IMMEDIATE EFFECTS OF TRANSCRANIAL ALTERNATING CURRENT ON QUADRICEPS ACTIVE
MOTOR THRESHOLD AND CENTRAL ACTIVATION RATIO

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ABSTRACT

Ariel M. Zaleski: Immediate Effects of Transcranial Alternating Current Stimulation on Quadriceps Active
Motor Threshold and Central Activation Ratio
(Under the direction of Brian Pietrosimone)

Quadriceps muscle dysfunction is commonly reported in individuals with knee joint injury. Decreased corticospinal excitability and activation are believed to be the neural causes of quadriceps dysfunction following knee joint injury. The purpose of this study was to examine if transcranial alternating current stimulation (tACS) can acutely alter corticospinal excitability and voluntary activation of the quadriceps in healthy individuals. Active motor threshold (AMT) and central activation ratio (CAR) were evaluated in a single-blinded, crossover study. Thirty-four participants were counterbalanced over 2 testing sessions to receive tACS or control. A dependent samples t-test was used to examine percent change scores for AMT and CAR between the two sessions. A post-hoc analysis was also run to evaluate any association between AMT percent change scores for the two sessions. No significant difference was found between the percent change scores and no association was found between the intervention and control sessions for AMT.

For my loved ones and all those who helped shape the athletic trainer I am today.
Thank you for your love, guidance, and support.

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LIST OF ABBREVIATIONS

ACL-R	Anterior Cruciate Ligament Reconstruction
AMT	Active Motor Threshold
CAR	Central Activation Ratio
EEG	Electroencephalogram
EMG	Electromyography
IKDC	International Knee Documentation Committee
MEP	Motor Evoked Potential
MVIC	Maximum Voluntary Isometric Contraction
OA	Osteoarthritis
SRTT	Serial Reaction Time Task
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
QAF	Quadriceps Activation Failure
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

CHAPTER 1: INTRODUCTION

Adequate muscle function of the quadriceps is necessary for ambulation and activities of daily living.[1] Quadriceps activation failure has been associated with a decrease in self-reported function in individuals with knee osteoarthritis (OA),[2] as well as in individuals following anterior cruciate ligament reconstruction (ACL-R).[3] Additionally, the decrease in physical function that is associated with quadriceps dysfunction is further linked to chronic comorbidities such as cardiovascular disease, obesity, and diabetes.[4,5]

Quadriceps muscle dysfunction is common following knee joint injury and surgery such as ACL-R[3,6-8] and meniscectomy,[9-11] as well in individuals with knee OA.[2,3,12] Quadriceps muscle dysfunction is defined as a reduction in voluntary muscle activation, and may persist for months to years following knee joint injury.[13] Quadriceps muscle dysfunction is influenced by alterations in cortical excitability.[3,7] Following joint injury, neuromuscular alterations manifest in the surrounding uninjured musculature.[1] Sensory nerve fibers of the injured joint relay information to the central nervous system, and may lead to decreased voluntary muscle activation that decrease forces placed on the injured joint.[1] Muscle contraction is generated through both spinal reflexive and voluntary descending pathways from the motor cortex, with decreased excitability in one or both pathways hypothesized to cause decreased quadriceps function.[1]

Alterations in neural excitability can be assessed with transcranial magnetic stimulation (TMS),[14] by providing information about the excitability of the descending corticospinal pathway,[14] or through the central activation ratio (CAR), providing information of the ability to recruit motor units during a maximal contraction.[15] A higher active motor threshold (AMT) is interpreted as lower corticospinal excitability while a lower AMT suggests higher corticospinal excitability of the primary motor cortex and the descending motor pathways.[14] To elicit a motor response, a stimulus must meet or exceed the motor threshold within the motor cortex.[1] If a motor response cannot be elicited, either a greater stimulus is required, or the

motor threshold must be lowered. Active motor threshold is greater in patients following ACL-R,[7,12] and may be a source for quadriceps muscle dysfunction in patients with knee joint injuries.

Greater voluntary activation (measured via CAR) is indicative of the ability to voluntarily recruit a greater number of motor units, potentially suggesting greater cortical excitability. Decreased voluntary activation within the quadriceps has been demonstrated in patients with ACL-R when compared to healthy participants,[15] in limbs with simulated knee joint effusion,[16] and in patients with a partial menisectomy.[17] It is hypothesized that decreased strength, decreased voluntary activation and diminished cortical excitability all occur concurrently in quadriceps muscle dysfunction.

Transcranial direct current stimulation (tDCS) is an intervention that has been previously used to increase corticospinal excitability.[18-21] This modality may cause alterations to oscillatory brain waves[19,22], which may alter cortical excitability.[18,20] Anodal tDCS has been demonstrated to increase cortical excitability, while cathodal tDCS has been demonstrated to inhibit cortical excitability in healthy individuals.[18] Anodal tDCS results in depolarization of neurons while cathodal tDCS results in hyperpolarization of neurons.[18] Transcranial direct current stimulation has been successful in increasing cortical excitability of the first dorsal interossei in stroke patients with hemiparesis and healthy individuals,[20] and in improving cortical excitability of the abductor digiti minimi in subcortical stroke patients and healthy subjects.[18]

Transcranial alternating current stimulation (tACS) is another form of transcranial current stimulation which has displayed improvement in symptoms for patients with depression,[23] insomnia,[24] pain,[25] and Parkinson's Disease[26], as well as modulating hearing and identifying auditory tones[27] and motor learning of fine motor tasks of the hand in healthy individuals.[28] While the mechanism that is responsible for the improvement in these various conditions is not clear in the literature, changes in the electrical pathways in the brain, causing oscillatory changes in brain waves[23,24,26-28] and hormonal and neurotransmitter changes within the brain[23,24] are hypothesized mechanisms for improvement of symptoms. Transcranial alternating current stimulation improved distal fine motor skills and altered oscillatory brain waves through de-synchronization of the beta wave form in Parkinson's disease patients.[26] Transcranial alternating current stimulation has also been demonstrated to improve motor learning at 10 Hz and 20 Hz when evaluated over given time points of the serial reaction time task.[28]

While tDCS has been used successfully in stroke patients to increase cortical excitability, and tACS has been used successfully in the treatment of Parkinson's disease,[26] transcranial current stimulation has not been examined in an orthopedic population who suffer from decreased corticospinal excitability.[1] Increasing corticospinal excitability in patients with quadriceps dysfunction may allow for improved muscle function, thereby improving self-reported function and physical activity. In order to determine if tACS can alter corticospinal excitability in the quadriceps of patients who have suffered joint injury, we must first determine if tACS can alter corticospinal excitability in the quadriceps of healthy individuals. Therefore, the purpose of this study was to examine if tACS can acutely alter corticospinal excitability of the quadriceps in healthy patients.

Research Questions and Hypothesis

1. To determine if tACS with concurrent voluntary isometric contractions can acutely alter quadriceps active motor threshold in healthy individuals compared to a control condition with voluntary isometric contractions.

   a. It is hypothesized tACS will acutely decrease AMT. Past literature has indicated that tACS has been a successful intervention for increasing corticospinal excitability,[23-27] thus suggesting active motor threshold can be decreased.

2. To determine if tACS with concurrent voluntary isometric contractions can acutely alter quadriceps central activation ratio in healthy individuals compared to a control condition with voluntary isometric contractions.

   a. It is hypothesized tACS will acutely increase CAR. Past literature has indicated that tACS has been a successful intervention for increasing corticospinal excitability, thus suggesting central activation ratio can be increased.

CHAPTER 2: REVIEW OF LITERATURE

Introduction

Adequate muscle function of the quadriceps is necessary for ambulation and activities of daily living. Quadriceps muscle dysfunction, which manifests as voluntary activation and strength deficits, is common following knee joint injury and surgery such as ACL-R[3,6-8,15], minescectomy,[9-11] and knee OA.[2,3,12] Diminished corticospinal excitability may be the underlying mechanism of quadriceps voluntary activation deficits[7,8,15] and strength deficits,[2,3,12] thus increasing quadriceps muscle dysfunction in patients following joint injury and surgery. Transcranial direct current stimulation (tDCS) is an intervention which as been studied in multiple pathological populations such as depression,[29] epilepsy,[30] and stroke,[31,32] and is believed to alter corticospinal excitability.[18-20,22,33] Transcranial alternating current stimulation (tACS) has also been studied in populations affected by depression,[23] insomnia,[24] pain,[25] and Parkinson's Disease.[26] This review of literature will examine 1) neuromuscular consequences of knee injury, 2) alterations in corticospinal excitability following knee joint injury, and 3) the capability of transcranial current stimulation to alter corticospinal excitability.

Neuromuscular Consequences to Knee Injury

Months and even years following knee injury, lingering quadriceps muscle dysfunction is present.[9,13] Quadriceps muscle dysfunction is commonly quantified through quadriceps voluntary activation[7,8,13,15-17,34-37], and quadriceps strength[6,13,16,17] following knee injury. Current neuromuscular theories suggest the cause of quadriceps muscle dysfunction is not a result of injury to the muscle itself, but rather alterations in the nervous system responsible for generating muscle contraction in the muscles surrounding the injured joint.[38] Specifically, alterations within in the primary motor cortex of the brain or altered cortical excitability, is thought to play a key role in mediating muscle dysfunction following joint injury.[3]

Voluntary Activation Deficits

The measurement of voluntary activation of the quadriceps is one method of quantifying neuromuscular effects of knee injury. Pietrosimone et al.[15] investigated spinal reflexive and corticospinal excitability differences between injured and uninjured limbs of ACL-R patients and healthy controls, as well as the relationship between voluntary activation, spinal reflexive excitability, and corticospinal excitability. Corticospinal excitability of the quadriceps was assessed in twenty-eight ACL-R participants and twenty-nine healthy participants. Pietrosimone et al.[15] demonstrated bilateral deficits in quadriceps voluntary activation in participants with ACL-R compared with the healthy controls. The mean CAR of the injured and uninjured limb of the ACL-R participants were both .88 while the healthy controls demonstrated a mean CAR of .96 and .95 of the matched injured to uninjured limb, respectively.[15] These findings contradicting the findings of a previous study conducted by Héroux and Tremblay.[7] However, there were no differences in voluntary activation demonstrated between limbs of the ACL-R group.[15,39]

A study evaluating simulated knee effusion and pain found diminished quadriceps activation when comparing normal knee conditions to various conditions of knee effusion and pain.[16] Fourteen participants were each tested under four conditions; normal knee, simulated effusion, simulated pain and simulated effusion and pain simultaneously. Quadriceps activation was quantified through the central activation ratio (CAR) via maximal voluntary isometric contraction (MVIC) testing. The results suggested that the normal knee condition had significantly better quadriceps strength and activation than the other three conditions collectively. No differences were found between the experimental conditions.[16]

The effect of a partial meniscectomy on voluntary activation has also been evaluated.[17] A sample of 32 male participants with a history of partial medial meniscectomy and no evidence of osteoarthritis and 32 healthy counterparts were assessed using twitch interpolation technique to determine voluntary quadriceps activation. The mean voluntary activation of the involved quadriceps of the injured sample was 80.9%; significantly lower than the healthy counterparts,[17] further supporting the presence of quadriceps muscle activation deficits following knee joint injury.

Strength Deficits Following Injury

Diminished quadriceps strength and subsequent impairments in physical function following acute and chronic knee injury has been well documented in previous literature.[3,9-12] Pietrosimone et al.[3] evaluated quadriceps strength and corticospinal excitability as measures to predict disability following ACL-R. Fifteen ACL-R participants were included in the study, which assessed quadriceps strength, quantified as MVIC, and corticospinal excitability, quantified as AMT. The International Knee Documentation Committee (IKDC) was used to determine self-reported knee disability following ACL-R. The investigators found a positive, strong correlation between quadriceps strength and knee disability, and a negative, weak correlation between AMT and knee disability; as strength increased, IKDC scores increased as well indicating less disability. The findings suggest quadriceps strength and cortical excitability are predictive measures of knee disability following ACL-R.[3]

Patients undergoing arthroscopic partial meniscectomy have also demonstrated diminished quadriceps muscle strength.[9-11] A systematic review of four studies found clinically significant reductions in quadriceps strength two weeks to four years following partial meniscectomy.[9] Ericsson et al.[11] evaluated long term muscle strength, functional performance, and self-reported outcomes an average of four years after partial meniscectomy. Fifty-six participants were included, in which functional performance, self-reported disability, and quadriceps muscle strength were evaluated. Functional performance was evaluated with three functional tests; the 1-leg hop test, the 1-leg rising test, and the square-hop test. Self-reported outcomes were evaluated with the Knee Injury and Osteoarthritis Outcome Score questionnaire. Quadriceps muscle strength was evaluated using MVIC. Muscle strength and performance on the 1- leg rising test were significantly lower on the operative limb compared to the contralateral limb. Self-reported function scores were significantly worse in the injured participants compared to healthy individuals from previous studies.[11]

Peak quadriceps muscle torque, quadriceps electromyography (EMG) activity, and muscle control as a result of muscle strength have also been examined following partial meniscectomy.[10] Fourteen subjects with varying times since surgery were evaluated for both isometric and isokinetic muscle torque, surface EMG for muscle activation of the vastus lateralis and vastus medialis, and a lower limb tracking-trajectory test for muscle control, which assessed multi-joint motor coordination. While no significant

findings were present for muscle control, both muscle torque and EMG activity were diminished on the involved limb compared to the uninvolved.[10]

Pietrosimone, Thomas, Saliba and Ingersoll[12] also evaluated the association between quadriceps strength and self-reported physical activity. Thirty-six participants diagnosed with knee OA and had voluntary quadriceps activation of less than 90% on the affected limb compared to the asymptomatic limb were assessed. Quadriceps strength was assessed via MVIC and physical activity was evaluated using the Godin Leisure-Time Questionnaire. The investigators found significantly higher quadriceps strength in participants with higher physical activity scores compared to those with low physical activity scores. The investigators also found significant correlations between quadriceps strength measurements and physical activity scores in participants with high physical activity scores, but no correlation was found between quadriceps strength and physical activity scores in participants with low physical activity scores.[12]

Another study[2] evaluated the influence of quadriceps activation failure (QAF) on the relationship between quadriceps strength and physical function of knee OA patients. One hundred five participants were included in the study. Quadriceps strength and level of muscle activation was measured via quadriceps MVIC and burst-superimposition, with CAR calculated to indicate the QAF. Physical function was evaluated using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) as well as the Get Up and Go test. The investigators found diminished quadriceps strength was associated with decreased physical function, while the QAF was associated with the WOMAC pain, WOMAC stiffness, WOMAC physical function subscales as well as the Get Up and Go time.[2] Overall, this study supported the association between poor quadriceps activation, diminished quadriceps strength and limited physical function. A direct relationship between diminished quadriceps activation and strength and limited physical function indicates the importance of high quadriceps activation. The use of interventions that specifically target diminished quadriceps activation and strength may help maintain an appropriate level of physical function in patients suffering from knee joint injury and pathology.

Corticospinal Excitability Alterations

Corticospinal excitability deficits have been cited in lower extremity orthopedic pathologies, such as ACL-R[3,7,15] and chronic ankle instability.[40] Deficits in corticospinal excitability, that have been

suggested to be associated with orthopedic injury, could lead to the long-term quadriceps strength and voluntary activation deficits that persist following joint injury. Determining interventions that alter corticospinal excitability may potentially improve clinical outcomes following knee injury as well as the physical disability and comorbidities associated with this orthopedic disease.

Transcranial magnetic stimulation (TMS) is commonly used to assess corticospinal excitability, in which a rapidly changing magnetic current is delivered through a coil placed on top of the scalp.[14] The magnetic current elicited from TMS causes an electrical current in the brain, resulting in brain stimulation through neuronal depolarization.[14] TMS can be used in many different ways to study the human motor cortex.[14] A magnetic current over the motor cortex creates an electrical current and causes neuronal depolarization. The neuronal depolarization results in a measureable motor evoked potential (MEP) in the targeted muscle group, which is a measurable outcome of corticospinal excitability.[14] The measurement of AMT and MEPs is important when considering diminished corticospinal excitability after orthopedic injury.[41] The amplitude of the MEP, measured through surface electromyography (EMG), indicates the effectiveness of the corticospinal pathway. The "hot spot" is identified as the location over the primary motor cortex that elicits the greatest MEP in the desired muscle group. The AMT of the muscle group is quantified as the lowest percentage of stimulus intensity needed to elicit a measureable MEP, with a higher AMT indicating a decreased corticospinal excitability.[41] Luc et al.[41] evaluated the reliability of TMS in measuring AMT and MEP outcomes in the vastus medialis oblique of the quadriceps and the fibularis longus over a 28-day period. Active motor threshold was defined as the lowest stimulus intensity which elicited 5 out of 10 measureable MEPs (>100µV), and the stimulus intensity directly below elicited 6 out of 10 MEPs measuring less than 100 µV. Luc et al.[41] found TMS outcome measurements to be reliable in the quadriceps and fibularis longus muscles of healthy participants up to 28 days.

Héroux and Tremblay[7] evaluated the corticospinal excitability of the lower extremity, comparing an ACL-R limb to a healthy limb and healthy subjects. The researchers evaluated corticospinal excitability through TMS testing of resting motor threshold of bilateral quadriceps in the ACL-R group and healthy group. A significant asymmetry in resting motor threshold between the involved leg and uninvolved leg of the ACL-R participants was found, with the resting motor threshold of the quadriceps of the involved limb

in ACL-R patients determined to be lower than the uninvolved limb.[7] Additionally, no asymmetry was found between limbs in the healthy participants.

Quadriceps corticospinal excitability and quadriceps strength have been suggested to be diminished following both acute and chronic knee injury. Some research has supported a relationship between cortical excitability and quadriceps strength. Also, quadriceps strength has been suggested to be an indicator of physical function.[3] Current research states that greater quadriceps strength may result in less knee disability and higher levels of physical activity. Future research should further evaluate the relationship between cortical excitability quadriceps strength in order to improve physical function following knee injury, as well as specific interventions to improve quadriceps strength.

Transcranial Current Stimulation

Transcranial direct current stimulation (tDCS) is an intervention that has been previously used to alter corticospinal excitability[18-22] and improve motor learning and function.[21,31,32,42] Transcranial direct current stimulation has been demonstrated to cause neuronal depolarization or hyperpolarization within the brain, thereby altering cortical excitability[20,22]. Anodal tDCS has been demonstrated to increase cortical excitability through neuronal depolarization, while cathodal tDCS has been demonstrated to inhibit cortical excitability, through neuronal hyperpolarization, in healthy individuals[18]. Transcranial alternating current stimulation (tACS) is another form of transcranial current stimulation which has displayed improvement for patients with depression,[23] insomnia,[24] pain,[25] Parkinson's Disease[26] and other conditions[27,28]. The cause of improvement of these various conditions is not clear in the literature, with changes in the electrical pathways in the brain, causing oscillatory changes in brain waves[23,24,26-28] and hormonal and neurotransmitter changes within the brain[23,24] as potential causes for the improvement in symptoms.

Early Research on Cortical Direct Current

Direct current over the motor cortex for the purpose of cortical evoked potential modulation has been studied since the mid-1900s[19,22]. Early studies involving the study of direct current and the brain were conducted on cats, as procedures involved transcortical stimulation, rather than transcranial

stimulation[19,22]. Creutzfeldt, Fromm and Kapp[19] examined the effects of transcortical direct current on neuronal activity in the motor and optic cortex. For transcortical stimulation, researchers performed a tracheotomy and cord transection on cats, then an incision was made into the scalp and a 5 mm hole through the cranium and duramater over the motor or optic cortex to allow for electrode placement 1-2 mm superficial to the pia mater. An electroencephalogram recorded neural activity around the electrode, and found ¾ of the cortical neurons in a 3mm radius around the electrodes had altered activity following stimulation[19]. Purpura and McMurtry[22] further investigated cortical direct current, but focused on cortical evoked potentials. The researchers used a similar procedure to Creutzfeldt et al.[19], however microelectrodes were used to record transcortical voltage and the evoked potentials. The researchers concluded that anodal direct current resulted in neuronal depolarization, thus transmitting an electrical current across the membrane, where cathodal direct current resulted in hyperpolarization, negating the effects of neuronal depolarization[22].

Patient Populations

Motor Cortex

Nitsche and Paulus[18] evaluated the effects of tDCS on cortical excitability in the human motor cortex in healthy individuals through a series of four experiments. A pair of saline-soaked, 35 cm^2 sponge electrodes delivered the current from an unspecified battery-driven stimulator, which could not exceed a current of 1 mA. The objective of the first experiment was to identify the optimal electrode configuration. Ten subjects were included and different electrode placements were tested to determine the best position and two sessions, one anodal and one cathodal, were conducted. Current intensity was not specified but identified as between .2 and 1 mA. Prior to tDCS intervention, 10 TMS evoked MEPs at .25 Hz were elicited as a baseline measurement. A 4 second tDCS intervention was applied, followed by 12 TMS evoked MEPs at .1 Hz. The 12 TMS evoked MEPs at .1 Hz were conducted again without the tDCS preceding. Researchers concluded after the first experiment that anodal stimulation resulted in an increase in cortical excitability when compared to cathodal stimulation. The increase in cortical excitability was only noted in the motor cortex-forehead arrangement. Other arrangements tested were not indicated. For experiments 2-4, electrodes were placed over the area of the motor cortex found best for stimulation

of the right abductor digiti minimi through TMS, as well as superior to the contralateral orbit. Similar to experiment one, baseline MEPs were recorded with 20 stimuli from TMS at .25 Hz, followed by the tDCS intervention. The objective of experiment two was to examine the effect of polarity (cathodal vs anodal electrode positioning). Nineteen subjects were used, and were provided tDCS with a current intensity of 1 mA for five minutes. Participants received both conditions of anodal and cathodal tDCS. Researchers concluded after the second experiment that anodal stimulation resulted in significantly increased MEPs, whereas cathodal stimulation resulted in significantly decreased MEPs. Results of the second experiment demonstrated MEP values returned to baseline values 10 minutes following the intervention. The objective of experiment three and four was to examine the after-effects of tDCS. Experiment three used 12 subjects, underwent anodal stimulation at a current intensity of 1 mA, with the duration varying from 1-5 minutes. Experiment four used 12 subjects, had varying current intensities between .2 and 1 mA for 5 minutes using anodal stimulation. MEPs were again recorded for 5 minutes with TMS at .25 Hz following the stimulation. MEPs were recorded for the final time with 20 TMS stimuli after a five-minute rest period. The results of the study suggest cortical excitability can be altered through tDCS and the excitability changes may last for several minutes following intervention as MEPs continued to increase through 5 minutes following the intervention before [18]

Visual Cortex

Previous animal studies have suggested tDCS can be used to alter functions in the visual cortex[33]. A study conducted by Antal, Kincses, Nitsche, Barfai and Paulus[33] examined the excitability changes in the visual cortex following tDCS intervention. Twenty healthy participants were included and visual evoked potential outcome was measured through electrodes placed on the scalp. The researchers utilized a battery-driven constant-current stimulator set at 1.0 mA. The current was transmitted through rubber electrodes placed within 5x7 cm water soaked sponges. Two different experiments were conducted within the study. The purpose of the first experiment was to determine the most effective electrode arrangement to elicit excitability changes. During the first experiment, electrode placement and current (anodal or cathodal) varied based on the conditions of the experiment and each tDCS intervention lasted ten minutes. The purpose of the second experiment was to evaluate the aftereffects of tDCS duration. During the second experiment, the electrode placement was kept constant, current varied

between anodal and cathodal and stimulation lasted either five or 15 minutes. A voltmeter measured constant current flow and at least 1 week separated each testing session. The results of the study agree with other literature findings[18,22], suggesting anodal tDCS results in greater cortical excitability, while cathodal tDCS results in decreased cortical excitability. Additionally, the researchers found optimal electrode placement affected the elicited response and the aftereffects were dependent upon the duration of the stimulation, with stimulation lasting 10 minutes or greater causing greater aftereffects when compared to five minutes of stimulation[33].

Motor Learning

Studies have examined the effect of tDCS over the primary motor cortex on motor learning. Nitsche et al.[21] examined the effect of tDCS on performance changes, specifically the serial reaction time task. The parameters for stimulation were not discussed in the manuscript. Electrode placement varied by three participant groups with placement over the premotor, primary motor and prefrontal cortices. Stimulation was provided while performing the task and every participant performed three sessions. Each session the participants were randomly assigned anodal, cathodal or noncurrent stimulation, so each participant was tested under each condition. Based on the data, the researchers suggested stimulation of the primary motor cortex resulted in the greatest improvement in reaction times during the serial reaction time task and anodal stimulation resulted in greater learning acquisition and consolidation[21].

A study conducted by Reis et al.[42] also evaluated the effect of tDCS on motor learning concerning online effects, offline effects and long-term retention. Online effect was defined as skill acquisition while performing the sequential visual isometric pinch task. Offline effect was defined as skill acquisition between performance sessions. To evaluate long-term retention, five sessions over three months were conducted and scores from the pinch task were compared between the groups and across time. Parameters for tDCS were not discussed in the manuscript. To evaluate online effects of tDCS, researchers used 12 subjects over five training sessions on consecutive days. Methods for evaluating offline effects were not discussed in the manuscript. Participants were split into two groups, receiving anodal tDCS or sham tDCS. No difference was found between the two groups through online effects, however a significant difference in offline effects was suggested. Data indicated anodal tDCS resulted greater total learning compared to sham tDCS. The researchers found both the anodal tDCS and sham

tDCS groups forgot how to perform the task at the same rate, but greater total learning over the first five days resulted in significantly higher skill in the anodal tDCS group at the end of the 85 days[42]. The two studies[21,42] discussed suggest anodal tDCS results in greater motor learning when compared to cathodal tDCS or sham tDCS.

A study conducted in 2015 evaluated the use of tACS to improve motor learning. Thirteen participants were used for a repeated measures study evaluating tACS at 10 Hz, 20 Hz and sham tACS with the serial reaction time task (SRTT), and sessions separated by 1 week.[28] TMS was used to evaluated MEP's over the motor cortex of the first dorsal interossei, to identify a hot spot and the location of one of the sponge electrodes.[28] The other electrode was placed superior to the contralateral orbit.[28] Participants received one of the three conditions of tACS while simultaneously performing the SRTT, a well-documented paradigm for motor learning that requires participants to follow cues on a screen about using specified fingers to tap keys. The SRTT follows a pattern, and the fact that there is a pattern is unknown to the participants. Only 2 of the 13 participants indicated they recognized a pattern, and were not asked to recall the pattern.[28] Stimulation was provided over the entirety of the SRTT, approximately 12 minutes.[28] Data from 4 time points were analyzed to represent motor learning.[28] The researchers found tACS facilitated effects of motor learning for both 10 Hz and 20 Hz stimulation. [28]

Stroke

Transcranial direct current stimulation has been used previously in an attempt to improve motor function in patients following stroke. Hummel et al.[32] hypothesized tDCS to the motor cortex would improve motor function following stroke. Six participants at least one year after the stroke were included in the double-blinded study. Following the conclusion of the first study, five of the six subjects also participated in additional testing evaluating corticospinal excitability. All participants suffered from the same type of stroke and had resulting upper arm motor paresis, but were able to complete activities of daily living. Motor function was evaluated with the Jebsen-Taylor Hand Function Test. Subjects participated in a familiarization session, followed by two testing sessions, one session of tDCS and one session of sham tDCS. Two saline-soaked, gel-sponge electrodes were used to deliver the stimulation. The anode was placed over the hand knob area of the primary motor cortex, identified through MRI, of the affected side of the motor cortex and the cathode over the contralateral supraorbital area. The

Phoresor II Auto (Model No. PM850; IOMED) stimulator was used for the study. A current of 1 mA was delivered for 20 minutes for tDCS and for 30 seconds then slowly turned off for sham tDCS. The five subjects who continued with a fourth testing session to evaluate corticospinal excitability followed the same parameters and stimulator set up as the previous sessions. TMS was used to evaluate motor threshold and MEPs of the first dorsal interossei of the affected hand just prior to receiving tDCS, immediately following tDCS and 25 minutes following tDCS. The researchers found significantly reduced time on the Jebsen-Taylor Hand Function Test with tDCS compared to sham. The researchers did not find a significant decrease in motor threshold following tDCS in the subjects[32].

Another study evaluated the after-effects of anodal and cathodal tDCS in stroke patients compared to healthy volunteers[20]. The researchers hypothesized cathodal tDCS would increase cortical excitability in the stroke patients. Nine healthy subjects and seven stroke patients with resulting hemiparesis were enrolled in the study. Two saline-soaked sponges were used as electrodes. In stroke subjects one was placed over the affected motor cortex, while the other was placed over the contralateral supraorbital area, while in healthy subjects one was placed over the left motor cortex, while the other was placed over the right supraorbital area. TMS was used to identify the optimal electrode placement by testing for the largest MEP over the first dorsal interossei. The CX-6650 battery driven stimulator (Rolf Schneider Electronics, Gleichen, Germany) was used to deliver the current. Each session was delivered at 1 mA for 10 minutes. Motor threshold of the first dorsal interossei of the affected side in stroke participants and the left side of healthy participants was evaluated through TMS. Corticospinal excitability was evaluated at 110% of the resting motor threshold of the subject, per the suprathreshold stimulation, and was tested before tDCS intervention, immediately following tDCS intervention, ten minutes following tDCS intervention and 30 minutes following tDCS intervention. MEPs were measured for each TMS testing time. The researchers found a significant difference in the resting motor threshold of the two groups, with the stroke group demonstrating a higher resting motor threshold. The data also suggests cathodal and anodal tDCS increases cortical excitability in stroke subjects, but cathodal tDCS decreases cortical excitability in healthy subjects[20]. Further research with larger subject pools is needed to support the use of tDCS in stroke populations.

Parkinson's Disease

Transcranial alternating current has also been evaluated in the treatment of Parkinson's disease. Krause *et al*[26] evaluated the effect of tACS at 10 Hz and 20 Hz on motor control of the more severely affect hand in 10 patients with Parkinson's disease. Pre-intervention and post-intervention measures were compared, as well as compared against 10 matched control subjects.[26] Participants with Parkinson's disease participated in 2 sessions, receiving each of the frequencies of tACS, while the control participants only participated in 1 session of 20 Hz tACS.[26] All sessions received 15 minutes of stimulation.[26] Neuromagnetic activity was tracked in the hand, as well as motor function, as measured by dynamic fast finger tapping. Sponge electrodes for stimulation were placed over the primary motor cortex of the involved side, as identified with a hot spot from TMS, and the contralateral orbit.[26] The researchers found a decreased beta band cortically and an improvement in distal fine motor skills in Parkinson's disease patients at 20 Hz tACS.[26] No changes were observed in healthy participants.[26]

Safety

A study that collected data from several studies on tDCS evaluated the safety of tDCS[43]. The data came from several studies involving tDCS where researchers had subjects complete a questionnaire following the intervention. The data included 567 sessions from 102 participants were used in the study; 77 healthy subjects, 9 migraine subjects, 10 tinnitus subjects and 6 post-stroke subjects. All subjects were blinded to the study, had current intensity at 1.0 mA and most subjects received anodal, cathodal and sham tDCS. Stimulation was between 9 and 15 minutes, with ramp time of 8-10 seconds, with the exception of sham. The most common effect, reported by 70.6% of participants, was mild tingling for 7.8% of the stimulation duration. Moderate fatigue was reported by 35.3% of participants, light itching under the electrodes was reported by 30.4% of participants during stimulation and 14.9% following stimulation and 21.6% of participants reported mild burning. All other effects were reported in less than 20% of the participants, which included mild pain (15.7%), difference in the types of stimulation (16.7%), difficulty concentrating during stimulation (10.8%) or after stimulation (3.9%), headache during stimulation (4.9%) or after stimulation (11.8%), nervous or overexcited during stimulation (4.9%), and nausea (2.9%). Other reported effects included 17.7% reporting the stimulation mildly unpleasant and one subject

reported sleep disturbances two days after stimulation. No further medical attention was needed, no changes in visual perception or hyperactivity were reported and no sessions were asked to be terminated[43]. Another study cited sensation of current flow has been noted as an itching sensation under both electrodes, and light flashes as the current is turned on and off[18]. Overall, tDCS has been found to be a safe intervention with few mild negative side effects.

Antal et al.[44] investigated the use of tACS versus tDCS for increasing cortical excitability. Fifty subjects participated in the study; 10 healthy individuals evaluated using TMS, 8 healthy individuals evaluated using electroencephalogram (EEG), 2 evaluated using both TMS and EEG, and 16 evaluated using implicit motor learning.[44] Electrodes for stimulation were placed over the motor cortex and the contralateral orbit.[44] For the TMS study, participants received both tACS and tDCS and MEP amplitude was the main outcome measure.[44] For the EEG study, participants outcome measure of EEG was taken prior to and following stimulation of both tACS and tDCS, comparing pre-intervention and post-intervention measures.[44] The implicit motor learning task was the SRTT, as studied by Krause *et al*[26] as well, and participants received either tACS or sham tACS.[44] The main finding of the study was an improvement in SRTT reaction times for 10 Hz tACS; no significant difference was seen between intervention and control for MEP amplitudes and EEG values.[44] The researchers noted small sample sizes for the MEP and EEG studies, and larger sample sizes may have resulted in significant findings.[44] Based on the data, the researchers believe tACS may be a valuable treatment in the future, pending further research.[44]

Conclusion

Neuromuscular alterations have been shown to be present in the quadriceps muscle group following knee joint injury[2,3,6-12]. Quadriceps voluntary activation deficits[7,15-17] and quadriceps muscle strength[2,3,10-12,36] deficits of the involved limb are believed to be the result of neuromuscular alterations, specifically decreased corticospinal excitability[3,7,40]. Altering corticospinal excitability may potentially improve quadriceps muscle dysfunction following knee injury and increase physical function[12]. Transcranial electrical current stimulation has been identified as a non-invasive intervention with the ability to alter cortical excitability,[18,20,21,32,33,42,43]. While there is research supporting the hypothesis that

transcranial electrical stimulation may alter corticospinal excitability in pathological populations, future research should evaluate the effectiveness of transcranial electrical current stimulation at altering corticospinal excitability in an orthopedic population experiencing quadriceps dysfunction, and further examining clinical effectiveness of reducing quadriceps muscle dysfunction by improving corticospinal excitability.

CHAPTER 3: METHODS

Research Design

This study utilized a single-blinded crossover design, where the investigator was blinded to whether the participant was receiving the control or intervention condition by stepping out of the room while the intervention or control was being administered. The independent variable was intervention condition (tACS or no tACS), and the dependent variables were the main outcome measures of cortical excitability and voluntary activation of the dominant quadriceps. Cortical excitability was measured as active motor threshold (AMT), via single pulse transcranial magnetic stimulation (TMS). Voluntary activation was assessed using the superimposed burst (SIB) technique and quantified using the central activation ratio (CAR).

The Institutional Review Board at the University of North Carolina at Chapel Hill approved the study prior to the start of data collection. All participants received and completed an informed consent form. Participants attended two data collection sessions, one intervention and one control session, one week apart at the same time of day. The order of intervention was counter-balanced, and was determined once the participant signed the consent form. The same investigator conducted all outcome measures during each of the two testing sessions.

Participants

Participants were a sample of convenience recruited at the University of North Carolina at Chapel Hill (See Figure 3 for demographics). Healthy participants between the ages of 18-40 with no history of knee or quadriceps injury within the past six months were included. Exclusion criteria included history of lower extremity surgery or ligamentous knee injury, chronic ankle instability, balance disorders, pregnancy, concussion or head injury within the past 6 months, Parkinson's Disease, Multiple Sclerosis, stroke, cardiac condition, epilepsy, fibromyalgia, diabetes, peripheral neuropathy (numbness, tingling, loss of sensation in hands or feet), migraine headaches, cranial neural surgery, cancer in the brain or

thigh musculature, diagnosed psychiatric disorder, cardiac pacemaker, implanted foreign metal objects, cochlear implants, implanted brain stimulators, aneurysm clip, or implanted medication pump.

In order to estimate sample size, we determined the standardized effect size (d=0.71) from reported motor evoked potential (MEP) amplitude means and standard deviations for corticomotor excitability following an electromyography biofeedback intervention. We estimated that 33 participants would be needed to detect a statistically significant difference between conditions with the smallest expected mean difference (5% AMT) with a moderate standardize mean difference (d=0.71), an alpha level of 0.05 and 80% power.[45]

Instrumentation

An isokinetic dynamometer (HUMAC Norm; CSMi, Stoughton, MA) was used to measure torque signal to determine quadriceps strength and voluntary activation, as well as provide feedback during the assessment of corticospinal excitability. The torque signal from the isokinetic dynamometer was exported at a gain of 1500, in order to prevent signal clipping, and was used for assessment of voluntary activation and corticospinal excitability.

Corticospinal Excitability

Transcranial Magnetic Stimulation (TMS) was used to determine AMT. Motor evoked potentials (MEP) were elicited using the Magstim Bistim (Magstim Company, Wales, UK) via a double cone coil (Magstim Company, Wales, UK) with a maximum stimulation output of 2 Tesla. All MEPs were measured in the vastus lateralis of the dominant limb, identified as the leg they would kick a soccer ball with, through electromyography (EMG) and were acquired using disposable, disk-shaped 10 mm pre-gelled Ag/AgCl (BIOPAC Systems, INC) electrodes. Acqknowldege Software (BIOPAC Version 3.7.3, BIOPAC Systems, Inc.) was used to visualize EMG signals, which were sampled at 1024 Hz and amplified at a gain of 1000 (EMG100C BIOPAC Systems, Inc.). The common mode rejection ratio of our EMG amplifier was 100 dB with input impedance of 2MOhms.

Quadriceps Voluntary Activation

For voluntary activation measurements the torque signal was exported from the dynamometer in real-time and a 16-bit, 1.25-MS/s A-to-D conversion board (National Instruments; USB-6251, Austin, TX) sampled the torque signal at 2000Hz in order to allow participants and investigators to visualize torque production in real time. The torque signal was displayed on a computer monitor placed in front of the participant and visualized using a custom built LabView program (v12.0; National Instruments, Austin, TX) with the ability to trigger the supramaximal stimulus delivered to the quadriceps during muscle activation assessment. A square-wave stimulator (S88, GRASS telefactor, W.Warwick, RI) and a stimulation isolation unit (SIU8T, W. Warwick RI) were used to produce a 100ms train of 10 stimuli at 100 pulses per second, with a 0.6ms pulse duration and a 0.01ms pulse delay.[46] Each participant was stimulated with approximately 125V.[46]

Transcranial Alternating Current Simulation

Transcranial alternating current stimulation (tACS) was administered with the Fisher Wallace Stimulator FW-100. The stimulator is FDA approved for the treatment of depression, insomnia and anxiety. The stimulator emits an alternating electrical current of 15 Hz[47] which was applied for 10 minutes.[20]

Procedures

Participants were recruited for 2 testing sessions; one session of tACS with quadriceps isometric contractions and one session with just quadriceps isometric contraction (Figure 1). Each of the 2 sessions followed the same testing order. Participants read and completed the informed consent form and exclusionary criteria questionnaire. Next we prepared the skin for EMG electrode placement and participant set up. Participants first completed the quadriceps strength assessment, as the MVIC measurement was used for AMT and CAR assessments, followed by voluntary activation and corticospinal excitability assessments. Either the intervention or the control was administered, followed by retesting AMT and CAR.

Figure 1. Order of Testing Procedures
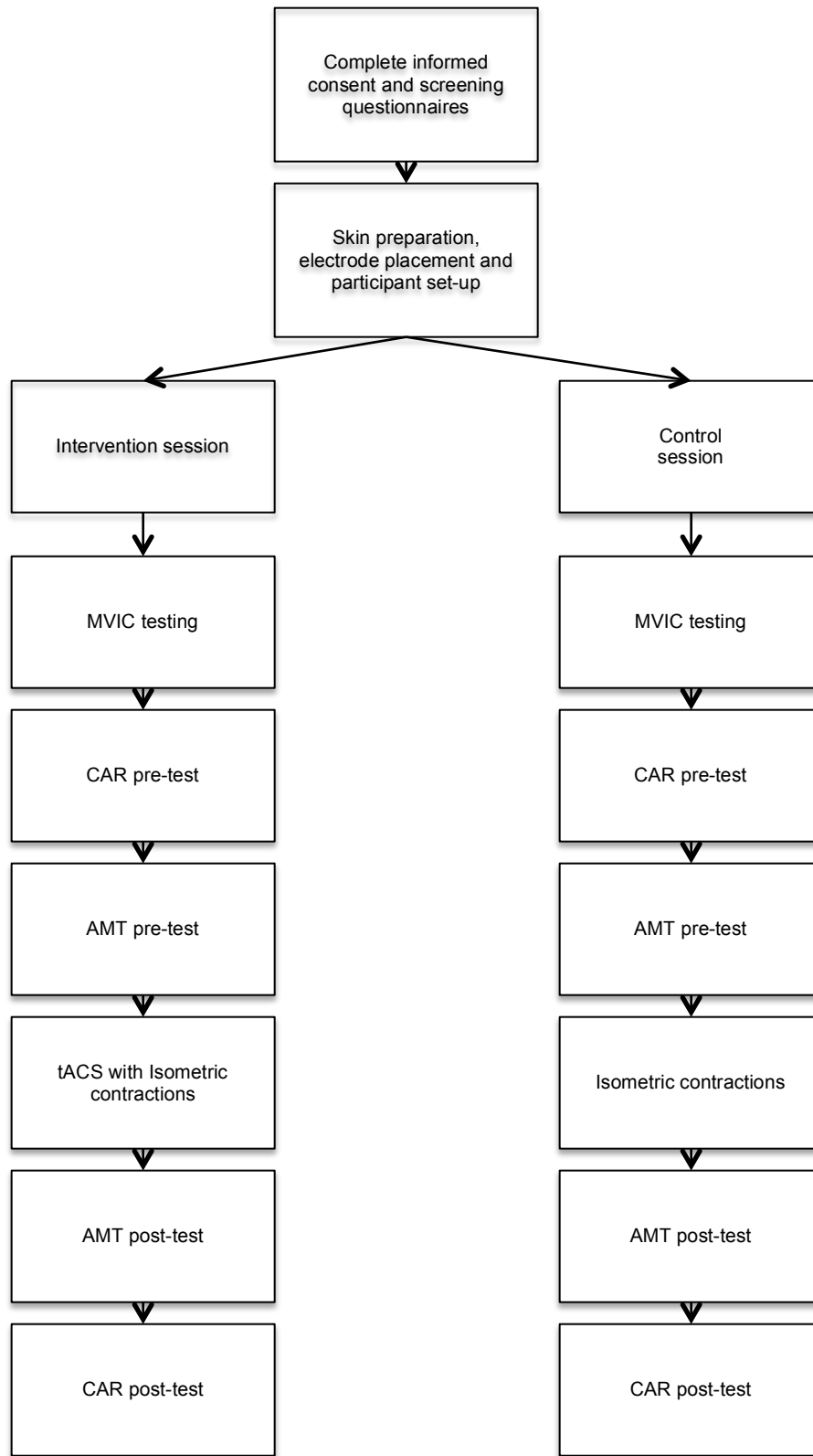
Hair over the sites of the EMG electrodes was shaved and skin was debrided and cleaned with an alcohol wipe. EMG electrodes were placed over the muscle belly of the vastus lateralis of the dominant limb, 1.75 cm apart, and a ground electrode was placed over the medial malleolus of the dominant leg. A lycra swim cap with a 20 cm x 20 cm grid was placed on the participant's head to locate the optimal area of stimulation for the quadriceps. Perpendicular lines on the swim cap were aligned from the bridge of the nose to the center of the occiput and from each external auditory meatus to ensure standardized positioning in the frontal and sagittal planes.[41] The perpendicular lines also served as a coordinate system for the grid to better identify the optimal hot spot for stimulation.

Outcome measures

Maximal Voluntary Isometric Contraction

Maximal voluntary isometric contractions were measured via a Humac dynamometer, and was used to calculate target lines for participants to ensure appropriate levels of muscle contraction during TMS testing, CAR testing and during the intervention. Each participant was seated on the isokinetic dynamometer with the hips flexed to 85° and the knees flexed to 90° with their arms folded across the chest. The pelvis and torso were secured to the dynamometer using adjustable straps in order to isolate the quadriceps muscle during maximal contractions.[48] The padded arm of the dynamometer was secured to the leg 3 cm proximal to the lateral malleolus and adjusted so the knee joint axis of rotation was aligned with the dynamometer axis of rotation. The locations of all dynamometer components were marked to assist with replication of the dynamometer position for the subsequent testing session. Once the participant was secured in the dynamometer, quadriceps MVIC was quantified to determine target activation levels that were used for both testing sessions. Participants were instructed to contract their quadriceps muscle and extend their knee into the padded arm of the dynamometer with as much force as possible, and to hold their maximum torque at a plateau for approximately 3 seconds. The maximum torque value for each trial was recorded. Participants continued to perform maximum voluntary isometric contractions, with at least 60 seconds of rest in between, until the maximum torque value from the trial no longer continued to increase by more than 10%.[49] The three MVIC trials that produced the greatest torque were averaged and used to create a threshold for the following testing procedures.

Active Motor Threshold

For all TMS testing, participants were instructed to sit and relax while maintaining a constant head and eye position with their arms crossed over their chest and to focus on the computer screen depicting real-time force in front of them. Participants were instructed to clear their mind of any additional thoughts, to focus on reaching the target line on the computer screen, and to remain awake and alert to control for mental-state variability throughout testing. Participants were seated in the dynamometer as previously described and the double cone coil was placed over the intersecting lines on the swim cap.[41] Each time a stimulus was delivered the participant was instructed to contract their muscle to 5% MVIC, which was depicted on the computer screen. Two stimuli were delivered at an intensity of 50% maximum stimulus intensity at each of the grid points on the swim cap, and the corresponding MEP elicited at each location were measured and recorded.[41] The "hot spot" for the quadriceps was identified as the location over the primary cortex, noted by points on a grid that elicited the greatest MEP in the desired muscle group. The hot spot was marked on the swim cap and the coil remained over the hot spot for the remainder of the session.

Active motor threshold was defined as the lowest TMS intensity required to evoke a measurable MEP (>100 µV) in 5 out of 10 consecutive measurements in the vastus lateralis.[41] Once 6 out of 10 measureable MEPs were elicited, the intensity level was decreased by 1% until a total of 5 out of 10 stimuli failed to elicit a measureable MEP amplitude greater than 100 µV.[41] Previously, dominant limb vastus medialis AMT has been found to have strong intersession reliability from baseline to 14 and 28 days ($ICC_{14\ days}$ = .963; $ICC_{28\ days}$ = 0.932). As electrodes for CAR stimuli were placed over the distal medial rectus femoris, the vastus lateralis muscle was evaluated via EMG.

Central Activation Ratio

Participants remained seated in the dynamometer and two 7 x 13 self-adhesive stimulating electrodes (Dura Stick II; Chattanooga Group, Hixson, TN) were positioned on the distal and proximal rectus femoris.[1] The distal electrode was positioned with the inferior edge 3cm superior to the patella and the lateral edge of the electrode in line with the midline of the patella. The proximal electrode was placed

over the rectus femoris with the superior edge at the height of the greater femoral trochanter and the midline of the electrode aligned with the anterosuperior iliac spine. Each participant underwent a graded warm-up to allow familiarization to the stimulus. Participants performed a series of submaximal contractions at 25%, 50%, and 75% of their perceived MVIC, which was paired with 25%, 50%, and 75% of the 125V maximum test stimulus.[1,46] Two visual feedback lines were depicted on a computer monitor in front of the participants. The first line was set at each participant's average MVIC value. The second was corresponding with 120% of the participant's average MVIC. Participants were instructed and verbally encouraged to contract their quadriceps and extend their knee into dynamometer arm with as much force and as fast as possible in order to attempt to increase their torque to the second feedback line in order to ensure a maximal effort. Two acceptable trials, with a 60 second rest between, were completed for each testing session. Previously, CAR with the SIB technique has been demonstrated to have a strong intersession reliability from baseline to 14 and 28 days ($ICC_{2,k}$ = 0.80, P = 0.001; $ICC_{2,k}$ = 0.85, P ≤ 0.001).[50]

Intervention

Participants remained in the Humac dynamometer during their intervention. Participants were tested in two conditions; one condition of isometric quadriceps contraction and one condition of tACS with isometric quadriceps contraction. The order of the conditions received was counterbalanced for each participant. During administration of the tACS one sponge electrode (30 mm round sponge electrode, Fisher Wallace, New York, NY) was placed over the hot spot and a second sponge electrode was placed superior to the contralateral orbit.[18] Location of the hot spot was measured using the coordinates of the hot spot and the measures taken from the bridge of the nose to the center of the occiput and from each external auditory meatus. The swim cap used for AMT was removed, however the grid lines on the swim cap was measured as perpendicular lines from the bridge of the nose to the center of the occiput and from each external auditory meatus. This measurement was recorded to ensure the swim cap is placed on the same location over the scalp following stimulation. The hot spot was identified for the sponge electrode placement after removing the swim cap. The measurements taken when centering the swim cap and the x,y coordinates found when identifying the hot spot were used to identify the location of the

hot spot over the scalp. The participants' hair was moved to allow the sponge electrodes to be placed as close to the scalp as possible. Participants received tACS via the Fisher Wallace Stimulator (FW-100, Fisher Wallace, New York, NY) for 10 minutes. The stimulatory intensity was turned up to a sensory level over the scalp, as reported by the participant. Participants felt light tingling along the scalp.[43] The intensity was then turned back to a sub-sensory level where the participant reported they no longer felt any stimulation. The Fisher Wallace Stimulator has been FDA approved.[47] While receiving the tACS, participants performed a series of quadriceps contractions, a total of 18, at 5% MVIC with a torque feedback and target line, holding an isometric contraction for 5 seconds, every 30 seconds for 10 minutes. The first contraction began when the timer started and the last contraction was at 9 minutes 30 seconds so the current could be turned off at 10 minutes.

Participants remained in the HUMAC dynamometer during the control session as well. The sponge electrodes were placed over the participants' scalp using the same method as the intervention session to ensure the sensory feedback from the Fisher Wallace Stimulator electrodes being placed over the scalp was not the cause of any changes in corticospinal excitability, however the device was never turned on during the control session. During the control session, participants performed a series of quadriceps contractions, a total of 18, at 5% MVIC with a torque feedback and target line, holding an isometric contraction for 5 seconds, every 30 seconds for 10 minutes. Immediately following the application of the tACS or the control, the swim cap was placed over the scalp again, using the same measures taken during the participant set up to ensure the swim cap was placed in the same location as it was prior to testing.

Data Analysis

A second LabView program was used for data analysis to determine MVIC peak torque and the CAR during each trial. The MVIC peak torque for each trial was determined as the average torque value over the 50ms prior to delivery of the SIB stimulus. MVIC peak torque was normalized to body mass (Nm/kg). The CAR was calculated by expressing the torque produced during the MVIC (T_{MVIC}) as a percentage of the total torque produced by the T_{MVIC} and the SIB stimulus (T_{SIB}) (Equation 1).
Equation 1.

$$CAR = \left(\frac{T_{MVIC}}{T_{MVIC} + T_{SIB}} \right) * 100$$

For each main outcome measure, a change score from pre-test to post-test was calculated. The percent change score for AMT was calculated using equation 2. A positive percent change score for AMT reflected a decrease in the AMT value from pre-test to post-test. A decrease in AMT from pre-test to post-test was the desired effect during the intervention session, reflecting an increase in corticospinal excitability.

Equation 2.

$$Percent\ Change\ = \frac{Pre\ test\ -\ Post\ test}{Pre\ test} * 100$$

Percent change scores for CAR was calculated using equation 3. A positive percent change score for CAR reflected an increase in the CAR values from pre-test to post-test. An increase in CAR from pre-test to post-test was the desired effect during the intervention session, reflecting an increase in activation.

Equation 3.

$$Percent\ Change\ = \frac{Post\ test\ -\ Pre\ test}{Pre\ test} * 100$$

Statistical Analysis

Prior to the primary analyses we evaluated intersession reliability for absolute agreement for AMT (ICC $_{3,1}$) and CAR (ICC $_{3,k}$) outcomes using the baseline measurements. A dependent samples t-test was used to evaluate differences in the percent change in AMT and CAR between pre-intervention and post-intervention between conditions. Separate dependent samples t-tests were used to determine differences between the intervention and control sessions for the change score calculated for each outcome measure. The α level was set a priori at 0.05. All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Version 19.0, IBM Corp., Somers, NY).

CHAPTER 4: RESULTS

A total of 39 participants were recruited for the study. Thirty-four participants completed both

testing sessions and were used for analysis (Fig. 1 & Fig. 2).

Figure 2. Participation Demographics
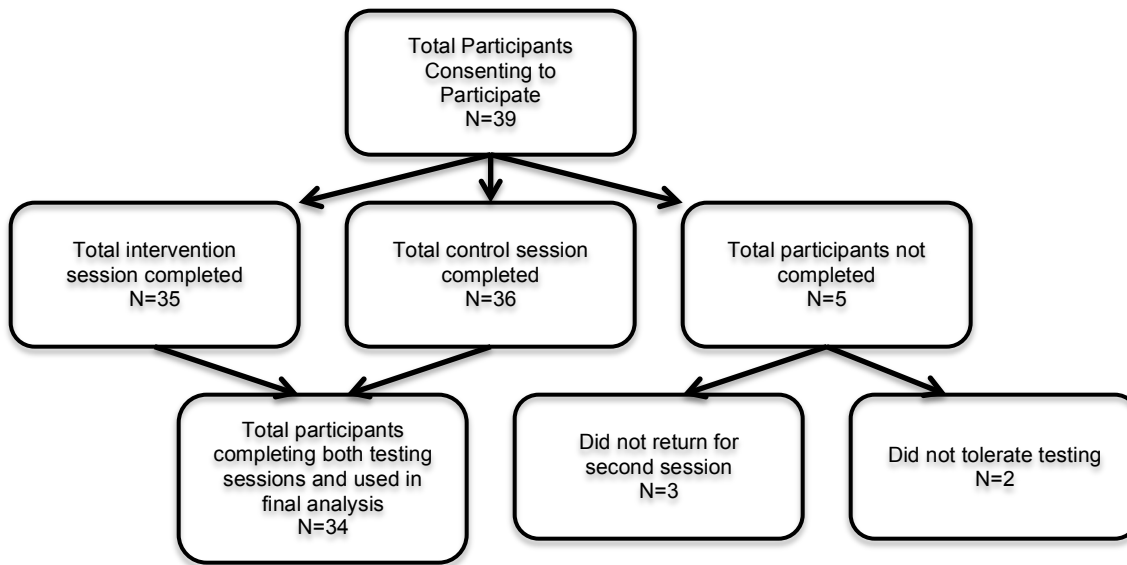
The pre-intervention, pre-control, post-intervention and post-control values for AMT and CAR are

presented in Table 1. First, intersession reliability was evaluated for baseline measures of AMT and CAR.

Active motor threshold demonstrated strong reliability ($ICC_{3,1}$ = .854) between testing sessions and CAR

demonstrated moderate reliability between testing sessions ($ICC_{3,k}$ = .734). There were no significant

differences in the percent change scores between the intervention and control conditions for AMT

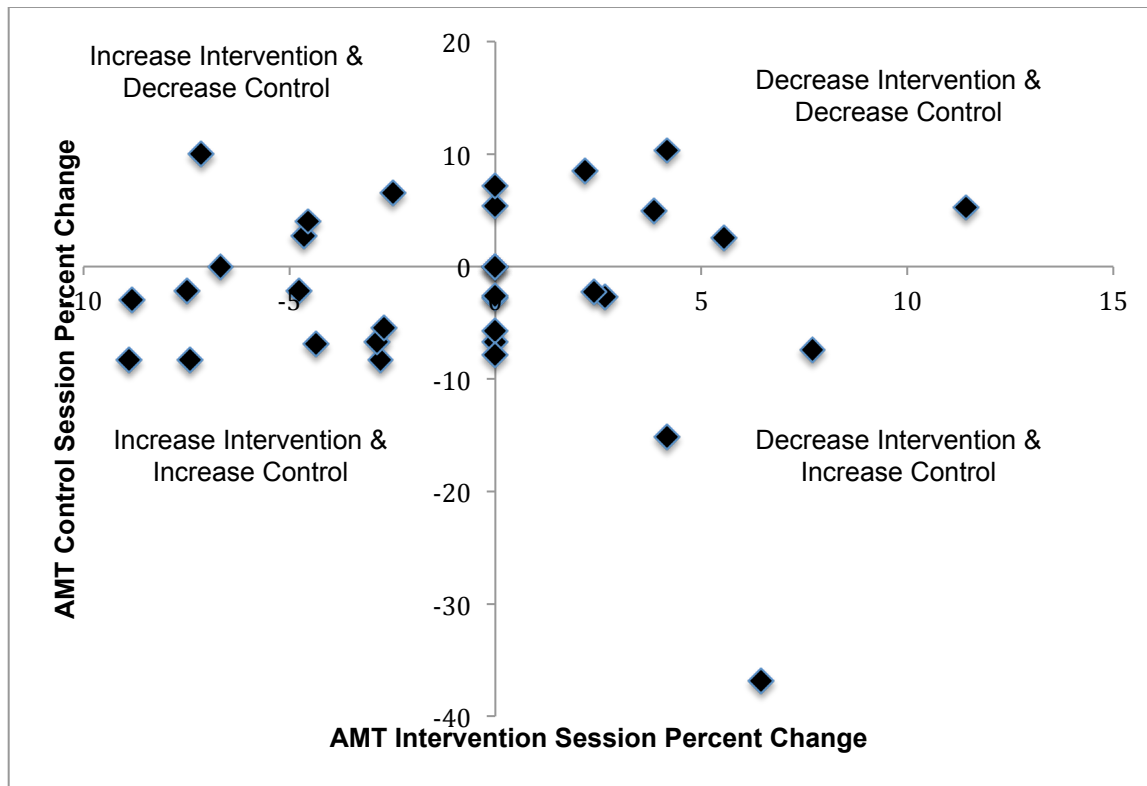(t_{33}=.813, P=.422)and CAR (t_{33}=-.449, P=.656).

Table 1. Participant Demographics

	Minimum	Maximum	Mean ± S.D.
Age (years)	18	27	20.35 ±1.76
Height (cm)	148	190	165.85 ±9.51
Weight (kg)	45	88	66.76 ±11.76
AMT Intervention Session			
Pre-test	20.00	75.00	35.15 ±10.07
Post-test	20.00	73.00	35.44 ±10.26
Percent Change	-8.89	11.43	-.733 ±4.89
AMT Control Session			
Pre-test	20.00	74.00	36.71 ±10.65
Post-test	18.00	76.00	37.62 ±11.60
Percent Change	-36.84	10.34	-2.17 ±8.67
CAR Intervention Session			
Pre-test	.73	1.00	0.92 ±0.07
Post-test	.86	1.00	0.94 ±0.05
Percent Change	-6.25	21.25	2.66 ±6.14
CAR Control Session			
Pre-test	.73	1.00	0.91 ±0.07
Post-test	.65	1.00	0.94 ±0.08
Percent Change	-31.58	21.25	3.31 ±7.71

Post-hoc Analysis

In order to better discuss our findings we conducted a post hoc analysis to determine the association between the percent change in AMT during the control session as well as during the intervention session to determine if any changes in corticospinal excitability could be attributed to the low level isometric contractions performed during the control and intervention session. We used a Pearson product moment correlation to assess two-tailed bivariate associations between percent change in AMT during the intervention session and the control session. There was no significant association between the percent change in AMT during the intervention session and the percent change in AMT during the control session (r = -0.003; P=0.987).

Figure 3. AMT Percent Change Control Session vs. AMT Percent Change Intervention Session

Scatter plot with x-axis labeled "AMT Intervention Session Percent Change" (ranging from -10 to 15) and y-axis labeled "AMT Control Session Percent Change" (ranging from -40 to 20). The four quadrants are labeled: "Increase Intervention & Decrease Control" (top left), "Decrease Intervention & Decrease Control" (top right), "Increase Intervention & Increase Control" (bottom left), and "Decrease Intervention & Increase Control" (bottom right).

CHAPTER 5: DISCUSSION

The purpose of this study was to evaluate the immediate effects of tACS on corticospinal excitability and voluntary activation of the quadriceps, as measured through active motor threshold and central activation ratio. In our sample of healthy participants, there were no differences in the percent change scores for AMT or CAR between the intervention and control sessions. Additionally, we determined there was no association between the percent change in AMT during the control session and the percent change in AMT during the intervention session, suggesting any changes in corticospinal excitability were not attributed to the low level isometric contractions.

Previous research using tDCS has demonstrated that the direction of the trancranial current passing through the primary motor cortex influences the change in cortical excitability. Cathodal tDCS hyperpolarizes cortical neurons, which leads to a decrease in cortical excitability[18]. Conversely, anodal tDCS causes cortical neuronal depolarization, which results in an increase in cortical excitability[18]. The Fischer Wallace Stimulator FW-100 tACS unit used in our study, however, emits an alternating current (tACS) which outputs current in both anodal and cathodal directions. The use of an alternating current rather than a direct current may influence the effectiveness of acutely altering cortical excitability and voluntary activation outcomes. Antal et al[44] evaluated the effect of tACS for altering MEPs of the first dorsal interossei, and found no significant changes in MEPs across five different frequencies of tACS and a sham tACS session. This finding supports the hypothesis that an alternating current working in both the anodal and cathodal direction may have an intracortical cortical affect that essentially cancel out any facilitatory or inhibitory effects of the current.

The tACS electrode placement in our study was similar to the placement used in previous studies evaluating the effect of transcranial current stimulation on cortical excitability. In previous studies one electrode is placed over the motor cortex of the investigated muscle and the other superior to the contralateral eye.[26,28,44,51,52] Previous literature cited a wide range of intensities used when applying tACS. For example, previous intensities cited in literature include 250 µA[44], 1 mA[26,51] and at varying intensities of 1 mA, 2 mA and 3 mA[52]. The Fischer Wallace Stimulator FW-100 is intended for general public use, so

the specific intensity is not visible on the device, however the manufacturers manual states the device emits an intensity of 1-4 mA. The stimulator intensity was set at a sub-sensory level, which may have been an intensity that was too low to induce robust acute alterations in corticospinal excitability. The Fischer Wallace FW-100 device does not indicate the current that is being emitted, and therefore the exact intensity that the intervention is applied at is not known. Additionally, the intensity in our study was based on the sensation that each participant perceived the stimulation. Therefore, the dose of the intervention may have been different for participants who may have perceived the sensation at different intensities. The Fischer Wallace FW-100 emits a set frequency of 15 Hz.[47] High and low frequency tACS has been evaluated in the literature, with inconclusive findings regarding the effectiveness of different frequencies.[44]

There were no significant associations between percent change in AMT during the intervention session and percent change in AMT during the control session. Thereby, an increase or decrease in a participant's AMT during the control session did not indicate a similar change during the intervention session. It could be hypothesized that if corticospinal excitability was affected similarly by repeated low-level muscle contractions, and that the tACS was ineffective, that change in in AMT would associate between sessions. While AMT for some participants either increased or decreased in both sessions, there were several participants whose AMT increased in one session, while decreasing in the other and visa versa (Figure 3). One outlier was present, whose AMT increased 36.84% during the control session, but the all other sessions were between 0% and 15%. This finding suggests that the response to tACS when evaluating AMT is a very individualized response. Varying degrees of baseline cortical inhibition and facilitation may alter the outcome of transcranial current stimulation and whether an increase or decrease in cortical excitability is demonstrated. Cortical excitability is believed to be the result of the balance between cortical inhibition and cortical facilitation. It is unclear if an individual in decreased cortical excitability is due to decreased facilitation within the motor cortex, or increased inhibition of intracortical neurons within the motor cortex.[53,54]

While the current study provides fundamental information regarding one tACS intervention technique, there were limitations to this study that should be considered when interpreting our findings. We assessed the intervention in healthy participants that may not have had significant deficits in cortical

excitability. Healthy individuals typically do not demonstrate lowered levels of cortical excitability compared to individuals with knee joint injury.[15] Previous studies [21,28,42] examining the efficacy of tACS on motor function have been conducted in conjunction with functional movements, i.e. the serial reaction time task, used for hand and finger function[18,21,28] and a sequential visual isometric pinch task, also used for the hand and fingers[42] rather than isometric muscle contractions. Another limitation is the measurement of AMT, which is assessed through single pulse TMS, and it evaluates a change in the excitability of the entire corticospinal pathway, from the primary motor cortex to the vastus lateralis.[14] By using AMT and evaluating the entire corticospinal pathway we do not know what is occurring in inhibitory and excitatory intracortical circuitry directly at the motor cortex where the intervention is being applied. Therefore we are unable to determine why some individuals may have responded to tACS while others did not respond to the intervention. Another limitation is that AMT is specific to the exact location of stimulation, and slight changes in coil placement may influence AMT. We used a swim cap positioned on the head of each participant that contained a grid which was used to identify the area of the scalp that elicited the greatest MEP.[41] However, in order to place the tACS sponge electrodes over the motor cortex as close to the scalp as possible the swim cap was removed during the intervention and was then placed back on the head following the intervention. While every effort was made to place the swim cap back in the same location over the scalp by measuring the cap and centering it on the head, the slightest shift in location of the swim cap and grid over the scalp could have influenced the posttest AMT. While we demonstrated strong reliability in AMT between sessions ($ICC_{2,1}$ = .854), our intersession reliability for CAR was moderate ($ICC_{2,1}$ = .734). The lower reliability that we found for CAR could be explained by the use of a manual trigger for the SIB stimulus that relies on the investigator to trigger the stimulus, rather than an automated system that triggers the stimulus based off of each participant's torque value.[55] While unlikely, lower CAR reliability may be influenced our assessment of quadriceps voluntary activation following the intervention.

The lack of association between the percent change in AMT following the intervention and the percent change in AMT following the control session suggests further studies should be conducted in order to determine if there is a specific population of individuals who respond to tACS. Previous research has suggested that injured individuals display lower cortical excitability following injury when compared to

healthy individuals and the uninjured limb.[1,15,34] Evaluating the effect of tACS on AMT and CAR in a pathological population where cortical excitability is decreased may result in greater changes in AMT and CAR than the changes determined in this study. Additionally, as anodal tDCS has been demonstrated to effectively increase cortical excitability as compared to tACS, future investigations should seek to determine the effect of tDCS on AMT.[18,20,33]

In conclusion, we found that tACS in conjunction with submaximal isometric contractions did not elicit acute changes in quadriceps AMT and CAR as compared to isometric contractions alone in healthy individuals. Additionally, as changes in AMT following the intervention session did not associate with changes in AMT following the control session, it is possible that the individual response to tACS can vary between individuals. Further research evaluating the effectiveness of transcranial current stimulation for the purpose of increasing cortical excitability in an orthopedic population displaying decreased quadriceps function is necessary. Targeting the neural origins of decreased muscle function in conjunction with traditional rehabilitation may allow for greater improvements in quadriceps strength following joint injury.

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