ORGANIZATION OF DETAILED PROTOCOL

Title: Effects of Transcranial Direct Current Stimulation (tDCS) and Transcranial Ultrasound (TUS) on the perception of pain and functional limitations due to Osteoarthritis of the Knee

Protocol 2016P000486

Date: 03/29/2019

I. BACKGROUND AND SIGNIFICANCE

The work to be completed and proposed herein is to be sponsored by the NIH National Center for Complementary and Integrative Health (NCCIH) to evaluate the use of transcranial direct current stimulation (tDCS) applied in conjunction with transcranial ultrasound (TUS) as a treatment for chronic pain due to Osteoarthritis (OA) of the knee. The work we present here is the second part of a 2-Phase plan [redacted]

Osteoarthritis (OA) of the knee is a leading cause of chronic pain and disability [1]. OA affects 13.9% of adults (aged 25yr +) and 33.6% of the elderly (65yr+), with the knee most commonly affected [1]. It is estimated that the total cost of OA is up to 2.5% of the US GDP [2], and the burden of OA has only been growing (due to aging and obesity) [1]. Conventional management of OA knee pain is based on a combination of pharmacological and non-pharmacological therapies including oral non-steroidal anti-inflammatory drugs, intra-articular injections, physical therapy, and opioid analgesics [3, 4]. Although these options can show some benefits, they are often associated with significant adverse effects [5, 6] and/or treatment benefits which decrease over time (due to therapy tolerance, disease progression, and/or

Functional and structural imaging studies support the notion that pain in OA is associated with increased activation of pain-related neural networks and that OA pain is correlated with thalamic gray matter volume decreases that can be reversed with decreased pain and increased function [9-17]. Recent evidence demonstrates the correlation between the experience of pain and brain states and recent studies have demonstrated a specific neural signature associated with chronic pain states, as can be indexed by EEG recording, noninvasive brain stimulation (NIBS) based assessments of cortical excitability, and imaging studies [12, 14, 18-26]. These assessments demonstrate specific subcortical-cortical changes in excitability and plasticity that are becoming useful in the development of novel therapies.

We hypothesize cumulative noninvasive brain stimulation sessions can induce neuroplasticity in brain circuits affected by OA and revert some of the maladaptive compensatory plasticity of the disease [27, 28]. Invasive and noninvasive brain stimulation methods [6, 7, 12, 14, 15, 18, 29] have been shown to be effective in treating chronic pain in some disease states, where it is thought that treatment induced changes in brain activity revert maladaptive plasticity associated with chronic pain sensation [12, 14, 30].

One candidate tool to modulate brain activity and to assess brain activity is transcranial direct current stimulation (tDCS) [39, 40]. Several well-conducted animal studies on the effects of tDCS have been performed in the 1950s and 60s [41-43]. These studies showed that tDCS is a powerful technique to modulate brain function. tDCS has shown some positive effects on chronic pain, most effectively via stimulation of the primary motor cortex (M1) which can lead to significant offline clinical improvements in OA pain based on long-lasting neuroplastic modifications [44-46] (i.e., stimulation effects can outlast the period of stimulation and lead to significant results from days to months following stimulation). However [47], tDCS often elicits suboptimal responses in magnitude and duration of clinical effect. It has been postulated that the inconsistent, suboptimal clinical effects result from the limited focality, penetration, and targeting control of tDCS, highlighting the need for improved noninvasive techniques for OA treatments [40, 48-51].

By combining tDCS with transcranial ultrasound (TUS) it is hoped that some or all of these limitations can be overcome. Transcranial direct current stimulation (tDCS) and transcranial ultrasound (TUS) (e.g., transcranial Doppler ultrasound (TDUS)) are powerful tools to modulate brain excitability and/or to assess brain anatomy (and hemodynamics). Transcranial Ultrasound (TUS) has been used clinically for over 20 years [52, 53] with long established safety guidelines [54], has been used for assessing anatomy and monitoring cerebral hemodynamics; and has been safely used in conjunction with neuromodulation methods like tDCS and transcranial magnetic stimulation (TMS) [55-57] [58-61]. TUS can aid in brain targeting.

In summary, there is a great-unmet need for non-invasive treatments for chronic knee pain due to OA. In this proposal, we will test whether tDCS applied in conjunction with TUS is effective in treating chronic knee pain from OA.

Sponsors

This study is to be funded by the National Institutes of Health (Highland Instruments SBIR). Highland Instruments is a company focused on developing neurostimulation technologies for treatment of neural pathologies.

II. SPECIFIC AIMS

In this study, we aim to assess the effects of tDCS in combination with TUS for the treatment of pain and functional limitations in subjects with OA of the knee. We will explore *Aim 1 Sub-aims 1.1- 1.4* with a parallel design (active tDCS+ active TUS (n=32), and sham tDCS+sham TUS (n=32)). The specific aims of this experiment are as follows:

Aim 1: **Assess the preliminary therapeutic effects of tDCS in conjunction with TUS in chronic OA knee pain.**

Sub Aim 1.1: *Safety of tDCS+TUS in subjects with OA of the knee.*

We hypothesize that tDCS in conjunction with TUS can be applied safely in OA subjects. To test this we will implement a comprehensive battery of safety and neurological tests in OA subjects following fixed doses of tDCS in conjunction with TUS provided over [redacted] for 10 days, 20 minutes/day. These assessments include EEG recordings, Visual Analog Mood Scale (VAMS), Mini Mental Status Exam (MMSE), 4-Choice Reaction Time, adverse events (AE) questionnaire and Neurological and physical examinations. We will compare active tDCS+ active TUS (n=32), and sham tDCS+sham TUS (n=32), inactive transducers. Follow-up evaluations will continue, while no further treatment is given, for 8 weeks (at 1, 2, 4, and 8 weeks) after the last stimulation.

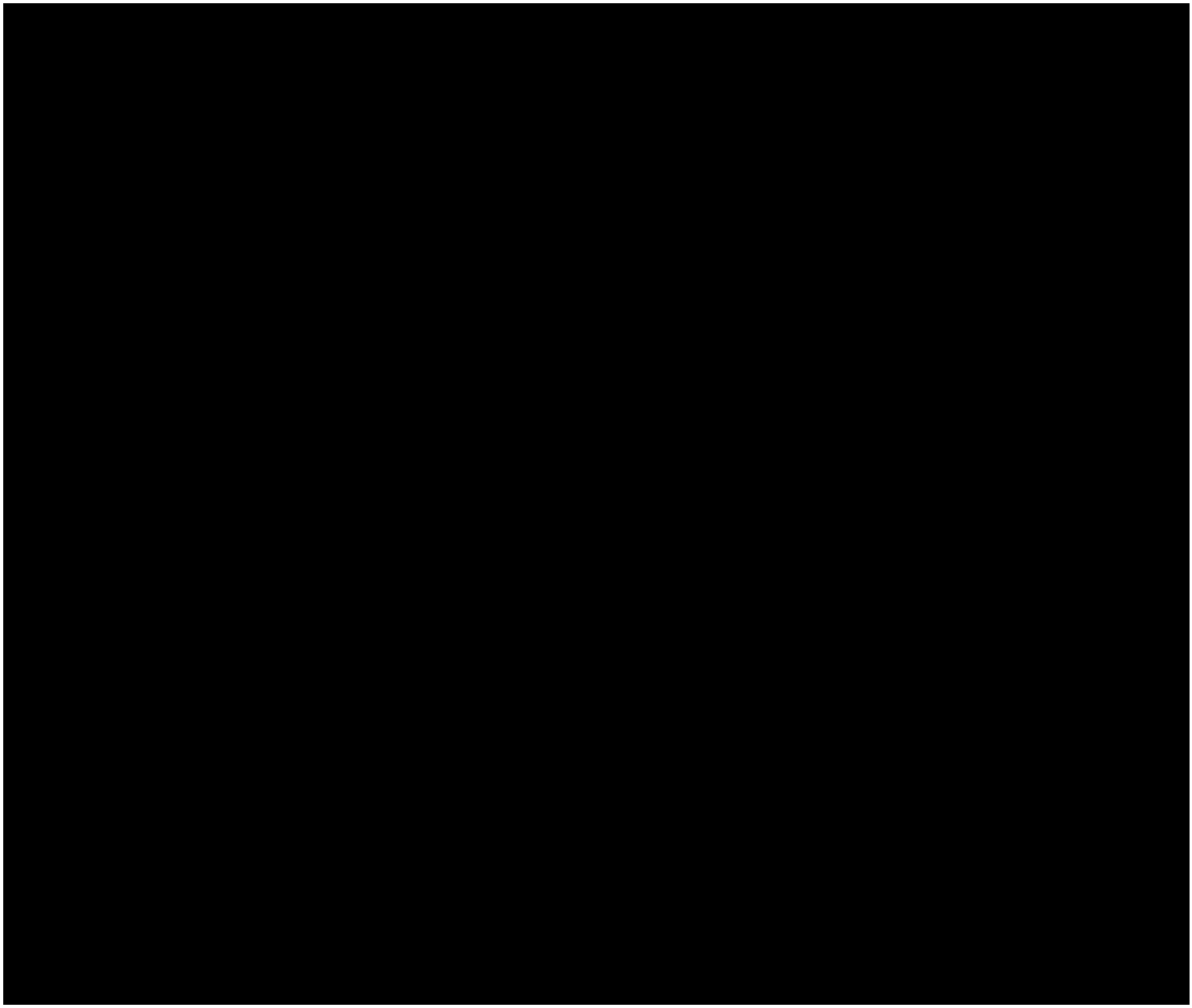
Sub Aim 1.2 *Effects of tDCS+TUS in pain perception in subjects with OA of the knee.*

We hypothesize that noninvasive tDCS in conjunction with TUS can be effective in decreasing the perception of pain in subjects with OA of the knee. To test this, we will use sensitive, currently validated outcomes for chronic pain subjects. A rater blind to the treatment arm will administer the following tests: Visual Analog Score (VAS) for Pain, Pain/Medication Diary, Von Frey assessment, and pain pressure tests (PPT) with algometer following stimulation detailed above, in SubAim 1.1. We will compare the stimulation conditions as above. Follow-up evaluations will continue, while no further treatment is given, for 8 weeks (at 1, 2, 4, and 8 weeks) after the last stimulation.

Sub Aim 1.3 *Effects of tDCS+TUS in biomechanical function in subjects with OA of the knee.*

We hypothesize that tDCS in conjunction with TUS can be effective in improving the biomechanical function in subjects with OA of the knee. To test this, we will compare the impact of active tDCS in conjunction with active TUS to the other stimulation conditions, as above, on markers of biomechanical function. We will use a biomechanical data acquisition and analysis system to specifically assess subject's knee biomechanical functionality and response to the intervention. For this purpose we will use a set of integrated sensors including accelerometers, gyroscopes, force sensors, and motion-capture cameras. Follow-up evaluations will continue, while no further treatment is given, for 8 weeks (at 1, 2, 4, and 8 weeks) after the last stimulation.

Sub Aim 1.4. *Effects of tDCS+TUS in the quality of life in subjects with OA of the knee*.
We hypothesize that tDCS in conjunction with TUS can be effective in improving the quality of life (QOL) in subjects with OA of the knee. To examine this aim, we will implement the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), as this is recommended as one of the most sensitive and specific instruments for quality of life assessment in OA [68] and physical functioning assessment including a wide range of daily activities, such as bathing and getting in or out of the car. We will also use a generic health status measure - the Medical Outcomes Study 36-Item Short Form (SF-36) [68]. We will compare the stimulation conditions as above. Follow-up evaluations will continue, while no further treatment is given, for 8 weeks (at 1, 2, 4, and 8 weeks) after the last stimulation.



[redacted]

IV. SUBJECT SELECTION

We will recruit up to 94 subjects for this study, with the intention of randomizing exactly 64 subjects with OA of the knee for this study. Once we randomize 64 subjects, we will stop recruiting subjects. Subjects will need to meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria:

1. Able to provide informed consent to participate in the study.
2. Subjects between 18-85 years old.
3. Diagnosis of chronic osteoarthritis with pain of either knee as self-reported. *Prior to beginning baseline procedures this must be confirmed by medical records or a letter from the subject's physician, or diagnosis by a study staff licensed physician. This document may either be brought in by the subject or retrieved by study staff after the subject signs a medical release form. If the subject is unable to obtain the medical record, a diagnosis by a study staff licensed physician will be accepted. After consent, if the subject fails to provide the necessary documentation, or if it cannot be retrieved by study staff, the subject will be considered a screen fail.*
4. Existing knee pain of at least 3 on a 0-10 VAS scale on average over the past 6 months.
5. Pain of at least 3 on a 0-10 VAS scale on average over the week prior to the first stimulation session.
6. Pain resistant to common analgesics and medications for chronic pain used as initial pain management such as Tylenol, Aspirin, Ibuprofen, Soma, Parafon Forte DCS, Zanaflex, and Codeine.
7. Having the ability to feel pain as self-reported.

Exclusion Criteria:

1. Pregnancy or trying to become pregnant in the next 6 months.
2. History of alcohol or drug abuse within the past 6 months as self-reported.
3. Contraindications to transcranial brain stimulation or TUS, e.g. implanted brain medical devices or implanted brain metallic devices.
4. Unstable medical conditions (e.g. uncontrolled diabetes, uncompensated cardiac issues, heart failure or chronic obstructive pulmonary disease).
5. Epilepsy.
6. Use of carbamazepine within the past 6 months as self-reported.
7. Suffering from severe depression (with a score of >30 in the Beck Depression Inventory).
8. History of unexplained fainting spells as self-reported.

9. Head injury resulting in more than a momentary loss of consciousness.
10. History of neurosurgery as self-reported.

V. SUBJECT ENROLLMENT

A total of 64 subjects, from 18 to 85 years of age, with a diagnosis of Osteoarthritis and pain of the knee will be randomized in this study. The safety of tDCS in pregnant populations (and children) has not been assessed, and therefore pregnant women (and children) will be excluded. Women of childbearing potential will be required to take a urine pregnancy test during the screening process.

Potential subjects will be identified by the following sources:

1. Attending physicians or therapists may refer their patients to the study. We will provide the physicians, therapists and clinics with study information sheets and flyers. Prospective subjects will be encouraged to contact the study co-investigators.
2. Flyers posted in public areas across the Boston-land region, in the outpatient specialist clinics, public posting boards (i.e., digital signage) and other private locations with given permission.
3. Internet, email and newspaper advertisements.
4. Advertising posted in public transportation (e.g., the subway-"The T").
5. Via the Partners Healthcare Research Patient Data Registry (RPDR).
6. Possible subjects might also be identified though their medical records (Meditech, LMR, etc.) and their physicians might be asked to inform the subjects about the study.
7. Presentations to outside sites (such as support groups, main rehabilitation centers from Spaulding and Partners Network, and others important institutions of the Massachusetts area, nursing/assisted living centers etc.)
8. Attending public forums, conferences, or events (such as walk-a-thons and association events) at which the co-investigator will distribute IRB approved recruitment materials.

We anticipate subjects will be primarily recruited through the Spaulding Rehabilitation Hospital/Network (one of the largest rehabilitation centers in the US). SRH provides outpatient services to the Boston area so we anticipate our recruitment procedures will reflect the distribution of races/ethnicities in the surrounding communities. In addition, we will approach colleagues at the other Harvard teaching hospitals, including Brigham and Women's Hospital (BWH), Massachusetts General Hospital (MGH) and outpatient clinics in the greater Boston area. Furthermore, we plan to advertise this clinical trial in the greater Boston area in a variety of media including on the Internet, via patient registries that are designed to promote research, and in local newspapers. We will also approach senior centers, assisted living facilities and support groups.

Eligible subjects will contact, or give permission to be contacted, by a co-investigator to obtain more information about the study. At the first point of contact (usually a phone call), study co-investigator will administer a phone-screening questionnaire. Once the phone screening process is complete, the information gathered by the co-investigator will be taken to the PI of the study for

further review to confirm eligibility. Data obtained from screening will be stored in a locked filing cabinet in the lab.

Informed consent will be obtained by the study PI and/or a co-investigator (not involved in the patient's care) at Spaulding Rehabilitation Network Research Institute (SRN-RI). The test procedures will be described and the testing equipment will be shown to the subject. Study co-investigators will clearly explain all the procedures and risks of the testing outlined in the consent form. The subject will be given the time needed to consider their decision and will be encouraged to ask questions, both during the initial phone interview and throughout the study. The PI or a co-investigator will answer any questions regarding the study at the time consent is given. Once enrolled, the subject may pause or terminate his/her participation at any time during the study.

In the case of Spanish-speakers, we will have a Certified Medical Interpreter present and have a translated copy of the informed consent form. The consenting process will be identical to the our standard consent process, expect there will be a Certified Medical Interpreter present to translate any information or questions made by the researcher or subject concerning the consent form or the research study. The subject will sign a copy of a translated consent form (translated by a Certified Medical Translator). All other documents Spanish-speakers receive will be translated into their language by a Certified Medical Translator, or validated Spanish versions of the assessments. In this protocol, we also have native Spanish-speaking co-investigators who can give instructions and screen participants in Spanish as necessary.

VI. STUDY PROCEDURES

We will recruit up to 94 subjects for this study, with the intention of randomizing exactly 64 subjects with OA of the knee for this study. Once we randomize 64 subjects, we will stop recruiting subjects. They will be randomly assigned to receive treatment with active tDCS+active TUS (n=32), or sham tDCS+sham TUS (n=32, inactive transducers), [redacted] Study procedures will be either done at Spaulding Rehabilitation Hospital or Spaulding Rehabilitation network (Neuromodulation Center).

Study Outline

Pre-screening Procedures:

During the pre-screening process, the subject will contact a co-investigator usually via a phone call. During this call, the co-investigator will discuss in greater depth the details of the study, explain the study procedures and encourage the subject to ask questions. In the privacy of the laboratory, the co-investigator will ask the subject questions from the following:

1) Phone screening questionnaire

Once this information is collected, the co-investigator will consult with the PI regarding the eligibility of the subject, who will then give approval for the subject to come to our laboratory for the screening procedure.

Visit 1
Screening Visit – (Approx Time: 45 mins)

Screening Procedures:
At Screening, the PI and/or a co-investigator will once more conduct a review of inclusion/exclusion criteria to determine the subject's eligibility for enrollment. Study procedures will be reviewed with the subject, and documentation of informed consent will be obtained.

At Screening the following procedures will be completed:

- Discuss study-specific procedures with the subject.
- Review inclusion and exclusion criteria.
- Obtain a signed and dated consent form.
- Conduct a Demographics Survey, Brief Medical History and the Beck Depression Inventory.
- Urine pregnancy exam (if applicable).

Confirmation of Osteoarthritis
Prior to the screening visit the subject will be asked if they can bring a document from their physician confirming the diagnosis of osteoarthritis of the knee. If the subject does not have it upon consenting, the subject will be asked to sign a medical release form allowing us to retrieve this document. If the subject is unable to obtain the medical record, a diagnosis by a study staff licensed physician will be accepted. This requirement has been outlined in the consent form. The baseline visit will only occur once confirmation of this diagnosis has been received.

Visit 2
Baseline Visit – (Approx Time: 2 hours)
This visit might be completed on the same day as the screening visit if time allows and the subject agrees.

Baseline assessments:

- Single Leg Standing Balance Test
- Step Test
- Functional Reach Test
- Timed Up and Go Test
- Knee Proprioceptive Test
- Knee Extensor/Flexor Force Test
- Knee Range of Motion (ROM)
- Visual Analogue Scale (VAS) for Pain

- Pain/Medication Diary
- Von-Frey Assessment
- Pain Pressure Test (PPT) with algometer
- Descending Noxious Inhibitory Control (DNIC)
- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
- Medical Outcomes Study 36-Item Short Form (SF-36)
- Visual Analog Mood Scale (VAMS)
- EEG recording
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Neurological and Physical Exam

Visit 3

Re-screening procedure-(Approx Time: 5 minutes)

Prior to performing any assessments for visit 3, the subject will be re-screened to determine if they comply with the following inclusion and exclusion criteria: "*Pain of at least 3 on a 0-10 VAS scale on average over the week prior to the first stimulation session"*. If the subject does not report a VAS of at least 3 on a 0-10 VAS scale on average over the week prior to the stimulation session they will be screened-out of the trial and provided with the reason. If the subject does report a VAS of at least 3 on a 0-10 VAS scale on average over the week prior to the stimulation session, then they will continue with the remainder of the study visits.

1st Stimulation Visit – (Approx Time: 3.0 hours)
This study will be scheduled ~1 week after the baseline measurements were done.

There will be 10 stimulation visits to be completed over 2 consecutive weeks (ideally Monday to Friday of each week). The subject will receive either 20 minutes of active tDCS in conjunction with active TUS, or sham tDCS in conjunction with sham TUS per day for the course of their stimulation visits.

Randomization:
Once eligibility and consent have been approved and completed, after all baseline evaluations and immediately before the first stimulation session, stratified blocked randomization will occur using the randomized list generated by an automatic web-based randomization program (www.randomization.com). A staff member who is not involved in any of the study procedures will be responsible for creating the randomization list and monitoring it during the study. Randomization will be stratified by baseline pain, using the median of Phase I as a cutoff (VAS of 7). Subjects will be randomly assigned to treatment or placebo groups in a 1:1 allocation ratio. We will use permuted blocks of variable sizes (4, 6, and 8) in a random manner to minimize the risk of study staff guessing at the next allocation. Randomization order will be kept in sealed envelopes.

Blinding:

All subjects and investigators will be blinded to the group assignment except for the co-investigators providing stimulation and staff involved in the randomization process (the latter would otherwise not be involved in study procedures). Only the staff involved in the randomization will be authorized to break the blind if there is a clinical need as requested by the PI, clinical care providers, Medical Monitor, or IMC. The patients will remain blinded until they have completed all visits (whether they complete all 16 visits or drop out early), or in the case of an adverse event necessitating un-blinding.

If the subject receives a sham stimulation, he/she may re-enroll into an open label portion of the study, where he/she will receive 10 days of active tDCS/TUS. Un-blinded co-investigators (e.g., who provided stimulation) will coordinate with subjects electing to enter into the open label phase, which will be conducted by the un-blinded study staff. The open-label study will be conducted without informing the blinded assessors of the patient enrollment. In the open label phase, we will complete the VAS assessments on Day 5 and Day 10 of stimulation. This collected data will not be analyzed with the main data collected in the study. This data will be used for exploratory analyses only.

Before stimulation, the subject will complete a series of assessments:

- Single Leg Standing Balance Test
- Step Test
- Functional Reach Test
- Timed Up and Go Test
- Knee Proprioceptive Test
- Knee Extensor/Flexor Force Test
- Knee Range of Motion (ROM)
- Visual Analog Scale (VAS) for Pain
- Pain/Medication Diary will be given to the subject
- Von-Frey Assessment
- Pain Pressure Test (PPT) with algometer
- Descending Noxious Inhibitory Control (DNIC)
- Visual Analog Mood Scale (VAMS)
- Neurological and Physical Exam

After stimulation subjects will complete the following assessments:

- Visual Analog Scale (VAS) for Pain
- Von-Frey Assessment
- Pain Pressure Test (PPT) with algometer
- Visual Analog Mood Scale (VAMS)
- Descending Noxious Inhibitory Control (DNIC)
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Adverse Events Questionnaire (AEs)

- EEG recording

Visits 4 – 6
Stimulation visits – (Approx Time: ¾ hour)

During the next 3 stimulation sessions – the subject will receive either 20 minutes of active tDCS in conjunction with active TUS, or sham tDCS in conjunction with sham TUS.
Pre-stimulation: immediately before stimulation, subjects will be asked to complete the following:

- Visual Analog Scale (VAS) for pain

After stimulation, the subject will complete a series of assessments:

- Visual Analog Scale (VAS) for Pain
- Visual Analog Mood Scale (VAMS)
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Adverse Events Questionnaire (AEs)

Visit 7
5th Day of stimulation visits – (Approx Time: 2 ¼ hours)

Pre-stimulation: immediately before stimulation, subjects will be asked to complete the following:

- Visual Analog Scale (VAS) for pain

After stimulation subjects will complete the following assessments:

- Single Leg Standing Balance Test
- Step Test
- Functional Reach Test
- Timed Up and Go Test
- Knee Proprioceptive Test
- Knee Extensor/Flexor Force Test
- Knee Range of Motion (ROM)
- Visual Analog Scale (VAS) for Pain
- Pain/Medication Diary
- Von-Frey Assessment
- Pain Pressure Test (PPT) with algometer
- Descending Noxious Inhibitory Control (DNIC)
- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
- Visual Analog Mood Scale (VAMS)
- EEG recording
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Adverse Events Questionnaire (AEs)

- Neurological and Physical Exam

Visits 8 – 11
6-9th Day of Stimulation visits – (Approx Time: ¾ hour)

Pre-stimulation: immediately before stimulation, subjects will be asked to complete the following:
- Visual Analog Scale (VAS) for pain

After stimulation, the subject will complete a series of assessments:
- Visual Analog Scale (VAS) for Pain
- Visual Analog Mood Scale (VAMS)
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Adverse Events Questionnaire (AEs)

Visit 12
10th Day of stimulation visits – (Approx Time: 2 ¼ hours)

Pre-stimulation: immediately before stimulation, subjects will be asked to complete the following:
- Visual Analog Scale (VAS) for pain

After stimulation subjects will complete the following assessments:

- Single Leg Standing Balance Test
- Step Test
- Functional Reach Test
- Timed Up and Go Test
- Knee Proprioceptive Test
- Knee Extensor/Flexor Force Test
- Knee Range of Motion (ROM)
- Visual Analog Scale (VAS) for Pain
- Pain/Medication Diary
- Von-Frey Assessment
- Pain Pressure Test (PPT) with algometer
- Descending Noxious Inhibitory Control (DNIC)
- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
- Medical Outcomes Study 36-Item Short Form (SF-36)
- Visual Analog Mood Scale (VAMS)
- EEG recording
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Adverse Events Questionnaire (AEs)

- Neurological and Physical Exam

Visit 13, 14, 15, and 16

Follow-Up Visit – 1 week, 2 weeks, 4 weeks, and 8 weeks post-stimulation – (Approx Time: 2 hours)

These visits will be scheduled ~1, 2, 4, and 8 weeks after the last stimulation session and the following assessments will be performed:

- Single Leg Standing Balance Test
- Step Test
- Functional Reach Test
- Timed Up and Go Test
- Knee Proprioceptive Test
- Knee Extensor/Flexor Force Test
- Knee Range of Motion (ROM)
- Visual Analog Scale (VAS) for Pain
- Pain/Medication Diary
- Von-Frey Assessment
- Pain Pressure Test (PPT) with algometer
- Descending Noxious Inhibitory Control (DNIC)
- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
- Medical Outcomes Study 36-Item Short Form (SF-36)
- Visual Analog Mood Scale (VAMS)
- EEG recording
- Mini Mental Status Exam (MMSE)
- 4-Choice Reaction Time
- Adverse Events Questionnaire (AEs)
- Neurological and Physical Exam

Stimulation protocol: Subjects will be randomized to undergo one of two different treatment conditions: 10 sessions of sham tDCS/TUS, or 10 sessions of active anodal tDCS/TUS (32 subjects per study arm).

An FDA approved assist device (Highland Instruments, Cambridge, MA) that can assist the stimulation provider in the synchronous application of TUS

and cranial electrical stimulation devices will be available if required (e.g., for operator fatigue the device can assist the operator in holding the TUS probe relative to the tDCS electrodes).

For sham tDCS/TUS, we will follow the exact procedure as above but use an inactive ultrasound transducer (i.e., the device will remain off during stimulation). For the sham condition, we will follow the exact procedure as above, however tDCS current will be applied for less than 30 seconds so that the sham subjects experience the same sensation of the device turning on as active subjects (the 'turning off' of the device is not perceived due to sensitization). It should be noted that less than 3 minutes of tDCS induces no lasting effects on cortical excitability [39]) and also using 30 seconds of sham is a reliable method of blinding, as shown by a randomized controlled study [69]. [redacted] Furthermore during the sham, the TUS will not be active during stimulation.

The schedule for the subjects can be seen in table 1, which illustrates the procedures that will be performed at each visit:

[redacted]

[redacted]

DESCRIPTION OF ASSESSMENTS:
A rater blind to the treatment arm will administer the following tests (also used in our previous study [70]:

Visual Analogue Scale (VAS) for Pain: The VAS is a common assessment used which asks subjects to self-reportedly measure their pain on a visual scale (i.e., unbearable to none). This will help us to monitor changes in subjects' pain levels when they come in for sessions.

Pain/Medication Diary: To help monitor pain levels, as well as safety, subjects will keep a diary listing their daily medications and pain levels when not at the laboratory.

Mechanical Detection Threshold (von-Frey MDT): This technique is adapted to evaluate light touch in small cutaneous regions [71]. The most used procedure is with the Von Frey monofilament (0.008g to 300g) via the method of limits. The examiner makes use of an ascending standardized sequence of varying stimulus intensities. In the case of ramping the stimulus up, the individual is asked to report when the sensation is first detected

Single applications of standardized monofilaments will be applied first to a reference region (thenar region, ipsilateral to the most painful knee), using the smallest weighted monofilament, followed by sequentially larger monofilaments until sensation is reported [71]. Following the reference region, the most painful knee will be tested. The monofilaments must be applied perpendicular to the skin and bow for a period of approximately 1.5 seconds [71, 72]. The threshold will be taken as the lowest force that causes a light touch sensation

Mechanical Pain Thresholds (von-Frey MPT): The evaluation of mechanical pain thresholds is performed using standardized, fine, blunt-tipped probes. The threshold to produce pain is recorded in this test. The force applied by the Von Frey monofilament is standardized by applying just the right amount of force to bow the monofilament for approximately 1.5 seconds [71, 72]. The subjects will be asked to say when they sense pain. The thenar region, ipsilateral to the most painful knee will be tested. Following the reference region, the most painful knee will be tested. The smallest monofilament that produces pain will be recorded.

Fixed Intensity hyperalgesia evaluation (von-Frey hyperalgesia): To study hyperalgesia the previously registered smallest monofilament to produce pain in the thenar region will be used [71]. A standard stimulus of fixed intensity will be applied over the thenar region, ipsilateral to the most painful knee, and the subject will be asked to rate the sensation of pain using a visual analogue scale for pain. The process will be done 3 times and the geometric mean of the visual analogous scale will be calculated. Following the procedure, the most painful knee will be studied and the subject will be asked to rate the sensation of pain using a visual analogous scale for pain. The process will be done 3 times and the geometric mean of the visual analogous scale will be calculated.

Pain pressure tests (PPT): PPT will be determined using blunt pressure delivered by a 1-cm2 hard-rubber probe using a FDA approved device (commander algometer – JTECH medical). During testing a series of discrete pressures are applied to a reference area, the thenar area ipsilateral to the most painful knee. Following the reference region testing, the most painful knee will be tested. The subject will let the investigator know when he/she feels any pain, and at that time the procedure will be stopped, and the value will be recorded. This procedure will be repeated 3 times. The test will take approximately 7-10 min to complete.

Descending Noxious Inhibitory Control (DNIC): Endogenous pain modulation is commonly evaluated in the laboratory using DNIC testing paradigms. These procedures incorporate a conditioning stimulus (a noxious stimulus that evokes DNIC activation) and a test stimulus (a noxious stimulus used to evaluate the analgesic response to the conditioning stimulus - this will be the PPT). This study will evaluate DNIC in pain patients using pressure as the test stimulus, and cold water as the conditioning stimulus. Pressure will be delivered using the same device as for the evaluation of PPT. DNIC will be induced approximately 1-min later by having subjects immerse their hand into a water bath maintained at 10-12˚C for approximately 1 min. Parallel to the last 30s of DNIC conditioning (cold water immersion), the same test stimulus will be reapplied (PPT procedure). DNIC will be evaluated as the mean difference in pain rating of the test stimulus applied before and during the conditioning stimulus. This value would typically be treated as a continuous variable if measured repeatedly in a clinical trial because it is unlikely that descending analgesic systems are either “on” or “off”, but rather function within a range of magnitudes across subjects.

EEG recording: One week prior to stimulation, on the first day of stimulation, at the end of each stimulation week, and during each follow up visit, EEG recordings will be taken to monitor brain activity and saved for post recording evaluation.

Neurological and Physical Exam: Trained study staff will conduct baseline and follow-up neurological examinations focused to ensure subject safety one week prior to stimulation, on the first day of stimulation, at the end of each stimulation week, and during each follow up visit [73].

Visual Analog Mood Scale (VAMS): This is a self-assessment scale in which subjects rate their own emotions, including anxiety, depression, stress, and sleepiness along a 100 mm line.

Mini Mental Status Exam (MMSE): This is a sensitive, valid and reliable 30-point questionnaire that is used extensively in clinical and research settings to measure cognitive impairment. This instrument will be used as a brief screening of cognitive abilities. The MMSE will be administered at the baseline evaluation and every subsequent visit.

4-Choice Reaction Time: Patients will be seated in front of a computer screen placed at eye level. One of four possible circles, each 4 cm in diameter, will be presented horizontally spaced on the screen and aligned above the appropriate response keys. The stimuli will be generated and response times recorded using Superlab pro v2.0 software (Cedrus Corporation, San Pedro, Ca). The patients have to push the key aligned with the circle that appeared using only the index finger of the dominant hand. The circle will not disappear until the correct button is pushed. The time between the appearance of the circle and the push of the correct response key will be defined as the choice reaction time. The 4-choice reaction time will be administered at the baseline evaluation and every subsequent visit.

Adverse Effects Questionnaire: At each session after stimulation begins, subjects will complete a questionnaire to evaluate potential adverse effects of stimulation (headache, neck pain, mood alterations, and seizures) on a 5-point scale. The scale will also be administered at the follow-up. The subjects will be asked whether they have experienced any side effects in an open-ended manner and they will then be specifically asked about headache, neck pain, scalp pain, scalp burns, tingling, skin redness, sleepiness, trouble concentrating, and acute mood change. If any side effects are reported, the degree of relatedness to the intervention will be assessed.

We will be investigating adverse effects using open-ended questions. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a treatment, whether or not considered related to the product.

Examples of AEs are as follows:

- Subjective symptoms offered by or elicited from the subject
- Objective signs observed by the Investigator or other study personnel

All concurrent diseases that occur after the start of the study, including any change in severity or frequency of preexisting disease will be investigated in several domains including: seriousness, severity, length of duration, and if any causal relationship exists with the intervention [30].

Single Leg Standing Balance Test: We will record the time for which a subject is able to stand unsupported on one foot while looking straight ahead with hands on hips [74]. During this test, the

subject will be standing on a force platform. We will also use an accelerometer/gyroscope sensors worn by the subject (e.g., on the subjects' ankle, above the most painful knee (thigh and shank), and/or on the back (~level L5)) and a Motion Capture system to record subject kinematics during the movements described above [75, 76] [77, 78]. No video images will be saved during the experiment, only positional coordinates of the subjects' body parts that are extracted in real-time from the video and/or sensor will be saved. Subjects will be informed regarding video equipment and informed consent will be obtained.

Step Test: Subject will be asked to stand unsupported with the feet parallel to each other and a block 5 cm directly in front of them. Subject will then be advised which leg is the stepping leg and asked to place the whole foot onto the block, then return it fully down to the floor. This procedure will be repeated as fast as possible. One completed step will comprise placing the foot fully onto the block and then on the floor [79]. During this test, the subject will be standing on a force platform. We will also use motion analysis system (e.g., motion capture camera, accelerometers/gyroscopes, force platform) to record subject kinematics during the movements.

Functional Reach Test: Subject will be instructed to stand next to, but not touch the wall, and position the arm that is closer to the wall at 90 degrees of shoulder flexion with a closed fist. The subject will be asked to reach forward as far as they can without taking a step, keeping their hands in a fist shape. Hand position will be recorded. The difference between the start and end position will be the reach distance [74]. During this test, the subject will be standing on a force platform. We will also use motion analysis system (e.g., motion capture camera, accelerometers/gyroscopes, force platform) to record subject kinematics during the movements.

Timed Up and Go Test: We will measure the time that the subject takes to rise from a standard arm chair, walk to a tape mark 3-meter away, turn around, walk back to the chair, and sit down [74]. Quantitative, objective metrics of gait function will include stride length and gait speed [75] [80, 81]. We will also use motion analysis system (e.g., motion capture camera, accelerometers/gyroscopes, force platform) to record subject kinematics during the movements.

Knee Proprioceptive Test: Proprioceptive acuity will be assessed by the ability to reproduce self positioning of the leg with eyes closed [74]. The subjects will be seated on a chair, with hips and knees at 90°; the full extension of the leg will represent 180°. The subject's leg will be passively moved and the angle of flexion will be measured at a randomly selected position with a goniometer that will be attached to the subject's leg, this should take approximately 5 seconds. Then the subject's knee will be returned to its original position by the examiner. The subject will then be asked to reproduce the criterion angle and to maintain it for approximately 5 seconds; this angle will also be measured with a goniometer. The difference between the criterion and reproduced angle will be taken as the measure of proprioception acuity. The procedure will be done 3 times. The average of the three procedures will be taken as the proprioceptive acuity for the knee [79].

Knee Extensor/Flexor Force Test: The subject will be seated on a chair with hips and knees flexed to 90 degrees and the maximal voluntary hip flexion and lower leg flexo-extension will be individually measured with a JTech Commander Manual Muscle Testing MMT Device (JTECH medical) on both legs. We will integrate the recorded data with that recorded via the motion

analysis system (e.g., motion capture camera, accelerometers/gyroscopes, force platform) to assess subject kinematics during the movements from the other tests (see above).

Knee Range of Motion (ROM): The knee range of motion (flexion/extension angle) will be measured with a goniometer. [82] The examiner will flex the subject's knee, measuring the maximum knee flexion obtained. The end of the range of knee flexion occurs when resistance is felt and attempt to overcome the resistance cause additional hip flexion. This procedure is validated and standardized as described by Norkin and White [83].

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC): The WOMAC is a self-reported questionnaire that measures three main components of the impact of OA on quality of life: pain (0-20 score), stiffness (0-8 score) and functional limitation (0-68 score). Physical functioning assessments include a wide range of daily activities, such as bathing and getting in or out of the car.
The Medical Outcomes Study 36-Item Short Form (SF-36): It measures 8-health related concepts using 36 questions and provides a profile of functional health and well-being scores. It also provides a psychometrical index of physical and mental health (note, as stated earlier in this and the subsequent phase of the research the administration of all exams will be completed by a rater blinded to the treatment arm).

VII. BIOSTATISTICAL ANALYSIS

Data forms and questionnaires will be coded in a standardized manner, and double-entered into our database. Digital measures/recordings will be similarly tracked in our database and regularly backed up. Analyses will be conducted using standard statistical software such as SAS and Matlab. We will use REDCAP as a database (http://projectredcap.org/), and personnel entering the data will be blinded.

Sample size calculations and data analysis:

Aim 1: Data forms and questionnaires will be coded in a standardized manner, and double-entered into our database. Digital measures/recordings will be similarly tracked in our database and regularly backed up. Analyses will be conducted using standard statistical software such as SAS and Matlab.

For planning sample size calculation for this study, we utilized the OARSI recommendations for the management of knee OA. In this recommendation [84], a panel of experts reviewed evidence for the most commonly used pharmacological and non-pharmacological interventions for OA pain, giving effect sizes for all these interventions. Examples of non-pharmacological interventions are: education, physical therapy, aerobic exercise, knee brace, footwear, thermal modalities, TENS and acupuncture. Examples of pharmacological treatments are: acetaminophen, NSAIDs, injections with corticosteroids and treatment with glucosamine. In this review, effect sizes vary from 0.06 to 0.72. In fact the largest effect size was shown with injection of corticosteroids. Based on these effect sizes and the effect size we found in our pilot data (1.4), we set the effect size for our sample size calculation considering a effect size smaller or equal than our pilot data and larger than the

larger effect size from this meta-analysis (0.72) [84]. For this particular protocol, we plan a sample size of 64 participants. We considered the same sample per group as four times the subjects in Phase 1: 32 subjects per group. This will be enough to detect a difference in effect size of 0.9 and thus an effect size that is superior than other treatments used in OA according to a large guideline on OA management [84], though we expect a larger effect size for pain. However it is important to be able to detect significance with smaller effect sizes in the Phase 2 work, as some of the neurophysiological outcomes require additional power. Note that the main aim here would still be the comparison of active ESStim vs. sham ESStim.

All analyses will be conducted according to the principle of intention-to-treat (using regression-based single imputation method). Intention-to-treat (ITT) population will include all randomized subjects. We will also perform an additional sensitivity analysis in which we will use the method of multiple imputation. The primary outcomes will be pain changes as indexed by VAS scores, biomechanical function changes as indexed with the motion analysis suite (i.e., our biomechanical data acquisition and analysis system), and QOL changes. Differences between the groups will be tested using Student's t-test, and additionally we will adjust for important baseline variables and test the time effect in general mixed longitudinal models. This model will be used for the primary and secondary outcomes (dependent variable (one model for each variable): VAS scores, biomechanical function changes as indexed with the motion analysis suite, and QOL changes, and independent variables will be treatment group and time of assessment (we will also adjust for baseline pain levels in this model)). Secondary analyses will be conducted in an exploratory manner (no correction for multiple comparisons). We will also conduct an EEG analysis to correlate EEG data with pain measures. Similar analysis will be conducted for the adverse effects measuring continuous outcome and for the categorical outcomes we will use Fisher’s exact test. In both cases, for safety analysis we will use uncorrected p-value to increase the likelihood of detecting detrimental adverse effects.