Approved protocol and statistical analysis plan for the study “Regulating Homeostatic Plasticity and the Physiological Response to rTMS” with personal information for the sub-investigators and the study coordinated was redacted.

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University of Arkansas for Medical Sciences (UAMS) Clinical Protocol

Study Title: Regulating homeostatic plasticity and the physiological response to rTMS

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Abbreviations/Definitions

- tDCS = transcranial direct current stimulation
- anodal tDCS = positive charge associated with cortical activation
- cathodal tDCS = negative charge associated with cortical inhibition
- TMS = transcranial magnetic stimulation
- rTMS = repetitive tanscranial magnetic stimulaiton
- MEP = motor evoked potential
- EEG = electroencephalography
- TMS/EEG = TMS evoked EEG
- TEP = TMS evoked response potential
- EMG = electromyography
- mA = milliamp

Protocol Summary

Background and Rationale: This device-study includes a pilot, physiological investigation of normal human subjects and a pilot investigation of patients with tinnitus. In both investigations, the aim is to determine how existing non-invasive neuromodulation devices affect brain circuitry as measured by EEG recording. Currently, clinical treatments that use non-invasive neuromodulation are rarely guided by detailed knowledge of how neural activity is altered in the brain circuits that are targeted for intervention.[1] This gap in knowledge is problematic for interpreting response variability, which is common, and for optimizing treatment dose. To address this gap, the current proposal aims to combine two forms of neuromodulation sequentially, transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS), to regulate homeostatic plasticity prior to rTMS delivery at different doses. Homeostatic plasticity, the initial activation state of a targeted circuit, is a key determinant of whether rTMS induces long term potentiation (LTP) or long term depression (LTD).[2;3] Yet, homeostatic plasticity is rarely measured or controlled in rTMS studies. We aim to control homeostatic plasticity by preconditioning the targeted circuits with tDCS prior to rTMS delivery.

Justification: Controlling homeostatic plasticity can reduce subject variability[2;3] and the knowledge gained can be used to optimize rTMS treatment. What is needed to move the field forward is a method for combining tDCS and rTMS and for measuring neuronal responses directly which we aim to establish in this study. The long term goal of our work is to optimize rTMS as a treatment for tinnitus. The project will examine the targeted effects of neurmodulation in normal subjects and in patients with tinnitus. The brain regions targeted for intervention include auditory areas in the temporal cortex (TC) that process sounds and functionally connected regions of the dorsolateral frontal cortex (DLFC) that mediate sensory habituation. Further, we aim to determine how neuromodulation suppresses sound perception.

Objectives: One overall objective of this pilot study proposal is to determine how different activation states of the TC and DLFC (their homeostatic plasticity) interact with different doses of rTMS (1 Hz and 10 Hz) to produce LTD and whether these physiological changes can affect tinnitus-like behavior in normal subjects. Transcranial direct current stimulation (tDCS) will be used to modulate the activation states of TC and DLFC prior to rTMS. The primary outcome measure is change in cortical excitability, which will be measured *directly* from TC and DLFC using TMS evoked EEG potentials (TEPs). A 128 channel EEG recording cap with embedded high density tDCS electrodes was specifically designed by our research team to interleave TMS/EEG recording with tDCS and rTMS delivery. A second objective, is to investigate how

tDCS preactivation of the DLFC prior to 1 Hz rTMS of the TC modifies cortical excitability (TEPs) and perception of tinnitus in a patient sample.

Hypothesis: Our central hypothesis for this pilot study is that increased cortical activation will facilitate the induction of LTD by rTMS, both at the treatment site in TC and in a network site in the DLFC. In patients with tinnitus, it is hypothesized that LTD induction will correlate with change in tinnitus perception.

Specific Aim 1: Determine in normal healthy subjects how increased activation of the TC modifies the induction of LTD by 1 Hz and 10 Hz rTMS. Analyses will examine difference scores between sham and active rTMS in the local mean field power (LMFP) of TEPs (∆ LMFP) recorded from TC and DLFC. Parallel groups of healthy subjects (n=5 each) will receive 1 and 10 Hz rTMS. Four contrasts are of interest – (1) sham vs active tDCS for 1 Hz rTMS, (2) sham vs active tDCS for 10 Hz rTMS, (3) 1 Hz vs 10 Hz rTMS for sham tDCS, and (4) 1 Hz vs 10 Hz rTMS for active tDCS. Our working hypothesis is that increased cortical activation will facilitate LTD following rTMS at both frequencies of stimulation, but the effect for 1Hz will be stronger for than for 10 Hz.

Specific Aim 2: Determine in normal healthy subjects how increased activation of the DLFC modifies LTD induced by 1 Hz and 10 Hz rTMS over the TC. New groups of subjects will be recruited. The design, analyses, and predictions are the same as for Aim 1. The difference is that circuits in the DLFC, a functionally connected network, are being preconditioned rather than TC. We predict that rTMS delivered to the TC will induce LTD in the DLFC after it has been preconditioned with tDCS.

Exploratory Aim. We will develop and test a behavioral model of sound perception in healthy participants who have normal hearing by placing them in a sound attenuated environment and measuring ratings of sounds played above threshold. We will examine how tDCS/rTMS modify these perceptions.

Specific Aim 3: Determine in patient with tinnitus how increased activation of the DLFC modifies LTD and tinnitus perception induced by 1 Hz rTMS over the TC. We will recruit ten subjects from a previous study of rTMS for tinnitus (109033) who agreed to be contacted for future research projects. We will examine change from baseline in TEPs (∆ LMFP) recorded from TC and DLFC and on ratings of tinnitus awareness, annoyance and loudness following three days of treatment (tDCS preconditioning of the DLFC followed by 1 Hz rTMS of the TC). Analyses will examine change from baseline for TEPs and tinnitus ratings. Change scores for tinnitus ratings will be compared to those for sham and active rTMS treatments from a previous study (109033).

Aims 1 & 2 Design: A randomized, blinded, sham controlled, mixed effects model with assignment to two treatment arms (1 or 10 Hz rTMS) and three experimental

conditions within each arm (Table 1). Subjects will be sequentially assigned to one of two parallel groups (1 Hz or 10 Hz rTMS).

Study endpoints: The primary outcome measure is the local mean filed potential calculated for electrodes over the temporal and dorsolateral frontal cortex that are measured at baseline and after sham and active treatment conditions. An exploratory outcome measure is ratings of auditory stimuli detected by participants before and after each of the three experimental conditions.

Statistical Plan or Data Analysis: Aims 1 & 2 examine the effect of treatment dose on one primary outcome measure – a difference score between sham and active rTMS in the LMFP of TEPs (∆ LMFP) recorded from TC and DLFC. The analysis plan is the same for both aims. Hierarchical Linear Models (HLMs) will be used due to the nested structure of the design. HLMs will fit the complex covariance structure of the dosing schedule – sham and active tDCS nested within rTMS treatment frequency (1 Hz and 10 Hz).

Trial design, endpoints and analysis for Aim 3. The design is an open label, feasibility study – a pre-post examination of change in the LMFP of TEPs (∆ LMFP) recorded from TC and DLFC and in the ratings of tinnitus awareness, loudness and annoyance after four days of treatment. Aim 3 is conceptually similar to Aims 1 and 2 in using tDCS preconditioning of rTMS and in using EEG to evaluate change; however, treatment length and the timing and methods of assessing tinnitus were adopted from a previous study so that the data would be comparable.

What are the test articles TMS and tDCS?

TMS will be delivered via a Magstim Super Rapid2 stimulator with sham and active 70mm coils (Magstim)**.** TMS is a non-invasive device that uses a coil held over the scalp to create a brief, focused magnetic field and a resulting small electrical field within the brain that can be delivered repetitively (rTMS)[4]. RTMS is well tolerated in most studies and it is FDA-approved for treating depression[5;6]. Repetitive TMS activates cortical tissue locally, beneath the stimulating coil, and this activity propagates to anatomically connected brain regions.[7] Association cortex in the temporal lobe (Brodmann's Area 22: BA 22) is accessible to rTMS by virtue of its close proximity to the scalp. In patients with tinnitus, rTMS may activate all of the cell assemblies in the primary auditory cortex and thereby enable the DLFC to being to habituate to these signals. For this proposal, however, we want to test the veracity of interactions between circuits in TC and DLFC. We want to determine whether LTD can be induced in the DLFC circuits by applying rTMS to circuits in TC. We aim to control homeostatic plasticity prior to rTMS delivery using tDCS.

tDCS will be delivered using a Soterix constant current stimulator and Soterix 1 cm diameter Ag/AgCl electrodes powered by two, 9 volt Alkaline batteries. Its current range is 0-2.0mA (in 0.5mA increments). In tDCS, a weak DC electrical current is passed across electrodes located on the scalp to modulate the resting membrane potentials of underlying neurons. The ability of tDCS to control MEP amplitudes in motor cortex is well established.[8] tDCS delivered at 1 mA and no longer than 20 minutes duration elicits an excitatory effect on underlying tissue beneath the anode electrode (where current is delivered) and an inhibitory effect beneath the cathode electrode (where current is received).[9-11] Multiple studies have used 1 mA tDCS to precondition motor cortex prior to rTMS.[2;3;12;13] Our approach is different in that 2 mA current will be passed between electrodes for 20 minutes to increase cortical excitability *between* electrodes. At 2 mA and no longer than 20 minutes duration, there is a net increase in excitability under *both* stimulating electrodes.[14;15] This approach is useful for avoiding the inhibitory effects located beneath the cathode. We adopted this approach to increase cortical excitability homogeneously between electrodes where rTMS will be applied. In this way, we aim to use tDCS to increase the modification threshold of targeted circuits prior to rTMS delivery.

Our expert consultant, Adam Woods, has used MRI-derived finite element models (Soterix HD-Explore software) to map the electrical fields produced by 2 mA tDCS for various electrode locations (Figure 4).[16-18] He determined that by placing the cathode electrode over anterior temporal cortex and the anode over the occipito-parietal junction, the mean field intensity at the site of rTMS delivery in TC can be raised to 0.3 V/m (tDCS often peaks at a field intensity of between 0.3 and 0.5 V/m).[15] By placing electrodes at the F3 and F4 EEG locations in the standard 10/20 system, the mean field intensity over DLFC can be raised to between 0.3 and 0.4 V/m. Importantly, there was minimal overlap between models. So, preconditioning of the TC should not increase activation of the DLFC and vice versa. Dr. Woods is currently using 2 mA tDCS for 20 minutes to produce a homogeneous impact on cortical excitability in a large, multisite, phase III clinical trial (The NIA funded ACT study; Woods, PI). So, our results will have direct relevance to the largest clinical trial of tDCS ever conducted. Our proposal will take the additional step of pairing tDCS preconditioning with rTMS.

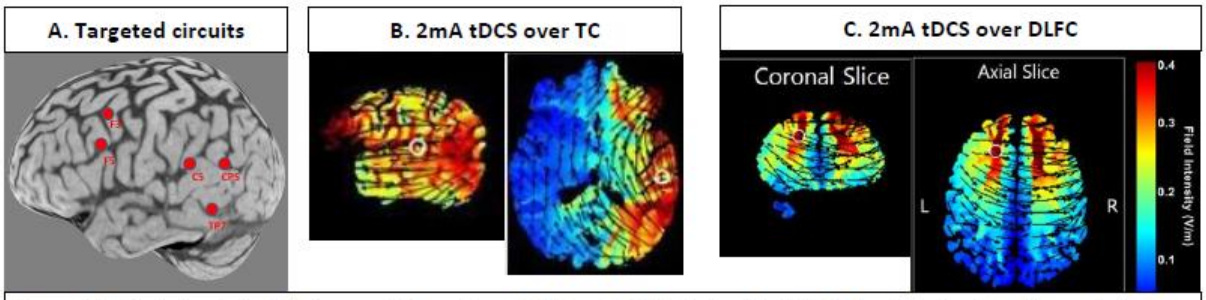

Figure 4. Models of electrical current flow through TC and DLFC during 2mA tDCS for 20 minutes. Figure A shows targeted circuit locations in TC and DLFC. Figure B shows relative field intensity in TC with anode located posteriorly and the cathode anteriorly. A white circle denotes approximate C5 electrode location. Figure C shows field intensity changes over DLFC, anode on left and cathode right.

Differences between tDCS and TMS include presumed mechanisms of action, with TMS acting as neuro-stimulator and tDCS as neuro-modulator**.** This is an important distinction. Whereas TMS stimulates cortical neurons causing them to fire; tDCS only makes it more or less likely that cortical neurons will respond to further stimulation.

What is the assessment tool TMS/EEG?

TMS/EEG is used in this proposal to measure the neural activity of targeted circuits. TMS/EEG is a method for probing superficial cortical brain regions to study intra-cortical neural circuits.[19] During TMS/EEG, single (or paired) TMS pulses are delivered repeatedly to a region of the brain while the EEG is being recorded. The data in this proposal will be collected off line, before and after tDCS and rTMS, respectively. TMS evoked EEG potentials (TEPs) can be used to measure cortical excitation/inhibition directly from the cortex. TEPs have high test-retest reproducibility, particularly when stereotaxic systems are used to precisely control the location of TMS delivery from session-to-session.[20;21] TEPs have been used in conjunction with MEPs to measure LTP induction in the motor cortex of healthy subjects.[22;23] TEPs are particularly well suited for use in this proposal to assess cortical excitability in non-motor areas, like the TC and DLFC, as well as multiple other locations within and between the cerebral hemispheres. TEPs have the characteristics of a good biomarker of change in neural dynamics. They are easy to obtain, have high test-retest reproducibility, and they have been validated in studies of motor and frontal cortex.[20-22] TMS/EEG data can also provide information about the ability of stimulated brain regions to generate oscillations in discrete frequency bands of the EEG[24] and about the sources and propagation of neural activity in functionally connected brain regions[25] which will be examined in exploratory analyses.

Scientific Premise

Individual responses to rTMS, both in normal subjects[26] and patient populations,[27] are variable, which is hard to interpret, in part, because homeostatic plasticity is rarely measured or controlled in rTMS studies.[27] Homeostatic plasticity refers to the initial activation state of a synapse, neuron, or population of neurons. rTMS is use specifically to change the initial activation state of neuronal populations – to increase or decrease cortical excitability. In general, high frequency rTMS (>3Hz) is expected to increase cortical excitability by inducing LTP, whereas low frequency rTMS (1 Hz or less) is expected to decrease cortical excitability by inducing LTD. In practice, however,[3;28] homeostatic plasticity actually determines whether or not rTMS, at any frequency, will induce LTD or LTP. The rationale is based on the Bienenstock-Cooper-Munro (BCM)[29] rule of synaptic modification, which holds that a prolonged reduction in post synaptic activity lowers the modification threshold and favors the induction of LTP;

whereas a prolonged increase in postsynaptic activity raises the threshold and favors induction of LTD. Therefore, either 1 or 10 Hz rTMS can have an excitatory or inhibitory effect depending on the previous activity history of the tissue and where the modification threshold is set at the time of rTMS delivery. These observations are the basis for our central hypothesis that raising the modification threshold, with 2 mA tDCS preconditioning, prior to rTMS delivery, at either 1 or 10 Hz, will facilitate the induction of LTD. The interaction between homeostatic plasticity and rTMS frequency can cause deviations from the usually assumed frequency dependent effects of rTMS (i.e., high frequency rTMS increases and low frequency rTMS decreases cortical excitability). These deviations have undoubtedly produced variability in individual responses to rTMS because the baseline level of cortical excitability is unknown. It is critical, therefore, to develop a method for measuring homeostatic plasticity, which we propose to do using TEPs, and for stabilizing neural activity prior to rTMS delivery, which we propose to do with tDCS preconditioning.

What are the safety concerns associated with using tDCS and TMS in human subjects?

Our protocol follows the guidelines that have been developed for the safe use of these techniques in human subjects. Four review and consensus articles have outlined the safety and ethical issues associated with tDCS and TMS. Two of these articles concerning tDCS have been uploaded with this protocol[30;31]. These articles show that the tDCS stimulation parameters used in this study have been used safely in human subjects. The risks and side effects associated with tDCS are listed further below in this protocol. Whereas tDCS is expected to be associated with sensations like scalp itching, tingling and rarely a burning sensation, it has not been associated with serious adverse events, like seizure, most probably because tDCS does not cause neurons to fire.

Two additional articles concerning TMS safety have also been uploaded with this protocol.[32;33] These articles provide a consensus opinion developed by leading experts on the parameters of TMS that can be considered safe for use in human subjects. Single pulse TMS has the best safety margin of all TMS applications. Single pulses of TMS are delivered during EEG recording. Repetitive TMS is also well tolerated when delivered within the established safety guidelines for human subjects and when subject are carefully screened for risk factors associated with rTMS. We propose to deliver stimulation at intensities that are within guidelines established for safe use in human subjects.[32] The associated risks and side effects of TMS are also listed further below in this protocol. We also screen subjects for any risk factors associated with rTMS. In summary, TMS carries greater risk to subjects than tDCS because some normal healthy subjects had seizures when receiving repetitive TMS (rTMS). Repetitive TMS means

that subjects receive many magnetic pulses in a row, such as either 1, 10 or 25 per second. Repetitive TMS is typically used in treatment studies, such as for depression, anxiety or tinnitus, where thousands of pulses may be given in a single session. Even with rTMS, adverse events are uncommon. For example, no serious adverse events were reported in a very large multisite study in patients with depression in which more than 10,000 cumulative sessions of high frequency rTMS were given[34]. The likelihood of an adverse event using single pulse TMS is much lower than when using rTMS. In a single pulse study, only one pulse is delivered at a time, and usually separated by at least 10 seconds in order to prevent a cumulative effect from occurring. Only one case of a normal subject who had either a seizure or a syncopal episode during single pulse TMS has been reported. This subject did not have identifiable risk factors for seizure and he was not taking medication that alters seizure threshold[35]. The following excerpt from the most current safety article on TMS (pg 19, Rossi[33]) provides the consensus opinion on the risk of seizure in rTMS studies:

> Considering the large number of subjects and patients who have undergone rTMS studies since 1998 and the small number of seizures, we can assert that the risk of rTMS to induce seizures is certainly very low.

This article goes further to classify TMS studies based on risk and to provide guidelines concerning the settings in which these studies may be conducted. Our protocol would fit in class 3 which is described as follows (pg, 2029[33]):

> – Class 3 (indirect benefit, low risk): studies in normal subjects and patients that are expected to yield important data on brain physiology or on safety, but have no immediate relevance to clinical problems. Normal volunteers should be permitted to participate in rTMS research when it is likely to produce data that are of outstanding scientific or clinical value.

The article indicates as well that it is reasonable to conduct class 3 studies using single pulse TMS in normal subjects in non-medical settings.

Several studies have been conducted that combine tDCS and rTMS delivery in the manner we propose for this protocol. No adverse events were reported in these studies. Further the studies confirmed that tDCS is capable of reliably establishing either a low or high modification threshold prior to rTMS.[2;3;12 36] All of these studies were consistently able to modulate homeostatic plasticity/modification thresholds using tDCS and all studies were consistent in showing that, in line with the BCM rule, homeostatic plasticity determines whether or not rTMS induces cortical inhibition (LTD) or excitation (LTP).

What is unique about our proposal?

Similar to the above mentioned studies our study uses tDCS to control modification thresholds of targeted brain regions prior rTMS delivery. It differs from these studies, however, in the way tDCS is applied (integrated in an EEG cap for Aims 1 & 2), how cortical excitability is being measured (TMS/EEG rather than MEP recordings), and where preconditioning is targeted (over temporal cortex and dorsolateral frontal cortex rather than over motor cortex). For Aim 3, whereas our previous studies have applied rTMS over the TC for the treatment of tinnitus; this study will add tDCS preconditioning of the DLFC and use a three-day treatment regimen of rTMS. Increasing the modification threshold of circuits in DLFC with tDCS is a means of controlling homeostatic plasticity in a way that promotes LTD induction upon further stimulation. Should stimulation of TC induce LDT in the DLFC, the predicted interaction of these circuits is confirmed. Finally, TMS evoked potentials (TEPs) will be used to physiologically confirm the effects of our interventions by measuring electrical activity directly from the cortex.

Measuring auditory perception before and after the experimental conditions is also a unique feature of this protocol. After 5 minutes in a silent environment, tinnitus-like-perception occurs in the majority of healthy subjects with normal hearing (between 65-94%). In this study, we want to explore whether neuromodulation can alter sound perception in normal healthy subjects. We also want to examine, in patients with tinnitus, how TEPs change in response to treatment as well as tinnitus perception.

Study Design, Population, Apparati and Investigational Plan

Aims 1 & 2.

Design: This is prospective, experimental design includes a block randomized, blinded, sham controlled, mixed effects model with sequential assignment to two treatment arms (1 or 10 Hz rTMS) and random assignment to three experimental conditions within each arm (Table 1).

Randomization: Subjects will be assigned sequentially to one of two parallel groups (1 Hz or 10 Hz rTMS: Table 1.5). Next, they will complete three experimental test sessions (Table 1). The order of test sessions is randomized. A minimum two week washout period will follow each session to minimize session to session carry forward effects.[15] Washout can exceed two weeks.

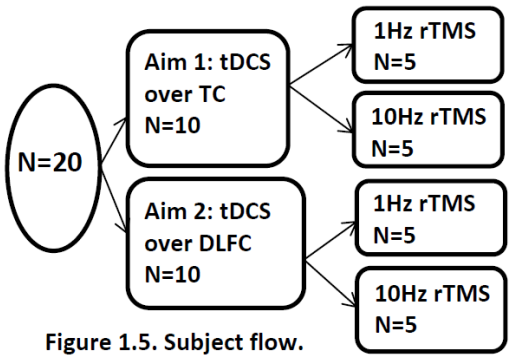

Figure 1.5. Subject flow.

Study visits: Study visits will be to the transcranial magnetic stimulation laboratory, room 654-2, located on the 6th floor of the Biomedical II research building on the UAMS

campus. In total, there will be 4 study visits - 1 consent/screening visit and 3 laboratory visits per subject. The 3 laboratory study visits will be separated in time by a minimum of two weeks. The time required to complete a study visit ranges from 1 hour, when TMS/EEG is not collected, to 3.5 hours, when TMS/EEG is collected. TMS/EEG is recorded immediately before and after tDCS preconditioning, immediately following rTMS and after a 20-30-minute delay to examine the acute and lasting effects.

Subject flow: Twenty healthy subjects who meet the entry criteria and pass safety screening for tDCS and TMS will be enrolled (Figure 1.5). Ten subjects will be tested in each aim, with two parallel groups of 5 subjects receiving either 1 or 10 Hz rTMS. Two randomization lists of 10, each balanced between tDCS over TC and tDCS over DLFC, were generated based on uniformly distributed random number generation in SAS v9.4. The first 10 recruited subjects will receive 1Hz rTMS treatment while the second 10 will receive 10Hz rTMS. The order of the conditions (sham/sham, sham/active, active/active) was also, independently randomized with uniformly distributed random number generation in SAS v9.4(Cary, NC). Power analyses suggest (see Analysis) that the sample sizes are sufficient to test our hypotheses. Subjects in Aim 1 will receive 2 mA tDCS preconditioning of the TC, whereas those in Aim 2 will receive 2 mA tDCS preconditioning of the DLFC.

Experimental conditions & TMS/EEG assessments.

Condition†	TMS/EEG	tDCS	TMS/EEG	rTMS	TMS/EEG	30 min delay	TMS/EEG
1		sham		sham			
2		sham		active			
3		active		active			
Time (mins)	0	20	40	60	80	100	130

Table 1. † The order of conditions is randomized. A 2 week washout separates each condition.

Study endpoints: The primary outcome measure of cortical excitability will be the local mean field power (LMFP) of TEPs obtained from TC and DLFC. All other measures are exploratory. Before and after each intervention, subjects will complete a hearing test designed to measure sound perception. All participants will complete a baseline puretone audiometry screening with threshold at 25 dB HL for both ears for octave frequencies between 250 and 8000 Hz in a sound attenuated booth. Before and after the tDCS and rTMS conditions, participants are seated in the sound attenuated booth in the TMS laboratory and given instructions for the listening experiments. Participants will sit quietly for a 5-minute session in the sound booth without any auditory stimulation. They are asked to report any sounds that they perceive by noting the time and by writing a brief sound description. Subjects will then listen to a series of tones, played randomly at different decibel levels over headphones. . Participants will rate the intensity of sounds they hear using numbers.

Aim 3

Up to ten subjects with tinnitus who participated in a previous rTMS study (i.e., 109033) and who agreed to be contacted for future studies will be invited to participate. The design of Aim 3 is an, open-label, feasibility study with pre and post assessments. All subject visits will be to the TMS laboratory and the Brain Imaging Research Center (BIRC) of the Psychiatric Research Institute. There will be a total of four study visits to the TMS laboratory, with one visit including a walk to the BIRC for the MRI scan, and three follow-up phone calls, emails or text messages used to obtain tinnitus ratings. The time required for each visit ranges from 1 hour when TMS/EEG is not measured to 3.5 hours when TMS/EEG is measured. During the first visit, subjects will complete the informed consent process and reestablish that they meet inclusion and do not meet the exclusion criteria. They will then complete a baseline TMS/EEG recording and make analogue ratings of tinnitus awareness, loudness and annoyance. An MRI image of the brain may be acquired as part of the first or second visit. During visits 2-4, subjects will be treated with tDCS over the DLFC (2 mA for 20 minutes) prior to receiving rTMS over the TC (1800 pulses delivered at 1 Hz and 110% of motor threshold). Following the treatment during visit four, subjects will have their second TMS/EEG recording. The study endpoints are the same as those in Aims 1 and 2 except that actual ratings of tinnitus are used in place of the procedure for measuring tinnitus like perception in healthy subjects.

Study Population

Aims 1 & 2

Study subjects will be recruited via advertisement. Twenty (20) healthy subjects between the ages of 19-65 years will be recruited. Screening measures include the Transcranial Magnetic Stimulation Adult Safety Screen (TASS).[37] A request for medical records either from UAMS or an external service provider may be necessary to ensure that subjects do not meet exclusion criteria.

Inclusion Criteria

- 1) complete the informed consent process
- 2) men and women, age: 19–65 years (a range reflective of our tinnitus samples, (e.g., CLARA IRB# 109639 *PET-CT to target and validate TMS as treatment for Tinnitus*)
- 3) negative pregnancy test (female subjects of childbearing age must take a pregnancy test).

Exclusion Criteria

- 1) a personal or family history of epilepsy,
- 2) severe head injury, aneurysm, stroke, previous cranial neurosurgery,
- 3) severe or recurrent migraine headaches,
- 4) metal implants in the head or neck, a pacemaker,
- 5) pregnancy,
- 6) medications that lower seizure threshold,

Aim 3

Up to ten subjects from a previous study (109033) will be invited to participate. Subjects enrolled in this study were between the ages of 21 and 85. Subjects will have already passed the following eligibility criteria for 109033 but they must indicate that they still meet the following eligibility criteria to be eligible for this study.

Inclusion Criteria

1) Report experiencing the presence of their phantom auditory perception for at least 6 months
2) Complete and pass the Transcranial Magnetic Stimulation Adult Safety Screen (TASS).
3) Female subjects of childbearing age must take a pregnancy test to rule out pregnancy prior to participating in this study.
4) Individuals taking SSRIs and benzodiazepines or for depression or anxiety related to tinnitus must be stable on doses of these medications for 3 months and not change medications during the course of the study.
5) Patients who agree to have an MRI scan must pass screening procedures for an MRI scan.

Exclusion Criteria

1) A clinical, personal or history of epilepsy, including a first degree relative diagnosed with epilepsy.
2) Head injury that resulted in the loss of consciousness for more than 10 minutes.
3) Aneurysm, stroke, previous cranial neurosurgery, diagnosed neurological or major psychiatric disorders (excluding depression or anxiety related to tinnitus).
4) Ferromagnetic metal implants in the head or neck, pacemaker.

5) Medications that lower seizure threshold or reduce cortical excitation. These medications include some antidepressants (i.e., tricyclic antidepressants and bupropion) and anticonvulsants.
6) Significant neurological disease, acoustic neuromas or glomus tumors, active Meniere's disease, or profound hearing loss (>90 dB at 4000 Hz).
7) Bipolar Disorder.
8) Patients who cannot speak English will be excluded because they will not be able to complete questionnaires and may not understand instructions.
9) Failing the claustrophobia screening questionnaire (exclusionary for fMRI only).
10) Abnormalities present on an acquired or existing CT or MRI image of the head.

Apparatus, Materials, and Procedures

Aims 1 & 2

Interventions: tDCS preconditioning and rTMS delivery

tDCS: A Soterix Model 1300A (Soterix 1x1) tDCS stimulator will be used to deliver tDCS at 2 mA for 20 minutes with 30 seconds of ramp-up and ramp-down. Current will be delivered through two Soterix 1 cm diameter Ag/AgCl electrodes placed in non-conductive electrode holders integrated into the 128-channel EEG cap. The highest current density for the electrodes is 2 (mA/cm^2) and the total charge per session is 2.4 C/cm^2 both of these values are well below the safety margins.[38;39] Electrode holders will be filled with electrical conductance gel (e.g., Signa Gel), the electrode placed into the holder, and then locked into position. Impedance will be <10 kilo ohms and will be matched between electrode positions to provide high quality and consistent delivery and return of current. Current passed through Ag/AgCl electrodes to electrical conductance gel contacting the scalp provides an efficient biochemical transfer of electrical current to the scalp with decreased sensation of active stimulation. This property improves the efficacy of sham procedures and subject blinding.[15] Since Ag/AgCl electrodes begin to corrode after two-six sessions of 2 mA current delivery, electrodes will be monitored and only be used for stimulation if they show no signs of corrosion before being replaced.[39]

Sham tDCS will involve the same ramp up/down as active stimulation, but will only deliver 30 seconds of active stimulation at 2 mA. As participants typically habituate to the sensation of stimulation within 30-60 seconds, this provides comparable and indiscernible sensation to active stimulation, but not the full duration of stimulation.[15]

rTMS: A MagStim Super Rapid2 will be used to deliver rTMS with two air cooled 70 mm coils – one active and one sham. The sham coil generates sounds identical to the

active TMS coil and has a similar weight and appearance. Thus, it mimics the look, sound, and feel of active rTMS necessary for subject blinding.

rTMS stimulation parameters. Similar to studies of preconditioning studies of the motor cortex,[2;3] 900 pulses will be delivered at 110% of the motor threshold (MT) over the TC at the rate of 1 Hz or 10 Hz. For 10 Hz rTMS, pulses will be delivered in 45, 2 second trains, with 20 seconds between pulse trains. The motor threshold (MT) will be determined during the first subject visit after the consent and screening information have been obtained. MT will be determined by placing the TMS coil over the cortical motor area and delivering single pulses of increasing intensity until the optimal area of stimulation is found. Threshold will be defined as the percentage of the maximum stimulator output (MSO) necessary to elicit a motor evoked potential (MEP) of 50 μvolts recorded from the thenar abductor pollicis brevis muscle of the contralateral hand in 3 of 6 stimulus trials. MEPs will be recorded with surface electrodes fixed on the skin with a belly-tendon montage. The EMG signal is filtered (10 Hz–1 kHz bandpass) and displayed on a computer screen. These rTMS parameters are well within the published safety guideline for use in human subjects[33;40] and were chosen to match those of our tinnitus studies, except that the overall number of pulses will be half of those given to patients.

Safety measures for post rTMS. A brief battery of validated neuropsychological tests[41;42] - The Digit Symbol Test, the Three Words at Five Minutes Test of memory recall, and the Finger Tapping Test - will be used, as in previous studies,[43-45] to assess for any adverse effects of rTMS on cognitive and motor function. Dr Mennemeier will oversee the administration and interpretation of these tests. Dr Mennemeier is a Clinical Neuropsychologist.

Methods for TMS targeting. A template MRI scan will be uploaded into the Brainsight frameless stereotaxy system (Rouge Research, Montréal, Québec, Canada) and used to track stimulation delivery for all subjects. Brainsight uses 3D infrared tracking position sensors to reference the MRI to anatomical landmarks on the subject's head and to the TMS coil. Digitized markers can be place on the MRI in Brainsight to ensure that the TMS coil is placed in the same locations from session to session. Brainsight will be used to target rTMS over the caudal two-thirds of the superior temporal gyrus (BA 22 in TC). During TEP recording, Brainsight will be used to target the TMS coil over BA 22 in the TC and to position the TMS coil over the convexity of the superior frontal gyrus where TEPs from the DLFC will be obtained. All stimulation sites will be marked in the Brainsight Stereotaxic system (RougeResearch) to ensure precise session to session targeting.

Methods for active and sham rTMS and study blinding. The MT will be established at the

Figure 5. Electrode location mock-up.

beginning of each test session. A technician will set up equipment for each test session while the subject is being prepped for EEG recording, to keep them blind to condition. To check integrity of the blind, subjects will be asked to guess whether they received active or placebo stimulation at the end of each treatment session. Subjects are not providing subjective responses to intervention, which minimizes the possibility of experimenter bias. Persons who process EEG data for analysis and the biostatistician will be kept blind to condition using session codes. These procedures were used successfully in previous studies.[45]

Aim 3.

The methods for tDCS preconditioning in Aim 3 is identical to that described for Aims 1 and 2 except that the high density stimulating electrodes will be placed in a cap specifically designed by the manufacture rather than the 128 channel EEG recording cap (as in Aims 1 & 2). Additionally, only active tDCS will be delivered.

Patients will be asked to have a structural MRI scan. For safety reasons and prior to the MRI scan, subjects may have a training session in the MRI simulator to help habituate and train them to the MRI environment. Per the MRI Policies and Procedures, the decision of who will be acclimated in the MRI Simulator will be made by the Research Coordinators and PI on a case-by-case basis. The PI for the study will have final say in this decision. Before the MRI scan, participants will be given an explanation of the study's procedures and screened with the MRI Safety Form for metal objects and claustrophobia. Participants will also be screened with the SAFESCAN® ferromagnetic detector according to MRI Policy and Procedures. The participant will then lie supine in the scanner. Participants will wear noise-cancelling headphones for communication and view visual stimuli through a mirror attached to the imaging head coil. Participants will undergo an anatomic scan.

The method for rTMS stimulation of the TC and for establishing the motor threshold is also identical to that described for Aims 1 and 2 except that 1800 pulses are delivered rather than 900 pulses and only active stimulation at 1 Hz will be used.

tDCS preconditioning of the DLFC will be delivered immediately prior to rTMS stimulation of the TC for three days within a one week period. rTMS will be targeted over the TC as described for Aims 1 and 2. The same safety measures will be performed before and after stimulation on each day of treatment. Additionally, analogue ratings (using a 0-100 scale) of tinnitus awareness, annoyance and loudness will be obtained at baseline and before and after each treatment session. The same ratings will also be obtained via phone, text or email on day 2, day 9 and day 16 following the last treatment. The necessary length of treatment and the timing and methods used to assess tinnitus were adopted from a previous study (109033) to ensure that the data

are comparable between studies.

To assess changes in cortical excitability, EEG without and with TMS will be recorded during the baseline visit and following treatment on visit four. The primary outcome measure for TMS evoked EEG and the equipment used to assess TMS evoked EEG are described next.

Assessments: procedures and signal processing.

Primary outcome measure. The local mean filed potential of TEPs are the primary outcome measure for Aims 1 & 2. Other measures are exploratory.

Equipment. TMS/EEG signals will be recorded (before and after tDCS and rTMS) using a 128-channel eego mylab EEG system (ANT Neuro HQ, Enschede, Netherlands) and a custom 128 channel, TMS compatible, Wavegaurd EEG recording cap with an extended, equidistant 10–20 system and linked mastoid reference. The cap will be embedded with high density tDCS electrodes located at the RD2 and RB6 (right temporal), the LD2 and LB6 (left temporal), and the LL6 and RR6 (left and right DLFC) electrode locations (Figure 5). The tDCS electrodes are fully compatible with our tDCS stimulator. As mentioned above under theoretical rationale, these electrode locations produce a homogeneous increase in cortical excitability between electrodes, in MRI-derived finite element models (Soterix HD-Explore software), when used with 2 mA tDCS delivered for 20 minutes.[16] These models were used by Mr. Chelette to diagram the 128 channel Waveguard cap that allows seamless integration of EEG recording with tDCS and rTMS delivery in the context of a single test session.

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TMS/EEG Acquistion. TMS-evoked EEG will be performed using the MagStim Super Rapid2 magnetic stimulator and the active 70 mm coil. Patients will wear earplugs playing noise to mask the sound of the TMS pulse in order to mitigate auditory evoked responses from click sounds produced by the coil.[24;24] Brainsight will be used with the anatomical MRI scan to a) target the center of the coil over the same locations in TC and DLFC from session-to-session. Adopting methods from previous studies,[20;21;24] TMS will be delivered at 110% of the motor threshold in order to elicit EEG responses with good signal to noise ratio.[24;46] Two hundred TMS pulses (100 eyes open and 100 eyes closed) will be delivered with a randomly jittered ISI of between 1500-2200 ms over target locations (i.e., 50 eyes open and 50 eyes closed over the TC and the DLFC). The time required to deliver 200 TMS pulses to both the TC and DLFC is ~15minutes.

Signal processing: mitigating volume conduction**.** The technician will process the EEG records and import artifact-free EEG data into MATLAB (The MathWorks, Natick, MA) with the use of the EEGLAB toolbox.[21;47] After rejecting any remaining muscle artifacts, signals will be filtered as mentioned above. Dr Govindan will download the de-identified EEG data for signal analysis.

Signal processing: calculating TEPs**.** Knowing that TMS pulses trigger a large, early TEP component (between 0-80 ms), consisting of a positive wave between 15 and 30 ms followed by a negative wave peaking around 50 ms, with maximum amplitude in the electrode at the TMS target;[21;24;46] analysis of the TEP signal will occur as follows: a) a region of interest (ROI) will be defined, b) the area of the early positive and negative waves triggered by the TMS pulse will be quantified for each electrode, and c) averaged across the ROIs to obtain the local mean field power (LMFP) of TEPs for the ROI. LMFP will be calculated as the square root of squared TEPs averaged across the ROI.[21] These analyses will be performed for each subject individually.

Risks and Benefits

Potential Risks

Risks Associated with TMS

A variety of potential risks of TMS have been identified during the last several decades. Procedures for minimizing these risks have been established[33]. The following risks have been identified:

Likely: Subjects may feel anxious about participation. This typically abates after the first one or two sessions. Subjects may experience minor discomfort associated with scalp muscle twitching. Subjects typically tolerate the discomfort better as the session progress. The coil position can be adjusted if pain occurs and stimulus intensity is reduced if pain persists.

Less likely: Head and neck pain related to stimulation of underlying muscle and nerves occurs in approximately 10% of subjects. The incidence and severity is a function of stimulus site and intensity but is most common over fronto-temporal regions. The symptoms are typically limited to the time of stimulation and can be treated with minor over-the-counter analgesics if necessary.

Rare: The following have rarely been identified:

1. Seizures: Seizure induction represents the most serious known risk of TMS[33]. Seizures have been reported more frequently in subjects with brain lesions (e.g., stroke) but have rarely been reported in subjects with no history of seizures or

neurologic disease. When seizures have been reported, they were almost exclusively in association with high frequencies of rTMS. Based on an extensive review of the literature, guidelines have been developed that specify the number of stimulations that may safely be given as a function of stimulus intensity (% of Motor Evoked Potential [MEP]) and frequency of stimulation[32]. Using these guidelines, there have been few published reports, to the PIs' knowledge, of seizures or evidence of after discharge or spread of excitation in normal subjects receiving repetitive TMS who did not have an identified risk factor that is exclusionary for the PIs' study[33]. The PI proposes to adhere to the published guidelines. Furthermore, as subjects with a history of seizures are more likely to experience seizures due to TMS, these subjects will be excluded using the TASS[37]. Finally, a trained nurse or study physician is present to carry out established rescue procedures in case of seizure caused by TMS.

2. Effects on Cognition: A number of studies have been performed to identify possible adverse neuropsychological consequences of TMS. There have been several studies in which a number of cognitive tasks were administered before and after TMS[32;48]. Few adverse effects of TMS on cognition are reported, and there is a trend for performance to be better on measures such as delayed story recall. Two studies, however, have demonstrated possible adverse effects lasting up to one hour. Greenberg et al. (cited in Wasserman 1998[32]) reported that task switching was impaired after 20-Hz stimulation of the right compared to the left dorsolateral frontal lobe. As there was no untreated condition, this effect may reflect an enhancement of function after the left prefrontal TMS rather than a decrement after right TMS (see Grafman et al.[49]). Flitman et al.[50] reported a significant decrease in logical memory one hour after testing after extensive stimulation using parameters that exceed guidelines for inter-train interval (150 trains of rTMS at 15 Hz, 750 msec duration, and 1.2 times the MEP).

3. Effects on Mood: Dysphoria with crying has been induced after left prefrontal stimulation[51]. In contrast, high-frequency stimulation of the right prefrontal cortex may transiently improve mood as rapid-rate rTMS has been shown to be a safe and effective treatment in patients with depression.

4. Effects on Hearing: Animals have shown permanent increases of the auditory threshold after single-pulse TMS[52], and humans have shown transient increases. Foam earplugs were effective in avoiding changes in the auditory threshold in a safety study of TMS[48]. As a precautionary measure, subjects will wear earplugs during both control and active rTMS. In the PI's preliminary studies, a decibel meter was used to test the click stimuli generated by the rTMS coil. These were between 65–75 dB the most intense stimulus measured was 88 dB. Although

OSHA guidelines allow exposure of individuals to 90-dB stimuli for up to 8 consecutive hours without protection. The PI's previous studies using 1 Hz rTMS have found no effect on hearing due to TMS stimulation.[53-57]

5. Scalp Burns: Rapid rate and high stimulus intensity TMS may cause the coil to heat and could possibly result in scalp burns in some situations[58]. The coil that is to be used in this proposal, however, is cooled and incorporates a temperature sensor in the coil; it will cease operation should the internal temperature of the coil exceed a set point (which is cool to the touch externally).

6. Histotoxicity: Studies from animals as well as a study of subsequently resected anterior temporal lobes of humans subjected to direct cortical stimulation or TMS have failed to demonstrate evidence of histotoxicity. For reasons reviewed by Wasserman 2002[59], there appears to be very little chance of histotoxicity. It is also noteworthy that MRI examinations done minutes and hours after occipital stimulation with rTMS sufficient to cause phosphenes have failed to demonstrate edema or diffusion changes[60].

7. Kindling: Kindling is a process by which repeated administration of an initially subconvulsive stimulus results in a progressive intensification of induced neuroelectrical activity resulting in a seizure. This has not been reported with TMS and appears unlikely for several reasons: Kindling is most readily obtained with high-rate repetitive stimulation (e.g., 60 Hz), requires a pulse duration of 1 msec (longer than that of TMS), and is easiest to produce in the amygdala and hippocampus. Kindling of the neocortex in animal models of epilepsy is very difficult to achieve. There is no evidence that kindling can be produced by TMS.

8. Exposure to Magnetic Fields: The maximal field strength generated by commercially available stimulators, such as the stimulator used in the proposed study's laboratory, is in the 2-Tesla range. The PI typically delivers TMS between 50% and 80% of the maximum output in their studies. The field is induced for a brief period only, and the strength of the field falls off rapidly with distance from the coil (negligible at >3 cm). There is no evidence of adverse effects from magnetic field exposure during TMS to our knowledge.

9. A seizure caused by rTMS could place subjects at financial risk secondary to cost of medical care. Having a seizure might also influence driving privileges, employment, and the ability to obtain insurance. Subjects are informed of these risks in the consent process. The PI would provide documentation that the seizure was triggered by rTMS, that it does not constitute epilepsy, and that seizures caused by rTMS have not resulted in future seizures. Seizures induced during electroconvulsive therapy (ECT) for depression, for example, do not cause

driving privileges to be revoked in the state of Arkansas. Like ECT, a seizure occurring after rTMS would not cause driving privileges to be revoked.

Risks Associated with tDCS

A weak electrical current is applied to the brain via the scalp during tDCS using two or more surface electrodes. Current evidence shows that tDCS applied to motor and non-motor areas according to the present tDCS safety guidelines[15;38] produces only minor adverse effects in healthy humans and patients with varying neurological disorders. One published safety study of tDCS, using stimulation parameters similar to this study, evaluated 103 subjects[30], and found no adverse effects on cognitive and psychomotor measures, nor EEG changes during or after 20 min of treatment. In a double-blind, sham-controlled study[61] it has been shown that comparing tDCS and sham stimulation of the motor cortex elicited minimal discomfort and difference in the duration of tingling sensations. Another study summarized adverse effects of 567 tDCS sessions over motor and non-motor cortical areas (occipital, temporal, parietal) in 102 subjects who participated in tDCS studies[62].

Likely: During tDCS a mild tingling sensation was the most common reported adverse effect (70.6%). Moderate fatigue was felt by 35.3% of the subjects. A light itching sensation under the stimulation electrodes occurred in 30.4% of cases.

Less likely: After tDCS, headache (11.8%), nausea (2.9%) and insomnia (0.98%) were infrequently reported.

Rare: Reviews of tDCS safety have not identified specific risks associated with electrodes; however, a scalp burn could occur via a loose connection if the plug came in contact with the scalp. We protect against this risk by using electrode holders that are embedded in the EEG cap which also ensure a good connection to the stimulator. The connectors are housed away from the scalp making the possibility of a burn highly unlikely.

Risks associated with EEG

Likely: Subjects experience discomfort associated with mild scalp abrasion to reduce electrode impedance. Minor wounds can occur due to abrasion.

Less Likely: The EEG cap holds electrodes in place via a chinstrap. Subject could experience pain at recording sites due to this pressure.

Rare: Abrasion might be sufficient to cause bleeding or cuts.

Risks associated with MRI

Likely: Subjects will recognize they are in a confined space.

Less likely: Subjects with claustrophobia may become uncomfortable in the close confines of the scanner. Subjects might have metal in their body that could contraindicate MRI scanning.

Rare: MRI can reveal an unknown medical condition.

Risks Associated with Loss of Confidentiality

Each subject's name, SSN, birth date, address, and phone number may be obtained for purposes of follow-up. Study personnel will ask for each subject's about their medical history and current drug use. Experimental data and questionnaire data will be collected. This information will be coded into the database. The PI, technician and study nurse will have access to subject identities in order to arrange follow-up and to call them as part of the research project. If subjects prefer e-mail correspondence, they will sign a form authorizing research personnel to send e-mails for scheduling, follow-up, and research-related communication

Likely: None.

Less Likely: None.

Rare: Confidential information about subjects may be accidentally disclosed. All data will be safeguarded in accordance with HIPAA. The investigators use codes to keep subject information secure. Data encryption software is required at UAMS for computers with confidential information. Protection Against Risks

Protection Against Risks Associated with TMS

Prevention: In rTMS studies, prevention through careful screening is the best means of protecting subjects against adverse events and this begins with the informed consent process. Subjects are fully informed about risk factors for adverse events during the informed consent process so that they understand the need to accurately report their medical history both during screening and throughout the course of the study. Careful screening for risk factors, medications and medical conditions that might increase the chance of an adverse event (covered under exclusionary criteria) is a very effective means of protecting subjects against risks associated with rTMS. The final protection against risk associated with rTMS is to have a comprehensive plan in place for managing and reporting adverse events like seizure should they occur. In the following section we describe common procedures for managing the unlikely event of a seizure.

Personnel: TMS treatment will be administered by trained technicians who are supervised by qualified professionals (i.e., Drs. Mennemeier and Dornhoffer at UAMS). A medical professional will be directly available to the study technician and to the subject during rTMS delivery in the form of a designated study physician (i.e., Dr Dornhoffer) who is on call at the time of the rTMS session and in the form of an

authorized registered nurse practitioner who is fully trained in rescue procedures and present during the active rTMS sessions and acting under standing orders of the study physician.

The following steps will be taken to minimize the risks associated with TMS. Additionally, subject exclusion criteria should eliminate subjects for whom risk is greater.

1. Subjects are fully informed of all risks during the informed consent process.
2. Prior to participation, subjects will be fully informed of the possibility of seizure, the plan for care in event of a seizure, and any foreseeable financial or medical consequences resulting from a seizure.
3. All stimulation procedures will fall within the guidelines recommended at the conclusion of the NIH Panel on TMS (see Wasserman[32;33]).
4. Pregnancy tests will be administered at the beginning of the study and prior to rTMS for women who are of childbearing age and for whom a possibility of pregnancy is indicated.
5. Subjects will be monitored by trained staff for any muscle contractions persisting after stimulation by inspection of body parts that might be affected (e.g., the left arm after right frontal stimulation) or for symptoms such as visual disturbances. Should these be observed, the session will be terminated. The TMS technician or nurse practitioner will contact the study physician and PI. The treatment parameters will be reviewed by the study physician and PI, if deemed causative of these events the subject will not be tested again using those stimulation parameters.
6. All subjects will wear earplugs during testing sessions.
7. Only approved personnel will administer and monitor the effects of rTMS.
8. The PI, study physician, or nurse practitioner will stock and maintain the laboratory with the emergency equipment and supplies as required.
9. If a subject were to have a seizure during a TMS session the TMS technician and nurse practitioner would attend to the subject and immediately administer standard precautionary procedures for seizures as follows:
    a. The stimulator coil will be removed from the subject's head.
    b. The subject will be supported to physically guard against injury.
    c. The subject will be placed on his/her side on a flat surface away from sharp edges.
    d. The subject will be observed for airway maintenance.

10. The study physician will be called immediately.
11. Emergency services, 911 or a hospital code, will be called and emergency personnel will determine the need for anti-seizure medication administration as well as the need to transport patient to the ER.
12. Subject's emergency contact will be called.
13. If subject refuses or there is no need to go to the ER, transportation back home will be arranged for the subject as he should not be allowed to drive back home.
14. After the seizure is over, the subject will be examined thoroughly by the study physician for injuries. A follow up appointment with a neurologist will be scheduled within 3 days. Follow up tests could include any or all of the following procedures which are at the discretion of the treating physician: a neurological exam will be completed. Routine studies, including calcium, magnesium, and prolactin, will be completed and urine will be sent for a drug screen. An MRI scan of the head will be performed if deemed necessary to rule out underlying epileptogenic pathology. An EEG will be performed with hyperventilation and anterior temporal leads if deemed necessary.
15. The subject will be advised that following a seizure provoked by TMS, the likelihood of further spontaneous seizures is not significantly increased unless other pathology is discovered. Any necessary documentation for the medical record or insurance providers will be provided by the study physician.

Protection Against Risk Associated with tDCS.

Subjects are fully informed of potential risks during the informed consent process. Subjects will be screened to using the TASS to reduce risks. Equipment will be inspected prior to use to ensure that all electrical contacts are secure to prevent the possibility of scalp burning.

Protection Against Risk Associated with EEG recording

Subjects are fully informed of potential risks during the informed consent process. Discomfort will be avoided by monitoring how the subject feels during electrode application and applying less pressure or loosening the cap if discomfort is noted.

Protection Against Risk Associated with MRI

Potential risks concerning MRI scan: One potential safety concern is participant internal metal during MRI scanning, which can be painful and dangerous. Screening procedures administered prior to the scan will be used to rule out any participants who

may potentially be at risk for harm as a result of internal metal objects. Another potential risk involved with imaging procedure is claustrophobia. Participants are able to quit the scan at any time by indicating to the technician that they wish to do so. Participants can undergo a "mock" scan to determine how well the can tolerate the real MRI environment. If the MRI scan revealed a previously unknown medical condition, such as a brain tumor, the subject will be fully informed of the condition and counseled by the study physician, Dr Dornhoffer, on appropriate follow-up care.

Protection Against Risks Associated with Loss of Confidentiality

Each subject's personal information will be de-identified in the database by using codes comprising the subject number and sequence of testing. All data will be safeguarded in accordance with HIPAA. The investigators use codes to keep subject information secure. Data encryption software is required at UAMS for computers with confidential information. All study staff will have current documentation of HIPAA and CITI training.

Potential Benefits

The PI anticipates that subjects will participate because they support the research effort underway. The study itself will allow the study team to gather important data with regard to the targeted effects of tDCS and rTMS on neural dynamics. Understanding how non-invasive neuromodulation alters neural activity is critical for using these techniques to treat disease and disability.

The benefit to society is that this research could lead to more effective treatments and the knowledge gained can be used to advance the development of these techniques. The risks of participation are reasonable given the knowledge to be gained and the potential for developing a new treatment.

There will be no direct benefits to the study participants in Aims 1 and 2; however, knowledge gained from the study could potentially benefit patients in the future. Participants in Aim 3 may experience a reduction in tinnitus perception that could be temporary.

Data Safety and Monitoring Plan

The PI is responsible for monitoring data confidentiality and the safety of the study's subjects. Confidentiality will be monitored through the use of checklists. Quality assurance will be monitored through a set of standard operating procedures that will be compiled and placed in study binders. Checklists will ensure that informed consent has

been obtained, that identifying information is placed in a designated secure location, and that the subject has been de-identified in the database. Checklists also confirm what has occurred during each test session and during each phase of recruitment and follow-up. Checklists are signed and dated either by the PI or relevant study personnel. The PIs' safety monitoring plan includes the following: 1) the PI is the designated responsible entity; 2) immediately following stimulation, subjects will be inspected for signs of twitching and movement indicative of after discharge; 3) in the event of seizure, the plan outlined under protection against risk will be followed by study personnel; 4) the PI will report any adverse events to the IRB and funding agency. Any seizure resulting from this study would immediately be published so as to add to the collective body of information on TMS. The PI will prepare regularly scheduled reports to the IRB, as required, in a timely fashion. The PI will report any adverse event related to tDCS, TMS or TMS/EEG. All staff involved in the conduct and/or monitoring of this study will have current UAMS Human Subject Protection and HIPAA Research Training.

Adverse Events Reporting and Evaluation

All adverse events occurring during the study, will be recorded on the Adverse Event Case Report Form. Special reporting procedures are required for certain adverse events.

Identification of Adverse Events

Anticipated adverse events that a subject may experience include the following:

- *Scalp tingling, itching or burning, and less so fatigue and nausea, have been associated with tDCS.*
- *Mild pain and headache related to scalp muscle twitching have been associated with TMS.*
- *Hearing loss, changes in cognition and mood, and seizure have rarely been reported with repetitive TMS and are even less likely with single pulse TMS. They are mentioned here to be comprehensive.*

Serious Adverse Events

Each adverse event will be assessed for its seriousness using the criteria outlined below. The term serious adverse event is not synonymous with a "severe" adverse event, which may be used to describe the intensity of an event experienced by the subject. An adverse event will be classified as serious if it meets any of the following criteria:

- Results in, or contributes to, a death

- Life-threatening (i.e., the subject was, in the opinion of the investigator, at risk of death at the time of the event, but it does not include an event that, had it occurred in a more severe form, might have caused death)
- Results in permanent disability or incapacity (i.e., permanent impairment of a body function or permanent damage to a body structure)
- Requires in-subject hospitalization or prolongs hospitalization
- Necessitates medical or surgical intervention to preclude a permanent disability or incapacity
- Results in a congenital anomaly or birth defect

A serious adverse event will be reported to the IRB within 24 hours of first learning of the event.

Non-serious adverse events are all events that do not meet the criteria for a “serious” adverse event.

The investigator must promptly notify its reviewing IRB of such an event as soon as possible, but no later than ten (10) working days after first learning of the event.

Severity

Each adverse event will be assessed for its severity, or the intensity of an event experienced by the subject, using the following.

1. **Mild:** Discomfort noticed, but no disruption to daily activity.
2. **Moderate:** Discomfort sufficient to reduce or affect normal daily activity.
3. **Severe:** Inability to work or perform normal daily activity.

Deaths

The investigator will notify the and IRB as soon as possible, preferably within 24 hours but in no event later than 48 hours, of learning of subject’s death.

Eliciting and Reporting Adverse Events

The investigator will assess subjects for the occurrence of adverse events at each study visit. *All* adverse events (serious and non-serious) reported by the subject must be recorded on the source documents and CRFs.

Independent data safety monitoring

All device studies (IDEs) operate under a mandatory, independent data and safety monitoring plan. The independent monitor for the study will be a monitoring specialist in the office of research and regulatory affairs which operates in conjunction with the Translational Research Institute (TRI).

1. The sponsor will conduct independent data safety and monitoring before the first subject is entered, after the first subject is tested, and following the next 3–5 subjects entered.
2. The Data and Safety Monitoring Plan describes operating procedures that will be in place to monitor compliance, study data validity and integrity, participant safety, individuals and/or entities (e.g., IRB) that will be involved in monitoring these procedures, and the frequency/regularity of this monitoring.
3. UAMS IRB regulations will be strictly adhered to in the conduct of the proposed research. Specifically, prior to implementation of any protocol changes, amendments will be submitted to the IRB for approval.
4. In terms of participant safety, if an adverse event occurs during the course of a study, guidelines in the UAMS IRB Investigator's Handbook for adverse event and serious adverse event reporting will be followed. The PI will report all such activities to the IRB and the sponsor (as appropriate). Additionally, the PI will inform the sponsor of any actions taken by the IRB resulting from its continuing review of this study.
5. In terms of reporting mechanisms of IRB actions to regulatory agencies, the following UAMS IRB policy (#2.6) applies: The IRB reports any unanticipated problems involving risks to human participants or others; any instance of serious or continuing noncompliance with the IRB regulations, requirements, or determinations; and any suspension or termination of IRB approval to the Food and Drug Administration (FDA), the Office for Human Research Protections (OHRP), and the Office of Research Oversight (ORO) according to appropriate regulations and the terms of the UAMS IRB Federal Wide Assurance (FWA).
6. Monitoring of the aforementioned procedures will also be overseen by the PI, study coordinator, and the IRB. These procedures will be reviewed regularly by the Project Director in a number of settings. For instance, issues pertaining to data validity and integrity, and subject safety will be addressed during regular research staff meetings. Moreover, the study coordinator and PI will meet on a regular basis to discuss these topics further. In addition, the IRB, in collaboration with the Office of Research Compliance (ORC), during its yearly continuing review process, will evaluate procedures in place to effectively monitor data integrity and validity, and participant safety.

ClinicalTrials.gov Requirements

If funded, this project will be registered in ClinicalTrials.gov.

Data Handling and Recordkeeping

Data collection will be accomplished via case report forms and electronic recording via the devices used in this study. The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. Participant's data will be de-identified using codes, personal identifiers will be kept separate from study data, and data will be locked in file cabinets or drawers in the TMS laboratory and in the PI's office, if hardcopy, or on a password-protected UAMS server, both located behind locked doors in a restricted access area of the UAMS campus. Only authorized individuals will have access to the code and information that identifies the subjects in this study. Following informed consent to participate, patient's hospital and outpatient records may be reviewed to identify appropriate candidates for the study. Any data obtained as part of the patient's clinical care may be reviewed to determine whether the subject meets inclusion and does not meet exclusion criteria.

Data Analysis

Analyses and Power calculations.

Aims 1 & 2 examine the effect of treatment dose on one primary outcome measure – a difference score between sham and active rTMS in the LMFP of TEPs (Δ LMFP) recorded from TC and DLFC. The analysis plan is the same for both aims. Hierarchical Linear Models (HLMs) will be used due to the nested structure of the design. HLMs will fit the complex covariance structure of the dosing schedule – sham and active tDCS nested within rTMS treatment frequency (1 Hz and 10 Hz). Four primary responses will be estimated, namely Δ LMFP for the sham vs active tDCS and the 1 Hz vs 10 Hz rTMS conditions. From these four responses, four contrasts of interest will be estimated: (1) sham vs active tDCS for 1 Hz rTMS, (2) sham vs active tDCS for 10 Hz rTMS, (3) 1 Hz vs 10 Hz rTMS for sham tDCS, and (4) 1 Hz vs 10 Hz rTMS for active tDCS. An additional 6 contrasts will be used to examine differences in the means for the rTMS post 1 (acute) and post 2 (lasting) time periods. The HLM methodology will estimate the means and standard errors for all these contrasts of interest with a Bonferonni comparison procedure to adjust for the multiple contrasts.

Power Estimates

One purpose of the pilot study is to collect TEP data for a power analysis because such data does not currently exist. To estimate power for the pilot study, effect sizes for the contrasts of interest in Aims 1 and 2 were estimated from the means and SDs of MEP amplitudes in two published studies that used anodal, 1 mA tDCS to precondition the motor cortex before rTMS was delivered a 1 Hz[28] or 5 Hz[3] (power estimates for 10 Hz stimulation were based on the 5 Hz study). As in our design, change in cortical

excitability was measured both immediately following rTMS (acute) and 30 minutes later (lasting effects). PASS 12 was used to calculate power by estimating power, conservatively, as two-sample t-tests (n=5 for all comparisons). For 1 Hz TMS, the power to detect a difference in MEP amplitude between the sham (mean=.89, SD=.08) and active (.64; .08) tDCS conditions was 99% acutely (effect size=2.95) and 48% at 30 minutes (effect size=95%). For the 5 Hz condition, power to detect a difference in MEP amplitude between sham (.99; .16) and active (.98; .16) tDCS was only 6% acutely (effect size=.06) but 100% at 30 minutes (effect size=4.80). Finally, the power to detect a difference in MEP amplitude between 1Hz (.64; .08) and 10Hz (.98; .16) rTMS (after active tDCS preconditioning) was 94% acutely (effect size=2.68) but only 8% at 30 minutes (effect size=.39). Importantly, anodal preconditioning induced LTD (a decrease in the MEP amplitude) in both the 1 and 5 Hz conditions but, consistent with the hypothesis for Aims 1 & 2, the effect was stronger for 1 Hz than 5 Hz rTMS. These estimates give confidence that the sample sizes of our pilot study are sufficient to test Aims 1 & 2.

Aim 3 is being conducted as an open label feasibility study. The data may be used to derive power estimates for future studies. Subjects in this study will have completed sham and active 1 Hz rTMS treatments for tinnitus in a previous study (109033). The length of treatment, time of assessments and ratings of tinnitus used for Aim 3 are based on the previous study (109033). Therefore, we will compare change scores from baseline derived for the ratings of tinnitus in Aim 3 to changes scores from baseline derived from the sham and active 1Hz rTMS treatments in study 109033. HLMs will be used to compare mean scores for each treatment condition to provide an estimate of effect size which can be used for power calculations.

To examine change in cortical excitability following treatment, the LMFP of TEPs derived for regioins of the DLFC and temporal cortex will be compared before and after treatment using paired t-tests.

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and

requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. This consent form must be signed by the subject or legally authorized representative, and the individual obtaining the consent. A copy of the signed consent will be given to the participant, and the informed consent process will be documented in each subject's research record. Subjects will be recruited through direct advertisement. Additionally, we plan to use the Translational Research Institute's ARresearch tool during the recruitment process. We intend to call potential research participants who have provided phone numbers and to send our study advertisement via email to potential research participants who have provided email contact information.

The office of research and regulatory affairs, acting on behalf of the sponsor, UAMS, monitors study procedures independently.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

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