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PRINCIPAL INVESTIGATOR: Gisela Chelimsky, MD

STUDY TITLE: A pilot study of a randomized sham-control auricular TENS unit stimulation to improve symptoms through vagal modulation in pediatric functional gastrointestinal disorders

STUDY PROTOCOL and STATISTICAL ANALYSIS PLAN

A. PURPOSE OF THE STUDY

The main innovation of this application is to begin to unravel a mechanism of action of vagal auricular stimulation. The known facts are that this type of stimulation improves symptoms significantly in FGID, and that it increases vagal cardio-modulation in this context. We know separately that the vagus has strong anti-inflammatory effects. However, we do not know if (**a**) it does so in patients with FGID and (**b**) if this is a potential mechanism of action for the observed benefit. This project will answer these last 2 questions, and do so in a rigorous way, since placebo effect in FGID is high[1]. It is critical to include a sham (tactile sensory) arm in a blinded study context.

Positive results from this study would move the FGID field forward significantly. Potential conclusions would include that (**1**) the vagal stimulator inhibits the neuroinflammatory cascade, (**2**) if the degree of inhibition correlates highly with clinical benefit, that this is a potential mechanism for the benefit it proffers; and (**3**) that neuro-inflammation plays a major role in pediatric FGID, a currently open question.

B. HYPOTHESIS / SPECIFIC AIMS

The overarching hypothesis of this proposal is that auricular vagal micro-stimulation reduces pain through a vagal anti-inflammatory mechanism. The 2 aims of this proposal are (1) to confirm the known cardiovagal modulatory rise and show that clinical improvement correlates with the magnitude of this rise and (2) to determine whether stimulation reduces microinflammatory indices. Comparing 4 weeks of auricular micro-stimulation with sham we will determine whether:

Aim 1: Cardiovagal modulation (as reflected in high frequency heart rate variability, hfHRV) increases, and correlates with clinical improvement (as reflected in the Functional Disability Inventory (FDI) and a 10 point-liker scale of the 5 worse symptoms (e.g.: abdominal pain, nausea, fatigue, headaches and generalized aches)

Objective 1: To determine if the auricular microstimulator produces the expected increase in hfHRV and determine if this rise parallels evidence of clinical improvement.

Expected outcome: Based on prior experience, in more than half the subjects, we expect hfHRV to improve by 10% from baseline to week 4, along with an FDI score improvement of >5 points, and an improvement in the worse 2 symptoms by 2 points.

Aim 2: Pro-inflammatory cytokines drop as assessed by LPS-induced cytokine production

Objective 2: If vagal modulation improves, we expect a decrease in inflammatory cytokine production, particularly TNF α, IL6 and IL8.

Expected outcome: We expect a significant reduction by 10% or more in TNF α (index cytokine) and some reduction in other cytokines as well.

C. BACKGROUND, SIGNIFICANCE, AND RATIONALE

Painful functional gastrointestinal disorders (pFGIDs) affect 10-20% of the pediatric population[2] and impair daily activity in 40%[3], yet studies in children are few[4,5]. The management has not been standardized as so few interventions show any benefit. These include dietary changes like the FODMAP diet[6,7], probiotics[8], hypnosis and cognitive behavioral therapy[9-12], peppermint oil[13] and recently, an auricular neurostimulator[14]. This neurostimulator improves abdominal pain[14]. For pain, opioids are still too frequently used, despite the absence clear benefit and significant risk of substance use disorder and side effects[15]. Furthermore, **opioids are proinflammatory[16], worsening the neuro-inflammatory process and the chronic pain syndrome**, and carry a significant risk of substance use disorder. This risk is great in adolescents for several reasons. First, brain immaturity increases the tendency to impulsivity and vulnerability to alcohol and other drugs with long-term behavioral and cognitive dysfunctions. Second, the family setting allows access to opioids from other family members in the household[17]. Third, even a few doses of opioids given in the post-operative setting can induce

substance use disorder in adolescents. Finally, having chronic pain as happens in FGID is a risk factor for opioid addiction in itself[18,19]. Given these facts, it is critical to find a low-risk, effective, non-invasive and accessible treatment that impacts the core pathophysiology of FGID, not just the symptoms.

Functional gastrointestinal disorders (FGID) affect 6-20% of the pediatric population however neither treatments nor pathophysiology are defined. In this age of the opioid epidemic, chronic pain and history of physical or psychological trauma are a well-known risk factor for substance use disorder[19]. FGID is currently considered to be a brain disorder with central sensitization. These 2 factors, chronic pain and history of trauma, are often present in FGID and other disorders with central sensitization[20-23]. Neuro-Inflammation plays an important role in the chronic pain of FGID[24,25]and other central sensitization syndromes[26,27]. These disorders often follow an infection or injury, with persistent low-grade systemic and mucosal inflammation after resolution of the original insult[28]. A neuro-inflammatory cascade likely contributes to the eventual chronic pain state[24] through central sensitization and inhibition of the vagal anti-inflammatory pathway[29].

We and others have demonstrated that cardiovagal modulation measured by heart rate variability (HRV) is decreased in FGID and other chronic pain syndromes. Increasing vagal modulation either through cognitive behavioral therapy (CBT) or external auricular neuromodulation[14] (manuscript in progress) decreases pain in children and adults with FGID. Another device, a cervical transcutaneous vagal stimulator, increased cardiovagal modulation and decreased production of TNF α[30]. A parallel device, an ear microstimulator, appears to provide similar benefit, while stimulating only the auricular cutaneous territory of cranial nerve X (vagus). Evidence is mounting that these external auricular devices may be helpful in substance use disorder[31]. Substance use disorder is known to have an inflammatory substrate as well, as opioids are strong agonists at the TLR4 receptor[32], and low doses of opioid antagonists such as naltrexone have strong anti-inflammatory effects[33]. It is therefore possible that the impact of the auricular stimulator device in substance use disorder is also an anti-inflammatory mechanism.

We will compare the differences between 4 and 8 weeks of active auricular microstimulation by including a second phase in the study. This is will also ensure that all participants are guaranteed to receive active therapy even if they are assigned to the sham group in Phase I of the study. This will both aid in study recruitment, as well as giving us an additional comparator of sham and active therapies.

D. DESIGN AND METHODS

We will enroll 30 subjects in this double-blind sham control study. Fifteen subjects will undergo sham stimulation for 4 weeks while the other 15 will have auricular microstimulation for 8 weeks. Then, the 15 subjects who were in the sham group will undergo stimulation for 4 weeks.

We will measure vagal modulation supine and standing utilizing heart rate variability, measure cytokines and mitochondrial bioenergetics through a Seahorse analysis at week 0, 4 and 8.

Inclusion Criteria:

- Female patients 12 – 18 years old with chronic idiopathic nausea, functional abdominal pain, dyspepsia and/or irritable bowel syndrome.
- English speaking

Exclusion Criteria:

- Patients who are unable to stand upright during the heart rate variability recording
- Patients with a known bleeding disorder
- Patients with swollen,infected, inflamed, or other skin eruptions on outer ear
- Patients with epilepsy
- Patients with any implanted cardiac pacemaker or defibrillator
- Patients with serious arterial circulatory problems in the lower limbs
- Patients with abdominal or inguinal hernia

- Patients who are pregnant
- Any unstable medical condition, such as renal disease, uncontrolled diabetes, etc.
- Requires new medication during the 8 weeks of the study that may affect the gastrointestinal symptoms, vagal modulation or immune response.
- Inability to answer questionnaires or report pain in a 0-10 visual analog scale.

Week 0 – Visit 1 (Enrollment)
A member of the research team will approach the patient and his/her legal guardian during his/her visit to the Functional and Gastrointestinal Disorders Clinic. If the patient and his/her guardian are unable to complete consent documents during the visit, the researcher may collect consent over the phone or at the study baseline visit in the Pediatric Translational Research Unit (TRU), prior to starting study procedures.

Following the completion of consent, assent and HIPAA documents, a study physician will perform a brief medical assessment to confirm study eligibility. The "MD Assessment" form will be utilized for this assessment and will also collect demographic information such as birth dates, race and gender. Please see the form included with this project submission for complete list of data to be collected. Only patients who continue to meet study eligibility will be allowed to continue with this study. Participants will be advised that they are not allowed to start any new medications while participating in this study. If a new medication is needed, they should first notify the research team prior to starting the new medication. Vitals such as height, weight, BMI and blood pressures will be collected either during the patient's clinic visit or via chart review.

Heart rate variability will be collected using the FAROS 360 device. Electrode leads will be placed according to the diagram found in section F. The patient will be asked to lie supine in a dark, quiet room for 15 minutes and then asked to stand for an additional 5 minutes. A member of the research team or TRU nurse will be present during this recording to document if the patient moves or coughs. The patient will be allowed to sit or lie down if he/she is unable to stand for the entire time however, this will also be documented.

A member of the TRU who is qualified to perform phlebotomy will collect the blood samples for the analysis of cytokines and flow cytometry. Up to about 59ccs (3-4 tablespoons) will be collected. The blood samples will be escorted by a member of the research team to Dr. Verbsky's lab (5th floor of the MACC Fund Building) where they will be prepared and frozen for later analysis by a member of the research team and/or by Dr. Verbsky's laboratory technologist. A member of the research team will initiate the processing of these samples and transfer them to coded tubes before giving them to Dr. Versky's lab.

Study participants will complete the following study questionnaires: Functional Disability Inventory-Child and Adolescents Form, Symptom Intensity Questionnaire, Pain Catastrophizing Scale and Revised Child Anxiety and Depression Scale. The participant's parent will also complete the Functional Disability Inventory-Parent Form. The participant will also be asked to complete the TENS Unit Daily Recording Diary to document when the device was used. A member of the research team will review the questionnaire instructions with the study participants and will advise them that they are allowed to skip any question that they are not comfortable answering. The researcher will review the questionnaires for any missed responses and will confirm with the participant if the question(s) was intentionally skipped.

Subjects will be randomized to 2 groups: **a) group 1**: 15 females who will only undergo active auricular TENS unit stimulation for the 8 weeks. **B) Group 2**: 15 females who will undergo for the first 4 weeks sham (tactile) stimulation, and the 4 weeks of active stimulation. The active settings for the TENS unit is micro mode, pulse width: 50 millisecond (ms), a frequency of 10 Hertz (hz), and intensity at sub-sensory to minimally perceived threshold. The study investigating physician will place the first TENS unit to the participant's inner part of each ear (conchae) using a clip that is provided. The investigator will train the participant on how to place the unit at home. Subjects will be asked to use the unit for at least 1 hour, twice a day. The patient will determine what time during the day that they will utilize the unit, depending on their schedule. To document the placement and the time of day that the unit is use, a data collection form will be provided. A study team member's contact

information will also be provided if the patient has any questions or concerns. A study team member will contact the patient 2 times each month as a check in and reminder. The sham stimulation will be done with non-conductive cotton cushion applied to the electrodes and the TENS unit set at an intensity of 0.02mA. The investigator and performing research coordinator will be blinded to the stimulation mode. It is possible and permitted that the performing research coordinator will become aware of which group the subject is in when checking in on the subject.

The participant will be asked to contact the research team if they have any complications with the device or experience any adverse events.

Week 4 – Visit 2:
Study participants will return to the TRU to complete HRV and the study questionnaires specified in section F of the protocol. The participant's pain score and blood pressure will be obtained and documented on the MD Assessment Form. Participants who were randomized to receive sham therapy during weeks 0 to 4 will receive an active device. The study investigator will instruct these patients to turn it up until they feel it, then reduce the intensity until it just disappears. They will be asked to use the unit for a minimum of 1 hour, twice a day, until they return to CHW at Week 8 – Visit 3. The patient will determine what time during the day that they will utilize the unit, depending on their schedule. To document the placement and the time of day that the unit is use, a data collection form (diary) will be provided. A study team member's contact information will also be provided if the patient has any questions or concerns.

Participants who were randomized to receive active microstimulation at Week 0 - Visit 1, will continue to receive active microstimulation until they return at Week 8 – Visit 3.

A member of the TRU who is qualified to perform phlebotomy will collect the blood samples for the analysis of cytokines and flow cytometry. The participant's pain score and blood pressure will be obtained and documented on the MD Assessment Form. The coded blood samples will be escorted by a member of the research team to Dr. Verbsky's lab where they will be prepared and frozen for later analysis by a member of the research team and/or by Dr. Verbsky's laboratory technologist.

Week 8 – Visit 3:
Study participants will return to the TRU where they will complete HRV, the study questionnaires specified in section F of the protocol and a blood draw for cytokines and flow cytometry. The participant's pain score and blood pressure will be obtained and documented on the MD Assessment Form. The coded blood samples will be escorted by a member of the research team to Dr. Verbsky's lab where they will be prepared and frozen for later analysis by a member of the research team or by Dr. Verbsky's laboratory technologist.

Participants will receive a $20 check following the completion of each of the first study visit, a $20 check after completing the second visit and $30 for completing the final study visit. All checks will be written in the participant's name.

E. TOTAL NUMBER OF HUMAN RESEARCH PARTICIPANTS PROPOSED FOR THIS STUDY AT THIS SITE AND GLOBALLY. WHAT ARE THESE NUMBERS BASED ON?

For this pilot protocol, we will enroll 30 subjects from the Functional and Gastrointestinal Disorders Clinics in Children's Hospital of Wisconsin. The PI and her nurse practitioner see 16 patients with FGID per week. We have been performing many studies in our clinic and patients are usually very eager to participate; therefore, we are not concerned about the feasibility of completing this pilot study in 1 year. Furthermore, the study was designed so that every subject gets the active intervention, in part to increase enrollment and retention.

F. DRUGS OR PROCEDURES

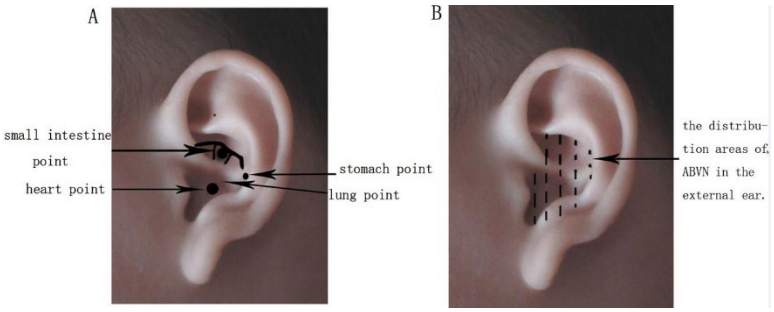
MD Assessment Form

Investigating physicians will perform a brief medical assessment with the participant to confirm study eligibility. This assessment includes a detailed history of gastrointestinal symptoms such as nausea, vomiting, abdominal pain, location of pain, duration & frequency of symptoms, bowel movements – frequency and consistency. In addition, this form will be used to evaluate non-gastrointestinal comorbid burden such as abdominal migraine and cyclic vomiting syndrome, temporomandibular joint disorder (TMJ), chronic fatigue, urinary symptoms, pelvic pain (including dysmenorrhea, endometriosis etc.), other chronic pain types, Raynaud's syndrome, syncope, postural tachycardia syndrome, chronic nausea or vomiting, complex regional pain syndrome, panic disorder, PTSD, anxiety and affective disorders and fibromyalgia (original criteria), evaluated by assessing 18 tender points described by the American College of Rheumatology[34]. Each symptom will be ranked as not present, possible, probable or definite based on the appropriate consensus criteria.

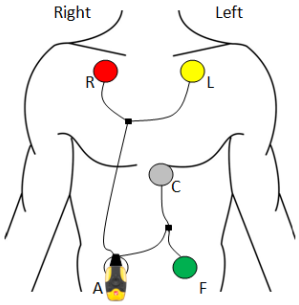
Blinding methodology: Patients will be informed that we are comparing 2 methods of ear stimulation, tactile and active. Both methods will be explained (either setting the machine at 0.02mA and using dry, non-conductive pads, or moving the device up until the stimulus is just experienced and then lowering until it is no longer experienced). Because the first stimulus is tactile, we will not use the word "sham" or "placebo", as we do not know that a tactile stimulus does not have some effect. In fact, ear piercings are popular for these symptoms. After both methods are explained the subject will demonstrate understanding of each. Once home, the patient will open a sealed envelope that was prepared by an independent research coordinator, and unknown to the investigator or the performing research coordinator, that instructs them as to which method to utilize. Only the independent research coordinator and the biostatistician will know the arm assigned. At the participant's Week 4 visit, the researcher will collect the previously used TENS unit device. The research coordinator will then provide all participants with another auricular device and letter which will include instructions on how to use the device during the remaining 4 weeks. It is remotely possible that some subjects will realize that the tactile form of the stimulation with dry cotton pads is actually a **sham** version of the stimulator. We think this is unlikely for 2 reasons. First, tactile stimulation of the ear is in fact sometimes referenced in the popular literature as a treatment (though this has not been formally tested). Second, neither version of the stimulator produces any conscious electrical sensation, so they will be quite comparable from an experiential perspective. In addition, the fact that tactile stimulation may have some effect will not alter the fundamental goal of this protocol, as we will be looking at the difference between the tactile and the electrical stimulating version. It is possible and permitted that the participant and the research team will become aware of which group the participant was initially assigned at the Week 4 visit.

TENS unit and Auricular Clip Placement

The TENS unit and auricular clip will first be placed by a qualified study investigator (physician) who will also provide teaching to the participant and family on how to place it at home. TENS unit applied to the inner part of each ear (conchae) by a qualified study investigator using a clip that is provided. The active settings will be set to the following: Micro mode, pulse width: 50 millisecond (ms), a frequency of 10 Hertz (hz), and intensity at sub-sensory threshold. This is the same process used for standard of care.



Heart rate variability (HRV) using the *Faros ECG 360*
The Faros ECG 360 is a remote electrocardiography monitoring system that is used for real-time HRV measurements. The ECG data will be transmitted from 3-5 electrodes (stickers) placed on the body (pictured below) to a mobile device through Bluetooth. The data can be retrieved in two ways: through a web browser for analyzing or with real time monitoring view on a computer. A password protected laptop will be used to import the data and then to import it into an HRV analysis software.



Heart rate variability will be collected using the FAROS 360 device. Electrode leads will be placed according to the diagram found in section F. The patient will be asked to lie supine in a dark, quiet room for 15 minutes and then asked to stand for an additional 5 minutes. A member of the research team or TRU nurse will be present during this recording to document if the patient moves or coughs. The patient will be allowed to sit or lie down if he/she is unable to stand for the entire time however, this will also be documented.

Questionnaires to be completed for research purposes
1. Functional Disability Inventory (FDI) will be completed by the participant (FDI-Child and Adolescent Form) and his/her parent (FDI-Parent Form). The FDI assesses the functional status of each subject. This brief 15-item instrument is widely used to assess functional disability in pediatric samples[35], including pediatric pain [36,37]. Both self- and parent proxy-reports will be used. The FDI has strong internal consistency (males $\alpha = 0.86$; females $\alpha = 0.91$), reliability, and validity[36]. Developed and validated by Walker[35], the FDI measures daily functions on a 5-point Likert scale such as ambulation, recreation, capacity for social interaction, performing chores, going to school, sleep, and eating. Validated for children with chronic abdominal pain, it shows excellent test-retest reliability within 2 weeks, and moderate at 3 months. It correlates well with school-related disabilities and both the child-report and the parent-report scores correlate with abdominal pain measures and other somatic symptoms[36,37].

2. Symptom Intensity Questionnaire score will be used to identify the most prominent 5 complaints rated in a 10 cm Likert scale the intensity form. Patients will write in each of their top 5 most severe symptoms on the corresponding line. They will then rate the symptoms severity from none (0 cm) to worst you can possibly imagine (10 cm) by placing a vertical line on the scale.

3. The Pain Catastrophizing Scale (PCS) will be completed by the participant (PCS-C) and his/her parent (PCS-P). The PCS-C is a modification of the adult Pain Catastrophizing Scale for use in children, measures pain-related cognitions and the dimensions of helplessness, rumination and magnification. This measure has both child-report and parent-report forms[38].

4. The Revised Child Anxiety and Depression Scale (RCADS) assesses children grades 3 to 12 containing subscales assessing for symptoms of Separation Anxiety Disorder, Social Phobia, Generalized Anxiety Disorder, Panic Disorder, Obsessive Compulsive Disorder, and Major Depressive Disorder.

5. Participants will be asked to complete the TENS Unit Daily Recording Diary while they are participating in this study. This diary consists of three columns; Date, Time 1, Time 2. Participants will update this diary daily to note when they used the TENS unit.

Blood for cytokines and flow cytometry
A qualified nurse from the Pediatric Translational Research Unit will collect up to 59ccs (3-4 tablespoons) of blood from each participant at study visit weeks 0, 4, and 8. A member of the research team who has completed laboratory training on the use of the centrifuge and hood, will initially process the blood prior to escorting the sample to Dr. Verbsky's lab in coded tubes. Members of Dr. Verbsky's lab will not be privy to any PHI. Due to decreased laboratory staffing/resources during Covid-19, samples will be frozen by a member of the research team after initial processing. These samples will remain viable until flow cytometry can be completed can be

	Week 0 Visit	Week 4 Visit	Week 8 Visit
Consent	X		
Pregnancy Test	X		
Device Randomization	X	X	X
MD Assessment	X	X	
TENS Unit and Auricular Clip Placement	X	X	X
HRV	X	X	X
FDI (Child and Adolescent Form and Parent Form)	X	X	X
Symptom Intensity Questionnaire	X	X	X
PCS-C	X	X	X
RCADS	X	X	
TENS Unit Daily Recording Diary	X	X	X
Cytokines and Flow Cytometry			

completed by Dr. Verbsky's lab. Primary blood mononuclearcells will be isolated by density-gradient centrifugation through Ficol/Hypaque (Pharmacia), suspended (8 ´ 106cellsml-1) in RPMI 1640 medium with 10% heat inactivated human serum (Gemini Bio-Products), and seeded in flasks. After incubation for 2h at 378C, adherent cells will be detached with 10mM EDTA, resuspended (106 cellsml-1) in medium supplemented with human MCSF (Sigma; 2 ngml-1), and seeded onto 24-well tissue culture plates (106cellsper well). Cells will be allowed to differentiate for seven days in the presence of MCSF. On day seven, fresh medium without MCSF will be added. LPS will be added to the cultures at a final concentration of 100ngml-1. Supernatants will be collected 4 and 20h after the addition of LPS and prepared for cytokine analysis. We will ship samples to Eve Technologies (located in Canada) where they will use their assay service to analyze the human cytokine array/Chemokine 42-Plex. We will use the high sensitivity assay due to low levels of cytokines in humans and will evaluate a panel consisting of EGF, Eotaxin-1, FGF-2, Flt-3L, Fractalkine, G-CSF, GM-CSF, GRO(alpha), IFNalpha2, IFNgamma, IL-1alpha, IL-1 beta, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-18, IP-10, MCP-1, MCP-3, MDC, MIP-1alpha, MIP-1beta, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, TGFalpha, TNFalpha, TNFbeta, VEGF-A.

Pregnancy Test

Participants will complete a urine pregnancy test at study baseline in the pTRU prior to beginning use of the TENS unit. Only participants who have a negative result will be eligible to continue with the study. Pregnancy tests will be administered by a qualified pTRU nurse.

Table of study procedures

G. RISK CATEGORY:

(1) 45 CFR 46.404 - Research not involving greater than minimal risk to the children.

H. RISKS AND THE PRECAUTIONS WHICH WILL BE TAKEN TO MINIMIZE RISK EXPOSURE

Breach of patient confidentiality is a risk to participants. All standard precautions concerning patient confidentiality will be taken. These include assigning the participants a code instead of their name and using that code to label their study documents. The research team will store the link to which code is assigned with which participant on a secure, encrypted database which only members of the IRB approved research team have access. Coded data will be entered into a REDCap database.

Coded heart rate variability (ECG) and psychological questionnaire data will be sent via encrypted email to study sub-investigator, Dr. Marcellus Merritt, at the University of Wisconsin Milwaukee, who will perform data

analysis. A member of the research team will ensure the data does not contain any identifiable information, such as name, medical record number or dates, prior to sending it to Dr. Merritt. Instead of sharing dates, we will label each with the study visit (week 0, week 4, week 8). Dr. Merritt will not be privy to any PHI.

It is possible that participants may develop skin irritation or scab(s) in the conchae of the ear from using the TENS unit. This is minor and, if experienced, the participant will be advised to move the placement of the auricular clip in a slightly different location. Participants may have pain or bruising from the blood collection.

Participants may temporarily experience some redness from the adhesive on the ECG surface electrodes. This redness, if experienced, is expected to resolve within a few hours.

Completion of questionnaires may be associated with mild distress in some participants. If parents and/or children become distressed during completion of study questionnaires, they are instructed in the consent and assent forms to inform the researchers. In turn, the researchers will notify the study investigators who will assess the distressed party, inform parents of children's distress, and provide referrals as needed and as dictated by law (e.g., suspected child abuse/neglect) to ensure patient and family safety and to address psychosocial needs.

The side effects from giving a blood sample for this project may include pain and bruising at the needle entry site. Rare complications of any venipuncture (drawing blood from a vein) include fainting, arterial puncture, peripheral nerve injury, local infection, and local blood clot. There may be other unanticipated risks, but every precaution will be taken to assure the participant's personal safety and to minimize discomfort. The person drawing the participant's blood will observe the participant for side effects. The participant will be asked to inform the study team if they experience any discomfort or feel faint.

The research team will send the coded blood samples to Eve Technologies where they will be analyzed. These samples will be labeled with the code that they study team assigns to the participants. No one at Eve Technologies will have access to any of the participant's identifying information.

Because assessment of child psychosocial functioning will be administered, the potential exists for clinically significant symptoms to be brought to light that the child or parent was not aware of previously. Any child endorsing clinically significant psychological distress or substance use problems on questionnaire-reported screening measures will be assessed by a qualified study investigator. Referrals will be provided as needed. The RCADS questionnaire will be scored within 5 business days. Dr. Gisela Chelimsky or study sub-investigator, Dr. Thomas Chelimsky, will contact study participants and their legal guardians if their score is 70 or higher to provide referrals as needed.

I. PROVISION FOR THE PROTECTION OF PRIVACY OF SUBJECTS AND TO MAINTAIN THE CONFIDENTIALITY OF DATA

As of October 2017 the NIH has updated their policy on Certificates of Confidentiality and are automatically issuing these for eligible studies that are NIH funded. They no longer issue a paper certificate, nor only submit on request.

Does this study qualify for automatic issuance of a Certificate of Confidentiality by the NIH?
☒No ☐Yes

For help in determining this see the IRB guidance document or visit the NIH website for information at: https://humansubjects.nih.gov/coc/index

Updated NIH policy can be found here: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-109.html

If you answered yes, and language regarding this is not included in the consent form(s) you will need to update the consent form to include this language (see NIH suggested consent language at https://humansubjects.nih.gov/coc/suggested-consent-language)

All research projects that collect electronic data must use appropriate security measures to ensure that data is protected from theft or loss in order to prevent breaches of confidentiality. You must indicate what encryption tools (or why they are not necessary) from the options below.

The IRB will not review this protocol unless you indicate the encryption tools being used to secure your research data. If you do not have encryption in place on your systems, please contact your Information Systems support to arrange for one of the encryptions options listed below.

The following encryption products employ cryptographic modules that the National Institute of Standards and Technology has certified as meeting FIPS 140-2 requirements. Children's Hospital and Health System endorsed the use of these products made to encrypt hard drives and removable media. All electronic research data must be encrypted using one or more of these products.

Please indicate which encryption tools you are using to secure your research data.

___ Credent Mobile Guardian (RS, PD)
___ GuardianEdge Hard Disk and GuardianEdge Removable Storage Encryption (HD, RS, PD)
_X_ IronKey encrypted flash drives (RS)
___ McAfee Endpoint Encryption (HD, RS)
___ Microsoft Bitlocker (HD, RS when used with Windows 7 and FIPS compliant algorithms are enabled)
___ PGP Whole Disk Encryption and PGP Portable (HD, RS)
___ SafeNet Protect Disk and SafeNet Protect File (HD, RS)
___ Seagate Secure Self-Encrypting Drives (HD when encryption option is enabled)
___ Symantec Endpoint Encryption (HD, RS, PD)
_X_ WinMagic SecureDoc encryption (HD) (for MCW owned computers)
___ Other (*add description*)

Does not apply because:

___ Data is de-identified – no PHI collected
___ Data is stored on paper only
___ Data is stored on CHW secured shared drives.
_X_ Data is stored on MCW secured shared drives.

Key

HD = Hard Drive
RS = Removable Storage (USB flash drive, CD, etc.)
PD = Portable Device (iPod; iPhone; PDA, etc.)

J. PROVISIONS FOR MONITORING DATA TO ENSURE THE SAFETY OF SUBJECTS; AND ADDITIONAL SAFEGUARDS TO PROTECT THE RIGHTS AND WELFARE OF SUBJECTS WHO ARE LIKELY TO BE VULNERABLE

Dr. Gisela Chelimsky will monitor the health of all patients in this study per standard clinical practice and she will also monitor protocol adherence and supervise data collection, entry, and analysis.

To protect subject's privacy interests consenting and research procedures will only be done in a private setting. Research team members will only collect necessary identified information to conduct the study and below we outline the measure in place to protect the interests of the subject.

We will store all records related to the child's involvement in this research in a locked file cabinet in the GI or Neurology office. We will indicate the child's identity on these records by a code number rather than by the child's name. The information linking this number with the child's identity will be kept separate from the research records in a password protected computer database or locked file cabinet. We will keep any information obtained from this research, as confidential as possible. We will see that the child's personal results of this research study are not part of the standard medical record. We will not identify the child's family by name in any publication of the research results. If such a need arises, we will obtain the child's written permission.

After the first 5 subjects have been enrolled a report to the IRB will be submitted by the study team to address the following questions:

- Has there been pain/sensitivity when using the device and ear clips?
- Has the subject used the device incorrectly (i.e., switching off the micro current setting)?
- Have other children in the household used/played with the device (or tried to)?

K. ANTICIPATED BENEFITS ASSOCIATED WITH THE PROTOCOL TO HUMAN RESEARCH PARTICIPANTS AND SOCIETY

If this auricular TENS unit device produces the expected results, it will: 1) provide a portable, affordable tool to decrease symptoms in FGID; 2) corroborate that increasing vagal modulation improves symptoms in FGID; and 3) if the pro-inflammatory cytokines decrease and mitochondrial bioenergetics increases, it will support the role of the vagus as an anti-inflammatory function in FGID and will expand the current understanding of the action of the vagus on the cardiac mitochondria to also the mitochondria in the blood cells.

L. STOPPING POINTS THAT WOULD NOT ALLOW THE STUDY TO CONTINUE AS PROPOSED

The stopping points for the study include the request to stop by the patient or parent/guardian as well as the inability to recruit participants or unexpected adverse events that might result in stopping the study.

M. IS THERE A DATA SAFETY MONITORING BOARD IN PLACE? WHO ARE IT'S MEMBERS? HOW OFTEN DO THEY MEET?

We will not use a DSMB for this project.

N. DESCRIBE HOW THE CONSENT AND ASSENT PROCESS WILL TAKE PLACE. INCLUDE A LIST OF APPROPRIATELY TRAINED PERSONNEL WHO WILL BE INVOLVED.

Written informed consent for participation will be obtained from parents for their child's participation. Written assent will be obtained from youth 12-13 years of age using the CHW assent form. Adolescents ages 14 and up will sign the assent line on consent form per CHW policy. Participants aged 18 years will sign the adult portion on the consent form. The study will be introduced to participants at the CHW GI clinic following their standard of care visit. Consent will be obtained by a study investigator or a member of the research team at the participant's visit to the CHW GI clinic, or at the baseline visit at the TRU prior to any study procedures. Participants will have the option of having the consent/assent document read aloud to them to facilitate understanding. Copies of signed consent/assent documents will be given to participants.

Although the MD Assessment form includes questions regarding whether the participant's various family members have been diagnosed with migraine, fibromyalgia, irritable bowel syndrome or if they are double jointed, we do not believe these various family members to be secondary subjects. The goal of collecting this information is to determine factors that may contribute to the subject responding or not to the microstimulation. For this protocol, we will only consent the child participant.

Patients who turn 18 years of age while participating in this study will be re-consented either in clinic at a routine visit or over the phone. If a participant is re-consented over the phone, a member of the research team

will first mail the "Letter to Reconsent Enrolled Participants" and a blank Consent/HIPAA form to the participant's address listed in the participant's medical record. A member of the research team will call the participant to complete the informed consent discussion over the phone. Once the participant signs the Consent/HIPAA form, he/she will mail it back to the research team member who completed the discussion and he/she will complete the corresponding signature portion. A completed copy will be mailed to the participant and a copy will be uploaded into the participant's medical record. The researcher will document this process on the informed consent discussion form which will be filed with consent form.

O. PROCEDURES TO BE EMPLOYED IN ANALYZING DATA AND THE ANTICIPATED SIGNIFICANCE OF THE PROPOSED STUDY

Data Management and Analysis The data gathered will be entered into RedCap data system with each ID having a unique identifier for each participant. REDCap is a secure, web-based application for building and managing online databases. All data will be de-identified, and samples will only have a unique identifier that will link the unique identifier with the patient's name.

Missing Data: Every effort will be made to avoid missing data. Using logistic regression, the pattern of missing data will be explored. Assuming the data are missing at random (MAR) multiple imputations for items will be used.

Randomization: we will randomize using Piantadosi randomization in blocks of 10. The sample size in this pilot study is small and it would likely result in bad imbalance of the two groups.

Data analysis overview: Summary statistics, such as mean, median, standard deviation, range and correlation will be used as a first step to examine data. To satisfy parametric assumptions, we may perform transformations with justifications if possible and otherwise use non-parametric tests. Statistical software employed for data analysis will be: Cytel StatXact, SAS version 9.4, SPSS Version 24. The power calculation was made using PASS 15.

Heart Rate Variability
The RR-interval time series recorded by the monitor will be detected from surface ECG signals at a sample rate of 1000 Hz and will be downloaded from the devices for further processing using custom HRV analysis software. The heart rate time series will be manually reviewed and processed in segments, excluding those from variability analysis in which artefacts or gaps account for > 15% of the recording. Shorter gaps will be replaced with intervals interpolated from adjacent normal beats. Frequency-domain analysis will be performed after constructing a detrended instantaneous RR-interval time series resampled at 5 Hz by piecewise cubic Hermite interpolation. The absolute and log-transformed power spectrum densities of consecutive segments will be computed for the frequency ranges of 0.04 to 0.15 Hz, designated as low-frequency power (LF), and 0.15 to 0.4 Hz, designated as high-frequency (HF) power. HF fraction will be computed as HF / (LF + HF) in addition to the LF/HF ratio. The HF power has been shown to result predominantly from parasympathetic modulation of heart rate. Time domain HRV parameters will include the standard deviation of all normal sinus beat (NN) intervals and the square root of the mean of the sum of the squares of differences between adjacent NN intervals. In addition to the inspection of the detected RR-intervals provided by the monitor, we will scan the recordings for non-stationary conditions employing Kalman-filter and wavelet-based spectrograms. Statistical analysis will be based on standard parametric or non-parametric group comparisons, as well as correlation computations were appropriate. The agreement between detected beats and the computed parameters for time-domain and frequency-domain HRV analysis between the monitors will be examined using Bland-Altman plots and summarizing using canonical correlations.

P. FINANCIAL RELATIONSHIPS

This study is supported by the 2020 CTSI Traditional Pilot Award. Participants will receive a total stipend of $70 for participating in this study via check ($20 after visits 1,2 and $30 after final visit).

Q. ADVERTISEMENTS / FLIERS

We will not use advertisements or flyers for this protocol.

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