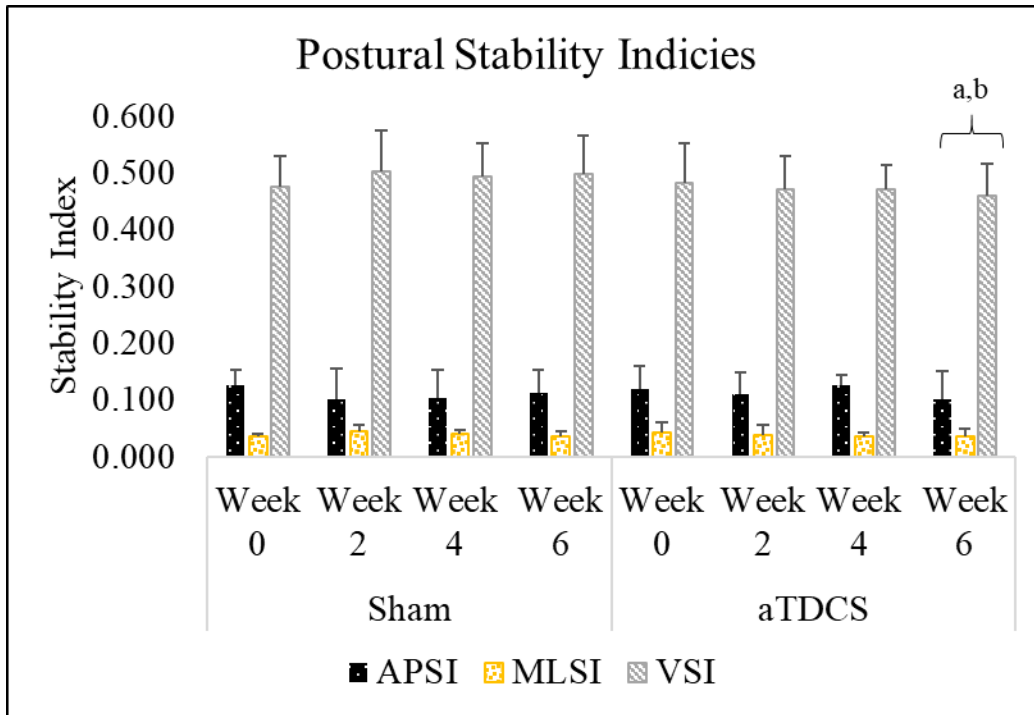[b] Significant difference in aTDCS from week-4 (p<0.05).

Figure 9. Postural stability indices standard deviation for APSI, MLSI, and VSI across group and time.

[a] Significant differences in aTDCS group from baseline (p<0.05)

[b] Significant differences in aTDCS group from week-4 (p<0.05)

Side-Hop Test:

There was a non-significant time-by-group interaction effect for the side-hop test ($F_{[3,60]}$=0.376; p=0.770; η_p^2=0.018). There was a significant main effect of time ($F_{[6,24]}$=5.272; p=0.003; η_p^2=0.209); and a non-significant main effect of group ($F_{[1,20]}$=0.619; p=0.441; η_p^2=0.030). Fisher's LSD comparisons revealed significant differences in both groups where baseline values were greater than values from all subsequent time points (week-2, p=0.024 week-4, p=0.016; week-6, p=0.032) (Figure 10).
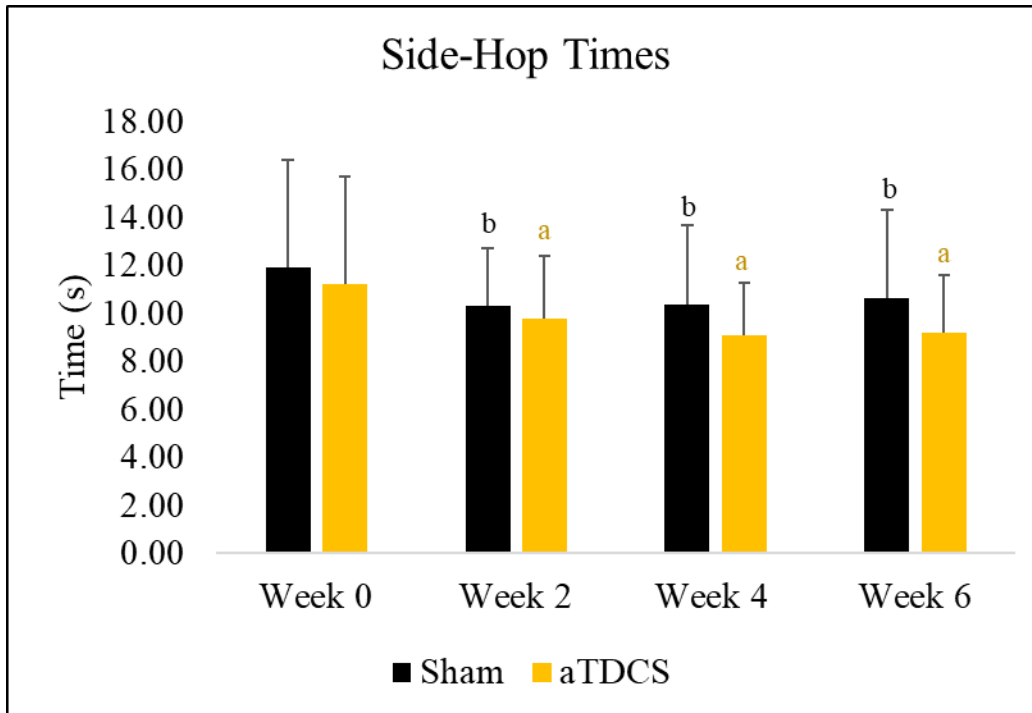
Figure 10. Side-Hop times across group and time.

[a] Significant differences in aTDCS group (p<0.05)

[b] Significant differences in Sham group (p<0.05)

Muscle Activation:

Tibialis anterior activation revealed non-significant time-by-phase-by-group ($F_{[3,60]}$=1.945; p=0.132; η_p^2=0.089), time-by-phase ($F_{[3,60]}$=1.408; p=0.249; η_p^2=0.066), and phase-by-group ($F_{[1,20]}$=0.077; p=0.784; η_p^2=0.004) interaction effects. There was a significant time-by-group interaction effect ($F_{[3,60]}$=3.524; p=0.020; η_p^2=0.150). There was a significant main effect of phase ($F_{[1,20]}$=91.468; p<0.001; η_p^2=0.821). Fisher's LSD comparisons revealed significant differences in the sham group, where activation decreased from baseline to all other time points (week-2, p=0.020; week-4, p=0.002; week-6, p<0.001). The sham group also significantly decreased TA

activation from week-2 to week-6 (p=0.036). Across all participants, TA activation increased from 250ms prior to landing compared to 250ms post landing (p<0.001) (Table 8 & Figure 11).

There was a significant time-by-phase-by-group interaction effect for peroneus longus activation ($F_{[3,60]}$=4.302; p=0.008; η_p^2=0.177). Fisher's LSD comparisons revealed significant differences in the aTDCS group, where activation increased in the post phase (250ms after landing) from baseline to week-6 (p=0.044). Significant differences were also observed in the sham group, where in the pre phase (250ms prior to landing), activation increased from baseline to week-2 (p=0.049) (Table 8 & Figure 12).

For soleus activation, there were non-significant time-by-phase-by-group ($F_{[3,60]}$=1.062; p=0.372; η_p^2=0.050), time-by-phase ($F_{[3,60]}$=0.514; p=0.674; η_p^2=0.025), phase-by-group ($F_{[1,20]}$=0.001; p=0.974; η_p^2=0.000), and time-by-group ($F_{[3,60]}$=0.547; p=0.652; η_p^2=0.027) interaction effects. There was a significant main effect of phase ($F_{[1,20]}$=11.760; p=0.003; η_p^2=0.370). Across all participants, SOL activation decreased from 250ms post landing compared to 250ms prior to landing (p<0.001). There were no significant main effects of time or group (Table 10).

EMG – Dynamic Balance (%Max)						
			Baseline	Week-2	Week-4	Week-6
TA	250-ms	Sham	0.32 (0.10)	0.27 (0.10)[a]	0.23 (0.07)[a]	0.25 (0.08)[ab]
	pre	aTDCS	0.31 (0.12)	0.22 (0.09)	0.22 (0.08)	0.26 (0.10)[b]
	250-ms	Sham	0.58 (0.11)	0.51 (0.12)[a]	0.48 (0.09)[a]	0.46 (0.12)[ab]
	post	aTDCS	0.46 (0.16)	0.47 (0.15)	0.49 (0.16)	0.49 (0.15)[b]
PL	250-ms	Sham	0.46 (0.12)	0.55 (0.12)[a]	0.52 (0.12)	0.50 (0.11)
	pre	aTDCS	0.49 (0.12)	0.52 (0.12)	0.48 (0.07)	0.48 (0.09)
	250-ms	Sham	0.56 (0.16)	0.57 (0.12)	0.57 (0.11)	0.58 (0.10)
	post	aTDCS	0.51 (0.12)	0.61 (0.10)[c]	0.58 (0.14)[c]	0.60 (0.11)[ac]
SOL	250-ms	Sham	0.66 (0.12)	0.61 (0.14)	0.60 (0.11)	0.57 (0.14)
	pre	aTDCS	0.58 (0.09)	0.59 (0.13)	0.63 (0.06)	0.59 (0.12)
	250-ms	Sham	0.51 (0.14)	0.45 (0.15)	0.46 (0.16)	0.44 (0.16)
	post	aTDCS	0.49 (0.16)	0.47 (0.19)	0.42 (0.17)	0.44 (0.21)

Table 10. Mean (Standard Deviation) for EMG activity values during the dynamic balance task across muscle, phase, group, and time.

[a] Significant difference from baseline ($p < 0.05$)

[b] Significant difference from week-2 ($p < 0.05$)

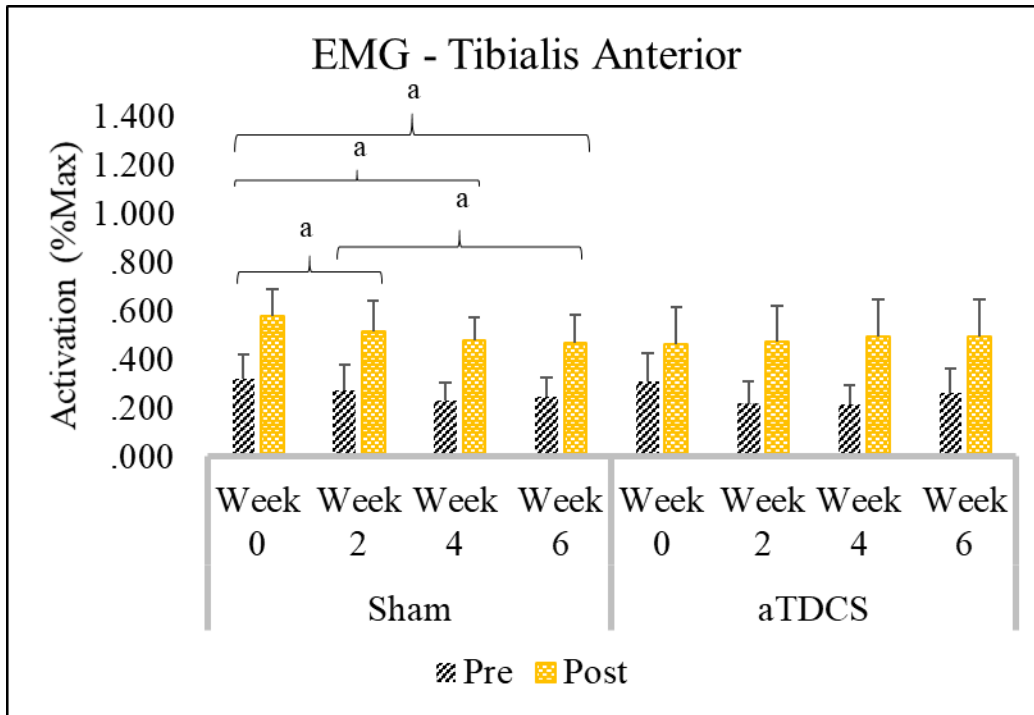[c] Significant increase from 250-ms pre to 250-ms post ($p < 0.05$)

Figure 11. EMG activity values of TA during the dynamic balance task across phase, group, and time.
[a] Significant differences in the Sham group regardless of phase (p<0.05).). Pre phase was significantly different from the post phase.
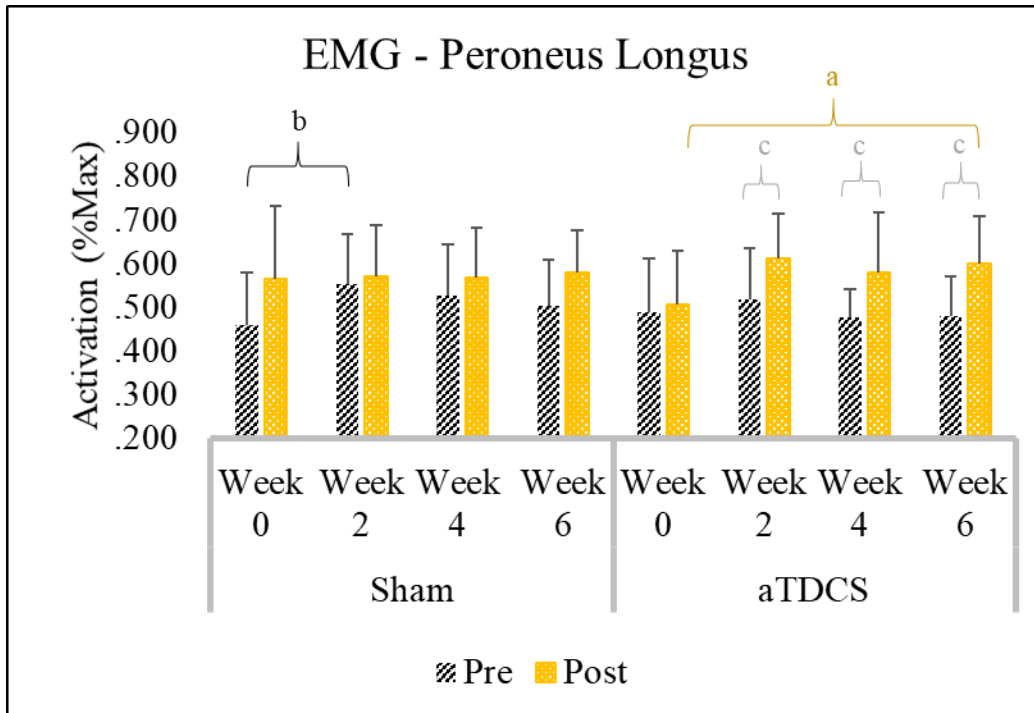
Figure 12. EMG activation values of PL during the dynamic balance task across phase, group, and time.

[a] Significant differences in aTDCS group (p<0.05)

[b] Significant differences in Sham group (p<0.05)

[c] Significant differences between phases (p<0.05)

Strength Measurements:

For concentric inversion strength there were non-significant time-by-speed-by-group ($F_{[3,60]}$=0.511; p=0.677; η_p^2=0.025), speed-by-time ($F_{[3,60]}$=0.411; p=0.746; η_p^2=0.020), speed-by-group ($F_{[1,20]}$=0.489; p=0.492; η_p^2=0.024), and time-by-group ($F_{[3,60]}$=0.317; p=0.813; η_p^2=0.016) interaction effects. There was a significant main effect of speed ($F_{[1,20]}$=5.201; p=0.034; η_p^2=0.206). There were no significant main effects of time or group. Inversion strength was greater at 30 degrees per second than 90 degrees per second (p=0.034) (Table 11).

For concentric eversion strength there were non-significant time-by-speed-by-group $(F_{[3,60]}=1.278; p=0.290; \eta_p^2=0.060)$, time-by-speed $(F_{[3,60]}=0.417; p=0.741; \eta_p^2=0.020)$, speed-by-group $(F_{[1,20]}=0.649; p=0.430; \eta_p^2=0.031)$, time-by-group $(F_{[3,60]}=0.216; p=0.885; \eta_p^2=0.011)$ interaction effects. There was a significant main effect of speed $(F_{[1,20]}=16.851; p=0.001; \eta_p^2=0.011)$. There was no significant main effects of time or group. Eversion strength was greater at 30 degrees per second than 90 degrees per second (p=0.001) (Table 11).

For eccentric inversion strength there were non-significant time-by-speed-by-group $(F_{[3,60]}=0.382; p=0.766; \eta_p^2=0.019)$, time-by-speed $(F_{[3,60]}=0.523; p=0.668; \eta_p^2=0.025)$, speed-by-group $(F_{[1,20]}=0.097; p=0.758; \eta_p^2=0.005)$, and time-by-group $(F_{[3,60]}=0.087; p=0.967; \eta_p^2=0.004)$ interaction effects. There were no significant main effects of speed, time, or group (p>0.05) (Table 11).

For eccentric eversion strength there were non-significant time-by-speed-by-group $(F_{[3,60]}=0.733; p=0.536; \eta_p^2=0.035)$, time-by-speed $(F_{[3,60]}=1.967; p=0.129; \eta_p^2=0.090)$, speed-by-group $(F_{[1,20]}=0.085; p=0.773; \eta_p^2=0.004)$, and time-by-group $(F_{[3,60]}=0.090; p=0.965; \eta_p^2=0.005)$ interaction effects. There were no significant main effects of speed, time or group (p>0.05) (Table 11).

		Baseline: Mean (SD)		Week-2: Mean (SD)		Week-4: Mean (SD)		Week-6: Mean (SD)		Time-by-Group Effect	
		30 °/sec	90 °/sec	30 °/sec	90 °/sec	30 °/sec	90 °/sec	30 °/sec	90 °/sec	F-value	p-value
aTDCS	Concentric Inversion	30.81(13.36)	29.47(15.21)	30.96(12.52)	28.80(14.39)	34.53(14.41)	30.90(13.41)	34.69(22.65)	34.69(27.29)	0.317	0.813
	Concentric Eversion	25.42(8.57)	20.86(7.55)	25.92(6.70)	25.03(9.76)	29.56(10.41)	29.31(10.49)	29.78(11.58)	26.50(10.67)	0.216	0.885
	Eccentric Inversion	30.60(6.69)	30.51(6.36)	30.34(6.20)	30.06(6.04)	30.10(3.11)	30.04(6.19)	30.02(6.46)	29.90(6.77)	0.087	0.967
	Eccentric Eversion	30.62(6.55)	30.34(6.28)	30.32(6.13)	29.98(5.99)	30.11(6.11)	30.04(6.20)	30.00(6.47)	29.85(6.76)	0.090	0.965
Sham	Concentric Inversion	30.40(17.47)	28.18(16.64)	31.10(12.27)	27.24(10.10)	33.11(10.57)	29.75(10.11)	31.36(10.05)	27.35(9.55)	0.317	0.813
	Concentric Eversion	26.46(11.77)	23.11(10.53)	29.32(10.63)	25.29(7.32)	29.97(7.05)	25.57(5.68)	30.08(10.80)	27.47(9.52)	0.216	0.885
	Eccentric Inversion	29.77(6.89)	29.09(6.14)	28.73(5.00)	28.34(4.33)	28.58(3.80)	28.64(3.73)	28.90(3.65)	28.87(3.80)	0.087	0.967
	Eccentric Eversion	29.66(6.64)	28.83(6.00)	28.71(4.47)	28.31(4.11)	28.61(3.74)	28.64(3.69)	28.95(3.67)	28.87(3.84)	0.090	0.965

Table 11. Mean (Standard Deviation) of peak torque at 30 and 90 °/sec for concentric and eccentric inversion and eversion motions across group and time. Additionally, time-by-group effect F and p values.

Chapter 5: Discussion

Introduction:

The purpose of this study was to determine the effects of eccentric training in conjunction with aTDCS on neural plasticity, functional performance, and strength measurements in individuals with CAI following a four-week intervention compared to sham intervention. These results suggest that eccentric training with aTDCS increased neural excitability, improved dynamic balance, and increased peroneus longus activity over the course of this study. These results align with our hypotheses that neural excitability would be enhanced with aTDCS intervention and dynamic balance would be improved potentially through increased muscle activation. Our hypothesis on side hop times was correct that participants did improve, however it is suggested that eccentric training was the mechanism behind this improvement, not the aTDCS intervention. These results also suggested that eccentric training had short-term effects on neural excitability. From baseline to week-2 of this study, the sham group had initial increases in their neural measures, but worsened or plateaued in improvements by the conclusion of training. It is currently nuclear as to why these observations in the sham group occurred. Our final hypothesis stated that strength would increase in both groups, however, both groups did not show any strength increases. This could be due to various factors such as a lack of motivation, the fixed range of motion of the dynamometer, or the difficult nature of training small ankle stabilizers in an everted direction. The implications of this study suggest that, in CAI patients, an eccentric training protocol will improve their functional performance, while the addition of aTDCS may increase neural excitability, improve dynamic balance, and enhance muscle activation beyond eccentric training alone.

Neural Measurements:

Cortical Excitability:

It has been previously observed that ligamentous injuries, such as injuries experienced by individuals with CAI, result in decreased cortical excitability seen six months post-injury, which can contribute to decreased strength and diminished voluntary muscle activation (A. Lepley et al., 2015; Lepley et al., 2018; Needle et al., 2017). There was a significant increase in cortical excitability in the aTDCS group at the week-6 testing session. This was observed through a leftward shift in the stimulus response curve resulting in lower RMT values and decreased I_{50} values (Figure 7 & 8). This leftward shift indicates that the recruitment threshold for cortical motor neurons decreased, resulting in easier activation of the peroneus longus (Devanne et al., 1997). The slope of the stimulus response curve did not change, indicating participants in the aTDCS group underwent a change in their resting motor threshold but not a change in the number of motor neurons recruited (Devanne et al., 1997; Needle et al., 2013). These results lend support to our hypothesis that neural excitability would increase. The mechanism for this increase is likely due to membrane depolarization creating LTP-like adaptations through an increase in synaptic strength (Stagg & Nitsche, 2011).

Interestingly, in the sham group there was a significant increase at the week-2 retest; however, those adaptations were not sustained over the following testing sessions (Figure 4 & 5). This observation contradicts the hypothesis that the sham group would increase in neural excitability over the course of the entire study just to a lesser extent than the aTDCS group. Both groups were expected to experience an increase in neural excitability due to the effects of eccentric contractions on diminishing neural inhibition (Hedayatpour & Falla, 2015). These results imply that after five eccentric training sessions neural adaptations can be observed;

however, they are potentially attenuated after the week-2 retest in the sham group. Conversely, the aTDCS group did not have an initial increase in neural excitability at week-2, potentially due to the addition of aTDCS (Figure 7 & 8). It is unclear as to why this occurred; it is possible that the aTDCS offset an initial neural adaptation due to a stabilizing effect of the current. The adaptations experienced by the aTDCS group, were significant at week-6, potentially because these were long-term changes that were augmented slowly due to the multiple sessions of aTDCS occurring over the course of several weeks (Jaberzadeh & Zoghi, 2013; Stagg & Nitsche, 2011). These findings were similar to previous findings stating that with anodal transcranial direct current stimulation, cortical excitability increased (Jaberzadeh & Zoghi, 2013; Schabrun et al., 2013; Stagg & Nitsche, 2011).

Reflexive Excitability & Intracortical Inhibition:

There were no significant changes in our measures of inhibition, observed through no changes in participant's reflexive excitability in both the sham and aTDCS group over the course of the intervention (Table 8). Eccentric training was hypothesized to enhance reflexive excitability because of the decrease of presynaptic inhibition leading to less overall inhibition (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002). This effect was suggested by Aagaard et al. (2002) in the soleus muscle; however, we may have observed contrasting effects because they trained their participants for 14-weeks instead of 4-weeks as in the present investigation (Aagaard et al., 2002). Another reason that this study did not see similar results compared to Aggaard et al.'s (2002) observation of the SOL, is because of the highly reflexive nature of the SOL muscle. The current study did not try to target the SOL muscle during the training sessions; this study focused on training the PL which has reflexive properties more

similar to the TA. Additionally, previous studies recorded H-reflex during maximal voluntary

contractions, which is suggested to elicit a more functional representation of the adaptations from

their training regimen, where presynaptic inhibition is taken into account (Aagaard et al., 2002;

Duclay et al., 2008; A. Lepley et al., 2015). Previous studies by Lepley et al. (2017, 2018)

showed there were significant differences in H-reflex post a bout of eccentric training while the

participant was at rest in CAI and ACL patients, which is why this study chose to have

participants at rest during this measurement (Lepley et al., 2017, 2018). Previous similar studies

saw a similar effect where after an eccentric training bout, reflexive excitability increased

(Lepley et al., 2018; Vangsgaard, Taylor, Hansen, & Madeleine, 2014). However, in these

previous studies the muscles they were examining were larger compared to the smaller

stabilizing muscles that were observed in this current study (i.e., trapezius and vastus medialis

compared).

There were also no differences in the cortical silent period which was similar to previous

findings (Table 7) (Cooney et al., 2015). However, according to Lepley et al. (2017) supporting

evidence suggests that following eccentric exercise there were adaptations to the neural pathways

eliciting a reduction in the silent period with improved corticospinal excitability (Lepley et al.,

2017). In the previous study they were also examining larger muscles which might express a

greater amount of adaptations due to the greater margin of potential strength and neural gains.

There may be a ceiling effect to the training adaptations in the muscles observed (TA, PL, and

SOL), due to their small size. It was observed in individuals with CAI, that reflexive inhibition

may occur after an ankle-joint injury or lateral ankle sprain (Kaminski & Hartsell, 2002). It is

also possible that some testing sessions throughout the training sessions (e.g., week-2 and week-

4) may have induced changes to inhibition offset by fatigue or soreness from the eccentric training.

Performance Measures:

Postural Stability Indices:

Overall there was a significant improvement in dynamic balance over the course of the study. The individual components of APSI, MLSI, and VSI showed a significant improvement over the six-week study in the aTDCS group's dynamic balance (Figure 9). These adaptations to the three balance components seem to take several weeks to become apparent. In the aTDCS group, positive adaptations to participants' dynamic balance became significant at the end of the intervention (week-4) and continued to improve at the 6-week retention test compared to baseline (Figure 9.). In previous studies, it was determined that individuals with CAI, have deficits in their dynamic balance (Brown, Ko, Rosen, & Hsieh, 2015; Gribble & Robinson, 2010; Rosen et al., 2013). These deficits have been attributed to a lack of activation from the peroneal muscles, tibialis anterior, and soleus (Rosen et al., 2013). Increases in cortical excitability have the potential to improve this activation, thus reducing their dynamic balance deficits. In another previous study conducted by Kaminski et al. (2016), they found that with aTDCS mediated changes, a dynamic balance tasks of standing on a stability platform while keeping it horizontal, in healthy individuals was improved (Kaminski et al., 2016). This was observed in the current results of this study, where after aTDCS stimulation was implemented participants improved their PSIs and their muscle activation of the PL was enhanced (Figure 9 & 12). Our results likely suggest improved balance was facilitated through increased EMG activation, as aTDCS participants increased their activation of PL - a preliminary stabilizer of the ankle - across a

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similar timeframe. There were significant improvements in dynamic postural stability indices values (which is the composite score of APSI, MLSI, and VSI); however, there were no post-hoc between group differences. The lack of differences seen here could be due to inter-subject variability, and may be minimized with a larger sample size.

It was expected that both groups would improve to some degree in their dynamic balance, with the aTDCS more than sham; however, there were no balance improvements in the sham group (Figure 9). It is possible that the eccentric training alone was not sufficient in order to decrease presynaptic inhibition, which would have elicited a more efficient activation in the PL; however, eccentric training with aTDCS seems to have facilitated increased activation, neural drive, and excitability evoking improved dynamic balance compared to eccentric training alone (Figure 9 & 12) (Aagaard et al., 2002; Hedayatpour & Falla, 2015; Sriraman et al., 2014). In a previous study by Lopez-Valenciano et al. (2018) discussed there was no relationship between eccentric strength and dynamic balance (López-Valenciano, Ayala, De Ste Croix, Barbado, & Vera-Garcia, 2018). This could be the reason behind the lack of improvements in the sham group who only received eccentric training. Conversely, another previous study found that there was a relationship between eccentric training and dynamic stability of the star-excursion balance test (Lockie, Schultz, Callaghan, & Jeffriess, 2013). These results lend support to the hypothesis that improvements in balance are aided with the use of aTDCS via enhanced muscle activation.

Side-Hop Test:

Across both the sham and aTDCS groups, there were significant improvements in the double-legged side-hop test at week-2 (participants completed the task faster) (Figure 10). It has been previously established in a meta-analysis conducted by Rosen et al. (2017) that CAI

individuals have decreased functional performance on side hopping tasks (Rosen et al. 2017). It was expected that there would be improvements in the participants' abilities to complete the side-hop test resulting from increased muscle activation and PL strength; the current study's results suggest that after five eccentric training sessions both group's PL activation increased as did their side-hop performance. This complements findings from Rosen et al. (2017) where they state the side-hop test required a large amount of activation from the PL and CAI individuals would be deficient in this muscle's activation (Rosen et al., 2017). The combination of the participant's potential initial deficits and adaptations in the PL from the eccentric training via greater activation of the PL, potentially influenced the improvement of their side-hop performance (Hall, Chomistek, Kingma, & Docherty, 2018; Lepley et al., 2017; Rosen et al., 2017). Interestingly, there was a plateau in improvements after the week-2 testing session, this could be due to a ceiling effect or learning effect (Figure 10). In a study conducted by Hall et al. (2018) they found after a six-week intervention their participants improved their side-hop performance at the week-6 testing session; however, they only tested their participants at baseline and at the conclusion of the study (Hall et al., 2018). If they had tested their participant's strength throughout their intervention, it would have been interesting to see if a similar ceiling effect early in the intervention was observed.

Muscle Activation:

The results from this study depicted significant differences in EMG activation in the PL in both the sham and aTDCS groups. In the aTDCS group, the intervention caused a significant increase in PL post-activation from baseline to week-6, and also caused an increase in activation

from pre- to post-landing at weeks-2, -4, and -6; whereas the sham group never increased activation across landing phases (Figure 12). These results show that eccentric training in addition to the aTDCS intervention enabled the PL to have higher activation in the post-phase from baseline to week-6. This finding may be extremely beneficial in relation to injury, as injury is likely to occur between 50 and 250ms after initial contact (Fong et al., 2009; Gehring, Wissler, Mornieux, & Gollhofer, 2013; Kristianslund, Bahr, & Krosshaug, 2011; Rosen et al., 2013). Therefore, these individuals may be able to activate their PL muscle with more precise timing in order to stress-shield the joint and reduce the amount of reinjuries and roll-over events they sustain to their ankle.

Previous studies have shown facilitatory effects of aTDCS on lower leg muscles. In a study conducted by Devanathan and Madhavan (2016), it was found that after a bout of aTDCS, participants exhibited an increase in muscle activation through EMG as evidenced by a quicker reaction time (Devanathan & Madhavan, 2016). Furthermore, a previous study by Hedayatpour and Falla (2015) concluded that in the early stages of an eccentric training protocol there is an increase in muscle activation of the muscles being trained (Hedayatpour & Falla, 2015) The combination of aTDCS and eccentric training enhances muscle activation, which enhanced dynamic stability and could therefore prevent future injuries through enhanced muscle activation of the PL after landing (Devanathan & Madhavan, 2016; Feger, Donovan, Hart, & Hertel, 2015; Hedayatpour & Falla, 2015; Rosen et al., 2013).

During the pre-phase in the sham group there was a significant increase in PL activation at week-2 compared to baseline (Figure 12). After this increase at week-2 there were no additional significant adaptations, suggesting that with some eccentric training there were neural adaptations, however, those adaptations were not sustained long-term. This is a similar result to

what was observed in the cortical excitability measurements. With the eccentric training there was an enhancement of neural drive to the PL, leading to these positive adaptations from baseline to the week-2 retest (Figure 12). It is currently unclear as to why no more improvements occurred or why the improvements were transitory in the sham group after the week-2 retest with their continued eccentric training. However, these results do seem to confirm a link between cortical excitability and performance in this CAI population.

There were also significant changes in the TA and SOL muscle activation from the pre-phase to the post-phase, which was expected due to the nature of the muscles themselves in relation to the hop-to-stabilization task (Table 10 & Figure 11). The enhanced activation of the TA after training in both the sham and aTDCS group shows that this muscle is trying to more efficiently stabilize the participants in the post-phase. The function of the TA is dorsiflexion and eccentric control of plantar flexion; if this muscle were not activated during landing the participant would be at a greater risk for injury due to the ankle potentially become unstable (Klykken et al., 2011). The decrease in SOL activation was expected because the SOL muscle plays a role in load absorption during landing, but is then inhibited post-landing allowing the TA to activate bringing the ankle to a more closed-packed position (Klykken et al., 2011; Pietrosimone & Gribble, 2012). The PL muscle changed over the course of the intervention because the eccentric training protocol was specifically targeting this muscle. If the training protocol had been altered to include plantarflexion and dorsiflexion motions it is likely that significant differences would have been observed in the TA and SOL.

Strength Measurements:

The results from this study showed no significant differences in strength across both sham and aTDCS groups (Table 11). These findings were surprising due to the fact that both groups underwent four weeks, or 10 sessions, of eccentric ankle training. The eccentric training protocol followed loading protocols from a previous study conducted by L. Lepley et al. (2015, 2018), where participants elicited at least 60% of their one-repetition maximum for four sets of ten repetitions, which should induce strength adaptations (L. Lepley et al., 2015, 2018).

The lack of strength adaptations is contrary to previous studies' results; however, those adaptations were typically observed in larger quadricep muscles compared to the smaller PL (L. Lepley et al., 2015, 2018). These lack of strength adaptations are also contrary to a study conducted by Hall et al. (2018) who did evaluate the PL muscle; however, they used resistance bands for their training method instead of a dynamometer which may have had a different effect on their adaptations (Hall et al., 2018). Resistance bands offer isotonic training as opposed to eccentric training, and allow the joint to move through a functional movement pattern rather than precise rotation. Additionally, Hall et al. (2018) trained their participants for six weeks instead of four, and included other stabilization exercises including proprioceptive neuromuscular facilitation with heel raises. Previous studies training the ankle using an isokinetic dynamometer have failed to elicit strength changes (Keles, Sekir, Gur, & Akova, 2014; Maeda et al., 2017). For the current study, the lack of adaptations could be due to various factors. Perhaps if participants underwent several more training sessions this potentially could have produced enhanced results. Additionally, the muscles being trained in this study are inherently small. There could be a ceiling effect to the amount of adaptations that these muscles can undergo. Furthermore, the ankle motion of true eversion is a very small movement and difficult to train

eccentrically. The dynamometer used here, forces the participants to go through a fixed range of motion instead of training them in their functional range of motion, which might have influenced strength measures. The dynamometer also would interrupt the stroke of the foot if the participant did not elicit enough torque to surpass the threshold of 60% of their one-repetition maximum, which could also have an effect on their results. Lastly, motivation could be a major factor that would explain the lack of significance. It was apparent over the course of several participant's training sessions that they were not producing maximal voluntary contractions for the 4 sets of 10 eccentric contractions despite the investigator's efforts to verbally encourage and motivate them.

Despite the lack of strength adaptations from both the sham and aTDCS groups, the neural, functional, and balance adaptations indicate that the eccentric training did have a positive effect on the participants even though they didn't express strength gains. As stated above there are several different variables that could have influenced the lack of strength increases in these participants. The lack of strength improvements was unexpected, however the benefits seen through these various dependent variables suggest that this training protocol was not futile.

Limitations:

Given the preliminary nature of this study, being the first to explore this intervention among a population with musculoskeletal injury, various limitations should be considered for future studies. First, there was no true control group or comparison to the standard of care (i.e., balance training). Both the sham and aTDCS both received eccentric training; a control group may illuminate any learning curves that might have influenced the results that were attributed to eccentric training. There was lack of double blinding in this study as the investigator conducting

the training and testing sessions knew which group the participants were in, which could have

contributed towards bias. The group differences between the sham and aTDCS group distribution

of males to females could also be a limitation. There were a greater number of females in the

aTDCS compared to the sham group. Additionally, the ankle motion eversion is a very small

movement and difficult to train eccentrically. Lastly, a limiting factor that is somewhat

uncontrollable is the state in which the subjects visit the lab for testing or training sessions.

Factors like hydration status and mental fatigue can alter the results of various measurements.

Furthermore, over the course of this study there were four participants that dropped out;

however, our power analysis did account for 25 percent attrition, and this fell far below that

value.

Chapter 6: Conclusion

This is the first study to examine the effects of aTDCS in a population with musculoskeletal injuries, as well as determining the effects of its combination with eccentric training on neural excitability and performance. Our results lend support to the hypothesis that aTDCS with eccentric training increases cortical excitability, enhances dynamic postural stability, and improves the timing and amplitude of muscle activation following four-weeks of training in patients with CAI. Eccentric training alone does elicit some benefits in functional performance as well as initial increases in cortical excitability and muscle activation in the PL; however, these positive results were expressed after the week-2 retest and were not sustained over the course of the entire six-week study. Overall, we posit that the improvements seen with the aTDCS intervention are beneficial in correcting the potentially maladaptive neuroplasticity occurring after ligamentous injury, and has the potential to improve health-related quality of life.

These results have important implications for the practicing clinician, although several steps stand between the current study and its use in a clinical setting. aTDCS is a simple to administer and relatively inexpensive therapeutic treatment; however, future randomized controlled trials are needed to establish its efficacy and gain federal approval. Additionally, while clinicians might not have access to an isokinetic dynamometer, resistance bands or manual resistance could replicate the eccentric training performed in this study. Future work in this realm should consider the addition of further adjuvant therapies beyond eccentric training (e.g., muscle stimulation, balancing, mobilization exercises, manual therapy), and utilize larger and more varied cohorts of injured individuals. Additionally, further testing should be implemented to determine the proper dosage and length of rehabilitation to maximize long-term outcomes as

well as understanding the mechanism behind the adaptations occurring in the sham group at week-2.

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Vita

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