Vagal Nerve Stimulation Protocol

Therapeutic potential and neuroimmune mechanisms of vagal nerve stimulation on gastrointestinal motility and inflammation
Stanford IRB Protocol

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1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization ICHE6), the Code of Federal Regulations Title 21 parts 803 and 812, and other applicable government regulations and Institutional research policies and procedures.

1.1 Background/Significance

The vagus nerve (VN) provides a bidirectional link between the brain and the gastrointestinal tract, and as such, is involved in maintaining the homeostasis of gut functions such as sensitivity, motility, and immunity both through its sensing and modulatory roles (Matteoli & Boeckxstaens 2013). The VN classically innervates the digestive tract from the esophagus to the splenic flexure, however for some anatomists, the VN innervates all the digestive tract in human. Dysfunction of the vagal tone has been observed in functional and inflammatory digestive disorders (Bonaz et al 2016 Rev). Being a major component in the control of upper gastrointestinal motility, low vagal tone has been associated with gastrointestinal motility disturbances (Bonaz et al 2016 Rev).

Gastroparesis is a disorder associated with delayed gastric emptying in the absence of mechanical obstruction. Patients with gastroparesis are often malnourished and experience a range of symptoms from nausea, vomiting, postprandial fullness, early satiety, and abdominal pain leading to significantly compromised quality of life and economic burden (Wang et al 2008; Talley et al 2001). Implicated etiologic factors include diabetes mellitus, although a third of the cases are idiopathic. Physiological vagal input to cholinergic enteric neurons is necessary to maintain a basal gastric tone and is important for gastric accommodation in humans (Lunding et al 2008; Azpiroz & Malagelada 1987). Antral dysmotility is a consistent finding in diabetics with delayed gastric emptying and impaired gastric autonomic innervation probably contributes to this dysmotility. An additional layer of complexity is provided by the fact that the digestive tract is exposed to a variety of luminal signals derived from the microbiota (Guarino et al 2016). Beyond the well-documented effects on gut immune responses, recent data suggest that dysbiosis of gut microbiota can affect gastrointestinal motility (Guarino et al 2016), although this has not been studied in gastroparesis.

Inflammation is increasingly recognized as playing an important role in gastrointestinal motility disorders such as gastroparesis (Spiller & Major 2016). Immune

cells such as macrophages and mast cells are found in close proximity to neurons within the enteric nervous system (Gabanyi et al 2015). Alterations in inflammatory mediators such as cytokines and number of leukocytes are found in gastroparesis and other gastrointestinal motility disturbances (Spiller & Major 2016). Moreover, leukocytes release mediators (e.g. cytokines, neurotransmitters) that alter neuronal activity and have been termed “neuroimmune synapse” leading to pro- and anti-inflammatory neural reflexes (Franco et al 2007). In addition, immune cells express receptors for neurotransmitters, including dopamine, acetylcholine, and norepinephrine, which can regulate leukocyte activity and differentiation. Thus neuroimmune interaction is a bidirectional process important in health and disease, particularly if inflammation is implicated (Franco et al 2007).

The VN is a major constituent of a neural reflex mechanism—the inflammatory reflex—that controls innate immune responses and inflammation during pathogen invasion and tissue injury. VN anti-inflammatory properties are mediated through the activation of the hypothalamic–pituitary–adrenal axis (HPA) by its afferents and by activating the cholinergic anti-inflammatory pathway locally through its efferents (rev by Tracy 2016). VN stimulation (VNS) has potent anti-inflammatory effects (Zhao et al 2012; Cialotto et al 2014) and can improve colitis in rats (Meregnani et al 2011; Ji et al 2014). In a small study with healthy volunteers, it was demonstrated that transcutaneous VNS enhances gastric motility and reduces somatic pain sensitivity (Frøkjaer et al 2016). VNS has further been shown to induce a clinical, biological, and endoscopic remission at 6 months in small cohorts of patients with active Crohn’s disease (Bonaz et al 2016, Haens et a. 2016). However, VNS mechanisms of anti-inflammatory responses in the gut have not been established, nor described in detail. Further investigations are needed to explore the intestinal component of digestive tract inflammation and motility under the modulation of vagal tone reinforcement.

Given that autonomic dysfunction has been frequently reported in patients with gastrointestinal motility disorders and inflammatory bowel disease, ***the central hypothesis*** of our project is that improving vagal tone can have pro-kinetic and anti-inflammatory effects of clinical relevance.We further ***hypothesize*** that gastroparesis is associated with improper immune interactions and microbial dysbiosis leading to disruption of neuronal function. In addition to supportive measures (e.g. dietary changes, glycemic control) and symptomatic control (e.g. anti-emetics), active treatment with pharmacologic agents and vagal/electrical stimulators has led to variable efficacy. Detailed molecular and neuroimmune studies are likely to identify patient heterogeneity and those that are likely to benefit from specific therapies – “precision medicine”. Thus with these in mind we propose the following specific aims (see below).

1.2 Specific Study Aims

Study Aim 1: Investigate molecular and neuroimmune signaling pathways in gastroparesis patients prior to treatment as compared to non-gastroparesis controls.

Study Aim 2: Investigate molecular and neuroimmune signaling pathways in gastroparesis patients post-treatment with VNS, and compared to pre-treatment.

Study Aim 3: Define microbial features and investigate microbial dysbiosis associated with gastroparesis.

2 Innovation

The etiology and pathophysiology of gastroparesis remains enigmatic despite evidence of underlying inflammation and dysautonomia. Additionally, therapies for delayed gastric emptying and its associated symptoms are limited. Given its extensive innervation of the stomach and its predominant role in parasympathetic regulation of inflammation and motility, the VN may be utilized as a powerful therapeutic target. As such, we are proposing to use VNS to induce pro-kinetic and anti-inflammatory changes of clinical relevance. Although gastric pacemakers are available as therapy for medically-refractory gastroparesis, these devices require surgery and have not demonstrated significant pro-kinetic effects (Ross et al, 2014; Yin et al 2012). VNS appears to be safe, well tolerated, and effective in the treatment of other inflammatory disorders with associated dysautonomia, such as rheumatoid arthritis (Koopman et al 2016) and Crohns disease (Bonaz et al 2016, Haens et a. 2016). The therapeutic effect of VNS in these disorders is based on stimulation of the cholinergic anti-inflammatory pathway (Tracy 2009; Howland 2014), which specifically targets pro-inflammatory macrophages in the myenteric plexus (Matteoli et al 2013), which have been implicated in the pathogenesis of gastroparesis. As such, VNS could potentially act as a disease modifier in gastroparesis. There are no therapies to date that have the potential to reverse underlying pathologic abnormalities in gastroparesis. VNS, through its anti-inflammatory and pro-kinetic effects, may offer a safe and well tolerated therapy for treatment of gastroparesis. In this study we propose establishing proof of concept for a novel therapy for gastroparesis. Additionally, we will attempt to fill the large gaps in pathophysiology of this disease by a detailed study of the neuro-immune interactions in patients with gastroparesis.

3 Study Design

3.1 General Design

This is a prospective interventional pilot study assessing the safety and tolerability, as well as efficacy of the use of VNS in patients with idiopathic and functional gastroparesis. We will also include patients with functional dyspepsia, which have almost identical clinical symptomatology and are believed to share similar underlying inflammation and dysautonomia. We plan to recruit a total of 45 patients, 15 patients of each subgroup, for 4 weeks of therapy with VNS. Patients will undergo baseline measures of symptoms, gastric emptying testing, vagal tone, and inflammation. After enrollment, patients will undergo two upper endoscopies (pre and post therapy). Pt will be seen in clinic for recruitment, day 0, for follow-up at 4 weeks, and again at 8 weeks, for a total of six visits including their appointed endoscopy days.

3.2 Study Objectives

1. Establish the effect of VNS on gastric emptying time and on cardinal symptoms of gastroparesis (nausea, vomiting, bloating, abdominal pain, and early satiety).
2. Assess the effects of VNS on various measures of quality of life, including global health and overall pain interference.
3. Investigate molecular and neuroimmune signaling pathways in different gastroparesis patients.
4. Determine the effects of VNS on measurable serum inflammatory markers and cytokines.
5. Determine the effects of VNS on measurable cytokines, nerve growth factors, and neurotransmitters in the gastric and duodenal submucosa.
6. To characterize the submucosal enteric nervous system in gastroparesis subtypes and identify novel neuro-immune interactions.
5. Determine if gastric emptying and symptom response to VNS is associated with changes in inflammatory markers and neuro-immune signaling.
6. Determine if there is a difference in response to VNS in patients with idiopathic vs. diabetic gastroparesis.
7. Evaluate the safety and tolerability of VNS therapy in patients with gastroparesis and functional dyspepsia.

3.3 Study Endpoints

I. Primary Study Endpoints

1.1 The primary outcome measure will be the effect of VNS on gastroparesis cardinal symptoms as measured by the gastroparesis cardinal symptom index (GCSI) questionnaire.
1.2 The second primary outcome measure will be the effect of VNS on gastric emptying time as measured by the gastric emptying spirulina breath test.

II. Secondary Study Endpoints

2.1 The effect of VNS therapy on overall pain interference as assessed by the PROMIS pain interference questionnaire
2.2 The effect of VNS therapy on overall wellbeing and health as assessed by the short form 12 (SF-12).
2.3. The safety and tolerability of VNS in patients with gastroparesis will be assessed by recording any side effects or adverse events.
2.4 The effect of VNS therapy on vagal tone as assessed bv heart rate variability (HRV) and respiratory variability using electrocardiogram (ASNAR) device and an HRV App called Heart Rate Variability Camera.
2.5 The effect of VNS therapy on systemic inflammation as measured by serum inflammatory markers and cytokines (as assessed by the Human 63-plex assay), as well as by activation markers in circulating leukocytes by flow cytometry.

2.6 The effect of VNS therapy on gastric and small intestine inflammation as measured by endoscopy biopsy tissue expression of inflammatory mediators (cytokines, chemokines, etc), and tissue infiltration of immune cells by flow cytometry and immunohistochemistry.

III. Exploratory Study Endpoints

3.1 Enteric neuro-immune interactions in distinct types of gastroparesis as measured by analyses of the submucosal plexus and infiltrating immune cells from endoscopy tissue biopsies via immunofluorescence, gene expression assays, and expression of neurotransmitters, nerve growth factors, cytokine receptors, and trafficking receptors.

3.2 Dysbiosis and bile acid metabolism in distinct types of gastroparesis as measured by stool sample 16S rRNA microbiome sequencing and metabolomics.

3.3 Assess the effect of VNS on neuro-immune interactions and dysbiosis in gastroparesis patients as measured in *3.1* and *3.2*.

3.4 Study Duration

1. Up to 8 weeks for screening period and baseline measures
2. Four weeks of active VNS therapy
3. Four weeks follow up period following discontinuation of therapy

4 Subject Selection and Withdrawal

4.1 Study Population

Adult patients, age 21 to 65 years old, with diagnosed functional dyspepsia, diabetic or idiopathic gastroparesis.

4.2. Sample size justification

- Total of 45 patients; 15 with functional dyspepsia, 15 with diabetic gastroparesis, and 15 with idiopathic gastroparesis.
- Primary comparison: Gastric emptying half time score and GCSI, pre and post VNS
- Error protection: Type I = 0.05 two-sided and Type II = 0.10 (80% power). To detect a clinically significant change of >30% in gastric emptying time, and a reduction in $\geq$1 GCSI aggregate.

4.3 Inclusion Criteria

1. Male or female.
2. Age 21-65 years old.
3. Established diagnosis of functional dyspepsia, idiopathic or diabetic gastroparesis as per AGA (American Gastroenterology Association) guidelines.
4. Patient is capable of giving informed consent and undergo upper endoscopy.
5. Patient is on stable doses of other medications for gastroparesis for preceding 4 weeks prior to enrollment (baseline measures).

4.4 Exclusion Criteria

1. Surgical-related gastroparesis
2. Extrinsic myopathy or neuropathy causing gastroparesis.
3. Use of narcotic pain medications in the preceding 2 weeks of study enrollment.
4. Patients with enteric feeding tubes or requiring parenteral nutrition.
5. Patients with severe flare requiring hospitalization.
6. Untreated significant depression or suicidal thoughts.
7. Pregnant or breast-feeding women.
8. History of gastric pacemaker implantation.
9. Patients with prior gastric surgery, including fundoplication, partial/total gastrectomy, or gastric bypass.
10. Patients with malabsorption of enteric, pancreatic, or hepatibiliary etiology.
11. Patients with primary pulmonary disorders that affect the spirulina breath test.
12. Patients with implantable electronic devices.
13. Patients with carotid artery atherosclerosis.
14. Patient with HX of embolic stroke or TIA, arterial vascular disease, active unexplained neurologic symptoms, or men 65 above who smoke tobacco.
15. Any patient who has had a botox injection in their abdominal region within the past 6 months prior to enrollment.

4.5 Subject Recruitment and Screening

Patients seen at the Stanford University Digestive Health Clinic in the Principal Investigator's clinical practice will be screened for appropriateness in the study and recruited. Those meeting eligibility criteria will be recruited. Informed consent will be obtained and the patients will be scheduled for first upper endoscopy and instructed on the use of the GCSI questionnaire and other surveys. After baseline endoscopy is completed, patients will return to clinic for blood sample collection, including complete blood count, comprehensive metabolic panel, serum or urine β-HCG (women of reproductive age only), ESR, CRP, serum cytokines (Human 63-plex Luminex assay custom-built by eBioscience), and two additional blood vials for flow cytometry and in-vitro re-stimulation assays, and a third vial for blood banking. Banked blood in the Habtezion lab will be used for subsequent studies of exploratory markers of inflammation, immunity, metabolism, and neurotransmission. No genetic testing will be done with fresh or banked patient samples. Stool collection and saliva collection will also occur. At this same visit, patients will undergo HRV testing and gastric emptying testing, which are non-invasive and office-based tests. Patients will then be given the gammaCore vagal nerve stimulator and be instructed on its use with a first time stimulation in the office and a repeat blood collection 90-120 minutes later. Patients will then self-administer daily VNS twice a day for total of 4 weeks' duration.

4.6 Early Withdrawal of Subjects

1. Patients may withdraw from the trial at any time.
2. Patients must be withdrawn from the trial for any of the following:
    - The patient withdraws consent.

- The patient develops serious adverse effect (none anticipated) during the study period.

5 Study Device

5.1 Description

GammaCore is a hand held device used for transcutaneous vagal nerve stimulation. The manufacturer, electroCore, has regulatory approval for the use of gammaCore therapy in the acute and/or prophylactic treatment of cluster headache, migraine and medication overuse headache throughout the European Union, South Africa, India, New Zealand, Australia, Colombia, Brazil and Malaysia. In Canada the therapy is indicated for the acute and/or prophylactic treatment of cluster headache and for the treatment of migraine. It is not FDA-approved in the USA, but is registered as an investigational new device in various clinical trials. GammaCore is CE marked and indicated for the acute and/or prophylactic treatment of Migraine, Cluster, Hemicrania Continua and Medication Overuse Headache in adults. Thus, the purpose of this protocol is to provide VNS to patients with gastroparesis and functional dyspepsia. Our IDE number is: [redacted]. Our NCT number is: NCT03120325

5.2 Treatment Regimen

1. Patients seen at Stanford University Digestive Health Center who meet study inclusion criteria will be screened for enrollment. Those patients with diagnosed functional dyspepsia, idiopathic or diabetic gastroparesis will be considered eligible. Those who do not meet any exclusionary criteria will then be actively enrolled in the study. Study subjects who sign informed consent to participate in the study will have a baseline history and physical exam, and baseline laboratory tests to be completed after baseline endoscopy and at the time of first (pre-treatment) gastric emptying testing.
2. Patients will complete the following forms at the time of study enrollment:
   - GCSI-DD (Gastroparesis Cardinal Symptom Index Daily Diary)
   - GI PROMIS belly pain questionnaire.
   - PROMIS Global Health Short Form questionnaire.
     PROMIS Pain Interference Short Form 8a questionnaire.
   - Short Form SF-12 questionnaire.
   - Compass 31 Survey
3. After successful baseline upper endoscopy and subsequent gastric emptying testing with associated baseline HRV, blood, urine, saliva, and stool tests, patients will start active VNS treatment. The first gammaCore device therapy will be done in the office to ensure proper use, tolerability, and address any questions or concerns patients may bring up. The dosing of therapy will be personalized for each patient at this first session (by asking subject to increase dose until they experience a tug on the ipsilateral side of their mouth). This dose will then be maintained for the duration of the study. Posteriorly, 90-120 minutes after the first stimulation, a repeat blood collection will take place (two vials for Human 63-plex Luminex assay and banking) to assess the acute effect of the VNS treatment on inflammatory markers.

4. Total treatment duration will be 4 weeks. GammaCore administration will occur twice daily, in the morning and evening. Each treatment will entail a 2-minute application to the R and L cervical vagus nerve, each side separated by 3-5 minutes, for a total of 4 applications daily (total of 8 minutes).
5. Patients will receive weekly calls to assess compliance, tolerability, and reminders for their symptoms questionnaires.
6. Patients will present to endoscopy for their post-treatment upper endoscopy 4 weeks after initiation of therapy. No later than 3 days after endoscopy, patients will present to clinic at which time post-treatment gastric emptying testing, HRV testing, and laboratory tests (blood and stool) will be obtained, and the aforementioned forms will again be completed.
7. Patients will be seen in clinic again at 4 weeks after discontinuation of therapy, and undergo a repeat medical history and physical exam. At this time gastric emptying testing, HRV testing, and laboratory tests (blood and stool) will again be obtained; and the aforementioned forms will again be completed to assess whether any change in symptoms and disease severity has occurred off therapy.

Study Assessment and Monitoring

1. **Spirulina Gastric Emptying Breath Test** (Viramontes et al 2001)**:** Gastric emptying times will be measured at baseline and after 4-week therapy. As part of standard of care procedures, diabetic patients will have their blood sugar measured prior to this test. They will need to have a blood sugar level of less than 275 mg/dl, as this high level will results in artificially delayed emptying. Patients will be asked to reschedule if this is the case.
2. **GCSI-DD[3]:** Gastroparesis-specific symptom severity scale. Obtained at baseline (1 week prior to randomization) and daily throughout the duration of the study.
3. **GI PROMIS[2]:** publically available patient related outcome (PRO) measure that covers 8 symptom domains (Abdominal pain, Gas/Bloating, Diarrhea, Constipation, Bowel Incontinence, Reflux, Nausea and Vomiting, Disruptive Swallowing) that can be used together or individually to assess GI symptoms in clinical practice and research. Symptoms are assessed via 7-day recall. The questionnaire will be filled out one week prior to starting therapy and at each follow up visit.
4. **PROMIS Global Health**
5. **PROMIS Pain Interference**
6. **Compass 31 Survey**
7. **Human 63-plex Luminex assay:** The Human Immune Monitoring Center (HIMC) at the Stanford Institute for Immunity, Transplantation and Infection has a standardized immunoassay for the detection of 63 human serum cytokines and chemokines using a Luminex assay.
8. **Flow Cytometry:** additional blood samples will be processed for storage, or for same-day in vitro re-stimulation assays in Dr Aida Habtezion research Laboratory at Stanford SOM.

9. **Dysbiosis:** stool samples will be frozen for storage until all subjects are enrolled. Subsequent metabolomics and 16S rRNA sequencing experiments will be done with collaborators of Dr Habtezion's Laboratory at Stanford SOM.
10. **Upper GI Endoscopy:** All patients will receive standard of care and standard precautions for GI endoscopy as per Stanford Outpatient Endoscopy guidelines. Patients will undergo a baseline endoscopy, and again after VNS treatment to assess the impact of therapy on the target organ, i.e. the stomach. During the procedure, detailed endoscopic assessment of the mucosa will take place, including tissue biopsies that will be collected and processed in Dr Habtezion's Laboratory at Stanford SOM.

	(baseline) Visit 1 Enrollment	≤ 8 wk prior (baseline) Visit 2 Endoscopy	Day 0 (start VNS) Visit 3 Clinic	Day 28 () Visit 4 Endoscopy VNS 4 Weeks	Day 31 End VNS) Visit 5 Clinic	Day 51 (Washout) Visit 6 Clinic
Inclusion criteria met, no exclusion criteria met	X					
Informed consent	X					
Medical History	X		X		X	X
Physical Exam	X		X		X	X
Medication Rev	X		X		X	X
Blood collection: CBC, BMP, LFT, A1C, β-HCG, ESR, CRP, 63-plex Luminex ,and Heparin vials for FACS analysis			X	X		X
			X	X		X
			X	X		X
			X	X		X
			X	X		X
			X	X		X
			X	X		X
Forms GI PROMIS pain	X		X		X	X
PROMIS Health	X		X		X	X
PROMIS Pain	X		X		X	X
SF_12_Health	X		X		X	X
COMPASS 31	X		X		X	X
GCSI-DD HRV-app	⟷ 8 weeks. (2 weeks before VNS, 4 weeks of VNS, 2 weeks after VNS)					
EGD		X		X		
HRV / EKG			X		X	X
Gastric Emptying			X		X	X
Stool collection			X		X	X
Saliva collection			X		X	

Adverse reactions	X	X	X	X	X	X

5.3 Implementation of Study Device

Patients will be instructed in the application of the gammaCore device in clinic, and will receive printed materials to guide them in the proper use and implementation of the device.

5.4 Subject Compliance Monitoring

Compliance will be assessed by weekly phone communications, during clinic visits at 4 weeks after initiation of therapy, and at study completion (8 weeks), by VNS device dosimeter reading.

5.5 Prior and Concomitant Therapy

Participants will be allowed to continue steady doses of medications they are on for gastroparesis for 4 weeks prior to study enrollment. No new scheduled medications for gastroparesis can be initiated during the study period and they must remain on stable doses of any pre-existing medications being used for gastroparesis. The following as-needed rescue medications for symptom control will be allowed for use on a PRN basis during the study: ondansetron, promethazine, diphenhydramine, and Tylenol, toradol. Patients on daily use of narcotic pain medications within the preceding 4 weeks of screening will be excluded from the study and patients will be prohibited from taking opioid medications during the study period.

6 Safety and Adverse Events

6.1 Risks

There is a low reported incidence of adverse side effects with the use of transdermal VNS using gammaCore. There have been no reported incidences of cardiac arrest, respiratory depression, or other serious adverse events. In an IDE, prospective, multi-centered pilot study, no adverse events were reported during or after stimulation. There were no significant changes in electrocardiogram, heart rate or systolic or diastolic blood pressure (Engel et al Poster). Reported side effects are mild and include hoarseness, shortness of breath, change in voice, & skin irritation.

6.2 Recording of Adverse Events

The principal investigator (PI) is responsible for monitoring the conduct of the study and the safety of the study subjects. The participants will be closely monitored by the PI for the development of any adverse events. If adverse events occur (whether expected or unexpected), the PI will evaluate the severity of the adverse event and will initiate immediate corrective measures. The PI will determine if any adverse event is attributed to the research study. All serious and/or unexpected adverse events will be reported to the IRB and appropriate federal agencies in a timely fashion. In particular, the investigator of an IDE such as this must notify in a written IDE safety report of any adverse experience associated with the use of the device that is both serious and unexpected. This notification must be made as soon as possible and no later than 15 calendar days after initial receipt of the information.

The PI will submit a yearly written progress report to the IRB for review. This report will also be sent to the FDA for review (see data collection section). This report will delineate the number of subjects entered into the study (in the past year and total), any

adverse events that have occurred in the past year, and the reason for any subject dropout/withdrawal from the study.

6.3 Reporting of Adverse Events

Within 60 days of the anniversary date that the IDE goes into effect (an IDE goes into effect 30 days after FDA receives it), a brief report of the progress of the investigation must be submitted to the FDA. This must include: 1) a brief summary of the status of each patient enrolled in the protocol; 2) the total number of subjects you plan to treat under the protocol; the number entered into treatment to date, and the number who dropped out of the study for any reason; 3) a description of the general investigational plan for the coming year.

7. Statistical Analysis Plan

The mean baseline GCSI aggregate score will be compared with the mean aggregate score at the end of each treatment week, and in the 4 subsequent weeks after therapy discontinuation. Paired Student T-tests will be done comparing baseline with all other subsequent time points. Gastric emptying times will be plotted before treatment, at the end of treatment, and four weeks after end of treatment and compared by ANOVA. Similarly, differences among the three subgroups of patients will be determined at baseline and after treatment.

Appendix

I. Layman's Narrative of Project:

Gastroparesis is thought to be caused by a mix of inflammation and neural dysfunction. The vagus nerve is a large nerve originating from the brain, which innervates the stomach and intestine, and plays a key role in maintaining normal gastrointestinal function. Research shows that a high vagal tone makes your body better at regulating blood glucose levels, reducing the likelihood of diabetes, stroke and cardiovascular disease. Low vagal tone, however, has been associated with chronic inflammation. Research also shows that patients with gastrointestinal motility problems, such as gastroparesis, have a low vagal tone. Further, in animal models, stimulation of the vagus nerve can have an anti-inflammatory effect in the the bowels, and improve gastrointestinal motility. Stimulation of the vagus nerve is an FDA-approved therapy for seizures and depression, but it has not been studied carefully in patients with gastroparesis. In this study, our aim will be to improve gastric motility and measures of inflammation by vagal nerve stimulation. We will stimulate the vagus nerve using a hand-held device which every study subject will be trained to use in their home. Stimulations will last only a few minutes and will be conducted twice a day for total duration of 4-8 weeks. To evaluate the potential benefits of vagal nerve stimulation, we will assess gastric emptying and inflammation by routine, standard of care tests such as upper endoscopy, breath testing, and sampling of blood, stool, urine, and saliva. We will also measure autonomic dysfunction via the Ansar autonomic functioning machine and the Heart Rate Variability Camera App. A recent autonomic evaluation of gastroparesis patients undergoing neurostimulation suggests that heart rate variability and systematic autonomic testing are both reliable methods of measuring autonomic changes before and after neurostimulation (Stocker et al, 2016). Utilizing mobile apps to measure heart

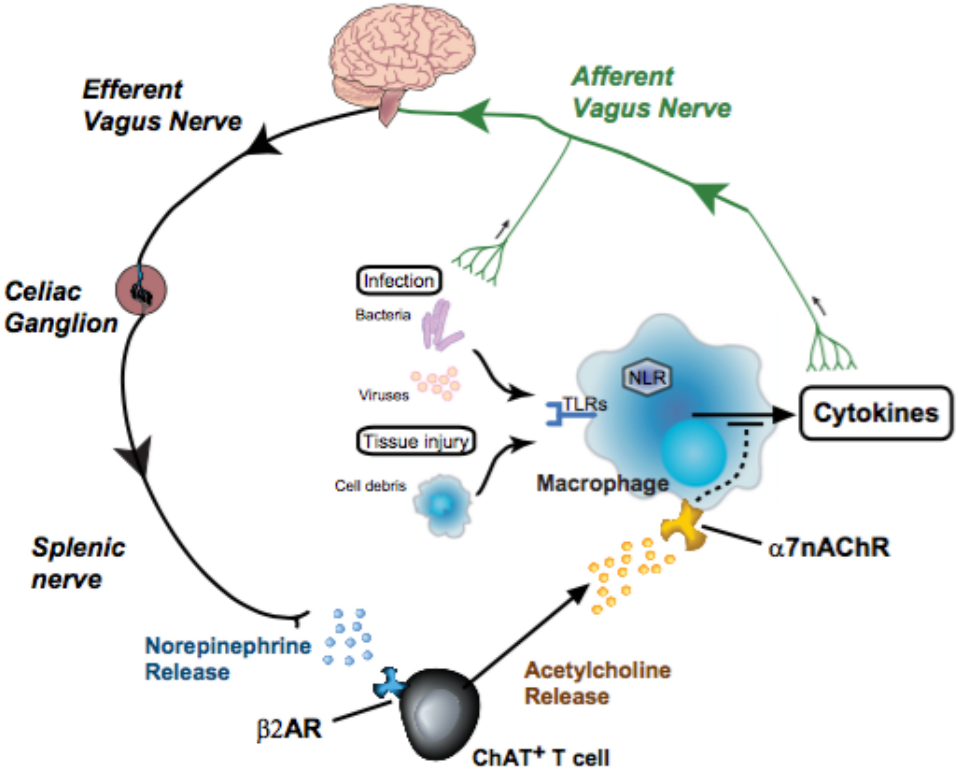
rate variability has been reliably tested (Scully et al 2012), and preliminary results from our studies specific mobile app suggests that the specific technology of photoplethysymography has provided accurate and non-invasive cardiac measures that can be integrated into research settings. (Altini et al, 2017; Plews et al 2017)

II. Gastric Emptying Breath Test (GEBT) is a non-radioactive, non-invasive, orally administered test for measurement of the rate of solid phase gastric emptying in adults. The GEBT has been validated against the reference method of gastric scintigraphy.

III. gammaCore VNS device:

Images provided courtesy of electroCore, LLC.

IV. Vagal Anti-Inflammatory Reflex:



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