Auricular Vagal Nerve Stimulation in Brain and Gastric Outcomes in Functional Dyspepsia Patients

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INTRODUCTION

Roughly 60-70 million people are affected by gastrointestinal (GI) disorders in the United States alone. It is estimated that Functional Dyspepsia (FD) affects 20% of this population and costs more than $8 billion in health care expenditures in the United States (Aro et al., 2011). These statistics clearly reflect that FD is a very common GI disorder. As a result of this, many professionals are trying to find a method that will help FD patients have a better quality of life by finding a treatment that will help alleviate their symptoms. Transcutaneous vagal nerve stimulation (tVNS) has been used previously for managing pain, depression, epilepsy, Inflammatory Bowel Disease, and gastric emptying, to name a few conditions. Currently, there is no previous knowledge on peripheral and central nervous system effects in vagus nerve stimulation (VNS) in FD patients. Without knowledge of tVNS working to help patients' symptoms, there is no way to know if the non-invasive procedure can help patients tolerate their symptoms better. Also, physicians need to gather a better understanding of the differences in the brain-gut axis functioning between FD patients and healthy controls (HC) as well as gather a better understanding of how transcutaneous vagal nerve stimulation (tVNS) affects the human brain and autonomic data. The goal for this study is to use gastric and autonomic measures combined with multimodal neuroimaging techniques to investigate the peripheral and central nervous system changes that accompany neuromodulation of brain-gut axis signaling by auricular tVNS in FD patients during a prandial challenge.

As previously mentioned, VNS has been used before in patients with epilepsy and other neurological conditions. First, it is important to address that the information from the stomach, pancreas, liver, bowels, heart, and lungs are delivered to the brain via sensory fibers in the vagus nerve (Zagon, 2001). Many studies have been done to evaluate the extent of the importance of the vagus nerve in finding possible treatments for different medical conditions. tVNS was first used in epilepsy to see if seizures would disappear or weaken with the continuous use of tVNS. Previous data has revealed a progressive increase overtime in seizure freedom after VNS therapy. 49% of the patients in the study responded to VNS therapy 0 to 4 months after implantation, with 5.1% of patients becoming seizure free (Englot et al., 2016).

The previous knowledge on VNS alleviating symptoms in epilepsy led questions to arise in other medical fields on whether VNS would alleviate symptoms in other disorders. Prior to using VNS on patients with disorders, Busch et al. in 2013 found that tVNS in healthy subjects increased mechanical and pressure pain threshold, the amount of pain an individual can tolerate. It was also found that there was a reduction of mechanical pain sensitivity, how fast an individual begins to feel pain. Also, active tVNS reduced pain ratings in the subjects during sustained application of a pain source versus the sham condition. When VNS was conducted on rats to determine its effect on gastric emptying using magnetic resonance imaging (MRI), it was found that VNS significantly increased gastric emptying, causing a

greater relaxation of the pyloric sphincter. It also increased antral contraction amplitude and peristaltic velocity. The degree to which VNS relaxed the pylorus was positively correlated with gastric emptying rate (Lu et al., 2018). Previous to this study, Meregnani et al. in 2011, applied tVNS on rats to evaluate the anti-inflammatory effects of chronic VNS on colonic inflammation. This study was done to see if tVNS would help patients with Inflammatory Bowel Disease (IBD). The study found that there is an anti-inflammatory role of tVNS when chronically performed on freely moving rats with ulcerative colitis, which is a type of IBD. Also, it was concluded that tVNS could be used as a potential therapeutic application for patients with IBD.

Apart from physical medical conditions, tVNS has also been used in treating depression and has had major positive effects. A study done by Hein et al. in 2012 tested non-invasive tVNS on depressed patients for the first time. The study instructed participants to complete two questionnaires before, during, and after the continuous use of tVNS. It was found that after the continuous use of tVNS, the scores on the two questionnaires decreased, thus proving that there is an anti-depressant effect on depressed patients with continuous use of tVNS. It was also found that tVNS has an effect on the autonomic nervous system.

A study by Buijs in 2013, wanted to assess the importance of homeostasis in the body regulated by the autonomic nervous system (ANS) through the central nervous system. They found that the brain sets the balance of the different parts of the ANS, causing its output to change its emphasis according to the situation. The study found that a disturbed balance, whether that be because of behavior or of disease of any of the organs, may lead to pathology affecting the functioning of the entire individual. Thus, the autonomic nervous system plays an important role in the homeostasis in patients with medical conditions. After many research studies were conducted to determine the effect of tVNS on various medical conditions, Yiloski et al., in 2017, decided to determine the autonomic effect of tVNS on patients with tinnitus. They wanted to investigate the acute effect of tVNS on autonomic nervous system imbalance, which often occurs in patients with tinnitus-triggered stress. Prior to the patients receiving active tVNS, the subjects' pre-treatment recording showed dominant sympathetic and reduced parasympathetic activity in about 73% of the subjects. After the continuous use of active tVNS, the variability of R peaks increased in 75% of patients. Thus, this study concluded that tVNS can induce a shift in autonomic function from sympathetic preponderance towards a parasympathetic predominance. Furthermore, since there was success using invasive tVNS, research aimed to determine the autonomic effect of non-invasive tVNS. It was found that in healthy participants, active tVNS significantly increased heart rate variability, indicating a shift in cardiac autonomic function toward parasympathetic predominance. It was concluded that tVNS can increase HRV and reduce sympathetic nerve outflow, which is desirable in conditions characterized by enhanced sympathetic nerve activity (Clancy et al., 2014). Another study testing the autonomic effects aimed to compare the cardiovascular effects of respiratory-gated tVNS in healthy

subjects and found that it is common for there to be an autonomic imbalance, specifically increased sympathetic and decreased parasympathetic activity, that drives the hearts functioning. After examining expiratory-gated, inspiratory-gated, and non-respiratory gated tVNS comparing active versus sham, it was concluded that expiratory-gated and non-respiratory-gated tVNS exert cardioinhibitory effects in healthy controls with high pre-stimulatory heart rate, whereas inspiratory-gated tVNS did not affect heart rate (Paleczny et al., 2019). As seen from the articles mentioned above, tVNS has had a positive effect in helping patients with medical conditions by correcting the "imbalance" between the parasympathetic and sympathetic nervous system in the autonomic nervous system.

As stated before, 20% of the population affected by GI disorders is affected by FD, and thus, these patients have a poor quality of life. There is currently a limited number of methods that help these patients tolerate their symptoms and thus, this study would help further close this gap of there not being a variety of effective possible treatments that are non-invasive or oral medication based. Society will benefit from this research through increased knowledge about potential mechanisms leading to symptoms in FD subjects. Also, this study could advance the study of the modulation of GI physiology by a vagal nerve stimulation procedure. With the knowledge made available by this research, FD patients could have an alternate non-invasive treatment that could help with their symptoms. The specific goal for this study is to investigate the effects of active versus inactive transcutaneous vagal nerve stimulation (tVNS) on enteric, autonomic, and central nervous system physiology and symptoms between HC and FD patients. With this in mind, several hypotheses were made: 1.) Active versus inactive transcutaneous vagal nerve stimulation will increase parasympathetic nervous system activity to "meet" sympathetic nervous system activity so that symptoms will be attenuated since FD patients have an increased sympathetic nervous system; 2.) With the application of tVNS, high frequency (HF) power will be increased by active tVNS relative to sham tVNS; 3.) FD patients will consume less Ensure during the nutrient drink test (NDT); 4.) Aversive symptoms during the NDT will be attenuated by active tVNS.

METHODS

To determine if tVNS has an effect on FD patients' symptoms, the lab staff recruited FD patients and healthy controls (HC) to be part of the Auricular Vagal Nerve Stimulation in Brain and Gastric Outcomes Study, executed by Massachusetts General Hospital in Boston, Massachusetts. The study lasted about one month for each participant and included a screening visit, two behavioral visits, and two Magnetic Resonance Imaging (MRI) visits (Figure 5). Although the MRI is used to study gastric emptying in FD subjects, my role in this study did not include doing any work with the MRI data. The behavioral visits were conducted by the staff and my mentor. The team, excluding myself, collected data such as: screening and questionnaires that came directly from the patients, executing the inactive and

active tVNS, and administering the NDT. My role, as the student researcher, was to review a plethora of literature, develop my own hypotheses, conduct data analysis on the behavioral visit data, evaluate the data, and consult with my mentor and the team.

Prior to consenting to be part of the study, there were inclusion and exclusion criteria that had to be assessed. These are seen in Table 1. Patients had to be between the age of 18 and 65. All FD patients had to be diagnosed with FD using the Rome III criteria, ass seen in Table 2. As of this writing, there are 17 HC and 11 FD patients enrolled. Of these, two FD and one HC dropped out. There is a smaller number of FD patients since these patients are harder to come by. Patients were recruited using Rally by Partners, referred by doctors, or were made aware of the study through social media advertisements. In total, the aim is to have upwards of 25 FD and around the same number of HC participants to match the FD subjects. HC subjects are matched for sex and age to the FD subjects. The sample size was estimated from previous studies which have shown differences in patient groups and normal volunteers with this level of sample size when comparing gastric emptying, accommodation, gastric motility, and secretions. This is an expected good sample size to generate pilot data to be used as justification for future research studies.

The first visit is a screening visit where the participant would give consent to be in the study. This included a variety of questionnaires, including the PROMIS- Anxiety, PROMIS- Depression, Brief Pain Inventory (BPI), Multidimensional Assessment of Interoceptive Awareness (MAIA), Short Nepean Dyspepsia Index, and the Patient Assessment of Gastrointestinal Disorders Symptom Severity Index (PAGI-SYM). All questionnaires can be viewed in Figure 1. PROMIS- Anxiety and PROMIS-Depression assess the anxiety and depression ratings of the participant and transfer the raw score to a t-score, which rescales the raw score into a standardized T-score with a mean of 50 and a standard deviation (SD) of 10. A score of 50 is the average for the United States general population with a standard deviation of 10 because calibration testing was performed on a large sample of the general population. Next, the BPI assesses for pain. Then, the MAIA assesses interoceptive awareness. Interoceptive awareness is defined as the sense of the physiological condition of the body, including the viscera. Furthermore, the Short Nepean Dyspepsia Index is a responsive disease-specific quality-of-life measure. Finally, the PAGI-SYM measures specific symptoms of patients with upper Gastrointestinal disorders. Apart from the questionnaires, a physician gave every participant a physical exam to approve that the patient was in good health to participate in the study.

Inclusion Criteria:	**Exclusion Criteria:**
● Volunteers, female and male, between 18 and 65 years of age ● For FD patients, diagnosis according to the Rome III criteria for both Postprandial Distress Syndrome (PDS) and Epigastric Pain Syndrome (EPS) subtypes ● Stable medical treatment for FD during the 1 month before the study and during the study period ● Avoidance of alcohol, nicotine, and caffeine for 24 hours prior to each study visit	● Previous GI surgery, electrolyte disturbances, kidney dysfunction, renal insufficiency or iron overload disorders ● Currently pregnant or breastfeeding or who have plans to become pregnant ● Medications that affect GI motility ● Medications or products containing tetrahydrocannabinol (THC) must be stopped at least 3 days prior to the start + for the duration of the study ● Mitochondrial disease, severe autonomic dysfunction, and small fiber polyneuropathy ● BMI (Body Mass Index) >32 and/or weight > 235 lbs ● Allergy to pineapple (high contrast meal during MRI) ● Any alternative treatment (e.g. acupuncture, hypnosis, CBT etc.) for FD two weeks prior/ during the study period ● Illicit drugs or opioid usage ● History of arrhythmias ● Implanted pacemaker ● Epilepsy or a prior history of seizures ● Inability to provide informed consent ● Contraindications for MRI

Table 1: Inclusion and Exclusion Criteria (1)

Rome III Diagnostic Criteria for FD:

At least 3 months, with onset at least 6 months previously, of 1 or more of the following:

- Bothersome Postprandial Fullness
- Early Satiation
- Epigastric Pain
- Epigastric Burning

AND

- No evidence of structural disease (including at upper endoscopy) that is likely to explain symptoms

Table 2: Rome III Criteria for FD (1)

Following the screening visit, patients would make an appointment in the Athinoula A. Martinos Center for Biomedical Imaging to complete their first behavioral visit. Subjects had to fast for at least 10 hours prior to their behavioral and MRI visits. Patients were treated with active or sham tVNS while completing a nutrient drink test (NDT). For the NDT, the tVNS was calibrated to 100 Hz and to a "moderate, non-painful sensation". The calibration rate of administering Ensure during the NDT was 30 milliliters per minute. The RAVANS device was utilized to conduct the tVNS. The application of tVNS is seen in Figure 2. Active or sham tVNS was administered at random so that no biases would occur. Prior to starting the NDT, the subject was attached to many wires which measured their autonomic data. This included an ECG and EEG. Respiration was also measured. After the tVNS, autonomic, and Ensure equipment was set up (Figure 3 A, B), the NDT would begin, and subjects were instructed to drink vanilla Ensure, a nutrition supplement drink, until they felt they reached their absolute MAX fullness. Vanilla Ensure (one bottle = 220 ml and 220 calories) was chosen because it is a densely caloric drink and it has been used in previous studies for conducting NDTs as well (Figure 4). Ensure was poured into a machine, as necessary, which transported the drink into a cup in front of the patient. The patient would consume the Ensure through a straw. Furthermore, every five minutes the patients were given a visual analog scale (VAS) where the patients would self-assess the five main symptoms of FD (early satiation, abdominal pain, nausea, bloating, and belching). Subjects were instructed to mark a 100 millimeter (mm.) scale for each symptom, respectfully. Another VAS was administered at the end time, as well as the 15 minute and 30 minute post NDT. After the subject has reached complete satiation, the autonomic measurement devices are removed from the subject's body and they are taken to another room to complete the same questionnaires as seen in the screening visit description above.

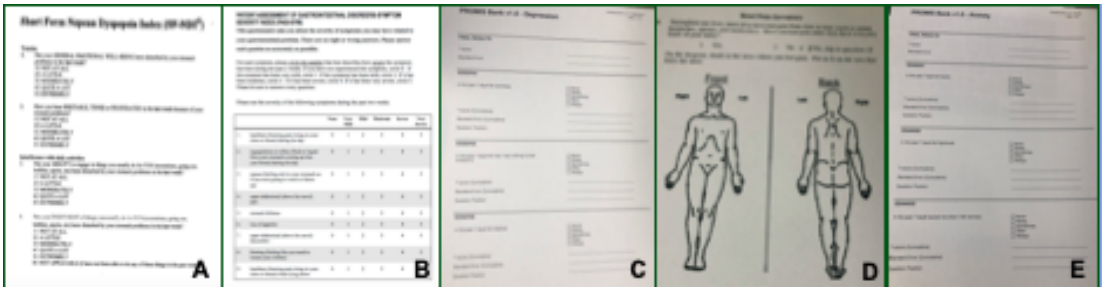

Figure 1: Short Form Nepean Dyspepsia Index, PAGI-SYM, PROMIS-Anxiety, BPI, PROMIS-Depression (from left to right) (1)

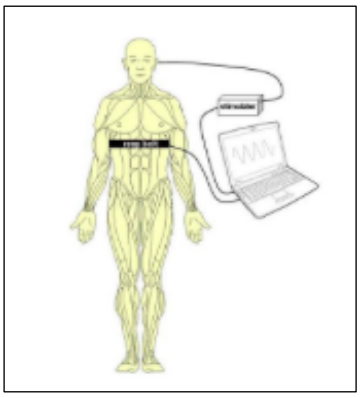

Figure 2: tVNS Set Up (1)

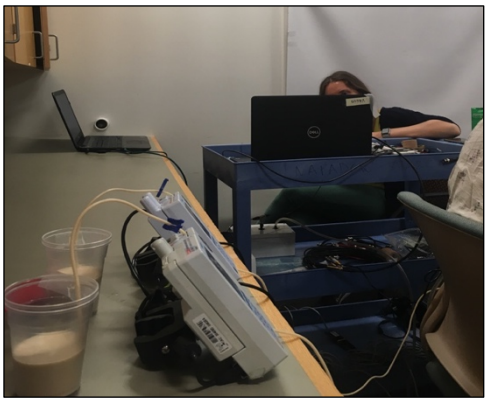

Figure 3 A (left) and B (right): Behavioral Visit Set Up (Source: Student Researcher)

Figure 4: Vanilla Ensure
https://static.abbottnutrition.com/cms-prod/ensure.com/img/ensure-original-vanilla-protein-shake.jpg

Although all measures were taken to ensure that the study would run smoothly, there were still some limitations. For the behavioral visits, limitations include how long the subject fasted prior to the NDT, how hungry the subject is when they arrive to the NDT, if symptoms are already present prior to the visits, air gulping due to consuming the Ensure through a straw, Ensure was spilled, the pumps transporting the machine to the patient cup sometimes malfunctioned, the onset time of symptoms differs for everyone, some patients did not reach max fullness, many disliked the taste of Ensure, and the description of fullness is subjective.

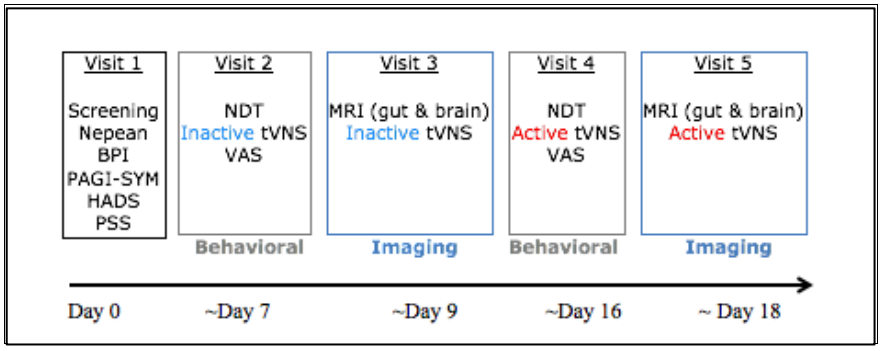

Figure 5: Study Timeline (1)

Data Analysis

As the student researcher, my role was to analyze the behavioral visits data, which included the questionnaires, tVNS, NDT, and autonomic data. Using the pre-existing data, I ran the following statistical analyses:

In order to determine if there was a positive effect using active tVNS relative to sham tVNS during the NDT (patients with FD vs. healthy control (HC)), a two-way ANOVA was performed to better understand the interaction between the two independent variables, FD and HC, on the dependent variable, the VAS score for each symptom. Furthermore, to be able to compare the heart rate variability (HRV) of HC and FD subjects, the first 6 minutes of the NDT were compared to the last 6 minutes of NDT comparing sham versus active. A non-parametric t-test was run in order to compare the means of the first and last six minutes for each group, FD or HC. The t-tests were run to determine if just session OR just group had an effect on the appropriate dependent variable. Moreover, in order to compare FD versus HC in regards to the six questionnaires, a Wilcoxon Rank Sum test and a Kruskal Wallis test were conducted. The tests were used because they use the median instead of the mean to generate the distribution of the data. This is necessary in small populations, which is true for the FD study population.

RESULTS/ DISCUSSION

With the data that has been collected thus far, certain trends have started to emerge.

Behavioral Questionnaire Data

Data analysis was run on the following questionnaires as a baseline to determine whether or not symptoms were affected by personal life or if symptoms affected personal life:

MAIA

The MAIA gathers information on how aware and hyper-focused patients are towards their bothersome symptoms. This relates to interoception, which means being aware of the internal state of the body. There are seven subscales for the MAIA: Noticing, Not-Distracting, Not-Worrying, Emotional Awareness, Self-Regulation, Body-Listening, and Trusting. The possible minimum score is 0 and the maximum score is 160 (without taking averages for each sub-scale). The higher the score, the more aware one is of their symptoms. For the population used in this study thus far, the scores have ranged from 6.94 to 36.68 after taking the averages of each sub-score and adding the averaged sub-scores together. When analyzing the data, two tests were run: Wilcoxon Rank Sum Test and Kruskal-Wallis Test. The Wilcoxon Rank Sum Test compared active versus sham conditions for all subjects and resulted in a p-value of 0.9265, which is not statistically significant. This means that there was no difference between sham and active tVNS in regard to interoceptive awareness and their symptoms. Furthermore, the Kruskal-Wallis also was statistically insignificant, with a p-value of 0.171. The overall average score (sham total score + active total score / 2) for HC was compared to the overall average score of FD subjects. This indicates that there is no major difference in the average scores and that both groups have similar awareness of their body and symptoms (Figure D).

PAGI-SYM

The PAGI-SYM measures specific symptoms of patients with upper gastrointestinal disorders. There are no sub-scales. The possible minimum score is 0 and the possible maximum score is 110. The score range for the study's population as of current is 0 to 69. Overall, three tests were run using two statistical tests- the Wilcoxon Rank Sum Test and the Kruskal-Wallis Test. First, the Kruskal-Wallis test was used to compare the difference in score (sham – active = difference) between HC and FD groups. The p-value, p=0.6385, was not statistically significant which means that there is no major difference between the difference scores for each group. Another Kruskal-Wallis test was run but it compared the average score of HC versus FD. As suspected, this test was extremely statistically significant with a p-value of 0.0006813. Thus, there is a major difference between the average score of both groups in regard to gastrointestinal symptoms. The last test that was run was a Wilcoxon Rank Sum Test, which compared all active scores for both groups to all sham scores for both groups. The p-value resulted in 0.6679, which is not significant, indicating that there is no major difference between active and sham scores (Figure A).

PROMIS-Anxiety

The Promis-Anxiety questionnaire assesses for an individual's anxiety ratings. There are no sub-scales. All the raw scores were converted to T-Scores, which rescales the raw score into a standardized

score with a mean of 50 and a standard deviation (SD) of 10. The possible minimum T-Score is 37.1 and the possible maximum T-Score is 83.1. The score range for the research study's population thus far is 37.1 to 60.4. It is important to note that there is an outlier in the data set. Three tests were run using the data but only two statistical tests were used: Wilcoxon Rank Sum Test and the Kruskal-Wallis Test. First, the Kruskal-Wallis test was used to compare the difference score (sham – active = difference) between HC and FD groups. The p-value, p=0.2602, was not statistically significant which means that there is no major difference between the difference scores for each group. Another Kruskal-Wallis test was run but it compared the average score of HC versus FD. Although close, this test was also not significant with a p-value of 0.09. Thus, there is no major difference between the average score of both groups. This means that the scores remained relatively consistent throughout the study. The last test that was run was a Wilcoxon Rank Sum Test, which compared all active scores for both groups to all sham scores for both groups. The p-value resulted in 0.3459, which is not significant. This indicates that there is no difference between active and sham scores. Thus, the sham versus active tVNS did not change the scores of the subjects (Figure B).

PROMIS-Depression

The Promis-Depression questionnaire assesses an individual's depression ratings. There are no sub-scales. All the raw scores were converted to T-Scores, which rescales the raw score into a standardized score with a mean of 50 and a standard deviation (SD) of 10. The possible minimum T-Score is 38.2 and the possible maximum T-Score is 81.3. The score range for the research study's population thus far is 38.2 to 66.8. It is important to note that there is an outlier in the data set. Three tests were run using the data but only two statistical tests were used- Wilcoxon Rank Sum Test and the Kruskal-Wallis Test. First, the Kruskal-Wallis test was used to compare the difference score (sham – active = difference) between HC and FD groups. The p-value, p=0.8964, was not statistically significant, which means that there is no major difference between the difference scores for each group. Another Kruskal-Wallis test was run but it compared the average score of HC versus FD. This test was significant with a p-value of 0.02. Thus, there is a difference between the average score of both groups, indicating that FD subjects have a higher depression score. The last test that was run was a Wilcoxon Rank Sum Test, which compared all active scores for both groups to all sham scores for both groups. The p-value resulted in 0.6347, which is not significant. This indicates that there is no difference between active and sham scores, meaning scores were relatively consistent (Figure C).

Short Form Nepean Dyspepsia Index

The Short Nepean Questionnaire examines the impact of the symptoms of FD on the quality of life of FD patients. The questionnaire has a total of 10 questions, asking subjects to assess their emotional well-being, their frustration, their abilities, and more. The score range for each question is from 1, where the patient had not been held back by their symptoms to feel a certain way or perform a certain activity during the previous week, to a 5, where the patient had been extremely impacted by their symptoms to feel a certain way or perform a certain activity the previous week. Scores when added up, as of thus far into the study, have ranged from 10 to 39. There are no sub scales for this questionnaire. As with the previous questionnaires noted above, three tests were run to analyze the data. First, a Wilcoxon rank sum test was run to compare sham and active scores. The p-value, p= 0.6725, is insignificant and thus, this means that there is no major difference between sham and active scores. Next, the Kruskal-Wallis test was run to compare the difference (S – A = diff.) by group (Sham or active). This also led to a statistically insignificant p-value, p=0.2086. This means that there was no major change between the active and sham scores, and that the scores remained consistent between sham and active scores. Lastly, another Kruskal-Wallis test was run, but this time, the test assessed the average scores of each group (HC vs. FD). This resulted in a statistically significant p-value of p= 0.00009091. Thus, this means that there is a big difference between the scores of FD patients compared to HC subjects, as suspected, in regard to their GI symptoms.

Nutrient Drink Test Data

First, group (FD vs HC) and Ensure consumed (mL) were compared between FD and HC to test the hypothesis: FD patients will consume less Ensure during the nutrient drink test (NDT). After running a two-way ANOVA, no main effect of stimulation (active vs. sham condition) or group was found. Although the hypothesis was made that HC subjects would consume more Ensure than FD subjects, this was found false since the p-value was 0.56240229, which was not statistically significant (Figure E).

Then, each of the patients' symptoms were analyzed. This data was analyzed to test the hypothesis: Aversive symptoms during the NDT will be attenuated by active tVNS. The group (FD or HC) and the Condition (active or sham) were compared in terms of the amount of ensure the subject consumed. The normalized data was analyzed instead of END point during the NDT. The normalized data was gathered dividing the END VAS score by Ensure consumed times 100. This represents how full the patient would get if all the patients drank the same amount at the same rate.

Satiation

After running the two-way ANOVA, a significant main effect was concluded. The p-value was 0.03018. This means that if all the patients drank the same amount at the same rate, the FD group would reach satiation faster. Also, with the use of active tVNS, FD subjects were able to reach a higher VAS score for satiation compared to the sham condition for FD subjects, suggesting the hypothesis stating tVNS would attenuate aversive symptoms is correct. This could mean that the onset of the symptom of satiation was delayed with the use of active tVNS (Figure F).

Abdominal Pain

Statistical significance was found for abdominal pain as well. The p-value was 0.002337. It was found that FD subjects, compared to HC subjects, had higher ratings for abdominal pain. It was also found that the sham condition for FD subjects had a higher rating for abdominal pain than the active condition for FD subjects, which also suggests that the hypothesis stating that tVNS would attenuate aversive symptoms is correct (Figure G).

Nausea

After running the two-way ANOVA, statistical significance was also found for the nausea symptom. The p-value resulted in 0.001799. Thus, this indicates that FD subjects do have a higher rating of nausea than HC subjects. Furthermore, with the use of active tVNS, FD subjects were able to consume more Ensure than with the use of sham tVNS. Thus, this could indicate that the tVNS is delaying the onset of nausea in FD subjects when they consume a meal (Figure J).

Bloating

The two-way ANOVA also showed statistical significance between group and condition compared to the normalized VAS score for the symptom of bloating. The p-value generated was .002852. Thus, FD subjects were able to consume more Ensure with the use of active tVNS rather than with the use of sham tVNS. This could mean that the tVNS is allowing the FD subjects to consume more tVNS because there was a delayed onset for the symptom of bloating (Figure H).

Belching

Out of all the symptoms, belching did not result in a statistically significant p-value when the two-way ANOVA was run. The p-value resulted in .1129. This result could be due to the fact that subjects were instructed to drink Ensure out of a straw and thus, subjects could have been gulping air

while consuming the Ensure. On average, the same amount of Ensure was consumed for both conditions for both FD and HC groups (Figure I).

Autonomic Data

Heart Rate Variability (HRV) data were analyzed in FD subjects but have yet to be analyzed in HC subjects. The autonomic data was analyzed in order to test the hypotheses: 1.) Active versus inactive transcutaneous vagal nerve stimulation will increase parasympathetic nervous system activity to "meet" sympathetic nervous system activity so that symptoms will be attenuated since FD patients have an increased sympathetic nervous system; 2.) With the application of tVNS, high frequency (HF) power will be increased by active tVNS relative to sham tVNS. Low frequency (LF) power, high frequency (HF), and the LF/HF ratio were analyzed and interpreted. For context, LF power ranges from .04 to .15 Hz and HF power ranges from .15 to .40 Hz. In order to evaluate the data, each power was analyzed separately. Condition (session) and spectrum power (ms^2) were compared for LF Power and HF Power, and for LF/HF, the condition was compared with the ratio. For each session (active or sham), the first and last 6 minutes of each subject's session was used for analysis. This was because the software that was used, Kubios, had a limit to the amount of data that was allowed to be interpreted and the subjects' sessions ranged from 6.00 to 58.50 minutes. Therefore, to keep the methods constant, only the first and last 6 minutes of all the subjects' data were used.

LF Power

When the first and last 6 minutes of data in FD subjects using the active condition was evaluated using a t-test, no significance was seen since the p-value was 0.3690. Likewise, no significance was shown when a t-test was run between the first and last 6 minutes when the sham condition was used. The p-value for this test was 0.3690. Throughout the entire session, it can be inferred that LF Power remains the same (Figure K).

HF Power

Just as in the LF Power analysis, no statistically significant difference was shown between condition (session) and spectrum power in either the first or last 6 minutes of each session. There was no main effect of stimulation on HF Power. The p-value for the active condition comparison was 0.6490 and the p-value for the sham condition comparison was 0.0857. Although not significant, there is a trend, between the first and last 6 minutes, toward lower HF power for sham but not for active (Figure L).

LF/HF Power

After running t-tests on the session compared to the ratio, it was proven that there is no statistical significance between the first and last 6 minutes of the active condition. The p-value resulted in 0.7004. In contrast, there was statistical significance between the first and last 6 minutes in the sham condition. The p-value for this test was 0.0068. As seen in the previous two analyses, LF Power remains the same and HF Power decreases. This causes the LF/HF ratio to increase. This is why the ratio is higher during the last 6 minutes of the session versus the first 6 minutes of the session. This is therefore suggesting that the parasympathetic nervous system increases or drives the balance between parasympathetic and sympathetic nervous system in the last 6 minutes of the session. Furthermore, this suggests that for FD subjects, tVNS results in an increase in the parasympathetic nervous system instead of the sympathetic nervous system during a NDT. The tVNS is acting on the effect of the meal ingestion. No comparison has been made between FD and HC (baseline) for the LF/HF Ratio as of yet. It is inferred, by looking at the data, that there is a difference between FD and HC but the meaning behind this is yet to be deciphered (Figure M).

Heart Rate

Apart from analyzing HRV, heart rate (HR) was also evaluated in FD subjects. Overall, during the NDT, HR increased in FD subjects in active and sham sessions. After running a t-test comparing both active and sham conditions against mean HR, a p-value of 0.0195 was derived from the active condition comparing the beginning six minutes to the end six minutes and a p-value of 0.0083 was derived from the sham condition comparing the beginning six minutes to the end six minutes. After further analysis, there was no difference found in mean HR between active and sham (p-value = 0.8119). Although, HR significantly increased from the beginning to the end in both active and sham sessions. Moreover, there was less of an increase seen during active tVNS than sham tVNS (Figure N).

For the questionnaires, very few differences between FD and HC subjects were found. Specifically, for the MAIA, no difference was found between sham and active tVNS in regard to interoceptive awareness and their symptoms. Furthermore, both groups have similar awareness of their body and symptoms. For the PAGI-SYM, as suspected, FD subjects had worse scores of GI symptoms than HC subjects. Also, no major difference between active and sham scores was found for either group. Moreover, for the PROMIS-Anxiety, all the scores across the population remained relatively consistent throughout the study and there was no major difference between FD and HC subjects. Also, sham versus active tVNS did not change the scores of the subjects. Furthermore, for the PROMIS-Depression questionnaire, FD subjects resulted in a higher depression score and no difference was found between active versus sham scores, meaning the scores stayed relatively consistent throughout the visits. In regard

to the Short Nepean Questionnaire, no statistical significance was found in the difference score by group nor for sham versus active. Despite this, there was statistical significance when a test was run on the average scores of each group. Thus, this implies that FD subjects do have worse symptoms than HC subjects in regard to their symptoms

For the autonomic data, HR, HF power, LF power, and LF/HF were analyzed. For HR, it was concluded that HR increase in FD patients for active and sham conditions during the NDT. No statistical significance was found between active and sham tVNS but, HR significantly increased from the beginning to the end for both active and sham conditions. It was also noted that there was less of an increase during active tVNS than sham tVNS. In regard to LF power, there was no main effect. LF power did not change through the study. Furthermore, there was no main effect of stimulation on HF power. Also, between the beginnings and ends of the behavioral visits, there is a trend towards lower HF power for sham but not for active tVNS. Thus, one could hypothesize that vagal withdrawal effect is consistent with the increase in HR. Moreover, since LF power did not change and HF power decreased in sham, the LF/HF ratio increased. This is the why the ratio is higher during the last 6 minutes of the session versus the first 6 minutes of the session. This therefore suggests that the PNS increases, or drives, the balance between PNS and SNS in the last 6 minutes of the session. To summarize, this result suggests that for FD patients, tVNS drives an increase in the PNS instead of the SNS during the NDT. Thus, the tVNS is acting on the effect of meal ingestion.

To relate the results to the hypotheses, it can be concluded that with the application of tVNS, HF power will be increased by active tVNS relative to sham tVNS. The hypothesis suggesting that FD patients would consume less Ensure than HC during the NDT was inconclusive. In fact, both FD and HC subjects consumed relatively the same amount of Ensure. The hypothesis suggesting that aversive symptoms during the NDT would be attenuated by active tVNS has yet to be determined. Although these are the answers to the hypotheses right now, there is not enough data currently and these results could change in the future.

Some limitations did arise when evaluating the data and thus, could lead to false positives or false negatives. The main limitations include a small FD population size, air gulping during the behavioral visits, not getting to max fullness, not liking the taste of Ensure, and the description of fullness is subjective.

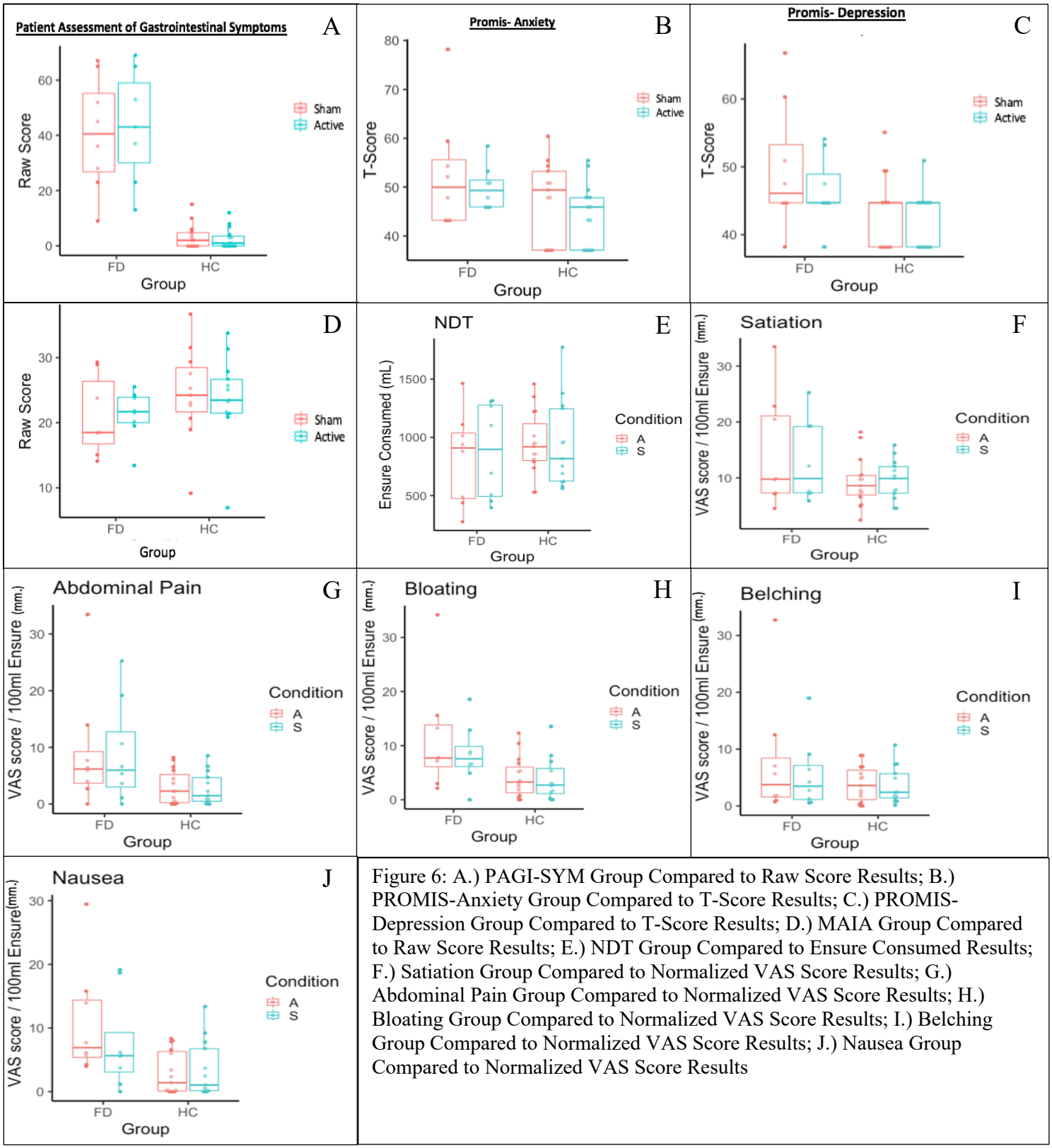

Figure 6: A.) PAGI-SYM Group Compared to Raw Score Results; B.) PROMIS-Anxiety Group Compared to T-Score Results; C.) PROMIS-Depression Group Compared to T-Score Results; D.) MAIA Group Compared to Raw Score Results; E.) NDT Group Compared to Ensure Consumed Results; F.) Satiation Group Compared to Normalized VAS Score Results; G.) Abdominal Pain Group Compared to Normalized VAS Score Results; H.) Bloating Group Compared to Normalized VAS Score Results; I.) Belching Group Compared to Normalized VAS Score Results; J.) Nausea Group Compared to Normalized VAS Score Results

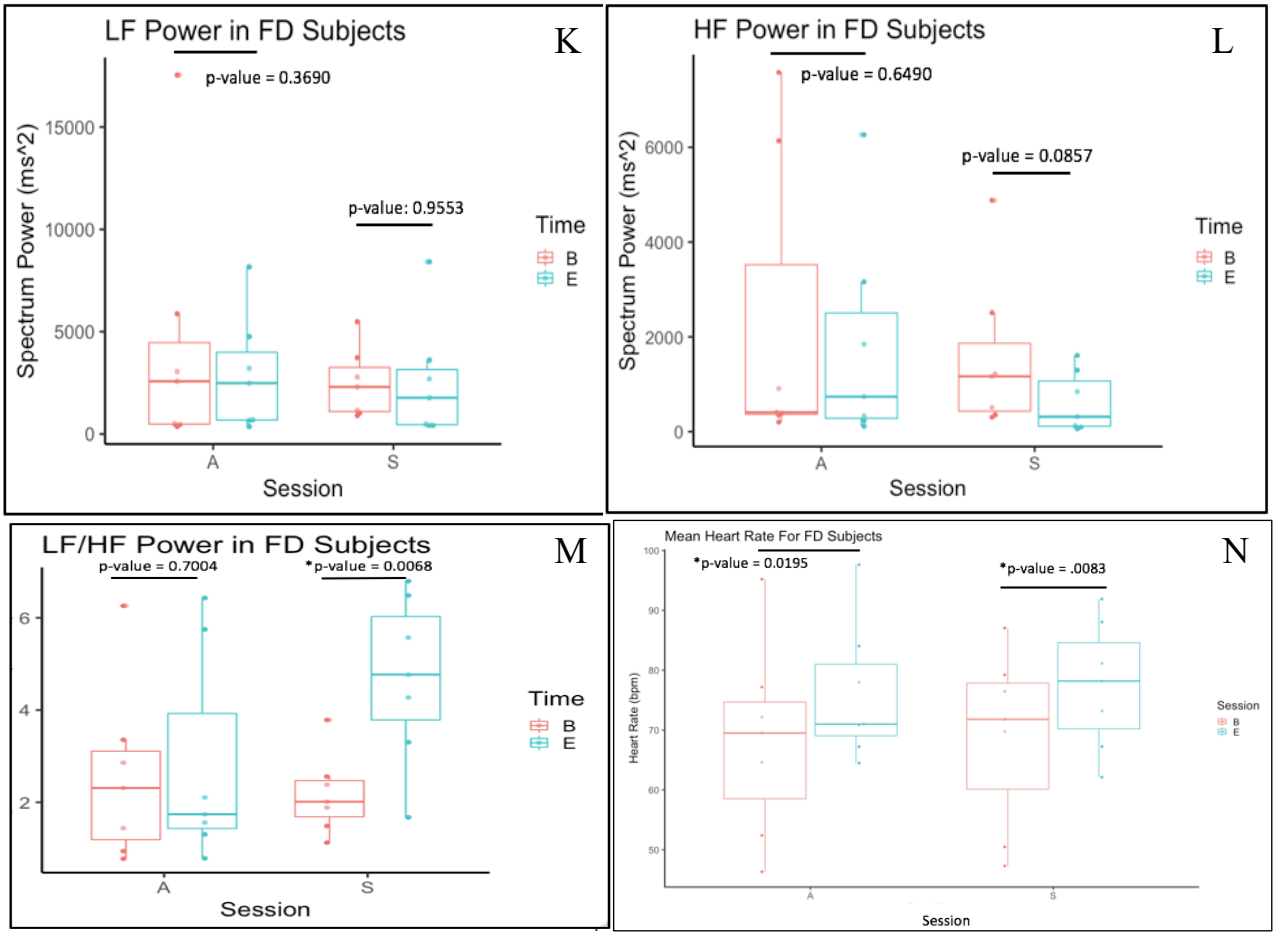

Figure 6 Continued: K.) LF Power in GF Patients Results; L.) HF Power in FD Patients Results; M.) LF/HF Ratio Power in FD Patients Results; N.) Mean Heart Rate in FD Patients Results

CONCLUSION

The goal of this study was to use gastric and autonomic measures combined with multimodal neuroimaging techniques to investigate the peripheral and central nervous system changes that accompany neuromodulation of brain-gut axis signaling by auricular tVNS in FD patients during a prandial challenge. This far into the study, there are 17 healthy controls enrolled, of which 1 dropped out, 1 had a screening fail, and 15 that provided complete data sets. There are also 10 FD patients enrolled in the study, including 2 drop outs, 1 screening fail, and 8 complete behavioral visits. Each subject completed 5 visits: a screening visit, 2 behavioral visits, and 2 MRI visits, where active or sham tVNS was applied. Although MRI's were conducted, my role as the student researcher did not include using the MRI data. Sham or active tVNS was administered randomly. For the behavioral visit, subjects were instructed to drink Ensure until complete fullness and every 5 minutes, a VAS would be administered to the patients. The subjects would mark how severe the symptoms of early satiation, nausea, abdominal pain, bloating, and belching were on a 100 mm. line, respectfully. After the behavioral visits, subjects completed a variety of questionnaires. Throughout the entire behavioral visits, autonomic and enteric physiological measures were monitored. At this point in the study, it can be concluded there are trends suggesting that tVNS does have an effect on the PNS on FD subjects and this increase in PNS activity helps alleviate some of the symptoms.

For the NDT data, significant differences were found between active and sham conditions and FD versus HC subjects. First, for the symptom of satiation, it was found that with the use of active tVNS, FD subjects were able to reach a higher VAS score compared to the sham condition for FD subjects. This could mean that the onset of the satiation symptom was delayed with the use of active tVNS. Next, for the symptoms of abdominal pain, it was found that FD subjects have higher levels of abdominal pain. It was also found that the sham condition of tVNS for FD subjects had a higher rating for abdominal pain than the active condition for FD subjects. For nausea, FD patients resulted in a higher rating for the nausea symptom than HC subjects. Furthermore, with the use of active tVNS, FD subjects were able to consume more Ensure than with the use of sham tVNS. Thus, this could imply that tVNS is prolonging the onset of nausea in FD subjects. Then, for bloating, FD subjects were also able to consume more Ensure with the use of active tVNS than with the sham condition. Thus, this could also imply that the tVNS is allowing the FD subjects to consume more due to the delayed onset of bloating. Lastly, no significant finding was found for belching between active or sham tVNS or FD and HC subjects. It is important to note that this could have been because subjects were drinking through a straw, and thus were gulping air.

The results thus far into the study suggest that tVNS does in fact increase the activity of the PNS, rather than the SNS, which could help alleviate some of the symptoms for FD subjects. Thus, this could lead to FD patients potentially having a better quality of life in the future after more studies are conducted

using the results of this study. Based off of the findings thus far, future research includes potentially using tVNS in other GI disorders, determining if tVNS is more effective in relieving symptoms in those with upper GI disorders or lower GI disorders, gathering a better understanding of the etiology and relationship between neuromuscular dysfunctions of the enteric tract and the underlying pathophysiology and symptoms, evaluating if the continuous use of tVNS will significantly decrease ,or completely eradicate, the symptoms related to FD, thus improving the quality of life, and evaluating depression and anxiety levels periodically months after the continuous use of tVNS. These possible research studies should be the next steps towards identifying if tVNS is a possible treatment plan for all GI patients because they could drastically improve the lives of these patients if their symptoms were alleviated or completely eliminated. It is currently unknown if the vagus nerve has a different effect on the upper or lower GI system, and thus, this could be a good way to decipher if tVNS as a better effect on upper GI or lower GI disorder patients. Moreover, patients impacted by medical conditions have a significantly higher rating of anxiety and depression ratings. It is possible that tVNS could help lower the ratings of these patients and thus, could also improve the quality of life of these patients. With the knowledge made available by this study, as well as many research studies to come, the 60 to 70 million people in the United States alone who are impacted by gastrointestinal disorders can actually start to live their lives, instead of getting held back by their burdensome GI disorders.

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