Children's Hospital and Health System™

INSTITUTIONAL REVIEW BOARD SUMMARY

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TITLE OF PROJECT: Defining Adolescent Nausea through Brain-Gut Physiology and Non-Invasive Neurostimulation Response

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STUDY TITLE: Defining Adolescent Nausea through Brain-Gut Physiology and Non-Invasive Neurostimulation Response

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A. PURPOSE OF THE STUDY

The main purpose of this study is to characterize adolescents with chronic nausea through non-invasive gastric motor function and autonomic tests, and subsequently evaluate brain connectivity changes induced by auricular neurostimulation in functional gastrointestinal disorders (FGIDs). Functional nausea is a poorly understood condition that is difficult to manage due to lack of diagnostic testing and targeted therapies. Symptoms often overlap with those of gastric motor disorders such as delayed gastric emptying (gastroparesis) and poor gastric compliance. We have recently demonstrated safety and efficacy of auricular neurostimulation for the treatment of FGIDs. Functional magnetic resonance imaging (fMRI) is an established technique for studying brain networks and their connections. fMRI-BOLD (blood oxygen level dependent) signals detect changes in blood flow as a measure of brain activity. Resting state functional connectivity (rsFC) analysis is based on studying the functional organization of large-scale brain networks when a subject is not engaged in any mental task. Certain brain regions have greater coherence of neural activity as measured by the spontaneous fluctuations of the fMRI-BOLD signal. Studies of adult patients with a variety of chronic pain conditions show altered resting state brain network activity.[1-4] There is convincing data that baseline or rsFC between brain networks is dysregulated in patients with FGIDs.[5,6] Our group has recently reported similar findings in children with irritable bowel syndrome.[7] The amygdala is a limbic brain structure involved in emotions, fear, anxiety and processing of visceral sensations. The medial prefrontal cortex (mPFC) interacts with the amygdala to mediate the behavioral responses to various stimuli.[8] We have recently demonstrated efficacy of non-invasive peripheral neurostimulation of the external ear in adolescents with FGIDs (IRB ID# 689519-17) (unpublished data). Preliminary animal studies from our group show significantly decreased neuronal signaling in the amygdala after auricular neurostimulation. The current proposal will phenotype patients with chronic nausea into various subgroups and then investigate intrinsic resting state brain connectivity in the functional nausea subgroup compared to irritable bowel syndrome and healthy controls before and after auricular neurostimulation therapy.

B. SPECIFIC AIMS / HYPOTHESIS

Specific aim #1: Define functional nausea (FN) as an entity subdivided by measures of arousal, anxiety and gastric motor function.

Hypothesis: Adolescents with chronic nausea form subgroups based on clinical, physiologic and psychological parameters: 1) FN: characterized by high anxiety and emotional arousal, autonomic imbalance/low vagal tone and absence of major foregut dysmotility, 2) gastroparesis, and 3) poor gastric compliance. We will separate FN from other groups by clinical questionnaires, autonomic tests (heart rate variability) and gastric motility tests (gastric emptying, accommodation tests).

Specific aim #2: Demonstrate that resting state functional brain connectivity is different in female adolescents with FN compared to gender and age-matched healthy controls.

Hypothesis: Adolescents with FN will have altered rsFC between brain regions associated with emotional arousal neurocircuits (amygdala) and the top-down regulation of these by the prefrontal cortex. There will be decreased resting state functional connectivity between the ventromedial prefrontal cortex (vmPFC) and amygdala in adolescents with FN compared to healthy controls.

Specific aim #3: Demonstrate that four weeks of auricular neurostimulation improves nausea while strengthening the vagal tone and the abnormal resting state brain connectivity in female adolescents with FN compared to baseline and to more resemble that of healthy controls.

Hypothesis: 3a) Compared to baseline (pre-stimulation), there will be reduction in nausea and concurrent symptoms in the active compared to sham neurostimulation group after 4 weeks of therapy. 3b) Active neurostimulation therapy will increase vagal tone compared to baseline and also increase the rsFC between the vmPFC and amygdala in female adolescents with FN.

C. BACKGROUND, SIGNIFICANCE, AND RATIONALE

Functional gastrointestinal disorders (FGIDs) affect a large percentage of children and negatively impact quality of life.[9] The childhood prevalence is rising and based on a European study, the annual cost per patient is approximately $2800.[10,11] In adult patients with irritable bowel syndrome (IBS), the annual direct cost is estimated at $1.35 billion.[12] Our recently published study showed that over 50% of children with FGIDs suffer from chronic nausea.[13] Most of them have a diagnosis of IBS or functional abdominal pain. The nausea is seldom recognized or targeted therapeutically. Nausea in children cause disability and frequent school absences. In most cases, the nausea is deemed functional, without a known cause and without clear understanding of mechanisms. Pharmacotherapy is therefore empiric and the typical drugs used are centrally acting agents such as tricyclic antidepressants, suggesting the involvement of higher brain centers. In children, these drugs still have marginal efficacy compared to placebo[14,15] and alternative strategies are generally as effective.[16] A recent systematic review concluded there is no evidence to support pharmacologic therapy in pediatric FGIDs.[17]

No prospective studies exist that characterize functional nausea (FN) clinically or mechanistically. FGIDs are currently defined using symptom criteria (Rome criteria) rather than through a mechanistic understanding of their pathophysiology.[18] Symptoms of FN often overlap with foregut motility disorders such as delayed stomach emptying (gastroparesis) and reduced stomach compliance (a 'stiff stomach') and are difficult to distinguish clinically. Current testing for gastric motor disorders is very limited in children, with lack of pediatric norms, using tests that expose children to radiation (nuclear gastric emptying scintigraphy). Non-invasive tests such as the ^{13}C gastric emptying breath test (a non-radioactive isotope) has recently been studied in children and correlates well with scintigraphy.[19] Similarly, the nutrient drink test (NDT) is an easy and non-invasive method that has been used in children to test sensitivity to gastric distention and impaired accommodation.[20,21]

Excessive emotional arousal, fears, stress and symptom-related anxiety is perhaps the most common and striking co-morbidity in patients with FGIDs.[22,23] Functional disorders are thus often presumed to stem from purely psychiatric disease. This may result in even further stress, astigmatism and mistrust in health care providers. We have shown that a majority of adolescents with functional nausea are adolescent females with co-morbid anxiety, sleep disturbances and more debilitating symptoms compared to those without nausea.[24,25] Others have shown that anxiety reduces the threshold for nausea[26] and that FGID patients with nausea have more severe GI and somatic symptoms, depression, low self-esteem, disability and family stress.[27]

The sympathetic and parasympathetic branches of the autonomic nervous system (ANS) connect with the enteric nervous system to modulate GI motor function and sensation.[28] Nausea elicits robust ANS changes indicated by accompanying diaphoresis, pallor, lightheadedness, palpitations, salivation etc.[29] Our studies show a strong association between FN and co-morbid symptoms of autonomic imbalance (dizziness, anxiety, exhaustion, and postural tachycardia syndrome).[24] Over 90% of adolescents seen in our Neurogastroenterology Clinic suffer from chronic nausea.[30] Improvement in nausea following treatment of orthostatic symptoms is also documented.[31] Others have found convincing associations between nausea, orthostatic disorders and anxiety.[32-34] Additionally, studies demonstrate low vagal (parasympathetic) tone in FGIDs, suggesting a possible pathophysiologic role.[35-37] The brain stem nucleus tractus solitarius (NTS) is a major relay center for afferent signals transmitted from the GI tract through the vagus (parasympathetic) nerve. The baroreflex is critical to maintain orthostatic control.[38] Baroreceptor signals travel directly along CN IX and X to the NTS.[39] The NTS projects to higher limbic brain regions (amygdala, hypothalamus) involved in arousal, emotions and *central autonomic control*.[40-42] Autonomic imbalance in adolescents is closely linked to reduced vagal baroreflex response and heart rate variability (HRV). [43] HRV is a measure of the ability to modulate cardiac autonomic outflow via baroreceptors.[44] A study of adolescents with chronic nausea showed decreased HRV, suggesting

decreased parasympathetic vagal modulation of the heart.[32] Cranial nerves IX and in particular X (vagus) may thus link ANS dysregulation with symptomatic nausea and indicate involvement of higher brain regions in FN. The emotional arousal neurocircuits are abnormally engaged in FGIDs, specifically during *expectation* of a visceral stimulus.[42,45]

The vast majority of nausea sufferers are adolescent females who suffer from anxiety. For unclear reasons, many suffer from intense morning nausea. A strong female predominance is well documented in FGIDs.[46] There are significant gender differences in the regions of the brain activated following pain.[6,47] Studies specifically point to gender-related differences in the rsFC of the insula and limbic regions with males demonstrating greater activation in insula while females showing more activation in limbic regions such as the amygdala and closely associated structures such as the anterior cingulate cortex (ACC).[45,47-49] Studies in adults suggest that females may have enhanced sensitivity in the brain regions that process cognitive/emotional aspects of GI sensation[45] and in response to somatic stimulation such as noxious heat.[50] The gender differences are proposed to stem from a greater activation in females of the emotional arousal circuits, specifically during the *expectation* of a visceral stimulus.[45,51] A concept thus emerges where female adolescent suffering from intense morning nausea may have an abnormal emotional arousal control with concurrent low vagal tone/ANS imbalance, that predisposes to symptoms upon morning arousal, sustained and exacerbated by fears and anticipatory anxiety of recurring symptoms. Aberrant connectivity in emotional arousal neurocircuits is increasingly recognized through functional magnetic resonance imagining studies (fMRI) in FGIDs.

Aberrant Brain Network Connectivity

The emerging concept in neuroscience emphasizes functional connections between brain areas as part of large-scale networks. Of particular importance to visceral sensation and emotion regulation in FGIDs is the emotional arousal neurocircuits (emotional arousal network), stimulus detection and the central autonomic network. fMRI studies show alterations in connectivity amongst affective (amygdala, insula), sensory and attentional brain regions in female FGID subjects.[52,53] Resting state functional connectivity (rsFC) studies show abnormalities in limbic/affective brain areas such as the amygdala and insula in adult FGID patients.[6,45,51,54] The amygdala is a limbic brain structure involved in emotions, fear, anxiety and pain processing.[8] The amygdala communicates directly with hypothalamus and brain stem nuclei that drive autonomic responses. There are also projections from the amygdala to all major neuromodulatory systems including dopaminergic, cholinergic, serotonergic and noradrenergic systems.[8] There are also consistent reports of sex differences in amygdala responses during the anticipation of aversive emotional stimuli in both healthy and IBS patients.[45,49,55,56] Further, there is solid data that amygdala interacts with the medial prefrontal cortex (mPFC), an area involved in higher cognitive processing to consolidate memories. RsFC analysis shows that the extent of amygdala coupling with the mPFC influences how a subject regulates their emotional response to a stimulus.[8] Brain areas such as the mPFC may attenuate sensory input such that it remains below the perception level.

The vmPFC regulates autonomic responses in which lower vmPFC activity reflects vagal withdrawal.[57] Numerous fMRI studies show increased vmPFC with concomitant decreased amygdala activity during successful emotional regulation, suggesting that the *strength of vmPFC-amygdala coupling predicts healthier regulation of emotions and anxiety*.[8,58-60] A developmental switch occurs during adolescence when there is a normal reduction in amygdala reactivity due to top-down mPFC control over amygdala.[61] Preliminary fMRI connectivity analysis by our group shows impaired vmPFC, amygdala and hippocampal connectivity with the hypothalamus in adolescents with FGIDs. The emotional arousal network with amygdala as a key node is activated by perceived or real perturbation of body homeostasis.[62] Abnormal emotional arousal with poor feedback inhibition of amygdala by the vmPFC and altered ANS output results in heightened anxiety, lower vagal tone and morning symptoms upon awakening. Nausea coupled with anxiety is thus likely a manifestation of dysregulated emotional arousal and resulting impaired corticolimbic modulation of gut sensations. **We hypothesize that there is aberrant top-down control by the vmPFC on amygdala activity, characterized by decreased rsFC between these regions in adolescent females with FN.** Modulating brain signals that communicate discomfort such as nausea by means of peripheral neurostimulation may influence these specific brain regions and provide clues to underlying pathophysiology and physiology-based treatment.

Neuromodulation such as gastric electrical stimulation is an emerging therapy for GI disorders.[63-65] These effects are believed to be mediated by increased vagal afferent input to the NTS, influencing ANS and higher brain centers.[66-68] Tracing studies confirm that the external ear contains branches of four cranial nerves (CN V, VII, IX, X) that project to the NTS.[69,70] Animal and human data shows that transcutaneous stimulation of the auricular branch of the vagus nerve in the ear transmits signals to brainstem medulla and NTS.[71-73] The crucial role of the vagal neurocircuitry in the genesis of nausea and vomiting is widely accepted.[74] Auricular neurostimulation may thus alter the sensation of nausea through NTS activation and signaling to limbic regions where ANS outflow and low vagal tone underpin nausea and anxiety.[75,76] Stimulation of the entire auricle may affect all four cranial nerves in the auricle that converge in the brainstem NTS.[69] Peripheral nerve field stimulation of the auricle is thus a plausible means of modulating the vagal afferents and arousal neurocircuits. Our unpublished data on a percutaneous auricular neurostimulator, the Electro Auricular Device (EAD), in adolescents with FGIDs show significant improvement in pain and global wellbeing (randomized, sham-controlled trial). Animal data from our group also shows that EAD alter the response characteristics of amygdala neurons and improves visceral hyperalgesia. **We hypothesize that ANS dysregulation and aberrant functional connectivity in emotional arousal circuits is the basis of an amplified perception of GI tract sensation, elevated anxiety, reduced vagal tone and negative emotional state observed in FN.** *We expect that peripheral stimulation of several cranial nerves* will improve symptoms in adolescents with FN by strengthening vagal tone and the top-down regulation of vmPFC on amygdala. Improved phenotyping and correlation of symptoms with physiologic measures is key to establishing a mechanistic basis for effective therapies. We therefore propose to define FN through clinical features and physiologic testing and underlying brain pathways and evaluate the response to a novel, non-pharmacological and noninvasive neurostimulation device.

D. DESIGN AND METHODS

Feasibility of comparing fMRI data across age groups

One of the primary concerns in interpreting fMRI data obtained in children and in making comparisons across age groups involves the significant developmental changes in brain morphology that occur during childhood and adolescence. Although the majority of brain growth occurs prior to the age of 5 years, it has been estimated that approximately 5-8% of cerebral growth occurs between the age of 5 years and adolescence.[41-44] Furthermore, brain maturation proceeds at different rates in different regions of the brain. Concerns have been raised regarding the possible effects of these developmental changes on the process of spatial normalization, which involves transformation of images to a common stereotactic space based on an adult-derived template. The latter process is necessary in order to make direct voxel wise comparisons across subjects and subject groups. Recent studies do support the feasibility of making such comparisons within a common stereotactic space, suggesting that anatomical differences between the atlas transformed brain morphology of children (aged 7 to 8 years) and adults are minimal, relative to the level of resolution possible with current functional imaging and that would not result in spurious functional differences.[45, 46] This study will include only adolescents who are at least 12 years of age, further minimizing any potential developmental effects.

Study Population

80-100 adolescents with chronic nausea and a subgroup of 15 patients with IBS as well as 15 healthy controls, between ages of 12-18 years, will be enrolled. All subjects will undergo gastric motor function and HRV testing at baseline followed by four weeks of auricular neurostimulation or sham therapy. By definition, all adolescents in this study will have no clear explanation for their symptoms after undergoing medical workup per standard of care.

*Inclusion Criteria (**Nausea and IBS Groups**):*

- Children aged 12 years to 18 years who provide written assent and whose parents provide written permission for participation

- English-speaking and able to verbalize their condition and concerns about nausea, pain and other symptoms
- Subjects will meet Rome IV criteria for functional nausea as determined by a pediatric gastroenterologist
- Subjects included in the IBS group will meet Rome IV criteria for Irritable Bowel Syndrome
- Patients must have an intact external ear that is free of infection or severe dermatological conditions, have stable vital signs for their respective age, no history of seizures and no currently implanted electrical device
- Subjects included in the FN group include those who meet Rome IV criteria for functional nausea and have:
  1) autonomic imbalance with low vagal tone defined as baseline resting supine RMSSD value 2 or more standard deviations below the mean on HRV measurements, and
  2) normal gastric motor function (absence of gastroparesis or impaired accommodation). The gastric motor function and HRV tests will be compared to normative values obtained through a separate, ongoing, IRB-approved protocol (# 1030543-2)

*Exclusion Criteria (**Nausea and IBS Groups**):*
- Other pain-related diseases or somatization disorder
- Mental retardation or pervasive developmental disorder or epilepsy
- Psychosis
- Genetic or chromosomal disorders
- Pregnancy
- Subjects who admit to substance abuse during screening
- Patients with findings of peptic ulcer disease, H.pylori gastritis, celiac disease, inflammatory bowel disease, allergic disorders, or any chronic condition or medication that may cause nausea or pain
- Patients who are treated with opioids or who had any changes in their medical regimen in the past four weeks prior to study
- Patients with a history of allergy to adhesives
- Patients with a lactose intolerance

Study Design Overview

Subjects will undergo medical workup per standard of care. Subjects refractory to current medical therapy may be enrolled without changes to their current medical treatment. No new pharmacological agents will be added during the 4-week study period and no changes in dosing or scheduling of current therapy will be allowed during the study period. Subjects will be screened weekly for any side effects by registered nurses who are part of the Pediatric Translational Research Unit (pTRU). All subjects will be asked to notify the PI or research team with any worsening in symptoms or side effects of therapy. Any subject without improvement in symptoms during the study will be able to discontinue the study. If symptoms worsen or fail to improve during the study or if their clinical presentation changes in any way, subjects will have the option to drop out any time, receive standard medical therapy and appropriate referrals. If per standard of care, a subject requires a change in one or several of their regular medications during the study, they will be asked to notify the research team. To monitor this, the subjects will be inquired about their medication list and doses during their weekly visits. If the medication change is determined to potentially affect the study results, the subject may be excluded from further study as determined by the PI.

Protocol and Procedures

Pre (Baseline):

The primary investigator, a co-investigator or research coordinator will introduce the study to eligible patients seen in the CHW Gastroenterology Clinic during their regularly scheduled clinic visit. A member of the research team will explain the details and purpose of the study during an informed consent discussion. Once the patient and caregivers have had enough time to consider participation, understand all of the risks and benefits and would like to participate, they will complete the informed consent, assent and HIPAA documents.

Medical and psychological history will be obtained to verify eligibility and screen patients for alcohol, nicotine, and drug abuse following the completion of consent/assent documents. All eligible subjects will complete the questionnaires related to demographic, medical, and psychological factors including nausea, abdominal pain, IBS symptoms (Rome IV), anxiety, disability and quality of life (Table 1). This will take about 30 minutes total to complete. Subjects will then undergo baseline heart rate variability (HRV) measurements in clinic. Children will perform a self-assessment of the Tanner stage of pubertal development using a validated self-assessment form.

Visit 1: Subjects will undergo the ^{13}C gastric emptying breath test (GEBT) (Table 1). See section on ^{13}C GEBT for details and attached protocol.

Visit 2: Subjects will undergo Satiety Drink Test (see SDT section and attached protocol).

Visits 3-6: Subjects will complete questionnaires as per Table 1 followed by neurostimulator placement by study physician. These visits will be repeated weekly and take about 20-40 minutes to complete. HRV will be measured before and after the EAD placement at Visit 3 and 6.

Post-neurostimulation #1: Subjects will not return but will complete questionnaires (Table 1) at the end of 4 weeks of therapy which will be mailed back to the research team. Participants in fMRI sub-study will undergo repeat fMRI scan (post-scan) at this visit and complete questionnaires per Table 1.

Post-neurostimulation #2: All subjects will return to clinic for their regularly scheduled clinic follow-up visit 2 months after end of therapy and will complete questionnaires as per Table 1.

Subsequent to the initial 4 weeks of therapy, subjects will undergo another 4 weeks of **active neurostimulation therapy (visits 7-10) with the exact same protocol as during the first round of treatment. A post-neurostimulation #3 questionnaire assessment will be completed (just as with post-neurostimulation #1) although no repeat fMRI scans will be performed at this time. Similarly, a post-neurostimulation #4 clinic visit will be scheduled 2 months after the end of the second 4 week of therapy.

Optional fMRI Sub-study

Participants who are interested in learning more about the fMRI sub-study will indicate so on the main study consent form. A member of the research team will explain the sub-study to the participant and guardian(s). If the participant is willing to participate, the sub-consent and assent forms will be completed. Dr. Silverman will complete a psychological history for all participants who agree to participate in the fMRI sub-study. Female participants in the experimental group (Nausea group; n=15 and IBS group; n=15) who are found to be eligible to participate in the sub-study will be scheduled to complete the fMRI scans at Visit 3 (baseline scan) prior to EAD placement and Post #1 (post-scan; day 5 after the last neurostimulator placement within one hour of removing the device). The post-scan will be compared to baseline and baseline scans from 15 age-and gender-matched healthy controls who will undergo one fMRI scan (no therapy). All ethnic categories will be included.

Healthy control participants will be recruited via word of mouth. Researchers will mention the study to peers who will be instructed to contact the research team to learn more about the study. A member of the research team will introduce the study either in person or over the phone and will explain the inclusion and exclusion criteria. If the participant is eligible, consent/assent documents will be completed. Participants will then be scheduled to visit the pTRU where they will meet with a study physician who will perform a brief medical examination to confirm participant eligibility. Eligible participants will be escorted to the MRI unit by a member of the research team where they will be introduced to the mock scanner. Participants will have the option of lying in the mock scanner prior to their fMRI scan. A medical professional who is a member of the research team will be present during the scan. Healthy controls will only have 1 fMRI visit which will be completed at their convenience.

*fMRI Sub-study inclusion (**FN and IBS Groups**):*
- meet general study inclusion criteria

*fMRI Sub-study inclusion (**Healthy Control Groups**):*
- Female children, aged 12 years to 18 years, who provide written assent and whose parents provide written permission for participation
- English-speaking
- Subjects who have no previous disorders or history, symptoms or signs of gastrointestinal disorder, fibromyalgia, chronic fatigue syndrome, migraine, chronic dizziness while upright, PTSD, panic disorder, functional gastrointestinal disorders, constipation or fecal incontinence, diagnosis of postural tachycardia syndrome, diabetes, asthma, neuropathy
- Subjects who have not seen by a gastroenterologist within 2 years of enrollment at the discretion of the PI.
- Subjects who have no chronic gastroenterology complaint

*fMRI Sub-study exclusion (**All Groups**):*
- Currently receiving any medication that will strongly affect fMRI results
- Patients with pacemakers, metal clips used in previous surgery or other device which are not compatible with MRI scanning (for sub-study group only)
- Inability to lie still in the scanner (for sub-study group only)
- Claustrophobia or inability to lie still in the scanner (for sub-study group only)
- Orthodontic braces or permanent retainers (for sub-study group only)
- Patients who are unable to tolerate noise produced by the MRI

Visit 3: Following completion of the questionnaires, subjects who wish to do a mock scanning, will be scheduled for a 30-minute test fMRI session in the mock scanner. If the subject tolerates this, they will complete the fMRI scan. When fMRI scan is completed, subjects in the experimental group will have the neurostimulator placed (see section on placement details) by a study investigator who is a certified MD.

Post #1: Subjects in the fMRI sub-study (FN or IBS groups) will return to the scanner to complete their final fMRI scan on day 5 of last week of therapy, after removal of the EAD device.

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

We aim to enroll 80-100 adolescents with chronic nausea for phenotyping with tests and clinical questionnaires followed by randomization to neurostimulation or sham therapy. A subset of 15 adolescent females and 15 age-matched females with IBS will enter a sub-study involving the same procedures as well as pre and post treatment fMRI scans. Baseline fMRI data will be compared to scans obtained in 15 healthy, age and gender matched healthy controls.

F. DRUGS OR PROCEDURES

Table 1.

	Pre	Visit 1	Visit 2	Visit 3	Visit 4-5	Visit 6	Post #1	Post #2
Screening & Consent	x							
Tanner staging	x							
^{13}C gastric emptying breath test		x						
Satiety Drink Test			x					
Heart Rate Variability	x			x[1]		x[1]		
Nausea Profile	x			x	x	x	x	x
BARF scale	x			x	x	x	x	x
SUDS scale	x			x	x	x	x	x

API (pain and nausea)	x			x	x	x	x	x
PROMIS anxiety & global health	x			x	x	x	x	x
FDI	x			x	x	x	x	x
Nausea Severity Scale	x			x	x	x	x	x
CSI	x			x	x	x	x	x
VSI	x			x	x	x	x	x
PAGI-SYM	x			x	x	x	x	x
ROME IV	x							
Symptom response scale					x	x	x	x
Neurostimulator (active or sham)				x	x	x		
fMRI scan (subset)				x			x	

NP=Nausea Profile;API=Abdominal Pain Index; PROMIS= Patient Reported Outcome Measurement Information System; FDI=Functional Disability Inventory; CSI=Children's Somatization Inventory; VSI=Visceral Sensitivity Index; PAGI-SYM=Patient Assessment of Upper Gastrointestinal Symptom Severity Index

x^1 HRV will be completed prior to and after neurostimulator placement.

All procedures will be performed in the Children's Hospital of Wisconsin (CHW) Pediatric Translational Research Unit (pTRU). Prepackaged kits (Cairn Diagnostics, Brentwood, TN, USA) for the gastric emptying breath test and nutrient drinks will be stored by research coordinators in locked offices within the Division of Gastroenterology and brought to the pTRU on the day of testing. The prepackaged kits for the gastric emptying test and neurostimulators will be shipped by manufacturer when requested by PI. A box labeled with subject ID and containing neurostimulators (four), a GEBT kit and a labeled folder with patient ID and relevant questionnaires will be premade and kept with each subject package. After the breath test is performed, research coordinators will ship the samples to Cairn Diagnostics within 2 days of collection.

Measurements and Data Collection

At the time of each neurostimulator placement, a member of the research team will inquire and document current medications and doses and if subjects experienced any side effects from the neurostimulator during the previous week. Subjects will also be asked if they wore the device for all five days and it will be documented if the device was returned to the research team.

Demographic Information

Data will be collected as part of the clinic visit and at enrollment. This includes child's date of birth, age, sex, ethnicity, and caretaker marital status. The physician assessment will also include questions about prior medical diagnoses, co-morbid symptoms and the characteristics of pain and nausea such as timing and triggers.

Symptom Information

Patients will complete the questionnaires as outlined above before, weekly at the time of application of the neurostimulator and after the study completion. These instruments have demonstrated reliability and validity and have been used for both clinical and research purposes in pediatric populations. Up to one year after study completion, the investigators my conduct brief follow-up phone calls to subjects to assess long-term effects.

Medical Information

Information recorded for patients is part of clinical care and includes weight, height, vital signs, medications, gastrointestinal symptoms, early life events, family history, surgeries and other medical co-morbidities.

Gastric Emptying Breath Test (GEBT)

The ^{13}C-GEBT is a validated, FDA approved and radiation free alternative in which the isotope ^{13}C bound to *Spirulina platensis* algae labels a test meal.[77,78] The ^{13}C-labeled solid is absorbed in the duodenum, metabolized and excreted as $^{13}CO_2$ in breath.[79] Breath samples are collected at specific intervals as gastric emptying is the rate-limiting step for the exhaled $^{13}CO_2$. Baseline ^{13}C-GEBT is performed on all subjects using a standardized

meal as previously performed in children and adults.[19,77,78] End-tidal breath samples are collected before and at regular intervals after ingestion (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min). Breath samples are analyzed for $^{13}CO_2$ content using isotope ratio mass spectrometry (Cairn Diagnostics, Brentwood, TN, USA) as previously described.[77] The time at which half of the $^{13}CO_2$ is excreted in the breath is defined as the gastric emptying half time ($t_{1/2}$). Subjects are classified as delayed ($t_{1/2}>90^{th}$ percentile) vs normal ($10^{th}< t_{1/2} \leq 90^{th}$ percentile) gastric emptying based on control data obtained through separate study.

Regulatory status

The ^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) is FDA approved (PMA #P110015) for use in adults (see attached forms and intended use statement below).

Intended Use Statement

The Gastric Emptying Breath Test (GEBT), to be used with the GEBT test meal, is intended for use in the measurement of the rate of gastric emptying of solids and as an aid in the diagnosis of delayed gastric emptying (gastroparesis) in adult humans who are symptomatic for gastroparesis. For these purposes, the test system utilizes a Gas Isotope Ratio Mass Spectrometer (GIRMS) for the measurement of the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath samples.

The GEBT procedure will be administered under supervision of a health care professional although no specialized facilities or specially licensed personnel are required.

Description of the Gastric Emptying Breath Test (GEBT)

The GEBT is a non-radioactive breath test that uses the stable isotope, carbon 13 (^{13}C).[80] ^{13}C stable isotope labeling is inherently safe as 1.1% of all the carbon in nature, our bodies and in the food we eat is ^{13}C, with the remaining 98.9% being ^{12}C. The test consists of a breath collection kit with subsequent analysis of the breath samples by Gas Isotope Ratio Mass Spectroscopy ("GIRMS"). The breath collection kit includes a standardized test meal that contains freeze-dried ^{13}C labeled Spirulina.

After an overnight fast, the patient consumes the test meal consisting of:

- a dried scrambled egg mix containing approximately 100 mg of ^{13}C-Spirulina. The 13C-labeled Spirulina/scrambled egg mix is rehydrated with potable water just before cooking in a microwave oven
- Six (6) Nabisco PREMIUM Saltine crackers
- Six (6) ounces of potable water

Breath samples, collected before and after the test meal, are sent to Cairn Diagnostic's CLIA certified laboratory for analysis by GIRMS to determine the ratio of $^{13}CO_2/^{12}CO_2$. This ratio is used to calculate the $^{13}CO_2$ excretion rate. By measuring the change in excretion over time, the rate of gastric emptying can be determined.

Principle of the GEBT

After providing two pre-meal breath samples, the individual being tested consumes the standard GEBT meal consisting of 27 grams of re-hydrated, pasteurized scrambled egg mix containing a dose of 43 mg of ^{13}C (provided by approximately 100 mg of ^{13}C-Spirulina), 6 saltine crackers, and 6 ounces of potable water. The caloric value of the meal is approximately 230 kCal. As the egg meal containing the ^{13}C-Spirulina is triturated by the stomach to a particle size of 1 – 2 mm, it passes through the pylorus into the intestine. In the intestine, the labeled products of ^{13}C-Spirulina digestion (proteins, carbohydrates, and fats) are absorbed and metabolized giving rise to ^{13}C-labeled carbon dioxide expired in the breath. Breath samples, collected periodically in capped glass tubes before and after test meal administration, are returned to a central laboratory for analysis by Gas Isotope Ratio Mass Spectrometry (GIRMS) to determine the ratio of $^{13}CO_2/^{12}CO_2$ in each sample. By measuring the change in this ratio over time as compared to the pre-meal value, the rate of $^{13}CO_2$ excretion can be calculated and the individual's gastric emptying rate determined. In addition, MLR equations[78] can be used to

calculate “breath test fraction emptied” values and a “breath test t½” value that are comparable with scintigraphic fraction emptied and scintigraphic half emptying times.

GEBT Configuration

The GEBT procedure is conducted over a 4-hour period. Following an overnight (or ≥8 hour) fast, duplicate pre-meal breath samples are collected from the test subject. Two pre-meal samples are collected to ensure that in the event of breakage, leakage or a lost sample, there is a “back-up” available in order to establish a subject’s baseline $^{13}CO_2$ level. The first intact sample of the two pre-meal samples will be analyzed to establish the subject’s baseline $^{13}CO_2$ value. Following pre-meal sample collection, the subject is administered the test meal. Single post-meal breath samples are subsequently collected at 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes from the end of test meal consumption.

List of all materials provided with the GEBT kit:

- 1 Pouch of Diagnostic Dosage (^{13}C-Spirulina/Egg Mix in a foil pouch with oxygen absorber)
- 6 Saltine crackers (3 packages, of two crackers each, repackaged in a foil pouch with oxygen absorber)
- 1 large (~8 oz) microwaveable cooking cup
- 1 filling cup (small (~3.5 fl oz) plastic cup with pour spout for transferring water)
- 1 plastic cutlery kit (knife, fork and spoon)
- 2 wrapped, plastic straws
- 8 barcode labeled, glass, breath collection tubes with screw caps
- 1 breath tube holder
- 1 mailer
- 1 GEBT requisition form
- 1 package insert

Shelf Life and Storage

The ^{13}C-GEBT Administration Kit will be stored at controlled room temperature*. The kit has an expiry date; the GEBT Kit will not be used beyond the expiration date displayed on the kit box.

Collected breath samples should be stored at controlled room temperature*. Breath samples need to be shipped to Cairn Diagnostics, in a sample mailer, within three weeks of collection.

*Controlled room temperature: a temperature maintained thermostatically that encompasses the usual and customary working environment of 20°C to 25°C (68°F to 77°F).

Subject Preparation

The GEBT should be administered after an overnight fast. No solid food should be consumed or vigorous activity undertaken within 8 hours prior to the test. The individual being tested may consume a small amount of water up to 1 hour before the test, but not more than 4 fluid ounces. Coffee may enhance gastric motility and should not be consumed within 8 hours prior to testing. Subjects should not smoke/use tobacco products (e.g. chewing tobacco, nicotine gum) before or during administration of GEBT.

The research team will confirm that the individual being tested does not have a known hypersensitivity to Spirulina, egg, milk, or wheat allergens; is not severely lactose intolerant; and has not taken medications known to influence the rate of gastric emptying (e.g., erythromycin, metoclopramide, opiates and anticholinergics) within 3 days of testing. The GEBT procedure will be performed in a comfortable environment within the CHW TRU after obtaining height and weight information on participants. The individual being tested will sit quietly and fast (with the exception of the test meal) for the duration of the test. Limited walking is allowed, when necessary, e.g., use of restroom.

Satiety Drink Test (SDT)

The SDT is a simple and non-invasive measure of sensation to gastric distention. It has good reproducibility and correlates fairly well with barostat studies of gastric accommodation.[81,82 20] The maximum tolerated gastric volume and satiety is recorded after subjects ingest a nutrient formula at a predetermined rate until reaching maximal satiety (see protocol below). The volume ingested when reaching a satiety score of 5 corresponds to the maximal ingested volume (i.e. drinking capacity). The SDT measurements will be compared to normative data from a separate study. A drinking capacity (kcal) of <5th percentile for age and gender will classify a patient as impaired gastric accommodation.

Studies will be carried out on a separated day from the above GEBT, also in the morning after an overnight fast. Height and weight of subjects will be recorded prior to testing. Subjects will ingest a room temperature liquid nutrient drink (Ensure Original Vanilla; Abbott Laboratories, IL, U.S.) containing 220kcal per 237ml, 18% protein, 11% carbohydrates and 9% lipids. One of two beakers will be filled with the drink by a research coordinator or nurse at a rate of 30 ml/min. The subject will be instructed to ingest the drink at the filling rate by alternating the beakers. Every 5 min, they will be asked to score their satiety and symptoms of nausea, bloating, pain, burning sensation and vomiting on a scale 1-5: 1= no sensation; 2=first sensation; 3=mild sensation; 4=moderate sensation and 5= maximum sensation. The maximum tolerated gastric volume (kcal), i.e. drinking capacity, is recorded after subjects reach maximal satiety with a score of 5 at which point they will cease drinking. At the same 5 min intervals, subjects will also be asked to circle their nausea on a validated pictorial nausea severity scale (BARF scale) 0-10.[83] Twenty minutes after completing the test, the subjects will again score the above symptoms on scale 1-5. A research coordinator or nurse will assist the children with completing the scores and carefully explain symptoms such as satiety as their fullness and opposite of desire to eat.

Prior to any study procedures, subjects will be carefully screened for any allergies to Spirulina, egg, milk, or wheat allergens, lactose intolerance as well as use of medications. Subjects will be instructed that some discomfort may be expected during the nutrient drink test. They will also be notified that they may experience symptoms of nausea, fullness, bloating or pain. However, since the test is designed to cease when maximum satiety is reached, we do not expect significant symptoms to occur.

Heart Rate Variability

HRV reflects the variability of the R-R interval in the electrocardiogram and its frequency domains.[84] HRV is an indirect measure of central autonomic control and an indicator of overall psychological well-being.[85] Reductions in resting HRV reflect decreases in vagal output. The high frequency range of HRV is considered representative of vagal (parasympathetic) activity and studies show altered vagal tone as measured by HRV in subjects experiencing nausea.[86] All subjects will undergo a 9 minute baseline HRV measurement (3 minutes sitting, 3 minutes standing and 3 minutes sitting) after 10 minutes of rest. This will be repeated 20 minutes into neurostimulation and following the end of therapy. Data will be compared to the healthy controls from IRB protocol (#1030543-5). Recordings will be performed supine, sitting and standing to assess baroreflex response. HRV indices such as Respiratory Sinus Arrhythmia (RSA), low frequency domains, low/high frequency ratio and the time domain RMSSD as a surrogate of vagal tone[84] will be analyzed using CardioEdit/Batch software. The RSA values will be the primary HRV outcome and compared to baseline pre-neurostimulation and previously collected normative data from age-matched, healthy controls.

Questionnaires:

All patients will complete the questionnaires listed below. Patients are allowed to skip any questions that they do not feel comfortable answering. A member of the research team will introduce each questionnaire and explain the instructions to each of them.

1. Nausea Profile (NP). The NP is a two page, 17-item questionnaire validated in adolescents which measures the subjective experience of nausea on a scale from 0 (not at all) to 9 (severely) across three dimensions: 1) somatic distress; 2) GI distress and 3) emotional distress.[87] The subjects will be asked to rate their nausea over the past week. Three subscale scores and a total score is generated which can be used to measure an individual's experience of nausea and how it changes over time.[88] This instrument has demonstrated

excellent internal reliability in adolescents (Cronbach's α=0.941).[32] Subjects will also rate their usual level of nausea on a scale from 0 to 10 (10 being the worst nausea imaginable).

2. Baxter Retching Faces (BARF): pictorial daily nausea severity rating scale (0-10)[83]
3. Subjective Unit of Distress Scale (SUDS): during active treatment (3x) and off days (1x) to measure amount of distress on a severity scale (0=peace to 10=nervous breakdown)[89] (weekly averages will be computed)
4. Abdominal Pain Index (API). The API is a short, one-page instrument validated in children with functional abdominal pain.[90] A revised form of this scale will be used to assess nausea (=Nausea Severity Scale).
5. Patient Reported Outcomes Measurement Systems (PROMIS) Pediatric Anxiety-Short Form scale:[91] Symptoms of anxiety will be assessed via the short, 8-item PROMIS anxiety short form. A Research Psychologist (PhD) will assist with interpretation of the psychometric data. Scores will be compared against healthy norms, and pediatric chronic pain patients.
6. Patient Reported Outcome Measurement Information System (PROMIS) Global healthy: A newly developed and validated global health/quality of life outcome measure that provides an efficient (7-item) summary of a child's physical, mental and social health (both self and parental reports will be assessed).[92]
7. Functional Disability Inventory (FDI): Symptoms of disability and reduced quality of life secondary to pain nausea will be assessed with the FDI child and parent report forms.[93] This is a one page, self-report measure of the degree that children experience difficulty in physical and psychosocial functioning due to impaired physical health. Patients and parents rate each of the 15-items on a five-point Likert scale indicating how much difficulty they have doing common childhood activities because of their physical health.
8. Children's Somatization Inventory (CSI): 24-item instrument assessing child and parent report of multisystem somatic complaints. The CSI has been validated in children with functional pain disorders.[94]
9. Visceral Sensitivity Index (VSI): The VSI is a short, one-page validated instrument assessing gastrointestinal symptom-specific anxiety.[95]
10. Subjects will also fill out a short validated overall symptom response scale.[96] Subjects will rate their symptoms as better, worse or no change based on a 15 point scale: -7 to -1= worse; 0 = no change; +1 to +7 = better). A score of +5 to +7 will be considered significant improvement.
11. Patient Assessment of upper Gastrointestinal Symptom Severity Index (PAGI-SYM): [97] Upper gastrointestinal symptoms including symptoms of gastroparesis will be assessed via this 20-item instrument.
12. Rome IV Diagnostic Questionnaire on Pediatric Functional Gastrointestinal Disorders: clinical classification into Rome IV criteria for functional GI disorders (baseline only)[98]

Neurostimulator

<u>Auricular Neurostimulation (EAD) treatment protocol</u>

Subjects will enter a 4-week, prospective randomized, double blind sham controlled treatment trial with the FDA-approved and commercially available EAD manufactured by Key Electronics (Jeffersonville, IN, USA) and distributed by Innovative Health Solutions (Versailles, IN, USA). The device is cleared under the BWK classification, with intended use in the practice of acupuncture by qualified practitioners of acupuncture as determined by the states. EAD is an ambulatory, neurological device which consists of a battery powered, externally affixed generator with 4 wire leads attached to 3 electrode/ needle arrays and one single point needle. The arrays are designed to produce a field effect similar to surgically implanted peripheral neurostimulators. The electrodes will be placed percutaneously in the external ear with the help of a transilluminator to visualize the neurovascular bundles. Three electrodes will be placed on the ventral and one on the dorsal aspect of the ear. The electrodes will be taped and secured behind the ear next to the generator itself which is secured to the skin with adhesive. The entire device may be covered by longer hair. The placement of the devices is within standard of care by properly trained medical doctors as already completed trial at CHW (IRB# 689519-17). Training of MDs has been performed by Dr. Chris Brown or Brian Carrico as representatives of Innovative Health Solutions. Training of other involved physicians may be performed if protocol approved. 40-50 subjects will receive the active EAD device while 40-50 subjects will receive an inactive device that is identical to the active device but lacking the battery.

Subjects will be randomized in a 1:1 ratio to either treatment or sham group. Randomization will be determined using a computer program based on random number generation in blocks of 10 subjects at a time. The manufacturer will provide the researchers with four devices per package (4 weeks per patient) and distribute 10 packages at a time to the investigators. Each package will have a serial number and only one research coordinator will have the key to which serial numbers represent active devices and which represent inactive ones. This research coordinator will keep the data in a secure database and will not be involved in any of the patient recruitment or study procedures. All other research coordinators, investigators, statistician and nurses involved will be blinded. Research coordinators within the division of gastroenterology will store the devices as pre-packaged by manufacturer and label the devices with a serial number and patient ID number. A labeled folder for each patient with all questionnaires needed for the entire study will also be kept with each subject package. The devices and folders will be stored in a locked office in the gastroenterology division, only accessible by research coordinators. Password protected iPads will be used to collect questionnnaire information from participants. No PHI will be loaded on these iPads.

A blinded research coordinator will bring each subject's numbered package to the research area (Translational Research Unit) when patient arrives. The package for the subject will be stored locked in the TRU until the subject finishes the 4 week therapy. The coordinator will ask the subject to complete the pre-placement questionnaires (Table 1; visit #3) and hand the device (numbered with serial # and patient ID only) to one of the certified MDs for placement. The devices will be "made to order" when requested by the PI and will be shipped packaged as above.

Patients will be told that they may or may not feel a slight flushing or tingling sensation after device placement. The electrode/needle arrays are implanted according to the individual's arterial and cranial nerve anatomy. The exact location of the implantation may vary slightly from person to person but is determined by both knowledge of auricular neuro-anatomy and visualization of the neurovascular bundles by trans-illumination (IHS, Versailles, IN, USA). The points will be targeted by four-point electrical stimulation using the EAD device after carefully disinfecting the ear. The small device will be positioned and secured behind the ear similar to a hearing aid, which may be covered by hair. Neurostimulation will be delivered below sensation threshold continuously for 5 consecutive days. The EAD device will be applied by a trained MD. Subjects will remove the device independently at home and discard them as they are non-functioning after 5 days (5 days battery life). If a device is lost or broken, the research team will attempt to schedule placement of a new EAD within 1 day if you wish. The devices are easily removed by removing the tapes and adhesives. Before and during the treatment trial, subjects and parents will complete questionnaires as shown in Table 1.

The EAD Surgical kit consists of :

- an alcohol swab
- prep and stay swab
- round fixation plasters
- fixation plasters to fasten the generator
- Steri-strip adhesive vial
- Sterile wire harness pack
- Generator
- Tweezers
- Surgical marker
- Transilluminator

EAD placement details:

1. A research coordinator in the GI office will be asked to open the package and obtain either a true device or a control device as outlined in a randomization scheme provided by the statistician. This research coordinator will document the patient ID, name and the device serial number in the study database and whether assigned to an active vs inactive device. This lead research coordinator will be the only unblinded person and she will not be involved in any patient contact or other parts of the study.

2. The neurostimulator placement will be performed as directed and per EAD training protocol instructions identically to prior CHW IRB approved trial.
3. Before neurostimulator placement, the subject should be advised that some discomfort is normal at first but should report if the discomfort persists or gets worse after a few minutes. The patients should be advised that they may feel a slight pulsing sensation and perhaps a warming sensation in the ear to which the electrodes are affixed. The pulsing and warming sensation may disappear after approximately 5 minutes. If the discomfort level increases the offending electrode can be slightly repositioned until the patient's discomfort level decreases to an acceptable level.
4. Device placement will take approximately 5 minutes. The patient will remain at rest for the next 5 minutes.
5. The subject will be advised not to immerse the device in water as the device is water resistant but not water proof. If showering or washing their hair, subjects will be instructed to place a dry wash cloth or plastic such as Press n' seal over the area to help protect the device. Subjects will be given a contact person (primary investigator and research coordinator) to call if they are having any problems with the device or if it falls off.
6. The patient will be instructed on removing the device after 5 days. If not comfortable doing so or unable to carry out the instructions, the patient may be seen that day for removal by one of the investigators.
7. At the end of the study (post-neurostimulation #1), subjects will be asked to guess their group allocation to test blinding.
8. The patient will be asked to return the used device for proper disposal when they return for their follow-up appointment.
9. The subject will be given a handout with information (attached) on device handling, exact date and time of device removal and date and time to return.
10. The subject will be advised to refrain from using any new or as needed anti-emetic or pain medications.
11. The subject is dismissed.

Optional fMRI sub-study

Participants will be given the option to participate in the *f*MRI sub-study. Post menarche patients must yield a negative urine pregnancy test on the day of each scan. Participants will then be escorted to the *f*MRI scanner in the lower level of FH where they will complete the safety screening questionnaire. The patient will be given the option of lying down in the mock scanner prior to their first fMRI scan.

The fMRI scans will be carried out using GE 3T short bore scanner. This scanner is located at the Froedtert Hospital. For each participant we will perform one baseline whole brain anatomical scan and one resting state fMRI scan before and after therapy. Healthy controls will have only one baseline fMRI scan. The healthy controls will also undergo a visual task where they are simply shown images while in the fMRI scanner. These images will be related to toilet training and this data will be used for healthy comparison data from separate study by our group (IRB #774802-6). We anticipate the whole session to last about 40 minutes. Echo planner images resolved to 64x64 pixels/slice at TR of 2s and echo time of 25 ms will be obtained.

<u>Resting State Functional Connectivity MRI</u>

Resting state functional connectivity (rsFC) analysis measures the temporal correlation between a predetermined seed region of interest and functionally related brain regions. After subgrouping and additional consenting, 15 post-pubertal female subjects classified as FN will undergo a 10 minute anatomical scan followed by a 6 min rsFC MRI scan at baseline prior to first EAD placement which is repeated after 4 weeks of therapy (after stimulator removed) (Table 1; post-neurostimulation #1). 15 female subjects classified as irritable bowel syndrome (IBS group) will undergo the same protocol. Resting state MRI scans will be performed per protocol below. The amygdala will be used as a seed region and the functional connectivity between the amygdala and PFC regions will be examined during rest at baseline. Data will be compared in treatment

responders (if possible) and non-responders in order to evaluate connectivity differences before and after therapy in these two groups.

Multimodal Imaging protocols

Research-dedicated GE MR750 3.0 Tesla whole-body scanners with 32-channel phase-array head coil will be employed and the scanner is located in the Froedtert Hospital (FH)/Medical College of Wisconsin (MCW). Our scanning protocol will focus on integrating the most recent advances in multimodal imaging technologies, including T1 SPGR, T2 FLAIR, R-fMRI, and ASL-CBF images. The justifications of utilizing these imaging protocols are: 1) to noninvasively characterize the structural, functional, and metabolic changes in subjects; 2) these imaging protocols will provide robust quality control, repeatable and reliable imaging datasets for imaging post-processing and statistical analyses; 3) most of these imaging protocols and pulse sequences have been tested and released as products by major scanner vendors (GE, Siemens, and Philips), therefore, they can be immediately translatable to the clinic setting.

Specifically, the integrated multimodal imaging protocols and justification are summarized below:

1) SPGR high-resolution images. Acquire T1 weighted spoiled gradient recalled echo (SPGR) images for high spatial resolution of anatomical images. The SPGR images can be employed for tissue segmentation and volumetric analysis using FreeSurfer, and image registration for functional neural network studies.

2) FLAIR images. Acquire T2 weighted fluid attenuated inversion recovery (FLAIR) images for general pathological detection such as for macroscopic white matter lesion quantification, and micro hemorrhage. In addition, the T1/T2 ratio can provide myelination information.

3) R-fMRI images. Acquire resting state functional magnetic resonance imaging (R-fMRI) datasets to study intrinsic blood oxygenation level dependent (iBOLD) signal. These iBOLD signals will be employed to study changes in neural networks of the disease and control brains. It is hypothesized that a) alterations in functional cortical networks are apparent; b) Network wide analysis can identify and classify functional disease status together with their comorbidity. We will utilize "seed"-based, module-based, and whole brain network-based analyses to characterize the changes in nausea/pain brains. For the image acquisition, we will employ our newly developed pulse sequence, named Spatially compensated Intra-shot Turbo keyhole (SCITH), based on multi-echo denoise approach for robust reliability and repeatability for functional connectivity analyses.

All these imaging protocols and data acquisition will be completed within one scanning sessions in 30 min for each subject.

Data Acquisition and Analysis

Data acquisition: The cerebral cortical activity will be monitored in all subjects with a blood oxygenation level-dependent (BOLD) fMRI technique. Gradient echo-planar imaging (EPI) data will be acquired on a Neuro-optimized 3.0 T MR system (General Electric, Milwaukee, WI, USA). A quadrature birdcage head coil will be used for radio frequency (RF) transmission and reception. One hundred and twenty T2*- weighted images depicting blood oxygenation level-dependent (BOLD) contrast (Ogawa et al., 1990) will be acquired over 6 min at each of 14 near-axial non-contiguous 4 mm thick planes parallel to the inter commissural (AC±PC) line: TE (echo time) = 25 ms; TR (repetition time) = 2 s; matrix size = 64 x 64; FOV (Field of view) = 240 mm; in-plane resolution = 3.75*3.75 mm^2; interslice gap = 0.7 mm.

Scanning protocol: A 6 min resting state scan will be performed at baseline. The same protocol is repeated at end of therapy with another scan after device is removed (Table 1; post-neurostimulation #1). This will allow us to examine functional connectivity changes induced by the stimulation. rsFC will be compared between baseline and end of treatment in both treatment responders and non-responders and compared to healthy controls and IBS patients. Brain activation will be quantified for each voxel by measuring percent change of BOLD signal relative to the baseline. Spatial smoothing of the response magnitude across voxels will be

conducted using a 6-mm full-width half-maximum (FWHM) Gaussian kernel filter to compensate for intersubject variability. In the group analysis, activation maps for each stimulation state and group will be derived by applying voxelwise one-sample *t*-tests of fitting coefficient from regression analysis. The obtained statistical maps were corrected for multiple comparisons using the probability and cluster thresholding technique (AlphaSim in AFNI).

fMRI-guided connectivity analysis: We have developed a novel *fMRI-guided connectivity analysis* in previous investigations by Dr. Li to determine the distribution of an arousal network by using a seed region in a specific brain area of interest. In this protocol, we will examine resting state functional connectivity with amygdala as the seed region of interest and its coupling with prefrontal cortex areas as well as insula and hypothalamus as key nodes of the emotional arousal neurocircuit and internal awareness.

Statistical Analysis

Data Management
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.

The REDCap clinical database will be maintained by the Department of Quantitative Health Sciences, at the Children's Research Institute under the direction of Pippa Simpson, PhD.

Missing Data Handling
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. 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. We will use CART and biological and physiological information to identify interactions when formulating regression models. Statistical software employed for data analysis will be: Cytel StatXact, SAS version 9.4, Salford System CART for trees, SPSS Version 24. The power calculation was made using PASS 13.'

Power

Aim 1. We will summarize characteristics by the groupings.
Aim 2. See fMRI analysis section below
Aim 3. *Hypothesis 3a): Four weeks of active neurostimulation will reduce nausea and concurrent symptoms from baseline compared to sham therapy as measured by weekly Nausea Severity Scale total score (primary outcome) after 4 weeks of therapy. Secondary outcomes include daily nausea (BARF) scores, and weekly nausea profile scores, upper GI symptoms (PAGI-SYM), abdominal pain (API), anxiety (SUDS, PROMIS and VSI), quality of life (PROMIS), disability (FDI) and co-morbid symptoms (CSI)*. Another secondary efficacy variable will be the change in Nausea Severity Scale score after the second 4 weeks of therapy in a subset of subjects. We will compare pre and post total nausea, GI symptoms, anxiety, disability, quality of life and co-morbid symptom scores using a two-sided paired t-test at a Bonferroni corrected alpha of 0.05. Power: with ~40 in each group initially, allowing for 12% dropout based on our prior study, we will have ~35 subjects and 80% power to detect a difference of at least XX standard deviations (SD). We will similarly compare the other variables. Further we will use regression and regression trees with final value as the outcome and demographics, baseline values and overall symptoms as covariates. *Hypothesis 3b: Adolescents with FN will have reduced vagal tone as measured by HRV. Active neurostimulation therapy will elevate vagal tone compared to baseline and sham therapy, resembling that of healthy controls*. We will similarly analyze the change in vagal tone in nausea subjects. Further we will compare

the final vagal tone to healthy using a two sample t-test of equivalence where equivalence will be defined as within 20%. See fMRI analysis section for details on resting state fMRI analyses.

Longitudinal Continuous Variables

The efficacy parameters of longitudinal continuous variables such as nausea and pain will be analyzed using a mixed effects covariance pattern model to utilize all the data collected over time with consideration of the variance-covariance matrix of the repeated measures. This method allows a general unstructured variance-covariance matrix and allows patients to have incomplete data across scheduled time points. The method applied is also known as mixed-effects repeated measures analyses (MMRM).

The primary model will include independent variables of the fixed, categorical effects of treatment, assessment visit, and treatment-by-assessment, interaction, along with the continuous effects of baseline and baseline-by-assessment interaction up to end of study. An unstructured variance-covariance structure will be used to model the within-patient errors and different variance-covariance structures will be tried for goodness-of-fit exploration.

Continuous/ Ordinal variables

Summary statistics such as mean, median, standard deviation, range and correlation plots and tree analysis will be used to examine distributions and interrelationships. Where necessary, for parametric assumption, we will employ appropriate transformations with justifications. If data cannot be appropriately transformed we will compare variables between the two groups using a Mann-Whitney test.

Additional Analysis

In addition to the above described primary analysis, the following analysis will be done.

1. The mean change from baseline in all efficacy variable scores will be summarized using descriptive statistics at all assessment time points.

2. To assess effects of dropouts, the dropout cohort analysis will be performed by summarizing the change of primary and secondary efficacy variables using different dropout cohorts. Dropout cohorts will be formed by patients that had their last primary efficacy measurement in the same assessment interval.

For all efficacy variables, nominal p-values will be presented without applying any adjustment for multiple comparisons.

GEBT Metrics

The preferred and FDA approved GEBT metric is the Percent Dose (abbreviated PCD) excreted at time t after consumption of the test meal. To provide a more convenient scale, we multiply PCD by 1000 to produce kPCD at any time, t.

$$kPCD_t = \left[\frac{DOB * CO_2PR * R_s * 13}{10 * dose} \right] * 1000$$

where:

- DOB = The measured difference in the ratio [$^{13}CO_2/^{12}CO_2$] between a post-meal breath specimen at any time (t-minutes) and the baseline breath specimen.
- CO_2PR = CO_2 Production Rate (mmol CO_2/min) calculated using the Schofield equations[97] which incorporate the patient's age, gender, height and weight.
- R_s = The ratio [$^{13}CO_2/^{12}CO_2$] in the reference standard (Pee Dee belemnite) for these measurements. Rs = 0.0112372
- 13 = the atomic weight of Carbon-13
- 10 = A constant factor for converting units

- *dose* = the weight (mg) of Carbon-13 in the dose of ^{13}C-Spirulina administered to the patient in the test meal. Since ^{13}C-Spirulina contains approximately 43% Carbon-13, a dose of 100mg ^{13}C-Spirulina corresponds to approximately 43 mg of Carbon-13.

The kPCD values will be used to estimate the fraction of meal emptied from stomach at the specific time points. We will then generate normative range values for kPCDs (^{13}C-excretion rates) at the different time points and also determine the half-life of gastric emptying: Breath test $t_{½}$ (BT_$t_{½}$)

BT_$t_{½}$ values comparable to scintigraphic half emptying times will be calculated.[78] $^{13}CO_2$ excretion rates (kPCD) are used to calculate "breath test fraction emptied" values using the multiple logistic regression equations published by Szarka et al which correct for age, gender, height and weight.[78]

T_{max}, the time of maximum $^{13}CO_2$ excretion rate, is another metric that will be used to interpret GEBT results: patients with delayed gastric emptying rates usually have a T_{max} of 240 minutes.

NDT

The endpoint of the nutrient drink test will be the volume and calories ingested at a satiety score of 5. Data will be expressed as median and interquartile ranges (IQR). Correlations between the endpoint of the satiety drink test and parameters such as weight, height, BMI, age and gender will be analyzed using Pearson's correlation. Differences will be compared using student's *t*-test or Mann-Whitney *U*-test and considered significant at the 5% level. The upper and lower limits of normal for the maximum tolerated volume/calories will be calculated as the 5th and 95th percentile for each age group.

For all efficacy variables, nominal p-values will be presented without applying any adjustment for multiple comparisons.

Safety Data

All safety variables (e.g., adverse events will be summarized for each assessment time (including follow-up) using descriptive statistics. Incidence of adverse events will be summarized by treatment.

fMRI analyses

Strategies for imaging data analysis and data fusion with clinical observation. The goal for analyzing results from integrated multimodal imaging protocols is provide structural, functional, and metabolic information for the insight into the mechanisms of pain/drug abuse brains in order to advance long-term prevention, diagnosis, prediction and treatment planning. Our strategy for analyzing these large complexes imaging datasets will involve four steps: **First step**, preprocess imaging datasets for individual subjects and then convert voxelwise imaging parameters into standard imaging matrices in MNI templates. **Second step**, conduct group-level statistical analysis based on each of imaging modalities, e.g. functional (R-fMRI) or ASL-CBF images. There are three levels of neural network analyses: 1) Seed-based network analysis; 2) ROI-based large scale network analysis; and 3) whole brain Graph theory analysis (e.g., Small-world graphical analysis). From the second step, we will obtain imaging parameters as independent variables for each subject such as brain atrophy, CBF and functional connectivity index (FCI) at seed, regions, and whole brain networks levels. **Third step**, construct a database containing demographic information and a battery of neuropsychological and behavioral testing scores. **Fourth step**, conduct Data fusion processes from step 2 and step 3 with **Multivariate analysis** and **Multivariate Linear Regression for establishing the neural biomarker for diagnosing and measuring treatment responses/efficacy.**

Multivariate analysis will be employed in determining the sensitivity and specificity in discriminating normal, pain, or drug abuse subjects as described in specific Aims. The imaging parameters ***P*** from step 2 (e.g. Functional connectivity index (FCI) and metabolic information (CBF) will be statistically combined to give a composite imaging Index, which will be employed in disease diagnoses. We propose to use a multivariate classification

technique, such as Fisher's Linear Discriminant Function. Assume that we have a training sample of n normal subjects, and m diseased subjects (nausea or pain). For each subject, we measure *P* parameters. Then, we can assemble this data into the matrices: $\mathbf{X_1} = [x_{11}, x_{12},\ldots, x_{1,n}]$ and $\mathbf{X_2} = [x_{21}, x_{22},\ldots, x_{2,m}]$,
where x_{ij} = vector of *P* measurements for subject j from group i (i=1,2 (normal and diseased, respectively). Let $\boldsymbol{\mu_1}, \boldsymbol{\mu_2}$ = sample mean vectors, and $\mathbf{S_1}, \mathbf{S_2}$ = sample covariance matrices, for the normal and diseased groups. The sample covariance matrices can be combined into a single, pooled estimate: $\mathbf{S_{pooled}} = [(n-1)\mathbf{S_1} + (m-1)\mathbf{S_2}] / (n+m-2)$.
From these quantities, Fisher's Sample Linear Discriminant Function is given by:
$y(\mathbf{x_o}) = (\boldsymbol{\mu_1} - \boldsymbol{\mu_2})\, \mathbf{S^{-1}_{pooled}}\, \mathbf{x_o}$, where $\mathbf{x_o}$ represents a vector of observations for a new subject. Define q as the midpoint between the transformed sample means:
$q = \frac{1}{2}[y(\boldsymbol{\mu_1}) + y(\boldsymbol{\mu_2})] = \frac{1}{2}(\boldsymbol{\mu_1} - \boldsymbol{\mu_2})\, S^{-1}_{pooled}\, (\boldsymbol{\mu_1} + \boldsymbol{\mu_2})$
Then, using Fisher's Linear Discriminant Function, we classify $\mathbf{x_o}$ as belonging to the normal group if $y(\mathbf{x_o}) > q$, and classify x_o as belonging to the diseased group if $y(\mathbf{x_o}) < q$.
Based on these values of $y(x_o)$, ROC curves can be constructed. Note: In order for the matrix inverse $\mathbf{S^{-1}_{pooled}}$ to exist, it is necessary that $n + m - 2 > P$.

Multivariate Linear Regression will be employed in determining the neural correlates of clinical parameters. The imaging parameters ***P*** from step 2 will be the independent variables and clinical parameters **M** from Step 3 will be the dependent variables. Therefore, in order to deal with multiple dependent and multiple independent parameters, across multiple subjects, we propose to use multivariate multiple regression models. Suppose that cognitive ability is measured as a vector of *m* components. That is, for each subject *j* in the study, we measure the vector *m* of cognitive attributes at time t:

$$\mathbf{Y}_j(t) = [Y_{j1}(t)\ \ Y_{j2}(t)\ \ldots\ Y_{jm}(t)].$$

We will assemble these measurements for each of the *n* subjects into the *n* x *m* matrix **Y.** The design matrix **X** is an *n* x *p* matrix, $\mathbf{X}_j(t) = [X_{j1}(t)\ \ X_{j2}(t)\ \ldots\ X_{jp}(t)]$. In this case, the regression coefficients, which relate the independent to the dependent variables, are represented by the *p* x *m* matrix **β.** Then, the multivariate linear regression model is given by: $\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon}$, where **ε** is the error matrix. The error terms corresponding to the *m* cognitive observations for the *j*th subject may be correlated. Likelihood ratio tests will be performed to assess the statistical significance of various components of the regression coefficient matrix **β**, in order to determine which subsets of the independent variables can be used to predict variations in cognitive ability across subjects.

MCW Research Team and imaging facility: MCW is a national leader with a well-established model for basic and clinical translational research. Our MCW research group was the first to discover and develop R-fcMRI methodology in 1995 and has been at the forefront in developing R-fcMRI applications for nearly two decades. Our group has previously used R-fcMRI to describe changes in neural networks in a variety of diseases and conditions, including cocaine/heroin abuse, mTBI, pain, Alzheimer's disease, depression, and anesthesia, etc. Also, MCI imaging research center, has a team of imaging physicists and imaging processing scientists and the-state-of-the- art GE MR750 3T scanner to conduct the proposed studies.

Outcome measure and Impact: The proposed study will provide biomarkers for the functional pathophysiology of functional GI disorders, as well as other comorbidities that can lead to improved diagnostic and treatment options for these debilitating conditions and ultimately improve the long-term health and welfare of functional GI disorders.

G. RISK CATEGORY:

Nausea and IBS patients - 45 CFR 46.405: Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child subjects
Healthy controls in fMRI sub-study - 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

Gastric function tests:
Involved risks are minimal. Possible risks involve allergies to contents of formula or premade meal for GEBT; although these will be carefully screened for prior to enrollment there is a possibility of unknown allergies. Patients may experience some discomfort, nausea and possible vomiting with satiety drink test. However, patients are instructed to cease drinking at point of satiety so extreme symptoms should not be experienced.

Questionnaires:
Patient distress during questionnaire completion: If children become distressed during completion of study questionnaires, they are instructed in the consent and assent forms to inform the researchers or nurse. The researchers/nurse will then contact the primary investigator who will place a referral to a psychologist.as necessary and as dictated by law (e.g., suspected child abuse/neglect) to ensure patient and family safety and to address psychosocial needs.

Patient/Parent report of clinically significant psychosocial or safety issues: Any child endorsing clinically significant psychological distress on questionnaire-reported screening measures will be referred to a psychologist. The PROMIS anxiety and global health scales will be scored by research coordinators within a week of completion. The raw score will be covered to a standardized T score where a T score of 50 represents the mean for a U.S. general pediatric population with a standard deviation of 10. Subjects scoring greater than 2 standard deviations below the mean will be reported to the PI. The PI will discuss the results with the family and provide them with a referral to a licensed clinical psychologist (Dr. Silverman) as necessary.

Heart Rate Variability:
Participants will be asked in advance if they have a history of skin allergy to adhesives to avoid provoking any skin irritation or rash. However, some redness from the adhesive on the ECG surface electrodes may temporarily appear.

Neurostimulation:
Overall risks/ discomforts involved are very minimal – Rare (event rate 1% - < 5%)

Possible risks/discomforts may involve:
a. Discomfort upon insertion of the electrodes for < 5 minutes - Rare (event rate 1% - < 5%)
b. Discomfort at the lead placement site > 5 minutes – Rare (1 % - < 5%)
c. Bleeding at the electrode site if the neurovascular bundle is penetrated - Rare (event rate 1% - < 5%)
d. Localized discomfort if the electrodes should become dislodged during the wearing of the device - Rare (event rate 1% - < 5%)
e. Localized dermatitis - Rare (event rate 1% - < 5%)
f. Drop in blood pressure - Rare (event rate 1% - < 5%)
g. Syncope (fainting) - Rare (event rate 1% - < 5%)

Adverse effects to supporting personnel
a. Skin piercing with percutaneous needles - Rare (event rate 1 % - < 5%)

**In our recently completed randomized trial of auricular neurostimulation for functional abdominal pain (unpublished data), there were no significant or serious adverse events noted. Of 115 enrolled subjects, 10 reported side effects for following reasons, none of which were serious: ear discomfort n=6 (three active/three placebo device); adhesive allergy n=3 (one active/two placebo); syncope due to needle phobia n=1 (placebo).

fMRI sub-study
1) Interviews and surveys: In this case that the subject becomes tired they will be permitted to rest.
2) Magnetic Resonance (MRI) Studies: MRI studies are associated with the following risks and discomforts:

a) An MRI machine acts like a large magnet. If the study subject has a pacemaker or any metal, such as an aneurysm clip, ear implant, or nerve stimulator in their body, they will not be allowed to have an MRI scan.
b) If a piece of metal (such as a tool, keys, or watch) is released into the scanner room, study subject may be injured. This chance is minimized by careful screening and by having only trained technicians or assistants in the immediate area, which is otherwise restricted.
c) Subjects will be required to wear ear protection to minimize noise injury in the scanner.
d) If the child brings credit cards, other magnetic media, or fine electronics or devices (such as a watch) into the MRI scanner room, they may be damaged by the strong magnetic field. The risk is minimized by proper screening before entering the scanner room.

3) Pregnancy Related Risk: Pregnant women will not take part in this research study. The effects of a MRI scanner on an unborn baby are not known. All female participants past puberty will take a urine pregnancy test prior to participating in the fMRI scan.

4) Fathering a child: The effects of a MRI scanner on an unborn baby are not known. Participants should not father a baby while in this study.

5) Claustrophobia: If the subject experiences fear of being trapped in a narrow space the scan will be stopped. If the subject wishes to re-attempt the scan another appointment can be made.

There may be other risks and side effects that we cannot predict. If these should occur the participant should discuss them with the researcher and /or their regular doctor.

Safety Monitoring Plan

All procedures will be performed by trained professionals within the standard of care under continuous medical supervision in the hospital setting (research unit). Side effects will be inquired about and documented every week during the study and at the post-study follow-up visit. Daily nausea ratings will be filled out by patients so this will be carefully monitored. If any serious harm or discomfort is identified by the subjects or the study personnel, the treatment will be discontinued. Any patient with worsening or no improvement in symptoms during the study will be able to drop out at any time and receive standard of care medical therapy. The skin of the external ear will be carefully disinfected to avoid any infection risks. Subjects will undergo vital sign screening before enrollment and vital sign assessment before neurostimulator placement.

Safety Analysis Plan

There are no dangerous interventions related to this study, and the short study timeline allows for discontinuation of treatment when it is proven to be ineffective.

Completion of psychometric questionnaires may be associated with mild distress in some participants. Because the questionnaires include assessment of patient psychosocial functioning, clinically significant symptoms that the patient or parent were unaware of may be brought to light.

Patient distress during questionnaire completion: If children become distressed during completion of study questionnaires, they are instructed in the consent and assent forms to inform the researchers or nurse. The researchers/nurse will then contact the primary investigator who will place a referral to a psychologist.as necessary and as dictated by law (e.g., suspected child abuse/neglect) to ensure patient and family safety and to address psychosocial needs.

Patient/Parent report of clinically significant psychosocial or safety issues: Any child endorsing clinically significant psychological distress on questionnaire-reported screening measures will be referred to a psychologist. Regarding psychological screeners, research assistants will score the PROMIS questionnaires within a week of completion. Any scores 2 or more standard deviations above mean will be reported to the PI. In turn, the PI will discuss the results with the family and provide them with a referral to a licensed clinical psychologist as necessary.

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

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)
___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)
___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.)

Each subject will be assigned a unique identifying number which will be the only identifier listed on the questionnaires, GEBT and neurostimulator kits and all data collection forms. Each subject's identifying number and related electronic data will be kept on a secured, MCW password-protected database that provides access only to the PI and research staff. Only authorized research personnel will have access to the database. A separate secure database will have the subject identification number linked to the patient's name and info. The GEBT kits and neurostimulator devices will be shipped directly from the manufacturer (Key Electronics) to the GI office research coordinator who will label each package of 4 neurostimulator devices with a patient ID and serial number with a prepared sticker label. A subject folder will also be prepared and labeled with the same ID and kept together with the device package. The devices will be stored in a locked room in the division of gastroenterology. The kits will be transported by a research coordinator to the TRU on the day needed. The remaining kits will be stored in a locked room in the GI clinic, labeled with the patient's ID. Only research coordinators within the division of gastroenterology will have access to the storage room. To track use and

proper disposal of the devices, subjects will be asked to return the used device the following week (at time of the next stimulator placement or follow-up clinic visit if week 4). The doctor or research coordinator will record that device was returned and dispose it properly. The research coordinator will log this data every week on each subject as devices are returned.

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

The PI will monitor the health of all patients in the study per standard clinical practice. The PI will monitor protocol adherence and supervise data collection, entry, and analysis.

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

There are numerous, possible direct and long-term benefits to the subjects in this study. Our recently completed study of EAD in children with various functional abdominal pain disorders demonstrate improved pain, global well- being and functioning after only 3 weeks of therapy with effects sustained at 2 months follow-up (manuscript under review). Since it is well documented that functional disability and quality of life is worse in adolescents with functional pain disorders with concurrent nausea than in those without nausea,[13,9] and as many subjects in the above trial were noted to have improvement in concurrent nausea, we postulate that some of the improvement in well -being and functioning may be due to reduction in nausea (along with pain). There is now also mechanistic data through heart rate variability studies before and after therapy (unpublished data), suggesting the EAD acts through peripheral vagal nerve stimulation effects. Given the known vagal pathways involved in nausea and vomiting, we anticipate a reduction in nausea in subjects suffering from chronic functional nausea. Our prior studies on functional nausea show a high prevalence of anxiety and co-morbid GI symptoms including bloating, fullness and discomfort.[13,24] Based on our prior data, we therefore also anticipate that concurrent symptoms such as bloating, post-prandial fullness, and anxiety may improve. Of 104 subjects analyzed, there were no significant side effects reported other than mild ear discomfort (n=6; n=3 in active and n=3 in placebo arm), adhesive allergy (n=3), and one subject with syncope due to needle phobia (placebo arm). Adolescents suffering from functional GI disorders thus have the opportunity to benefit from a non-pharmacological and non-invasive therapy without the adverse effects of pharmacological agents. The results of this study may provide important insights to the medical field regarding a poorly characterized group of patients and the underlying mechanisms.

As an added incentive, all participants who complete the study will receive a $75 check mailed to their address on file. Those who complete the fMRI portion will receive $125. Subjects who do not complete all parts of the study, will still receive a prorated stipend of $10 per each week of therapy they completed.

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

Stopping points for the study include achieving adequate information on enough of the goal subjects, unanticipated adverse events, and inability to obtain enough data or patients/caregivers electing to discontinue the study.

Reporting Adverse Events and Unanticipated Problems

Expected adverse events that are not serious will be reported on the Continuing Review Progress Report. Continuing Review will be performed on a 12-month cycle, starting at time of protocol's initial approval. More frequent progress reports will be submitted at the request of the IRB.

Serious Adverse Events: The PI, within 24 hours, will report all serious adverse events occurring in any enrolled subjects to the IRB. Unexpected (but not serious) adverse events occurring in enrolled subjects which,

in the opinion of the PI, are possibly related to participation in the protocol will be reported by the PI within 5 working days to the IRB.

Unanticipated problems involving risks to subjects or others will be reported to the IRB within 24 hours.

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

As this is a low risk protocol, no data safety monitoring board will be appointed unless requested.

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

Written informed consent for participation will be obtained from the parents and children for their child's participation. Written assent will be obtained from youth 12 and 13 years of age using the CHW assent form. Patients ages 14 and above will sign the assent line on the consent form per CHW policy. Consent will be obtained by a study investigator or member of the research team at the patients' appointment in the GI Clinic. Volunteers' consent will allow for accessing information collected for program evaluation/clinical purposes. Participating children and parents 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. When consent is given for a minor child to participate in a research study by one or both parents, and the child reaches the age of majority (18 years) the child will be re-consented as an adult to continue participation in the study. If age of majority is reached while the participant is active and continues to receive study interventions per the study protocol, he/she will be re-consented using the most current approved IRB informed consent document and HIPAA form. If age of majority is reached after all study interventions have been completed and the participant remains in the study for purposes of data collection for outcomes only a separate "Age of Majority" informed consent will be obtained.

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

Analyses will be conducted with SPSS and SAS software programs. Probability levels of $< .05$ will be used as cut offs for statistical significance.

The key significance of this study is the identification of a successful, non-invasive therapy for chronic nausea and to study the underlying brain connectivity that may be altered by this therapy. With this study, we hope to lay the foundation for the application of auricular neurostimulation for pediatric functional GI disorders by demonstrating effects on brain network. This is an innovative and novel treatment, which will enhance our knowledge on the brain-gut neural connectivity underlying functional disorders and ultimately improve patient care.

P. FINANCIAL RELATIONSHIPS

The EAD devices will be supplied by the distributing company (IHS) at manufacturing rate. The gastric emptying breath test kits and analysis will be supplied by distributing company (Cairn Diagnostics) at a discounted rate. Internal funds from Department of Pediatrics have been received to support this study and grant funds have been received from Children's Reasearch Institute (CRI). A mentored K23 NIH grant is also pending.

Q. ADVERTISEMENTS / FLIERS

Not applicable.

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