EFFECT OF ELECTRICAL STIMULATION OF THE AURICULAR BRANCH OF THE VAGUS NERVE (ABVN) ON CERVICAL VAGUS NERVE COMPOUND ACTION POTENTIALS- Phase 2

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BACKGROUND

It is known that the vagus nerve is one of the main determinants of gastrointestinal motility and gastric emptying. For example, truncal vagotomy produces gastroparesis in both animals as well as human subjects. It is speculated but never proven that neuropathy of the vagus nerve is the cause of the delay in gastric emptying that is characteristic of that disorder.

The typical symptoms of gastroparesis are nausea, vomiting, weight loss, early satiety, abdominal pain and bloating. The initial medical treatment consists of dietary alterations and medications such as phenothiazines (Phenergan), 5HT3 receptor agonists (Zofran) and cannabinoids (Marinol). Treatment with prokinetic agents such as metoclopramide, domperidone, erythromycin and cisapride can be considered but the prolonged use of these agents is limited by the frequent neurologic and cardiac side-effects of these agents. In severe cases patients require placement of a gastrojejunostomy feeding tube (GJ) tube which bypasses the gastroparetic stomach or even intravenous feeding such as total parental nutrition (TPN) in which the patient's entire nutrition is dependent on intravenous fluids.

Gastric electrical stimulation (GES) has been shown to be an effective treatment of nausea and vomiting in patients with gastroparesis (1). However, how GES works is unclear, as patients who undergo GES show resolution of nausea and vomiting before any improvement in gastric emptying is recognized. It has been proposed, but never proven, that GES may influence vagal outflow via afferent fibers that terminate in the CNS.

The vagus nerve is composed of both myelinated and unmyelinated fibers and one of the specific unknown factors is precisely which elements of the nerve are activated by stimulation with various output settings of the stimulator. Stimulation of a peripheral or cranial nerve with a mixed fiber population generates a compound nerve action potential (CNAP) composed of various nerve fibers which conduct the electrical impulse at various rates.

Our current investigations show that it is possible to measure vagus nerve CNAPs during gastric electrical stimulation with cutaneous ECG electrodes placed on the neck. Our studies show that gastroparetic patients who exhibit high-amplitude CNAPs show significant improvement in symptoms such as nausea, vomiting and early satiety than patients who demonstrate lower amplitude vagal nerve CNAPs. The PI currently has an active IRB protocol designed to further evaluate the effect of vagal nerve CNAPs on symptom control in gastroparetic patients (IRB# 1206008988). Another study that is currently undergoing is the effect of the ABVN stimulation on the vagal nerve CNAPs (IRB number 1809683671). These investigations indicate that generation of vagus nerve CNAPs appear to be factorial in symptom control in gastroparetic patients. The study in healthy volunteer is still under data analysis, but the preliminary results do look promising.

Despite its efficacy in some patients, gastric electrical stimulation of the stomach has its drawbacks. These are 1) its expense; 2) it is an invasive procedure and requires surgical implantation of a foreign body into the patient's abdomen; 3) it is not universally effective and it is difficult to predict with any precision which patient will respond to treatment and which will not; 4) the device battery life is finite and patients typically require another surgical procedure to replace the battery within five to ten years; 5) as the device is metallic, patients are unable to undergo MRI scans for any other medical conditions. Given these considerations another less invasive modality to electronically stimulate the vagus nerve would be highly desirable.

Recent investigations have demonstrated the feasibility of physiological access to the vagus nerve through its auricular branch; namely, the auricular branch of the vagus nerve (ABVN) which in human subjects is located near the external auditory meatus. Both the nucleus tractus solitarius (NTS) and the spinal trigeminal nucleus receive somatosensory afferents through the auricular branch (2-4) and neuroimaging studies show that transcutaneous stimulation of the ABVN modulates brainstem and cortical areas in a manner similar to classical vagal nerve stimulation (5-7). A recent clinical trial suggested that auricular branch vagal nerve stimulation (ABVN)) also reduces the frequency of migraine episodes (2), while another independent investigation demonstrated that stimulation of the ABVN influences respiratory variation of heart rate in human subjects (8). This finding supports the notion that this technique can be useful in modulating parasympathetic outflow of the vagus nerve.

COLLABORATIVE ARRANGEMENTS

The current proposal is generated at the behest of Dr. Felicia Quashu of the NIH Office of Strategic Coordination. Both the PI Dr. Nowak and Dr. Ward (Indiana University and Purdue University, respectively) have active funding from the NIH SPARC program (Stimulating Peripheral Activity to Relieve Conditions) to evaluate vagally mediated compound nerve action potentials in gastroparetic patients. The other coinvestigators named in this proposal, Drs Napadow, Hubbard and Kuo, all from Harvard University, likewise have active research support from the NIH. Their research involves electrical stimulation of the auricular branch of the vagus nerve as described in the paragraphs above. As one of the goals of the SPARC program is to promote collaborative research among institutions Dr. Quashu suggested that Indiana, Purdue and Harvard University combine their efforts to evaluate the effects of noninvasive ABVN stimulation on vagal nerve function.

Both the PI and Co-PI (Drs Nowak and Ward) travelled to Boston in July 2018 to meet with the Harvard investigators and to formulate a study plan and a cooperative effort. The technique of ABVN stimulation as described above is adapted from the protocol in

place at Harvard University. A letter of collaborative support from Dr. Napadow is included with this application (Appendix 2).

STUDY OBJECTIVES

The research described in this protocol is an extension of our current protocols which is designed to measure cervical compound vagal nerve action potentials in patients who have an implanted gastric electrical stimulation device (IRB# 1206008988) and also the protocol to measure the effect of ABVN stimulation and vagal maneuvers in healthy volunteers. Healthy volunteer subjects and gastroparetic subjects will be recruited and studied for this project, volunteer subjects will undergo transcutaneous stimulation of the auricular branch of the vagus nerve (ABVN) to determine whether this modality will also produce cervical vagus nerve compound action potentials and changes in the gastric hormones. Gastroparetic subjects who have not had a gastric stimulator placed will also undergo the ABVN stimulation to look for changes in vagal nerve CNAPs and gastric hormones.

Additionally, as there are currently no physiological methods that can record and measure gastric vagal activity in human subjects, a separate component of this study will be to measure the gastric vagal activity in humans. If the proposed study shows that it is indeed possible to record a vagal "signature" in response to electrical stimulation, then we anticipate further investigations which examine the vagal response to other gastric stimuli.

Due to the low signal-to-noise ratios observed from compound vagal nerve activity in response to GES, we are interested in measuring the vagal response to maneuvers which are known to stimulate the vagus nerve. The implementation of these vagal maneuvers will allow comparison of ABVN stimulation to traditional methods of vagal stimulation.

Based on our heathy volunteer study and our proposal with NIH, we would like to propose some additional features. We would like to study the effect of transcutaneous stimulation of the auricular branch of the vagus nerve (ABVN) in gastroparetic patients with symptomatic nausea and vomiting who have not undergone gastric electrical stimulation device implantation.
A total of 40 gastroparetic patients will be enrolled with 20 diabetic and 20 idiopathic patients.

Also, we propose to study the effect on gastric hormone before, during and after auricular stimulation.
The proposal is to enroll 30 healthy volunteers with no gastric symptoms to undergo transcutaneous stimulation of the auricular branch of the vagus nerve and also undergo

blood draw before, after stimulation and later a third draw after a period of no stimulation.
This is based upon the results obtained in our previous IRB approved study where blood was drawn in patients who had gastric electrical stimulation device implanted. Blood was drawn to study the effects of stimulation on gastric hormones. (Cardiac Vagal Effects of Gastric Electrical Stimulation and Vagal Nerve Action Potentials in Vagus nerve in Patients with Gastroparesis- IRB 1206008988)

The next part is also to enroll 40 gastroparetic subjects to undergo blood draw before, after transcutaneous stimulation of the auricular branch of the vagus nerve, and a third draw after a period of no stimulation. Another part is to enroll 40 gastroparetic subjects to undergo vagal maneuver that includes food study using the bipolar (EKG electrodes).

Another part is to enroll 10 healthy volunteers to undergo vagal maneuver using the Multi Array Electrodes (MEA)

An additional 15 subjects who have no GI symptoms (otherwise healthy) but have undergone Vagal Nerve Stimulation (VNS) Therapy for the treatment of partial or focal seizures will be recruited for the study.

INVESTIGATIONAL PLAN

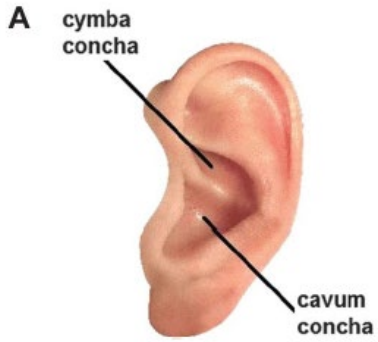
Healthy human subjects ranging in age between 18 to 80 years and who are able to give informed consent are considered candidates for study. Pregnant subjects are excluded from study.
The consent may be administered by the study team either in person or virtually using RedCap platform.

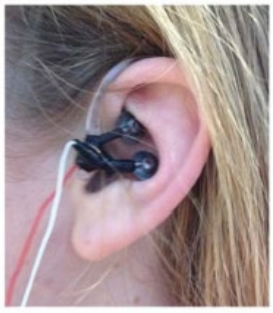
A modality of therapy currently being utilized to treat focal or partial seizures that do not respond to seizure medications is called as VNS Therapy (Vagus Nerve Stimulation). This has been approved by FDA as an effective treatment for seizure disorders. Our aim is to study the HRV in patients undergoing this therapy as part of their regular mode of treatment. The specific aim is to measure the HRV in patients undergoing vagus nerve stimulation therapy.
During VNS treatment the stimulator is surgically implanted in the upper chest and stimulation electrodes are implanted in the left cervical vagus nerve.

Technique of transcutaneous auricular vagus nerve stimulation

Two electrodes are placed in the auricle of the left ear at loci described by the (1) the cymba concha and (2) the slope between the antihelix and cavum concha (Figs. A and B).

These locations are chosen based on auricular sub regions with the greatest probability of vagal innervation of the human auricle, as has been previously determined by cadaver dissection (9) Electrodes are passed through the penetration panel with inline low-pass radio frequency filtering (80 MHz). Electrical stimulation to these electrodes is provided by a constant-current stimulator with 100 V compliance (FE 180 Stimulus Isolator, AD Instruments, Boston MA). Stimuli consist of rectangular pulses with 450 µs pulse width, delivered at 30 Hz, and pulse train duration of 0.5 seconds, as previously described in studies of chronic pain patients (10). Stimulation is gated, with 0.5-second delay, after peak inhalation (i.e., during exhalation) or after peak exhalation (i.e., during inhalation).





Respiratory gating for stimulation requires real-time evaluation of the respiratory cycle. We use a respiratory belt transducer designed to measure changes in chest diameter resulting from breathing (Fig. C). This pneumatic belt is placed around the subject's lower thorax at the level of maximum respiratory expansion (TN 1132/ST Respiratory Belt, AD Instruments, Boston MA). The belt is pretensioned slightly. The belt contains a capacitive sensing element and custom electronics that respond linearly to changes in length. The transducer is connected to a PowerLab pod port (Power Lab, AD Instruments, Boston MA). The transducer contains a flexible sensing element bonded to the inside of the fabric belt.

Once electrode placement in the ear is validated, subjects are asked to rate stimulation intensity of 0 to 10 (0: no sensation, 10: mild discomfort). Current intensity is set to achieve moderate to strong (but not painful) sensation (target score: 4/10), and this current intensity is used on subsequent stimulation runs. For SHAM baseline study, the electrode in the ear remains in place, but the leads are disconnected from the stimulator.

Measurement of cervical vagal nerve compound actions potentials and electrogastrogram (EGG)

The Principal Investigator has an active IRB protocol which examines cutaneous cervical vagal nerve compound nerve action potentials (CNAPS) and the cutaneous electrogastrogram (EGG) in patients with gastroparesis who have undergone implantation of a gastric electrical stimulation device. We refer to IRB protocol “Cardiac Vagal Effects of Gastric Electrical Stimulation and Vagal Nerve Action Potentials in Vagus nerve in Patients with Gastroparesis” (IRB# 1206008988). . Briefly ECG electrodes are

placed on the chest and arms or legs of each subject as well as on the neck overlying the right and left vagus nerves. To measure the cutaneous electrogastrogram (EGG) a set of three ECG electrodes are placed over the upper abdomen in a line parallel to the longitudinal axis of the stomach. Again, we refer to the protocol itself for further details.

ABVN Phase 2 Outcome Measures/Endpoints

1. To measure the amplitude of vagal nerve compound action potentials in gastroparetic subjects with symptoms of nausea and vomiting with skin electrodes over right and left vagus nerve during ABVN stimulation and also during the vagal maneuvers phase
2. To measure respiratory changes in heart rate during ABVN and also during the vagal maneuvers phase.
3. To measure changes in the amplitude of the surface electrogastrogram during ABVN and also during the vagal maneuvers phase.
4. To measure the Heart rate variability(HRV), vagal nerve compound action potentials and electrogastrogram in subjects undergoing VNS Therapy for focal or partial seizures.
5. To measure the effect of ABVN stimulation on the gastric hormones in healthy and gastroparetic subjects.
6. To see the effect on the data if we change the regular ECG electrodes to MEA electrodes in the neck.
7. To see the effect of COVID 19 on HRV in healthy volunteers diagnosed with COVID-19

The study has 4 parts to it;
Healthy human subjects;

Healthy human subjects ranging in age between 18 to 80 years and who are able to give informed consent are considered candidates for study. Pregnant subjects and those taking medications known to affect gastrointestinal motility are excluded from study. Approximately 30 healthy volunteers will be recruited for this next phase of the study. All previously enrolled healthy volunteers in the earlier phase of the study (IRB# 1206008988) can also enroll in this phase of the study. The study team may reach out to them to see if they would like to receive more information about this new phase of the study. If they are willing, then information will be provided to them and if they are interested then they will be contacted for a screening visit. The team feels that by reaching out to the previously enrolled subjects with the possibility of repeating some parts of the study as dictated by the protocol, they would be able to verify the previous results and thus help in the research.
If the previously enrolled subjects are not reachable or not interested, then new healthy volunteers will be enrolled.

Inclusion Criteria

- Healthy volunteers with no gastric symptoms or conditions (except due to COVID 19)
- Aged 18-80 years
- Willing to have electrodes placed in the external ear (ABVN arm)
- Willing to perform vagal activity maneuver (Vagal arm)
- Willing to have 1 tablespoon (15 ml) of blood drawn at 3 time points

Inclusion criteria for VNS Therapy subjects

1. Volunteers with no GI symptoms
2. Ages 18-80
3. Undergoing VNS Therapy as part of clinical treatment for focal or partial epilepsy or seizure
4. Willing to have MEA electrodes placed on the neck and bipolar electrodes placed on chest and stomach

Exclusion Criteria

- Unable to provide consent
- Having gastric motility issues (not due to COVID 19) as determined by the PI or Sub I
- Taking medications affecting gastric motility
- Pregnant females
- Prisoners

Study Procedures:
Healthy volunteers who meet the inclusion and exclusion criteria will be enrolled in the study. They will complete a Health History questionnaire that asks in detail about their health history along with any medications that they may be on. If they have been diagnosed with COVID 19 then they will be asked to complete another questionnaire about their symptoms. If they meet all the requirements for the study, then they will do the following

EAR STIMULATION GROUP:
This is a onetime visit only that involves baseline and test procedures.

1. Two MRI compatible electrodes will be placed in the auricle of the left ear, fixed by a plastic armature that wraps around the ear. The leads from the electrodes are connected to a stimulator.
2. A pneumatic belt may be placed around the lower thorax. Pneumatic tubes connect this belt to a pressure transducer that in turn sends signal to a laptop-controlled device that acquires the signals.

3. Two ECG electrodes are placed on both sides of the neck overlying the area near the carotid artery where the vagus nerve is superficial. This is to measure the vagal action potentials in the neck.
4. Two ECG electrodes are placed one on each arm and one on the chest for measurement of the electrocardiogram (ECG)
5. Three ECG electrodes are placed on the abdomen in a line parallel to the longitudinal axis of the stomach to record the electrogastrogram (EGG).

Electrical stimulation of the electrodes placed in the ear is provided by a current-constant stimulator. The stimuli consist of rectangular pulses with 450 μs pulse width, delivered at 30 Hz, and pulse train duration of 0.5 seconds. Stimulation is gated, with 0.5-second delay, after peak inhalation (i.e., during exhalation) or after peak exhalation (i.e., during inhalation).

Baseline procedure:

A butterfly catheter is inserted under aseptic conditions into a peripheral vein. Fifteen ml (1 tablespoon) of blood is withdrawn and placed on ice. After this the baseline recording will be started as described below.

During the baseline recording the stimulating electrodes are placed in the auricle of the subject but no current is delivered. Cervical neck electrodes are placed over the right and left vagus nerve to record any electrical activity that might occur during the baseline period. Baseline ECG and EGG recordings are also performed during this interval.

Test procedure:

Stimuli consisting of rectangular pulses with 450 μs pulse width, delivered at 30 Hz, and pulse train duration of 0.5 seconds are then delivered to the subject. Current intensity is set to achieve moderate to strong (but not painful) sensation, and pulse frequency/duration is set following pilot testing to achieve a subjectively comfortable stimulus sensation. Starting current is 0.1mA and the maximum current that can be given will not exceed 5.0 mA. This is 4 out of 10 and is designated “100 per cent.” The current amplitude is then increased again by 10 per cent and ABVN is applied again for 60 seconds. This sequence is repeated until the “100 percent” level (4/10) is reached. Based on the previous study we find that the target of 4/10 may correlate with a current of somewhere between 1.0 and 3.5 mA, but this is variable from subject to subject. Sensation of 10/10 may correlate with close to 10.0 mA. The time that may elapse from the start to reaching the 100% target is very variable and may range from 5 minutes to 15 minutes. It depends upon how soon the subject reaches the subjective 4/10 sensation. These stimuli are delivered during expiratory phase of respiration as investigations have shown this to be most effective in producing vagal stimulation (8).

Once the target stimulus intensity has been achieved, stimuli are delivered during the expiratory phase for a total of two minutes. Another 15 ml (1 tablespoon) of blood will be collected 20 minutes after stopping the stimulus.

The subject will be allowed to keep on resting and 20 minutes after the second blood draw, another 15 ml (1 tablespoon) of blood will be drawn. This is the final draw.
The catheter will then be withdrawn, and the subject will be allowed to go home

ECG and EGG recordings are continued during auricular branch vagal nerve stimulation.

VAGAL MANEUVER GROUP:

Healthy volunteers will be recruited initially to see the effect of these maneuvers on the vagal activity.

A total of 10 healthy volunteers will be recruited. These subjects can be the same subjects that enroll in the ear stimulation group of the study or can be entirely new subjects.
At the beginning of the study, a baseline recording of 10- 20 minutes will be made following which the maneuvers will be conducted. Cervical neck electrodes used in this part of the study will include the multi electrode array (MEA) that are currently being used for a different protocol (IRB 1907095542). The MEA can be placed on either side of the neck over the area of the vagus nerve to record any electrical activity that might occur during the baseline period. Baseline ECG and EGG recordings are also performed during this interval by placing the electrodes over the chest and over the stomach area for ECG and EGG recordings.
In a previous protocol (IRB 1809685671) healthy volunteers have already been recruited using the bipolar ECG electrodes for the vagal nerve recording in the neck, hence a total of 10 healthy volunteers to be recruited using the MEA electrodes for comparison of data.

After 10 seconds of each maneuver, another 10-20 minutes of recording will be made before doing the next maneuver.

For the eating maneuver, there is no time restriction and the subject will drink it as per their capacity.
We will include the following maneuvers:

Cough; Each subject will be asked to generate approximately 6 to 8 forceful and sustained coughs for 10 seconds.

Cold stimulus to face: We propose to place a washcloth soaked in ice water on each subject's face for about 10 seconds. This creates a physiological response to a person being submerged in cold water (Diver's Reflex)

Carotid Massage: This technique is performed with the subject's neck in an extended position, the head turned away from the side being massaged. Only one side is massaged at a time. Pressure is applied underneath the angle of the jaw in a gentle circular motion for about 10 seconds. The subject is monitored throughout.

Gagging: A tongue depressor is briefly inserted into the subject's mouth for about 10 seconds touching the back of the throat, which causes the person to reflexively gag. The gag reflex stimulates the vagus nerve.

Valsalva maneuver: The subject is instructed to bear down as if they were having a bowel movement. The subject is asked to blow through a mouthpiece that is hooked to a device used in clinical spirometry until the pressure reaches 10mmHg. Alternatively, we can have the subject blow through the barrel of a 10 ml syringe for 10 seconds. This maneuver increases intrathoracic pressure and stimulates the vagus nerve.

Eating: Subjects will be asked to drink water or/ and Ensure Original that has 220 calories after fasting overnight to see the effect on vagal activity during eating. The subjects take their time to drink the water or ensure and the time to finish the drink is noted.

All the maneuvers described above will be conducted for duration of 10 seconds each except wherever stated otherwise and the recordings will be made for duration of 5-10 minutes after each maneuver. If it is not possible to do all the maneuvers in one visit, then they will be conducted over two visits. Sometimes not all maneuvers will be conducted, and this will depend on the discretion of the investigator.

VNS Therapy group:

Volunteers who do not have gastrointestinal symptoms and undergoing VNS Therapy as part of treatment for focal or partial seizure will be recruited for the study. A total of 15 subjects will be recruited for the pilot study. This will help us determine if HRV is affected by VNS treatment.
Volunteers will be recruited from the IU Health Physicians Neurology clinic where the patients are being seen for a routine follow up clinical visit after the VNS placement.

1. Cervical neck electrodes used in this part of the study will include the multi electrode array (MEA) that are currently being used for a different protocol (IRB 1907095542). The MEA can be placed on either side of the neck over the area of the vagus nerve to record any electrical activity that might occur during the baseline period.
2. Two ECG electrodes are placed either one on each arm or on the chest for measurement of the electrocardiogram (ECG)

3. Three ECG electrodes are placed on the abdomen in a line parallel to the longitudinal axis of the stomach to record the electrogastrogram (EGG).

A onetime baseline recording will be made for 20 minutes after which the subject will be discharged from the study. There will be no follow up visit for these subjects in the pilot study.

Gastroparetic subjects without implanted gastric stimulators;

Gastroparetic subjects ranging in age between 18 to 80 years and who are able to give informed consent are considered candidates for study. Pregnant subjects are excluded from study.
Approximately 40 gastroparesis subjects will be recruited for this new phase of the study.

Inclusion Criteria

- Gastroparesis subjects with a confirmed diagnosis of gastroparesis from a GI physician
- Have a formal diagnosis of gastroparesis or are seeing the GI physician for symptoms related to gastroparesis
- Aged 18-80 years
- Willing to have electrodes placed in the external ear (ABVN arm)
- Willing to perform vagal activity maneuver (Vagal arm)
- Willing to have 1 tablespoon (15 ml) of blood drawn at 3 time points

Exclusion Criteria

- Unable to provide consent
- Pregnant females
- Prisoners

Study Procedures:
Gastroparesis subjects who meet the inclusion and exclusion criteria will be enrolled in the study.

VAGAL MANEUVER GROUP:

Gastroparetic subjects will be recruited initially to see the effect of these maneuvers on the vagal activity.

A total of 40 gastroparetic subjects will be recruited. These subjects can be the same subjects that enroll in the ear stimulation group of the study or can be entirely new subjects.
At the beginning of the study, a baseline recording of 10- 20 minutes will be made following which the maneuvers will be conducted. Cervical neck electrodes used in this

part of the study include the bipolar EKG electrodes. The bipolar electrodes can be placed on either side of the neck over the area of the vagus nerve to record any electrical activity that might occur during the baseline period. Baseline ECG and EGG recordings are also performed during this interval by placing the electrodes over the chest and over the stomach area for ECG and EGG recordings.

After 10 seconds of each maneuver, another 10-20 minutes of recording will be made before doing the next maneuver.

For the eating maneuver, there is no time restriction and the subject will drink it as per their capacity.

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Eating: Subjects will be asked to drink water or/ and Ensure Original that has 220 calories after fasting overnight to see that effect on vagal activity during eating. The subjects take their time to drink the water or ensure and the time to finish the drink is noted.

All the maneuvers described above will be conducted for duration of 10 seconds each except wherever stated otherwise and the recordings will be made for duration of 5-10 minutes after each maneuver. If it is not possible to do all the maneuvers in one visit, then

they will be conducted over two or more visits. Sometimes not all maneuvers will be conducted, and this will depend on the discretion of the investigator.

EAR STIMULATION GROUP:
This is a onetime visit only that involves baseline and test procedures.

1. Two MRI compatible electrodes will be placed in the auricle of the left ear, fixed by a plastic armature that wraps around the ear. The leads from the electrodes are connected to a stimulator.
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Baseline procedure:

A butterfly catheter is inserted under aseptic conditions into a peripheral vein. Fifteen ml (1 tablespoon) of blood is withdrawn and placed on ice. After this the baseline recording will be started as described below.

During the baseline recording the stimulating electrodes are placed in the auricle of the subject but no current is delivered. Cervical neck electrodes are placed over the right and left vagus nerve to record any electrical activity that might occur during the baseline period. Baseline ECG and EGG recordings are also performed during this interval.

Test procedure:

Stimuli consisting of rectangular pulses with 450 μs pulse width, delivered at 30 Hz, and pulse train duration of 0.5 seconds are then delivered to the subject. Current intensity is set to achieve moderate to strong (but not painful) sensation, and pulse frequency/duration is set following pilot testing to achieve a subjectively comfortable stimulus sensation. This is 4/10 and is designated “100 per cent.” The current amplitude

is then increased again by 10 per cent and ABVN is applied again for 60 seconds. This sequence is repeated until the "100 percent" level (4/10) is reached. These stimuli are delivered during expiratory phase of respiration as investigations have shown this to be most effective in producing vagal stimulation (8).

Once the target stimulus intensity has been achieved, stimuli are delivered during the expiratory phase for a total of two minutes. Another 15 ml (1 tablespoon) of blood will be collected 20 minutes after stopping the stimulus.

The subject will be allowed to keep on resting and 20 minutes after the second blood draw, another 15 ml (1 tablespoon) of blood will be drawn. This is the final draw. The catheter will then be withdrawn

The subject will then undergo stimulation at the "100 percent" designated level for 2 minutes and a 5-minute post stimulation recording may be made. This will be followed by another 2-minute stimulation followed by 5 minute of post stimulation recording.

At the end of the 5-minute post stimulation recording. The subject will be allowed to go home

ECG and EGG recordings will be continued during auricular branch vagal nerve stimulation.

If the blood collection is not possible due to any reasons the subjects will still go ahead with the auricular branch stimulation of 2 minutes and 5 minutes recording made after that.

Risks:

There is no pain associated with this protocol. Prior to the study, subjects are asked at what threshold they experience discomfort and subsequent stimuli are delivered below the discomfort threshold. There is a small possibility of slight and temporary discomfort at the site where the electrodes are placed. Potential vaso-vagal reactions to stimulation resulting in dizziness or light-headedness will be promptly recognized and treated by a study physician or clinically trained personnel with prompt removal of the electrodes or other vagal stimulus and repositioning the subject into a supine position. In some subjects, the experience of electrical stimulation may cause anxiety in which case the electrodes will be removed. Subjects will be required to remain under supervision until all symptoms subside. A study physician will be available at all times to discuss the study with subjects should they become concerned.

The electrical stimulation procedure is without significant safety concerns. The electrical stimulator, checked by Biomedical Engineering, has a current limiter preventing harmful stimulation levels. Further, during the electrical stimulation, we will be closely

monitoring that the current received by the subject is well tolerated. Throughout the measurement sessions, participants will be repeatedly asked if they are well and comfortable. Should the subject feel any discomfort he or she will be advised to let the researcher know and the study will be immediately interrupted/terminated.

There may be a risk of transient ischemic attack (TIA) or mini stroke during the carotid massage maneuver.

The patient experiences some pain during venipuncture. An indwelling catheter is used to decrease the discomfort of multiple venipuncture. The procedure is performed using aseptic technique to minimize the risk of infection. Pressure and a bandage are applied over the venipuncture site to minimize the risk of bleeding.

There is no additional risk as part of the recording for VNS Therapy subjects that are research related.

Data Safety Monitoring:

The study team along with an independent person (Dr. John Wo, MD) will be responsible for all the data safety and monitoring. The PI along with the study coordinator, personnel from Purdue along with Dr. John Wo, MD will look at the data after the first subject is enrolled. Thereafter they will meet every month for monitoring until the last subject is enrolled. After that they will meet quarterly till it is deemed unnecessary by the study team. As part of the Data Safety Monitoring Plan (DSMP) data quality, subject recruitment, accrual, retention, outcome and adverse event data, assessment of scientific reports, results of related studies that may impact subject safety, and procedures designed to protect the privacy of subjects will be evaluated.

Study withdrawal/ Discontinuation:
The subject can withdraw the consent any time during the study. The PI or the Sub I can withdraw the subject if they feel that subject safety may be affected by the participation of that subject.

Data Analysis

The CNAPs are visually analyzed and given a score based on their amplitude. Mean values for each level of stimulation are then determined. A comparison between the baseline value (SHAM stimulation) and the amplitude at each level of stimulation is then determined. The amplitudes of the various nerve fibers at each level of stimulation are also examined to determine if a particular nerve fiber is particularly susceptible to stimulation of the ABVN. Statistical analysis is performed using analysis of variance (ANOVA).

The blood samples will be sent as deidentified to Purdue lab for processing and data will be analyzed by the Purdue team.

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Appendix 1: Previously approved protocol: 1206008988

Cardiac Vagal Effects of Gastric Electrical Stimulation and Vagal Nerve Action Potentials in Vagus nerve in Patients with Gastroparesis

Appendix 2: Letter of Support

Thomas Nowak
Professor of Clinical Medicine,
Gastroenterology & Hepatology
Indiana University School of Medicine

September 1, 2018

Dear Dr. Nowak:

This Letter is in full support of our collaborative research study, evaluating the neurophysiology of transcutaneous vagus nerve stimulation. We have been conducting similar research, in a safe and reliable manner for more than 5 years, and I am happy to continue to advise your group in setting up your research studies.

Please do not hesitate to contact me or my team with any further queries, and I look forward to continued collaboration.

Best regards

Vitaly Napadow, PhD LAc
Director, Center for Integrative Pain Neuroimaging (CiPNI)
Associate Professor, Martinos Center for Biomedical Imaging
Massachusetts General Hospital, Harvard Medical School
Boston, MA

Appendix 3: Previously approved protocol for healthy volunteers

EFFECT OF ELECTRICAL STIMULATION OF THE AURICULAR BRANCH OF THE VAGUS NERVE ON CERVICAL VAGUS NERVE COMPOUND ACTION POTENTIALS: 1809683671

Appendix 4: Previously approved study to use MEA electrodes: 1907095542

High Resolution, Noninvasive Measurement and Functional Classification of Vagal Nerve Response Patterns in Relation to Gastroparesis Symptom Management using Gastric Electrical Stimulation Therapy