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The effects of low-and high-frequency non-invasive transcutaneous auricular vagal nerve stimulation (taVNS) on gastric slow waves evaluated using in vivo high-resolution mapping in porcine

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Abstract

Backgrounds: Gastric motility is regulated by an electrophysiological activity called slow-wave and neuronal innervations by the vagus nerve. Transcutaneous auricular vagal nerve stimulation (taVNS) has been demonstrated to have therapeutic potential for a wide range of medical conditions, including the management of gastric dysfunctions. The main objective of this study was to gain a better understanding of how non-invasive neuromodulation influences gastric slow wave under in vivo conditions.

Methods: TaVNS protocols were applied in conjunction with 192-channel gastric bioelectrical mapping in porcine subjects under general anesthesia. The spatiotemporal profiles of gastric slow wave were assessed under two different taVNS protocols at 10 and 80 Hz.

Key Results: The taVNS protocols effectively altered the interval and amplitude of gastric slow waves, but not the velocity or the percentage of spatial dysrhythmias. In the subjects that responded to the protocols, the 10 Hz protocol was shown to normalize slow-wave propagation pattern in 90% of the subjects, whereas the 80 Hz protocol was shown to inhibit slow waves in 60% of the subjects.

Conclusions and Inferences: Chronic responses of gastric motility and slow waves in response to taVNS should be investigated using non-invasive means in conscious subjects in future.

KEYWORDS

electrical stimulation, gastric slow wave, gastrointestinal (GI) tracts, neuromodulation, transcutaneous auricular vagal nerve stimulation

1 | INTRODUCTION

Gastric motility is regulated by an electrophysiological activity called slow waves, which are generated by the interstitial cells of Cajal (ICC). Under normal circumstances, gastric slow waves entrain to a single frequency and are one of the governing factors of motility.[1] In addition, ICC act as intermediaries to neural innervation of gastric slow waves, and damages to either ICC and/or the enteric nerves are associated with a number of gastric disorders such as gastroparesis, chronic nausea and vomiting, and functional dyspepsia.[2]

Yusuf Ozgur Cakmak and Peng Du equal contribution.

Vagus nerve stimulation (VNS) has been utilized to treat a number of gastrointestinal (GI) dysfunctions by improving motility and reducing somatic pain sensitivity,[3-5] treatment of refractory gastroparesis,[6] and management for depression in epilepsy.[7] The vagus nerve is a major component of the parasympathetic nervous system (tenth cranial nerve) that innervates broadly in thoracic and abdominal viscera for regulation and sensation of viscera. The nucleus tractus solitarius (NTS) is the primary viscerosensory nucleus of the vagus, which gathers vagal afferent input modulates the activity of the dorsal motor nucleus of the vagus (DMV) to alter visceromotor (parasympathetic) output. The left vagal fibers predominantly innervate the anterior surface of the corpus and fundus of the stomach where the natural gastric pacemaker region is localized.[8,9] VNS includes two modalities: cervical and auricular stimulations, both of which have been applied using transcutaneous and cutaneous devices.[10,11]

The detailed mechanism of action of VNS on gastric slow waves is an ongoing area of research. While suppression of gastric slow-wave frequency has been shown in vivo with activation of the enteric inhibitory neurons via the vagus nerve,[12] vagotomy in humans does not appear to alter gastric slow-wave activity.[13] A number of recent studies have also demonstrated the involvement of the auriculo-vagal pathway in VNS.[14-16] Non-invasive electrostimulation of vagal auricular branch at lower frequencies, for example, 2 and 25 Hz, improved gastric transit time and ameliorated gastric dysfunction in rats.[4,16] In addition, electrostimulation of the vagal auricular branch at 100 Hz resulted in decreased gastric hypersensitivity in rats.[17] Finally, human studies have shown that taVNS at 10 and 80 Hz has opposite effects on autonomic nerve and sensory processing functions.[18-20] Based on these studies, we hypothesized that left taVNS at 10 and 80 Hz would also alter gastric slow-wave activity.

The main objective of this study was to gain a better understanding of the effects of the non-invasive high- and low-frequency stimulation of the left auricular vagal nerve on gastric slow-wave patterns with the aid of high-resolution (HR) mapping under in vivo conditions. In the context of the underlined auriculo-gastric neural networks, we applied transcutaneous high- and low-frequency stimulation of the left auricular vagus nerve protocols in conjunction with HR mapping in porcine subjects and assessed the changes in the spatiotemporal profiles of the gastric slow waves.

Key Points

- Auricular stimulation is known to improve gastric motility and reduce pain sensitivity. It is unclear how gastric slow waves, which govern motility, are altered in this process.
- High-resolution mapping on the serial surface and auricular stimulation are combined to show that the stimulation effectively altered the characteristic of slow waves depending the protocol and/or baseline condition.
- Chronic responses of gastric motility and slow waves in response to auricular stimulation should be investigated using non-invasive means in future.

2 | METHOD

2.1 | Animal preparation

Ethical approval was granted by the University of Auckland Animal Ethics Committee (Reference: 002021), and the International Guiding Principles for Biomedical Research Involving Animals were followed. The protocols of animal preparations and recordings were mostly based on previous established experimental protocols.[21] A total of nine healthy white cross-breed weaner pigs (female, weight 40.5 ± 4.4 kg) were housed individually and fasted overnight before the experiments to avoid compounding effects of ingestion. Each subject was infused with 6% Voluven saline (Fresenius Kabi Freeflex, USA) and general anesthesia with Zoletil (tiletamine HCL 50 mg/mL and zolazepam HCl 50 mg/mL) and isoflurane (2.5%-5%) with an oxygen flow of 400 mL within a closed-circuit anesthetic system.

Vital signs (blood pressure, heart rate, and temperature) were monitored continuously throughout the experiments. The femoral artery was cannulated in the right limb for blood pressure. A rectal thermometer was deployed to measure temperature. Endotracheal intubation was performed via using Normocap 200 Oxy to monitor

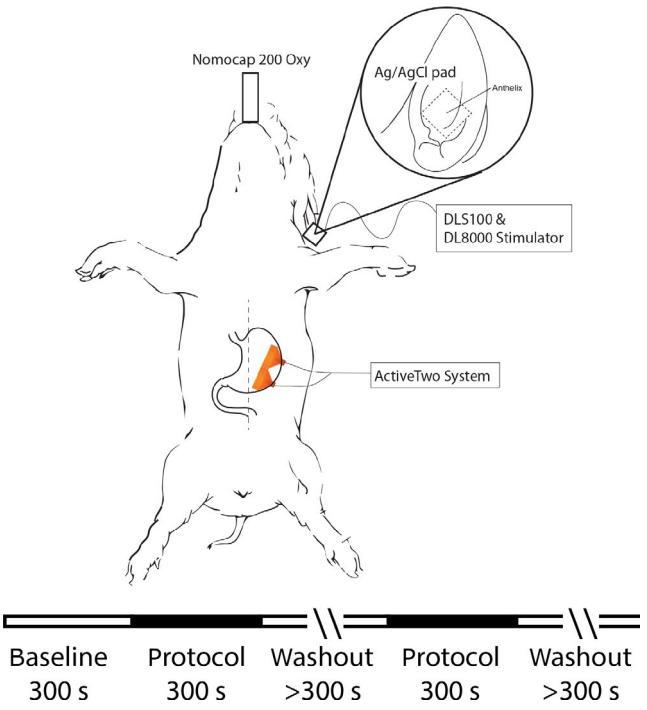

FIGURE 1 Experimental setup. A total of 192 FPC electrodes (32 × 6 array; 4 mm spacing) were placed directly against the anterior serosa of the stomach. Two conventional ECG electrodes were used as stimulation pads on the left ear. Auricular vagal stimulation was delivered in either as 10-Hz and 80-Hz protocols, with the order alternated between subjects

O_2, CO_2, and N_2O levels. The subjects were maintained in a normal physiological range during the experiments by a respiration pump (Harvard Apparatus, co, Inc) and a heating lamp (38.5-39.5°C).

A middle line laparotomy was performed to access the stomach of the subjects for HR mapping. 192-channel (32 × 6 electrodes; 4-mm spacing between electrodes; coverage 155 mm^2) high-resolution (HR) flexible printed circuit (FPC) electrodes (FlexiMap) were used to record gastric slow waves, mostly from the corpus and antrum regions of the anterior stomach, as shown in Figure 1. After placing the FPC electrodes, warmed (39°C) saline-soaked gauzes were placed on top of the electrodes to keep it in position. The incision was then sutured for the duration of the experiment. At the end of the experiments, the subjects were euthanized with a bolus injection of 50 mL of magnesium sulfate.

2.2 | Stimulation protocols

Two stimulating ECG electrodes (210 Series Foam, Kendall MediTrace) were placed oppositely onto the anthelix of the left ear. A WPI DL 8000 stimulator (WPI) with a DLS100 isolator (WPI) was used to deliver electric pulses with 10 mA, a pulse width of 10-80 ms, different frequencies (10 Hz or 80 Hz), and 10-minute duration if a change in the spatiotemporal profile was observed; otherwise, the protocol was allowed to continue for a further 10 minutes in each subject.

The order of the two different frequency of stimulation (10 and 80 Hz) protocols was alternated between each subject. The four pigs were stimulated with 10-Hz before 80-Hz stimulation while applying 80 Hz before 10-Hz stimulation protocols in the other five pigs. At least 30 minutes was allowed in between stimulus to allow recovery of baseline activity.

2.3 | Data acquisition and processing

The FPC electrodes were connected to an ActiveTwo System (Biosemi) via a 32-way ribbon cable for isolating external noises and amplifying the signals. The ActiveTwo System was in turn connected to a notebook via a fiber-optic cable. BioSemi software (written in LabView v8.2, National Instruments) was then used to monitor and record the amplified signals at a sampling frequency of 512 Hz.

The first 300 seconds of baseline, stimulation, and washout periods was taken from each protocol and analyzed in the Gastric Electrical Mapping Suite (GEMS v1.7, The University of Auckland, New Zealand) via MATLAB (R2018b, MathWorks).[22] The activation time of each slow wave was automatically marked and then grouped into discrete wavefronts (cycles), followed by manual review and correction. Regular antegrade propagation was classified as normal slow waves from the fundus to the antrum, as defined in a previous baseline study.[23] Spatial dysrhythmias were defined as directional deviations from the typical antegrade propagation. The average amplitudes, velocity, and time interval from the preceding cycle were quantified for every cycle of analyzed slow waves.

Statistical analyses were performed in one-way ANOVA to investigate the effects of 10-Hz and 80-Hz stimulations on the characteristics of slow waves, that is., interval, amplitude, and velocity, quantified by the mean, standard error of the mean (SEM), and *P*-value. A *P*-value of < .05 was considered to be statistically significant. A Tukey Kramer follow-up test was used to quantify the difference where statistical significance was found.

3 | RESULTS

Slow-wave recordings with adequate coverage for mapping propagation were obtained from all subjects prior to auricular neuromodulation. Serosal HR mapping exhibited regular slow wave from the anterior of the stomach, and the normal propagation pattern was comparable to previous HR mapping studies in porcine subjects (Figure 2).[21,23] A total of 117 cycles of slow wave, on average 14.6 ± 5.3 cycles per subject, were analyzed prior to neuromodulation, and a total of 423 cycles of slow wave, on average 12.44 ± 5.6 cycles per subject, were analyzed following to each neuromodulation protocols (12.2 ± 6.0 cycles per subject at the 10-Hz protocol and 12.7 ± 5.8 cycles per subject at the 80-Hz protocol).

The summary results and baseline and auricular neuromodulation are shown in Table 1. On average, 13 ± 12% of the baseline recordings demonstrated some level of spatial dysrhythmias, while similar levels of spatial dysrhythmias were observed during the 10-Hz and 80-Hz protocols at 11 ± 9% and 9 ± 9%, respectively (*P* > .05). On average, the first change of spatial propagation was observed 9.7 ± 6.6 minutes after the application of the 10-Hz protocol and 1.6 ± 1.0 minutes after the application of the 80-Hz protocol.

The statistical analysis of the baseline and neuromodulation data is shown in Table 2. The interval of baseline gastric slow waves was on average 17.4 ± 0.9 seconds, which was significantly lower than both the 10-Hz and 80-Hz protocols (21.7 ± 0.7 and 20.9 ± 0.7 seconds, respectively; *P* < .05). However, there was no difference in the interval between the two protocols. The amplitude of the slow waves in the 10-Hz and 80-Hz protocols (0.93 ± 0.02 and 0.91 ± 0.02 mV respectively) was lower than the baseline (1.00 ± 0.02 mV; *P*-value < .05). The 10-Hz protocol had no effect on the amplitude of the slow waves compared to the 80-Hz protocol (*P*-value ~ .69). This amplitude analysis is consistent with the interval analysis. There was no difference in the velocity between the baseline and the protocols.

When the baseline was distinguished between normal activity and those containing spontaneous spatiotemporal dysrhythmias. In general, the interval and velocities between the normal and abnormal baselines were different. Both the 10-Hz and 80-Hz protocols had significantly altered the interval of slow waves when the baseline was abnormal, but only the 80-Hz affected the interval of the normal baseline. Both protocols also had a significant impact on the amplitude of slow waves in both normal and abnormal baselines. Interestingly, the 10-Hz protocol changed the

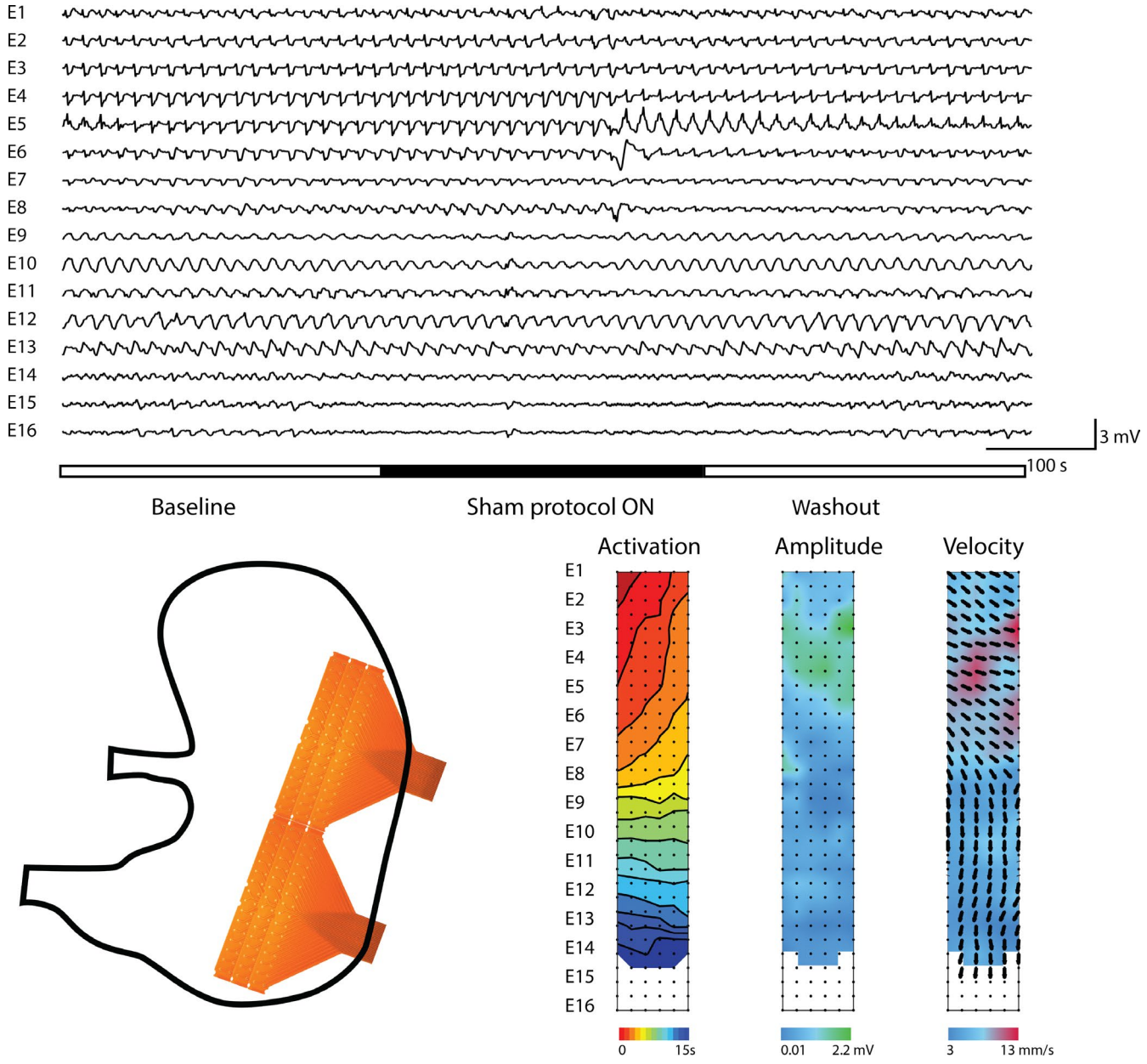

FIGURE 2 An example of stable baseline slow wave over a 900 s period, with a sham TaVNS protocol. All of the waves in this period propagated in the antegrade direction towards the pylorus as demonstrated by the activation map. The amplitude and velocity information are also shown

	Amplitude (mV)	Velocity (mm/s)	Interval (s)	Spatial Dysrhythmias %
Baseline	1.00 ± 0.02	6.12 ± 0.11	17.40 ± 0.91	13 ± 12
10 Hz	0.93 ± 0.02	6.09 ± 0.08	21.73 ± 0.67	11 ± 9
80 Hz	0.91 ± 0.02	6. 09 ± 0.08	20.91 ± 0.66	9 ± 9

Note: The protocol periods included the washout periods.

TABLE 1 Summary of gastric slow-wave amplitude, velocity, interval, and percentage of spatial dysrhythmias during baseline, 10-Hz, and 80-Hz protocols

velocity of slow waves of the normal baselines but not the abnormal baselines, while the 80-Hz protocol had the opposite effect.

The responses to stimulation protocols were heterogeneous. In over 55% (5/9) of the subjects tested, the protocols were able to induce a temporal change in slow-wave frequency, as demonstrated in Figure 3A, B. In the case of the 10-Hz protocol (Figure 3A), slow-wave interval became regular approximately 400 seconds following the onset of the stimulus and remained regular after the stimulus was turned off, so that the slow waves were completely restored from an abnormal baseline activity. The

TABLE 2 ANOVA results of amplitude, velocity, and time interval in gastric slow waves between baseline, 10-Hz, and 80-Hz taVNS protocols

	Group comparison		lower 95% CI for mean	Diff. In mean	Upper 95% CI for mean	*P*-value
Interval (s)	Normal	Abnormal	−5.87	−3.07	−0.28	.03
	Normal	10-Hz	−1.41	0.48	2.37	.62
	Normal	80-Hz	0.33	1.11	1.88	.006
	Abnormal	10-Hz	−15.51	−7.85	−0.18	.04
	Abnormal	80-Hz	−36.34	−24.26	−12.18	.0001
	10-Hz	80-Hz	−1.90	0.19	2.28	.98
Amplitude (mV)	Normal	Abnormal	−0.11	−0.02	0.08	.73
	Normal	10-Hz	0.08	0.14	0.19	2.14E-06
	Normal	80-Hz	0.44	0.55	0.65	1.06E-10
	Abnormal	10-Hz	0.04	0.12	0.21	.006
	Abnormal	80-Hz	−3.02	−2.32	−1.62	1.76E-10
	10-Hz	80-Hz	−0.03	0.02	0.07	.69
Velocity (mm/s)	Normal	Abnormal	0.88	1.24	1.60	6.47E-10
	Normal	10-Hz	−0.88	−0.63	−0.38	2.35E-06
	Normal	80-Hz	−0.02	0.22	0.46	.07
	Abnormal	10-Hz	−0.33	0.06	0.45	.76
	Abnormal	80-Hz	−1.19	−0.88	−0.56	4.71E-08
	10-Hz	80-Hz	−0.27	0.00	0.27	1.00

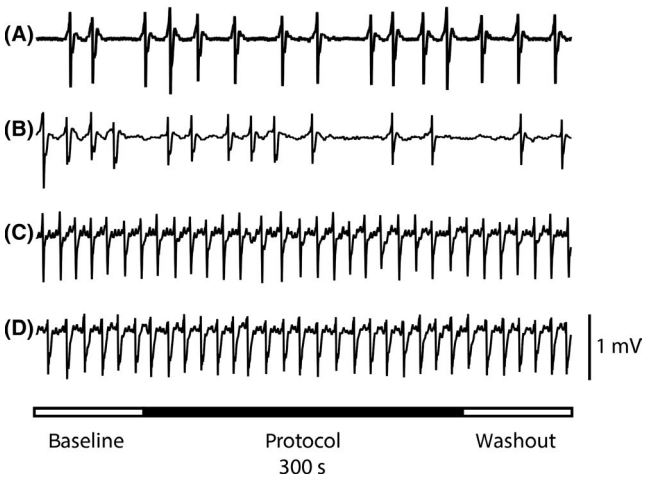

FIGURE 3 Examples of the effects of taVNS on gastric slow waves. (A) and (B) demonstrate examples where changes in the slow waves in response to 10-Hz and 80-Hz protocols, respectively, were observed. (C) and (D) demonstrate examples where neither protocols had an effect on the frequency of slow waves

normalizing effect of the 10 Hz was demonstrated in 44% (4/9) of the subjects or 90% (4/5) of the subjects that responded to the protocol. On the other hand, the 80-Hz protocol produced an inhibitory effect on the underlying slow waves (Figure 3B), where the regular events during the baseline were inhibited approximately 340 seconds following onset of the protocol. The inhibitory effect of the 80 Hz protocol was observed in 38% (3/9) of the subjects or 60% (3/5) of the subjects that responded to the protocol. Not all the subjects responded to the neuromodulation protocols however, as demonstrated by the two examples in Figure 3C (10 Hz) and D (80 Hz). In both cases, the neuromodulation protocols had no noticeable effect on the frequency of the slow waves (baseline vs protocol: 10 Hz, 3.1 ± 0.5 vs 3.0 ± 0.5, $P > .5$; 80 Hz: 3.5 ± 0.1 vs 3.5 ± 0.2, $P > .5$).

An example of the effect of 10-Hz protocol on spatial dysrhythmias is illustrated in Figure 4. In this particular subject, a persistent ectopic pacemaker had developed preceding the onset of the stimulus. The location of the ectopic pacemaker was in the mid-corpus of the stomach, which occurred at a bradygastric frequency of 0.8 ± 0.4 cpm. The focus of the ectopic pacemaker also exhibited higher amplitude (1.0 ± 0.4 mV) and velocity events (10.5 ± 1.6 mm/s) as a signature of gastric dysrhythmias.[21] The ectopic pacemaker persisted over 2 cycles (~138 seconds) following the onset of the 10-Hz protocol, after which the stomach was quiescent for up to 59 seconds. The first instance of antegrade propagation occurred at approximately 283 seconds following the onset of the stimulus. The high amplitude slow wave in the focus (around E10) was eliminated by the antegrade wave. Although the antegrade propagating activity was preserved, the interval of the slow waves was not stable after the stimulus was switched off. There was an immediate lengthening in the interval at the start of the washout period (wave 4), after which the intervals were more regular over six cycles of slow waves (waves 4-9; 33 ± 2 seconds), before another lengthening in interval occurred between waves 9 and 10.

An example of normal slow-wave response to the 80-Hz protocol is illustrated in Figure 5. The antegrade propagation was

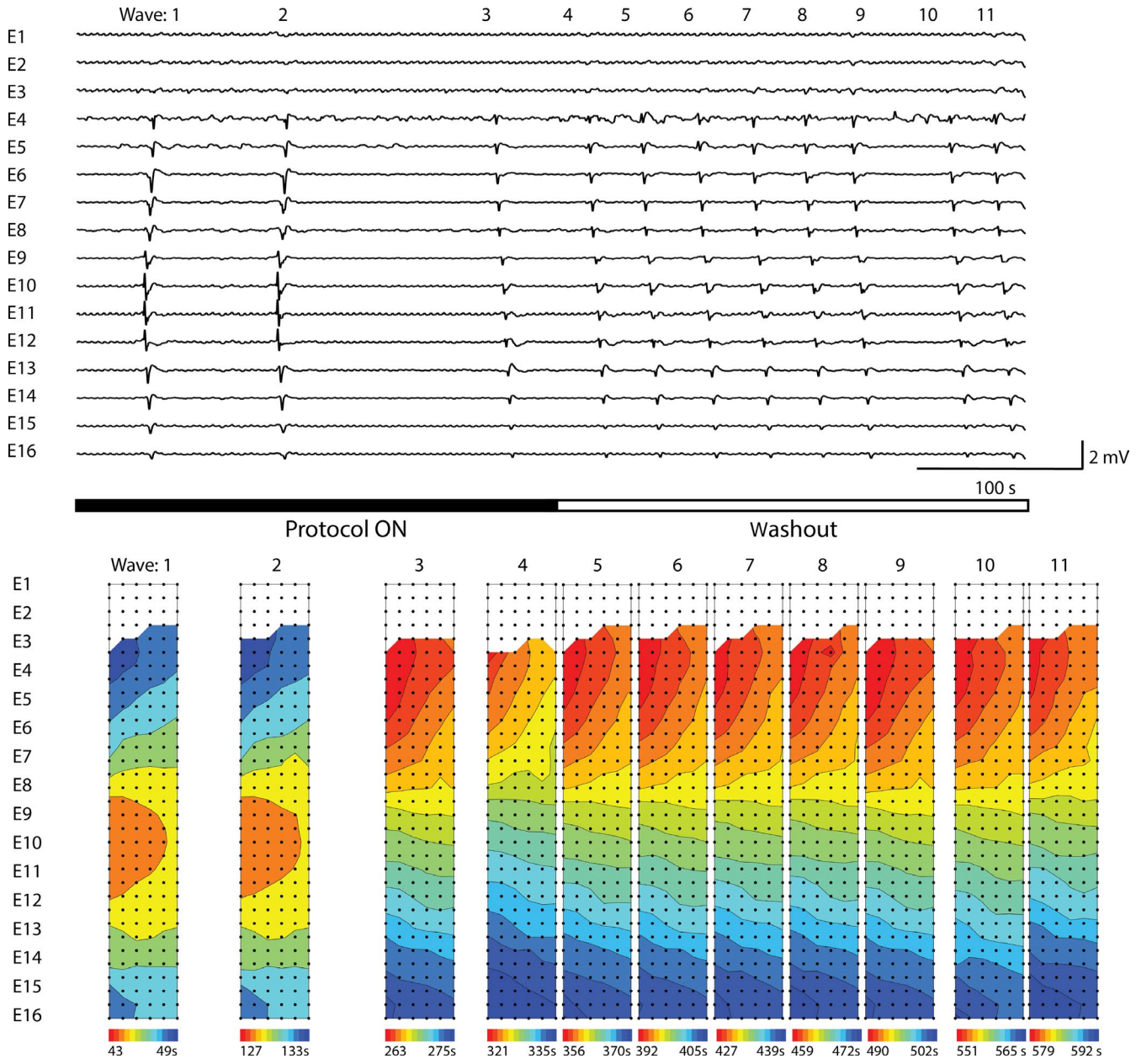

FIGURE 4 An example of normalization of gastric slow-wave propagation pattern by taVNS protocol at 10 Hz over 600 s of recording. The baseline immediately preceding the onset of the stimulus produced consistent ectopic pacemaking pattern in the corpus of the stomach at bradygastric frequency range. The direction of propagation was restored at the end of the on-period and persisted in the antegrade direction after the stimulus was turned off

captured over the entire length FPC array, which took on average of 18.8 ± 0.6 seconds over 124 mm. Only one cycle of antegrade activity occurred following the onset of the stimulus, after which the stomach remained quiescent for the duration of the on-period. Antegrade propagations returned 43 seconds after the stimulus was switched off and persisted throughout the rest of the recording session; however, the frequency/interval of the antegrade activities was irregular and bradygastric. The velocity of the waves during the washout period was comparable to the baseline values (6.7 ± 0.1 vs 6.9 ± 0.7 mm/s; $P \sim 0.52$).

4 | DISCUSSION

This study demonstrated the effects of the transcutaneous auricular vagus nerve stimulation (taVNS) on the characteristics of the gastric slow waves in the pig stomach, evaluated by applying high-resolution (HR) mapping from the serosa of the stomach. The effects of the two different stimulation protocols were compared to baseline and the washout periods following the stimulation. The main finding was that the characteristics of the slow waves changed significantly in the subjects that responded to the stimulation protocols but the effects were not consistently observed in all of the subjects studied.

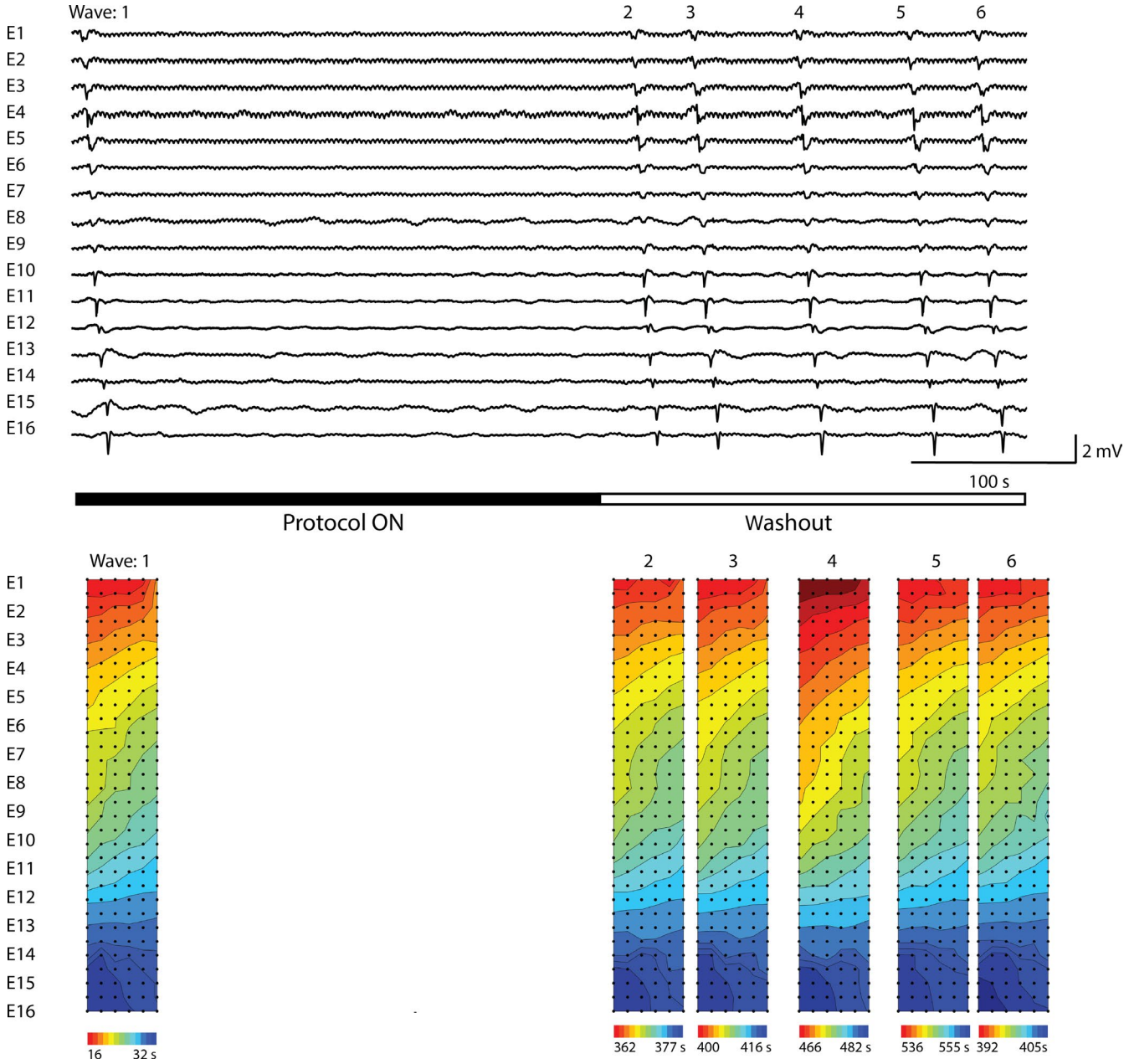

FIGURE 5 An example of inhibition of gastric slow wave by taVNS protocol at 80 Hz over 600 s of recording. The baseline activity in this case was propagating in the antegrade direction of the stomach consistently preceding the onset of the stimulus. Slow wave was inhibited following stimulation and reoccurred at irregular intervals after the stimulus was turned off. The direction of propagation did not change between the baseline, on-period, and washout period

To the best of our knowledge, this is the first time the effects of neuromodulation protocols have been demonstrated to alter gastric slow waves using HR mapping. The one previous HR mapping study gathered intraoperative data in patients with refractory gastroparesis undergoing gastric neuromodulation and found no effects on the incidences of dysrhythmias and/or other slow-wave characteristics during the stimulation on- and washout periods.[24] There are several differences that distinguish the present study from the previous HR mapping study on neuromodulation. Firstly, the previous study used direct stimulation on the serosa of the stomach, whereas the present study targeted the auricular branch of the vagus nerve, which is consistent with the standard vagus neuromodulation protocols.[3,17] Secondly, the present study used control subjects without known degradation in ICC and/or gastric dysfunctions, whereas the previous HR mapping study was conducted on a group that is known to exhibit significant nerve damage, which could have prevented the subjects from effectively responding to neuromodulation.[25] Thirdly, the present study implemented continuous stimulations during the on-periods with continuous stimulation parameters, whereas the previous HR mapping study relied a commercial stimulator device with variable amplitudes and pulse-width settings at each frequency, and more

importantly with relatively short on-off periods (0.1-4 seconds on; 1-17 seconds off over 290 seconds).[24] One of the critical findings from the present study was that response to stimulation generally required a sustained on-period (Figure 4) before any response could occur.

A number of previous investigations have demonstrated variations in slow-wave characteristics among species.[23,26,27] However, taVNS with similar protocols enhanced gastric motility in most species. In humans, a combination of the electrical (taVNS with 0.1-10 mA, pulse width of 250 μs, and frequency of 30 Hz) and physiological (deep slow breathing) improved antral contraction in the postprandial state.[3] In contrast, the frequency of antral contractions (4.9 ± 2.4 per 3 minutes) in patients with chronic pancreatitis could not be improved using a taNVS device compared to prior to a stimulation.[28] Blocking of the vagus using direct high-frequency stimulation of the anterior and posterior vagal trunks near the gastro-esophageal junction has also been proposed to promote satiety and weight loss.[29] In the hound, 2 seconds pulse trains of 25 Hz increased the percentage of regular slow waves (standardizing at 5 cpm) and gastric motility in the postprandial state.[30] In mice, a protocol of 1 mA and frequency of 2 Hz were conducted via the left conchae for 15 minutes and demonstrated improvements of gastric motility by 14% compared to the control group.[31] The present study demonstrates the potential mechanistic link between taVNS and changes in gastric slow waves, which is in agreement with the outcomes of the aforementioned studies.

Spontaneous slow-wave dysrhythmias were recorded in a number of baseline recordings. Four of the nine subjects demonstrated some levels of spontaneous spatial and/or temporal dysrhythmias, which was comparable to the rate reported previously in the same animal model (8/16).[32] It was notable that the response to taVNS was not consistent among subjects. There could be a number of contributing factors to the rate of response. The detailed mechanisms underpinning the occurrence of spontaneous gastric dysrhythmias are multi-factorial, which could be influenced by temperature, hormones, and/or mechanosensitivity.[33,34] Visceroception of gastric distention is also a significant process of communication via vagal mechanoceptors and higher centers. Gastro-vagal-NTS-insula axis has been documented for the visceroception neural network of the stomach.[35-37] Furthermore, the insular cortex is not only a passive gastric monitoring center but it also innervates the DMV.[38] This neural axis is also postulated as ongoing monitoring and regulation of gastric pacemaker activity.[39] It has been shown that gastric distention activates insula, which is the center of interoception, whereas the meal ingestion results with the deactivation of insula.[39,40] In this context, it can be postulated that the inflated or relatively deflated states of the stomach during the same stimulation parameters may explain the inconsistent results of the same stimulation parameters among subjects.

There are a number of limitations in the present study. At present, the relatively low presence of spontaneous spatial dysrhythmias during baseline meant that a fair comparison between response of normal and abnormal baselines to TaVNS could not be established. To address this issue, a reliable gastric dysrhythmic animal model would have to be utilized, either through direct electrical stimulation or drug-induced dysrhythmias.[41-43] Motility measurements were not performed in response to the stimulus protocols as gastric motility is not typically observed under anesthesia. It is likely that functional motility measurements will be a significant metric in conscious subjects, which can be explored in future studies. It should also be noted that general anesthesia agents such as nitrous oxide together with isoflurane and handling could reduce the frequency of slow waves,[44] as well as potentially containing anti-inflammatory properties and activations of vagal afferents. Given the relatively organized nature of the slow-wave wavefronts even during dysrhythmia, one potential solution is to apply a telemetric system for measuring low-channel slow-wave and motility measurements in conscious subjects, or alternatively with non-invasive means of measurements.[45,46] Direct stimulation and/or measurement from the vagal trunk near the stomach would also offer a more conclusive evidence of the activation/inhibition of vagal nerves. While the surgical access to obtain such recordings in the animals would be more traumatic, laparoscopic delivery of the recording/stimulation electrodes could be utilized to minimize the level of handling to the stomach. Finally, the subjects underwent overnight fasting prior to the studies, which could have impacted the response of the stomach compared to shorter period of fasting.[47]

In conclusion, the main finding of this study was that taVNS protocols had significant effects on the characteristics of gastric slow waves in control pig subjects, with varying responses depending on the state of the baseline activity. Chronic responses of gastric motility and slow waves in response to taVNS should be investigated using non-invasive means in future.

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DISCLOSURE

Authors PD and AG hold intellectual property in the field of gastrointestinal electrophysiology. PD is also a shareholder in FlexiMap Ltd. YC is a shareholder in Stoparkinson Healthcare Systems LLC.

AUTHOR'S CONTRIBUTION

AS and PD performed the experiments, analyzed the data, and wrote the paper; PD and YC designed the research study; YC also provided neurological perspectives to the paper; PK analyzed the data; and AG and PK edited the paper.

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