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PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Treating Deep Seizure Foci with Noninvasive Surface Brain Stimulation		
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B1. PURPOSE OF PROTOCOL

The overall goal of this study is to open up the promising treatment of repetitive transcranial magnetic stimulation (rTMS), which has been shown to be effective against seizures in patients with surface neocortical foci, to a much larger population of patients with mesial temporal lobe epilepsy (MTLE) and other forms of epilepsy with deep foci, who are not currently considered good rTMS candidates.

We hypothesize that rTMS can modulate the hyperexcitable state in patients with deep seizure foci by targeting its usage to accessible cortical partner regions. In this study we aim 1) to map the functional connectivity of the epileptogenic mesial temporal lobe in patients with medically refractory mesial temporal lobe epilepsy; and 2) to perform a randomized controlled assessment of repetitive transcranial magnetic stimulation protocols applied to specific neocortical targets in mesial temporal lobe epilepsy. The methods used in this study will include magnetic resonance imaging (MRI) of the brain, electroencephalography (EEG), and transcranial magnetic stimulation (TMS).

Our proposal, if successful, would open up this promising treatment to the large population of patients with MTLE, who are not currently considered good rTMS candidates.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Overview

Neuromodulation with rTMS has been effective in seizure patients with cortical foci

Early clinical trials of rTMS as a therapeutic modality in medically refractory epilepsy showed only mixed seizure outcomes, but they varied substantially in stimulation protocol, determination of target location, and selection of epilepsy patients. [1,2,3] More recently, it has become clear that patients with discrete seizure foci at the neocortical surface, particularly those with anatomically visible dysplastic lesions that allow for straightforward targeting of TMS, can dramatically benefit from repeated sessions of low-frequency (0.5 Hz or 1 Hz) rTMS, with striking improvements in seizure and IED frequency that remain sustained for many weeks.[4,5]

Unfortunately, many refractory forms of epilepsy involve deeper areas of the brain

MTLE is the most common new-onset focal epilepsy in adulthood, and frequently leads to medically uncontrolled seizures. Resective surgery may be a good therapeutic option for some patients, but not for all, due to potential postoperative neurological compromise, bilateral seizure onset, or patient

preference. Focal neuromodulation could theoretically be of significant benefit in MTLE, but handheld rTMS has not demonstrated efficacy in this condition[2], despite the marked benefit seen in patients who have neocortical foci that are more easily accessible to rTMS targeting.[4] This is unsurprising given that the physiological effect of TMS on current densities in the brain may not extend more than 1 cm deep to the cortical surface.[6]

Cortical targets for TMS can be identified based on connectivity imaging in a model epilepsy disorder
The unique developmental brain malformation of periventricular nodular heterotopia (PNH) offers an opportunity to study epileptogenic circuits and focal hyperexcitability in an anatomically well-characterized and often genetically determined disorder that leads to seizures after a latency of 20 years from birth, on average.[7] We have demonstrated that the subcortical nodules of heterotopic gray matter in PNH are structurally and functionally connected to discrete foci of overlying cerebral cortex, as established by diffusion tensor tractography and resting-state functional connectivity imaging.[8] The strength of abnormal connectivity is higher among patients with longer durations of epilepsy, suggesting a link between the development of these circuits and the process of epileptogenesis. In addition, we have shown that periventricular nodules can be metabolically coactivated with overlying cortical regions during the performance of specific cognitive tasks, supporting the notion that heterotopia become integrated into functional cortical circuits.[9]
We have recently identified altered physiological responsiveness in the cerebral cortex of patients with PNH; these changes appear to be specifically limited to cortical regions that have aberrant connectivity to deep regions of gray matter heterotopia. The nature of these functional abnormalities, which are characterized by an augmented late response to single-pulse TMS, is most consistent with cortical hyperexcitability, and supports the hypothesis that aberrant connectivity leads to epileptogenesis through changes in network physiology.

There is clinical precedent for using rTMS to modulate networks involving deep gray matter
Currently, rTMS is approved for therapeutic usage in patients with medically refractory depression. Specifically, targeting the dorsolateral prefrontal cortex (DLPFC) with rTMS over repeated sessions can decrease symptoms and signs of depression in those who have not been helped sufficiently by medications. The neurobiological basis of this effect is thought potentially to rest on the known connectivity between the DLPFC and other gray matter regions (including the deep subgenual cortex of the cingulate gyrus, for example) in networks important for mood regulation. [10.11] Therefore, there is already precedent for using surface-targeted rTMS to exert a modulatory influence on deeper brain structures, as we propose to do in patients with medically refractory MTLE.

The optimal rTMS protocol to achieve a reduction in brain hyperexcitability has not been determined
While low-frequency rTMS is known to decrease local cortical excitability, high-frequency rTMS increases such excitability. Low-frequency rTMS has had mixed results in clinical epilepsy trials, but dramatic improvement has been seen in patients with accessible cortical foci.[5,4] When rTMS is used in medication-refractory depression, high-frequency rTMS is typically used for the left DLPFC, while low-frequency rTMS is used for the right DLPFC as an alternative option. Based on our preliminary data with rTMS in PNH and in normal individuals, we hypothesize similarly that using differential rTMS protocols for different cortical targets in MTLE, identified based on the nature of their connectivity to the epileptogenic mesial temporal lobe, will successfully downmodulate hyperexcitability in MTL circuits and thus treat the epileptic state.

Alternate rTMS protocols may be directly compared in some individuals to determine their effect on

brain hyperexcitability
In some individuals, the brain excitability data acquired after a particular rTMS protocol may be compared directly to the data acquired after an alternate rTMS protocol in the same individuals, depending on brain connectivity, thus allowing for additional information on the intraindividual variability of response to rTMS.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Overview

The overall goal of this study is to open up the promising treatment of repetitive transcranial magnetic stimulation (rTMS), which has been shown to be effective against seizures in patients with surface neocortical foci, to a much larger population of patients with mesial temporal lobe epilepsy (MTLE) and other forms of epilepsy with deep foci, who are not currently considered good rTMS candidates. We plan to recruit thirty subjects with MTLE from our Comprehensive Epilepsy Center and through referrals from other clinicians. Subjects will undergo a series of noninvasive investigations, including high-resolution brain MRI, EEG, and rTMS. We plan to obtain blood oxygenation level-dependent (BOLD) functional magnetic resonance (MR) images in 30 patients with refractory MTLE during the task-free resting state. We will create a map for each patient of selected neocortical foci that are highly correlated and anti-correlated to the epileptogenic MTL by using a population-based map of normal resting-state MTL connectivity as a seed network of interest. We will conduct a controlled assessment of two different rTMS protocols applied to specific cortical targets (derived from connectivity imaging results) in the 30 patients with medically refractory MTLE. We will randomize subjects into three treatment groups: In Group 1, we will target low-frequency rTMS to cortical regions that are highly correlated with the epileptogenic MTL. In Group 2, we will target high-frequency rTMS to cortical regions that are highly anti-correlated with the epileptogenic MTL. Group 3 will receive sham rTMS as a control intervention. At 12-week follow-up, outcome measures will be seizure frequency (primary) and interictal epileptiform discharge (IED) frequency (secondary). All subjects will have EEG recordings prior to the first TMS session, after the last session, and at 12-week follow-up. Subjects will be blinded to their treatment group assignment. Subjects and family members or caregivers will be asked to maintain diaries of all seizures and possible adverse effects, which will be reviewed by an investigator blinded to treatment group assignments. For each subject, the MRI will be performed in one 1-hour session on one day, the EEGs will be performed in three 1-hour session on three separate days (pre-rTMS, post-rTMS and 12 weeks after rTMS) and rTMS will occur over ten daily 30-minute sessions.

Overall Study Design

Subject Enrollment
We will recruit 30 subjects from our Comprehensive Epilepsy Center and through referrals from other clinicians. The patients may be initially informed on of several ways:

- The investigator will briefly explain this study to them, and ask if they would like to hear more about it.
- The potential subject may be approached at the time of a clinic appointment. At that time, the investigator may briefly explain the study.
- The potential subject may receive a telephone call from an investigator on this study. At the time of the telephone call, the investigator may briefly explain the study.

In either case, a "voided copy" of the consent form will be mailed to the potential subject, or handed to them in person if possible. This will allow adequate time to read the consent form in detail, record questions, and consider the option of enrollment.

At the time of consent, randomization will occur based on order of enrollment into the study using a computer-generated randomization table. Subjects will be randomized into three treatment groups. In Group 1, we will target continuous low frequency rTMS (1 Hz, at 95% resting motor threshold (RMT), over ten daily 30-minute sessions) to cortical regions that are highly correlated with the epileptogenic MTL. In Group 2, we will target high-frequency rTMS (10 Hz, 110% RMT, over ten daily 30-minute sessions) to cortical regions that are highly anti-correlated with the epileptogenic MTL. Group 3 will receive sham TMS as a control intervention (using a sham coil over a highly correlated cortical region, over ten daily 30-minute sessions).

Subjects will be blinded to their treatment group assignment. Subjects and family members or caregivers will be asked to maintain diaries of all seizures and possible adverse effects, which will be reviewed by an investigator blinded to treatment group assignments.

1. MRI scanning and data analysis (One hour to complete, on one day)

After subjects have been randomized, they will undergo brain connectivity imaging. We will obtain BOLD functional MRI in 30 patients with refractory MTLE during the task-free resting state. We will systematically determine for each patient the neocortical foci that are highly correlated and anticorrelated to the epileptogenic MTL by using a population-based map of normal resting-state MTL connectivity as a seed network of interest. This will allow us to selectively identify regions of relevant correlation to the MTL that fall within easily accessible areas for rTMS based on our technical experience (including most of the frontal, parietal, and lateral temporal cortex, while avoiding the frontopolar and occipital regions due to head positioning and the need for eyeglasses worn to calibrate the TMS neuronavigation system). **Subject time: 1 hour.**

2. Baseline seizure diary (12 weeks)
Subjects will keep a baseline seizure diary for 12 weeks, during which time connectivity analyses and tMS target creation will be performed by the investigators. **Subject time: 12 weeks.**

3. Pre-rTMS EEG (One hour to complete, on one day)
EEG recording of interictal epileptiform discharge (IED) frequency will occur prior to first TMS session. **Subject time: 1 hour.**

4. Experimental Design for rTMS (On average one and a half hours to complete, on ten consecutive weekdays)
We will conduct a controlled assessment of two different rTMS protocols applied to specific cortical targets (derived from connectivity imaging results) in the 30 patients with medically refractory MTLE. The cohort had been randomized into three treatment groups. In Group 1, we will target continuous low-frequency rTMS to cortical regions that are highly correlated with the epileptogenic MTL. In Group 2, we will target high-frequency rTMS to cortical regions that are highly anti-correlated with the epileptogenic MTL. Group 3 will receive sham TMS as a control intervention. Each subject will have ten 30 minute rTMS sessions over a two-week period, ten consecutive weekdays. **Subject time: 1.5 hours per rTMS session. 10 rTMS sessions.**

5. Post-rTMS EEG (One hour to complete, on one day)
EEG recording of interictal epileptiform discharge (IED) frequency will occur after the last TMS session. **Subject time: 1 hour.**

6. Follow-up seizure diary (12 weeks)
Subjects will keep a follow-up seizure diary for 12 weeks post rTMS. **Subject time: 12 weeks.**

7. Final EEG (One hour to complete, on one day)
EEG recording of interictal epileptiform discharge (IED) frequency will occur 12 weeks after the last rTMS session. **Subject time: 1 hour.**

At 12-week follow-up, the primary outcome measure will be seizure frequency compared to baseline, and the secondary outcome measure will be IED frequency compared to baseline. Subjects will be blinded to their group assignment, as will the investigator who reviews seizure diaries and determines IED frequency on EEG. Adverse events will be tracked closely throughout the study protocol.

Follow-Up
The investigators will conduct a controlled assessment of the response to the initially chosen rTMS protocol and determine if the subject may be appropriate for further rTMS. Investigators may invite subjects back to repeat the study procedures 2-7. The follow-up rTMS protocol chosen by investigators will depend on the subject's response, or lack thereof, to the previously performed protocol.

Subjects who are invited back for further rTMS sessions and choose to return will undergo the following specific procedures:

1. Baseline seizure diary (12 weeks)
Subjects will keep a baseline seizure diary for 12 weeks, during which time connectivity analyses and tMS target creation will be performed by the investigators. **Subject time: 12 weeks.**

2. Pre-rTMS EEG (One hour to complete, on one day)
EEG recording of interictal epileptiform discharge (IED) frequency will occur prior to first TMS session. **Subject time: 1 hour.**

3. Experimental Design for rTMS (On average one and a half hours to complete, on ten consecutive weekdays)
Based on analyses of the subject's response to the initially assigned rTMS protocol, the alternative protocol (either low-frequency rTMS to cortical regions that are highly correlated with the epileptogenic MTL or high-frequency rTMS to cortical regions that are highly anti-correlated with the epileptogenic MTL) will be used. Each subject will have ten 30 minute rTMS sessions over a two-week period, ten consecutive weekdays. **Subject time: 1.5 hours per rTMS session. 10 rTMS sessions.**

4. Post-rTMS EEG (One hour to complete, on one day)
EEG recording of interictal epileptiform discharge (IED) frequency will occur after the last TMS session. **Subject time: 1 hour.**

5. Follow-up seizure diary (12 weeks)

Subjects will keep a follow-up seizure diary for 12 weeks post rTMS. **Subject time: 12 weeks.**

6. Final EEG (One hour to complete, on one day)
EEG recording of interictal epileptiform discharge (IED) frequency will occur 12 weeks after the last rTMS session. **Subject time: 1 hour.**

Additional Follow-Up Analyses
At 12-week follow-up after the second rTMS protocol, the primary outcome measure will be seizure frequency and the secondary outcome measure will be IED frequency. Both of those measures will be compared both to the pre-rTMS baseline and to the measures following the initial rTMS protocol. Subjects will be blinded to their group assignment, as will the investigator who reviews seizure diaries and determines IED frequency on EEG. Adverse events will be tracked closely throughout the study protocol.

Details of methods to be used

Brain image acquisition
Thirty subjects with MTLE will be imaged on a dedicated research 3.0-Tesla magnet (General Electric Signa VH/i, GE Healthcare, Waukesha, WI) with a commercial head coil. High resolution structural whole-brain images will be acquired using a T1-weighted sequence with 128 slices per slab, a 256 x 256 matrix, a field of view (FOV) of 256 mm, a slice thickness of 1.33 mm with 0.63 mm interslice gap, repetition time (TR) of 2530 ms, inversion time of 1100 ms, echo time (TE) of 3.39 ms, and flip angle of 7 degrees. Resting-state functional image acquisition will be performed while subjects are asked to rest quietly (acquisition time 6.4 min), using an echo-planar sequence sensitive to BOLD contrast with TR 6000 ms, TE 30 ms, FOV 256 mm, voxel size 2.0 x 2.0 x 2.0 mm, and flip angle 90 degrees.

rTMS protocol
Stimulation will be performed with a Nexstim eXimia TMS stimulator with real-time MRI neuronavigation (Nexstim NBS software version 3.2.1, Nexstim Ltd, Helsinki, Finland) and a figure-of-eight coil (mean diameter 59 mm, outer diameter 70 mm). Resting motor threshold (RMT) will be determined via surface electromyography recorded using pre-gelled disposable Ag/AgCl electrodes, with the active electrode over the first dorsal interosseus (FDI) muscle, the reference electrode over the metacarpophalangeal joint, and the ground electrode over the wrist. RMT will be conventionally defined as the minimum stimulus intensity that elicits a motor evoked potential of at least 50 μV in at least five out of ten trials. Low-frequency rTMS will employ a 1-Hz, 95% RMT protocol for 30-minute sessions delivered once each day over ten consecutive weekdays. High-frequency rTMS will employ a 10-Hz, 110% RMT protocol delivered similarly over ten days. Sham TMS will employ a specially fabricated coil that provides no magnetic stimulation but has a similar appearance and creates an auditory artifact that mimics TMS, and will be targeted toward a highly correlated cortical target.

EEG analysis
EEG will be recorded using a 60-channel TMS-compatible system (eXimia EEG, Nexstim Ltd, Helsinki, Finland). EEG signals will be referenced to an additional electrode on the forehead, filtered (0.1 – 500 Hz) and sampled at 1450 Hz with 16-bit resolution. Two extra sensors will be used to record electrooculogram, and electrode impedance will be kept below 5 kΩ at all times. All recordings will be at least 60 minutes in duration, and subjects will be kept awake to control for the effects of

sleep on IED frequency. Each EEG recording will be inspected visually and IEDs will be counted for the total duration of the artifact-free EEG by an investigator board-certified in clinical neurophysiology and blinded to subjects' treatment group assignments.

B. Statistical Considerations

Sample Size Justification:

Our expected statistical analyses will be based on intention to treat and will include an analysis of variance (ANOVA) with the factors Group (low-frequency vs. high-frequency vs. sham) and Time (baseline vs. follow-up) with repeated measures on time, to assess both seizure frequency (primary outcome) and IED frequency (secondary outcome). If indicated, post hoc analyses, corrected for multiple comparisons using the Bonferroni method, will be performed. Based on results from the two most directly relevant rTMS epilepsy studies in the literature[5,4], we estimate the effect size of a potential decrease in mean seizure frequency at 56%. To achieve 80% power, with a statistical threshold of $\alpha < 0.05$ and three equal treatment groups, the total sample size needed would be 27 subjects[12], so we plan to enroll 30 subjects.

According to the Epilepsy Foundation, temporal lobe epilepsy accounts for approximately 60% of all patients with epilepsy and MTLE accounts for 80% of all patients with temporal lobe epilepsy. MTLE is the most common form of refractory epilepsy in the patient population at BIDMC. At BIDMC, there are more than 3500 outpatient epilepsy visits annually with refractory epilepsy accounting for an estimated 1166 of those patients. Accordingly, we would estimate approximately 560 MTLE outpatient visits annually, likely representing more than 140 unique MTLE patients. Therefore, even accounting for refractory MTLE patients who are not eligible or who decline to participate, we anticipate that we would be able to recruit and enroll 30 subjects over the projected multi-year time period of this study.

Data Analysis.

We will obtain BOLD functional MRI in 30 patients with refractory MTLE during the task-free resting state. We will systematically determine for each patient the neocortical foci that are highly correlated and anticorrelated to the epileptogenic MTL by using a population-based map of normal resting-state MTL connectivity as a seed network of interest. This will allow us to selectively identify regions of relevant correlation to the MTL that fall within easily accessible areas for rTMS based on our technical experience (including most of the frontal, parietal, and lateral temporal cortex, while avoiding the frontopolar and occipital regions due to head positioning and the need for eyeglasses worn to calibrate the TMS neuronavigation system).

We expect to demonstrate pathological connectivity involving the MTL and specific cortical regions; this will be a major step in understanding MTLE pathogenesis and in defining targets for stimulation. We will use an in-house functional connectivity software toolbox for analysis, as we have done for our Preliminary Data. Briefly, our steps include realigning, normalizing, and coregistering functional images, followed by segmentation and BOLD signal extraction. Band-pass filtering and smoothing will be applied, and possible confounders removed. A population-based map of MTL connectivity (derived from normal individuals), with rTMS-accessible regions removed, will be used as a seed network to create color-coded functional connectivity maps identifying rTMS-accessible areas of significant correlation and anti-correlation to the average BOLD time-series within the MTL network,

subject to a voxel-wise statistical threshold and cluster thresholding.

We will then conduct a controlled assessment of two different rTMS protocols applied to specific cortical targets (derived from connectivity imaging results) in the 30 patients with medically refractory MTLE. The cohort will be randomized into three treatment groups. In Group 1, we will target continuous low frequency rTMS (1 Hz, at 95% resting motor threshold (RMT), over ten daily 30-minute sessions) to cortical regions that are highly correlated with the epileptogenic MTL. In Group 2, we will target high-frequency rTMS (10 Hz, 110% RMT, 10 daily sessions) to cortical regions that are highly anti-correlated with the epileptogenic MTL. Group 3 will receive sham TMS as a control intervention (using a sham coil over a highly correlated cortical region). At 12-week follow-up, the primary outcome measure will be seizure frequency compared to baseline, and the secondary outcome measure will be IED frequency compared to baseline. Subjects will be blinded to their group assignment, as will the investigator who reviews seizure diaries and determines IED frequency on EEG. Adverse events will be tracked closely throughout the study protocol.

We expect to downmodulate the excitability of the epileptogenic MTL, thus reducing seizure and IED frequency. Although it is difficult to predict the magnitude of such an effect given the unique nature of our targeting based on connectivity findings, prior rTMS studies in epilepsy have shown seizure frequency reductions in the 53-80% range, durable over at least eight weeks of follow-up, and IED frequency reductions in the 14-66% range. [4,5] Our use of EEG recordings immediately pre- and post-rTMS, as well as at the end of the follow-up period, will allow for an estimation of immediate and delayed/durable effects on the epileptic state in these patients. We expect our results to prove the principle that patients with deep seizure foci can be safely treated by targeting accessible cortical regions and to provide guidance on which active rTMS protocol (high- or low-frequency) is optimal for MTLE.

Our analysis following the second rTMS protocol, in those subjects who participate in that portion of the study, will provide further evidence of the relative efficacy of the two rTMS protocols in downmodulating cortical excitability.

Brain image acquisition

Anatomical and functional connectivity MR images will be acquired using a dedicated research 3.0-Tesla magnet (General Electric Signa VH/i, GE Healthcare, Waukesha, WI) with a commercial head coil. Highresolution structural whole-brain images will be acquired using a T1-weighted sequence with 128 slices per slab, a 256 x 256 matrix, a field of view (FOV) of 256 mm, a slice thickness of 1.33 mm with 0.63 mm interslice gap, repetition time (TR) of 2530 ms, inversion time of 1100 ms, echo time (TE) of 3.39 ms, and flip angle of 7 degrees. Resting-state functional image acquisition will be performed while subjects are asked to rest quietly (acquisition time 6.4 min), using an echo-planar sequence sensitive to BOLD contrast with TR 6000 ms, TE 30 ms, FOV 256 mm, voxel size 2.0 x 2.0 x 2.0 mm, and flip angle 90 degrees.

Functional connectivity analysis

Functional connectivity imaging analyses will be performed using an in-house software toolbox, according to methods previously described. [8] Briefly, functional images will be realigned, normalized, and co-registered to an anatomic image. Functional images will be segmented and BOLD signal extracted. A band-pass frequency filter ($0.01 \text{ Hz} < f < 0.1 \text{ Hz}$) will be applied, and Gaussian smoothing performed (6 mm full width at half maximum). Several possible confounding sources of noise will be removed. A population-based map of right or left MTL connectivity derived from normal

individuals, with rTMS-accessible regions of cortex removed, will be used as a seed network of interest for analyses. Color-coded functional connectivity maps will be created for each subject, showing correlations to the average BOLD signal time series of the MTL network, subject to a voxel-wise statistical threshold of $p < 0.001$ and cluster thresholding with an intensity cutoff.

Stimulation target creation

For each subject, a neocortical target for TMS will be created based on the functional connectivity results and the subject's group assignment (significantly correlated region for Groups 1 and 3, and significantly anti-correlated region for Group 2). The target will be manually outlined using MRICroN software[13] as a discrete region of cortex that demonstrates significant connectivity to the MTLE network seed, based on the analyses derived above. Targets will then be transferred directly into the TMS neuronavigation software for visualization during the stimulation paradigm.

rTMS protocol

Stimulation will be performed with a Nexstim eXimia TMS stimulator with real-time MRI neuronavigation (Nexstim NBS software version 3.2.1, Nexstim Ltd, Helsinki, Finland) and a figure-of-eight coil (mean diameter 59 mm, outer diameter 70 mm). Resting motor threshold (RMT) will be determined via surface electromyography recorded using pre-gelled disposable Ag/AgCl electrodes, with the active electrode over the first dorsal interosseus (FDI) muscle, the reference electrode over the metacarpophalangeal joint, and the ground electrode over the wrist. RMT will be conventionally defined as the minimum stimulus intensity that elicits a motor evoked potential of at least 50 μV in at least five out of ten trials. Low-frequency rTMS will employ a 1-Hz, 95% RMT protocol for 30-minute sessions delivered once each day over ten consecutive weekdays. High-frequency rTMS will employ a 10-Hz, 110% RMT protocol delivered similarly over ten days. Sham TMS will employ a specially fabricated coil that provides no magnetic stimulation but has a similar appearance and creates an auditory artifact that mimics TMS, and will be targeted toward a highly correlated cortical target.

EEG analysis

EEG will be recorded using a 60-channel TMS-compatible system (eXimia EEG, Nexstim Ltd, Helsinki, Finland). EEG signals will be referenced to an additional electrode on the forehead, filtered (0.1 – 500 Hz) and sampled at 1450 Hz with 16-bit resolution. Two extra sensors will be used to record electrooculogram, and electrode impedance will be kept below 5 kΩ at all times. All recordings will be at least 60 minutes in duration, and subjects will be kept awake to control for the effects of sleep on IED frequency. Each EEG recording will be inspected visually and IEDs will be counted for the total duration of the artifact-free EEG by an investigator board-certified in clinical neurophysiology and blinded to subjects' treatment group assignments.

C. Subject Selection

All subjects will be between 18 and 64 years old and have a diagnosis of MTLE based on the combination of clinical semiology, neuroimaging findings, and EEG results. Subjects with ≥ 1 seizure with loss of awareness per 4-week period, on average, despite the use of antiepileptic drugs (AEDs), will be eligible. AEDs will not be adjusted during the study period, except in compelling clinical circumstances. Exclusion criteria are: 1) prior brain surgery or exposure to TMS; 2) rapidly progressive brain lesions (but low-grade gliomas, cystic lesions, mesial temporal sclerosis, or other static or slowly progressive lesions would be eligible); and 3) inability to tolerate MRI or TMS, or

specific MRI or TMS contraindication as set forth in standard protocols of our institution. Women of childbearing potential will be asked if they may be pregnant, questioned about the use of contraceptives if they are sexually active, and asked to identify the date of their last menstrual period. If investigators deem necessary, urine pregnancy testing may be performed before enrollment in the study.

The planned enrollment demographics for our study are derived from the demographic representation of the Greater Boston area (U.S. Bureau of the Census, 2000), from which the majority of our human subjects will be drawn. We thus expect to enroll approximately 10% Hispanic or Latino subjects and 90% non-Hispanic/Latino. Among racial categories, we expect to enroll approximately 75% white subjects, 10% black or African-American, and <5% each American Indian/Alaska native, Asian, and Native Hawaiian or Other Pacific Islander. No sex/gender or racial/ethnic groups will be excluded from this study.

Our subject selection criteria are based purely on age (18-64); diagnosis of MTLE; and nonfulfillment of specific exclusion criteria. MTLE appears to be evenly distributed across racial/ethnic lines. One of our exclusion criteria is pregnancy (due to lack of definitive information on fetal safety in MRI), but we do not believe this will independently affect the sex/gender composition of our enrolled subjects in any significant way.

We will recruit subjects from our Comprehensive Epilepsy Center and through referrals from other clinicians. Randomization will occur based on order of enrollment into the study using a computer-generated randomization table. Subjects will be blinded to their treatment group assignment. Subjects and family members or caregivers will be asked to maintain diaries of all seizures and possible adverse effects, which will be reviewed by an investigator blinded to treatment group assignments. All subjects will provide written informed consent in accordance with research protocols that will be approved by the institutional review board of Beth Israel Deaconess Medical Center.

B4. POSSIBLE BENEFITS

We do not necessarily expect this study to yield direct benefits to the participating subjects. However, repetitive transcranial magnetic stimulation (rTMS) has been shown to be effective against seizures in patients with surface neocortical foci; therefore, there is a possibility that a larger population of patients with mesial temporal lobe epilepsy and other forms of epilepsy with deep foci would benefit from improved seizure control as a result of treatment of rTMS. Additionally, a thorough understanding of when and how to treat with rTMS would lead to improved treatment options.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

Possible Risks

Brain imaging

There are no known or foreseeable risks or side effects associated with conventional MRI except to those people who have electrically, magnetically, or mechanically activated implants (such as cardiac pacemakers) or to those who have clips on blood vessels in their brain. Subjects will therefore be screened very carefully to exclude the possibility that they have any such devices and/or implants and will be excluded from participation if they do. We will employ our institution's standard research MRI safety questionnaire in advance of enrollment.

There are no known additional risks associated with functional MRI. Both the conventional and functional MRI systems have been approved by the FDA and will be operated within the standards reviewed and accepted by the FDA. The Center for Biomedical Imaging's usual safety guidelines concerning MRI will be in place at all times.

MRI might be uncomfortable if subjects are prone to claustrophobia, do not like to lie still for a period of time, or do not like banging or beeping sounds. The investigator will explain the procedure and if potential subjects express any doubt about these issues, they will not be included in the study. Investigators will remain in continual verbal contact with all subjects while in the scanner; if subjects indicate that they feel uncomfortable during the scan, they can activate the emergency button and can be taken out of the scanner at any time.

Scalp EEG Recording

Routine EEG is performed by applying electrodes to the scalp using a standard removable adhesive. In our experimental protocol, Ag-Ag/Cl scalp electrodes that are TMS-compatible and have been used extensively in human subjects[14] will be employed. On rare occasions patients may have an irritative scalp reaction to the electrodes or adhesive, but this is very uncommon, and would result in discontinuation of the test if necessary. No electrical current is passed through the electrodes, and the recording of cortical potentials occurs painlessly without the need for any particular effort on the subjects' part other than quiet rest. No activation procedures, such as intermittent photic stimulation or hyperventilation, will be used. Our institution's usual clinical safety guidelines concerning scalp EEG recording will be in place at all times.

Transcranial magnetic stimulation

TMS has been used in a growing number of laboratories worldwide since 1984. A series of adverse events have been identified. Guidelines for the safe use of rTMS were published in 1993 by Pascual-Leone et al. and were updated at the First International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation held in June 1996 in Bethesda, Maryland. At this workshop, a revised table for maximum single rTMS train duration, depending on rTMS frequency and intensity, for the safe application of rTMS, was agreed upon.[15] The proposed study uses rTMS parameters well within these safety guidelines. However, the following side effects require mention:

1. Up to 20-40% of individuals undergoing TMS experience headaches or neck pain, which are

believed to be due to excessive muscle tension. In the case of such an event, subjects will be offered acetaminophen, which in prior cases of headache or neck ache induced by TMS has promptly resolved the discomfort.

2. TMS produces a loud clicking sound when the current is passed through the stimulation coil. This loud click can result in ringing in the ears and short-term decreased hearing if no protection is used. In order to prevent this potential adverse effect, subjects will be given earplugs. Animal and human studies have demonstrated that earplugs can effectively prevent the risk of hearing disturbance due to TMS.

   In one case a subject's hearing protection fell out and resulted in permanent hearing loss during a 1Hz rTMS session at 120% of the motor threshold. The authors were using a special TMS coil called the H-coil in which the subject's head is inside a large helmet. However, the authors claimed that the loudness of the H-Coil they were using was not different from other coils.[16]

   In order to better inform and protect the subjects, we will take the following precautions:
   a. Advise subjects of the risk of permanent hearing loss if an earplug should loosen, become detached, or fall out.
   b. Advise subjects that they should immediately report to the investigator any loosening or detachment of an earplug during TMS.
   c. Inform subjects that investigators will immediately stop TMS if the subject reports or if an investigator observes that an earplug has loosened or has fallen out.
   d. Prompt referral for auditory assessment of all individuals who complain of hearing loss, tinnitus, or aural fullness following completion of TMS.

3. The risk of seizure during TMS is a well-studied one. In normal subjects, it is an extremely rare occurrence. Repetitive TMS can induce a seizure in the absence of pre-existing brain lesions, epilepsy, or other seizure risk factors, both in patients and healthy subjects. From the several thousands of studies that have used TMS to date, a total of 16 cases have been reported, of which 9 cases occurred after the 1998 safety guidelines. Based on the available data, the reported risk of seizures is less than 1 in 1000 for repetitive TMS. In individuals with a known history of seizures, the occurrence of a seizure temporally proximate to TMS is very uncommon; the overall rate of any seizure associated with single- and paired-pulse TMS in those with known epilepsy is only 0.8-1.8%, and the risk if no changes were made in anticonvulsant medications is only 0.0-0.4%. Similarly, even with rTMS in patients with known epilepsy, the crude risk of seizures associated with TMS was about 1.4%, and almost all seizures were identical to those typically experienced by the individual, making it unclear whether seizures were causally related to the TMS or not.[17] Almost all reported seizures predated the advent of current safety guidelines, to which our proposed study will adhere closely. No instances of status epilepticus have ever been reported in association with TMS.
4. Syncope can occur due to anxiety and psycho-physical discomfort during testing and treatment with TMS. This is reported less than seizure activity but the true number may be higher due to underreporting. Subjects will be monitored for feeling any signs or symptoms of a pending syncopal event (i.e. feeling "dizzy, lightheaded or going to pass out"). TMS will immediately be stopped and the subject will be assisted.
5. TMS could induce short-term changes in memory, attention, and other cognitive functions. This is a theoretical risk, as none of the safety studies conducted has found such side effects. However, because the understanding of brain connectivity in the population of patients with PNH is limited, the risk for these patients in particular is not clear.

6. Acute psychiatric effects such as mania and delusions have been described in patients with medically refractory depression or bipolar disorder who received rTMS. This seems to be a rare complication (incidence <0.15% of patients), and individuals with a current diagnosis of a mood disorder will be excluded from this study.
7. The possibility of dental pain during rTMS has been reported. This potential adverse effect would occur during the stimulation itself. Subjects will be asked to report any such discomfort immediately to the investigator, who would terminate the stimulation session. The subject will be encouraged to seek a dental evaluation, since this is a very rare occurrence but may point to the presence of a cavity that may require care. This adverse effect is not expected to lead to any lasting problems or complications.

Protection Against Risks

As described above, no significant health risks are expected from the neuroimaging procedures in this study. Standard research MRI safety procedures in place at Beth Israel Deaconess Medical Center will be used, including safety questionnaires prior to scanning (asking about surgeries, metal implants, shrapnel, etc.).

Multiple safety precautions are in place for the TMS portion of the study, including the design of the stimulation protocol in a way that is well within guidelines for rTMS safety, is consistent with prior studies that have demonstrated safety in a population of patients with refractory epilepsy.[4] All TMS sessions will be conducted in the presence of a board-certified neurologist who has been trained in the safe and efficient administration of TMS and in basic life support and in the recognition and treatment of seizures, syncope, and other neurological and medical emergencies. A fully equipped and regularly checked "crash cart" is available at Beth Israel Deaconess Medical Center for potential emergencies. This emergency equipment also includes oxygen supply, IV line supplies, and emergency medications in the event of a convulsion. Subjects will be monitored in detail during and after delivery of TMS, using an approach drawn directly from suggested guidelines.

Confidentiality will be preserved at all times, using standard HIPAA-compliant clinical measures already in place at the institution. As described above, only the PI and PI's research assistant will be aware of individual subject identities and have access to code number assignments.

The PI, a board-certified neurologist and clinical neurophysiologist, will be responsible for the patient's medical welfare should any unexpected adverse events occur during these research activities, and will intervene as appropriate.

Risk-Benefit Analysis

Given the limited foreseeable risks described above, the procedures to be put in place to protect against these risks, and the potential benefits to the subjects and others as described above, the risk/benefit ratio is acceptable for subjects.

Data and Safety Monitoring

All adverse effects, regardless of attribution to study procedures, will be collected from the start of the experimental protocol to the end of study participation, using standard adverse event forms. Subjects will be asked in an open-ended way about the presence of any adverse events; intensity will be graded

for each adverse event; and the likelihood that the event is or is not related to study participation will be noted.

A data safety monitoring board, comprising three investigators with expertise in the field, experience in conduct of human subject research and statistical knowledge, independence from the direct management of this study, and freedom from conflict of interest or commitment, will be appointed by the PI. A chairperson will be appointed and will be responsible for overseeing the meetings, developing the agenda, and summarizing the meeting. The chairperson will be the contact person for the DSMB.

The DSMB will meet prior to the enrollment of the first subject to review the research protocol, informed consent documents and plans for safety and data monitoring of the study. The DSMB will determine the risks and benefits to research subjects, protection and safety of the subjects and offer suggestions for improving the study design. In addition, the Board will reach agreement on the data that will be required for review. Determination of the schedule of future meetings, appointment of the chair and voting members, who receives minutes, and the signing of conflict of interest statements will occur during pre-enrollment meeting. The pre-enrollment Board meeting may result in modification of the safety plan provided in the CCI application. If the DSMP is changed at the first meeting of the Board, the new plan will be submitted to the BIDMC Committee on Clinical Investigations (CCI).

The DSMB will meet at least annually, and may decide to meet more frequently if necessary. The chairperson may call ad hoc meetings depending on safety or efficacy concerns. Meetings may be conducted by teleconference or over e-mail at the request of Board members. The DSMB will review interim data to detect evidence of efficacy or adverse effects to determine if the trial should continue as originally designed, should be changed or should be stopped based on the data. The DSMB will evaluate the progress of the trial, including periodic assessments of data quality/completeness, recruitment goals, protocol adherence, accrual and retention of participants and other factors that may affect the study outcome. The DSMB will protect confidentiality of the study participants, trial data and results of the monitoring.

Specifically, at each meeting, the PI will provide the Board with the information that was determined at the pre-enrollment meeting. The Board may then, according to a plan determined at the pre-enrollment meeting, review the following:

a. determine adherence to treatment plan
b. review interim analysis, if applicable, and determine specific data to be analyzed
c. evaluate end point/stop point rules
d. review protocol violations and deviations to assess adequacy of study
e. ensure documentation of informed consent
f. enrollment
    (1) followed eligibility criteria
    (2) enrollment numbers
    (3) visit compliance
    (4) screening failure information
g. review IND/IDE information
h. discuss investigator or key personnel changes
i. review completeness and quality of data collection forms
j. evaluate the aggregate analysis of adverse events/serious adverse events

k. review vital signs, clinical tests, etc.
l. review confidentiality

The major outcomes following data review include:

1. continuing the trial unchanged
2. modify the protocols and/or consent form
3. terminate the trial

Minutes from the meeting will be maintained. The investigator will not be present for at least part of this meeting. Following the Board meeting, a report will be provided to the investigator, the BIDMC CCI, and if necessary, study participants. The report will indicate whether the study should continue as originally designed, whether the study should be modified to protect patient safety or whether the study should be terminated.

All records regarding this research project will be stored in the locked office of the PI, in the Comprehensive Epilepsy Center of BIDMC. The data will be stored on paper and on secure computers protected with passwords. The subjects' identity will not be disclosed in publications or presentations. The records of the research project are open only to authorized monitors from the BIDMC CCI, the DSMB, and the FDA. All applicable local regulatory requirements relating to the reporting of adverse events will be followed during this study.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

We will recruit subjects from our Comprehensive Epilepsy Center and through referrals from other clinicians. The patients may be initially informed on of several ways:

- The investigator will briefly explain this study to them, and ask if they would like to hear more about it.
- The potential subject may be approached at the time of a clinic appointment. At that time, the investigator may briefly explain the study.
- The potential subject will receive a telephone call from an investigator on this study.

In either case, a "voided copy" of the consent form will be mailed to the potential subject, or handed to them in person if possible, prior to meeting for informed consent. This will allow adequate time to read the consent form in detail, record questions, and consider the option of enrollment.

Consent

Subjects will be contacted and introduced to the concept of the study. Those potential subjects who do not express interest in the study will be thanked and no further recruitment efforts will be pursued. For those who do express interest in the study, they will be given a copy of the consent form. The study procedure, risks and benefits, and alternatives will be explained by an MD designated to obtain consent on the study, and subjects will be encouraged to ask questions and discuss any concerns. The Investigator will then obtain informed consent and each subject will be given a copy of the signed informed consent form. Potential subjects will be reminded that participation in the study is completely voluntary and that they are free to terminate the study at any time, and that the Principal

Investigator will be available to contact for any questions that may arise during the study period.

Subject Protection
No vulnerable populations are included in this study.

B7. STUDY LOCATION

Privacy

In all portions of the study, provisions will be made to ensure that the privacy of the subjects are protected throughout. Recruitment of the subjects will take place by phone or in person in a private examination room with the door closed in a secure clinical area. If subjects wish not to speak on the phone or to be contacted at a different number or at a later time, their preferences will be accommodated. The information collected during recruitment will be limited only to the minimum amount of data necessary, namely to confirm that the subjects actually are eligible for the study according to the inclusion/exclusion criteria. The consent process will occur in person in a private examination room with the door closed in a secure clinical area.

Physical Setting

Recruitment and consent will occur at the Comprehensive Epilepsy Center of Beth Israel Deaconess Medical Center (PI's location).

MRI scanning and TMS experiments will be performed at the Berenson-Allen Center for Noninvasive Brain Stimulation at Beth Israel Deaconess Medical Center.

Data analysis will occur at BIDMC (PI's location), and the Berenson-Allen Center for Noninvasive Brain Stimulation at BIDMC (laboratory of Mouhsin Shafi, MD, PhD (Co-investigator). For data analysis, imaging datasets will be transferred either in person to the collaborating sites or electronically using secure Web-based file transfer protocols

B8. DATA SECURITY

Data obtained from out experiments will be assigned specific identifying code numbers so that they can be processed, analyzed, and reported without direct access to subjects' identities. Only the following individuals at Beth Israel Deaconess Medical Center need to know individual subject identities, and will have access to the code number assignments: the PI and the PI's research assistant. Electronic data containing PHI will be kept on a secure server behind the BIDMC firewall.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☐ No ☒ N/A

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☐ No ☒ N/A

B10 Dissemination of Research Results

The study staff will verbally thank research subjects at the conclusion of each study visit. At the end of the study, study staff will ask the subject if he or she is interested in knowing the results of the study. If the participant is interested, preliminary results will be reviewed during a phone call scheduled with the Principal Investigator.

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