THE EFFECT OF SHORT-TERM ANKLE IMMOBILIZATION ON JOINT STIFFNESS AND NERVOUS SYSTEM FUNCTION

A Thesis
by
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Abstract

THE EFFECT OF SHORT-TERM ANKLE IMMOBILIZATION ON JOINT STIFFNESS AND NERVOUS SYSTEM FUNCTION
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Ankle sprains are the most common musculoskeletal injury observed in the physically active, with high rates of recurrent injury tied to neuromechanical alterations. While immobilization is often employed in the treatment of initial ankle sprains, debate remains regarding its beneficial and detrimental effects. Previous research has identified alterations in corticospinal excitability following upper extremity immobilization; however it remains unknown how immobilization affects neuromechanical function at the ankle. Therefore, the purpose of this study was to determine the effects of short-term immobilization on stiffness and reflexive and cortical excitability of the ankle joint. Twelve able-bodied volunteers (22.5±1.4yrs, 173.05±17.5cm, 71.6±12.7kg) walked on a treadmill for 30 minutes while wearing either an ankle immobilizer, pneumatic leg brace, or no external support. Joint stiffness, cortical & reflexive excitability were evaluated via ankle arthrometry (maximum anterior/posterior displacement, total inversion), transcranial magnetic stimulation (motor evoked potential at 90, 110, 150% of active motor threshold), and the Hoffman reflex (H_{max}:M_{max} Ratio), respectively, before and after walking. Findings revealed no significant change in cortical or reflexive excitability across time, conditions, and muscles. These results lend support to the hypothesis that short-term immobilization allows for the joint to be protected from potentially deleterious loading while possibly presenting alterations in corticospinal excitability. Further research is needed to examine how longer bouts of immobilization effect cortical and reflexive excitability.

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Chapter 1: Introduction

Over a lifetime, 60 percent of people have experienced an ankle sprain and up to 74 percent of people develop residual symptoms (Anandacoomarasamy & Barnsley, 2005; Attenborough, Hiller, Smith, Stuelcken & Greene, 2014; Hiller, Nightingale & Raymond, 2012). These symptoms include pain, weakness, swelling, and instability which could lead to recurrent ankle sprains (Hertel, 2002). In an effort to negate the risk of recurrent ankle sprains, initial treatment typically consists of the ankle being immobilized in combination with functional exercises. A recent position statement from the National Athletic Trainers Association provided recommendations that severe ankle sprains be immobilized for up to 10 days, while Grade I and II sprains would benefit from functional rehabilitation instead of immobilization (Kaminski, et al., 2013). However, these outcomes are largely based on return-to-play rather than long-term function and it is unclear how immobilization affects mechanical and nervous system function as well as long term joint stability.

Joint stability depends on the ability of static and dynamic stabilizers to protect the ligamentous structures from injurious loads (Freeman, 1965). Both feed-forward (preparatory) and feedback (reactive) muscular activity must be coordinated by the nervous system in order to avoid injury. Function of the peripheral and central nervous systems have been assessed with measures of reflexive and cortical excitability, respectively, documenting the contributions of spinal reflexes and the primary motor cortex in providing joint stability (Johansson, 1991; McVey, Palmieri, Docherty, Zinder, & Ingersoll, 2005). A relationship between joint stiffness and this neurological function, termed *neuromechanical coupling*, has been described throughout the central and peripheral nervous systems (Needle, Palmer, Kesar, Binder-Macleod & Swanik, 2013). While joint injury variably affects mechanical and neurological function, it might lead to neuromechanical *decoupling*. The exact causes for this decoupling has eluded researchers, forcing shifts in current paradigms of joint stability.

Two well established ways to directly quantify central nervous system changes secondary to injury are Transcranial Magnetic Stimulation (TMS) and the Hoffmann Reflex (H-reflex). TMS allows for direct investigation of cortical excitability and inhibition (Hallett, 2007) while the H-Reflex evaluates reflexive excitability (Johansson, 1991; Needle, Baumeister, Kaminski, Higginson, Farquhar & Swanik , 2014).

One factor with the potential to alter neuromechanical coupling is joint immobilization. It has been suggested that immobilization protects the joint, leading to improved healing and better long-term outcomes after ankle sprain (Palmieri, Hoffman & Ingersoll, 2002). However, immobilization has also been associated with harmful changes to bone, muscle, ligament and neurological function (Lamb, Marsh, Hutton, Nakash, & Cooke, 2009). These differing viewpoints causes a stark contrast between basic science research and those investigating clinical outcomes following injury. Limited studies have documented the effect of immobilization on central nervous system function

Lateral ankle sprains present a problem to public health due to both a high occurrence and recurrence rate leading to negative effects on lifelong physical activity and health (Lundbye-Jensen & Nielsen, 2008; Valderrabano, Hintermann, Horisberger, & Fung, 2006). While initial treatment of ankle sprains often utilize immobilization, little is known about how different forms of immobilization such as pneumatic leg splints or boot immobilizers affect mechanical function, and cortical and reflexive excitability. As treatment of initial ankle sprains often relies on the use of immobilization; the distinctive effects of immobilization devices on joint stability may contribute to our understanding of why half of patients develop recurrent problems, while others are able to successfully heal following their injuries. Therefore the purpose of this study is to determine the effect of immobilization on neuromechanical coupling, as quantified through passive ankle stiffness, cortical excitability, and reflexive excitability.

CHAPTER 2: Review of Literature

Introduction

Suffering a lateral ankle sprain has been associated with lower activity level and health related quality of life thus presenting a problem to public health (Hubbard-Turner & Turner, 2015). Lateral ankle sprains have a high incidence rate both on the athletic field and in everyday life (Boyce, Quigley, & Campbell, 2005; Bridgman, Clement, Downing, Walley, Phair & Maffulli, 2003; Fong, Man, Yung, Cheung, & Chan, 2008; Waterman, Owens, Davey, Zacchilli & Belmont, 2010; Swenson, Collins, Fields, & Comstock, 2013). Immobilization is the most commonly used treatment intervention; however, it has not been deemed the gold standard of treatment. There is an established link between increased stiffness of the ankle joint and immobilization; however, the effects of immobilization on cortical and reflexive excitability have yet to be examined. It has been previously researched how immobilization of the ankle affects functional outcomes, but not how immobilization affects neurological function that controls the ability of the joint to maintain stability. It is unclear if immobilization is beneficial or detrimental to nervous system excitability, which is a vital part of maintaining joint stability. The purpose of this review of literature is to review the neuromechanical aspects of joint stability and the potential effects of ankle immobilization on these factors.

Prevalence and Recurrence of Ankle Sprains

About 625,000 lateral ankle sprains occur every year in the United States (Waterman et al., 2010; Fong et al., 2008; Hootman, Dick, & Agel 2007) and make up 14% of musculoskeletal injuries seen in accident and emergency departments as well as 15% of injuries in NCAA sporting events (Fong et al., 2008; Hootman et al., 2007). Though ankle sprains are viewed as a mild injury, they are the most common reoccurring injury and present a big problem to overall public health (Waterman et al., 2010; Hubbard-Turner & Turner, 2015; Houston, Lunen & Hoch, 2014).

Sixty percent of people have sprained their ankle and up to seventy percent of them develop residual symptoms. These symptoms may include pain, weakness, swelling and instability (Anandacoomarasamy & Barnsley , 2005; Hiller et al., 2012; Hertel, 2002). *Chronic ankle instability*

(CAI) is described as repetitive episodes of the ankle giving way as well as self-reported functional limitations following at least one significant ankle sprain (Gribble et al., 2014). Symptoms of CAI include pain, weakness, and recurrent ankle sprains/giving way (Verhagen, de Keizer, & van Dijk, 1995). Recurrent ankle sprains can increase the risk of long term degeneration of the joint and has been found to have a higher prevalence rate when the individual has suffered trauma to the ligaments of the ankle (Struijs & Kerkhoffs, 2010; Valderrabano et al., 2006). Valderranabano et al. (2006) investigated 36 patients with ankle instability and found ankle osteoarthritis in 78% of cases (Valderrabano et al., 2006). A recent study by Hubbard-Turner examined activity level of those with chronic ankle instability compared to those without, and found a decrease in steps per day with the CIA group. This decrease exemplifies the potential health risk of those who have CAI (Hubbard-Turner & Turner, 2015). Most individuals who suffer a lateral ankle sprain return to medical professionals due to residual symptoms, reinforcing the importance of early and effective treatment of a lateral ankle sprain (Anandacoomarasamy & Barnsley, 2005).

Maintenance of Joint Stability

Neurological and mechanical components of joints must work together to prepare for and react to a potentially injurious load. During potentially injurious loads, static and dynamic stabilizers are utilized to protect ligamentous structures and maintain stability. Muscle contractions generate stiffness via musculotendinous units, which in turn provide dynamic protection of joints. In the case of lateral ankle sprains for example, the peroneus longus and brevis control supination and thus help to protect against lateral ankle sprains (Hertel, 2002). *Joint stiffness* is defined as resistance to stretch by the joint and its supporting structures including joint capsule, ligaments, muscle and skin. Ultimately determining the amount of force required to cause an injury (Needle et al., 2013).

Neuromuscular function is hypothesized to be a vital component of joint stability (Johansson, 1991). Protecting a joint from injury requires the nervous system to coordinate both feedforward and feedback muscle activity. The feedforward component is a preparatory mechanism. During gait, the musculature of the ankle is preactivated before and during the stance phase, and it is theorized that

muscle spindle sensitivity is increased. Following the mechanoreceptors sensing a stretch from the forced inversion (in the case of lateral ankle sprains) and sends an afferent signal to the spinal cord (Gutierrez, Kaminiski & Douex, 2009). Regardless of whether or not this afferent stimulus is enough to cause a monosynaptic reflex and initial motor response via muscle twitch, it will continue to ascend in the central nervous system. An efferent signal is sent to the gamma motor neuron of the muscle spindle of the peroneal muscles and sensitizes the muscle spindles (Gutierrez et al., 2009). The sensory information will ultimately be interpreted by the medulla, pons and cerebellum. A reflexive response will come from the cerebellum; however, a volitional response to stabilize the joint will be formed in the primary motor cortex. The volitional response is considered the feedback component (Needle et al., 2013).

Peripheral and central nervous system function is assessed via reflexive and cortical excitability, which depicts the contributions of spinal reflexes and motor cortex in maintaining joint stability (McVey et al., 2005; Needle et al., 2014). In order to examine reflexive excitability, the Hoffman reflex (H-Reflex) is evaluated via stimulating the nerve directly. The H-reflex estimates the excitability of alpha motor neurons, with the maximum value representing the maximum reflexive excitability response (Hmax) (Zher, 2002). Transcranial magnetic stimulation (TMS) is a way of assessing cortical excitability by introducing a brief magnetic field to the targeted area of the brain, usually the motor cortex and will either excite or inhibit the targeted area. When the motor cortex is the targeted area, the response is measured through a muscle twitch known as the motor- evoked potential (MEP) (Hallett, 2007). This relationship between excitability and joint stiffness is known as neuromuscular coupling. If the injurious loads are too great the reflexive response will be activated immediately to aid in the stability of the joint (Needle et al., 2013). Injury may alter this relationship and lead to neuromechanical decoupling. The cause for this decoupling mechanism eludes researchers.

Ankle Sprain Effects on the Nervous System

Lateral ankle sprains result in adverse changes to the neuromuscular system that lead to a decrease in proprioception and neuromuscular control that ultimately contribute to the reoccurrence of lateral ankle sprains (Hertel, 2008; Khin, Ishii, Sakane, & Hayashi, 1999). When the ankle is sprained, ligament integrity is compromised causing a decrease of afferent input accuracy. This results in sensorimotor adaptations, perceptual changes and structural adaptations. Many authors have hypothesized that there is a cascade effect in the development of CAI (Wikstrom & Brown, 2014).

It is hypothesized that changes to the neuromuscular system, ligamentous injury cause a dual cascade of neuromechanical changes to the joint. Cascade #1 is categorized as the initial injury damage forces causing structural adaptations and spinal reflex inhibition as well as residual symptoms which occur in the days following the injury. These adaptations and inhibition can be attributed to the increase in pressure caused by inflammation as well as chemical mediator release which decreases muscle spindle sensitivity. The pattern of athrogenic inhibition and increased joint laxity present in cascade #1 appear to be consistent with all individuals that suffer a lateral ankle sprain. Within about two to four weeks a secondary cascade of neurological changes will occur. Cascade #2 can result in either successful or not successful adaptations. If the individual is a coper (successful adaptations and no residual symptoms), the cascade will stop and normal function will resume. If the individual is not a coper, then unsuccessful adaptations will alter joint loading and supraspinal motor control mechanisms. The development of CAI and the continuous negative feedback loop which reverts to another injury (Wikstrom & Brown, 2014). Though the divergent outcomes following cascade #1 have been established, it is unclear how treatment interventions such as immobilization affect these factors and potentially the cascades, despite immobilization being the most commonly used treatment method.

Arthrogenic Inhibition

Arthrogenic muscle inhibition consists of ongoing inhibition of musculature that surrounds a joint following damage to the structures of the joint that is related to pain or joint effusion (McVey et

al., 2005). In order to evaluate if arthrogenic inhibition is present in a joint, the Hoffman reflex is often tested by estimating the alpha motor neuron excitability. The peak value is the maximal reflex activation (Zehr, 2002). Arthrogenic inhibition is represented by a decrease in the Hmax:Mmax ratio. This ratio represents the total number of motor neurons able to be activated compared to the total number of motor neurons. This means that the reflexive output capacity of the muscle is minimized, thus overall muscle activity is depressed (Matthews, 1966).

Myers, Reimann, Hwang & Lephart, (2003) investigated the effects of lidocaine and saline injections into the lateral ligaments of the ankle and found a decreased response following each injection when inversion loads were applied (Myers et al., 2003). A study conducted by McVey et al. (2005) evaluated the H-reflex in healthy individuals both with unilateral ankle instability and without ankle instability. This study found that a depressed H: M ratio in the soleus and peroneus longus of the unstable ankle compared to the stable ankle. These results contribute to the notion that neuromuscular deficits are present after an injury to the ankle joint. It is known how an injury affects the values of the H-reflex and H: M ratio. It is unknown how different treatment interventions affect these values in the lower extremity.

Treatment Methods of Lateral Ankle Sprains

A position statement from the National Athletic Trainers Association provides guidelines that Grade I and II sprains would benefit from functional rehabilitation over immobilization and that severe ankle sprains be immobilized for up to ten days. These outcomes are based on time to return-to-play instead of long-term function of the joint and it is unclear how different modes of immobilization affects nervous system function of the joint (Kaminski et al., 2013). The most common forms of immobilization for the ankle joint are a Bledsoe boot, Aircast and compression wrap/tubular bandage.

Functional treatment (early immobilization and external support) improves both stability and function of the ankle compared to immobilization alone (Struijs & Kerkhoffs, 2010). The general consensus is that immobilization is a more effective treatment method compared to no treatment. Eiff,

Smith, & Smith (1994) compared early mobilization with immobilization (nonweight-bearing cast for ten days) and found that the early mobilization group returned to functional activities sooner and reported less pain than the immobilization group (Eiff et al.,1994). Lamb et al. (2009) investigated functional outcomes following a ten day below the knee casting and found notable improvements in ankle function, pain and swelling at three months when compared to the AirCast and tubular bandage. However nine months following the immobilization period there was no notable difference between the interventions (Lamb et al., 2009).

Effects of Immobilization

It has been found that immobilization for greater than four week will decrease symptoms but also decrease function of the joint (Struijs & Kerkhoffs, 2010). Functional deficits following a period of immobilization include decrease in range of motion and balance, while also contributing to atrophy of the musculature. Separate from atrophy, functional deficits can also be explained by alterations in nervous system function. It has been found that immobilization causes a decrease in central activation of muscle (Clark, Taylor, Hoffman, Dearth & Thomas, 2010) while also increasing reflex excitability (Lundbye-Jensen & Nielsen, 2008). Immobilization also decreases maximal motor neuron firing rate (Seki, Kizuka & Yamada, 2007). The overall consequence is decreased ability to activate skeletal muscle via the nervous system (Clark et al., 2010). Though there is limited research on effects of immobilization on the neurological function of the lower extremity, there is research on how immobilization affects the neurological function of the upper extremity.

After casting the wrist in eleven healthy subjects for three weeks, it was found that wrist flexion strength decreased significantly and remained depressed fifteen percent after a week's recovery. Central activation remained significantly decreased after one week recovery. The H reflex increased following immobilization and remained elevated after one week of recovery (Clark et al., 2010). A different study also found muscle strength deficits following a week of wrist immobilization in ten subjects. Maximum voluntary isometric contraction torque decreased. Decreases in strength and central activation despite hypersensitivity of the H-reflex remain constant despite recovery time

(Lundbye-Jensen & Nielsen, 2008). This may contribute to recurrent injuries to the joint following immobilization.

When comparing an Aircast and a tubular bandage, subjects wearing the Aircast had significantly better joint function at both ten days and one month following an ankle sprain (Boyce et al., 2005). Functional outcomes of casting compared to compression bandage were significantly better after three months but show no significant differences by the nine month mark following injury. There is no significant benefit in using the Bledsoe boot over the compression wrap (Lamb et al., 2009). Though functional outcomes are vital in evaluating the effectiveness of an immobilization intervention, consideration of the effects on the H-reflex and H:M ratio should be taken into account. Findings after casting below the knee are consistent with the finding of casting the wrist (Clark et al., 2010). There was an increase in H reflex activity and a decrease in maximum voluntary contraction (Lundbye-Jensen & Nielsen, 2008). The change in muscle activity and H reflex demonstrates the effect of below the knee casting on neurological function.

These changes in both functional outcomes and neuromechanical measures can also be observed during short – term immobilization (i.e. bracing and taping). Ankle braces worn during functional tests decrease muscle activity in the lateral gastrocnemius, anterior tibialis, and peroneus longus (Feger, Donovan, Hart, & Hertel, 2014). This decrease in muscle activity can be a contributing factor to recurrent ankle sprains. In a high school athlete population, 10% of ankle sprains that took place while the athlete was wearing a brace (Swenson et al., 2013). When reflexive excitability of the soleus while subjects wore an ankle brace while standing on both an unstable and stable surface was investigated there was no effect on SOL reflex depression. This illustrated that short-term immobilization may decrease the dependence on the motorneuron pool while also increasing ankle stability on an unstable surface (Sefton, Hick-Little, Koceja & Cordova, 2007).

Though immobilization is a widely used intervention for lateral ankle sprains, there is limited research on the neurological effects of immobilization in the ankle. It is unknown if the decrease function of the joint leaves an individual more at risk for a recurrent ankle injury.

Conclusion

Lateral ankle sprains present a problem to public health due to both a high occurrence and recurrence rate. Immobilization is the most commonly implemented technique. It is known how immobilization of the ankle affects functional outcomes but it is unknown how immobilization affects neurological function of the joint. The purpose of this study is to determine the effect of immobilization on cortical and reflex excitability in the ankle joint. It is hypothesized that ankle immobilization will increase ankle stiffness as well increase reflexive excitability and decrease cortical excitability. It is also hypothesized that immobilization changes the relationship between laxity and neuromechanical variables.

Chapter 3: Methodology

Experimental Design

The purpose of this study was to determine the effect of immobilization on mechanical function, and cortical & reflexive excitability in the ankle joint. This study employed a pre-test post-test design with repeated measures. The independent variables were immobilization device (pneumatic leg splint, boot immobilizer, or no intervention), time (pre and post-walking) and, with regards to excitability measures, muscle (gastrocnemius, tibialis anterior and peroneus longus). Dependent variables will include measures of passive joint stiffness, cortical excitability (motor threshold, maximum response) and reflexive excitability (H_{max}:M_{max}).

Participants.

Twelve (22.5±1.4yrs, 173.05±17.5cm, 71.6±12.7kg) able-bodied and physically active males and females without a history of ankle sprains volunteered to participate in this study. Exclusion criteria were current leg injury or history of any fracture or surgery to the legs. TMS exclusion criteria included metal or electronic implant, history of seizure, concussion within the past 6 months, currently pregnancy or being treated for a psychiatric or neurological disorder. These were confirmed via the Physical Activity Readiness Questionnaire (PAR-Q) and TMS exclusion questionnaire (Rossi, Hallett,Rossini & Pascual-Leone, 2009).

Instrumentation.

In order to assess ankle laxity, an instrumented ankle arthrometer (Blue Bay Research, Milton, FL) consisting of a loaded cell connected to an instrumented handled as well as a footplate connected to a shin pad by means of a six degrees-of-freedom kinematic linkage system was used. The arthrometer will assess anteroposterior force as well as inversion-eversion force. Participants' cortical excitability was evaluated using transcranial magnetic stimulation (TMS, Magstim 20-2 LTD, Wales, UK) with a double conical coil that targets the lower extremity. A DS7AH Constant Current Stimulator (Digitimer LTD, Hertfordshire, England) connected in series with a bar electrode was used to assess reflexive excitability. Electromyographic activity from surface electrodes on the tibialis

anterior, gastrocnemius and peroneus longus was recorded using a Bagnoli-4 EMG system (Delsys, Boston, MA) (Kovaleski, Hollis, Heitman, Gurchiek & Pearsall, 2002).

Procedures

Following approval from the Appalachian State University Institutional Review Board, participants were asked to report for a total of 3 testing sessions 2 to 7 days apart. Each testing session lasted approximately 2.5 hours in duration. During the first testing session, after providing informed consent, participants were asked to complete a health screening questionnaire that the investigator will review with the subject to determine study eligibility prior to each session (Appendix). During each testing session, participants' ankle laxity, dorsiflexion range of motion and cortical and reflexive excitability were tested before and after walking on a treadmill for 30 minutes at 1 m/s.

Mechanical Measures.

Ankle laxity was assessed using an instrumented ankle arthrometer (Figure 1). Laying supine on a table, the arthrometer was affixed to the participant's ankle and 5 anterior-posterior translations to 125 N (50N/s); followed by 5 inversion-eversion rotations to 4.2 Nm (1Nm/sec) (Kovaleski et al., 2002). Peak laxity and stiffness across groups were extracted for analysis.

Functional dorsiflexion range of motion was assessed using a weight bearing lunge (Figure 1). The participant placed two fingers from each hand on the wall to help keep balance. The participant then place his/her foot on a tape measure on the ground with their great toe on the line marked zero inches and lunge towards the wall by bending their ankle until the knee is in contact with the wall. Once completed the participant gradually moved back. The participant kept their heel in contact with the ground to be able to move to the next measurement back. The greatest distance reached was recorded (Chisholm, Birmingham, Brown, Macdermid & Chesworth, 2012).

Excitability Measures.

For measurement of cortical and reflexive excitability (Figure 2), participants were instrumented with electromyography sensors (Delsys Inc., Boston, MA) over the tibialis anterior, peroneus longus, and soleus muscles of each leg. The area where the electrodes were placed was

shaved (if necessary), cleaned with an alcohol swab, and abraded to ensure a quality signal (Basmaijian,1967). Cortical excitability was assessed using a Magstim 200-2 Magnetic Stimulator with a double-conical coil (MagStim LTD, Wales, UK). Participants were seated in a chair with a tightfitting cap on the head and provided earplugs to wear throughout testing. Prior to testing, we quantified the maximum voluntary muscle activity by having the subject evert their ankle maximally for 2 seconds, repeated up to 3 times. TMS was delivered under 2 conditions – with the muscles relaxed and with the subject voluntarily contracting their muscles at 10 percent of maximal effort (with visual feedback via a lab tablet to aid consistent effort). The "hotspot (location of maximum peak-to-peak MEP) was located by first identifying the vertex of skull then moving the coil lateral and anterior 1 cm. Intensity of the pulses was then gradually increased until a small muscle contraction was visible. Next the coil was moved in approximately 5-mm radius in order to determine where the largest MEP was observed (Conforto, Z'Graggen, Kohl, Rosler & Kaelin-Lang, 2004). This location was then marked on the cap. Motor threshold was determined by stimulating over a range of intensities with the subject relaxed. After determination of motor threshold and the hotspot, the coil was placed on the hotspot and 10 pulses of 90, 110 and 150 percent of the resting motor threshold (30 pulses total) were applied, while EMG activity was collected at 2000 Hz. All data were collected and intensities were triggered using customized LabVIEW software (National Instruments, Austin, TX).

For testing the Hoffmann reflex, a probe electrode was applied behind the knee, in the superolateral corner of the popliteal fossa. The location of the sciatic nerve proximal to its bifurcation in tibial and common peroneal divisions were assessed by applying brief pulses and identifying the location that is able to generate the greatest muscular response across all 3 muscles at the lowest stimulation intensity. Brief electrical pulses (1ms) were applied beginning at a low intensity, while the current was gradually increased by 2mA until a maximal response was observed from the muscles. The direct muscle activation (M-wave, 10-40ms) and the reflexive response (H-wave, 50-

100ms) was identified and peak-to-peak values were extracted. Electromyography data was collected at 2000 Hz.

Following the measures being taken, the subject walked on the instrumented treadmill for 30 minutes either barefoot; with a pneumatic leg splint (Aircast Air Stirrup Ankle Brace, DJO Global, Vista, CA); or with an ankle immobilizer (AirCast PF Walker Boot, DJO Global, Vista, CA). The order of immobilization type was randomized for each participant. The measures were then repeated immediately after walking.

Data Reduction and Analysis

In order to calculate cortical excitability, peak-to-peak amplitudes muscle activity was normalized to the largest observed MEP and plotted against the stimulus intensity to form a stimulus-response curve. A curve was fitted to these data using a Levenberg-Marquardt nonlinear fit with a modified Boltzmann equation:

$$y = \frac{MEP_{min} + (MEP_{max} - MEP_{min})}{1 + e^{[m(I_{50} - x)]}}$$

From this equation, the maximum response (MEP_{max}) was extracted as our measure of cortical excitability. From the Hoffman reflex, stimuli were analyzed for peak-to-peak amplitude from 10-50ms and 60-100ms after the stimulus to identify the direct and reflexive muscle response. The maximal reflexive response was normalized to the maximal direct response to determine reflexive excitability.

All data was analyzed in custom LabVIEW software. Total anterior-posterior displacement, inversion-eversion rotation, and dorsiflexion rotation was extracted, as well stiffness in the first, middle, and last second of force application (N/mm or N/deg). Muscle activation during TMS pulses were visually inspected for artifacts and peak-to-peak values of motor evoked potentials were averaged for each stimulation intensity. Similarly, M-waves and H-waves from Hoffman reflex testing were inspected, and the maximum M-wave and maximum H-wave were determined separately for each muscle. The ratio of Hmax to Mmax was extracted and used for analysis. Laxity and

dorsiflexion range-of-motion were assessed using 2-way analyses of variance (ANOVA) with 2 within-subjects factors (time, 2 levels; device, 3 levels). Cortical and reflexive excitability variables was assessed using 3-way ANOVA's with 3 within-subjects factors (time, 2 levels; device, 3 levels; and muscle, 3 levels).

Chapter 4: Results

Mechanical Measures.

Mechanical values of joint stiffness and dorsiflexion range of motion are presented in Table 1. There was no significant main effect of condition ($F_{[2,16]}$=.133, p=.876) or time ($F_{[1,8]}$=4.737, p=.061) on maximum displacement. A significant effect however, was detected for immobilized side ($F_{[1,8]}$=8.934, p=.017). Significance was not found for interaction effects of condition*time ($F_{[2,16]}$=.103, p=.902) , time*side ($F_{[1,8]}$=1.468, p=.260) or 3-way interaction condition*time*side ($F_{[2,16]}$=.640, p=.541). A significant interaction effect of condition*side was observed ($F_{[2,16]}$=.102, p=.026). Total inversion yielded no significant main effect of condition ($F_{[2,20]}$=.110, p=.896), time ($F_{[1,10]}$=.580, p=.464) and side ($F_{[1,10]}$=.424, p=.530). There was also no significant interaction effect between condition*time ($F_{[2,20]}$=3.078, p=.068), condition*side ($F_{[2,20]}$=1.635, p=.220), time*side ($F_{[1,10]}$=.133, p=.723) or 3-way interaction condition*time*side ($F_{[2,20]}$=.114, p=.893)

Functional Dorsiflexion Range of Motion.

Weight bearing lunge yielded no significant effect of condition ($F_{[2,20]}$=.132, p=.877), time ($F_{[1,10]}$=.974, p=.347) or side ($F_{[1,10]}$=2.918, p=.118). Interaction effect also showed no significant effect between condition*time ($F_{[2,20]}$=.204, p=.817),condition*side ($F_{[2,20]}$=1.738, p=.201) but significance was found between time*side ($F_{[1,20]}$=6.328, p=.031). 3-way interaction of condition*time*side ($F_{[2,20]}$=2.294, p=.127) elicited no significant effect. Fisher's LSD pairwise comparison revealed no significant difference of time pre or post (p=.083, p=.181).

Reflexive Excitability.

H_{max}:M_{max} ratio values are presented in Table 2. Though there was no significant effect of condition ($F_{[2,16]}$=.243, p=.787), there was a significant effect of time (post) ($F_{[1,8]}$=6.337, p=.036) and muscle (soleus) ($F_{[2,16]}$=14.614, p=.000). There was no interaction effect significance detected between condition*time ($F_{[2,16]}$=.016, p=.985), condition*muscle ($F_{[4,32]}$=.106, p=.980), time*muscle ($F_{[2,16]}$=.676, p=.523) and 3-way interaction of condition*time*muscle ($F_{[4,32]}$=.566, p=.689).

Cortical Excitability.

MEP size at 90, 110 and 150% of active motor threshold values are presented in Table 3. Across all three muscles (TA , PL, and SOL) there was no significant effect of condition [TA ($F_{[2,16)}$=1.733, p=.208), PL ($F_{[2,14]}$=2.188, p=.149), SOL ($F_{[2,16]}$=1.338, p=.290)] or time [TA ($F_{[1,8]}$=3.379, p=.103), PL ($F_{[1,7]}$=.069, p=.800),SOL ($F_{[1,8]}$=.135, p=.723)]. There was however a significant effect of intensity [TA ($F_{[2,16]}$=43.88, p=.000), PL ($F_{[2,14]}$=50.064, p=.000),SOL ($F_{[2,16]}$=7.520, p=.005)] observed in all three muscles (90%<110%<150%). Interaction effects of condition*time [TA ($F_{[2,16]}$=.224, p=.802),PL ($F_{[2,14]}$=.425, p=.662),SOL ($F_{[2,16]}$=2.105, p=.154)]condition*intensity [TA ($F_{[4,32]}$=1.152, p=.350), PL ($F_{[4,28]}$2.039, p=116), SOL ($F_{[4,32]}$=1.077, p=.3840] time*intensity [TA ($F_{[2,16]}$=2.181, p=.145),PL ($F_{[2,14]}$=1.410, p=.277), SOL ($F_{[2,16]}$=.182, p=.836)] and 3-way interaction of condition*time*intensity [TA ($F_{[4,32]}$=.192, p=.941), PL ($F_{[4,28]}$=.864, p=.498), SOL ($F_{[4,32]}$=.133, p=.876)] yielded no significant results in across the three muscles.

Chapter 5: Discussion

Introduction

The purpose of this study was to assess alterations in joint stiffness and corticospinal excitability following an acute bout of immobilization of the ankle joint. It was hypothesized that joint stiffness and reflexive excitability would increase while cortical excitability would decrease after walking in an immobilization device for 30 minutes. While prior research has provided evidence of immobilization-induced neuroplasticity at the cortical and segmental levels, the current investigation revealed no significant change in joint stiffness or reflexive and cortical excitability following 30 minutes of ambulation using a pneumatic leg splint (Aircast) or ankle immobilizer (boot). While several explanations for discrepancies from prior research will be presented, these data suggest that alterations to stiffness and nervous system excitability observed following longer bouts of immobilization are likely not due to short-term changes in neurological function, but rather a combination of tissue contracture and long-term potentiation.

Mechanical Measures.

Clinicians typically exercise caution in utilizing immobilization devices, citing tissue contractures contributing to adhesions and subsequent decreased range-of-motion as a key limitation (Kaminski et al., 2013). This is largely due to the lack of normal stresses being placed on tissue, leading to decreased strength and increased stiffness of the collagen structures comprising the ligament and joint capsule (Järvinen, 1977). However, it has also been hypothesized that alterations in afferent feedback may serve to modify fusimotor activity responsible for the regulation of muscle tone (Needle et al., 2013). In the present investigation, no significant changes in anterior displacement, total inversion-eversion laxity or functional dorsiflexion range of motion were observed following 30 minutes of immobilization. Several explanations may be hypothesized for the lack of mechanical changes following immobilization but notably this may be explained by the inclusion of a 30-minute walking task, which could have caused a temperature rise that raises

collagen elasticity as opposed to the contractures expected (Miller, Needle, Swanik, Gustavsen & Kaminski, 2012).

Following periods of immobilization up to 2 weeks, Landrum et al. (2008) found injured subjects displayed decreased anterior-posterior displacement. Dorsiflexion range of motion has also been found to decrease following prolonged ankle immobilization (Freeman, 1965). Some clear methodological differences may explain the discrepancies in results between these studies and the current investigation. For instance, the inclusions of pathological populations have typically been incorporated to decrease undue burden on otherwise healthy individuals; yet these pathologies may have contributed to joint contracture. Additionally, these investigations have looked at long-term total immobilization or casting that would clearly indicate a more severe stimuli than 30 minutes of less restrictive devices.

Reflexive Excitability.

Reflexive excitability describes the strength of the motor response elicited from stimulation of Ia afferents. This is analogous to the stretch reflex, and is determined by sensitivity of muscle spindles as well as the size and excitability of the alpha motor neuron pool at the segmental level. We hypothesized that reflexive excitability would increase following a 30-minute period of immobilization. By restricting joint motion, the lack of peripheral stimuli to peripheral mechanoreceptors would decrease the threshold needed to evoke a reflexive response ultimately increasing the excitability of the reflex (Johansson, 1991). Contrary to our hypothesis, there was no significant change in reflexive excitability following acute immobilization. One potential explanation is the degree of immobilization used for the present study allowed for some degree of movement at the joint and thus proprioceptive feedback that served to negate the inhibitory influences expected.

These results were conflicting with a previous study conducted by Lundbye-Jensen & Nielson (2008) that detected an increase of reflexive excitability of the hand and wrist musculature following one week immobilization. Differences in extremity (leg versus arm), time of immobilization (30 minutes versus 2 weeks) and also intensity of immobilization (splint versus cast)

may be able to account for the differences. Sefton et al., (2007) examined reflexive excitability of the soleus while subjects wore an ankle brace while standing on both an unstable and stable surface and found no effect on SOL reflex depression. The intensity and duration of immobilization were comparable to our investigation. Since the soleus is a postural muscle reflexive excitability may not be influenced in acute bouts of immobilization or activity.

Cortical Excitability.

The excitability of corticospinal pathways assessed via transcranial magnetic stimulation provide a measure of the "ease" of volitional contracture to target musculature from the primary motor cortex . Although we observed a decrease in corticospinal excitability following walking, this occurred across all muscles and under all conditions, and not specific to immobilization device as hypothesized. As expected, excitability increased with stimulus intensity; however immobilization did not affect the modulation of cortical excitability. While multiple factors, including medications and injury, have been observed to modify corticospinal excitability, it was hypothesized that this property would decrease secondary to decreased sensory feedback from peripheral mechanoreceptors surrounding the ankle joint. The decreased input to the somatosensory cortex would then serve to lessen the input to the primary motor cortex from supplementary motor areas.

A single prior investigation has discussed cortical excitability as it related to lower extremity immobilization. Leukel et al. (2015) examined cortical excitability following eight weeks of ankle casting, noting *increases* in overall cortical excitability. Key differences of intervention and time immobilized may serve to explain why a change was not seen in our study. Devices used in our study (pneumatic leg splint and ankle immobilizer), allowed for some accessory and mild physiologic movement of the ankle joint that would not be permitted in a cast. The increase in cortical excitability seen in eight week casting of the ankle joint may be due to an elimination of sensory stimulus as well as elimination of lower leg musculature activation via the motor cortex. Authors hypothesized that the increase in cortical excitability was caused by pathway-specific adaptations over the 8-weeks that may not be sufficiently observed across 30-minutes.

The most notable difference between the current investigation and those previous models of cortical excitability in the upper and lower extremities is the duration of immobilization. Long-term immobilization would cause neuroplasticity secondary to long-term potentiation. However, an aim of this study was to determine whether immobilization was capable of modifying synaptic plasticity via post-tetanic potentiation (PTP): a transient effect of motor learning due to an excess amount of calcium within the synapse. A 30 minute treadmill walk was not enough to induce changes in cortical excitability, lending support to the hypothesis that PTP did not occur. Another crucial factor to consider is that walking is a subcortical task; yet, has been observed to cause changes in cortical excitability when a novel element is added. Barthelemy, Alain, Grey, Neilson & Bouyer , (2012) induced cortical excitability changes by causing an adaption to force fields while walking. Though time walking was comparable, walking while immobilized was not sufficient enough to induce plasticity and ultimately post-tetanic potentiation (PTP) because minimal adaptations were needed to complete the task in comparison to walking with the force field. This may mean that using the ankle immobilizer does not contribute to maladaptation. Further research of long term effects is needed to support this notion.

Limitations.

There were several limitations to this investigation. Time of immobilization (thirty minutes) and walking speed (1m/s) may not have been high enough to induce post-tetanic potentiation and ultimately changes in corticospinal excitability. Cortical excitability was assessed for lower leg muscles that directly affect movement of the ankle joint but excitability may have been more likely to change at either the gluteal or the quadriceps muscles. Furthermore, there was no control variable pre measures such as time of day and caffeine intake which effect excitability due to both subject and lab scheduling constraints. Time of day and caffeine may have contributed to variable pre measures (Cerqueira, Mendonca, Minez, Dias & Carvalho, 2006).

Conclusion

Our data suggest that short-term immobilization of the ankle does not induce significant changes in joint stiffness or nervous system excitability. When considering the deficits observed in patients with chronic joint instability, it could be theorized that decreases in sensory feedback caused by immobilization would not be beneficial. Yet depravation of feedback would be beneficial for individuals with acute inflammation experiencing nociception and increases in intracapsular pressure. Our results indicated that for these effects to potentially occur, a longer period of time and/or a more restrictive immobilization device must be utilized. Lamb et al. (2009) proposed that a short period of immobilization (2-3 weeks) in a below-knee cast leads to fastest recovery. Our results lend support to the hypothesis that short-term immobilization allows for the joint to be protected from potentially deleterious loading while possibly preventing alterations in corticospinal excitability.

A recent position statement from the National Athletic Trainers' Association recommended limiting immobilization and incorporating functional rehabilitation for grade I and II ankle sprains; and at least 10 days of immobilization with a rigid brace or below the cast for grade III (Kaminski et al., 2013). Our data does not support modification of the recommendation, however growing evidence in this field suggests neuromechanical adaptations that occur after immobilization may be vital in correcting deficits in chronically injured joints. In order for further treatment recommendations to be made, studies must be conducted with pathological populations and across varying device and time-frames. Future research is needed to investigate the long-term outcomes following short-term immobilization

References

Anandacoomarasamy, A., & Barnsley, L. (2005). Long term outcomes of inversion ankle injuries. *Br J Sports Med, 39*(3), e14; discussion e14. doi: 10.1136/bjsm.2004.011676

Attenborough, A. S., Hiller, C. E., Smith, R. M., Stuelcken, M., Greene, A., & Sinclair, P. J. (2014). Chronic ankle instability in sporting populations. *Sports Med, 44*(11), 1545-1556. doi: 10.1007/s40279-014-0218-2

Barthélemy, D., Alain, S., Grey, M. J., Nielsen, J. B., & Bouyer, L. J. (2012). Rapid changes in corticospinal excitability during force field adaptation of human walking. *Exp Brain Res Experimental Brain Research, 217*(1), 99-115. doi:10.1007/s00221-011-2977-4

Basmajian, J. V. (1967). Electromyography: Its Structural and Neural Basis. *International Review of Cytology,* 129-140. doi:10.1016/s0074-7696(08)60813-x

Boyce, S. H., Quigley, M. A., & Campbell, S. (2005). Management of ankle sprains: a randomised controlled trial of the treatment of inversion injuries using an elastic support bandage or an Aircast ankle brace. *Br J Sports Med, 39*(2), 91-96. doi: 10.1136/bjsm.2003.009233

Bridgman, S. A., Clement, D., Downing, A., Walley, G., Phair, I., & Maffulli, N. (2003). Population based epidemiology of ankle sprains attending accident and emergency units in the West Midlands of England, and a survey of UK practice for severe ankle sprains. *Emerg Med J, 20*(6), 508-510.

Cerqueira, V., Mendonça, A. D., Minez, A., Dias, A., & Carvalho, M. D. (2006). Does caffeine modify corticomotor excitability? *Neurophysiologie Clinique/Clinical Neurophysiology, 36*(4), 219-226. doi:10.1016/j.neucli.2006.08.005

Chisholm M. D., Birmingham T. B., Brown J, Macdermid J, Chesworth B. M. (2012). Reliability and validity of a weight-bearing measure of ankle dorsiflexion range of motion. *Physiother Can*,64(4):347355. 20.

Clark, B. C., Taylor, J. L., Hoffman, R. L., Dearth, D. J., & Thomas, J. S. (2010). Cast immobilization increases long-interval intracortical inhibition. *Muscle Nerve, 42*(3), 363-372. doi: 10.1002/mus.21694

Conforto, A. B., Z'Graggen, W. J., Kohl, A. S., Rosler, K. M., & Kaelin-Lang, A. (2004). Impact of coil position and electrophysiological monitoring on determination of motor thresholds to transcranial magnetic stimulation. *Clin Neurophysiol, 115*(4), 812-819. doi: 10.1016/j.clinph.2003.11.010

Eiff M. P., Smith A.T., Smith G.E., Early mobilization versus immobilization in the treatment of lateral ankle sprains. (1994). Am J Sports Med.22(1):83–88.doi: 10.1177/036354659402200115

Feger, M. A., Donovan, L., Hart, J. M., & Hertel, J. (2014). Lower extremity muscle activation during functional exercises in patients with and without chronic ankle instability. *Pm r, 6*(7), 602-611; quiz 611. doi: 10.1016/j.pmrj.2013.12.013

Fong, D. T., Man, C. Y., Yung, P. S., Cheung, S. Y., & Chan, K. M. (2008). Sport-related ankle injuries attending an accident and emergency department. *Injury, 39*(10), 1222-1227. doi: 10.1016/j.injury.2008.02.032

Freeman, M. A. (1965). Instability of the foot after injuries to the lateral ligament of the ankle. *J Bone Joint Surg Br, 47*(4), 669-677.

Gribble, P. A., Delahunt, E., Bleakley, C. M., Caulfield, B., Docherty, C. L., Fong, D. T., . . . Wikstrom, E. A. (2014). Selection criteria for patients with chronic ankle instability

in controlled research: a position statement of the International Ankle Consortium. *J Athl Train, 49*(1), 121-127. doi: 10.4085/1062-6050-49.1.14

Gutierrez, G., Kaminski, T., & Douex, A. (2009). Neuromuscular Control and Ankle Instability. *Pm&r, 1*, 359-365. doi:10.1016/j.pmrj.2009.01.013

Hallett, M. (2007). Transcranial Magnetic Stimulation: A Primer. *Neuron,* (15), 187-199. doi:10.1016/j.neuron.2007.06.026

Hertel J. (2002) Functional Anatomy, Pathomechanics, and Pathophysiology of Lateral Ankle Instability. *J Athl Train*.37(4):364-375.

Hertel, J. (2008). Sensorimotor deficits with ankle sprains and chronic ankle instability. *Clin Sports Med, 27*(3), 353-370, vii. doi: 10.1016/j.csm.2008.03.006

Hiller C.E., Nightingale E.J., Raymond J., et al. (2012) Prevalence and impact of chronic musculoskeletal ankle disorders in the community. *Arch Phys Med Rehabil*. 93(10):1801-1807.

Holder G.E., Celesia G.G., Miyake Y., Tobimatsu S., Weleber R.G. (2010). International Federation of Clinical Neurophysiology: recommendations for visual system testing. *Clin Neurophysiol*, 121(9):1393-1409.

Hootman, J. M., Dick, R., & Agel, J. (2007). Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. *J Athl Train, 42*(2), 311-319.

Houston, M., Lunen, B., & Hoch, M. (2013). Health-Related Quality of Life in Individuals with Chronic Ankle Instability. *Journal of Athletic Training*, *49*(6), 758-763. doi:10.4085/1062-6050-49.3.54

Hubbard- Turner, T., & Turner, M. (2015). Physical Activity Levels in College Students With Chronic Ankle Instability. Journal of Athletic Training.

Järvinen, M. (1977). Immobilization effect on the tensile properties of striated muscle: An experimental study in the rat. *Archives of Physical Medicine and Rehabilitation, 58*(3), 123-127.

Johansson H. (1991). Role of knee ligaments in proprioception and regulation of muscle stiffness. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*. 1(3):158-179.

Kaminski, T., Hertel, J., Amendola, N., Docherty, C., Dolan, M., Hopkins, J., . . . Richie, D. (2013). National Athletic Trainers' Association Position Statement: Conservative Management and Prevention of Ankle Sprains in Athletes. Journal of Athletic Training, 48(4), 528-545. doi:10.4085/1062-6050-48.4.02

Khin Myo, H., Ishii, T., Sakane, M., & Hayashi, K. (1999). Effect of anesthesia of the sinus tarsi on peroneal reaction time in patients with functional instability of the ankle. *Foot Ankle Int, 20*(9), 554-559.

Kovaleski, J. E., Hollis, J., Heitman, R. J., Gurchiek, L. R., & Pearsall, A. W. t. (2002). Assessment of Ankle-Subtalar-Joint-Complex Laxity Using an Instrumented Ankle Arthrometer: An Experimental Cadaveric Investigation. *J Athl Train, 37*(4), 467-474.

Lamb, S. E., Marsh, J. L., Hutton, J. L., Nakash, R., & Cooke, M. W. (2009). Mechanical supports for acute, severe ankle sprain: a pragmatic, multicentre, randomised controlled trial. *Lancet, 373*(9663), 575-581. doi: 10.1016/s0140-6736(09)60206-3

Landrum, E. L., Kelln, B. M., Parente, W. R., Ingersoll, C. D., & Hertel, J. (2008). Immediate Effects of Anterior-to-Posterior Talocrural Joint Mobilization after

Prolonged Ankle Immobilization: A Preliminary Study. *Journal of Manual & Manipulative Therapy, 16*(2), 100-105. doi:10.1179/106698108790818413

Leukel, C., Taube, W., Rittweger, J., Gollhofer, A., Ducos, M., Weber, T., & Lundbye-Jensen, J. (2015). Changes in corticospinal transmission following 8weeks of ankle joint immobilization. *Clinical Neurophysiology, 126*(1), 131-139. doi:10.1016/j.clinph.2014.04.002

Lundbye-Jensen J, Nielsen JB.(1985) Central nervous adaptations following 1 wk of wrist and hand immobilization. *J Appl Physiol*, 2008;105(1):139-151.

Lundbye-Jensen, J., & Nielsen, J. B. (2008). Immobilization induces changes in presynaptic control of group Ia afferents in healthy humans. *The Journal of Physiology, 586*(17), 4121-4135. doi:10.1113/jphysiol.2008.156547

Matthews, W. B. (1966). Ratio of maximum H reflex to maximum M response as a measure of spasticity. *J Neurol Neurosurg Psychiatry, 29*(3), 201-204.

McVey, E. D., Palmieri, R. M., Docherty, C. L., Zinder, S. M., & Ingersoll, C. D. (2005). Arthrogenic muscle inhibition in the leg muscles of subjects exhibiting functional ankle instability. *Foot Ankle Int, 26*(12), 1055-1061.

Miller, H., Needle, A. R., Swanik, C. B., Gustavsen, G. A., & Kaminski, T. W. (2012). Role of External Prophylactic Support in Restricting Accessory Ankle Motion after Exercise. *Foot Ankle Int Foot & Ankle International, 33*(10), 862-869. doi:10.3113/fai.2012.0862

Myers, J., Riemann, B., Hwang, J., & Lephart, S. (2003). Effect Of Peripherial Afferent Alteration Of The Lateral Ankle Ligaments On Muscle Activity During Dynamic

Activity. *Medicine & Science in Sports & Exercise, 31*(4), 498-506. doi:0363-5465/103/3131-0498$02.00/0

Needle, A. R., Palmer, J. A., Kesar, T. M., Binder-Macleod, S. A., & Swanik, C. B. (2013). Brain regulation of muscle tone in healthy and functionally unstable ankles. *J Sport Rehabil, 22*(3), 202-211.

Needle A. R., Baumeister J., Kaminski T.W., Higginson J.S., Farquhar W.B., Swanik C.B. (2014) Neuromechanical coupling in the regulation of muscle tone and joint stiffness. *Scand J Med Sci Sports*.24(5):7 37-748. 10.

Palmieri R. M., Hoffman M.A., Ingersoll C.D. (2002) Intersession reliability for H-reflex measurements arising from the soleus, peroneal, and tibialis anterior musculature. *Int J Neurosci*. 112(7):841-850.

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol, 120*(12), 2008-2039. doi: 10.1016/j.clinph.2009.08.016

Sefton, J., Hicks-Little, C., Koceja, D., & Cordova, M. (2007). Modulation of soleus H-reflex by presynaptic spinal mechanisms during varying surface and ankle brace conditions. *Neurophysiologie Clinique/Clinical Neurophysiology, 37*(1), 15-21. doi:10.1016/j.neucli.2007.01.007

Seki, K., Kizuka, T., & Yamada, H. (2007). Reduction in maximal firing rate of motoneurons after 1-week immobilization of finger muscle in human subjects. *J Electromyogr Kinesiol, 17*(2), 113-120. doi: 10.1016/j.jelekin.2005.10.008

Struijs, P. A., & Kerkhoffs, G. M. (2010). Ankle sprain. *Clin Evid (Online), 2010*.

Swenson, D. M., Collins, C. L., Fields, S. K., & Comstock, R. D. (2013). Epidemiology of U.S. high school sports-related ligamentous ankle injuries, 2005/06-2010/11. *Clin J Sport Med, 23*(3), 190-196. doi: 10.1097/JSM.0b013e31827d21fe

Valderrabano, V., Hintermann, B., Horisberger, M., & Fung, T. S. (2006). Ligamentous posttraumatic ankle osteoarthritis. *Am J Sports Med, 34*(4), 612-620. doi: 10.1177/0363546505281813

Verhagen, R. A., de Keizer, G., & van Dijk, C. N. (1995). Long-term follow-up of inversion trauma of the ankle. *Arch Orthop Trauma Surg, 114*(2), 92-96.

Waterman, B. R., Owens, B. D., Davey, S., Zacchilli, M. A., & Belmont, P. J., Jr. (2010). The epidemiology of ankle sprains in the United States. *J Bone Joint Surg Am, 92*(13), 2279-2284. doi: 10.2106/jbjs.i.01537

Wikstrom, E. A., & Brown, C. N. (2014). Minimum reporting standards for copers in chronic ankle instability research. *Sports Med, 44*(2), 251-268. doi: 10.1007/s40279-013-0111-4

Zehr, E. P. (2002). Considerations for use of the Hoffmann reflex in exercise studies. *Eur J Appl Physiol, 86*(6), 455-468. doi: 10.1007/s00421-002-0577-5

Tables

Table 1

Descriptive Statistics of Mechanical Measures

Max Displacement				
		Barefoot	Pneumatic Leg Splint	Ankle Immobilizer
Imm	Pre/Post	9.53/10.06	8.63/10.36	8.78/8.76
	STD	2.891/3.19	2.33/3.24	3.51/3.82
Non	Pre/Post	8.32/8.95	8.45/8.64	9.21/10.51
	STD	2.753.91	3.22/2.28	3.265/2.47
Total Inversion				
		Barefoot	Pneumatic Leg Splint	Ankle Immobilizer
Imm	Pre/Post	27.18/27.09	25.16/27.62	30.20/26.07
	STD	10.25/10.2	9.94/10.09	13.24/12.86
Non	Pre/Post	27.60/26.58	27.91/29.41	30.65/26.91
	STD	11.39/8.94	11.73/12.41	12.201/9.97
Weight Bearing Lunge				
		Barefoot	Pneumatic Leg Splint	Ankle Immobilizer
Imm	Pre/Post	11.27/11.45	11.55/11.27	11.19/11.27
	STD	4.10/4.03	4.27/4.13	3.92/3.85
Non	Pre/Post	10.45/10.63	10.45/10.81	10.64/10.92
	STD	3.36/3.12	3.14/3.34	3.53/3.18

Table 2
Descrptive Statistics of H_{max}:M_{max} Ratio

H:M Ratio				
		Barefoot	Pneumatic Leg Splint	Ankle Immobilizer
TA	Pre	0.19	0.22	0.22
	STD	0.10	0.06	0.07
	Post	0.17	0.22	0.19
	STD	0.06	0.07	0.04
PL	Pre	0.24	0.22	0.25
	STD	0.17	0.12	0.12
	Post	0.20	0.21	0.22
	STD	0.13	0.13	0.11
SOL	Pre	0.40	0.43	0.41
	STD	0.18	0.19	0.16
	Post	0.36	0.35	0.38
	STD	0.15	0.15	0.16

Table 3

Descriptive Statistics of MEP size Based on Condition and Time

		Tibialis Anterior MEP			Peroneus Longus MEP			Soleus MEP		
		Barefoot	Pneumatic Leg Splint	Ankle Immobilizer	Barefoot	Pneumatic Leg Splint	Ankle Immobilizer	Barefoot	Pneumatic Leg Splint	Ankle Immobilizer
90% AMT	PRE	0.09	0.07	0.04	0.12	0.11	0.09	0.03	0.02	0.01
	STD	0.10	0.06	0.05	0.11	0.22	0.06	0.06	0.01	0.00
	POST	0.07	0.06	0.04	0.13	0.13	0.09	0.03	0.02	0.01
	STD	0.09	0.05	0.05	0.11	0.07	0.05	0.05	0.03	0.01
110%AMT	PRE	0.19	0.21	0.14	0.21	0.22	0.17	0.05	0.05	0.02
	STD	0.19	0.15	0.14	0.15	0.09	0.10	0.07	0.06	0.01
	POST	0.16	0.14	0.11	0.22	0.26	0.16	0.03	0.06	0.02
	STD	0.17	0.10	0.11	0.15	0.17	0.09	0.06	0.08	0.02
150%AMT	PRE	0.35	0.38	0.28	0.28	0.40	0.27	0.16	0.17	0.05
	STD	0.24	0.18	0.15	0.17	0.16	0.14	0.27	0.24	0.03
	POST	0.29	0.32	0.24	0.30	0.37	0.24	0.09	0.20	0.04
	STD	0.15	0.14	0.18	0.19	0.15	0.12	0.10	0.29	0.02

Figures

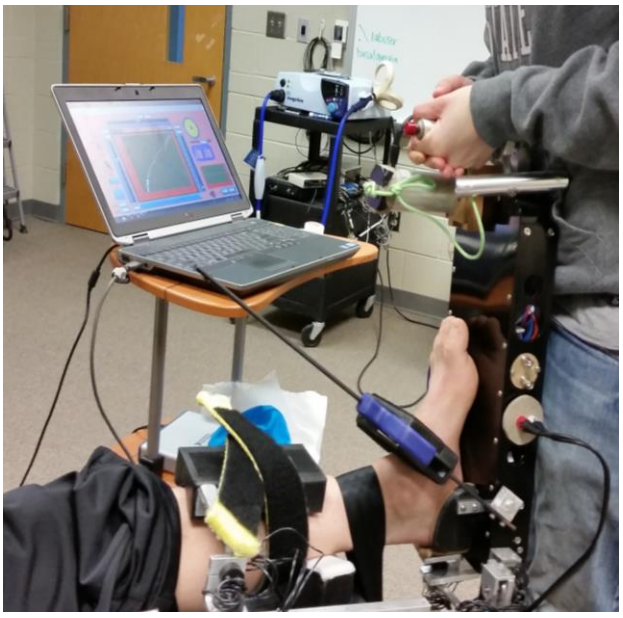

Figure 1: Ankle Arthrometer assessment of Anterior-Posterior displacement and total inversion via Instrumented Ankle Arthrometer

Figure 2: Assessment of functional dorsiflexion range of motion via Weight Bearing Lunge

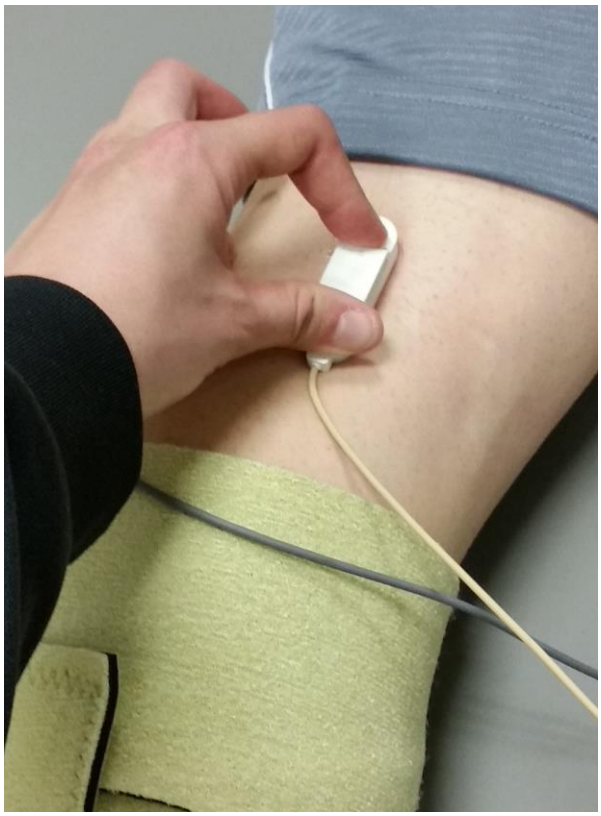

Figure 3: Hoffman Reflex setup

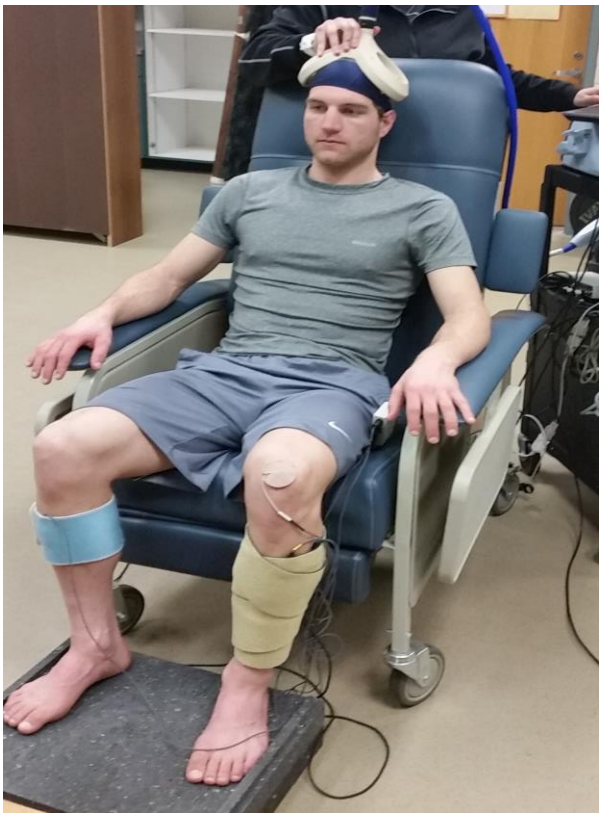

Figure 4: Transcranial Magnetic

Stimulation setup

Appalachian STATE UNIVERSITY

Consent to Participate in Research
Information to Consider about this Research

Neuromechanical Adaptations after Short-Term Ankle Immobilization
Principal Investigator: Alyssa Stirling
Department: Health and Exercise science
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What is the purpose of this research?
Immobilization is the most common treatment after ankle sprains, in order to protect the joint from further injury. Several changes to the nervous system has been observed after immobilization of the upper extremity; however, it is not clear how these changes pertain to the lower extremity, or how immobilization impacts joint stability.

You are invited to participate in a research study that will measure ankle stability and nervous system activity following walking on a treadmill with and without an ankle immobilization device. Findings from this study may allow us to determine what changes and subsequent treatments may lead patients to better outcomes following injury.

Why am I being invited to take part in this research?
You are invited to participate because you are an able-bodied volunteer between the ages of 18-35 that has no history of ankle sprain, or fracture or surgery to your injured leg; and have no current or past history of neurological disorder. If you volunteer to take part in this study, you will be one of about 30 people to do so.

Are there reasons I should not take part in this research?
You ~~should~~ cannot participate in this research if you have any current or past history of cardiac issues , seizure or epilepsy or have an immediate relative with epilepsy; are hearing impaired or have ringing in your ears, have implanted medical devices including cochlear implants, metal in the brain or skull, an implanted neurostimulator, pacemaker, or a medication infusions device; are or may be pregnant; have a history of concussion within 6 months; experience recurrent bouts of fainting or syncope, or migraines; have a history of skull fracture or any skull abnormalities; or have a history of surgery to the brain or heart. The use of (or withdrawal from) several medications may also exclude you from participating in this study. The principal investigator will present you with a screening questionnaire and a list of medications that will determine your eligibility for this study. You will also not be allowed to participate in this study if you have a history of fracture or surgery to either leg.

What will I be asked to do?
Complete participation in this study will entail a total of 3 sessions over the next 3 weeks. Each session will take up to 2.5 hours in duration. All testing will take place in the Injury Neuromechanics Lab in the HolmesConvocation Center Room 011 and/or the Biomechanics & Neuromuscular lab (Convocation Center Room 083). On the first day you will complete the physical activity readiness questionnaire (PARQ), foot and ankle disability index (FADI) and the transcranial magnetic stimulation questionnaire. The following measures will be taken in a random order.

Ankle Laxity
An ankle arthrometer will tell us about the amount of give, or laxity, in your ankle. To test this, a specialized device will be secured to your ankle with 2 clamps and a Velcro strap and the investigator will pull on your ankle 3 times, and then turn your ankle in and out 3 times. The device will be secured tightly, but should not be uncomfortable.

For the other 2 measures, electrical sensors will be placed on your leg. We may need to shave, clean, and lightly abrade your leg at the locations the sensors will be placed. These sensors will allow us to monitor your muscles' activity.

Functional Dorsiflexion range of motion
The weight bearing lunge will tell us about the flexibility of your Achilles tendon. You will be asked to place one foot on a measuring tape so that your knee touches the wall without your heel leaving the ground. If this is done successfully you will move back until you are unsuccessful.

Cortical Excitability
Transcranial magnetic stimulation (TMS) will be used by the faculty advisor to administer magnetic pulses over your head. By measuring your muscles' responses to the pulses, we can determine the strength of the connections between your brain and your ankle. You will be asked to wear a tight fitting cap so that measurements may be made on your head and earplugs to decrease the sound of the machine. You will then be familiarized with the magnetic stimulator which sends very short (less than half a second long) pulses through a large coil. The coil will touch the top of your head during the stimulation. Once familiarized, we will deliver one pulse every 5 seconds at different locations in approximately a 3-cm radius on your head. We will target the areas of your brain that control the muscles being measured. Up to 50 pulses may be delivered at varying intensities to obtain your motor threshold. Next you will receive 30 pulses at 90, 100 and 150% (10 at each intensity) of your motor threshold. The set-up, device, and coil are pictured in the attached handout.

While TMS pulses are being delivered, you will be asked to remain seated with either your muscles relaxed or contracted slightly with the heel out (into foot eversion). You will also be asked to stand on one foot. You will hear a click every time the TMS pulse is delivered. The TMS pulse will feel like a tap on your head and will cause twitching of your leg muscles. At higher intensities, the TMS pulse may cause your forehead or face to twitch.

Spinal Excitability
A final measure will be using electrical stimulation to study the strength of the reflexes surrounding your ankle joint. A stimulating electrode will be placed behind your knee and one will be placed on the front of your thigh. Brief electrical pulses (less than half a second), will be applied while the muscle activity is recorded in your legs. The pulses will begin at a low intensity and will be increased in intensity as the muscle contraction in your leg in response to the electric pulses is recorded. The intensity of the pulses will be increased until a maximal muscle contraction is observed. These pulses will produce a tingling sensation with a muscle contraction that will go away shortly after the stimulation.

What are possible harms or discomforts that I might experience during the research?

There is a mild risk of skin irritation at the location where the muscle sensors are placed, but this will usually go away after the sensors are removed.

Rare cases of seizures during or immediately after TMS have been reported. A recent review found only a few cases of seizure in subjects without a previous history of seizure or neurological disorder using the type of stimulation being used in this study (Groppa et al 2012, p866). Individuals who have a history of seizures or have been diagnosed with epilepsy will be excluded from this study. Metal objects close to the coil may be damaged during magnetic stimulation; we will therefore exclude individuals who have implants in their head. Some medications may contribute to an increased risk of seizure and will also result in exclusion from the study. You may feel twitches in the muscles of your arm, leg, or face during the magnetic stimulation, but these twitches should not be painful.

There is a possibility of headaches, scalp discomfort, or lightheadedness associated with both TMS testing. If they occur, these effects are usually mild and short-lasting. In rare cases, fainting may occur. There may be some minor irritation of the skin around the site of the electrodes following the experiment.

During electrical stimulation, the pulses applied will cause a muscle twitch and tingling sensation shooting down the leg that may be uncomfortable; however, each pulse will last less than one second and every effort will be made to minimize the amount of pulses that must be applied. There may be some discomfort associated with measuring ankle stiffness; however, this is minimized by correct use of the device. Please let the investigators know as soon as possible if the device feels uncomfortable and adjustments may be made to maximize comfort.

Are there any reasons you might remove me from the research?
There may be reasons we will need to remove you from the study, even if you want to stay in. If you experience an injury to either lower extremity between testing sessions, it will be at the discretion of the principal investigator whether to allow you to remain in the study. Additionally, if you experience any of the adverse reactions mentioned above, we will immediately terminate your participation in this study.

What are possible benefits of this research?
There are no direct benefits to volunteers, and you are free to end your participation at any time. It is our hope that your participation in this project will improve our understanding of how protecting the ankle after an injury may benefit the nervous system.

Will I be paid for taking part in the research?
There is no compensation for participating in the study.

What will it cost me to take part in this research?
You are responsible only for arranging transport to and from the laboratory for testing.

How will you keep my private information confidential?
Your identity will remain confidential and will not be revealed in any publications resulting from this work. All data will be stored on a secure long-term storage medium. The data will not have any identifiers linking information to you. The results of this study may be used for teaching, publications, or presentations at scientific meetings. If your individual results are discussed, your identity will be protected by using a study code rather than your name. Following completion of

this project, the data will be destroyed or transferred to a long-term storage medium for use during future research studies. Retained data will be stored on an encrypted secure server.

With your permission, photos may be taken during the study and used in scientific presentations of the research findings. Your identity will not be revealed when the photos are presented. Please indicate whether or not you agree to having your photo taken for use in scientific presentations (without name identification):

- ☐ Yes, I grant permission for my photo to be taken and used in scientific presentations
- ☐ No, I do not grant permission

Whom can I contact if I have a question?

The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at 828-262-4039.If you have questions about your rights as someone taking part in research, contact the Appalachian Institutional Review Board Administrator at 828-262-2692 (days), through email at irb@appstate.edu or at Appalachian State University, Office of Research Protections, IRB Administrator, Boone, NC 28608.

Do I have to participate?

Your participation in this research is completely voluntary. If you choose not to volunteer, there is no penalty or consequence. If you decide to take part in the study you can still decide at any time that you no longer want to participate. You will not lose any benefits or rights you would normally have if you do not participate in the study.

I have decided I want to take part in this research. What should I do now?

If you have read this form, had the opportunity to ask questions about the research and received satisfactory answers, and want to participate, then sign the consent form and keep a copy for your records.

Participant's Name (PRINT) Signature Date

Appalachian
STATE UNIVERSITY

Photography and Video Recording Authorization

With your permission, still pictures (photos) and/or video recordings taken during the study may be used in research presentations of the research findings. Please indicate whether or not you agree to having photos or videos used in research presentations by reviewing the authorization below and signing if you agree.

Authorization

I hereby release, discharge and agree to save harmless Appalachian State University, its successors, assigns, officers, employees or agents, any person(s) or corporation(s) for whom it might be acting, and any firm publishing and/or distributing any photograph or video footage produced as part of this research, in whole or in part, as a finished product, from and against any liability as a result of any distortion, blurring, alteration, visual or auditory illusion, or use in composite form, either intentionally or otherwise, that may occur or be produced in the recording, processing, reproduction, publication or distribution of any photograph, videotape, or interview, even should the same subject me to ridicule, scandal, reproach, scorn or indignity. I hereby agree that the photographs and video footage may be used under the conditions stated herein without blurring my identifying characteristics.

_____

Participant's Name (PRINT) Signature Date

Data Collection Sheet

NAME:_____ DATE:_____

HEIGHT:_____in. WEIGHT:_____lbs. AGE:_____

PHYSICIANS NAME:_____ PHONE:_____

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

	Questions	Yes	No
1	Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?		
2	Do you feel pain in your chest when you perform physical activity?		
3	In the past month, have you had chest pain when you were not performing any physical activity?		
4	Do you lose your balance because of dizziness or do you ever lose consciousness?		
5	Do you have a bone or joint problem that could be made worse by a change in your physical activity?		
6	Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?		
7	Do you know of any other reason why you should not engage in physical activity?		

If you have answered "Yes" to one or more of the above questions, consult your physician before engaging in physical activity. Tell your physician which questions you answered "Yes" to. After a medical evaluation, seek advice from your physician on what type of activity is suitable for your current condition.

FOOT AND ANKLE DISABILITY INDEX

Please answer each question with one response that most closely describes your condition within the past week. If the activity in question is limited by something other than your foot or ankle, mark N/A.

	No difficulty at all	Slight difficulty	Moderate difficulty	Extreme difficulty	Unable to do	N/A
1. Standing						
2. Walking on even ground						
3. Walking on even ground without shoes						
4. Walking up hills						
5. Walking down hills						
6. Going up stairs						
7. Going down stairs						
8. Walking on uneven ground						
9. Stepping up and down curves						
10. Squatting						
11. Sleeping						
12. Coming on your toes						
13. Walking initially						
14. Walking 5 minutes or less						
15. Walking approximately 10 minutes						
16. Walking 15 minutes or greater						
17. Home responsibilities						
18. Activities of daily living						
19. Personal care						
20. Light to moderate work (standing, walking)						
21. Heavy work (push/pulling, climbing, carrying)						

22. Recreational activities						
23. General level of pain						
	No pain	**Mild**	**Moderate**	**Severe**	**Unbearable**	**N/A**
24. Pain at rest						
25. Pain during your normal activity						
26. Pain first thing in the morning						
	No difficulty at all	**Slight difficulty**	**Moderate difficulty**	**Extreme difficulty**	**Unable to do**	**N/A**
27. Running						
28. Jumping						
29. Landing						
30. Squatting and stopping quickly						
31. Cutting, lateral movements						
32. Low-impact activities						
33. Ability to perform activity within your normal technique						
34. Ability to participate in your desired sport as long as you would like						

NAME_____

Appalachian State University
Transcranial Magnetic Stimulation Screening Questionnaire

1. Do you have **epilepsy** or have you ever had a convulsion or seizure?	◻ Yes	◻ No
2. Do you have any *immediate* family members with a history of epilepsy?	◻ Yes	◻ No
3. Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s)?	◻ Yes	◻ No
4. Have you ever had head trauma that was diagnosed as a concussion or was associated with loss of consciousness? If yes, how long ago was your most recent concussion?	◻ Yes	◻ No
5. Do you have any hearing problems or ringing in your ears?	◻ Yes	◻ No
6. Do you have cochlear implants?	◻ Yes	◻ No
7. Are you pregnant or is there a chance you might be?	◻ Yes	◻ No
8. Do you have metal in the brain, skull, or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, specify the type of metal.	◻ Yes	◻ No
9. Do you have an implanted neurostimulator (e.g. DBS, epidural/subdural, VNS)?	◻ Yes	◻ No
10. Do you have a cardiac pacemaker or intracardiac lines?	◻ Yes	◻ No
11. Do you have a medication infusion device?	◻ Yes	◻ No
12. Do you frequently suffer from migraine headaches?	◻ Yes	◻ No
13. Do you have a history of skull fracture or any present skull abnormalities?	◻ Yes	◻ No
14. Have you ever had surgery to the brain or heart?	◻ Yes	◻ No
15. Are you taking any medications?	◻ Yes	◻ No
If so, do they match any of the medications listed on the opposite side of this page?	◻ Yes	◻ No
16. Did you ever undergo TMS in the past?	◻ Yes	◻ No
If so, were there any problems?	◻ Yes	◻ No
17. Did you ever undergo MRI in the past?	◻ Yes	◻ No
If so, were there any problems?	◻ Yes	◻ No

List of Potentially Hazardous Drugs for TMS

CLASS A

Imipramine	**Amitriptyline**	**Doxepine**	**Nortriptyline**
Maprotiline	**Chlorpromazine**	**Clozapine**	**Foscarnet**
Ganciclovir	**Ritonavir**	**Amphetamines**	**Cocaine**
MDMA *(ecstasy)*	**Phenycyclidine** *(PCP, angel dust)*	**Ketamine**	**Alcohol**
Theophylline	**Gamma-Hydroxybutyrate (GHB)**		

CLASS B

Mianserin *(Bolvidon, Norval, Tolvon)*	**Fluvoxamine** *(Luvox)*	**Paroxetine** *(Aropax, Paxil)*	**Sertraline** *(Zoloft)*
Citalopram *(Celexa, Cipramil)*	**Reboxetine** *(Edronax, Vestra)*	**Venlafaxine** *(Effexor)*	**Duloxetine** *(Cymbalta, Yentreve)*
Bupropion *(Wellbutrin, Aplenzin)*	**Mirtazapine** *(Remeron, Avanza, Zispin, Reflex)*	**Fluphenazine** *(Prolixin)*	**Pimozide** *(Orap)*
Haloperidol *(Haldol)*	**Olanzapine** *(Zyprexa, Zydis, Relprevv)*	**Quetiapine** *(Seroquel)*	**Aripiprazole** *(Abilify)*
Ziprasidone *(Geodon)*	**Risperidone** *(Risperdal)*	**Chloroquine** *(Aralen)*	**Mefloquine** *(Lariam)*
Imipenem *(Primaxin)*	**Penicillin**	**Cephalosporins** *(Cephalosproium)*	**Metronidazole** *(Flagyl)*
Isoniazid *(Laniazid, Nydrazid)*	**Levofloxacin** *(Levaquin)*	**Cyclosporin** *(USAN, BAN)*	**Chlorambucil** *(Leukeran)*
Vincristine *(Oncovin)*	**Methotrexate** *(Trexall, Rhumatrex)*	**BCNU** *(Carmustine)*	**Lithium** *(Lithoboid, Eskalith)*
Anticholinergics *(i.e. Atrovent, Albuterol, Combivent, DuoNeb)*	**Antihistamines** *(i.e. Allegra, Claritin, Benadryl)*	**Sympathomimetics** *(i.e. ephedrine, amphetamine, Ritalin)*	**Ampicillin** *(Omnipen, Polycillin, Principen)*
Cytosine arabinoside *(Cytarbine)*	**Fluoxetine** *(Prozac)*		

Additionally, you should *not* participate in this study if you are undergoing **withdrawal** from *alcohol, barbiturates, benzodiazepines, meprobamate, or chloral hydrate*

For Investigator Use Only:
If subject answered yes for any question, explain below:

_____
_____
_____
_____
_____

Vita

Alyssa Marie Stirling was born in Arad, Romania to James and Kathleen Stirling. She graduated from Coatesville Area Senior High School in Pennsylvania in 2009. The following fall, she enter into Immaculata University and graduated in 2014 with a Bachelor of Science degree in Athletic Training. In the fall of 2014, she accepted a research assistantship in the injury neuromechanics lab at Appalachian State University and began study toward a Master of Science degree. The M.S. was awarded in May 2016.