Immediate Effects of rTMS on Excitability of the Quadriceps with Knee Osteoarthritis

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PROTOCOL TEMPLATE: INTERVENTIONAL STUDY

Complete Title: Immediate Effects of rTMS on Excitability of the Quadriceps with Knee Osteoarthritis
Short Title: rTMS for Quadriceps and Knee OA
Sponsor: North Carolina Translational and Clinical Sciences Institute
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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
rTMS	Repetitive transcranial magnetic stimulation
TMS	Transcranial magnetic stimulation
MVIC	Maximum voluntary isometric contraction
OA	osteoarthritis
WOMAC	Western Ontario and McMaster Universities Arthritis Index
BMI	Body mass index
ACL	Anterior cruciate ligament
MCL	Medial collateral ligament
NPRS	Numeric Pain Rating Scale
TUG	Timed up and go
PPT	Pressure pain threshold
AMT	Active motor threshold
SICI	Short interval inhibition
ICF	Intracortical facilitation
CAR	Central activation ratio
PCS	Pain catastrophizing scale
ST	Superimposed Torque
MEP	Motor evoked potentials
AMT-MEP	Active motor threshold motor evoked potential

PROTOCOL SYNOPSIS

Study Title	Immediate Effects of rTMS on Excitability of the Quadriceps with Knee Osteoarthritis
Funder	North Carolina Translational and Clinical Sciences Institute
Clinical Phase	Pilot
Study Rationale	The purpose of the study is to identify things that influence the ability to “turn on” the thigh muscle (quadriceps). The thigh muscle tends to be underactive with knee osteoarthritis, which may make it difficult to strengthen the muscle. We are also testing a new technology called repetitive transcranial magnetic stimulation (rTMS) to determine whether it may help “turn up” activity in the underactive thigh muscle immediately after its application. rTMS uses a targeted pulsed magnetic field, similar to what is used in an MRI (magnetic resonance imaging) machine to send an electrical signal from the brain to the thigh muscle.
Study Objective(s)	Aim 1 is a descriptive study to compare the neural (cortical and corticospinal) excitability of the quadriceps in the symptomatic knee to the asymptomatic knee in participants with knee osteoarthritis. We will examine the associations between neural excitability and clinical measures of pain, strength, function, and coping styles. Aim 2 is a double blind, crossover study design. Each participant will partake in two testing sessions, spaced 1 week apart. We will evaluate outcome measures prior to and following the "true" intervention versus the "sham" intervention. The true intervention is rTMS + exercise. The sham is sham rTMS + exercise. The primary outcome measures are quadriceps strength, as measured from a maximal isometric voluntary contraction, and quadriceps activation percent, as calculated from the torque values from the voluntary strength measurement and a brief, intense electrical stimulus. Secondary measures are clinical measures of pain and function.
Test Article(s) *(If Applicable)*	rTMS at 10 Hz (5 sec on, 55 sec off) and light quadriceps isometric exercise (5% MVIC)
Study Design	Double-blind, randomized, cross-over assignment
Subject Population key criteria for Inclusion and Exclusion:	Inclusion Criteria 1. Subjects age 40 – 75 years 2. Symptomatic knee osteoarthritis involving one leg Exclusion Criteria

	1. Subjects with conditions that are of concern for using rTMS based on the published guidelines. 2. Orthopedic conditions affecting the leg other than knee OA or could limit their ability to detect sensation or pain 3. Cardiac conditions that might be unsafe for exercise 4. Pregnancy, planning to get pregnant or not on reliable method of contraception
Number Of Subjects	20
Study Duration	Each subject's participation will last 3 weeks. Each subject will attend 3 sessions spaced 1 week apart. The baseline session takes about 2.5 hours. The two intervention sessions take about 1.5 hours. The entire study should take 12 - 16 months to complete.
Study Phases Screening Study Treatment Follow-Up	(1) Preliminary Screening: screening for eligibility by phone (2) Baseline Session: screening for eligibility at lab, obtaining consent, and collection of comparison data from the symptomatic knee and the less asymptomatic knee for Aim 1. (3) Randomization (4) Intervention (double-blind, crossover): Arm 1: Session 1 = rTMS and exercise; Session 2 = sham rTMS and exercise Arm 2: Session 1 = sham rTMS and exercise; Session 2 = rTMS and exercise
Efficacy Evaluations	Quadriceps maximal voluntary isometric contraction (MVIC) Change in Quadriceps Central Activation Ratio (%)
Pharmacokinetic Evaluations	NA
Safety Evaluations	Adverse events will be determined using the NC TraCS Safety Monitoring Plan guidelines for Minimal Risk Studies. The Grading Scale is as follows: Mild - Event results in mold or transient discomfort, not requiring intervention or treatment; does not interfere with daily activities (e.g. transient muscle soreness) Moderate - Event is sufficiently discomforting so as to limit or interfere with daily activities; may require additional interventional treatment (e.g. musculoskeletal injury such as a muscle strain) Severe - Event results in significant symptoms that prevent normal daily activities; may require invasive intervention In the event that two adverse events are classified as either moderate or severe the study will be placed on hold in order to reassess the study procedures in order to decrease the risk of another adverse event.

Statistical And Analytic Plan	Aim 1: We will compare between limb differences and use correlation analyses to examine associations between measures of neural excitability, pain, pain sensitization, quadriceps function, timed-up-and-go test, and pain catastrophizing. Aim 2: We will use a repeated measures analysis of variance (ANOVA) to compare the pre- and post-intervention outcomes.
DATA AND SAFETY MONITORING PLAN	In the event that two adverse events are classified as either "moderate" or "severe", based on the NC TraCS Safety Monitoring Plan guidelines, the PI will place the study on hold in order to reassess the study procedures to decrease the risk of another adverse event. Individuals will be withdrawn from the study if they experience any of the following: 1) Signs of musculoskeletal injury 2) Failure or inability to comply with the study procedures 3) Any sign of moderate or severe adverse effects associated with TMS or rTMS. 4) Pregnancy

1 BACKGROUND AND RATIONALE

Quadriceps weakness is one of the most modifiable risk factors for knee osteoarthritis, but strengthening efforts are limited by neural mechanisms. Inhibition implies a reduced level of neural excitation to the muscle. Neural inhibition may contribute to persistent quadriceps weakness and pain in people with knee arthritis. Interventions that affect neural inhibition may serve as valuable adjuncts to exercise for quadriceps weakness. However, there is a gap in our understanding of how to effectively treat knee arthritis-related quadriceps inhibition. Also, we lack understanding of how critical factors such as pain, pain sensitization, and coping styles interact with neural inhibition to influence quadriceps strength, voluntary activation, and function in knee arthritis.

The objective of this research is to overcome natural barriers to strengthening quadriceps muscles weakened with knee arthritis and to provide laboratory and clinical methods to evaluate deficits and treatment efficacy. Our research will study repetitive transcranial magnetic stimulation (rTMS), a modality which that increase neural excitability in conditions such as stroke and can relieve chronic pain. However, rTMS has not been studied to determine if it can increase neural excitability in the quadriceps of people with knee arthritis. This research is a critical first step to establish a comprehensive set of measurements to explore factors that contribute to persistent quadriceps weakness with knee arthritis and to evaluate the potential for rTMS as a modality to improve quadriceps strength and voluntary activation.

Introduction

The study will take place the Neuromuscular Research Laboratory in Fetzer Hall at the University of North Carolina at Chapel Hill.

1.1 Name and Description of Investigational Product or Intervention

rTMS will be delivered using the Super Rapid2 (Magstim, Jali Medical) and 110 mm Double Cone Coil over the motor cortex. Pulses will be delivered at 10 Hz, 5 seconds on and 55 seconds off for 15 minutes while the subject performs a light quadriceps exercise (5% MVIC).

1.2 Non-Clinical and Clinical Study Findings

Potential Benefits: Repetitive transcranial magnetic stimulation (rTMS) with exercise has been shown to increase corticospinal excitability and motor function in people recovering from stroke and spinal cord injury. rTMS has also led to reductions in pain in chronic pain conditions. If rTMS is able to increase neural excitability in the thigh muscle of people with knee osteoarthritis, then it may prove to be a useful modality as an adjunct to traditional rehabilitation interventions to ameliorate inhibition and pain. Future studies would be needed to investigate the necessary dosage of rTMS and determine the characteristics people and conditions that would respond best to this intervention.

Likelihood of Direct Benefit: It is possible that the subjects will experience a short-term improvement in the neural excitability of the thigh muscle and reduction in pain. If so, there may be a transient increase in the strength of the quadriceps and a minor improvement in the ability to perform daily activities with less pain and dysfunction. We expect any transient benefits of rTMS to "wash out" within hours. To be conservative, we have scheduled sessions 2 and 3 to be a week apart.

Procedures to Minimize Risks of Consequences of breach of Confidentiality: There is a rare chance there will be a breach of confidentiality during this study. All contact with the subject will be conducted in private areas and all data will be kept in locked files, in locked offices, and on password protected computers. Code numbers will be used, rather than names on all documents that are collected for study purposes. Participants will be identified only by this subject ID# to which their personal information will not be linked. No form of dissemination of this study's findings will include any subject's identifiable information.

Procedures to Minimize Risks of Pain, Discomfort, or Injury:
Very common: The electrical stimulation used during the Quadriceps Voluntary Activation assessment, though extremely brief (less than 1/1000 of a second), may commonly cause discomfort – not pain – during testing.

Likely: The noise from the rTMS device will likely be uncomfortable to the subjects. Humans exposed to TMS have shown temporary increases in auditory threshold (especially at high frequencies) lasting at least 5 minutes and less than 4 hours. We will provide earplugs to reduce the noise level.

Infrequent: Muscle soreness and knee joint soreness from contracting the quadriceps may occur, but will be minimal and the soreness should go away within a day. The thigh muscle contractions during the muscle strength testing may infrequently result in muscle soreness or, in extremely rare cases, muscle injury. Participants will be provided with warm-up exercise before completing these tasks and provided adequate rest breaks between tasks in order to reduce the risk of injury.
Some people report mild discomfort when the TMS pulses are applied over the scalp. A small number of people (~5%) report headache following rTMS. However, the headaches are temporary and manageable with common over-the-counter pain remedies.

Rare: There is a possibility of discomfort associated with prepping the areas where electromyographic (EMG) sensors will be placed (i.e. shaved, lightly abraded, and cleaned with alcohol).
Syncope is defined as a momentary loss of awareness and postural tone. It typically has a rapid onset, short duration, and spontaneous recovery. Although syncopal episodes are very rare with TMS (less than 1%), they typically occur during the motor threshold procedure before the rTMS treatment has begun. Individuals that are sleep deprived and have low or unstable blood pressure are at greater risk. The participant's blood pressure and pulse will be taken prior to data collection. Fainting due to a sudden drop in blood pressure is called syncope. Syncope with TMS and rTMS is rare and occurs at a rate similar to other medical procedures such as drawing blood. However, anyone with low blood pressure (< 90 systolic and < 60 diastolic) or slow heart rate (< 50 beats per minute) or feeling light headed will be excluded from continuing on to the TMS testing.

Regarding sleep deprivation, in our intake Knee History Questionnaire, the participant is asked about their sleep in the past 24 hours to determine if he/she may be sleep deprived. The questions specifically are: How many hours of sleep did you get last night? _____ Is this normal for you? No Yes

If the subject reports less than 7 hours of sleep we further explore the sleep pattern. We will ask the participant if this is a normal amount of sleep for him/her and if he/she had a nap in the past 24 hours. If the total reported amount of sleep is off by 2 hours or more compared to their normal pattern, we will consider this a sign of sleep deprivation and the subject will be rescheduled to return for testing on another day.

There is a very rare risk of seizure. TMS stimulates the neurons in the brain at a level below what is required to cause a seizure, although eight seizures have been noted in the literature in the past 20 years. Six of them have been in healthy volunteers (without any history of seizures, brain masses or traumatic brain injuries). The risk of seizure induction is related to the intensity, duration, frequency and rest interval of stimulation. Following the adoption and widespread use of the safety guidelines, 1 seizure has been reported since 1997 and it involved parameters of higher settings then the safe range (Rossi, 2009). The stimulation with the parameters and settings we propose to use are adjusted to the participant's motor threshold and we have included a long "on" to "off" ratio during the repetitive TMS intervention. Our ratio of 1:11 well exceeds the safety guideline of 1:2. To be safe, we have excluded individuals with a history of seizures or epilepsy. We have also excluded people with a history of head injury and medications that could lower the seizure threshold. By following all of the published safety guidelines, the risk of seizure is rare.

There are always possibilities that unknown side effects may occur during or following testing.

Participants will be monitored throughout the study for any adverse events and will be withdrawn from the study if they report any adverse events associated with either the testing or the intervention. In the event that a participant sustains an injury during the study, they will be referred to the appropriate medical personnel on campus (i.e. Campus Health Services or the UNC Hospitals Emergency Room).

Rossi S, Hallett M, Rossini PM, Pascual-Leone A; Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009 Dec;120(12):2008-39

1.4 Relevant Literature and Data

Background

A significant number (9.29%) of US adults are diagnosed with knee OA by age 60.[1] The lifetime risk of knee OA is as high as 23.87% in obese women.[1] Over half of adults in the US diagnosed with knee OA will undergo a total knee replacement (TKR)[2] with the number of cases projected to increase 673% by 2030[3] with consequent increases in revisions due to loosening, instability, infection, and other complications.[4] TKR is an irreversible surgical procedure, with attendant morbidity and mortality, that is likely premature or unnecessary in a proportion of individuals that would benefit from restored or enhanced quadriceps strength.

Significance

Quadriceps weakness is associated with greater knee pain and reduced physical function in persons with knee OA.[5, 6] Quadriceps weakness can occur in the absence of pain and muscle atrophy with knee OA and is considered an etiologic factor for the development of knee OA.[6-8] The quadriceps function as a shock absorber during gait, so the increased impulsive forces with weakness can contribute to the development and progression of knee cartilage damage.[6, 9-12] Our data show that the subjects with knee OA, compared to healthy individuals, have a 26% deficit in production of voluntary quadriceps isometric torque (MVIC).[13] Quadriceps torque was influenced by physical (activation deficits and muscle morphology), sensory (pressure pain threshold) and personal factors

(pain catastrophizing).[13, 14] Low quadriceps voluntary activation severely limits strength training.[14]

Strong quadriceps muscles are associated with less knee OA pain and improved function.[15, 16] Although there is presently no cure for knee OA, a quadriceps that is stronger[15] and of greater cross-sectional area[17] may attenuate progression of cartilage loss over time. Slowing the progression of OA through early intervention can prevent disability and improve the long-term health of the knee. However, neuromuscular inhibition[18] is a common complication of knee OA that impairs strengthening[19] and that may explain why traditional rehabilitation approaches are often ineffective in ameliorating quadriceps muscles weakness in knee OA.[20, 21],[22]

Quadriceps neuromuscular inhibition after knee injury is commonly viewed as an acute, protective mechanism that likely serves the function of limiting muscle forces acting across the knee, but the chronic persistence of inhibition is detrimental to recovery of strength and function.[5, 20, 23-25] Pain can contribute to quadriceps inhibition, but inhibition can occur without pain.[24, 26] Quadriceps inhibition is associated with a decrease in spinal reflex excitability[26, 27] and a concurrent decrease in corticospinal excitability.[28, 29] Finding effective ways to overcome inhibition and sufficiently strengthen the quadriceps may delay the development and progression of knee OA and TKR surgery. To date, interventions such as transcutaneous electrical stimulation,[30] electromyography biofeedback,[31] and vibration[18] have met with variable success, have not been specifically and widely tested in knee OA, and long-term efficacy is unknown. rTMS has been shown to produce long-lasting changes in corticospinal excitability.[32] Using rTMS to augment a strengthening exercise intervention has the potential to target both central and peripheral factors contributing to persistent quadriceps weakness with knee OA.

Pain is modulated by both spinal and supraspinal mechanisms; therefore, it is also possible that changes in supraspinal mechanisms also occur in response to knee OA pain. Pain with knee OA correlates poorly with radiographic indices of disease severity.[33] Alteration of central mechanisms (sensitization) may contribute to the development of chronic, widespread pain with knee OA.[34-36] rTMS over M1 is thought to reduce pain by activating distant brain areas involved in pain integration and modulation.[37]
Human pain studies cannot ignore the powerful influence of psychological factors. One of the more robust factors is pain catastrophizing, a maladaptive cognitive style of coping with pain.[38] Our data indicate that higher levels of pain catastrophizing are associated with greater knee pain, pain sensitization, quadriceps inhibition, and slower TUG performance in people with knee OA.[13, 14] Pain catastrophizing coupled with altered CNS pain processing (e.g., diminished endogenous pain inhibition) and sensitization may serve as a central mechanism that promotes the development, continuation, and exacerbation of persistent pain.[38]

Our data indicate that coping behaviors such as catastrophizing must be considered for their influence on pain, sensitization, quadriceps strength, and function.[13, 14] The current proposal is significant because it 1) determines whether rTMS shows immediate beneficial effects with people with knee OA as found with other chronic neuromuscular and painful conditions and 2) evaluates a comprehensive set of factors that will be used in future studies to evaluate the efficacy of interventions for knee OA aimed at increasing quadriceps strength, reducing pain, and improving function.

2 STUDY OBJECTIVE

The purpose of the study is to identify things that influence the ability to "turn on" the thigh muscle (quadriceps). The thigh muscle tends to be underactive with knee osteoarthritis, which may make it difficult to strengthen the muscle. We are also testing a new technology called repetitive transcranial magnetic stimulation (rTMS) to determine whether it may help "turn up" activity in the underactive thigh muscle immediately after its application.

2.1 Primary Objective

To determine the immediate (within day) effects of rTMS + exercise on the ability to activate the quadriceps muscle and clinical outcomes in people with knee arthritis. The primary outcome variables are quadriceps strength, (maximum isometric voluntary contraction) and quadriceps voluntary activation percent (calculated from strength and a brief intense electrical stimulation). Secondary outcome variables are measures of neural excitability from the contralateral motor cortex and hemispheres, pain, pain sensitization, and function (based on the Timed-Up-and-Go test)

2.2 Secondary Objective

To compare the neural (motor cortex and corticospinal tract) excitability (turning the muscle on) of the anterior thigh muscle (quadriceps) in the symptomatic knee to the asymptomatic knee in participants with knee arthritis. We will examine the associations between neural excitability and clinical measures of pain, pain sensitization, quadriceps strength, walking and balance function, and coping styles.

3 INVESTIGATIONAL PLAN (brief overview)

3.1 Study Design

Aim 1 is a descriptive observational study comparing the symptomatic to the asymptomatic knee and obtaining data to characterize the participant's clinical condition. This will be collected in the first data collection session (Session 1).

Aim 2 is a double blinded, crossover, repeated measures design. Each participant will partake in 2 sessions. (Sessions 2 and 3). One session is the control (sham rTMS + exercise) and one is the intervention (rTMS + exercise). Participants will be randomized into blocks of 3 in an A:B, B:A order, where A is the "true" intervention session and B is the "sham" control session. Half the subjects will be A:B order and half B:A. The randomization list will be created by the biostatistician.

Screening: Individuals who express an interest in participating in the study during the recruitment process, either in person, email, or by phone, will be contacted by a researcher.

3.2 Allocation to Treatment Groups and Blinding (if applicable)

A research assistant who is responsible for data collection will be blind to the intervention or control. Subjects will be blind to whether they are receiving rTMS or sham rTMS**.**

3.3 Study Duration, Enrollment and Number of Subjects

Four study contacts (1 screening, 3 laboratory sessions). Each laboratory session will be 1.5 to 2 hours in duration for a total of 6 hours. The sessions are spaced 1 week apart so that the study is completed within 3-4 weeks total time. A total of 20 subject will be recruited.

3.4 Study Population

Subjects will be between 40 and 75 years old. Participants must exhibit symptomatic knee osteoarthritis involving one leg, which we will define as a normalized, person based, Western Ontario and McMaster Universities Arthritis Index (WOMAC) function subscale score of 10 or more points or 14.7% (out of 100 %, indicating most dysfunction). When radiographs of the knee are examined for osteoarthritis, the arthritis will be judged to be between a 2 to 4, based on the Kellgren – Lawrence scale. The opposite knee should be relatively asymptomatic with WOMAC pain score ≤ 5. If radiographs are available, the opposite knee should score ≤ 2 on the Kellgren-Lawrence scale.

Potential participants will be excluded if they have orthopedic conditions affecting the leg other than knee osteoarthritis, conditions that could limit their exercise tolerance, pregnancy, conditions that could alter sensation and pain processing, and conditions that are of concern for using repetitive transmagnetic stimulation (rTMS). We have incorporated published safety guidelines for repetitive transcranial magnetic stimulation (TMS) to screen subjects (Wasserman, 1996; Rossi, 2009; Rossi, 2011; Rossini, 2015;):

The full list of exclusion criteria, including those for transcranial magnetic stimulation, for this research includes:

- A body mass index (BMI) > 35
- Low blood pressure (< 90 systolic, 60 diastolic) or heart rate (< 60 beats per minute)
- Pregnant or planning to become pregnant in the next 3 months
- Require a cane, walker, or crutches to walk
- Heart condition or disease that restricts exercise
- History of low back surgery in the past year
- History of leg orthopedic surgery in the past year
- Injection (corticosteroid or hyaluronic acid) in either knee in the past 4 weeks
- Knee replacement surgery
- Knee ligament tear that did not undergo reconstruction (ACL, MCL)
- Severe arthritis in both knees
- Knee ligament tear that did not undergo reconstruction (ACL, MCL)
- Cancer in the thigh area (bone or muscle)
- Fibromyalgia
- Uncontrolled diabetes with numbness and tingling in legs or arms
- Peripheral neuropathy (numbness, tingling in extremities)
- A balance disorder
- Had an adverse reaction to an MRI or Transcranial Magnetic Stimulation (TMS)?
- Seizures or epilepsy
- Have had a stroke
- Have had a head injury (including neurosurgery)
- Have metal in the head (outside of the mouth) such as shrapnel, surgical clips, cochlear implant, or fragments from welding or metalwork
- Have an implanted devices such as a cardiac pacemaker, medical pump, or intracardiac lines

- Suffer from frequent or severe headaches
- Have had any other brain-related condition (Parkinson's, swelling, multiple sclerosis, cancer)
- An illness that caused brain injury in the past
- Immediate family (parents, brothers, sisters) history of epilepsy
- Currently withdrawing from a drug or alcohol
- Have had little sleep in the past 24 to 48 hours (sleep deprived)
- History of fainting spells (syncope)
- Taking medications known to lower the seizure threshold potential. These include: Imipramine,amitriptyline, doxepin, nortriptyline, maprotiline, chlorpromazine, clozapine, foscarnet, ganciclovir, ritonavir, theophylline, phencytidine, ketamine, gamma hydroxybutirate, amphetamine cocaine, MDMA (Ecstasy)
- Inability to consistently comprehend and repeat back directions regarding details of the study

Excluding pregnant women, justification, and type and timing of pregnancy testing to be used. We will exclude pregnant women, women who are trying to get pregnant or plan to get pregnant, or women who are not using a reliable method of contraception during the study. The vast majority of the females in our study will be post-menopausal as the median age of knee OA diagnosis is 55 years old (Losina et al. 2013), and the average age of menopause is 51 according to the National institute on Aging. All female participants of childbearing age and who have not undergone hysterectomy or tubal ligation will undergo urine pregnancy testing at baseline and before each study visit. A positive test will exclude further participation in the study. Women included in the study must be on a reliable method of contraception, including oral contraception, barrier methods, or long-acting reversible contraception.

TMS and rTMS Safety References:

1. Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalography and clinical neurophysiology. Jan 1998;108(1):1-16.
2. Rossi S, Hallett M, Rossini PM, Pascual-Leone A; Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009 Dec;120(12):2008-39
3. Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Screening questionnaire before TMS: an update. Clin Neurophysiol. 2011 Aug;122(8):1686.
4. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol. 2015 Jun;126(6):1071-107.

4 STUDY PROCEDURES (what will be done)

Recruitment

Participants will be recruited through:

1) referral from family medicine, orthopaedic and rheumatology physicians - these doctors will provide flyers regarding the study but will not be responsible for screening or enrollment of subjects

2) ongoing studies of people with knee OA in the Neuromuscular Research/Sports Medicine Research Laboratory - we currently have a list with over 20 individuals with knee arthritis who have agreed to be contacted to participate in research of this nature

3) emails to the UNC listserv and patients identified in the Carolina Data Warehouse

Physicians in the Department of Medicine (Divisions of Family Medicine, Orthopaedics and Rheumatology) at the University of North Carolina at Chapel Hill may provide study flyers to patients with knee arthritis but these physicians will not screen or enroll the participants. Subjects who have been screened for participation in other studies or have participated in research in the Neuromuscular Research/Sports Medicine Research Laboratory and agree to be contacted for future studies also will be provided study flyers. For example, Dr. Pietrosimone is currently excluding nearly 50% of subjects with knee osteoarthritis for one of his current IRB approved projects (#15-1150) because of their WOMAC function score or quadriceps function. The inclusion/exclusion criteria for this study are not as stringent as Dr. Pietrosimone's study. Therefore, we expect to recruit most subjects from this subject pool. Finally, we will use the UNC email system and emails to potential participants identified in the Carolina Data Warehouse.

4.1 Screening/Baseline Visit procedures

Screening: Individuals who express an interest in participating in the study during the recruitment process, either in person, email, or by phone, will be contacted by a researcher. The prospective subject will be asked if he/she is interested in learning more about the study and to be screened for inclusion to the study. This screening will follow a standardized format approved by the IRB. We expect most of the time it will take place by phone, but it could also be done in person. The prospective subject will be asked if he/she is willing to provide basic demographic information (gender, age). The researcher will read the list of exclusion criteria and ask if there is anything on this list that could apply to him or her. The subject does not need to disclose the reason, only that there is at least one item that would be applicable. With the subject's verbal permission, the researcher will continue and ask about medications to screen for medications that could lower the seizure threshold. Subjects will then provide ratings of pain and function using the Pain and Functional Subscales of the Western Ontario and McMaster Universities Arthritis Index (WOMAC). This screening process will reduce the chance of participants coming to the lab for further screening if he or she would be excluded based on demographics, health history, medications, and level of physical function. Following the phone or in person screening, eligible participants will be invited to the Neuromuscular Research Laboratory and scheduled for an appointment.

<u>Laboratory Session 1</u>

The goals of the study and procedures will be explained to the subjects. The consent form will be reviewed and the PI or research assistant will address any questions by the subject. After the consent form is completed and signed, the subject will complete additional forms and questionnaires.

Subjects will complete the following forms:
1. Knee injury history and screening form
2. Screening form for inclusion/exclusion criteria including transcranial magnetic stimulation (TMS), repetitive TMS (rTMS), and electric stimulation contraindications
3. Pain numeric rating scale for current pain and pain over the past week
4. Pain Catastrophizing Scale
5. UCLA Activity Score
6. Western Ontario and McMaster Universities Arthritis Index (WOMAC)

The subject will then undergo baseline testing – see study evaluations and measurements in Section 5.

4.2 Intervention/Treatment procedures (by visits)

Laboratory Sessions 2 and 3

Sessions 2 and 3 will be scheduled 1 week apart to allow for wash out of any effects from repetitive TMS (rTMS). The only difference between Session 2 and Session 3 is whether the subject receives rTMS + Exercise or sham rTMS + Exercise. After completing the session 2 pre-testing protocol, the subject will be randomly assigned to receive either the true rTMS or sham rTMS + Exercise intervention. The randomization schedule for the 20 subjects will be generated prior to the onset of the study with assignments only known by the biostatistician. Treatment allocation will be revealed to an investigator performing the rTMS + Exercise intervention after
collection of the session baseline measurements. If the subject is allocated to the sham rTMS intervention for Session 2, the subject will receive the "true" rTMS intervention in Session 3 and vice versa.

Measurements from the symptomatic leg only will be made pre and post intervention (true or sham) using the previously evaluations and measurements described in Section 5. Specifically, the pre and post measurements include:

- Pain numeric rating scale (NPRS) for current pain.
- Timed-up-and-go (TUG) - repeated 3 times each time measured
- Pressure pain threshold (PPT) at the 4 leg sites and arm site - repeated 3 times at each site location each time measured
- Thigh muscle function: quadriceps isometric strength (MVIC) and central activation ratio (CAR),
- Single-pulse and double-pulse TMS for cortical and subcortical excitability -
- active motor threshold motor evoked potential (AMT-MEP), cortical inhibition (SICI), and cortical facilitation (ICF)

Repetitive TMS (rTMS) + Exercise Protocol

The protocol for the rTMS is based on a careful review of the literature to optimize the stimulus parameters. As previously described, we will use the single-pulse TMS to find the "hot spot" or optimal location for eliciting the thigh muscle response and to establish the active motor threshold (AMT). Keeping the TMS coil in position, we will follow guidelines established for rTMS (Rossini, 2015). To deliver pulses in a series over time (repetitive TMS), we will use the Magstim Rapid2 device. The device will be set to deliver a total of 750 pulses over a period of 15 minutes. The frequency of the repetitive pulses is set to 10 Hz. The intensity is set at 70% of active motor threshold (AMT). The pulses will be delivered for 5 seconds (on) and 55 second off. During the on time, the subject will perform an isometric exercise for the thigh muscle at 5% of their maximum (MVIC). The subject will rest during the off interval to minimize fatigue. The ratio of "on" to "off" stimulus of rTMS well exceeds the recommended safety guidelines for rTMS maximum safe duration (Wassermann, 1998). These guidelines recommend that the "off" time be at least twice as long as the duration of the "on" time. We are using a 1:11 ratio.

For sham rTMS + Exercise we will maintain the position of the TMS coil for localizing the optimal thigh muscle response. Mu-metal will be placed between the coil and the scalp. The mu-metal serves as a shield for preventing most of the magnetic energy transmission. The coil will be encased in a black mesh bag to allow cooling but hide the mu material. During the "true" rTMS condition, we will also use the mesh bag over the coil to blind the condition. This procedure is similar to one being used by Mark Bowden, PT, PhD, an investigator at the Medical University of South Carolina (MUSC). Dr. Bowden designed this in consultation with Mark George, PhD, who has conducted a number of studies using sham rTMS. The immediate effects of rTMS + exercise on the symptomatic knee will be assessed by repeating the outcome measures for the post-test. For session 3, subjects

will return to the lab 1 week after session 2. This will provide sufficient time to "wash out" the effects of the rTMS.

A considerable body of research exists for the use of rTMS for cortical stimulation for rehabilitation of stroke (Le, 2014; Chieffo, 2014; Pollack, 2014), spinal cord injury (Tazoe, 2015; Boldt, 2014) and chronic pain (Galhardoni, 2015; Klein, 2015). Dr. Givens has received training rTMS from the National Center of Neuromodulation for Rehabilitation, an NIH sponsored center at the Medical University of South Carolina. The Magstim Rapid2 has FDA clearance (510k) for treatment of drug resistant Major Depressive Disorder and for peripheral stimulation. Magstim devices are considered to be investigational for rehabilitation use in the USA for cortical stimulation, yet we have requested an IDE for this device as it has been safely and successfully used on clinical and healthy populations. We are conducting this study to determine if rTMS + exercise can acutely alter corticospinal excitability of the quadriceps in people with knee OA. Improving corticospinal excitability in patients with quadriceps dysfunction secondary to knee OA may allow for improved muscle function, thereby improving self-reported function and physical activity. This is a first step in testing a neurophysiological principle. According to the Magstim company, in 4 out of 5 carried out on TMS, the researchers have used Magstim products. An number of areas of study and references are available for review.

4.3 Follow- up procedures (by visits)

N/A

4.4 Unscheduled visits

N/A

4.5 Concomitant Medication documentation

N/A

4.6 Rescue medication administration (if applicable)

N/A

4.7 Subject Completion/ Withdrawal procedures

Participants may withdraw at any time in the study. Participants will be monitored throughout the study for any adverse events and will be withdrawn from the study if they report any adverse events associated with either the testing or the intervention. In the event that a participant sustains an injury during the study, they will be referred to the appropriate medical personnel on campus (i.e. Campus Health Services or the UNC Hospitals Emergency Room). Most women enrolled will not be of childbearing age. If patients are pregnant or plan to be become pregnant during the study we will exclude these patients from the study as these changes may have an effect on the physiology that could alter the response to the rTMS and other measures.

Individuals will be withdrawn from the study if they experience any of the following:
1) Signs of musculoskeletal injury
2) Failure or inability to comply with the study procedures
3) Any sign of moderate or severe adverse effects associated with TMS or rTMS.
4) Pregnancy

4.8 Screen failure procedures

Subjects will be screened initially by phone or in person interview. If they are not eligible to continue, they will not be scheduled for a laboratory session. Those who pass the pre-screening will be scheduled to come to the laboratory for session 1. The subject will complete the baseline questionnaires that will

be reviewed to verify the subject continues to meet the inclusion and exclusion criteria. If this screening detects an issue, the subject will not continue with the study.

5 STUDY EVALUATIONS AND MEASUREMENTS (how measurements will be made)

All data will be collected from subjects in the laboratory setting.

Pain numeric rating scale for current pain and pain over the past week

The intensity of any current knee pain and average knee pain over the past week will be rated using the Numeric Pain Rating Scale (NPRS) (Huskisson 1974). Pain is marked on the scale from 0 mm representing "no pain" to 100 mm representing "maximal pain." The NPRS has moderate to good reliability (ICC= .71-99) and for measuring musculoskeletal pain (Good, Stiller et al. 2001; Kahl and Cleland 2005) and a MCID of 18 mm in individuals with knee osteoarthritis (Singh, Luo et al. 2014).

Pain Catastrophizing Scale

The PCS is a 13-item questionnaire to quantify pain catastrophizing, a maladaptive cognitive style of coping with pain that includes the tendency to magnify the threat of pain, to feel helpless in the context of pain, and to ruminate (Sullivan 1995; Quartana, Campbell et al. 2009). Items are rated, based on the degree that the person has thoughts or feelings when experiencing pain, from 0 "not at all" to 4 "all the time". The range for the total score is from 0 to 52 with higher scores associated with higher pain catastrophizing. The PCS components and total score have been shown to have acceptable reliability (α= .74-.91) and validity in both clinical and non-clinical populations (Osman, Barrios et al. 1997; Van Damme, Crombez et al. 2002; George, Valencia et al. 2010). In people with knee OA, a PCS cutoff score of 16 or higher before surgery predicted poor outcomes after knee arthroplasty (Riddle, Wade et al. 2010).

UCLA Activity Score

The participant rates their level of physical activity. The range is from 1, defined as "no physical activity, dependent on others" to 10, defined as ""regular participation in impact sports." It has been shown to have construct validity for monitoring physical activity with knee OA (Terwee, Bouwmeester, et al 2011).

Western Ontario and McMaster Universities Arthritis Index (WOMAC)

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (Bellamy, Buchanan et al. 1988) (Hawker, Wright et al. 2001) (Mahomed, Liang et al. 2002) (Hawker, Wright et al. 1998) (Bullens, van Loon et al. 2001) is one of the most commonly used patient-reported outcome measures for knee OA, provides ratings of pain severity during various positions or movements, severity of joint stiffness, and difficulty performing daily functional activities (Collins, Misra et al. 2011). Each item is scored from 0 to 4 with higher scores indicating worse pain, stiffness, or physical function. The total score for each subscale is determined by adding the items. A total score from 0 (no problems) to 96 (extreme problems) can be calculated. The WOMAC has moderate to good reliability for the three subscales (ICC> .75) (Gandek 2015) and an MCID of 4-9 points (Williams, Piva et al. 2012).

Vital Signs

The participant's blood pressure and pulse will be taken prior to data collection. Fainting due to a sudden drop in blood pressure is called syncope. Syncope with TMS and rTMS is rare and occurs at a rate similar to other medical procedures such as drawing blood. However, anyone with low blood pressure (< 90 systolic and < 60 diastolic) or slow heart rate (< 50 beats per minute) or feeling light headed will be excluded. In our intake Knee History Questionnaire, the participant is asked about their sleep in the past 24 hours to determine if he/she may be sleep deprived.

How many hours of sleep did you get last night? _____ Is this normal for you? ? No ? Yes

If the subject reports less than 7 hours of sleep we further explore the sleep pattern. We will ask the participant if this is a normal amount of sleep for him/her and if he/she had a nap in the past 24 hours. If the total reported amount of sleep is off by 2 hours or more compared to their normal pattern, we will consider this a sign of sleep deprivation and the subject will be rescheduled for another time.

Timed-Up-And-Go Test (TUG)

The Timed-Up-And-Go Test is a measure of function, balance, and walking ability. Participants will be seated in a standard chair with arm rests and instructed to complete the test and quickly and as safely as possible.

Instructions will be to rise from the chair, walk to a line 3 m away, turn, walk back to the chair and return to a sitting position in the chair. Time to complete the task will be measured using a stop watch and recorded. The test will be repeated 3 times and the Timed-Up-And-Go scores will be averaged.

Pressure Pain Threshold (PPT)

Pressure Pain Threshold is a quantitative sensory test. The device is pressed on the skin with increasing amount of pressure until the subject indicates the stimulus is painful. It will be measured using the AlgoMed Computerized Pressure Algometer. Pressure Pain Threshold will be determined at 4 sites for the leg of the symptomatic and asymptomatic knee. The sites are: knee medial joint line, knee lateral joint line, 12 cm distal to knee lateral joint line (area of tibialis anterior muscle) and the ipsilateral heel plantar surface. We will also measure 1 site at the lateral elbow, bilaterally. Each site will be marked using an indelible marker. Each location will be measured 3 times each site. The tip of the pressure algometer will be applied with increasing pressure until the participant indicates a change from a pressure sensation to a painful sensation. Pressure Pain Threshold will be the average of 3 trials for each location.

Quadriceps Function: Strength (MVIC), Central Activation Ratio (CAR), Superimposed Torque (ST)

We will follow the same procedures for the symptomatic and asymptomatic knee. We will be following a well established protocol that has been used in many studies within the UNC Neuromuscular and Sports Medicine Research Laboratories (Luc et al, 2016, Pietrosimone et al, 2015, Lepley et al, 2015). Participants will be seated on a device used to measure muscle strength called an isokinetic dynamometer (Humac). Straps will be used to secure the torso, thigh, and lower leg to the device with the knee in 70° of flexion. The moveable arm of the dynamometer will be fixed in place because we are taking measurements of quadriceps strength when the muscle is performing an isometric contraction. An isometric contraction means that the leg does not move.

Participants will be asked to contract the quadriceps maximally and as quickly as possible by "kicking out" against the dynamometer in response to a verbal cue while torque data are sampled. This represents the quadriceps maximal voluntary isometric contraction (MVIC). To calculate the capacity of the anterior thigh muscle (quadriceps) to produce more torque (force times distance), we will have electrodes placed on the thigh muscle and apply a brief intense electrical stimulus to a maximally contracting muscle (superimposed burst of electrical stimulation), which activates all the muscle's motor units in the area. This produces a measurement we call the superimposed torque (ST), which is the voluntary strength plus more elicited with the strong electrical stimulus. The torque resulting from the superimposed burst of electrical stimulation (ST) and the maximal voluntary isometric contraction (MVIC) are used to calculate the level of quadriceps voluntary activation as a percentage, referred to as the Central Activation Ratio (CAR) People without knee problems may produce a CAR from the thigh muscle of about 95%, whereas people with knee arthritis may produce only 80% to 90%. Participants will perform 3 to 5 practice trials to become familiar and comfortable with these procedures. Three trials will then be recorded from which the measurements will be averaged for statistical analyses.

A Grass electrical stimulator is used to create the brief intense electrical stimulation to the anterior thigh muscle. The Grass stimulator consists of two parts: the S48 Stimulator and SIU8T isolation units. Both the S48 Stimulator and SIU8T isolation units meet the follow safety standards: UL 3101-1, CSA C22.2 No. 1010-1, EN 61010-1. In this study, we are not assessing the clinical safety or efficacy of the Grass stimulator. We are using the Grass stimulator to measure a physiological principle, which is the level of voluntary activation of the quadriceps muscle. Therefore, the use of the Grass stimulator for quantification of voluntary activation is defined as non-invasive as well as a non-significant risk device under the FDA Code of Federal Regulations, Title 21, Section 812.3.

Additionally, the Grass stimulator was approved by UNC Health and Safety on January 26, 2015. Catherine Brenenn from Health and Safety inspected the Grass and cleared it for use. (see IRB# 15-1150) The Grass stimulator will be isolated from fluctuations in building's electrical power supply via a stimulus isolation unit. The Grass stimulator, methods, and stimulus characteristics have been was approved for use in other investigations (conducted on subjects with knee OA and healthy controls) in the UNC Neuromuscular and Sports Medicine Research Laboratories., IRB# 12-0189).

Neural Excitability (Motor Cortex and SubMotor Cortex): Measurements from Transcranial Magnetic Stimulation (TMS), Single-pulse and Double-pulse Stimuli

We will follow the same procedures for the symptomatic and asymptomatic knee. The TMS stimulus is produced and controlled with the Magstim BiStim Unit. The stimulation intensity can be increased or decreased by turning a dial on the unit. When one stimulus is sent by this TMS unit to the stimulating coil, this is referred to as single-pulse TMS. Measurements made using single-pulse TMS represents the excitability of both the brain (cortex) and subcortical regions of the central nervous system (Rossini, 2015). Recording electrodes placed over the belly of a muscle, referred to as electromyography, shows the outcome of the TMS stimulus when the appropriate motor area of the brain is stimulated. The muscle's motor threshold is the defined as the lowest single-pulse TMS intensity that elicits motor evoked potentials (MEPs) from the muscle in 50% of trials (George and

Belmaker, 2007). If the muscle is active during the test, it is referred to as the active motor threshold (AMT), which is defined as the stimulus required to elicit a peak-to-peak amplitude of >100 μV with the muscle activated (George and Belmaker, 2007). We will obtain measurements of active motor threshold motor evoked potentials (AMT-MEP) from the anterior thigh muscle from both the symptomatic and asymptomatic knee in Session 1. In Sessions 2 and 3, these measurements will be made before and after the intervention and only from the symptomatic knee.

The above single-pulse TMS measurements have been obtained using the Magstim BiStim unit in previous investigations in the Neuromuscular and Sports Medicine Research Laboratories. The procedures have been implemented with no adverse events (IRB #15-1345, IRB #14-1978, IRB #13-2314, IRB #13-3228). The results have been disseminated in peer reviewed literature (Gibbons et al, 2010; Pietrosimone et al, 2015; Luc et al, 2014; Thomas et al, 2015).

The subject will be prepared for the TMS testing by pacing a lycra swim cap with a 20 cm x 20 cm grid on the head to locate the optimal area of stimulation for the quadriceps. The lycra swim caps used can be washed following each session. Perpendicular lines from the bridge of the nose to the center of the back of the head (occiput) and from ear (external auditory meatus) to ear will be measured to ensure the cap is centered and placed in the same location for each testing session. The perpendicular lines also serve as a coordinate system for the grid, to better identify the optimal "hot spot" in the motor area of the brain for TMS stimulation of the anterior thigh muscle.

For all TMS testing, participants will be instructed to sit and relax and to focus on the computer screen in front of them depicting the force produced by their quadriceps. Participants will be instructed to clear their mind of any additional thoughts and to remain awake and alert to control for mental-state variability throughout testing.

We will use the Magstim Bistim unit to establish the active motor threshold (AMT). During this test, the participant will be instructed to contract their thigh muscle to 5% of their previously measured maximum (MVIC). The level of torque to achieve 5% maximum will be depicted by a line on the computer screen in front of them. The magnitude of the active motor threshold (AMT) will be determined by muscle responses (motor evoked potentials) that are >100 μV (peak-to-peak). Participants will wear a Lycra swim cap with two perpendicular lines that intersected at the vertex of the cranium, thus sectioning the head into quadrants (11). The swim cap is positioned such that one line connected the center of the occiput and nose, and the second line connected the left and right external auditory meatus (11). The quadrants posterior to the intersection of the two lines denoted the approximate location of the motor cortex (11). A 11 x 8 cm grid consisting of points located 1 cm apart is drawn on the posterior quadrants of swim cap in to allow for movement of the coil in a systematic pattern to locate the optimal point to apply TMS. Initial stimuli are provided at 60% of the maximum stimulator output to locate the optimum coil placement to elicit maximal motor evoked potentials (MEPs). Two stimuli are delivered at each grid location of the swim cap. After systematically stimulating the points on the motor cortex, contralateral to the limb being tested, the grid location producing the greatest MEP amplitude is determined to be the "hot spot" and is marked on the swim cap for subsequent testing (14). The orientation of the coil is traced on

the swim cap in order to ensure exact placement and positioning of the coil each time a stimulus is delivered. AMT is defined as the lowest TMS intensity required to evoke a measurable MEP (>100 μV) in 5 out of 10 consecutive measurements (15). Once 5 out of 10 measurable MEPs are elicited, the intensity level will be decreased by 1% until a total of 6 out of 10 stimuli fail to elicit an MEP amplitude greater than 100 μV (15). The peak-to-peak amplitude of the signals from the thigh muscle will be measured and averaged for the AMT-MEP outcome variable.

After the AMT-MEP is established, 10 stimuli are first delivered at a stimulus intensity of 120% of AMT in order to normalize the MEPs elicited via paired pulse paradigms; paired-pulse paradigms were completed immediately after establishing the normal magnitude of MEPs elicited at 120% of AMT. These 120% AMT measurements are needed for assessing motor cortex inhibition and facilitation. The order of the paired pulse paradigms (intracortical inhibition (SICI) or intracortical facilitation (ICF)) will be randomized and determined once a participant is enrolled into the study.

For ICF, a conditioning stimulus was applied at 80% of AMT 15 ms prior to the testing stimulus, which was applied at 120% of AMT (16). For SICI the conditioning stimulus was applied at 80% of AMT 3 ms prior to the testing stimulus, which was applied at 120% of AMT (16). 10 stimuli were delivered for both SICI and ICF. AMT, SICI, and ICF are reliable measures of corticospinal and intracortical excitability (17, 18). Peak-to-peak MEP amplitudes were measured and recorded for each testing paradigm (120% of AMT, SICI and ICF), and the 10 MEP amplitudes elicited from each testing paradigm were averaged within each participant. Average MEP amplitudes elicited via SICI and ICF paradigms were then normalized to the average MEP amplitude elicited at 120% of AMT for each participant. A lesser conditioned response, or lesser amplitude elicited via the SICI paradigm indicates greater intracortical inhibition, whereas a greater conditioned response, or greater amplitude elicited via the ICF paradigm indicates greater intracortical facilitation.

5.1 Efficacy Evaluation (if applicable) NA

5.2 Pharmacokinetic Evaluation (if applicable) NA

5.3 Safety Evaluations

We will describe any potential physical risks, including the category of likelihood and severity, and what will be done to minimize these risks. We describe the likelihood of the risks occurring, using the following terms:

Very Common (approximate incidence > 50%)
Common (approximate incidence > 25 - 50%)
Likely (approximate incidence of > 10 - 25%)
Infrequent (approximate incidence of > 1 - 10%)
Rare (approximate incidence < 1%)

We Describe severity of risks using the following grading scale:

Mild- No disruption to the subject's ability to perform daily activities; may include non-prescription intervention only
Moderate- Temporary interference with daily activities; may include prescription intervention
Severe- Interference with daily activities; medically significant but not life threatening
Life threatening

Very common: The electrical stimulation used during the Quadriceps Voluntary Activation assessment, though extremely brief (less than 1/1000 of a second), may commonly cause discomfort – not pain – during testing.

Likely: The noise from the rTMS device will likely be uncomfortable to the subjects. Humans exposed to TMS have shown temporary increases in auditory threshold (especially at high frequencies) lasting at least 5 minutes and less than 4 hours. We will provide earplugs to reduce the noise level.

Infrequent: Muscle soreness and knee joint soreness from contracting the quadriceps may occur, but will be minimal and the soreness should go away within a day. The thigh muscle contractions during the muscle strength testing may infrequently result in muscle soreness or, in extremely rare cases, muscle injury. Participants will be provided with warm-up exercise before completing these tasks and provided adequate rest breaks between tasks in order to reduce the risk of injury.
Some people report mild discomfort when the TMS pulses are applied over the scalp. A small number of people (~5%) report headache following rTMS. However, the headaches are temporary and manageable with common over-the-counter pain remedies.

Rare: There is a possibility of discomfort associated with prepping the areas where electromyographic (EMG) sensors will be placed (i.e. shaved, lightly abraded, and cleaned with alcohol).
Syncope is defined as a momentary loss of awareness and postural tone. It typically has a rapid onset, short duration, and spontaneous recovery. Although syncopal episodes are very rare with TMS (less than 1%), they typically occur during the motor threshold procedure before the rTMS treatment has begun. Individuals that are sleep deprived and have low or unstable blood pressure are at greater risk. The participant's blood pressure and pulse will be taken prior to data collection. Fainting due to a sudden drop in blood pressure is called syncope. Syncope with TMS and rTMS is rare and occurs at a rate similar to other medical procedures such as drawing blood. However, anyone with low blood pressure (< 90 systolic and < 60 diastolic) or slow heart rate (< 50 beats per minute) or feeling light headed will be excluded from continuing on to the TMS testing.

Regarding sleep deprivation, in our intake Knee History Questionnaire, the participant is asked about their sleep in the past 24 hours to determine if he/she may be sleep deprived. The questions specifically are:
How many hours of sleep did you get last night? _____ Is this normal for you? No Yes
If the subject reports less than 7 hours of sleep we further explore the sleep pattern. We will ask the participant if this is a normal amount of sleep for him/her and if he/she had a nap in the past 24 hours. If the total reported amount of sleep is off by 2 hours or more compared to their normal pattern, we will consider this a sign of sleep deprivation and the subject will be rescheduled to return for testing on another day.

There is a very rare risk of seizure. TMS stimulates the neurons in the brain at a level below what is required to cause a seizure, although eight seizures have been noted in the literature in the past 20 years. Six of them have been in healthy volunteers (without any history of seizures, brain masses or traumatic brain injuries). The risk of seizure induction is related to the intensity, duration, frequency and rest interval of stimulation. Following the adoption and widespread use of the safety guidelines, 1 seizure has been reported since 1997 and it involved parameters of higher settings then the safe range (Rossi, 2009). The stimulation with the parameters and settings we propose to use are adjusted to the participant's motor threshold and we have included a long "on" to "off" ratio during the repetitive TMS intervention. Our ratio of 1:11 well exceeds the safety guideline of 1:2. To be safe, we have excluded

individuals with a history of seizures or epilepsy. We have also excluded people with a history of head injury and medications that could lower the seizure threshold. By following all of the published safety guidelines, the risk of seizure is rare.

There are always possibilities that unknown side effects may occur during or following testing.

Participants will be monitored throughout the study for any adverse events and will be withdrawn from the study if they report any adverse events associated with either the testing or the intervention. In the event that a participant sustains an injury during the study, they will be referred to the appropriate medical personnel on campus (i.e. Campus Health Services or the UNC Hospitals Emergency Room).

Rossi S, Hallett M, Rossini PM, Pascual-Leone A; Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009 Dec;120(12):2008-39

6 STATISTICAL CONSIDERATION

6.1 Primary Endpoint

Immediate effects of the rTMS within a laboratory session

6.2 Secondary Endpoint

All effects are evaluated within a laboratory session. Participants will be monitored throughout the study for any adverse events and will be withdrawn from the study if they report any adverse events associated with either the testing or the intervention. Individuals will be withdrawn from the study if they experience any of the following:

1) Signs of musculoskeletal injury
2) Failure or inability to comply with the study procedures
3) Any sign of moderate or severe adverse effects associated with TMS or rTMS.
4) Pregnancy

6.3 Statistical Methods

Aim 1. To compare the neural (motor cortex and corticospinal tract) excitability (turning the muscle on) of the anterior thigh muscle (quadriceps) in the symptomatic knee to the asymptomatic knee in participants with knee arthritis. We will examine the associations between neural excitability and clinical measures of pain, pain sensitization, quadriceps strength, walking and balance function, and coping styles.

As a preliminary analysis step, all data will be cleaned and inspected for outliers, missing data and distributional irregularities. Although general linear and mixed linear modeling methods are generally robust to minor violations of distributional assumptions, appreciable departures from normality can compromise statistical tests and estimators. Where error distributions potentially deviate from normality or heteroscedasticity is suspected, or for outcomes expected to be non-normally distributed, departures from normality will be addressed using power transformations where appropriate and/or the use of alternative methods that do not assume normality of outcomes (e.g. nonparametric analyses. We will compare between limb differences and use correlation analyses to examine associations between measures of neural excitability, pain, pain sensitization, quadriceps function, timed-up-and-go test, and catastrophizing. If nonparametric statistical tests are indicated, we will use

two-tailed Wilcoxon Signed Rank tests to examine differences between limbs and two-tailed Spearman Rank-Order correlations to examine associations between variables. The threshold for statistical significance is $p < .05$.

Aim 2. To determine the immediate (within day) effects of rTMS + exercise on the ability to activate the quadriceps muscle and clinical outcomes in people with knee arthritis. The primary outcome variables are quadriceps strength (maximum isometric voluntary contraction) and quadriceps voluntary activation percent (calculated from strength and a brief intense electrical stimulation). Secondary outcome variables are measures of neural excitability from the contralateral motor cortex and hemispheres, pain, pain sensitization, and function (based on the Timed-Up-and-Go test)
We will use a repeated measures analysis of variance (ANOVA), conducted under a mixed modeling framework, to compare outcome variables pre- and post-intervention. Because of its superior statistical properties with small sample sizes, restricted maximum likelihood with Kenward-Roger standard errors will be used in these analyses. The threshold for statistical significance is $p < .05$.

6.4 Sample Size and Power

The sample size for this experiment was explored for the primary variables of interest. These variables are thigh muscle strength from a maximal voluntary isometric contraction (MVIC) and percent voluntary activation. We based the estimates from our preliminary data and available data in the literature (Gibbons, 2010). For muscle strength, the estimated effect size was 0.84 (95% CI: -0.11, 1.7). For central activation ratio, the estimated effect size was 0.84 (95% CI: -0.13, 1.7).

Based on a calculated effect size of 0.84, an α of 0.05 and a power (1-β) of 0.8, we estimated that 11 subjects are needed. Our measurements may have larger variances than previous studies that used single-pulsed TMS to investigate changes in thigh muscle function. Therefore, we inflated our sample to 20 subjects.

Gibbons CE, Pietrosimone BG, Hart JM, Saliba SA, Ingersoll CD. Transcranial magnetic stimulation and volitional quadriceps activation. J Athl Train. Nov-Dec 2010;45(6):570-579.

6.5 Interim Analysis

No interim analysis is planned for the statistical analyses. For safety, in the event that two adverse events are classified as either "moderate" or "severe", based on the NC TraCS Safety Monitoring Plan guidelines, the study will be placed on hold in order to reassess the study procedures in order to decrease the risk of another adverse event.

7 STUDY INTERVENTION (drug, device or other intervention details)

Repetitive TMS (rTMS) + Exercise Protocol delivered via pulses in a series using the Magstim Rapid2. The frequency of the repetitive pulses is set to 10 Hz, 5 seconds on and 55 seconds off for 15 minutes to deliver a total of 750 pulses. The intensity will be set at 70% of active motor threshold (AMT). During the on time, the subject will perform an isometric exercise for the thigh muscle at 5% of maximum (MVIC). The subject will rest during the off interval to minimize fatigue. The ratio of "on" to "off" stimulus of rTMS well exceeds the recommended safety guidelines for rTMS maximum safe

duration (Wassermann, 1998). These guidelines recommend that the "off" time be at least twice as long as the duration of the "on" time. We are using a 1:11 ratio. The coil will be encased in a black mesh bag with a plastic mesh between the coil and the scalp to mimic the sham condition. This will allow to allow cooling but hide the intervention condition.

For sham rTMS + Exercise the protocol will be the same with the exception that Mu-metal will be placed between the coil and the scalp. The mu-metal serves as a shield for preventing most of the magnetic energy transmission. The coil will be encased in a black mesh bag to allow cooling but hide the mu material. During the "true" rTMS condition, we will also use the mesh bag over the coil to blind the condition.

8 STUDY INTERVENTION ADMINISTRATION (if applicable)

Participants will be randomized into blocks of 3 in an A:B, B:A order, where A is the “true” intervention session and B is the “sham” control session. Half the subjects will be A:B order and half B:A. The randomization list will be created by the biostatistician.

A research assistant (RA) who is responsible for data collection will be blind to the intervention or control. The RA will leave the room during the rTMS intervention. The second RA will be provided the subject’s group assignment by the PI and will then fit the appropriate mesh bag with or without the Mu metal over the TMS coil. The subject will be blind to whether they are receiving rTMS or sham rTMS.

9 SAFETY MANAGEMENT

The principal investigator, Deborah Givens, will oversee data collection monitoring and all issues related to participant safety, data collection protocols, and participant confidentiality. Dr. Givens will personally collect any adverse event that occurs during the data collection sessions in the Sports Medicine Research Laboratory.

The participant's vital signs will be taken before any active testing, TMS, or rTMS. If the blood pressure or heart rate is low (< 90 systolic, < 60 diastolic, < 50 beats/min), the participant will be advised of the higher risk of syncope and excluded. If the participant reports being light headed or sleep deprived, the data collection session will be stopped and rescheduled.

All researchers involved with TMS and rTMS will be trained on management of syncope and seizures. We will minimize risk of seizure by screening participants for a seizure history and tailoring each person's TMS intensity to their motor (and indirectly seizure) threshold. We will exclude subjects if they are taking medications that are linked to reducing seizure thresholds. We will minimize hearing damage from rTMS by having all participants wear earplugs to protect their hearing during the TMS sessions.

Before maximum voluntary isometric contraction (MVIC) testing participants will be given a warm-up. Participants will also go through a graded warm up to acclimate to the stimulation needed for quadriceps voluntary activation testing.The 5-minute warm-up prior to testing is designed to reduce the risk of muscle soreness and/or injury during testing. The electrical stimulator will always be connected to the stimulus isolation unit to ensure that the subject is isolated from fluctuations in the building's power supply. Additionally, most members of the research team are certified/licensed health

care providers (Licensed Physical Therapist or Certified Athletic Trainer), and all are trained and certified to provide First Aid and CPR. Any safety concerns (i.e. musculoskeletal injury) would likely be recognized immediately during testing and the research team will monitor participants for adverse events during both testing and intervention sessions.

The research team will refer any individuals who experience adverse events to the Stallings-Evans Sports Medicine Center (located in the building adjacent to Fetzer Hall where the Neuromuscular and Sports Medicine Research Laboratories are located) or Emergency Medical Services depending on the nature and severity of the adverse event issues.

Adverse events will be determined using the NC TraCS Safety Monitoring Plan guidelines for Minimal Risk Studies. The Grading Scale is as follows:
Mild - Event results in mold or transient discomfort, not requiring intervention or treatment; does not interfere with daily activities (e.g. transient muscle soreness)

Moderate - Event is sufficiently discomforting so as to limit or interfere with daily activities; may require additional interventional treatment (e.g. musculoskeletal injury such as a muscle strain)

Severe - Event results in significant symptoms that prevent normal daily activities; may require invasive intervention

In the event that two adverse events are classified as either moderate or severe the study will be placed on hold in order to reassess the study procedures in order to decrease the risk of another adverse event.

10 DATA COLLECTION AND MANAGMENT

The principal investigator will oversee data collection monitoring and all issues related to participant safety, data collection protocols, and participant confidentiality. All adverse events will be reviewed and investigated by Dr. Givens, a licensed physical therapist. Data will be reviewed by two investigators to ensure quality at all stages of reduction, post processing and imputation into statistical software packages. All data will be de-identified and stored in secure password protected computers. The principal investigator will randomly check data reduction, post-processing, and imputation at time points throughout the study. We will utilize the REDCap software to secure data management.

Each subject and his/her associated information will be identified by a subject identification number. Code lists linking the subject identification number and his/her name and email address will be viewed by the research team only, and will be secured in the Neuromuscular Research Laboratory on a password-protected computer. Email addresses will be obtained solely for the purpose of contacting participants to schedule testing. All data will be de-identified and stored in electronic format on both the data collection computer and the principal investigator's personal computer, as well as a number of backup storage devices. Computer access will be protected via confidential passwords, and backup devices will be stored in the Neuromuscular Research Laboratory, which is secured via keycard entry.

11 RECRUITMENT STRATEGY

A multi-pronged approach will be utilized to identify study participants. Participants will be recruited through: 1) direct referral from physicians, 2) subjects who have been screened for participation in Dr.

Pietrosimone's research study but were excluded based on their central activation ratio or pain score, and 3) A search of patients with knee osteoarthritis will be conducted using the Carolina Data Warehouse (CDW) or via practice records systems such as EPIC.

Physicians in the Department of Medicine (Divisions of Family Medicine, Orthopaedics, and Rheumatology) at the University of North Carolina at Chapel Hill will provide study flyers to knee OA patients treated in the clinics. Dr. Porras and Dr. Berkoff, co-investigators on this study, have appointments in Family Medicine and Orthopedics. Dr. Porras and Dr. Berkoff will not screen or enroll subjects in the study but will provide flyers to the prospective participants and ask interested patients for permission to share contact information with the study team. Approved study personnel will be in constant contact with physicians and clinic personnel to collect knee OA patient contact information and follow-up with OA patients treated in their clinics who have indicated willingness to be contacted. We will remind participating physicians and clinic administrators about the need for patient referrals with regular email alerts that report accrual rates to date.

We expect the majority of the participants will come from a list of prospective subjects from Dr. Pietrosimone's lab. Subjects with knee osteoarthritis who have been screened by Dr. Pietrosimone and others in the Neuromuscular Research Laboratory but were excluded from his study (IRB# 15-1150) will be identified. Dr. Pietrosimone has more stringent inclusion criteria for pain and level of central activation deficits that are not required for this study. Subjects who have participated in research and indicated they would be willing to return for other studies will be contacted by phone or email to invite to participate. In his ongoing study, subjects who are excluded by Dr. Pietrosimone will be offered a flyer. If interested, the subject will be scheduled to return to the lab for screening and participation in this study**.**

Secondary recruitment methods will include searching the Carolina Data Warehouse for Health. Study personnel will identify potential patients using knee OA ICD-9/10 codes, and send an authorized recruitment letter to each participant. A recruitment method using EPIC, the healthcare software used at UNC Hospitals that provides past and current medical information, will also be employed. EPIC will also be used to identify potential participants from the broad list of result obtained from the Data Warehouse. Authorized study personnel, who have been EPIC and HIPAA trained will obtain medical record numbers that correspond to OA ICD-9 and 10 codes from Data Warehouse personnel. By reviewing medical records using EPIC, authorized study personnel will determine which participants have X-rays within the past 6-months and a confirmed OA diagnosis. The primary care doctor of prospective participants will be contacted for approval before a letter is sent to the potential subject. Potential participants found in this way will be sent an authorized recruitment letter which will indicate that the patient's primary care doctor was consulted regarding the patient's participation in this research project.

Emails, flyers, and recruitment letters will have the contact information for the study. Those who have indicated an interest in participating in knee arthritis research, either from the physicians' clinics, Dr. Pietrosimone's lab, or responding to these materials will be contacted for screening. Subjects will be contacted by the PI or the study coordinator by telephone or in person in the laboratory or clinic.

12 CONSENT PROCESS

All consenting interviews, exams, questionnaires, and study procedures will be done in a private room or area in a room adjacent to the laboratory. Dr. Givens, Dr. Pietrosimone, and all research assistants will obtain consent for participation.

Upon reporting to the laboratory, and prior to screening and data collection procedures, all subjects will be required to read and sign the approved informed consent document. Members of the research team will oversee this process to ensure any questions subjects have are answered prior to providing written informed consent.

Participants will be preliminarily screened either over the phone, in the clinic, or lab. Patients will be asked if they are interested in learning about the study. After telling them about the study we will ask if they are interested in coming to the laboratory (if on the phone or in the clinic) to be screened for inclusion into the study. If they say "yes", we will verbally ask if they would be willing to provide information about health history, medications, and knee disability. We will read the list of exclusion criteria and ask if there is anything on this list that could apply to him or her. The subject does not need to disclose the reason, only that there is at least one item that would be applicable. We will ask about medications to ensure they are not taking ones that should not be used with TMS and rTMS. Subjects will then provide ratings of pain and function using the Pain and Functional Subscales of the WOMAC.

We will explain to interested individuals that this process will decrease the chance of patients attending a lab screening session if they have a high likelihood of being excluded from the study. Following the preliminary screening, eligible participants will be invited to the Neuromuscular Research. The data from the phone screening will not be shared with anyone outside of the research team. We will explain to these patients that they will have to provide written consented before screening in the laboratory and eventual inclusion in the research pilot study.

Subjects are always reminded that their participation in a research study is voluntary and that declining will not in any way affect their health care at UNC. Similarly, UNC-CH employees will be informed that their participation or the lack thereof will not influence their employment status.

We will offer to answer any questions during the process. Subjects will be advised that they may withdraw from the study at any time.

13 PLANS FOR PUBLICATION

The results of the study will be submitted for publication in peer reviewed journals that are of interest to physical therapists such as the Journal of Orthopedic & Sports Physical Therapy.

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STATISTICAL ANALYSIS PLAN

Statistical Methods

Aim 1. To compare the neural (motor cortex and corticospinal tract) excitability (turning the muscle on) of the anterior thigh muscle (quadriceps) in the symptomatic knee to the asymptomatic knee in participants with knee arthritis. We will examine the associations between neural excitability and clinical measures of pain, pain sensitization, quadriceps strength, walking and balance function, and coping styles.

As a preliminary analysis step, all data will be cleaned and inspected for outliers, missing data and distributional irregularities. Although general linear and mixed linear modeling methods are generally robust to minor violations of distributional assumptions, appreciable departures from normality can compromise statistical tests and estimators. Where error distributions potentially deviate from normality or heteroscedasticity is suspected, or for outcomes expected to be non-normally distributed, departures from normality will be addressed using power transformations where appropriate and/or the use of alternative methods that do not assume normality of outcomes (e.g. nonparametric analyses. We will compare between limb differences and use correlation analyses to examine associations between measures of neural excitability, pain, pain sensitization, quadriceps function, timed-up-and-go test, and catastrophizing. If nonparametric statistical tests are indicated, we will use two-tailed Wilcoxon Signed Rank tests to examine differences between limbs and two-tailed Spearman Rank-Order correlations to examine associations between variables. The threshold for statistical significance is $p < .05$.

Aim 2. To determine the immediate (within day) effects of rTMS + exercise on the ability to activate the quadriceps muscle and clinical outcomes in people with knee arthritis. The primary outcome variables are quadriceps strength (maximum isometric voluntary contraction) and quadriceps voluntary activation percent (calculated from strength and a brief intense electrical stimulation). Secondary outcome variables are measures of neural excitability from the contralateral motor cortex and hemispheres, pain, pain sensitization, and function (based on the Timed-Up-and-Go test)

We will use a repeated measures analysis of variance (ANOVA), conducted under a mixed modeling framework, to compare outcome variables pre- and post-intervention. Because of its superior statistical properties with small sample sizes, restricted maximum likelihood with Kenward-Roger standard errors will be used in these analyses. The threshold for statistical significance is $p < .05$.

1.1 Sample Size and Power

The sample size for this experiment was explored for the primary variables of interest. These variables are thigh muscle strength from a maximal voluntary isometric contraction (MVIC) and percent voluntary activation. We based the estimates from our preliminary data and available data in the literature (Gibbons, 2010). For muscle strength, the estimated effect size was 0.84

(95% CI: -0.11, 1.7). For central activation ratio, the estimated effect size was 0.84 (95% CI: -0.13, 1.7).

Based on a calculated effect size of 0.84, an α of 0.05 and a power (1-β) of 0.8, we estimated that 11 subjects are needed. Our measurements may have larger variances than previous studies that used single-pulsed TMS to investigate changes in thigh muscle function. Therefore, we inflated our sample to 20 subjects.

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