Vagus Nerve Stimulation and Stress Reduction Training for Migraine

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Clinical Intervention Study Protocol

FULL PROTOCOL TITLE

Boosting mind-body mechanisms and outcomes for chronic pain

A randomized, placebo-controlled longitudinal trial evaluating the effects of combined Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) and Mindfulness Meditation (MM) in Migraine patients.

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IND#142546 for the use of [^{11}C] PBR28 in PET/MR imaging

Tool Revision History

Version Number: 1.0
Version Date: October 8, 2018
Summary of Revisions Made: first draft

Version Number: 1.2
Version Date: December 23, 2018
Summary of Revisions Made:

- Updates based on OCRA edits to randomization strategy

Version Number: 1.3
Version Date: June 1, 2019
Summary of Revisions Made:

- Study roster section was updated to include two new research coordinators
- Following recommendations from our EAB the inclusion and exclusion criteria for both healthy controls and migraine patients have been modified
- RAVANS tVNS stimulation parameters have been updated
- Consenting procedures have been modified to include a nurse practitioner or an MD in order to follow Partners IRB recommendations.
- The Education Control protocol has been modified to better match the duration and activities from the MBSR intervention
- A new activity (ambulatory ECG and actigraphy measurement) has been added to provide exploratory data on heart rate variability and motor activity during the intervention
- The list of psychometric assessments has been modified according to EAB recommendations
- A new task (Quantitative Sensory Testing) has been added to the Autonomic Assessment session to provide an additional measure of pain processing in study subjects
- Information about PBR affinity testing for PET imaging has been added
- StudyTRAX will be the electronic data capture system used in the study instead of RedCAP to follow with FDA regulations (21 CFR Part 11)

Version Number: 1.4
Version Date: August 2, 2019
Summary of Revisions Made:

- Incorporated changes from OCRA,Westat, and NCCIH.
- Updated participant randomization to occur before the baseline assessment.
- The Baseline visits have been changed from 2 weeks to 3 weeks.
- PET-MR criteria has been separated from the general exclusion and inclusion criteria.
- Added a more detailed explanation of what happens when participants are no longer eligible for the A-line after genotype testing.

- Study roster section was updated to include a phone number for new study staff and removed staff no longer involved in the study.
- The inclusion and exclusion criteria for both healthy controls and migraine patients have been modified to reflect IRB changes.
- Adherence for groups and booster sessions has been updated.
- Added a daily diary eligibility procedure.
- Added the Rally website as an additional resource for recruitment.
- Updated welcome letter details.

Version Number: 1.5
Version Date: August 22, 2019
Summary of Revisions Made:

- Included safety reporting process specific to an IND-regulated study.
- Clarified MD/NP procedures at screening.
- Updated study staff personnel.

Version Number: 1.6
Version Date: September 5, 2019
Summary of Revisions Made:

- Updated the age of inclusion criteria for healthy volunteers to match the inclusion criteria of migraine subjects.
- Updated information about procedures of the QST session.
- The forehead stimulation procedure in fMRI assessments has been clarified.
- A breath-hold task has been added to the fMRI visits as well as a section on breath-hold risks and a new exclusion criterium for the study related with this test.
- A toxicology screening has been added to the PET-MR visit.
- A saliva draw for the genotype test during screening has been added.
- Added nature-based questionnaires to baseline and post-treatment visits.
- Removed if the participant would prefer consent of MD or NP to correspond with IRB protocol updates.
- Minor grammar and rewording edits.

Version Number: 1.7
Version Date: November 26, 2019
Summary of Revisions Made:

- Exclusion criteria have been updated following FDA recommendation.
- Details about the fMRI session have been clarified.
- An exploratory heartbeat detection task has been added to the procedures of the first and last booster sessions.
- An exploratory Motion Coherency Test (MCT) has been added to the PET/MR visit.
- PET blood draw procedure specifications have been added.

- QST procedures have been clarified.
- Questions about pain ratings have been added to the baseline and post-treatment visits as well as Booster sessions.
- An Aura Information Handout has been added to the screening visit to improve fidelity of daily diary completion.
- Minor grammar and rewording edits.
- Added note on daily diary completion under adherence.

Version Number: 1.8
Version Date: March 23, 2020
Summary of Revisions Made (submitted to NCCIH for review prior to COVID-19):

- Updated study staff personnel.
- Updated information regarding the qualifications of anesthesia residents and nurses placing A-lines due to IRB approval.
- The A-line blood collection amount has increased slightly due to a correction of a previous calculation error.
- The cold water bath device has been updated to a newer model.
- Minor grammar and rewording edits.

Version Number: 1.9
Version Date: May 11th, 2020
Summary of Revisions Made (submitted to NCCIH for review during COVID-19):

- Updated study staff personnel.
- Updated the intervention to include an option for virtual group MBSR and Nature education sessions, which may be more in demand due to COVID-19.
- Added safety risks for video conferencing.

Version Number: 2.0
Version Date: July 13th, 2020
Summary of Revisions Made (after EAB review):

- Updated footnote to the 'Schedule of Evaluations' table
- Added remote screening visits due to COVID-19
- Clarified intervention details

Version Number: 2.1
Version Date: August 14th, 2020
Summary of Revisions Made:

- The use of RAVANS has been added to the already scheduled daily home practice for subjects randomized to both MBSR or education control interventions.

Version Number: 2.2
Version Date: November 11th, 2020

- Updated study staff.
- Added behavioral task during PET-MRI visit.
- The already approved Recent Medical History survey will be given to participants at all baseline and posts visits.
- The exclusion criteria for weight has been clarified to also exclude participants with a BMI higher than 34 due to restrictions with the MRI machines.
- Added COVID-19 antibody serology testing.
- The A-line monitoring time has been changed to reduce the time participants will need to be at the center due to COVID-19.

Version Number: 2.3
Version Date: August 30th, 2021

- Updated booster visits to allow for more flexibility as to in-person vs. virtual visits.
- Updated study staff.
- Updated recruitment methods per MGH IRB guidelines.

Version Number: 2.4 and 2.5
Version Date: February 28th, 2022

- Updated headache day eligibility criteria following discussion at External Advisory Board meeting
- Updated screening visit procedures per recommendation by Westat
- Updated study staff
- Added more recruitment methods

Version Number: 2.6
Version Date: April 27th, 2022

- Added new policy for EMR review for SAEs per Westat guidelines
- Clarified 1 month of diary completion
- Clarified remote procedures due to COVID-19
- Added BDI-II to screening visit in addition to PET visits
- Updated study staff

Version Number: 2.7
Version Date: May 24th, 2023

- Updated Study staff
- Increased healthy subject enrollment total to 50
- Clarified exclusion criteria

- Corrected maximum headache days each month to 20 throughout the document
- Updated healthy subject daily diary requirement
- Clarified remote consent visit information
- Added information about text message communication
- Added Aura Questionnaire at Screening Visit
- Clarified information regarding blood draw requirements at PET-MR visit
- Added information about withholding from Triptan related medications prior to the PET-MR visit

Version Number: 2.8
Version Date: July 3rd, 2023
Updated participant attrition rate, completion, and randomization totals for migraine participants

Version Number: 2.9
Version Date: November 3rd, 2023
- Updated exclusion criteria
- Updated study staff roster

Version Number: 3.0
Version Date: December 26th, 2023
- Updated the table reflecting the correct questionnaires given at the 3 month and 6-month follow-up
- Eliminate COVID-19 antibody testing
- Include urine pregnancy testing
- Updated study staff roster

Version Number: 3.1
Version Date: November 25th, 2024
- Update Study Staff Roster
- Included data sharing language for Dr. Brusaferri and Tohyama that was required by the MGB IRB

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PRÉCIS

Study Title

Boosting mind-body mechanisms and outcomes for chronic pain

Objectives

Primary objective: To evaluate the effects of combined Mindfulness Meditation (MM) + Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS tVNS) on brainstem and cortical response to trigeminal sensory afference, peripheral parasympathetic activity and neuroinflammation levels in migraine patients

Secondary objective: To evaluate differences in brainstem and cortical response to trigeminal sensory afference, peripheral parasympathetic activity and neuroinflammation levels between migraineurs and healthy controls.

Design and Outcomes

Our proposal will employ a multi-modal approach to evaluate synergistic effects on migraine pathophysiology following MM and RAVANS tVNS. We will apply ultrahigh field (7T) fMRI, H-MRS, PET/MRI neuroimaging and autonomic testing at baseline and following 8-weeks of therapy in a 2x2 randomized longitudinal trial design with groups receiving RAVANS or sham tVNS, and MM training or education control.

Interventions and Duration

MM training will utilize Mindfulness-Based Stress Reduction (MBSR), which focuses on purposely cultivating an attitude of non-judgmental awareness towards present-moment experiences, through formal and informal mindfulness meditation practices. MBSR will be provided following commonly accepted standards of practice and will be adapted into weekly sessions (attended virtually, i.e. online) that last up to 2 hours. In addition, each week, there will also be weekly 30-minute booster visits with a focused attention, mindfulness of breathing recording while receiving RAVANS or sham. The education control will be didactic and include viewing of nature videos that are matched in duration to the amount of meditation dose provided during each MBSR group.

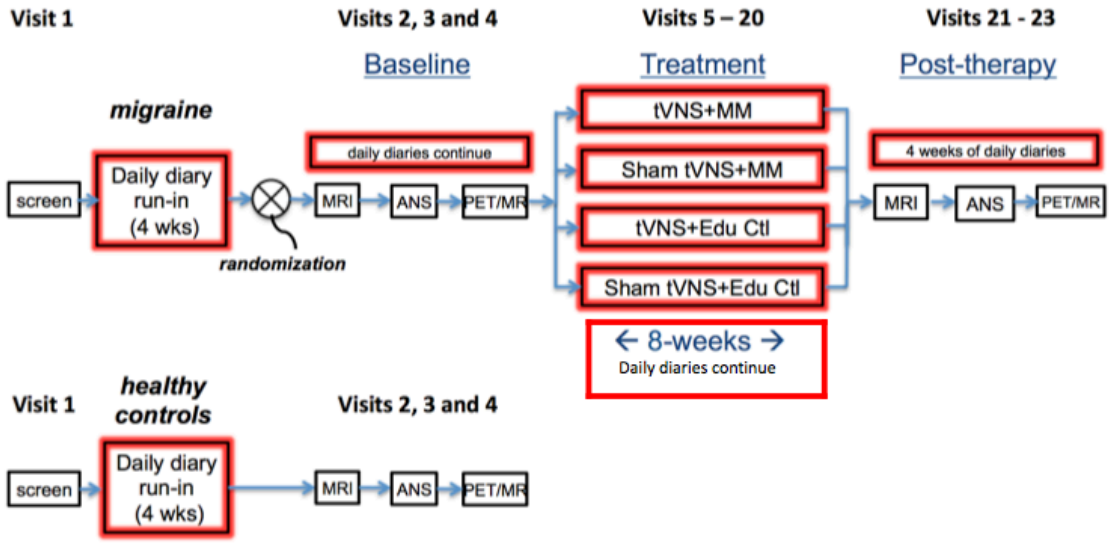

Figure 1. Study Schema for 2x2 longitudinal neuroimaging study evaluating RAVANS tVNS (RAVANS) and Mindfulness Meditation (MM)

Sample Size and Population
Episodic migraine subjects (N=96) who fulfill the International Classification of Headache Disorders – Third-beta edition criteria for the diagnosis of migraine will be enrolled and randomized to one of the 4 N=24 groups. A group of 50 healthy controls will also be enrolled for baseline comparisons.

1. STUDY OBJECTIVES

1.1 Primary Objectives

For this Program Project Grant, there will be 3 Projects, each evaluating a single primary outcome:

Project 1:

- Evaluate the effects of combined MM + RAVANS tVNS on brainstem and cortical response to trigeminal sensory afference in migraine patients

Hypothesis: Combined MM + RAVANS tVNS, compared to monotherapy and control interventions, will significantly reduce cortical amplification ratio to trigeminal afference (posterior insula to spinal trigeminal nuclei percent signal change) in migraine patients.

Project 2:

- Evaluate the effects of combined MM + RAVANS tVNS on peripheral parasympathetic activity of migraine patients

Hypothesis: Combined MM + RAVANS tVNS, compared to monotherapy and

control interventions, will significantly increase High Frequency-Heart Rate Variability (HF-HRV) response to sensory/affective stressors.

Project 3:

- Evaluate the effects of combined MM + RAVANS tVNS on neuroinflammation in migraine patients

Hypothesis: Combined MM + RAVANS tVNS, compared to monotherapy and control interventions, will significantly reduce PET [^{11}C]PBR28 ligand binding in the posterior insula of migraine patients.

1.2 Secondary Objectives

Project 1:

- Evaluate differences in cortical amplification ratio and habituation in response to trigeminal sensory afference between migraine patients and healthy controls

Hypothesis: At baseline, interictal episodic migraine patients will present increased cortical amplification ratio to trigeminal afference and reduced habituation in thalamus, hypothalamus and insula cortex compared to healthy controls.

-Investigate the effects of combined MM + RAVANS tVNS on brain glutamate levels of migraine patients

Hypothesis: Combined MM+RAVANS tVNS, compared to monotherapy and control interventions, will result in significant reduction of glutamate concentrations in thalamus and insula in migraine patients.

-Evaluate if changes in brain response to trigeminal afference are associated with glutamate concentrations

Hypothesis: Individuals showing the most adaptive changes in cortical amplification and habituation will also demonstrate the greatest reductions in glutamate concentrations.

Project 2:

- Evaluate differences in HF-HRV response to sensory/affective stressors between migraine patients and healthy controls

Hypothesis: At baseline, interictal episodic migraine patients will present lower HF-HRV response to sensory/affective stressors in comparison to healthy controls.

- Evaluate central autonomic network (CAN) response to sensory/affective stressors in migraine patients and its response to combined MM + RAVANS tVNS

Hypotheses:

-At baseline, as compared to healthy controls, migraine patients will demonstrate larger amygdala response to sensory/affective stressors.

- Combined MM+RAVANS tVNS, compared to monotherapy and control interventions, will result in a significant decrease in amygdala response to sensory/affective stressors in migraine patients.

- Evaluate the relationship between CAN activation and peripheral parasympathetic response to sensory/affective stressors in migraine patients and its response to combined MM + RAVANS tVNS

Hypotheses:
-At baseline, as compared to healthy controls, migraine patients will show reduced association between activity of CAN regions (insula, MCC, mPFC) and HF-HRV response to sensory/affective stressors.

- Combined MM + RAVANS tVNS, compared to monotherapy and control interventions, will result in a significant increase in the correlation between CAN activation and HF-HRV response to sensory/affective stressors in migraine patients.

Project 3:

- Evaluate differences in neuroinflammation levels between migraine patients and healthy controls

Hypothesis: At baseline, Interictal episodic migraine patients will present elevated PET [^{11}C]PBR28 ligand binding in the posterior insula compared to healthy controls.

- Evaluate the effects of combined MM + RAVANS tVNS on neuroinflammation-linked low frequency fluctuations in brain fMRI signals (fALFF)

Hypotheses:
- Combined MM+RAVANS tVNS, compared to monotherapy and control interventions, will result in a significant fALFF signal reduction in migraine patients.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Migraine is a highly prevalent disease (36 million Americans)[1]. A recent study examining disability-adjusted life years (DALYs)[2] has concluded that migraine is the 3rd most prevalent medical disorder on the planet, and that migraine accounts for 30% of the global burden and more than 50% of the disability burden attributable to all neurological diseases worldwide. Overall, migraine is the 4th ranking cause among women and the 7th ranking case of all disease-associated disability worldwide. In the US, migraine costs employers about $14.5 billion a year because of missed workdays

and impaired work function[3]. Migraine is a condition with multifactorial neurobiological underpinnings that are steadily being characterized. Migraine is a neurovascular disorder characterized by central sensitization[4,5], specifically in the brainstem trigeminal sensory complex[6-9], leading to up-regulation of cortical excitability[10,11]. Behaviorally, central sensitization can manifest as hyperalgesia, allodynia, and impaired habituation, which have been commonly reported in migraine patients, even during the interictal phase (between attacks)[12,13]. This excitability is also associated with dysregulation of the autonomic nervous system[14,15] and both may stem from and up-regulate neurogenic neuroinflammation mediated by microglia and/or astrocyte activation[16].

Migraine management is a challenging issue, no single therapy has proven to be completely successful for alleviating migraine, and while many mind-body therapies have shown promise for chronic pain disorders, including migraine headache, the efficacy of any single-modality treatment is typically modest. Finding a way to boost clinical outcomes for these safe and well-accepted therapeutic modalities is a crucially important goal. It is well documented[17-19], and recommended in the recent Institute of Medicine (IOM) report on pain[20], that a multimodal approach is optimal for chronic pain management. The concept behind a multimodal approach for chronic pain is to target several convergent pathways, thereby enhancing the benefits of each individual approach[17-19]. Mind-body therapies can involve either or both "mind" and "body" elements. To date, however, better integration of "top-down" and "body-based" therapies has been hampered by a lack of mechanistic understanding of how these interventions might interact, presenting a knowledge gap that, when filled, could help optimize care by determining approaches most likely to have a synergistic salubrious effect.

2.2 Study Rationale

Mindfulness meditation (MM) is a top-down intervention that encourages non-judgmental awareness and acceptance towards inner experiences (thoughts, emotions) and physical sensations. MM encompasses various techniques, including focused awareness of sensory, emotional, and cognitive events, and open monitoring[21,22]. As pain experience involves unpleasant sensory and emotional experiences, and is strongly influenced by negatively-valenced evaluative processes[23,24], MM has significant potential to shape the chronic pain experience. In fact, the first MM research trial targeted chronic pain, and was published by Jon Kabat-Zinn in 1982[25]. Recent large N clinical trials have shown promise for reducing pain[26-30]. MM training has also been successfully applied for migraine headache, by our group and others[31-33]. However, the neural mechanisms supporting MM-mediated pain relief are poorly understood[34], and may differ from those supporting placebo analgesia[35,36].

MM likely operates by top-down mechanisms; indeed, multiple fMRI studies suggest that applying mindfulness techniques during painful stimulation enhances activation of cortical pain-inhibitory circuits involving ACC/mPFC, and reduces pain-related activity in the insula and thalamus[36,37]. Additionally, attention to breath has been demonstrated to increase activity in prefrontal cortical regions[38], including

mPFC[39], and MM training strengthened mPFC connectivity with posterior insula during an attention to breath task[40]. Importantly, mPFC is key in linking context (e.g. allostatic stressors) with adaptive emotional responses and is characterized by a dense array of subcortical connections[41]. Hence, mPFC is a key region for top-down modulation of subcortical and cortico-limbic hyperexcitability. A recent MR spectroscopy (H-MRS) study also suggested that experienced meditators show reduced glutamate levels in the thalamus[42]. As animal models of chronic pain show reduced glutamate in mPFC[43] and diminished inhibitory control of subcortical regions such as amygdala and thalamus. Moreover, recent reviews on MM and pain highlight the ability of meditation-based approaches to reduce sensitivity to pain[21,34,44]. Behaviorally, indicators of central sensitization are often measured using Quantitative Sensory Testing (QST), which applies calibrated noxious thermal and/or mechanical stimuli to evaluate the effects of an intervention or manipulation on pain responses. To date, more than a dozen QST studies have revealed that long-term MM practitioners (many with thousands of hours of training) exhibit reduced pain sensitivity compared to non-practitioners[45-48], and that even brief training in MM principles and breathing practices lowers pain sensitivity in subjects[35-37,49-56]. While much of this research has been conducted in healthy volunteers, MM has also been shown to reduce indices of central sensitization (e.g., post-MM, patients report less pain in response to administration of noxious stimuli) in chronic low back pain[57] and fibromyalgia patients[58]. The time frame for MM effects has not been definitively elucidated; brief sessions (e.g., 15-20 minutes) of breath-focused mindfulness training reduce sensitivity to pain in healthy subjects[52], suggesting rapid benefits. However, practice appears to facilitate MM's capacity to reverse sensitization processes[34,59], as individuals with more MM experience are most able to reduce intensity and unpleasantness of noxious stimuli[48,60], and most effectively modulate insula[46] activity. Collectively, MM may reduce CNS sensitization by recruiting mPFC pain-inhibitory pathways and reducing activation in pain-processing areas that frequently exhibit enhanced excitability in persistent pain[34,59,61].

The current study proposes to enhance the relaxing and self-regulatory effects of MM by incorporating a targeted bottom-up neuromodulatory intervention, transcutaneous vagus nerve stimulation (tVNS). While pharmacological options for migraines have targeted brainstem neuromodulatory centers, including serotonergic (raphe nuclei) and noradrenergic (locus coeruleus) nuclei[62,63], novel neuromodulation stimulation therapies as tVNS have also been proposed[64,65]. The vagus nerve is regarded as the main parasympathetic conduit of the autonomic nervous system (ANS)[41], and consists of both afferent and efferent fibers. Most vagal fibers (~80%) are afferents and carry sensory information from the head, neck, abdomen, and thorax to (mainly) the nucleus tractus solitarii (NTS)[42–44]. NTS information is then communicated to neuromodulatory brainstem nuclei for diffuse monoamine systems – i.e. locus coeruleus (LC) and raphe nuclei[66,67]. VNS has demonstrated efficacy in migraine prevention and reduction of headache severity[68-70], and while the precise analgesic mechanisms of VNS are unknown, vagal afference relayed to NTS in the medulla may modulate cortical/subcortical excitability and connectivity with higher brain structures[69] via these monoaminergic neuromodulatory systems. For instance, LC could be involved in vascular regulation of migraine through direct projections to

both intra- and extra-cranial vasculature[71], while both serotonergic and noradrenergic systems are known to suppress cortical/subcortical hyper-excitability and cortical spreading depression[63], a slowly propagating wave of sustained strong neuronal and glial depolarization that underlies migraine aura and activates downstream inflammatory and nociceptive pathways[72]. Indeed, a previous animal study[73] has shown that VNS significantly suppresses cortical spreading depression susceptibility in rat occipital cortex. Despite the therapeutic potential of VNS, adverse events associated with surgery and chronic stimulation limit broad applicability[74]. Importantly, the NTS also receives somatosensory afference via the purely afferent auricular branch of the vagus nerve (ABVN)[75,76], which innervates the auricle (outer ear), most consistently at the cymba conchae. Non-invasive (transcutaneous) methods of ABVN stimulation (tVNS) have been proposed[77], and preliminary neuroimaging studies have found that tVNS modulates brainstem and cortical areas similar to classical VNS[78-80]. Commercial tVNS devices have been approved for treatment of migraine in Europe, and one company is currently seeking FDA approval (but is not yet available) in the US, following publication of a clinical trial suggesting that tVNS reduces migraine frequency[81]. Interestingly, it is well known that the dorsal medullary vagal system (which includes NTS) operates in tune with respiration (e.g. respiratory sinus arrhythmia). Second-order NTS relay neurons receive afference from pulmonary stretch receptors and aortic baroreceptors. NTS also receives inhibitory inputs from medullary ventral respiratory group (VRG) nuclei during inspiration, and facilitation during expiration[82-84]. For these reasons, our group has proposed that ABVN stimulation gated to expiration may optimize brainstem targeting[85], and developed the Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) technique. In addition to enhanced targeting due to VRG influence on NTS, by supplying afference with intermittent, naturally irregular stimulation, RAVANS tVNS may also limit the neural habituation occurring with constant high-frequency stimulation, common with most VNS and tVNS applications[86].

Combining MM with RAVANS tVNS is promising on both a conceptual and neurophysiological level. We propose that respiratory-coupled pathways for MM mechanisms will be potentiated by RAVANS' respiratory-gated, bottom-up neuromodulation converging on overlapping brain circuitry. This will then manifest in synergistic improvement for migraine pathophysiology, and ultimately, clinical outcomes.

3. STUDY DESIGN

We will employ a multi-modal approach to evaluate the effects on migraine pathophysiology following MM and RAVANS tVNS in a 2x2 randomized longitudinal trial design. Patients that complete the 4-week diary lead-in prior to the baseline sessions will be randomized into one of 4 possible treatment groups: 8 weeks of (1) active tVNS and mindfulness meditation (MM), (2) inactive tVNS and MM, (3) active tVNS and education control (EduCtl), or (4) inactive tVNS and EduCtl. Twenty fourpatients will be randomized in each treatment group, for a total of 96 patients available for analysis (Figure 1). Subjects will be asked to come to the Martinos Center and Cambridge Health Alliance (CHA) Center for Mindfulness and Compassion for a total of 23 visits: 4 visits

prior to treatment (1 screening visit, 1 behavioral/Autonomic Nervous System Testing (ANS) session, 1 fMRI and 1 PET/MRI sessions, all at the Martinos Center), 16 treatment visits at CHA or remote using Zoom over 8 weeks, 3 imaging/ANS visits post treatment, and 2 online follow-ups. Patients not willing to perform PET as part of the PET/MR visits will not check the related PET box on the consent form. They will be able to perform other study procedures and stay in the study. Additionally, 50 healthy volunteers will be enrolled to complete baseline procedures only (Visits 1-4).

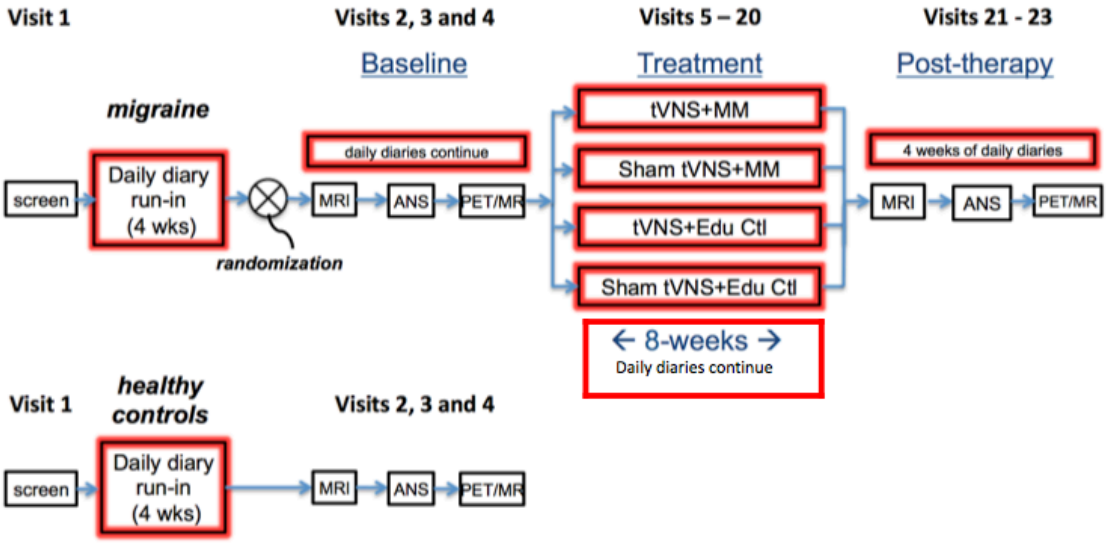

Figure 1. Study design for 2x2 longitudinal neuroimaging study evaluating RAVANS tVNS (RAVANS) and Mindfulness Meditation (MM)

Primary outcomes will include the changes post-therapy compared to baseline in the cortical amplification ratio to trigeminal afference as assessed by fMRI, the HF-HRV response to sensory/affective stressors measured in the ANS sessions and the [^{11}C]PBR28 PET ligand binding in posterior insula, as quantified in the PET/MR sessions.

We will conduct at least 2 MM and 2 education control groups per year, with staggered start dates over the years to achieve relative balance among seasons. We will ensure that MM group leaders are balanced across tVNS and sham groups to reduce the impact of leader variability. We will aim for 8 participants per group (minimum:6, maximum: 10) with a goal of 6-10 MM and 6-10 education control patients running concurrently on different days of the week (we are prepared for more if enrollment schedule dictates).

Given the logistics of recruiting and enrolling subjects in a group-intervention for which groups of 6-10 need to be formed and subjects will likely drop out if required to wait several months for a new group to form, we will recruit in a temporal sequence with fully randomized order of treatment. We will not able to stratify patients, however, to reduce the chances of sampling bias, defined narrowly as systematic differences across

randomized groups, we will employ a modified version of covariate adaptive randomization. In covariate adaptive randomization, a new participant is sequentially assigned to a particular treatment group by considering specific covariates and previous assignments of participants. However, in this study, individuals must be enrolled in the nearest temporal cohort in relation to their screening. To accommodate this fact, each pool of currently eligible individuals will each be scored based on their potential to balance the experimental groups, conditional on previous assignments and their characteristics. Assignments will be based on a solution that places the maximum number of individuals currently under consideration into the current cohort while assigning the fewest possible to the next cohort (i.e., a two-variable optimization). This is important as any delay from when subjects express desire to enroll in the study and actually receiving therapy (at absolute minimum, the time would already be 1 month of daily diaries plus 3 weeks of fMRI and PET-MR scans and behavioral sessions), impacts dropout. Thus, at worst subjects would join the next group cohort and the method of minimization will be used to reduce the difference in groups if the current participant was assigned to the current group or the next available group. The variable considered for balance will be monthly headache frequency. This variable will be assessed during the daily diary run-in period.

All patients and study staff will be blinded, except for the study coordinator, who will execute the randomization scheme, and Cambridge Health Alliance (CHA) study staff directly involved in the delivery of this behavioral intervention (i.e. MM trainers). Recruitment and outcome assessment will be performed by blinded personnel.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet all of the following inclusion criteria to participate in this study:

1. Subjects must be between 18 and 65 years of age.
2. The diagnosis must come from a neurologist or other qualified MD and meet International Classification of Headache Diagnosis, Version 3 (ICHD-3) beta criteria for migraine according to either of the categories:
   ICHD-3 beta 1.1: Migraine without aura
   ICHD-3 beta 1.2: Migraine with aura
3. The frequency of migraine attacks must be at least 4 migraine-days per month.
4. The duration of the disease must be of at least one year.
5. Willingness to attend twice-weekly treatment sessions, complete daily diaries throughout study, and engage in 40-60 minutes of home practice or equivalent (if randomized to EduCtl) per day during treatment.
6. Able to give written consent and participate in group interventions in English.

Inclusion criteria for healthy volunteers:

1. Subjects must be between 18 and 65 years of age.

2. Ability to give written consent.

4.2 Exclusion Criteria

All candidates meeting any of the following exclusion criteria at baseline will be excluded from study participation:

1. Greater than 20 headache days per month
2. Major systemic illness (e.g., brain tumor, severe hypertension) or unstable medical/psychiatric condition (e.g., suicide risk, requiring immediate treatment or that could compromise protocol adherence.
3. Evidence of relevant co-morbidity, systemic or not, with potential to substantially interfere with the phenotype of the conditions studied in the present protocol.
4. A history of neurological disease or injury, including a history of seizures or significant head trauma (i.e., extended loss of consciousness, neurological sequelae, or known structural brain lesion), which may confound the results of the study as determined by the Clinical Core.
5. Symptoms that could impact protocol adherence or be disruptive to group participation (e.g., Tourette's, schizophrenia with current psychosis, bipolar 1 disorder, severe OCD, current severe episode of major depression, dissociative disorder, severe PTSD, severe personality disorder, traumatic brain injury, severe agoraphobia, etc.) as determined by the Clinical Core.
6. Medication overuse, using ICHD-3 beta criteria framework for medication overuse headaches, as determined by Clinical Core.
7. Other chronic pain disorder whose intensity exceeds the intensity of pain during migraine headaches.
8. Moderate or high levels of opioid use (>60mg Morphine equivalents).
9. Participation in other concurrent therapeutic trials.
10. Current training or training in the past 2 years in mindfulness (MBCT, MBSR) or in the Relaxation Response.
11. Relevant mindfulness meditation practice for an average of 10+ minutes a day in the past 3 months.
12. Conditions of skin or anatomy that affect left auricle or forehead skin and could impact placement of electrodes for tVNS or forehead stimulation.
13. Severe Raynaud syndrome or peripheral neuropathy will prompt Clinical Core review to determine eligibility.
14. New pharmacotherapy started within 4 weeks of the screening visit that could impact study as determined by the Clinical Core.
15. Subjects who are prescribed preventative calcitonin gene-related peptide (CGRP) medications that include CGRP antibodies and oral CGRP receptor antagonists and have been taking them for <3 months before screening.
16. Unwilling to maintain consistent pharmacotherapy for the duration of the study that could impact study as determined by the Clinical Core.
17. Meets criteria for active, non-remitted, DSM-V Moderate or Severe Substance Use Disorder, or demonstrates regular substance use (assessed by self-report and/or saliva screening) in a manner which would impact protocol or be disruptive to group as determined by Clinical Core.

18. General fMRI safety exclusion criteria listed below.
19. Any impairment, activity or situation that in the judgment of the Study Coordinator or Principal Investigator would prevent satisfactory completion of the study protocol. This includes unreliable or inconsistent completion of daily diaries, as determined by the Clinical Core.
20. A diagnosis of a significant pulmonary disease, e.g., COPD that could impact participation in breathing tasks as determined by Clinical Core
21. Diagnosis of significant cardiovascular or cerebrovascular disease [e.g. congestive heart failure, stroke, cardiac conduction disorders (including: bundle branch block, heart block, long Q-T syndrome), history of asystole or non-sustained ventricular tachycardia] as determined by Clinical Core.
22. Bradycardia (defined as resting heart rate <50 bpm), or
23. Hypotension (defined as blood pressure <90/60 mmHg) that would impact study participation as determined by the Clinical Core.
24. History of COVID-19 infection within the past 3 months WITH neurological symptoms that required hospitalization as determined by the Clinical Core.

Exclusion criteria for healthy volunteers:

1. The same as for patients, with the addition of current or past history of migraine or chronic pain.
2. First degree relatives with a history of migraine.

Combined fMRI and PET-MR safety exclusion criteria:

1. Electrical implants such as cardiac pacemakers or perfusion pumps
2. Ferromagnetic implants such as aneurysm clips, surgical clips, prostheses, artificial hearts, valves with steel parts, metal fragments, shrapnel, metallic tattoos anywhere on the body, tattoos near the eye, or steel implants ferromagnetic objects such as jewelry or metal clips in clothing
3. Pregnant, suspected to be pregnant or actively trying to become pregnant (a negative STAT quantitative serum hCG pregnancy test is required before the subject can participate for PET-MR visits)
4. Breastfeeding
5. Pre-existing medical conditions including a likelihood of developing seizures or claustrophobic reactions, and any greater than normal potential for cardiac arrest;
6. Is unable to lie comfortably on a bed inside a PET-MR or fMRI scanner with their head in the field of view for at least 90 minutes as assessed by physical examination and medical history (e.g. back pain, arthritis)
7. Less than 18 years of age
8. > 300 lbs (weight limit of the fMRI table) or BMI >34.

PET-MR safety exclusion criteria

1. History of Type I or Type II diabetes mellitus; unless well managed
2. Use of certain antibiotics such as minocycline

3. Routine use of benzodiazepines or any use in the 2 weeks prior to the scan(s), *except* clonazepam, lorazepam, and alprazolam, which show very low binding affinity to TSPO

Exclusion Criteria for placement of arterial line (an optional choice for patient subjects and healthy volunteers):

Contraindications to placement of arterial line (abnormal result on modified Allen's test on both hands; Raynaud syndrome; bleeding disorder; use of anticoagulants such as Coumadin, Plavix or Loven

3.3 Study Enrollment Procedures

We will utilize a mixed methods approach to recruitment. MGH Headache and Neuropathic Pain Unit, directed by Dr. Cheng, a co-investigator of the study, has 5 neurologists and a nurse practitioner who see an average of more than 500 migraine headache patients per month. The BWH's Division of Headache and Pain has 7 attending neurologists and two Headache Medicine fellows who together see an average of 210 migraine headache patients per month. Potential subjects will also be given study flyers at their subsequent physician visits. We will also use the Partners clinical studies website (http://clinicaltrials.partners.org/), the Partners Rally website, and the Partners Research Patient Data Registry (RPDR). We will also use information booths and Dr. Cheng's patient symposium for recruitment. MGH has adopted a new system to replace RODY which allows the use of Research Invitations sent through Patient Gateway or regular mail to recruit patients without physician permission. Under this new approach, all patients are "opted in" to receive Research Invitations but have the option to opt out of receiving these notices. Study staff will make sure that patients who opt out are filtered out of the recruitment list (e.g., from RPDR, Epic, etc.) and they are not sent the Research Invitation. Study staff will also use social media ads and patient recruitment services or registries (such as Trial Facts or Research Match) to reach more participants. The PI will conduct ongoing monitoring of patient responses to ensure that the selection criteria are identifying the right patients, and complaints about this method of recruitment will be submitted to the IRB as an "other event". Furthermore, MGH & BWH hospitals have a database system for clinical research (RSVP) that can identify patients with migraines, who have agreed to be contacted for research studies. Additionally, advertisements for the study will be posted in CHA neurology and primary care sites.

If necessary, as back-up, we have found that a combination of standard methods (e.g. provider referral, flyers, print ads) as well as relatively new methods (e.g., internet, targeted Facebook ads) can significantly boost recruitment, using IRB-approved language. To encourage retention, once subjects complete 1 month of daily diaries and pass inclusion criteria, they will be given a proposed study visit schedule and will not be allowed to proceed to neuroimaging sessions unless they can commit to the proposed schedule and daily home practice of 40-60 minutes for both arms. Once participants reach on month of daily dairies, before the baseline visits, unblinded members of the Clinical Core will review eligibility criteria. If the participant has less than 4 or more than 20 headaches in that time frame they would

be considered no longer eligible to move onto the baseline visits. Migraine prevalence is higher amongst women, so we expect to have an unequal recruitment between sex.

To mitigate subject attrition, we will offer a study specific schedule upon completion of the daily diaries clearly illustrating the logistics of the subjects' involvement. We will schedule subjects for all of their visits at the beginning of the study and follow up with reminder phone calls and/or e-mails 24-48 hours prior to each visit; we have found that this method reduces the number of withdrawn subjects and supports protocol compliance. We will implement several innovative patient-centered engagement practices that support retention, including offering twice monthly engagement phone calls to subjects in all arms with brief informal interviews about study experience, ongoing participant advisory board meetings twice yearly to get focus group feedback about procedures that enhance retention[87,88]. Our experience was that these methods increase retention by 40%.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

RAVANS procedures

Experimental intervention sessions will take place at the Cambridge Health Alliance Center for Mindfulness and Compassion (CMC), directed by Zev Schuman-Olivier. RAVANS tVNS will be performed with electrodes attached to the surface of the auricle. A small current will be delivered with a safe battery-powered, constant-current, portable stimulator. Current amplitude will be set from 0.1 to 10 mA based on subject's perception – i.e. 'moderately strong but not painful". Stimuli will consist of a 1.5-sec train of monopolar rectangular pulses with 300 μS pulse width, delivered at 100 Hz, initiated 0.1 seconds following peak inspiration. Respiratory gating for stimulation will require real-time evaluation of the respiratory cycle. For subjects randomized to sham stimulation groups, the electrodes in the ear will remain as described above, but a "Sham" stimulation mode will be selected in the stimulation device, which does not provide electrical stimulation. Subjects will be told that stimulation will be at a fixed level and will be told that they "may or may not feel anything at the ear," and asked if the stimulation is "painful or not", to promote compliance with the therapy and maintain blinding using a Credibility Questionnaire[89]. These tVNS procedures will be used in conjunction with MM training or EduCtl, as described below.

Mindfulness Based Stress Reduction Intervention (MBSR)

MM training encourages nonjudgmental awareness and acceptance of inner experiences (thoughts, emotions) and physical sensations. Our mindfulness training intervention (MBSR) is a semi-standardized behavioral intervention that consists of 8 weekly 2-hour classes and a 4-hour retreat with an instructor. MBSR is a highly studied method for training people to practice MM. MM may enhance self-regulation and reduce stress through cognitive and emotional processing mechanisms.

Subjects will participate in an intervention which will include virtually (i.e. online) attended group sessions and in-person/online booster visits. The group sessions will take place once per week, with a virtual connection using a secure video conferencing computer application (i.e., Zoom). The study has CHA IT-approved iPads that could be given to participants during the study to help facilitate participation if needed. During the virtual MBSR sessions, breakout rooms will be introduced with different group members as well as movement breaks at least every 60 minutes to ensure subjects' engagement. In order to ensure fidelity of the MBSR intervention, we will have 3 layers of quality assurance. The first is ensuring all MBSR groups will be led by experienced MBSR teachers who have received individual supervision from supervisors affiliated with the University of Massachusetts Center for Mindfulness (the originators of MBSR). Second, teachers will have ongoing regular weekly supervision from members of an expert MBSR consultative team consisting of certified MSBR teachers and experts who have led MBSR for patients with migraines. Third, subjects will consent to having each session recorded with a digital video camcorder (both education control and MBSR). We will record all of the MBSR and education control sessions and have an independent auditor watch, listen to, and evaluate 10% of the sessions. We will use a validated process for measuring both fidelity and adherence in mindfulness-based programs and all teachers will be rated for competence using the Mindfulness-Based Interventions: Teaching Assessment Criteria (MBI:TAC). On a monthly basis when a group is being implemented, group supervisors will review selected footage (e.g., delivery of specific experiential components, inquiry period, etc.) from at least 1 of the sessions and give the group leader fast and routine feedback to prevent drift.

Individual "mindfulness of breathing" booster sessions including RAVANS-tVNS administration will occur twice a week for 8 weeks. This choice of mindfulness of breathing was supported by a recent dismantling study that reported focused attention practice on breath was associated with faster improvement in affective disturbances compared with open monitoring practices[90]. In addition, use of RAVANS or Sham tVNS with breathing mindfulness will be included in home practice sessions and will represent 20-30 minutes of the 40-60 minutes of daily home practice. Note that home practice is to be encouraged on 4 out of 7 days per week, and is not required on days where the participant will participate in a MBSR session. The daily administration of tVNS was selected based on a previous RCT that found this intervention to be safe and beneficial for the reduction of headache days in patients with episodic migraine after three months of treatment.[81] The compliance and duration of home practice will be recorded on StudyTRAX with morning and evening daily headache diaries or with a paper form filled out by the participant, and will also be tracked objectively by a logfile automatically generated by the device.

Education Control Intervention

In order to keep participants blind to their cohort assignment and to have a control group that includes enough content to be matched to MBSR (eight 2-hr visits) on attention and practice doses, we propose using an innovative education control training. Visits in the EduCtl condition will be matched for the amount of professional

contact and will include information about the impact of nature on physical and mental health. An equal number of meetings will take place on a weekly basis, and a one-time 4-hour retreat will also be included. Subjects will participate in an intervention which will include virtual group sessions and in-person/online booster sessions. The virtual group sessions will take place on a secure video conferencing computer application (i.e., Zoom).

The education control program will include inspirational discussion and activities related to encouraging people to get connected to nature to reduce stress. This control has acceptability among migraineurs because they are aware of the tendency of different artificial environments to provoke migraines. The education control will be didactic and include viewing of nature videos that are matched in duration to the amount of meditation dose provided during each MBSR group. The nature videos will occur once per session and last approximately 30 minutes with approximately 3-minute long sections, which will vary in content with both pleasant/neutral (e.g., calm lake, ocean cliffs, alpine meadows, sunsets, peaceful animals) and unpleasant (e.g., fox eating prey, crawling insects, snakes slithering, bees swarming) videos of nature. An equal ratio of pleasant, neutral, and unpleasant sections will be used in each video, in an attempt to mimic the experience of mindfulness meditation, which is not always relaxing/pleasant. Education content will not be related to etiology or triggers of migraine headaches or stress reduction/health behavior change to prevent confounding. During the virtual sessions, breakout rooms will be introduced with different group members as well as movement breaks at least every 60 minutes to ensure subjects' engagement. The EduCtl program will not include specific instruction in breathing techniques, mindfulness, or cultivation of non-judgmental awareness. Interpersonal and group-building exercises will be conducted to parallel the interpersonal and social support aspect of MBSR. Readings or other nature related content will be assigned each week that will be equal in time commitment to the daily mindfulness home practice assignments. EduCtl group sessions will be led by experienced group leaders with at least a master's-level degree or with an equivalent level of group leadership experience.

Individual EduCtl booster sessions with RAVANS tVNS will occur twice a week for 8 weeks. During the tVNS sessions on booster sessions, participants will watch 30 minutes of nature videos. In addition, similarly to the MBSR arms, the use of RAVANS or Sham tVNS will be included in home practice sessions for the Education control intervention and will represent 20-30 minutes of the 40-60 minutes of daily home practice. The compliance and duration of home practice will be recorded on StudyTRAX with morning and evening daily headache diaries or with a paper form filled out by the participant, and will also be tracked objectively by a logfile automatically generated by the device.

5.2 Handling of Study Interventions

MBSR is a standardized, well-defined and systematic patient-centered educational meditation practice. MBSR generally includes an orientation session, but given the RCT structure of the study, the orientation will be modified and incorporated into the

study consent process, which will focus on commitment to home practice, confidentiality, and openness to experience. MBSR is traditionally provided in a heterogeneous population, but studies have shown efficacy in homogenous populations. Mindfulness practice instruction will be offered during experiential parts of the sessions. Dyadic and group dialogue will be fostered, and didactic elements and group exercises will be presented. MBSR provides teachers flexibility in the curriculum structure and prioritizes effective use of the present moment; a general overview of session topics is below (Table 1).

Week	MBSR Session Content Focus	Edu Ctl Session Content Focus
1	What is Mindfulness?	What is Nature?
2	Perception/ Beginner's Mind	The Natural World and the Mind
3	Mindful Movement	Confronting Fears of Nature
4	What is Stress?	How Can Nature Improve Health? (Pt. 1)
5	Coping with Stress	How Can Nature Improve Health? (Pt. 2)
6	Communication	Your Relationship with the Natural World
Retreat	Deepening Practice	Deepening Immersion in Nature
7	Cultivating Kindness	Caring about our Natural World
8	The Rest of Your Life	Setting up your Return to Nature Plan

Table 1. Description of MSBR and Education Control sessions

Ambulatory HRV and Actigraphy

The day of the booster sessions a body-worn, Bluetooth-enabled, soft, flexible, water resistant patch (~2.5" x 1.3" x 0.2" medical grade silicone) with polarized electrodes (BioStamp nPoint, MC10), will be placed on the chest and will be used to collect ambulatory ECG and actigraphy measurements. The data will be recorded during the participant's first and last booster session. The data may also be recorded during booster sessions in the even weeks of the intervention (weeks 2, 4, 6, and 8). . Patches will be programmed for both ECG and activity using a tablet-based mobile app. Activity recordings will be used primarily for filtering and interpretation of HRV data. However, data will also be of interest in exploratory analyses of motor activity, restlessness and sleep migraine symptoms, including types of activity in different periods of the day and night (lying down, sitting, walking, total activity).

Heartbeat Detection Task

The Heartbeat Detection Task (HBDT) will be completed during the first and last booster sessions. Participants will be asked to count their heart beat during three varying time intervals (30s, 30s, 60s). In parallel, individuals' heart-rate will be measured by Kardia Mobile technology. Participants will place their index and middle fingers of both hands-on top of the Kardia Mobile device and will be instructed to count their heartbeat for a period of 30 or 60 seconds. The readings from this device are linked to the Kardia Mobile App, which will be on a screen faced away from the

participant. Participant data stored in the Kardia Mobile app will be identified with the Study participant ID, which will not be linked to any identifying information.

Intervention Questionnaires

The following questionnaires will be given at specific points during the intervention, and will be given to both the MBSR and EduCtl Intervention groups:

- Group Climate Questionnaire: This questionnaire will be administered at weeks 4 and 8 of the intervention to evaluate perceived atmosphere of group environments in terms of engagement, positivity, and conflict.

- Credibility Questionnaire: This questionnaire will be administered at weeks 1 and 8 of the intervention to measure the degree to which subjects' find the treatment they are receiving to be credible and having the potential to be efficacious.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

Enrolled participants will be screened for concomitant medications and are required to have been on stable doses for at least 30 days prior to starting the trial. They will also be asked to maintain medications/dosage regimes while participating in the trial.

5.3.2 Required Interventions

Non-Applicable.

5.3.3 Prohibited Interventions

Any participants with moderate or high levels of opioid use (>60mg Morphine equivalents) will be excluded from the study. Individuals that have current training or training in the past two years in mindfulness (MBCT, MBSR) or in the Relaxation Response or participants with relevant mindfulness meditation practice for an average of 10+ minutes a day in the past 3 months will be excluded from the study. However, if such interventions, though prohibited, are initiated during the study period, we will record this information but continue to collect outcome data.

5.4 Adherence Assessment

Adherence will be defined as completing at least 5 out of the 9 group treatment sessions including the retreat. This will be confirmed by research assistants, who will attend all treatment visits and will verify and track participant attendance. Booster sessions can be made up at alternative times and do not count towards the 5 out of 9 treatment session attendance requirement. Migraine subjects' adherence to daily diaries will be deemed complete (i.e. no protocol deviation) if they perform at least 75% of their diaries. Healthy subjects will be asked to complete 4 weeks of daily diaries but they are not required to reach 75% completion to continue in the study.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment / Procedure	Screening: Visit 1 (Day -42 to day -28)	Daily diary run-in (Day-28 to Day 0)	Baseline (fMRI-ANS-PET-MR) Visits 2-4 (W1 to W2)	Treatment Visits 5-20 (W3 to W10)	Post-Treatment fMRI (fMRI-ANS-PET-MR) Visits 21-23 (W11 to W12)	3- and 6-month Follow-up
Informed Consent	X*					
Demographics	X*					
Medical history	X*					
Inclusion/Exclusion Criteria	X*					
Headache diary		X*	X*	X	X	
Pain ratings			X*	X	X	
HIT6			X		X	X
MIDAS			X		X	X
MSQL			X		X	
PCS			X*		X	X
PROMIS-29			X*		X	
Perceived stress scale-14			X*		X	
BDI-II	X*		X*		X	
Aura	X*					
FFMQ			X*		X	
Freiburg-14			X*		X	
SCS-SF			X*		X	
MAIA 2			X*		X	X
DERS			X*		X	
HMSE			X		X	X
Brief Resilience scale			X*		X	

Assessment / Procedure	Screening: Visit 1 (Day -42 to day -28)	Daily diary run-in (Day-28 to Day 0)	Baseline (fMRI-ANS-PET-MR) Visits 2-4 (W1 to W2)	Treatment Visits 5-20 (W3 to W10)	Post-Treatment fMRI (fMRI-ANS-PET-MR) Visits 21-23 (W11 to W12)	3- and 6-month Follow-up
Expectation for relief scale			X*			
Widespread Pain Index			X*		X	
Measuring love and care for nature			X*		X	
The connectedness to nature scale			X*		X	
PET Recent Medical History Survey			X*		X	
Menstrual phase			X*		X	
fMRI screening form	X*		X*		X	
PET safety form	X*		X*		X	
Session attendance log				X		
Concomitant medications	X*	X*	X*	X	X	X*
Group Climate Questionnaire				X		
Ambulatory ECG/Actigraphy				X		
Credibility questionnaires				X		
Adverse events			X*	X	X	

X* These procedures/items will also be completed by healthy controls in the study.

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Consenting Procedure

Potential subjects interested in participating will be contacted by study staff in charge of enrollment. After explaining the study protocol, study staff will then ask for verbal consent before prescreening procedures are performed. Potential subjects will then answer a brief list of questions pertaining to inclusion/exclusion criteria, which may also be confirmed from the medical record. After completing the pre-screening process, they will be asked to come in for written informed consent and an initial screening visit.

The screening visit will begin with informed consent, which will be conducted by a Nurse Practitioner or MD. The informed consent form will be an IRB approved form with language both specific to Partners Healthcare Research and the study protocol itself. Two copies of the consent form will be signed by both the patient and the study staff, one for each individual to keep. Documentation of signed consent and any modifications made will be logged and submitted for approval within the Partners Research IRB website. The subjects will be informed about the PET procedure and the risks associated with exposure to radiation and A-line, as well as risks of tVNS and behavioral treatments. They will be informed about minimal risks of routine high magnetic field and non-ionizing RF radiation involved in MR imaging. Subjects will also be informed about small space within the magnet and noises made by switching gradients. Subjects will be informed that if they feel uncomfortable with the study, they can choose to terminate the study at any time. They will be informed that their refusal to participate in the study or choosing to terminate it at some point will have no effect on care and treatment received by them at MGH now or in future. The subjects will be informed that their personal information will be protected as per the HIPAA guidelines. Before each testing session the participant will be told about the procedures for the experiment and we will only proceed if verbal assent is given. Patients will be given the time they desire to complete consent forms.

Remote Consenting

In order to comply with public health efforts to address COVID-19, informed consent may be obtained remotely. This will be done via electronic consent (e.g. Partners REDCap e-consent), or a remote consent process where the participant will be asked to sign the consent form and return back by email or mail. In either case, the consent discussion will occur identically to an in-person visit (as outlined above), but held over phone call using secure Cisco Jabber software or video conference. A physician member of the staff or a NP from the Clinical Translational Research Unit in the Charlestown Navy Yard will obtain this remote consent as well as a remote medical history (and migraine medical history for migraine participants) and documentation of concomitant medications. Following the informed consent process, a copy of the signed consent document will be provided to the patient (electronically if e-consent was used, and via mail if a physical copy).. The REDCap e-consent template being utilized is equivalent to written consent and is FDA compliant. As is with in-person consent, the study team will obtain and document informed consent before the participant is enrolled and any study procedures begin. All forms that cannot be completed electronically will be completed at the first in-person baseline visit instead. These screening forms include the MRI safety and PET safety forms, the physical, and the Modified Allen's test. The saliva genotyping test will be mailed to participants during their daily diary eligibility period to minimize in-person visits.

Screening

Screening will take place within the first study visit of the trial, after informed consent. The participant will undergo a screening examination by the study staff, including:

- Demographic and socioeconomic questionnaires
- MRI safety screening questionnaire
- PET safety screening questionnaire
- BDI-II
- Aura Questionnaire
- Concomitant medication questionnaire
- Participants will be review instructions about how to complete the daily diaries and information about aura with migraines.
- MD/NP will review Inclusion/Exclusion criteria
- MD/NP will perform medical history and physical, modified Allen's test, and blood/saliva draw (PBR affinity test)
- PBR affinity test: Unless patients have been previously genotyped for the Ala147Thr TSPO polymorphism (during the course of our previous experiments), either venous blood (up to 10 ml) or a saliva sample will be collected at the Screening Visit from all subjects considered for potential participation in a [^{11}C]PBR28 scan in order to have them genotyped for the Ala147Thr polymorphism in the TSPO gene (rs6971). Due to COVID-19, the saliva kit may be mailed to the participant after the Screening visit is complete. Participants will complete the test at home and mail it back to study staff. Participants found to be low affinity binders will still be considered eligible for behavioral testing and fMRI scanning, but will not complete the study's PET-MR imaging component. If the participant agrees to an A-line and after the PBR affinity test is found to have the genotype and unable to complete the PET-MR, they will be notified within their 4 weeks leading up to the baseline visits.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

Upon completing informed consent and passing the screening eligibility criteria, participants will be enrolled in the study. This date will be considered the enrollment date and will be recorded and kept in the enrollment log.

Following the screening visit, subjects (migraine patients and healthy controls) will complete daily morning and evening online electronic diaries to chronicle any experience with migraine headaches or pain for 4 weeks. These diaries will include daily assessment of migraine headache, severity, perceived trigger, medication use, sleep quality, and other related symptoms. These diaries will allow us to assess whether headache frequency and severity of migraine patients is indeed consistent with our inclusion / exclusion criteria as well as provide a source for baseline measures that can be used for clinical outcomes. Electronic diaries will be administered using StudyTRAX. Study participants will receive reminders to open a link to their personal StudyTRAX account. When participants click the link on their phone's home screen (placed there under supervision of research assistants during the screening visit), they will open the daily diary within their account. A research

assistant will monitor the StudyTRAX database for daily diary completion and subjects will have remuneration incentive to complete as many diaries as possible.

Text Message Communication

Participants will be asked at the phone screen if they consent to receiving unencrypted text messages. Participants will be asked again at the screening visit to reconfirm their consent. Participant consent will be documented on REDCap. Subjects will be able to opt out of receiving text messages at any time throughout their study participation. Text messages will only be sent for appointment reminders, scheduling confirmation, and daily diary reminders.

Pre-intervention Assessments

Upon confirming patient eligibility after the 4-week daily diary run-in period (between 4 and 20 headache days for migraine patients), subjects will attend a series of 3 pre-intervention evaluations within a period of 3 weeks including:

- Pre-intervention fMRI assessment: a pre-therapy assessment to compare with post-outcomes after the patient's undergoes the 8-week intervention period. Each fMRI scanning session will take approximately 3 hours and subjects will be in the scanner for up to 2 hours. Participants will be exposed to a forehead stimulation task (non-painful trigeminal nerve branch stimulation to the forehead) similar to our previously validated experimental model [91] in order to assess fMRI hyperexcitability in the brainstem and higher brain regions. This location targets the ophthalmic (V_1) spinal trigeminal nerve branch. Stimulation will be delivered to the subjects using a block fMRI design. After the fMRI experiment, the intensity of sensations during the scan will be rated by subjects on a numerical rating scale (NRS) of 0 to 10 (0: no sensation, 10: pain detection threshold, i.e. on the verge of painful sensation). We have used a similar standardized stimulation procedure in previous IRB-approved protocols (#2016P001009). This task will be repeated before and after a RAVANS tVNS stimulation fMRI scan run. Additionally, structural and resting state fMRI scans will be collected.

  During the fMRI session we may employ a breath-holding task during one of the scans. CO2 is a potent vasodilator of the cerebrovasculature and can be used to test the responsiveness of the autoregulatory system in migraine patients. The outcome of the cerebrovascular reactivity using breath-holding methods has been shown to be comparable with 5% CO2 inhalation [92]. Subjects will be instructed to have breath-holding and normal breathing paced by visual cue. The paradigm consists of 2 consecutive phases (normal breathing and breath-holding) repeating 5 times. The normal breathing phase

will last no less than 60 seconds, while the breath-holding phase lasts 30 seconds or less. The phases will be paced by the software Presentation (Neurobehavioral Systems) and the task will last 10 minutes. The participant will be told to stop if there is any complaint of respiratory discomfort, chest pain, or neurologic symptoms. Participants will also be asked to refrain from smoking for at least 3 hours prior to procedure.

- Pre-Intervention PET/MR assessment: Prior to the scan session, subjects will be asked to provide a saliva sample for a toxicology screen. If the results of this test show the presence of drugs that were not previously disclosed and that could interfere with the trial (such as substances of abuse) or with the PET scan, then the subject may be dropped from the study after consultation with a staff physician. The toxicology screen includes testing for the following kinds of drugs: alcohol, cocaine, cannabis, opioids (including oxycodone and fentanyl), amphetamines, methamphetamines, phencyclidine, benzodiazepines, barbiturates, and methadone. The results of the toxicology testing, while documented in the research records, will not be entered into participants' medical record/chart.

  At the beginning of the scan session, an intravenous catheter will be placed in the participant's antecubital vein of the left or right arm, prior to going to the scanning area. The radioligand [^{11}C]PBR28 will be used to determine whether patients with migraine exhibit evidence of glial activation. During IV placement, up to 10mL of blood may be drawn to test complete blood count, serum chemistries, interleukins, and TNF-alpha. Female participants of childbearing age will also be asked to have ~5mL of their blood drawn in order to perform a serum pregnancy test or complete a urine sample pregnancy test on the day of the scan (blood will be sent to the core lab for super stat testing). Participants that do not receive the PET injection (due to genotype or other personal reasons) will not have blood drawn or urine sample for pregnancy.. The presence of antibodies will be used to explore the possible effects of prior exposure to the coronavirus on neuroinflammation, in exploratory analyses. Up to 10 ml of blood will be drawn for both healthy volunteers and migraine patients. This testing will be performed through a third party vendor or through the MGH core lab. Furthermore, blood pressure, pulse, and body temperature will be measured to document the presence of a fever or any other abnormalities with the subject that could present a risk for their safety in the scanner.

  If the participant opted for an arterial line, it will be placed in a radial artery with local anesthesia (20 or 18 gauge cannula, 2-5 ml of lidocaine 1% intradermal and subcutaneous) using sterile techniques, if the participant has

consented to this procedure. The placement of an arterial line will be presented as optional to the participants, and we will ask for the participants' consent each time. The arterial line will be placed in the arm contralateral to the intravenous line that is used for the [^{11}C]PBR28 radiotracer injection. The arterial line will enable blood sampling (1mL to 12mL) at various times during the imaging study for at most 154mL of blood. The collected arterial blood will be used to compute metabolite-corrected arterial input function for kinetic modeling analyses. The catheter will be placed by an experienced staff physician (i.e., board-certified anesthesiologist, fully licensed anesthesia resident, or certified registered nurse anesthetist), monitored throughout and accessed by an experienced research nurse. Residents will be eligible as long as they are at least half way through PGY2 year and have completed a cardiac anesthesia rotation, have a full license, are personally approved for proficiency by the residency program director, and they operate under the supervision of an attending. Either a physician or a nurse practitioner will discontinue the catheter at the end of the study visit.

In addition, participants will be exposed to a sensory/affective stressor (IAPS task) during this visit. The IAPS task uses a large set of standardized, emotionally-evocative, internationally-accessible, color photographs that includes contents across a wide range of semantic categories. The IAPS is being developed and distributed by the Center for Emotion and Attention (CSEA) at the University of Florida. It is highly validated and has been shown to reliably elicit autonomic responses and self-reported valence and arousal ratings from male and female subjects [93-99]. The images will be presented in a block fMRI designs.

During the scan, participants will also complete an interoception task where they will be asked to focus on the sensation of their heart, lungs, sight, and sound. After focusing on these areas, participants will be asked how strongly they felt each sensation.

After the scan session, participants will complete the Motion Coherency Task. They will sit in front of a computer screen and will be presented with white moving dots. A group of these dots will move coherently either toward the center or away from the center. The rest of dots will move randomly. Participants are expected to look at a fixation object, presented at the center of screen, and to report the direction of coherently moving dots (toward vs. away from the center) by pressing one of the two keys on the keyboard. Response time and accuracy are both important and will be measured during the experiment. Each test will start with few practice trials (< 20 trials) during which the participant will become familiar with the stimuli and task.

The overall test (including the practice trials) is expected to take between 5-10 minutes.

In most cases, this visit will require up to 6 hours for completion: ~45 min for preparation, ~30 min for A-line placement, if applicable, ~120 min for scanning procedures and ~60 min for filling out questionnaires and observation, plus an additional ~1.5 hr to perform pregnancy test in women of childbearing age.

- Pre-Intervention autonomic (ANS) assessment: This visit will take approximately 3 hours and will include the collection of electrocardiography (ECG), skin conductance level (SCL), and respiration data at 500 Hz using an MRI-compatible, noninvasive BIOPAC MP150 system (BIOPAC Systems, Goleta, California) or Chart Data Acquisition Software (ADInstruments, Colorado Springs, CO) on a laptop. The following tests will be performed for evaluation of cardiac autonomic function in the subjects included in the study:

  a) Paced Breathing Task. Subjects sit quietly while pacing their breathing with the aid of visual cues on a computer screen as in [100]. Three different paces within the traditional HRV HF and LF frequency ranges will be used (0.2, 0.25, and 0.3 Hz) for 3 minutes each (9 minutes in total). This test measures the vagally-mediated responses to changes in breathing rates [101-104].
  b) Sit-stand Test. Three minutes after the paced breathing task, a sit-stand test (5 min duration) will be performed. The hemodynamic response to standing is a commonly performed measure of autonomic function[105-108]. Active standing causes an abrupt increase in heart rate that peaks at approximately 3s followed by a more gradual increase that peaks at approximately 12s after standing. Heart rate and blood pressure return to a new baseline after approximately 30s. The '30:15 ratio' assesses the ratio of the heart rate increase that occurs 15s after standing relative to 30s after standing.

  c) Quantitative Sensory Testing (QST), including:
  - *Mechanical pain:* Responses to punctate mechanical stimuli will be measured using a standard set of weighted probes that provide estimates of pain threshold and mechanical temporal summation. Series of 10 stimuli (with 1-second inter-stimulus intervals) will be used to assess the temporal summation of pain that occurs with rapid administration of identical stimuli.

A Wagner pressure algometer will be utilized to assess responses to pressure stimulation at several anatomical sites. Pain pressure thresholds (PPT) will be determined twice at each of the following sites on the right and left sides of the body: the medial fat pad of the trapezius and frontalis. Mechanical pressure will be applied using a 0.5-cm^2 probe covered with 1mm polypropylene pressure-transducing material; pressure is increased at a steady rate of 30 kPA/s until the subject indicates that the pressure has become painful. Participants are informed that they may terminate the procedure at any time.

- *Cold Pain Assessment*: Responses to noxious cold will be evaluated using a repeated cold pressor task (CPT), which involves immersion of the hand in a circulating water bath (Arctic Series) maintained at a temperature of 8°C. This test has been used to investigate autonomic response in stress, the relationship between psychological disorders and autonomic responsivity, as well as cortical influences on autonomic function[101,102,109-115]. Participants will undergo a series of three cold pressor tasks, with the first 2 consisting of serial immersions of the hand for 15 sec, with 2 min between immersions. Conditioned Pain Modulation (CPM, which refers to the phenomenon of one noxious stimulus inhibiting the pain of a second noxious stimulus) is measured during these 2 cold pressor trials by assessing PPTh during the immersion. The pressure pain threshold assessment will be done twice on the trapezius. The 3rd and final CPT involves an immersion of the hand lasting until a participant reaches pain tolerance (or a 3 min maximum). Participants will rate the intensity of the cold pain on a 0-100 scale ("no pain" to "most intense pain imaginable") every 15 sec.

In addition, the following validated psychometric assessments will be administered before the autonomic testing session:

- Headache Impact Test-6 (HIT-6)[116]: assessment of headache-related disability
- 1-month Migraine Disability Assessment (MIDAS)[117]: assessment of headache-related disability
- Migraine Specific Quality of Life Questionnaire, version 2.1[118]: assessment of quality of life
- Pain Catastrophizing Scale [119]: measures pain-related

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- Patient Reported Outcomes Measurement Information System (PROMIS-29): a 29-item used to assess patient-reported health status for physical function, anxiety, depression, fatigue, sleep disturbance, ability to participate in social roles and pain.[120]:
- Perceived Stress Scale[121]: PSS-14 stress assessment
- Five Facet Mindfulness Questionnaire[122]: mindfulness assessment
- Freiburg Mindfulness Inventory – 14-question mindfulness assessment.
- Short-form Self-Compassion Scale (SCS-SF) [123]: an abbreviated 12-item form of the original 26-item Self-Compassion Scale.
- Multidimensional Assessment of Interoceptive Awareness (MAIA 2) [124]: a 37-item self-report scale designed to assess multiple aspects of interoception and interoceptive awareness.
- Difficulties in Emotion Regulation (DERS) Scale [125]: a 36-item self-report scale designed to assess emotional dysregulation.
- Headache Management Self-Efficacy Scale[126]: self-efficacy assessment
- Brief Resilience Scale: a 6-question assessment of resilience
- Expectation of Relief: measures the extent to which subjects expect the treatment to relieve their migraine pain
- Demographics form asking for participants' gender, race, ethnicity, sexual orientation, socioeconomic data (annual household income), and languages spoken.
- Widespread Pain Index: assessment of pain across the body[127]
- Measuring love and care for nature[128]
- The connectedness to nature scale[129]

Remote Questionnaires

All questionnaires typically collected during the in-person screening visit or behavioral visit may be collected remotely, as they are already completed on secure online platforms (i.e. StudyTrax) with built in identification systems to ensure they are completed by the subject. This will minimize the safety risks between participants and study staff due to the COVID-19. All other procedures for the screening visit including the physical and modified Allen's test, will be completed at the first in-person visit. All other procedures for the behavioral visit including the Autonomic Assessment, Paced Breathing Task, Sit-Stand Task, and Quantitative Sensory Testing will be completed at the in-person behavioral visit.

Pain Ratings

Before and after each Baseline visit, Booster visit, and Post-Treatment visit, participants will be asked about their headache pain, non-headache pain, and anxiety. These questions will be on a scale from 0 to 100, where 0 is no pain and 100 is the worst pain imaginable.

Randomization

Patients will be randomized before completing their pre-intervention evaluations. This will be at least 4 weeks after their screening visit, and after it has been determined that the participant's frequency of migraines is within the range specified in our inclusion/exclusion criteria (between 4 and 20 headache days per month). Study staff conducting the pre-intervention assessments will be blinded to the treatment arm assignment.

6.2.3 Blinding

All patients and study staff will be blinded, except for the study coordinator and the statistician, who will execute the randomization scheme, and Cambridge Health Alliance (CHA) study staff directly involved in the delivery of this behavioral intervention (i.e. MM trainers). Outcome assessment will be performed by blinded personnel. For RAVANS administration at home practice sessions, the study coordinator will program the stimulation devices to active or sham stimulation according to each subject allocation. The device instructions and display will be the same for active and sham groups, with the only difference being that subjects in the sham arm will not receive electrical stimulation. Subjects in the sham group will also be told that stimulation will be at a fixed level and that they "may or may not feel anything at the ear".

The blind will be unlocked only after the last subject has completed their participation and the database is locked. The recruitment and the randomization order will be maintained by the research coordinator and study staff. Information regarding the blind will be kept in a secure file, accessible by this staff member.

6.2.4 Treatment Visits

Sixteen treatment visits will be performed in the 8 weeks following the completion of pre-intervention evaluations. Measurements and procedures in each visit will include:

- Visits 5-20:
  - MBSR or Education control intervention
  - RAVANS tVNS or Sham tVNS intervention
  - Session attendance log
  - Concomitant medications questionnaire
  - Credibility questionnaire (Weeks 1 and 8 only)
  - Group climate questionnaire (Weeks 4 and 8 only)
  - Adverse events form
  - Headache diaries
  - Ambulatory ECG and Actigraphy measurements
  - Pain ratings

6.2.5 Post-intervention Evaluation

Final evaluations will be performed in visits 21-23 and will include the following procedures:

- Post-treatment fMRI session:
  - fMRI safety form
  - fMRI scan (including forehead, RAVANS tVNS stimulation, and breath-hold task)
  - Concomitant medications questionnaire
  - Recent medical history survey
  - Adverse events form
  - Headache diaries
  - Menstrual phase questionnaire
  - Pain ratings

- Post-treatment PET-MR session:
  - PET-MR safety form
  - fMRI safety form
  - Menstrual phase questionnaire
  - Beck Depression Inventory-II
  - PET-MR scan (including IAPS task and interoception task)
  - Motion Coherency Task
  - Concomitant medications questionnaire
  - PET recent medical history survey
  - Adverse events form
  - Headache diaries
  - Pain ratings
  - Participants will be given: Post PBR28 Instructions and Post Arterial Line Instructions (if applicable)

- Post-treatment ANS session:
  - Behavioral questionnaires (including HIT6, MIDAS, MSQL, PCS, PROMIS-29, Perceived stress scale, BDI-II, FFMQ, Freiburg-14, SCS-SF, MAIA-2, DERS, SECD-6, HMSE, Brief Resilience Scale, WPI, Love and Care for Nature, Connectedness to Nature)
  - Autonomic tasks (including paced breathing, sit/stand test, QST)
  - Concomitant medications questionnaire
  - Recent medical history survey
  - Adverse events form
  - Headache diaries
  - Pain ratings

6.2.6 3- and 6-month Follow-up (home-based Patient Reported Outcomes):

- Questionnaires (including HIT6, MIDAS, PCS, PROMIS-29, HMSE, Concomitant medications questionnaire)

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

fMRI, PET-MR safety considerations

The FDA considers investigations of fMRI software and hardware operating within FDA specific parameters as non-significant risk device studies. All studies will adhere to these (non-significant risk) FDA approved safety levels for the Siemens system. These safety parameters include static magnetic field, time varying magnetic fields (dB/dt), specific absorption rate (SAR), and acoustic noise levels. Risks already established for fMRI include claustrophobia due to confinement of the patient in the system, the malfunction of electromagnetic implants caused by interaction with the magnetic fields, projectiles and tissue burns caused by metallic tattoos or implants, pregnancy, surface burns due to interaction of metallic system components or surface adhesives with the patients skin, slight hearing impairment due to high acoustic noise levels generated by the system, and slight neuromuscular twitching (for the higher field strength systems). However, system safeguards have been designed and operating guidelines have been provided to minimize any of the aforementioned risks.

PET-MR safety considerations

The FDA recently gave the first regulatory clearance of a hybrid PET-MR scanner in the U.S. An intravenous catheter will be placed for this study. The subject will feel a slight pinprick, similar to a bee sting, and may feel some discomfort and have some bruising or bleeding at the site where the needle goes in. Depending on the length of time the catheter is in place, a bruise may last for a day or so. Rarely an infection may occur at this site. If infection does occur, it will be treated. Also, for participants who agree to an arterial line each time, an intra-arterial catheter will be placed by an anesthesiologist, on the arm opposite of the radioligand injection line, for blood draws during the PET study (1-2ml of 1% lidocaine will be injected as needed to numb the area). Local infection, swelling, and redness could occur at the sites of line placement, as well as temporary loss of pulse at the wrist. This area may have a bruise or feel uncomfortable for 2-3 days after the catheter is removed. The risks associated with having blood drawn include: bruising, local discomfort, or infection at the site of the needle puncture. Rarely an infection may occur at this site, and if an infection does occur, it will be treated. Inserting an arterial line (A-line) can hurt more than having a regular IV or having blood drawn with a needle. We will place the A-line under local anesthesia (i.e., lidocaine), which may cause an allergic reaction. Even if we numb the wrist area first, the insertion may still hurt. Once the A-line is in place, it usually does not hurt. We will ask subjects not to take triptan or ergotamine related drugs (dihydroergotamine, or Cafergot, or sumatriptan etc.) 72 hours before the PET visit. Refraining from taking these medications could increase the risk of pain and discomfort from headache. If the headache pain becomes worse

and the subject needs to use these kinds of medications within 72 hours prior to the PET visit, we will reschedule the visit if possible. Other medications such as Tylenol and other over-the-counter pain medications will be allowed.

The subject may experience:

- Pain, bleeding, swelling or redness at the wrist. This could mean infection, but infection is rare (less than 1 in 100) and can be treated.
- Short loss of pulse at the wrist if blood flow in the artery is briefly stopped (for example, because of a clot or spasm of the artery).
- Damage to the artery wall or nearby nerves.
- Catheter breaking or falling out.

There have been reports of decreased blood flow to the hand, which resulted in the need for surgery. This is very rare and has not been reported when catheters have been in place for only a few hours for research.

After the catheter has been removed by MD or NP:

- We will ask the subject to stay for at least 30 minutes so we can check on him/her.
- The subject may have a bruise or feel tenderness for 2-3 days around the area where the catheter was placed.
- We will instruct the subject to avoid lifting anything heavier than a small bag of flour for a day.
- Approximately 24 hours after the beginning of the imaging procedures, we will give the subject a phone call to determine whether he or she is experiencing study related issues.
- We will instruct the subject to call us if:
    - Bleeding occurs after the subject leaves (rare).
    - The wrist area is painful or red or swollen.

At least a Nurse Practitioner and a Registered Nurse will be present for at least 30 minutes following radiotracer injection.

The radiation exposure in this study will be small and there is no evidence that it represents a major health risk. If subjects have participated in other research studies in the past 12 months that have involved radiation exposure, they will be asked to inform the investigators or study staff (by writing initials on the consent form verifying that they have not been exposed to other radiation in the past 12 months). If it is determined that their prior radiation exposure exceeds our current guidelines, it is possible that they will not be allowed to participate in this study.

We will use [^{11}C]PBR28 produced by the cyclotron/radiochemistry/ radiopharmacy facility at the A. A. Martinos Center for Biomedical Imaging. The Radioligand injection will be administered by a licensed Nuclear Medicine Technologist. Should there be an adverse event during PET/MR, Drs. Hadjikhani or Loggia will be responsible for communicating with the IRB within the stipulated time frame. Steve Stufflebeam, MD, PhD, is a radiologist and will review any abnormal results that are discovered on the images and refer subjects for follow-up and treatment if needed.

Mindfulness Meditation Safety considerations

The mindfulness training intervention given in this study is a behavioral intervention and can be considered minimal risk. The main risk is of increased psychological discomfort, due to increased attention to unpleasant thoughts, feelings or body sensations that can lead to an increase in undesirable feelings. Since commonly encountered psychological discomforts will be discussed as a regular part of trainings (e.g. noticing the frequency of negative thoughts or emotions, becoming aware of unusual habits or thought patterns, etc.), some participants may experience psychological discomfort during discussions. The trained therapist has extensive experience with minimizing discomfort in patient populations going through this training.

Educational Control Intervention risks

The control intervention given in this study is a behavioral intervention and can be considered minimal risk. The main risk is of increased psychological discomfort, due to increased attention to unpleasant images, that can lead to an increase in undesirable feelings. The trained therapist has experience minimizing discomfort in patient populations going through this training.

Virtual Group Visit Risks

Patients will attend group sessions virtually, using a computer-based video conferencing computer application. Video conferencing risks include an increased expense from cellular carrier if participants have a limited data plan and rely on a mobile data, instead of home internet, for videoconferencing. All internet-based technology, like the mobile application, is open to eavesdropping. Because of this we have made every effort to reduce the chance of outside people gaining access to protected data. The system is very secure with updated security protection technology, but no system is perfectly secure.

RAVANS tVNS Stimulation risks

There is a small possibility of slight and temporary discomfort at the site where the electrodes are placed. Potential vaso-vagal reactions to stimulation resulting in dizziness or light-headedness will be promptly recognized and treated by a study physician or clinically-trained personnel with prompt removal of the electrodes and repositioning the subject into a supine position. In some subjects, the experience of electrical stimulation may cause anxiety. Subjects will be required to remain under supervision until all symptoms subside. A study physician will be available at all times

to discuss the study with subjects should they become concerned. The electrical stimulation procedure is without significant safety concerns. The electrical stimulator, checked by MGH Biomedical Engineering, has a current limiter preventing harmful stimulation levels. Further, during the electrical stimulation, we will be closely monitoring that the current received by the subject is well tolerated. Throughout the measurement sessions, participants will be repeatedly asked if they are well and comfortable. Should the subject feel any discomfort he or she will be advised to let the researcher know and the stimulation will be immediately interrupted. Subjects will be trained at the first booster session on the use of the device at home and study personnel will be available by phone or video in case subjects present any question or concern. In addition, log stimulation files extracted from the device will be assessed weekly by unblinded study personnel to assess compliance and the appropriate use of the device.

Breath-Hold Task risks

No serious risks are associated with the levels and durations of CO2 to be induced in the breath hold task. Elevated PCO2 can sometimes cause light headedness during breath holding and shortness of breath immediately after breath holding. These symptoms are also time-limited and resolve spontaneously without need for medical treatment. Most importantly, the subjects have the full control to restore normal breathing whenever they wish.

Sensory Testing risks

There may be increased pain from some of the sensory tests (QST). The increased pain will usually decrease and go away within minutes. There is a slight chance of mild transient bruising associated with use of the probes or algometer. In our experience, this is quite rare (< 5 % of cases). Electrode gel used for stimulation and monitoring may cause minor skin irritation that would resolve within a few days.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

We will follow our institutional and NIH policies for reporting adverse events. As the study intervention is minimally invasive and generally safe (i.e. tVNS), the safety profile for this study is not high risk.

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recording regardless of their relationship to the study intervention.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

Examples of adverse events for this study would be dizziness, nausea, headache resulting from tVNS stimulation and pain, swelling or bruising resulting from the A-line from the PET/MRI scan, however safeguards have been put in place to minimize the risk of such events.

All SAEs will be confirmed in the participant's electronic medical record. If the participant does not have a MGB record, study staff will inquire with the participant and ask for documentation (e.g., patient portal, discharge summary, doctor's note, etc.).

7.4 Reporting Procedures

In accordance with Partners Healthcare Research Committee regulations, all unanticipated problems including serious adverse events will be reported within 5 working days/7 calendar days. In the case of SAEs, copies of the written follow-up report will also be sent to the PHRC office as well as any additional follow-up evaluations. The investigator will report for up to one month all SAE's that occur after a subject has discontinued or completed the study.

Non-serious adverse events will be reported in writing to PHRC. All AEs, SAEs, and protocol deviations will be kept in logs in the study's regulatory binder.

In addition, since this is an IND regulated study (IND#142546 for the use of [11C]PBR28 to assess neuroinflammation with PET imaging), reporting procedures will be in compliance with the Federal Food, Drug, and Cosmetic Act (FDCA) (21 U.S.C. 301 et. seq.) as well as the implementing regulations [Title 21 of the Code of Federal Regulations (CFR)]. These procedures will include

- Reporting any unexpected fatal or life-threatening suspected reactions to the FDA no later than 7 calendar days after initial receipt of the information. [21 CFR 312.32(c)(2)].
- Reporting any (1) serious, unexpected suspected adverse reactions, (2) findings from other clinical , animal, or in-vitro studies that suggest significant human risk, and (3) a clinically important increase in the rate of a serious suspected adverse reaction to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting [21 CFR 312.32(c)(1)].
- Submitting annual progress reports within 60 days of the anniversary of the date that the IND became active (the date the clinical study was permitted to begin) [21 CFR 312.33].

7.5 Follow-up for Adverse Events

All AEs and SAEs will be monitored until resolution. Additionally, subjects whom have experienced an AE will be followed up with one month following the event.

7.6 Safety Monitoring

An independent Data and Safety Monitoring Board, forming the Independent Monitoring Committee (IMC), will be organized for all proposed research. The DSMB for this study will be a 3-member multidisciplinary group whose members include a neurologist, a pain researcher, and a statistician; Drs. Zev Schuman-Olivier and Bruce Rosen will coordinate these DSMB interactions. All adverse event reporting will be coordinated by Dr. Schuman-Olivier who, as noted, will be informed of any adverse events immediately after or during their occurrence. Regular meetings will be held annually; however, in the event of severe adverse events, Dr. Schuman-Olivier and the committee will convene immediately.

8. INTERVENTION DISCONTINUATION

Intervention will be discontinued for subjects who:

- Experience AEs that interfere with study completion
- Are unable to complete study visits
- Cannot follow protocol
- Are deemed unable to continue the trial based on judgement of the PI

Possible reasons for discontinuation are:

- Inability to understand questionnaires/study protocol
- Claustrophobia
- Scheduling conflict
- Concomitant medications

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

We will employ a multi-modal approach to evaluate the effects on migraine pathophysiology following MM and RAVANS tVNS. We will apply fMRI, PET, and H-MRS neuroimaging at baseline and following 8-weeks of therapy in a 2x2 randomized longitudinal trial design with groups receiving RAVANS or sham tVNS, and MM training or education control. Following screening and 4 weeks of daily migraine diary completion and baseline behavioral/ANS and PET/MRI sessions, a total of N=96 episodic migraine patients will be randomized considering a 15% attrition rate for the goal of N=96 migraine participants in 4 N=24 groupsPatients will return post-therapy for PET/MRI and behavioral/ANS evaluation.

Our primary outcomes are the longitudinal treatment group contrasts (post-treatment vs baseline) among Migraineurs in the following variables:

- Cortical amplification ratio to trigeminal afference as assessed by fMRI (specifically, pINS to Sp5 fMRI percent signal change).

- HF-HRV response to sensory/affective stressor.

- [^{11}C]PBR28 PET ligand binding in pINS

Our secondary outcomes are the baseline contrasts between Migraineurs and healthy controls in the aforementioned variables.

9.2 Sample Size and Randomization

Study outcomes will be evaluated using a similar statistical approach (generalized linear model) with identical contrasts. As such, they share similar power calculations for their primary outcomes. These power calculations were conducted using the 'pwr' and 'pwrFDR' packages in R and are presented below:

Primary Outcomes: Longitudinal treatment group contrasts among Migraineurs

Assuming 15% attrition after randomly allocating N = 96 individuals in a 1:1 fashion to each study arm (n = 24), an evaluable group of individuals with migraine (N = 96; n = 24/arm), with a two-tailed alpha = 0.05, a moderate correlation between pre-treatment and post-treatment scores (i.e., r = 0.50), and using pairwise between-groups contrasts, the study will have power = 0.80 to detect an effect size as small as $d \geq 0.86$ after adjustment for all six possible comparisons across intervention arms using a false discovery rate (FDR) of 0.10.

Secondary Outcomes: Baseline contrasts between Migraineurs and Healthy Controls

Assuming an evaluable (i.e., excluding attrition) group of individuals with migraine (N = 96) and healthy controls (N = 50, considering a 50% attrition rate for healthy controls), with a two-tailed alpha = 0.05, and a between-groups GLM contrast, the study will have power = 0.80 to detect an effect size as small as $d \geq 0.65$. For each of the projects, the observed pilot data exhibited large effect sizes (ranging from $d \sim 0.90$ to 1.2) that will be sufficiently powered given the proposed sample size.

9.2.1 Treatment Assignment Procedures

To reduce the chances of sampling bias, defined narrowly as systematic differences across randomized groups, we will employ a modified version of covariate adaptive randomization. In covariate adaptive randomization, a new participant is sequentially assigned to a particular treatment group by considering specific covariates and previous assignments of participants. However, in this study, individuals must be enrolled in the nearest temporal cohort in relation to their screening. To accommodate this fact, each pool of currently eligible individuals will each be scored based on their potential to balance the experimental groups, conditional on previous assignments and their characteristics. Assignments will be based on a solution that places the maximum number of individuals currently under consideration into the current cohort while assigning the fewest possible to the next cohort (i.e., a two-variable optimization). This is important as any delay from when subjects express desire to enroll in the study and actually receiving therapy (at absolute minimum, the time would already be 1 month of daily diaries plus 3 weeks of MRI/PET scan and behavioral sessions), impacts dropout. Thus, at worst subjects would join the next group cohort and the method of minimization will be used to

reduce the difference in groups if the current participant was assigned to the current group or the next available group. The variable considered for balance will be monthly headache frequency.

9.3 Definition of Populations

Care will be taken to reduce the manual corruption of data, but it is inevitable that certain data will be corrupted (e.g., equipment failure), missing (e.g., attrition), or excluded due to a priori designations (e.g., poor treatment compliance). A full accounting of data will be recorded in all cases and included in the reports of the analyses. Readers of the research reports will be specifically informed of the enrolled samples/patients and the amount of missing data for any reason. Where appropriate, attempts will be made to examine the impact of missing data on the analyses.

A modified intention to treat (mITT) philosophy will be applied to the primary analyses for participants enrolled in the clinical trial. Any participant who is enrolled, randomized, is observed for the baseline testing session, and engages in a single treatment session will be incorporated into the mITT analysis set. A per protocol (PP) set will also be designated as those individuals who complete all testing sessions as well as complete their treatment assignment.

To account for missing data in either analysis set, an imputation strategy will be utilized that is appropriate to the extent of missing data. The PP set, by definition, will exhibit missing data only due to corruption of measurements or equipment failure. Thus, it is expected that missing data will be very limited, and if <15% of total measurements, will be viewed as missing completely at random and ignored in the analysis. If missing data >=15%, multiple imputation strategies will be used to estimate missing variable distributions from which to generate unbiased inferences. For the mITT set, we will rely on techniques recommended by White et al. (2011) including adjusted analyses relying on multiple imputation (MI) and inverse probability weighting (IPTW). For both the MI and IPTW models, we will examine the pattern of missing data for evidence of violations of the missing at random assumption (e.g., headache frequency progression leading to enhanced drop-out).

9.4 Interim Analyses and Stopping Rules

There are no interim analyses planned for this clinical trial. Although the data will be monitored by the PIs for safety outcomes, and the DSMB will be provided with summary level data, no formal superiority or futility analyses will be conducted prior to the completion of the study. When the enrollment targets have been met, the plan of analysis will be conducted as outlined below.

9.5 Outcomes

9.5.1 Primary Outcomes

Project 1:

- Effects of combined MM + RAVANS tVNS on brainstem and cortical response to trigeminal sensory afference in migraine patients

Longitudinal analyses will use post-treatment amplification ratio contrasted

between the four treatment arms using a generalized linear model. This model will evaluate post-treatment levels of cortical amplification ratio to trigeminal afference as the dependent variable, treatment group main effect as the predictor, and baseline amplification ratio as a covariate (i.e., an ANCOVA). A statistically significant main effect will be interpreted as evidence that treatment assignment induces a differential change in amplification ratio. These changes will be further evaluated using planned contrasts designed to evaluate the impact of group assignment in Bonferroni corrected pairwise contrasts.

Project 2:

- Effects of combined MM +RAVANS tVNS on peripheral parasympathetic activity of migraine patients

Longitudinal analyses will use post-treatment HF-HRV response to sensory/affective stressor contrasted between the four treatment arms using a generalized linear model. This model will evaluate post-treatment HF-HRV response as the dependent variable, treatment group main effect as the predictor, and baseline HF-HRV response as a covariate (i.e., an ANCOVA). A statistically significant main effect will be interpreted as evidence that treatment assignment induces a differential change in HF-HRV response. These changes will be further evaluated using planned contrasts designed to evaluate the impact of group assignment in Bonferroni corrected pairwise contrasts.

Project 3:

- Effects of combined MM +RAVANS tVNS on neuroinflammation in migraine patients

Longitudinal analyses will use post-treatment PET [11C]PBR28 SUVR signal contrasted between the four treatment arms using a generalized linear model. This model will evaluate post-treatment [11C]PBR28 SUVR signal in the posterior insula as the dependent variable, treatment group main effect as the predictor, and baseline pIns SUVR as a covariate (i.e., an ANCOVA). A statistically significant main effect will be interpreted as evidence that treatment assignment induces a differential change in PET signal. These changes will be further evaluated using planned contrasts designed to evaluate the impact of group assignment in Bonferroni corrected pairwise contrasts.

9.5.2 Secondary Outcomes

Project 1:

- Differences in cortical amplification ratio and habituation in response to trigeminal sensory afference between migraine patients and healthy controls

The cortical amplification ratio to trigeminal afference at baseline will be contrasted between the MIG and HC using a generalized linear model (GLM) with the amplification ratio as the dependent variable (and appropriate link function) and migraine group (MIG vs HC) as a fixed factor. Habituation ratios for all subjects will be calculated by linear fit for individual fMRI response estimates over 11 stimulus blocks modeled as continuously scaled fixed effects. The slopes will be evaluated using the same GLM as the primary outcome.

- *Glutamate levels in MIG and longitudinal response to MM + tVNS and association in trigeminal afference response with neurotransmitter concentration*

The analysis plan will use a LCModel incorporating a voxel-wise analysis with H-MRS-derived maps of Glx (combined glutamate and glutamine) concentration, as analyzed by GLM. An additional model will examine an amplification x habituation interaction term in predicting glutamate response.

Project 2:

- *Differences in HF-HRV response to sensory/affective stressors between migraine patients and healthy controls*

The HF-HRV response to sensory/affective stressor at baseline will be contrasted between the MIG and HC using a generalized linear model (GLM) with the HF-HRV response as the dependent variable (and appropriate link function) and migraine group (MIG vs HC) as a fixed factor.

- *Central autonomic network (CAN) response to sensory/affective stressors in migraine patients and its response to combined MM + RAVANS tVNS*

Using amygdala and other CAN regions' (i.e., insula, MCC and mPFC) response to negative images (in a region-of-interest analysis), we will test for group differences between migraineurs and HCs using GLM as described above. We will also test for treatment group differences between migraineurs using GLM as described above.

- *Relationship between CAN activation and peripheral parasympathetic response to sensory/affective stressors in migraine patients and its response to combined MM + RAVANS tVNS*

Post-treatment changes in amygdala response will be regressed on PRE-POST change in HF-HRV during IAPS provocation with negative images in migraine patients receiving MM and/or RAVANS tVNS. Using PRE and POST data in migraine patients from the 3 active interventions, we will test for association between PRE-POST change in amygdala response and PRE-POST change in HF-HRV during negative image presentation using GLM. The same analyses will be conducted for neutral images, and for other brain regions within the CAN.

Project 3:

- *Differences in neuroinflammation levels between migraine patients and healthy controls*

The [11C]PBR28 SUVR maps and fALFF-slow 4 maps at baseline will be contrasted between the MIG and HC using a generalized linear model (GLM) with [11C]PBR28 SUVR maps or fALFF-slow 4 maps as the dependent variable (and appropriate link function) and migraine group (MIG vs HC) as a fixed factor. In a secondary analysis, we will use an alternative strategy to remove the effect of potential confounders (age, sex and genotype) that may not be fully statistically corrected for. In this case, a subset of migraine patients, from the available pool of 96 patients recruited in the study, will be identified and compared with the controls in a matched pairs design, in which each patient will be matched to a TSPO

polymorphism-, age- and sex-matched control subject. The differences between these matched pairs on *baseline* [11C]PBR28 SUVR and fALFF-slow maps will be evaluated using GLM.

9.6 Data Analyses

All of the analyses conducted under the aims and hypotheses for all projects will be conducted using the generalized linear model. This approach allows flexibility in modeling the outcome distributions using link functions (e.g., normal, log-normal) while maintaining the statistical power of a parametric approach. Additionally, using this approach over a non-parametric equivalent, allows the estimation of the adjusted models, formal statistical interaction, and allows for the study of individual change.

Each of the primary, secondary, and adjusted models will be conducted utilizing the experimental groups and manipulations as fixed effects. For example, one set of comparisons evaluates the participants with migraine versus healthy controls (Mig vs HC). For models involving individual change (i.e., longitudinal models) across the four experimental groups, group assignment will be entered as a fixed effect, with baseline levels of the outcome variable entered as a covariate, and the post-therapy measurements will be specified as an outcome using a link function appropriate to distribution under study. This approach is analogous to an ANCOVA for which Vickers & Altman (2001) illustrate as being very advantageous when there is at least a modest within-individual correlation between measurement occasions. When a statistically significant main effect is observed, protected post hoc testing will be conducted to contrast the impact of each treatment to examine the additive (single modality treatments) and interactive (both treatments combined) in relation to each other. The adjusted models will be conducted in the same way but with an additional covariate entered into the model to adjust the associations for baseline migraine severity (number of headache days per month).

Several of the outcomes examine the association between interval-scaled fixed effects, and these will also be conducted using generalized linear models. For these models, the outcome of interest (or change in the outcome of interest) will be regressed on the predictor of interest (or change in the predictor of interest). Estimating the associations using this approach allows for flexible outcome distributions, additional covariates in the adjusted models, and produces association metrics in the original units of the variables under study.

All analyses will be conducted using the most recent version of R and R studio. Given our commitment to conducting reproducible analyses, we will utilize the R language to capitalize on its advanced capabilities for generating publication quality outputs that allow a reader to evaluate the results and the code that produced them. There are several methods that are primarily used to make analyses verifiable and more transparent
(see: https://cran.rproject.org/web/views/ReproducibleResearch.html). Each of the analyses will be disseminated to the investigators using these data driven documents.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data will be collected by the study staff who will also be operating the MRIs. These individuals will be blinded to treatment allocation. Questionnaires will be completed and stored electronically using the StudyTRAX database.

10.2 Data Management

All participant records and information are de-identified and given a code in order to protect patient confidentiality. All electronic records will be secured within the Partners password protected StudyTRAX database. All written records will be kept in a coded folder, filed and locked away.

Dr. Tohyama at the University of Toronto and Dr. Brusaferri at London South Bank University will access data from the current study using remote linux machines and MGB Dropbox. The data will include imaging from positron emission tomography / magnetic resonance imaging (PET-MR) and/or MRI imaging and demographic data (i.e., information such as age, binding affinity, and scan date). All participant information will be labeled with a study code such as mig001.

In the research consent form, participants will be prompted to give their permission for us to share their samples and health information for other research. Data will only be shared for those participants that indicate “yes” to the question, “Do you agree to let us store and use your samples and health information for other research related to chronic pain?”.

10.3 Quality Assurance

10.3.1 Training

All study staff have undergone MRI safety training and lead MRI operators have passed a certification exam to operate the magnet. All members of the study staff are CITI certified and have been trained to follow Good Clinical Practice (GCP). Additionally, study staff have undergone radiation safety training through the Massachusetts General Hospital Radiation Department.

10.3.2 Quality Control Committee

QC for all data will be completed by research assistants to ensure accurate completion and imaging data quality.

10.3.3 Metrics

We will use verified head motion QC metrics including frame displacement and root-mean-square measures for all imaging data.

10.3.4 Protocol Deviations

All protocol deviations are documented in the deviation log and are reported during scheduled Continuing Reviews through the Partners IRB Committee.

10.3.5 Monitoring

Monitoring will be performed as needed by the institution and NIH oversight personnel.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This study protocol and informed consent documents and all amendments/modifications will be submitted and reviewed by the Partners Human Research Committee IRB.

11.2 Informed Consent Forms

Study staff will have two copies of the Informed Consent on hand, one for themselves and one for the subject. They will go through each portion of the form with the subject and answer any questions before obtaining written consent. The subject and the study staff will sign, date, and time both documents. Fluency in English is an inclusion criterion, and subjects will need to be able to understand all study procedures in order to be eligible for the trial. Therefore, the subject must be able to read through the form with the study staff and provide their own written consent.

11.3 Participant Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NCCIH, and the OHRP

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

11.5 Virtual Group Visits

We have established a relationship with the HIPAA-compliant Zoom videoconferencing solution (https://zoom.us/). Zoom provides HD video and voice conferencing as well as resistance to video-freezing. All videos are protected with Secure Socket Layer (SSL) while in transit, using AES 128-bit encryption. Access is

controlled by establishing a "Host" of each Zoom meeting. The meeting will start and end exactly when the "Host" (the instructor) enters and leaves the meeting. Meetings are represented by unique URLs generated by the Zoom API at the instructor's discretion. The "Join link," which allows a participant to join the meeting, is sent from the study staff to the Participant at the time of meeting creation through Partners secure email. Participants will receive a new password each week to access the group meeting.

Furthermore, the host can (and must) explicitly allow or disallow access to anyone attempting to enter the meeting. As part of the fidelity measurement, the sessions will be recorded to allow for 10% of all video encounters to be assessed for quality assurance. The center of the intervention, the Center for Mindfulness and Compassion, currently owns the appropriate hardware and software components for the use of Zoom.

12. COMMITTEES

Non applicable

13. PUBLICATION OF RESEARCH FINDINGS

Study findings will be published in compliance with all institutional and journal policies.

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Appendix 1. Elaboration of Randomization Procedures

The randomization scheme used in the trial is designed to accommodate the complex interplay of the logistical considerations of delivering the interventions (e.g., the mindfulness intervention is administered in groups), the reality of scheduling scanner time months in advance, and best ensuring balance in the groups on baseline headache frequency. To obtain proper randomized allocations while accommodating these restrictions, two randomization procedures are combined to generate allocations:

Permuted blocks randomization:

A permuted block randomization strategy will first be used to generate a sequence of intervention groups denoted by their temporal order (e.g., the first through Nth intervention assignment). The 2 x 2 design yields 4 intervention conditions that are each conducted at scheduled times with groups of participants that have been recruited and have established eligibility for inclusion (n = 6 to 10 per group). A single block size, 8, will be used to maintain the masking of randomization order. Approximately 16 group cohorts will be conducted (i.e., 16 randomization assignments are expected to be required to fulfill sample size targets). This strategy will maintain a strict balance of intervention group assignments after each block of 8 groups has been conducted (though the participant sample sizes might differ across groups, see below). A restriction on the randomization will be imposed such that sequential randomization assignments will not be allowed to be identical. In this way, back-to-back group cohorts cannot be assigned the same combination of interventions (i.e. RAVANS tVNS and MM). These assignments will be conducted using the 'randomizeR' package in R.

Covariate adaptive randomization

Because of the importance of balanced group assignment to this project, an adaptive randomization will be conducted to specifically balance all four groups on baseline headache frequency. Headache frequency, or the number of headache days per month, is a variable that is itself associated with medication intake, headache-related disability, and affective distress. Thus,

balancing all four intervention groups on baseline headache frequency will better ensure balance on a host of variables that are likely to impact outcome.

The study will be conducted by selecting the next two available treatment allocation cohorts at each scheduled time. These two cohorts are referred to as 'yoked cohorts' because they will be conducted nearly simultaneously and because they will share the same pool of eligible participants for covariate adaptive randomization. Because of the restrictions in the randomization order (above), there will be two separate intervention groups in each yoked cohort and this will allow for the degrees of freedom required to perform covariate adaptive randomization.

Each participant in the yoked cohort pool will be assigned a sequential number based on the temporal order of his or her signed consent (1 to 24). Then, the covariate adaptive randomization procedures will be used to allocate sequential participants to one of the two interventions based on the assignment that minimizes the imbalance between these two groups on baseline headache frequency. This procedure is based on estimating a statistical test ($\chi 2$ goodness-of-fit) and considers all of the participants that have been randomized to these two groups up to the current assignment (i.e., cumulatively). The assignment that minimizes the imbalance is allocated to that participant and the next participant on the list is then considered until all participants have been assigned to one of the two yoked cohorts. This process is repeated for each yoked cohort and will produce a balance in baseline headache frequency across all four intervention groups. These assignments will be conducted using the 'SeqAlloc' package in R.