Neurogastroenterology & Motility

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ORIGINAL ARTICLE OPEN ACCESS

Cardiac Vagal Efficiency Is Enhanced by Percutaneous Auricular Neurostimulation in Adolescents With Nausea: Moderation by Antidepressant Drug Exposure

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Received: 22 May 2024 | **Revised:** 30 November 2024 | **Accepted:** 6 January 2025

Funding: This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases. Chaja Foundation. Children's Hospital of Wisconsin Research Institute.

Keywords: disorders of gut–brain interaction | functional gastrointestinal disorders | functional nausea | neuromodulation | neurostimulation | vagal efficiency

ABSTRACT

Objectives: Percutaneous electrical nerve field stimulation (PENFS) is an effective treatment for disorders of gut–brain interaction (DGBI), proposed to influence vagal pathways. Cardiac metrics such as respiratory sinus arrythmia (RSA) and vagal efficiency (VE) can noninvasively assess parasympathetic output. Commonly used antidepressant drugs inhibit vagal signaling and may interfere with PENFS. This study examined immediate effects of active compared to sham PENFS on cardiac vagal function in adolescents with chronic nausea with and without concurrent drug therapy.

Materials and Methods: Participants ($n = 84$) were randomized to active (3.2 V, 1–10 Hz) or sham PENFS within an 8-week prospective, double-blind clinical trial. Subjects underwent posture challenges to elicit a vagal response before and after PENFS device placement mid-way through the study. RSA, mean heart period (HP), and VE were calculated from electrocardiogram recordings. Exposure to antidepressant drugs was recorded.

Results: The mean (SD) age was 15.61 (2.07) years (83% female). Fifty percent were treated with antidepressants. PENFS neurostimulation enhanced VE in patients without antidepressant exposure (mean increase after PENFS stimulation =7.56 [95% CI: 0.26, 14.86], $d = 0.30$, 17% increase) but not in those treated with antidepressants (mean change = −5.30 [95% CI:−14.28, 3.68]). Sham PENFS did not produce significant VE changes regardless of medication use (both $p > 0.40$). There were no significant effects on RSA or HP.

Conclusions: Acute enhancement of cardiac VE is demonstrated with PENFS in patients not exposed to chronic antidepressant drug therapy. Findings indicate that VE is a sensitive metric for rapid assessment of PENFS effects but raise concern for possible interaction or interference by standard of care medications.

Trial Registration: Clinicaltrials.gov #: 1064187–2

Summary

- Percutaneous electrical nerve field stimulation (PENFS) is an effective therapy for disorders of gut–brain interaction (DGBI) presumed to act by enhancing vagal signaling.
- Acute enhancement of cardiac vagal function is demonstrated with active compared to sham PENFS.
- This effect is not found in children chronically exposed to antidepressants with anticholinergic and serotonergic effects.

1 | Introduction

Neuromodulation is being explored as an intervention for a variety of psychological and physical conditions, with vagus nerve stimulation as a common target. The vagus innervates organs throughout the body, including the digestive tract, and provides a channel for afferent activity from the visceral organs to the brainstem [1]. Neurostimulation is an emerging therapy for many gastrointestinal (GI) conditions governed by disordered brain–gut signaling [2]. Non-pharmacological and alternative medicine therapies are frequently sought by families of children suffering from disorders of gut–brain interaction (DBGI), many who have experienced adverse reactions to drug therapies [3]. Neurostimulation therapies are thus increasingly explored due to the concerning side effect profile of many pharmacological agents coupled with a lack of mechanistic support and insufficient evidence for their use in children [4–6].

DGBI are stress-sensitive conditions with clinical features and comorbidities suggesting heightened autonomic reactivity and visceral sensitivity [7]. Meta-analyses and systematic reviews demonstrate that functional impairment of vagal regulation is implicated in the pathophysiology of several DGBI, functional somatic syndromes, and anxiety disorders [8–10]. Vagal regulation can be indexed using power spectral analysis of heart rate variability [11], providing specific, rapid, and noninvasive assessment of neuromodulation effects on the vagus. Cardiac vagal tone can be measured by the amplitude of respiratory sinus arrhythmia (RSA; also known as high-frequency heart rate variability; HF-HRV), an established and validated index that reflects changes in the signaling of the myelinated cardiac vagal pathway to the heart [12, 13]. More recently, the coupling strength of RSA to average heart period (HP) (time between heart beats) during physiological challenges was measured in pediatric DGBI using the vagal efficiency (VE) index [14]. Reduced VE was documented in cohorts of children with both abdominal-pain-related DGBI and cyclic vomiting syndrome, indicating a possible mechanism and therapeutic target [14, 15]. A reduced VE was also found in a subset of DGBI patients with a therapeutic response to auricular percutaneous electrical nerve field stimulation (PENFS) [16]. However, studies are needed to examine whether vagal function indices such as VE are acutely responsive to stimulation.

Auricular PENFS is a novel treatment that targets cranial afferent nerves with an electrical current applied across the external ear [17]. PENFS is demonstrated effective for several different pediatric DGBI. This includes efficacy for abdominalpain-related DGBI based on randomized, sham-controlled trials [18, 19], and several observational studies of pediatric functional dyspepsia and cyclic vomiting syndrome [20–23]. A large-scale, multicenter registry study also reported improvement in abdominal pain, nausea, and functional disability when applied per standard of care for any pediatric DGBI [24]. Importantly, several studies are notable for significant improvement in not only GI symptoms but also in physical functioning, quality of life, and psychological comorbidities [18, 20, 23, 25, 26]. The device is cleared by the Food and Drug Administration for the treatment of abdominal pain associated with irritable bowel syndrome in children and adolescents [27]. Despite the surge of clinical outcomes data, knowledge of the mechanisms underlying PENFS effects remains unclear.

To better target treatment, it is critical to establish mechanisms of action. Auricular neurostimulation effects on DGBI are hypothesized to be mediated via the vagus by afferent projections to the nucleus of the solitary tract, which signals to the dorsal motor nucleus of the vagus (DMNV) [28–30]. The DMNV sends efferent vagal signals to the GI tract as well as the nucleus ambiguus, which produces outflow that can be measured with RSA [31, 32]. These known neural projections coupled with the positive clinical effects on syndromes and comorbidities associated with altered vagal regulation are suggestive of vagal nerve stimulation effects [20, 25, 26]. Further, the finding that reduced VE can predict PENFS treatment success suggests that VE is a promising metric to investigate a mechanism of action [16].

Cardiac vagal effects of neurostimulation can likely be influenced by medications. The vagus nerve uses acetylcholine (Ach) as a primary postganglionic neurotransmitter; thus, medications with anti-cholinergic properties may directly inhibit stimulation response [33]. This has been demonstrated in animal studies using other neurostimulation modalities and various anticholinergic agents [34, 35]. Drugs such as tricyclic antidepressants (TCAs), 5-HT_{2A} receptor antagonists (i.e., cyproheptadine), and selective serotonin reuptake inhibitors (SSRIs) are commonly used to manage symptoms and comorbidities of DGBI in children despite insufficient evidence of efficacy [4, 6, 36]. TCAs, 5-HT_{2A} receptor antagonists, and certain SSRIs exert anticholinergic properties [37, 38]. In addition to the overlapping noradrenergic and serotonergic effects of these different drugs, some SSRIs may decrease parasympathetic function [38, 39]. Therefore, the potential interactions and impact of these drugs on concurrent PENFS therapy need to be explored.

Vagal pathways, particularly afferents, are essential components of the neural mechanisms that induce nausea and vomiting [40, 41]. While chronic nausea is a common but poorly defined entity included under the umbrella of DGBIs, emerging data implicate that at least a large subset suffers from comorbidities indicating ANS dysfunction [42–44]. Multiple studies have demonstrated that a large fraction of adolescents with chronic, unexplained nausea deemed "functional" in origin have altered parasympathetic response, abnormal tilt table testing, and orthostatic intolerance [45–47]. We hypothesized that active auricular neurostimulation via PENFS would acutely enhance cardiac vagal activity in adolescents with nausea compared to

a sham intervention and that the effect could be moderated by chronic anticholinergic drug exposure. The aim of this study was to test the acute effect of auricular neurostimulation in a sample of adolescents with chronic nausea with and without medication exposure.

2 | Methods

2.1 | Population

Data were collected from subjects participating in a double-blind, randomized, sham-controlled clinical trial of auricular PENFS in adolescents with chronic nausea. Results of efficacy outcome data are pending analysis. Data collection was conducted between April 2018 and February 2022. Eligible patients suffered from predominant chronic nausea several times per week for at least 2 months. Patients were eligible if they were ages 11–18 years and proficient in English. Exclusion criteria were organic health conditions or medication use that may explain GI symptoms, developmental delay, pregnancy, illegal drug use, and the presence of any implanted electrical device. Patients were also excluded if they received therapy with opioids or had changes to their medical regimen in the 4 weeks prior to enrollment. Information on demographics, medical history, comorbidities, and medication use was collected from medical records and by surveying participants and their legal guardians. The study was approved by Children's Wisconsin (CW) hospital Institutional Review Board (#1030543). An informed consent and/or assent, approved by CW IRB, was reviewed and signed by all children and one legal guardian prior to study activities.

2.2 | Protocol

Subjects were randomized to receive 4 weeks of active or sham PENFS therapy in a double-blind design, followed by another 4 weeks of open-label, active PENFS therapy.

Medication use. Chronic medication use was defined as a minimum of 2 months of daily therapy with at least one prescribed pharmacological agent with known anticholinergic properties (tricyclic antidepressants, cyproheptadine) [37, 48] or SSRIs. Subjects taking medications with cardiac inhibitory effects (e.g., beta blockers) were excluded.

Comorbidities. Examined comorbidities included recurrent dizziness/lightheadedness, migraine headaches, recurrent syncope, sleep problems, concentration difficulties, and self-reported joint hypermobility. Clinical diagnoses examined included hypermobile Ehlers–Danlos Syndrome, hypermobile spectrum disorder, gastroparesis, and dysautonomia.

Stimulation. The PENFS device (Neuraxis, Carmel, IN) was attached behind one ear per standard procedures (Figure 1a) by certified medical personnel as previously described [18]. The device contained four electrode arrays, each with a small, sterile 2-mm titanium needle. Prior to placement, the ear was cleaned with an alcohol wipe and transilluminated to visualize the ear's vascular branches. The electrode arrays were placed percutaneously in close proximity to the neurovascular branches with three electrodes on the ventral and one on the dorsal side of the ear, providing field stimulation of the entire ear [49]. The device was continuously worn for five consecutive days each week (5 days on/2 days off), with a new device fitted weekly by trained and certified research personnel per the same protocol as in prior studies [18, 23, 25, 49, 50]. The active PENFS device delivered standard stimulation parameters (bipolar, alternating 1–10 Hz, 3.2 V). The sham device did not deliver an electric current but was otherwise identical and applied using the exact same procedure as the active device.

Cardiac vagal metrics. Data were collected at the last (4th) randomized device placement. This timing avoided possible interfering effects from patient anxiety about initial placements. Situational anxiety can have a dampening effect on cardiac vagal activity, which is documented in children with nausea and vomiting [51], and thus may interfere with vagal responses. At the time of recordings, patients had already undergone three prior device placements. In addition, per standard protocol (see above), participants had not worn the device during the preceding 2 days.

Electrocardiogram (ECG) data were collected in a quiet room without interactions with other people or access to electronic devices. Subjects were instructed to avoid caffeine on the day of the recordings. ECG data were collected via two electrodes spanning the precordium, using the Firstbeat Bodyguard 2 (Firstbeat Technologies Ltd., Jyväskylä, Finland). The Firstbeat quantified inter-beat intervals between R-waves sampled at 1000 Hz and has been shown to have high accuracy [52]. After a 10 min period of quiet sitting rest, recordings were conducted during cued sitting, standing, and sitting posture shifts with 3 min in each position. Movement from sitting to upright standing increases gravitational demand on blood circulation and reduces fluid pressure on carotid baroreceptors, causing compensatory heart rate change mediated by autonomic circuits [53, 54]. During the following 10 min, active or sham devices were placed on the subject's ear. The subject then again took part in a 10-min period of quiet, sitting rest during active or sham neurostimulation, followed by repeat posture shifts.

Resulting inter-beat interval cardiac data were inspected for artifacts, arrhythmias, and missed beats to avoid bias and invalidation of heart rate variability measures [55]. Missed beats were reconstructed and noise artifacts were removed to produce an uninterrupted signal using CardioEdit+ Software (Brain–Body Center for Psychophysiology and Bioengineering, University of North Carolina at Chapel Hill). Cardiac measures were quantified in 15-s epochs using CardioBatch software (Brain–Body Center for Psychophysiology and Bioengineering, UNC-Chapel Hill).

2.3 | Measures

Mean heart period (HP). Mean HP is the average timing between successive heart beats measured in milliseconds. Changes in HP reflect a composite measure of overall neural influences on the heart's pace, including vagal and sympathetic outflow.

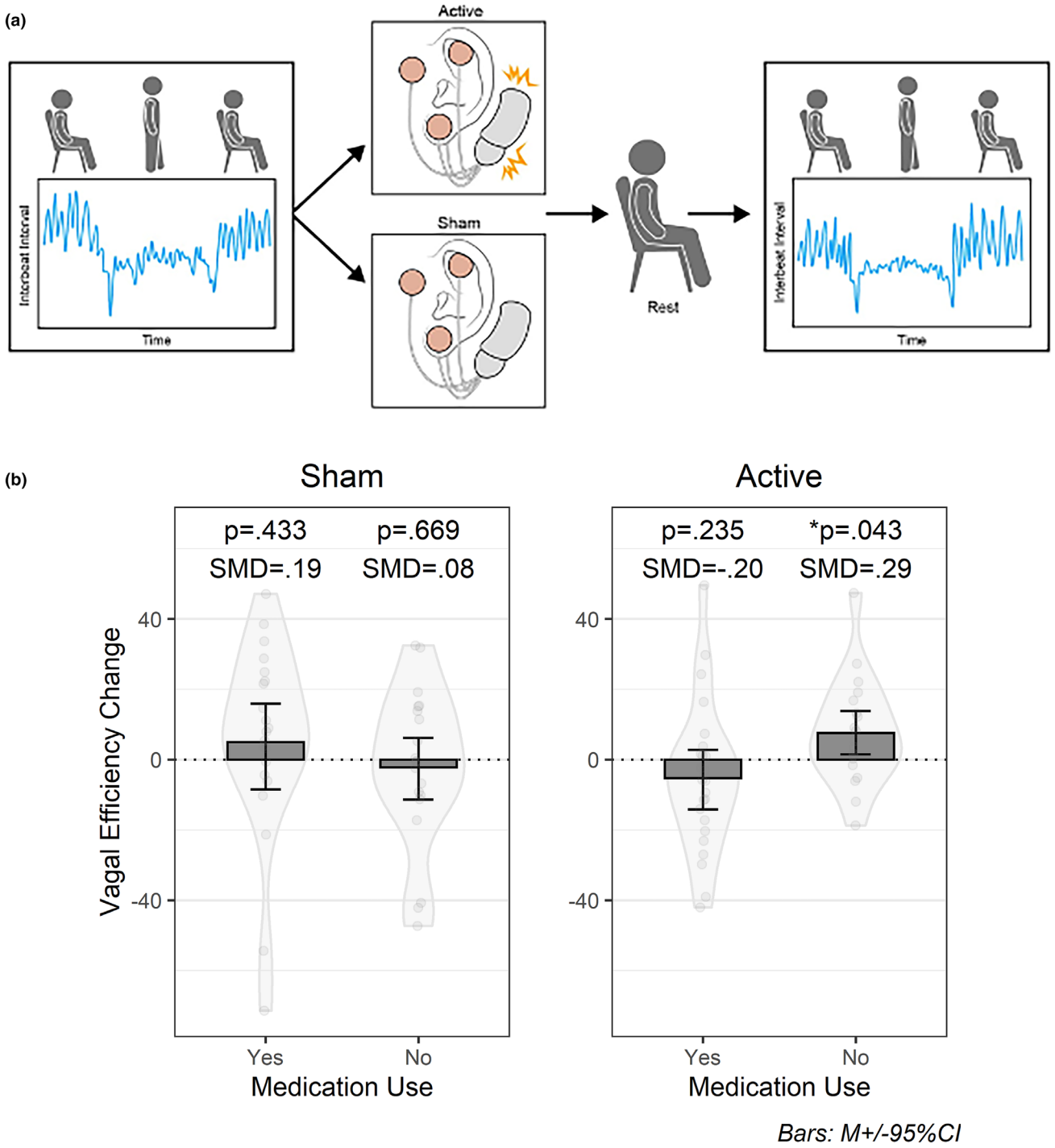

FIGURE 1 | (a) Study design. (b) Change in cardiac vagal efficiency during active and sham conditions with and without anti-cholinergic medication use. One-sample *t*-tests comparing change scores to zero. $p = p$ value, SMD = standardized mean difference.

Respiratory sinus arrhythmia (RSA) amplitude. RSA is a validated index of cardiac vagal tone, the effect of the myelinated vagus nerve on the heart's pacemaker [12]. Cardiac vagal tone was quantified by heart rate variability within the frequency band of spontaneous breathing in adolescents (0.12–1.02 Hz) using a 21-point moving polynomial filter to remove slow periodic frequencies and nonstationary aperiodic trends [13, 56, 57]. The resulting metric was natural log transformed to reduce skew.

Vagal efficiency (VE). Cardiac VE is a measure of the coupling of changes in cardiac vagal tone and heart rate. Per previous studies [14, 16, 58, 59], it was quantified as the slope of regression analyses between the entire set of synchronous 15-s epoch HP and RSA across the sequence of posture shifts for each subject.

2.4 | Statistical Analysis

Analysis was conducted in R 4.2.0 and RStudio 2022.07.01 [60]. Chi-square and *t*-tests were used to assess simple group differences. Data was analyzed with mixed-effects modeling using the lme4 package [61]. For VE, which is calculated across all

postures, we ran a mixed effect model testing a medication group * medication use * device placement (pre/post) interaction. For HP and RSA, which are calculated individually within each posture, we included an additional interaction term with posture (sitting 1, standing, sitting 2). Prior to running the final models, we explored interactions and direct effects of variables prior to device placement. For all models, support for parameters as nonzero was indicated by 95% confidence intervals that were calculated using parametric bootstrapping with 5000 samples. Marginal means were calculated using the emmeans R package [62]. Percent change and standardized mean difference effect sizes were calculated using pooled mean and standard deviation data.

3 | Results

A total of 105 adolescents completed the study procedures. Of these, 92 had complete inter-beat interval data for both pre and post PENFS device placement time points and high-quality data from multiple postures at each measurement. Of the resulting participants, eight were excluded because of the use of beta blockers, which have a direct impact on cardiac receptors [63]. This group was too small to test for specific interactions with intervention and was thus excluded from the main analyses. Descriptive statistics for the final analysis dataset ($n = 84$) are presented in Table 1. There were no significant demographic differences in the medication vs. no medication groups.

Medication use. Fifty percent of subjects were chronically treated with SSRIs or antidepressant drugs that exert anticholinergic effects; SSRIs ($n = 24$), TCAs ($n = 12$), 5-HT_{2A} receptor antagonists ($n = 12$), and as needed use of various anti-spasmodic or anti-emetic agents including dicyclomine, hyoscyamine, diphenhydramine, hydroxyzine, meclizine, and scopolamine. All subjects in this medication group were exposed to polypharmacy (two drugs $n = 37$; three drugs $n = 4$; four drugs = 1). Medication use was not significantly associated with age, gender, or body mass index (all $p > 0.05$). Medication use was also not associated with any of the examined comorbidities (recurrent dizziness/lightheadedness, migraine headaches, recurrent syncope, sleep problems, concentration difficulties, and self-reported joint hypermobility), total comorbidity count or other clinical diagnoses including mental health diagnoses (all $p > 0.05$). Prior to device placement, patients in the medication group had lower VE (VE = 39.59 vs. 50.93; $t(82) = 2.03$, $p = 0.046$), lower RSA across all postures, and shorter mean HP during sitting (Tables S1 and S2; Figure S1).

Treatment group comparisons before device placement. Treatment and sham groups did not significantly differ on VE ($t(82) = 0.079$, $p = 0.937$), RSA, or HP metrics before device placement (interaction parameters include 0; Tables S3 and S4).

Device pre/post placement effects on autonomic metrics. There were no significant effects of treatment versus sham on RSA or mean HP (Tables S5 and S6). However, results showed that there was a 3-way interaction of medication use, treatment arm, and pre/post device placement on VE (Table 2). We examined this interaction by probing pre/post stimulation change by medication use and treatment arm (Figure 1b). The sham device did not produce significant VE changes in either the medication or no medication group. PENFS neurostimulation enhanced VE in patients without chronic medication use (mean increase after stimulation = 7.56 [95% CI: 0.26, 14.86], $t(18) = 2.18$, $p = 0.043$, SMD = 0.30, average 17% increase) while patients who were on chronic medications did not show an

TABLE 1 | Demographics and descriptive statistics of total sample and medication versus no medication groups.

Variable	Total sample	Antidepressant drugs	No antidepressant drugs	Difference tests
n	84	42	42	
Age (yrs)	14.92 ± 2.18	15.32 ± 2.04	14.52 ± 2.27	$t(81.061) = -1.705$, $p = 0.092$
Age Range	11.00–18.70	11.00–18.70	11.00–17.70	
Female	68	35	33	$\chi^2 = 0.077$, df = 1, $p = 0.781$
BMI	22.89 ± 4.95	23.54 ± 5.15	22.23 ± 4.71	$t(81.377) = -1.215$, $p = 0.2281$
BMI range	14.15–38.76	14.15–38.76	14.80–35.93	
*Race and/or Ethnicity**				$\chi^2 = 0.851$, df = 1, $p = 0.356$
Asian	1	—	1	
Asian, Caucasian	1	—	1	
White	79	41	38	
White, Hispanic or Latino	3	1	2	

*Difference test compares binary groups (white and non-white).

TABLE 2 | Mixed effect results for interactions of medication use, treatment arm, and device placement predicting VE. There was a significant three-way interaction (Type II Wald $\chi^2(1) = 4.36$, $p = 0.037$).

Term	Estimate	SE	*t*	95% CI low	95% CI high	Excludes 0
(Intercept)	52.868	5.96	8.87	41.271	64.639	*
Med Group (Ref = No Med)	−13.875	8.328	−1.666	−29.973	2.77	
Treatment (Ref = Sham stimulation)	−1.473	8.539	−0.173	−18.62	14.704	
Device placement (Ref = Pre)	−1.534	3.534	−0.434	−8.354	5.494	
Med Group * Arm	3.62	11.677	0.31	−18.607	26.204	
Med Group * Device Pre/Post	5.022	4.938	1.017	−4.648	14.377	
Arm * Device Pre/Post	6.876	5.063	1.358	−3.384	16.718	
Med Group * Arm * Device Placement	−14.11	6.924	−2.038	−27.938	−0.608	*
Residual SD	24.199	—	—	—	—	—

acute response to stimulation (mean change = −5.30 [95% CI: −14.28, 3.68], $t(23) = -1.221$, $p = 0.235$, SMD = −0.14, −12%; Figure 1b). A sensitivity analysis to exclude the effects of one outlier (3.21 SD below mean) in the sham stimulation/medication condition resulted in consistent results (see Supporting Information).

4 | Discussion

This study demonstrates a moderate, acute enhancement of cardiac VE within 20 min of PENFS neurostimulation only in patients *not* exposed to antidepressant drugs. The results support VE as a potential measurement tool for examining the mechanistic effects of neuromodulation. To our knowledge, no prior research has examined acute VE response to neuromodulation. With the availability of scalable, noninvasive, and accurate methods for collecting inter-beat interval cardiac data, VE is a metric that could be integrated into both research protocols and clinical care for monitoring vagal activity. Potential applications may include predicting treatment response and personalizing neurostimulation parameters. However, the study findings also raise questions about standard-of-care drugs as possible candidates for interacting or interfering with vagal regulation or with neurostimulation therapy.

By estimating the coupling of vagal output with overall heart rate, cardiac VE provides a distinct measure of the efficiency of the vagal brake on the heart induced by postural stress that triggers autonomic adjustments [16]. Our findings are in line with large-scale, meta-analysis data, which suggest that auricular stimulation enhances vagal signaling [64]. Assessment of ANS reactivity using heart rate variability has been adopted for many chronic diseases as a measure of overall health or to assess the physiologic effects of interventions [51, 65]. However, protocols and measurement approaches vary widely and many confounding factors may influence results, including day-to-day stressors, duration of measurements, concurrent drug exposure, demographics, and body mass index [66]. Due to confounders such as day-to-day stressors, chronic ANS alterations induced by neurostimulation interventions may be particularly difficult to assess with a single point-in-time measure. Therefore, acute assessment of neurostimulation effects on vagal outflow in response to posture stressors in a controlled experimental setting may provide more reliable physiologic information. Further, VE is a dynamic measure of cardiac vagal outflow that has already been demonstrated to be a metric of ANS activity and PENFS response in children with DGBI in response to posture stressors [14–16].

In contrast to stimulation effects on VE measured across posture shifts in participants without medication use, our study did not find a significant effect of PENFS on RSA, a resting measure of beat-to-beat dynamics of the heart that can be reliably used to measure the cardiac outflow of the central autonomic network [67]. While some studies using transcutaneous auricular stimulation in healthy controls find effects on resting indices [68, 69], null results are also observed in studies of DGBI [70, 71]. Similarly to our findings, a study of acute effects of transcutaneous auricular vagal nerve stimulation (taVNS) in adults with functional dyspepsia did not show an increase in high-frequency heart rate variability, a metric related to RSA, after 30 min of stimulation during rest but did demonstrate significantly higher vagal activity compared to sham after a nutrient drink challenge [70]. Similarly, another study of taVNS in healthy adult men did not find significant effects of 30 min of resting state stimulation on root mean square of successive differences (RMSSD), another measure of parasympathetic activity [72], regardless of stimulation parameters (10, 40, or 80 Hz), though the 40 Hz condition did induce an increase in RMSSD after a drink meal [71]. These studies indicate that vagal effects of transcutaneous stimulation may need to be induced by robust symptom triggers.

Neuromodulation effects on the ANS may be spread across one or more nodes in the autonomic feedback systems. These include homeostatic set-points, sensitivity of specific autonomic sensory pathways, synthesis of multimodal sensory information, or gain of input/output feedback leading to change in efferent output magnitude [73]. Different neuromodulation intervention modalities also may impact these nodes to different degrees. This implies that neuromodulation studies should be rigorously evaluated for changes in both resting levels of autonomic activity and, critically, for changes in the dynamic control of the ANS in response to challenges such as posture shifts. Extensions of this

research may explore non-posture challenges to the ANS such as a meal challenge to trigger the underlying symptoms and elucidate the mechanism of action for various neuromodulation interventions.

Although VE was a moderately sensitive metric for auricular stimulation in this study, patients exposed to anticholinergic drugs and SSRIs did not demonstrate a similar change. These findings align with animal data using other neuromodulation techniques and demonstrating that anticholinergic drugs inhibit the neurostimulation response. Two studies in dogs and guinea pigs demonstrate that various anticholinergic agents effectively block the contractile urinary bladder response to pelvic nerve stimulation [34, 35]. The inhibitory effects of drugs with anticholinergic properties on gut motility should also raise concerns for their use in DGBI, disorders that overlap with motility disturbances [74–76]. There is in fact limited data to support the use of pharmacologic therapy for children with DGBI, and the anticholinergic properties are associated with risks of side effects [77]. A recent study calls into question the efficacy of commonly used drugs to treat pediatric DGBI, specifically cyproheptadine, which was found inferior to PENFS therapy [21]. While mostly retrospective studies demonstrate the efficacy of cyproheptadine for DGBI, these studies also consistently report high rates (30%) of adverse events including somnolence, weight gain, and mood changes [78, 79]. Conversely, no serious adverse events have been reported in large-scale studies of PENFS [18, 24]. While these findings raise concerns about antidepressants moderating neurostimulation response, it is important to note that these results require further study and that drugs may induce beneficial neuromodulation response in some patients.

It remains unclear why chronic anticholinergic and/or serotonergic drug exposure moderates the acute neurostimulation response in this pediatric population. PENFS is likely to exert central effects via stimulation of vagal pathways projecting to the brainstem [29, 30, 80]. Both animal and human studies suggest that altering cholinergic and serotonergic neurotransmission may affect neural signaling and cognition [81–83]. While it remains speculative, many of these agents have anticholinergic properties that inhibit vagal signaling [33] and may therefore affect neurostimulation response.

The strengths of our study include a large sample size, a sham control condition, and measurements performed after acute neurostimulation intervention in a controlled experimental setting. However, the interpretation of the study should also be made in the context of its limitations. Medication use was observed rather than randomized. We were unable to control for specific dosing, drug effects such as specific anticholinergic strength [48], interactions from combination drug therapy, or the sole effects of specific drug agents. However, all subjects in the drug-exposed group were treated with SSRIs and/or drugs exerting anticholinergic effects at the time of the study. Though anticholinergics have well-established effects on the vagus, SSRIs have more mixed evidence, and the potential effects of SSRIs and the possible different effects of various SSRIs need additional studies [84]. While anticholinergics affect signaling throughout the vagus, which relies on acetylcholine, beta blockers may more directly influence cardiac metrics while being less likely to affect gastric signaling via the vagus [63]. Therefore, the exclusion of patients treated with beta blockers was necessary to eliminate possible confounding effects of these drugs.

It is also possible that medication burden or comorbidities not examined may be an indicator of underlying differences in symptoms or disease complexity. However, we did not find a difference in demographics, symptom severity, additional diagnoses, or comorbidity burden in subjects with medication compared to those without medication use. In addition, the ECG measurements were conducted after 3 weeks of chronic PENFS therapy, before and after acute neurostimulation. Thus, it is unclear whether the same effects (or even greater) would be observed at the first PENFS device placement when subjects were naïve to prior neurostimulation. Though the current approach allowed some control of confounding anxiety effects with the first electrode needle insertion, which would affect ANS reactivity, follow-up studies are needed to also investigate the effects on initial neurostimulation. This will be important for applications where stimulation parameters may be modulated based on the individual's stimulation response.

In summary, DGBI affects a large proportion of the population and is associated with impaired quality of life largely due to lack of effective therapies [85, 86]. Modulating autonomic reactivity via brainstem circuits by auricular neurostimulation is a promising therapeutic avenue [87]. Due to the crosstalk between brainstem nuclei controlling both GI and cardiac efferent signals, VE measured during physiological challenge may be an effective surrogate marker of GI dysfunction and ultimately, a biomarker of therapeutic response. This study demonstrates an acute enhancement of cardiac VE in response to active PENFS neurostimulation, an effect not observed by sham stimulation. It also suggests that exposure to antidepressant medications that are frequently used to treat DGBIs in children may moderate the enhancement of cardiac VE during PENFS treatment.

Author Contributions

J.K.: study design, analysis and interpretation of data, statistical analysis, drafting, and critical revision of the manuscript. J.K. has approved the final submitted manuscript. O.K.R.: processing and editing of data, assistance with data analysis, drafting, and critical revision of the manuscript. O.K.R. has approved the final submitted manuscript. G.F.L.: interpretation of data, drafting, and critical revision of the manuscript. G.F.L. has approved the final submitted manuscript. K.K.: study concept and design, acquisition of data, interpretation of data, obtained funding, administrative support and study supervision, drafting, and critical revision of the manuscript. K.K. has approved the final submitted manuscript.

Acknowledgments

Research reported in this publication was supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under award number K23DK116969. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Jacek Kolacz's work on this manuscript was supported by the Chaja Foundation. Funding in support of this work was also provided by the Children's Research Institute, Children's Wisconsin Hospital. Study sponsors had no role in the study design, collection, analysis, or interpretation of data. The authors wish to thank Ilona Ovestrud, Jingyi Zhang, and Ani Koppaka for their inspection and cleaning of the raw interbeat interval files. Katja

Karrento has served on the Medical Advisory Board for Abbvie and as a consultant for Takeda Pharmaceuticals and Neurogastrx.

Disclosure

This work was previously published in abstract form at the American Neurogastroenterology and Motility Society annual meeting. Reference: Abstracts of the 2023 ANMS Annual Meeting Scientific Program: Advancing patient care through cutting-edge research August 11–13, 2023 Austin, Texas, USA. *Neurogastroenterology and Motility*. 2023;35(S1):e14642. doi: https://doi.org/10.1111/nmo.14642.

Conflicts of Interest

Katja Karrento has served on the Medical Advisory Board for Abbvie and as a consultant for Takeda Pharmaceuticals and Neurogastrx.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.