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Autonomic and neural responses to varying transcutaneous cervical electrical stimulation parameters

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Abstract

Background Transcutaneous cervical electrical stimulation (TCES) offers a noninvasive approach to modulate the autonomic nervous system (ANS), but optimal stimulation parameters remain undefined. This pilot study aimed to identify optimal TCES parameters by evaluating autonomic and neural responses across varying frequencies, current intensities, electrode montages, and durations, using heart rate variability (HRV) and electroencephalography (EEG) alpha-band power as biomarkers of parasympathetic activity.

Methods Twenty healthy adults completed four testing sessions, each examining one stimulation parameter. Autonomic data were collected including electrocardiography, non-invasive blood pressure, pupillometry, photoplethysmography, and dry-electrode EEG. Four frequencies (10, 25, 40, 150 Hz), three current intensities (sub-sensation threshold, sensation threshold, supra-sensation threshold), three electrode montages (bilateral, left-only, right-only), and two durations (4, 20 min) were tested. Root mean square of successive differences (RMSSD) and global EEG alpha-band power were primary outcomes. Parameters were sequentially optimized across visits based on individual RMSSD responses.

Results No single frequency produced a significantly higher RMSSD or alpha-band power response. However, each participant exhibited a personalized preferred frequency yielding a mean 41% RMSSD increase in visit 1. This individualized frequency was selected for further visits varying current intensity and electrode montage. Supra-sensation threshold intensity was most effective, with 60% of participants responding strongest at this level. Left-sided stimulation resulted in a decrease in both RMSSD and alpha-band power, while right-sided and bilateral montages resulted in similar increases for these biomarkers. Due to decreasing cardiac vagal response in successive sessions, the preferred frequency was reevaluated before testing duration. The mean RMSSD response increased 54% upon recalibration in visit 4, though the preferred frequency shifted in 75% of participants. Autonomic vitals did not significantly modulate more with longer stimulation duration; pulse rate variability during 20-min stimulation revealed oscillatory autonomic dynamics with peak parasympathetic responses emerging around 4 min.

Conclusions TCES can modulate cardiac vagal and cortical responses as measured by RMSSD and EEG alpha-band power, respectively, and a personalized, biomarker-guided approach to TCES parameter optimization is essential for future clinical applications targeting autonomic dysfunction.

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Trial registration This study was registered with Clinicaltrials.gov, identifier NCT04100486, on September 16, 2019.

Keywords Vagus nerve, Noninvasive, Cervical stimulation, Stimulation parameters, Stimulation frequency, Autonomic nervous system, Parasympathetic, Heart rate variability, Alpha band power, Personalized medicine

Background

The autonomic nervous system (ANS) is essential for maintaining and coordinating the physiology of various organ systems, including cardiovascular, pulmonary, gastrointestinal, and ocular systems, as well as the spleen (Berthoud and Neuhuber 2000). The vagus nerve (VN), a key component of the ANS, has branches extending through the neck and ear to the brain, where it modulates the locus coeruleus-norepinephrine system via the nucleus tractus solitarius pathway (Pavlov et al. 2018; Rodenkirch et al. 2022; Yuan & Silberstein 2016). A complex network of afferent and efferent vagal signals regulates homeostatic reflexes (Jayaprakash et al. 2023). This has led to the development of bioelectronic and neuromodulation therapies, utilizing both implanted and transcutaneous vagus nerve stimulation (VNS). VNS has demonstrated safety and efficacy in treating epilepsy (The Vagus Nerve Stimulation Study Group 1995), depression (Rush et al. 2000), and facilitating stroke recovery (Dawson et al. 2021). Furthermore, VNS has shown promise in managing heart failure and arrhythmias (De Ferrari et al., 2017; Huang et al., 2015; Nasi-Er et al. 2019; Stavrakis et al., 2015; Yamaguchi et al. 2018), pulmonary hypertension (D. Ntiloudi et al. 2019; Yoshida et al. 2018), tinnitus (Tyler et al. 2017), Alzheimer's disease (Merrill et al. 2006), rheumatoid arthritis (Koopman et al. 2016; Addorisio et al. 2019), Crohn's disease (Bonaz et al. 2016a, b), lupus (Aranow 2018), peripartum depression (Deligiannidis et al., 2022), and post-traumatic stress disorder (Schwartz et al. 2022; Debnath et al. 2026) and other conditions with inflammatory components (Guiraud et al. 2016).

There is an abundance of recent research that has prioritized noninvasive methods attempting to stimulate the VN. In particular, transcutaneous auricular VNS (taVNS) attempts to target the auricular branch of the VN, while noninvasive transcutaneous cervical VNS (tcVNS or ncVNS) attempts to stimulate afferent and efferent fibers of the cervical branch (Yuan & Silberstein 2016; Frangos and Komisaruk, 2017; Jigo et al. 2024). Although there is still no definitive evidence regarding the quantity or specificity of vagus nerve fibers activated by either of these noninvasive stimulation methods, studies using ncVNS have demonstrated anti-inflammatory effects (Lerman et al. 2016; Brock et al. 2017; Tarn et al. 2019) and potential for treating migraine (Chen et al. 2016) and post-traumatic stress disorder (PTSD) (Gurel et al. 2020; Bremner et al. 2020; Wittbrodt et al. 2020). However, the mechanisms of both noninvasive VNS methods and their impact on the ANS remain unclear, with inconsistent results observed depending on stimulation parameters and location (Burger et al. 2020; Yap et al. 2020; Debnath et al. 2021a).

Stimulation parameters in both invasive and noninvasive methods have varied in neuromodulation studies and clinical devices, and they likely have a prominent impact on efficacy. The most commonly discussed parameters include frequency, current intensity, electrode placement, and durations for both sessions and therapy periods. Other less-reported parameters include pulse shape, pulse width, and electrode type, size, or attachment method; indeed there is no standardized approach to determining how these factors that can impact administration of electrical stimulus. In taVNS studies alone, frequency has ranged from 1 to 30 Hz and currents of 0.1 to 50 mA, with most studies stimulating at intensities above a perceptual threshold at one or both ears (Thompson et al. 2021); these studies often utilized On/Off protocols, with stimulation on for 0.5 to 30 s and off for 30 to 270 s, with therapy durations up to 9 months (Vonck et al. 1999; Busch et al. 2013; Hein et al. 2013; Clancy et al. 2014; Kreuzer et al. 2014; Capone et al. 2015; Frangos et al. 2015; Hasan et al. 2015; Straube et al. 2015; Bauer et al. 2016; Fang et al. 2016; Rong et al. 2016; Yakunina et al. 2017; Tu et al. 2018). Current ncVNS devices on the market have pulse frequencies ranging from 20–30 Hz, intensities above a sensory threshold, and promote daily 2 to 4-min stimulations, usually on the left side of the neck only. There does not seem to be a consensus best set of parameters, nor a standard measurement to determine a personalized optimal set of parameters based on patient or disease. There may be a wide range of parameters that is able to induce physiological effects, but there needs to be a better way to target a patient's symptoms and autonomic dysfunction by measuring baseline and post-therapy ANS function.

ANS function depends on a balance between two subsystems, the sympathetic and parasympathetic nervous systems. Disruptions in this balance are implicated in various disorders, including neurological conditions like spinal cord injuries (Krassioukov et al. 2012) and stroke (Dütsch et al. 2007), systemic illnesses such as sepsis (Badke et al. 2018; Ferreira & Bissell 2018), rheumatoid arthritis (Koopman et al. 2016), Crohn's disease (Engel et al. 2015), and diabetes mellitus (Verrotti et al. 2014; Serhiyenko & Serhiyenko 2018), various heart conditions (Broadstone et al. 1991; Kishi 2012; Carthy et al., 2013; Vinik et al. 2013; Shen & Zipes 2014), and psychiatric

disorders such as PTSD (Agorastos et al. 2013; D'Andrea et al.; 2013; Schneider & Schwerdtfeger 2020). While monitoring ANS activity via implanted electrodes in cranial and peripheral nerves is possible (Barman & Yates 2017; Cracchiolo et al. 2019; Devor et al. 1994; Masi et al. 2019; Yoshimura et al. 1994; Zanos 2019; Zanos et al. 2018), noninvasive methods are often preferable clinically (Gibbons et al. 2014; Weimer 2010). Established noninvasive techniques assess ANS-dependent vitals through measures like heart rate (HR) and non-invasive blood pressure (NIBP), while calculated measures from electrocardiography (ECG), pupillometry, photoplethysmography (PPG), and electroencephalography (EEG) can provide further insights (Debnath et al. 2021b). A calculated measure of autonomic function from collected vitals is heart rate variability (HRV) derived from ECG, which quantifies inter-beat interval variations and can be associated with parasympathetic or sympathetic activity. In particular, root mean square of successive R-R interval differences (RMSSD) reflects beat-to-beat variability driven by vagal efferent activity, while standard deviation of N–N intervals (SDNN) can provide information about overall autonomic function (Shaffer & Ginsberg, 2017). These measures are reliable indices even in short intervals (Salahuddin et al. 2007; Baek et al. 2015).

Indices of parasympathetic activity can be used to quantify vagal tone, which describes strength and efficiency of communication between the brainstem and heart to regulate cardiac function and homeostasis (Porges 1995). Decreased vagal tone, measured by reduced HRV, is associated with impaired or delayed recovery of cardiovascular, endocrine, and immune markers in healthy participants (Weber et al. 2010), as well as a biomarker for stress and stress vulnerability (Porges 1995). Lowered or impaired vagal function has also been associated with increased fasting glucose (Thayer & Sternberg 2006), increased overnight cortisol (Thayer 2009), gastrointestinal dysfunction (Bonaz et al. 2016a, b), and increased inflammatory markers (Sloan et al. 2007; Pellissier et al. 2014). Meanwhile, interventions to increase HRV may improve individual health; increased vagal tone has been linked with treatment efficacy in major depression (Chambers & Allen 2002), improved emotional self-regulation (Goldstein et al. 2016), and better sleep quality (Werner et al. 2015). Because it reflects cardiac vagal modulation, is reliable in short and very short recording windows, RMSSD can be used as a target biomarker for interventions in this study focused on increased vagal tone and parasympathetic activity (Laborde et al. 2017; Thomas et al. 2019).

EEG studies show that cortical oscillations are affected by neuromodulation; transcranial alternating current stimulation (tACS) has been shown to modulate alpha oscillations reflecting task-dependent changes during arousal and attention (Kemmerer et al. 2022; De Koninck et al. 2023), while vagus nerve stimulation studies have reported alpha, delta, and gamma synchronization changes as predictors of responsiveness in epilepsy patients (Madhani et al. 2022). Brain–heart interactions can be captured by heart-evoked potentials (HEP) that show how cortical activity relates to individual heartbeats under autonomic control (Baranauskas et al. 2021), while cortico-cardiac dynamics have been characterized during sleep (Lyu et al. 2025). In particular, EEG alpha-band (8–13 Hz) activity is associated with parasympathetic tone; alpha power increases during parasympathetic-dominant states characterized by high vagal tone (Kawashima et al. 2024), while it is suppressed during sympathetic activation (Rho et al. 2023). Acute ncVNS has been shown to increase alpha wave brain activity, and these effects were not observed with taVNS intervention (Ahmed et al. 2026). While context-dependent and potentially driven by distributed network dynamics (Magosso et al. 2019), cortical alpha rhythms and HRV metrics can both be used to track autonomic state.

Due to the absence of definitive VN engagement from other noninvasive stimulation methods, the approach in this work is referred to as transcutaneous cervical electrical stimulation (TCES). This pilot study aims to identify optimal stimulation parameters for a novel TCES protocol by evaluating cardiac vagal modulation, hemodynamics, and neural responses across varying frequencies, intensities, electrode montages, and durations. The study device was configured to perform stimulation using custom settings to stepwise determine the most efficacious and personalized therapy based on recording noninvasive ANS-dependent vitals and evaluating increases in RMSSD and EEG alpha-band power, biomarkers for parasympathetic activity. Optimizing stimulation parameters to target the ANS is essential for effectively enhancing vagal tone in clinical applications, as well as understanding how stimulation settings can be adjusted to target specific vagus nerve fibers and autonomic organs.

Methods

Human participants

The study recruited 20 healthy, able-bodied individuals between the ages of 18 and 60. The mean age (± s.d.) was 31.2 (± 5.8) at the time of their first session, with 11 males and 9 females. Participants were asked to fast and refrain from caffeine for at least 4 h before the testing session. Exclusion criteria were history of neurological disease or psychiatric illness, cardiac arrhythmia, coronary artery disease, autoimmune or chronic inflammatory disease, anemia, malignancy, diabetes mellitus, renal disease, or any other chronic medical condition, current tobacco or nicotine use, pregnancy, and existing

implantable electronic devices. The study was approved by the Northwell Health Institutional Review Board, IRB #19–0461, and registered with Clinicaltrials.gov, identifier NCT04100486.

Autonomic testing stimulation and sessions

Testing sessions occurred in a laboratory setting without external noise or distraction. Lighting was set at an ambient level such that significant pupil change could be detected. Each participant attended four morning sessions lasting one to two hours (starting between 7 and 11 am), with at least one week between sessions. Each visit tested multiple options for a single stimulation parameter (Fig. 1A); every condition was tested in a 12-min stimulation block, with 4 min of baseline recording, 4 min of stimulation at a given condition, and 4 min of post-stimulation recording. Participants were still and silent, but awake, for the entire testing block. Visit 1 tested four

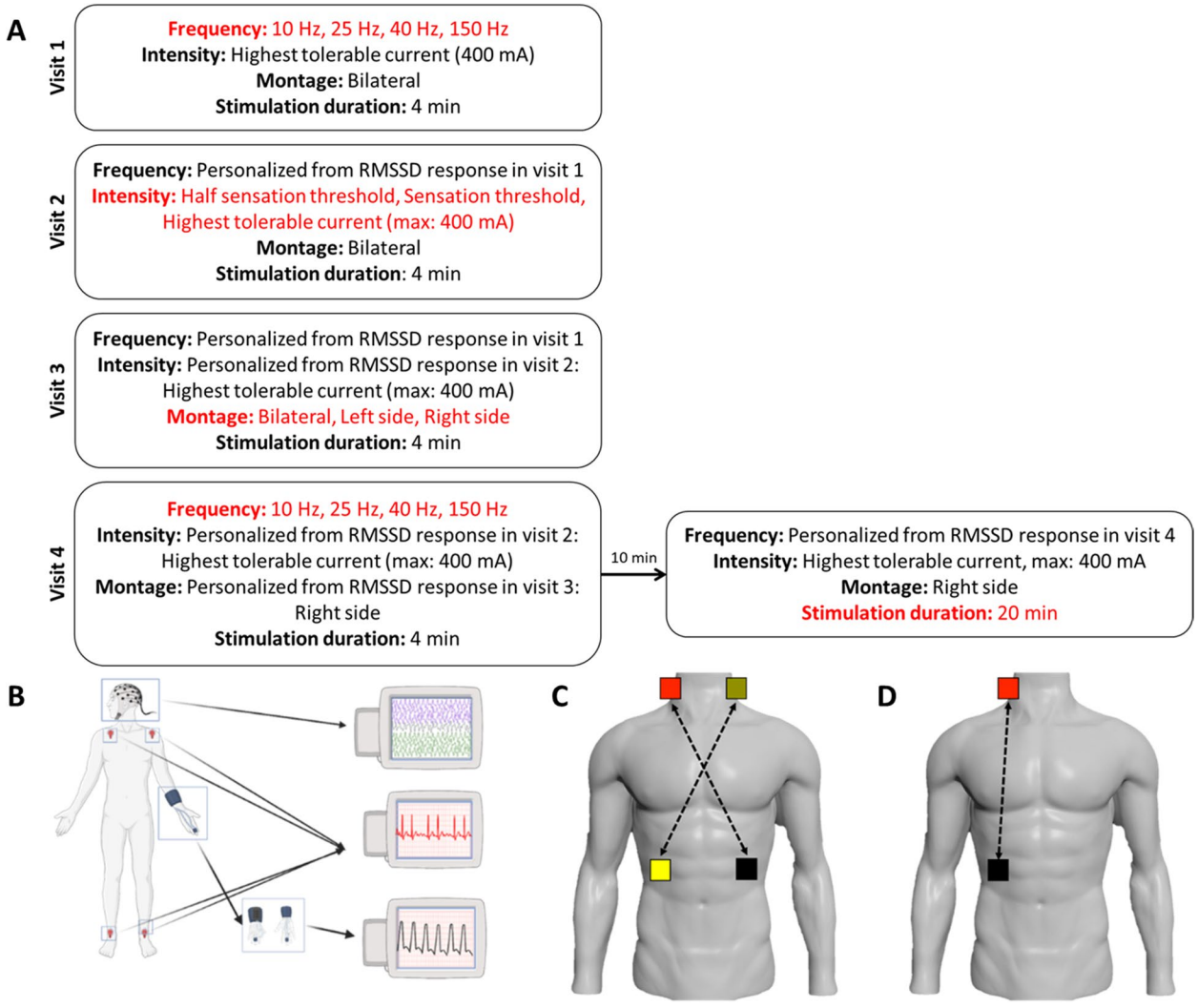

Fig. 1 **A** Descriptions of testing sessions. For each of four visits, one stimulation parameter was varied, shown in red, while all others remained constant. In subsequent visits, the next parameters were chosen based on the largest RMSSD responses in previous testing sessions. For each condition, a 12-min block consisted of baseline recording for 4 min, stimulation for 4 min, and a post-stimulation recording for 4 min. Based on the number of options for each parameter, participants went through 3 to 4 of these blocks per visit. In visit 4, frequency was varied like in visit 1; the frequency that resulted in the largest RMSSD response was then used to test stimulation duration in a 20-min stimulation block. **B** Physiological monitoring. Signals for each participant were recorded by a six-lead electrocardiogram (in red, wires attached to four foam adhesive electrodes placed at each shoulder and each ankle) and noninvasive blood pressure (in blue, small inflatable cuff on middle phalanx of middle finger). A dry electroencephalography system was placed on the participant's head, while eye tracking glasses were used to record pupil diameter and gaze location. **C** Bilateral stimulation montage. Adhesive electrodes were placed on each side of the neck, between the ear and jawline, and each side of the torso, below the ribcage on the midclavicular line. The return for each neck electrode was on the contralateral torso electrode. **D** One-sided stimulation montage. Adhesive electrodes were placed on one side of the neck, between the ear and jawline, and the return electrode was on the ipsilateral side of the torso, below the ribcage on the midclavicular line. Shown is the right side only

different frequencies, visit 2 tested three intensities based on perceptual threshold, visit 3 tested electrode montage and laterality (left only, right only, both sides), and visit 4 tested effects of duration.

Physiological recording

In each of the four testing sessions, participant autonomic data were continuously captured by noninvasive sensors (Fig. 1B) and transmitted through a data acquisition system (PowerLab 16/35, ADInstruments, Sydney, Australia) and software (LabChart, ADInstruments, Sydney, Australia), sampled at 1 kHz. Autonomic data recorded included six-lead ECG attached to four foam adhesive electrodes placed at each shoulder and ankle and continuous NIBP from a small inflatable cuff wrapped around the middle phalanx of the middle finger on the right hand. For visit 4 only, finger PPG was added. Additionally, eye tracking glasses (Tobii Pro Glasses 2, Tobii Pro AB, Stockholm, Sweden) were used to capture pupillometry (pupil diameter and gaze location), and EEG was measured by a dry electrode system (DSI-24, Wearable Sensing, San Diego, California, USA), sampled at 300 Hz; both devices have their own data acquisition software. All signals were marked and synchronized with experimental cues aligned with baseline and stimulation onset.

Stimulation

The TCES device used to stimulate was a handheld microcurrent machine (MEND Wellness, MEND Technology, Clackamas, Oregon, USA) with adhesive electrodes placed on each side of the neck, between the jawline and hairline just beneath the ear, and each side of the torso, below the ribcage on the midclavicular line (Fig. 1C). Current setpoint started at 0 μA and was increased in 10 μA increments, depending on the stimulation parameter condition.

For all visits, the conditions were randomized per participant (Fig. 1A). In visit 1, four frequencies were tested: 10, 25, 40, and 150 Hz. For each frequency tested, intensity was increased as high as possible while remaining tolerable and neither painful nor uncomfortable, with an upper limit of 400 μA. In visit 2, three intensities were tested: sensation threshold, 50% of sensation threshold, and a maximum intensity that was tolerable and neither painful nor uncomfortable. Intensity was increased from 0 μA until there was any sensation or perception at all; this value was used as the sensation threshold value. In visit 3, electrodes were placed on both sides of the neck (with return electrodes on the contralateral torso, Fig. 1C), left side of the neck only (with return on the left torso), or right side only (with return on the right torso, Fig. 1D). In visit 4, the frequency testing from visit 1 was repeated, and 1 frequency was selected based on the highest RMSSD change to be applied for a 20-min stimulation block, with similar 4-min baseline and post-stimulation periods before and after, respectively.

Signal processing

All signal processing, analysis, and statistical evaluation were performed using MATLAB (MathWorks, Natick, MA). A 60 Hz notch filter was applied to remove line noise from raw cardiovascular data signals.

Autonomic vitals. The average HR, mean arterial pressure (MAP), RMSSD, and SDNN were calculated in the baseline and post-stimulation periods. Effects were normalized for each feature per participant and per condition by dividing the post-stimulation values by the baseline mean. During stimulation, cardiovascular data signals were overwhelmed by stimulation artifact; because of this, in visit 4, PPG data was used to calculate continuous HRV during the stimulation period from a moving 1-min window.

Pupillometry. Pupil diameter data was calibrated based on gaze location; for all tests, participants were reminded to keep their eyes open and continue looking forward. Since illumination and perceived brightness can influence pupil size, measured pupil diameter was normalized to extract the autonomic response. Participants were not focused on any nearby objects, so pupil reactions due to accommodation were expected to be minimal and removed through averaging. The RGB values of the gaze location were converted to relative luminance values, and this was linearly fit to pupil diameter. By dividing by the slope of the line, the pupil sizes were normalized to account for effects of luminance.

Electroencephalography. Firstly, A1, X3, X2, X1, A2, and CM were excluded. Channels A1 and A2 are reference electrodes located at the left and right mastoid, respectively, while X3, X2, and X1 were not recorded from in the context of this work. CM is only for noise reduction during recording. All missing data points on other channels were filled using a 5-sampling mean window. The signals were high pass filtered at 1 Hz to remove slow drifts, and a notch filter was applied at 60 Hz using a Hamming window to eliminate electrical line noise. Large artifacts were removed using the clean_artifacts function from EEGLAB (Swartz Center for Computational Neuroscience, University of California-San Diego, La Jolla, CA, USA), with only the burst criterion removal option, setting a threshold cutoff of one standard deviation (1σ). Removed data was interpolated using artifact space reconstruction (ASR). Channels were then referenced to the average signal. Binary infomax independent component analysis (ICA) decomposition was used to isolate statistically outlying components of the signal that occur due to eye blinks, eye movements, or face and neck

muscle movements. Missing or bad channel components are interpolated using spherical spline interpolation.

Electrodes were divided into eight clusters, which included bilateral temporal, parietal, occipital, and frontal areas. Electrodes positioned at the skull midline were assigned to clusters on both sides. Power of the average signal for each cluster was calculated using Morlet wavelet decomposition for frequencies between 1 and 100 Hz. The logarithm of the mean percent power change was determined for each of these groups for the selected time interval for physiologically relevant EEG frequency bands, including delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz). Power changes were averaged across all clusters for a single global alpha power; while region-specific changes have been found resulting from non-invasive VNS studies (Konjusha et al. 2022), vagal indices from HRV have been directly correlated to spectral power in most electrodes across the brain (Rajan et al. 2024). Early analysis separating the eight defined clusters did not show any significant differences and measured effects were topographically uniform. Alpha power changes can lead to overall cortical power changes and suffices in providing a cortical index per condition and per participant (Colombo et al. 2023).

Null simulation for personalization gain. To ensure effects of selecting an optimal frequency were from real personalization rather than statistical artifact, a simulation was built to estimate the expected personalization gain under the null hypothesis that frequency has not effect on RMSSD. For each of visits 1 and 4, the null distribution was estimated by computing the observed mean and standard deviation of all response data. For 20 simulated participants, four values were drawn from this distribution and the maximum value was selected as the simulated optimal frequency response. The average of these 20 maxima was taken as the expected personalization gain if frequency had no effect at all. This simulation was repeated 10,000 times, and the RMSSD increases due to selecting an optimal frequency in the observed participant data was compared with the expected personalization gain distribution.

Results

Autonomic vitals, pupil size, and EEG signals were synchronously recorded for healthy participants during four stimulation sessions that included variations in frequency, current intensity, electrode montage, and duration. Stimulation parameter conditions were completed in a randomized order per session. Raw recordings for ECG and BP were used to calculate HR, RMSSD, SDNN, and MAP; EEG power was calculated for four frequency bands at eight spatially clustered electrode groups. Continuous pupillometry was tracked from baseline through stimulation and the post-stimulation periods; there was no significant difference between the responses in left and right eyes, so only left-sided pupil diameter (PD_L) was reported. All data were averaged during the baseline and post-stimulation periods then normalized by the baseline mean to determine effects due to stimulation and parameter tuning. No single frequency produced a statistically significant response in RMSSD or alpha-band power. A high, but tolerable, intensity and right-sided stimulation produced larger RMSSD responses; however, these selected parameters did not consistently outperform other conditions.

Frequency analysis

In visit 1, four frequencies were tested per participant: 10, 25, 40, and 150 Hz. Current intensity was increased as high as possible while remaining tolerable and comfortable, with an upper limit of 400 μA, and electrodes were placed in a bilateral montage. Fig. 2 (top row) shows the normalized post-stimulation autonomic responses for each frequency; there was no significant difference in any autonomic responses for any one frequency. Specifically, there were no significant effects of stimulation frequency on RMSSD (Fig. 2C), a target metric to evaluate cardiac vagal activity. However, each participant did have a personalized ranked RMSSD response (Fig. 3A), in that different individuals had varying increases in RMSSD response based upon frequency. This favored frequency occurred at random during the testing visit (Fig. 3B); it did not, for example, always occur in the first or last stimulation block. By leveraging this favored stimulation frequency for each individual, there was an average increase of 41% in RMSSD, compared to a 4.6% mean change for any single stimulation frequency used on all subjects (Fig. 3C). The personalized RMSSD increase exceeded the expected gain from selecting the maximum of four independent measurements from the distribution of all recorded responses (null expectation: $3.68\% \pm 0.1655$, $p = 0.0003$).

Neural responses to varying frequencies are also shown in Fig. 2 (bottom row). As with the autonomic responses, there were no significant responses in any EEG frequency band from any stimulation frequency. Focusing on alpha band power response as a parasympathetic index (Fig. 2H), there again was a preferred stimulation frequency per participant (Fig. 3D) that led to an average increase of 24%, compared to a maximum of 11% increase due to 40 Hz stimulation and less for all others (Fig. 3F). Personalizing the stimulation frequency based on alpha band power increase was not as effective as personalizing based on the RMSSD response.

Current intensity analysis

In visit 2, three stimulation intensities were tested per participant: sensation threshold, 50% of sensation

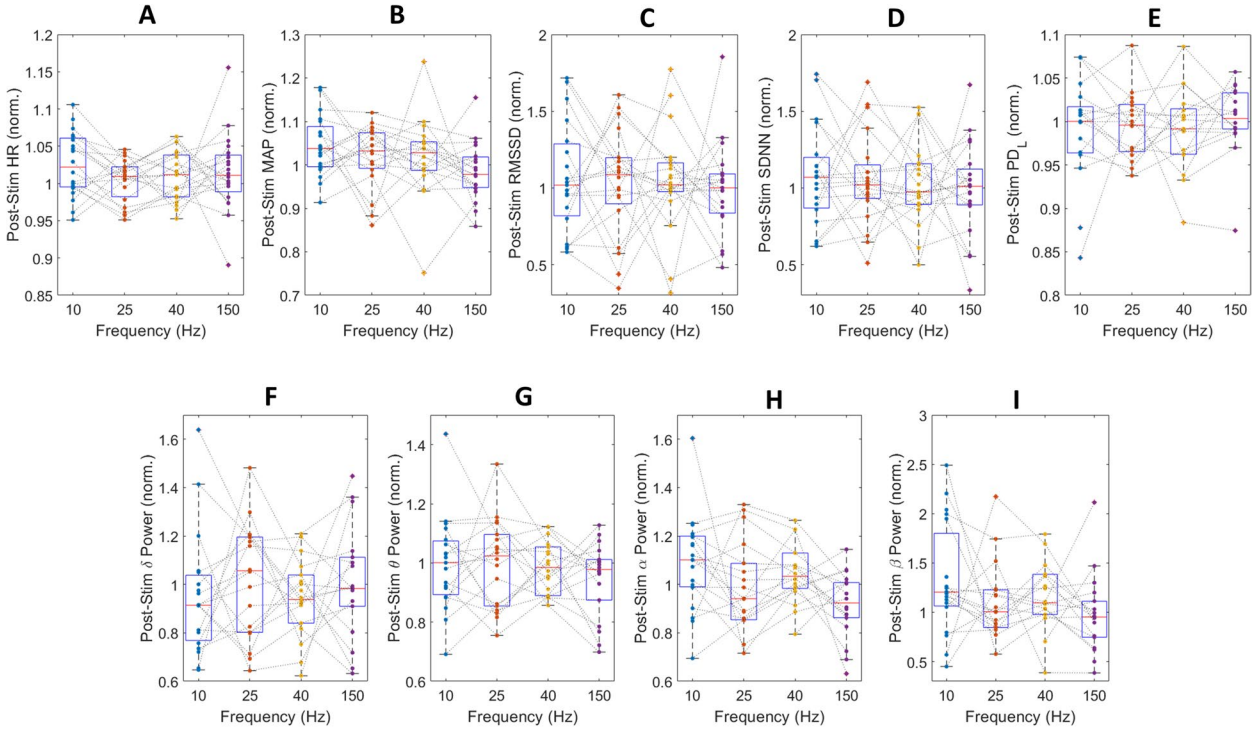

Fig. 2 Normalized post-stimulation responses to varying frequencies, visit 1. Boxplots show normalized post-stimulation responses for **A** HR, **B** MAP, **C** RMSSD, **D** SDNN, **E** PD_L, **F** delta, **G** theta, **H** alpha, and **I** beta band power. For each boxplot, the red horizontal line denotes the median, while the box is determined by the 25th to 75th percentiles. Whiskers extended to the maximum and minimum values that are less than 1.5 times the interquartile range away from the bottom or top of the box; outliers are beyond this limit. Individual responses are displayed by the colored points and connected by dotted lines

threshold, and a high intensity that remained tolerable and comfortable (same values as in visit 1). Each participant only received the frequency that generated the greatest RMSSD response in visit 1, while electrode remained in a bilateral configuration. In general, the high intensity stimulation in visit 2 closely matches the responses in visit 1 with matched stimulation parameters in both autonomic and neural features (Fig. 4). There was a much lower response, especially in RMSSD (Fig. 4C), at sensation threshold and 50% of sensation threshold (below any level of perception). In 12/20 participants (60%), the high intensity stimulation produced the largest RMSSD response (Fig. 5A), compared to 3 and 5 for sensation threshold and 50% of sensation threshold, respectively. In 10/20 participants (50%), the high intensity stimulation produced the largest alpha band power increase (Fig. 5D), compared to 4 and 6 for sensation threshold and 50% of the sensation threshold, respectively.

The RMSSD response (Fig. 4C) from visit 1 at a favored frequency per participant could be replicated, on average, in visit 2 by stimulating at a high intensity that remained tolerable and without pain. While sub- and at sensory threshold stimulation might represent more bearable intensities in a clinical population, to maintain an increased RMSSD, a majority of participants responded strongest to supra-sensation threshold. Similarly, the average alpha band power response (Fig. 4H) was similar between visit 1 and visit 2 with matched stimulation parameters. There was more variability in the visit 2 measures. However, it can be noted that the mean visit 2 RMSSD and alpha band power responses were lower than that from visit 1, even at the highest stimulation intensities. Among other EEG frequency bands, all visit 2 responses, regardless of stimulation intensity, were higher in delta and lower in beta compared to visit 1.

Laterality and electrode montage analysis

In visit 3, three electrode montages were tested: bilateral, left side only, and right side only. In the montage applying bilateral stimulation, the return was placed across the body on the other side of the neck electrode. In the left and right side only montages, the return was placed on the torso on the same side as the neck. In this visit, each participant received high intensity stimulation, based on visit 2 results, at the frequency that generated the highest RMSSD response in visit 1. Figures 6 shows the normalized post-stimulation autonomic and neural responses for each montage, preceded by the matched parameter responses in visits 1 and 2 (matched

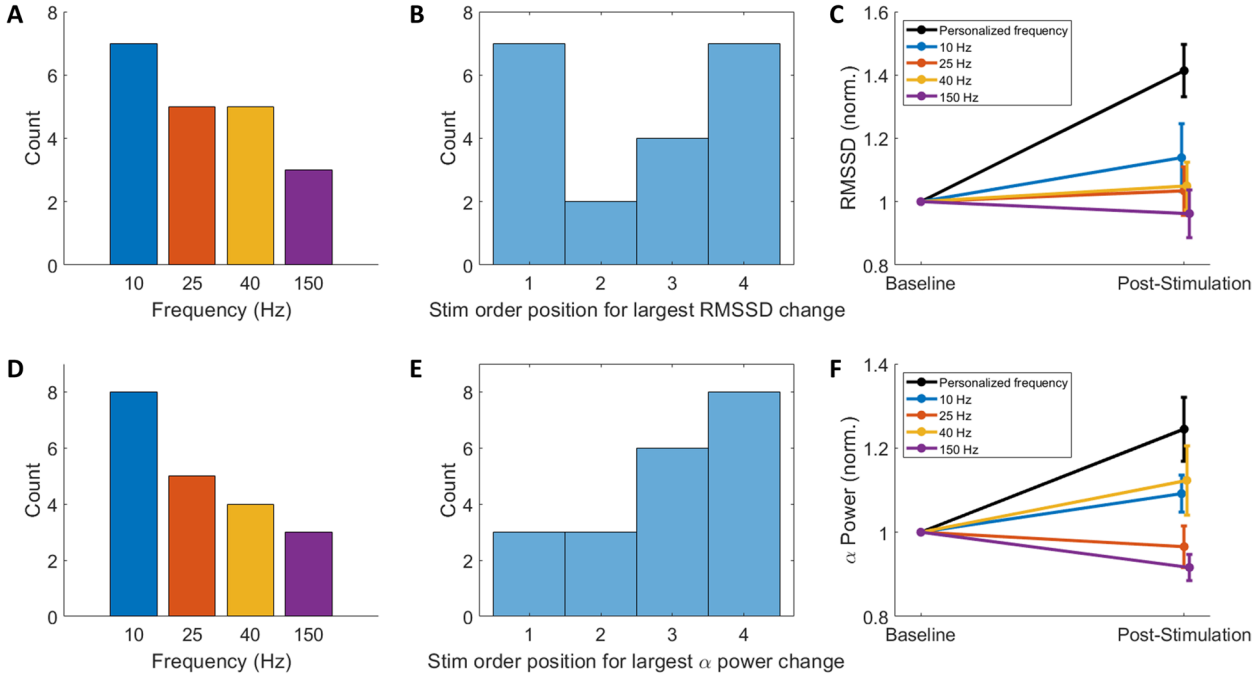

Fig. 3 Personalizing stimulation frequency, visit 1. **A** Histogram of preferred frequencies based on the largest RMSSD increase. **B** This favored frequency occurred at random during the testing visit, though it most often was the first or last stimulation block. **C** Shown are the average RMSSD responses at each frequency (colored lines) compared to the average RMSSD response to the favored frequency for each individual (black line). **D** Histogram of preferred frequencies based on largest alpha band power increase. **E** This favored frequency occurred at random during the testing visit, though it most often was the third or last stimulation block. **F** Shown are the average alpha band power responses at each frequency (colored lines) compared to the average alpha band power response to the favored frequency for each individual (black line)

by favored frequency, high intensity, and bilateral stimulation). Looking at only visit 3 montage-related RMSSD responses, there was slight increased average RMSSD from bilateral stimulation and a larger RMSSD response from right-sided stimulation (Fig. 6C). Although, left cervical VNS is commonly used in clinical applications for targeted autonomic effects and to reduce potential cardiac risk, left only stimulation by this montage decreased RMSSD responses compared to baseline. In 11/20 participants (55%), right-sided stimulation produced the largest RMSSD response, compared to 6 and 3 from bilateral and left-sided stimulation, respectively (Fig. 7A). Similarly, left only stimulation did not affect the average alpha band power response, despite the increased variability (Fig. 6H). Both bilateral and right-sided stimulation increased alpha band power, with the largest responses observed in 7 (35%) and 9 (45%) of participants, respectively (Fig. 7D). However, it can be noted that the visit 3 responses were lower than that of visit 1 and 2 when matching all previous stimulation parameters. Even though higher stimulation intensity and bilateral/right-sided stimulation produce higher RMSSD and alpha band power responses than other parameter options, there is a fading response when frequency is kept constant over time. This leads to the possibility that the favored frequency to modulate the parasympathetic nervous system is not durable and may need to be recalibrated over time.

Re-personalizing stimulation frequency

Despite finding the most effective options for stimulation intensity and electrode montage, there was a decreased RMSSD response while frequency was kept constant in visits 2 and 3. In visit 4, the frequency testing was repeated using high intensity and right-side only stimulation in a 4-min block; after RMSSD calculations were performed during the participant's visit, the favored frequency was selected and used in a 20-min stimulation block. In the initial 4-min block, autonomic responses can be compared for the favored frequency with visit 1 results (Fig. 8, top row), and while again, there was no significant difference between frequencies nor the number of participants for each personalized frequency, the average RMSSD response in visit 4 was higher than visit 1 (Fig. 8C). Other autonomic data remained stable. As with visit 1, this favored frequency occurred at random during the testing visit (Fig. 9B); it did not, for example, always occur in the first or last stimulation block, and at a different distribution than visit 1. The average RMSSD increase with favored frequency of 54%, compared to a 4.8% mean change for any single stimulation frequency

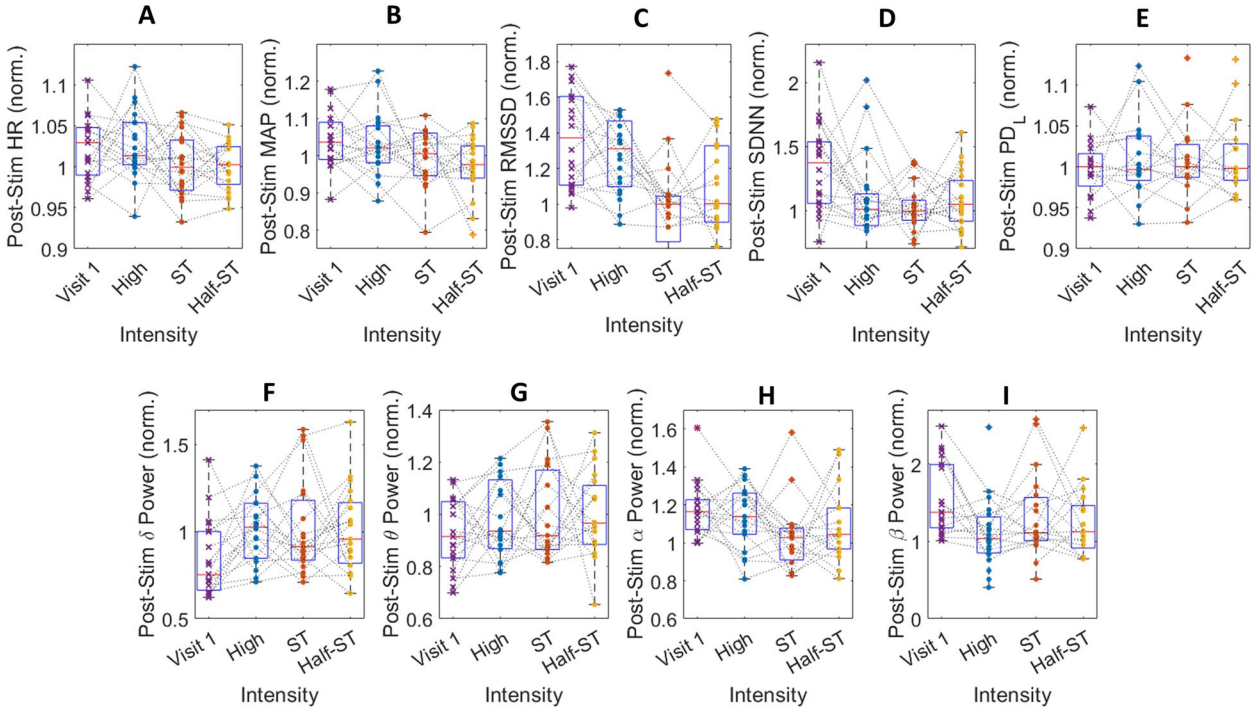

Fig. 4 Normalized post-stimulation responses to varying intensities, visit 2. Boxplots show normalized post-stimulation responses for **A** HR, **B** MAP, **C** RMSSD, **D** SDNN, **E** PD_L, **F** delta, **G** theta, **H** alpha, and **I** beta band power. For each boxplot, the red horizontal line denotes the median, while the box is determined by the 25th to 75th percentiles. Whiskers extended to the maximum and minimum values that are less than 1.5 times the interquartile range away from the bottom or top of the box; outliers are beyond this limit. Individual responses are displayed by the colored points and connected by dotted lines

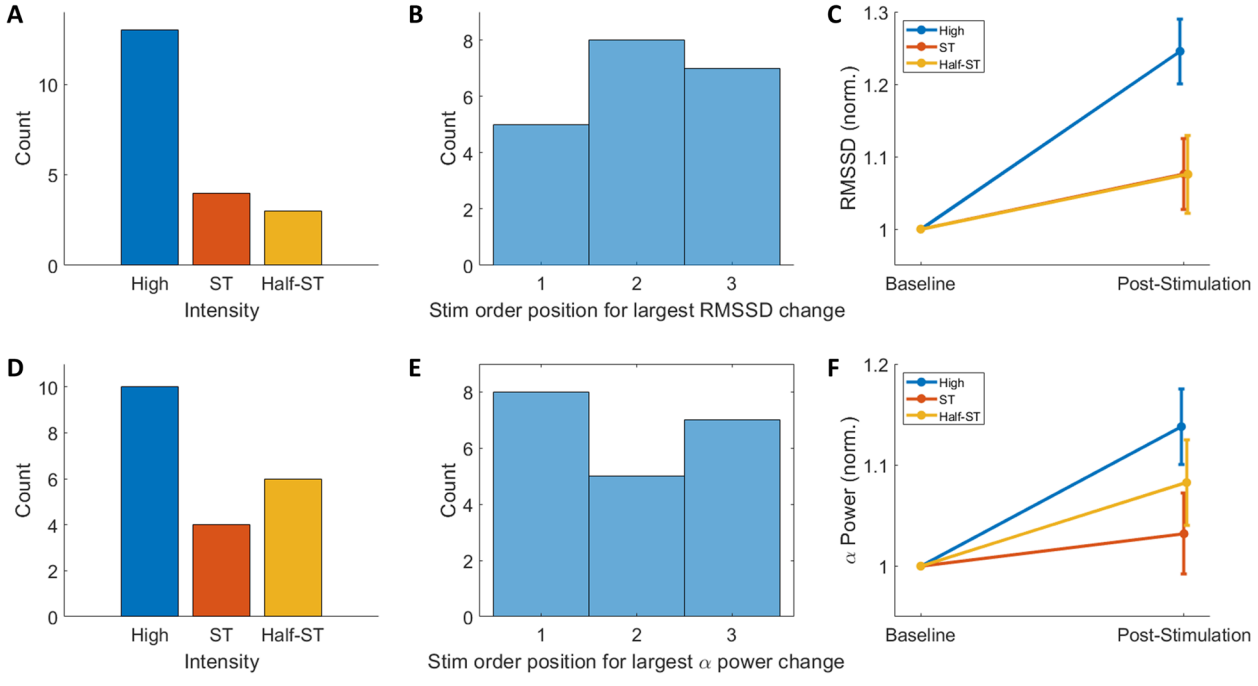

Fig. 5 Comparing stimulation intensity. **A** Histogram of stimulation intensities with the largest RMSSD increase. **B** This RMSSD response occurred at random during the testing visit. **C** Shown are the average RMSSD responses at each intensity (colored lines) at each individual's favored frequency. **D** Histogram of stimulation intensities with the largest alpha band power increase. **E** This alpha band power response occurred at random during the testing visit. **F** Shown are the average alpha band power responses at each intensity (colored lines) at each individual's favored frequency

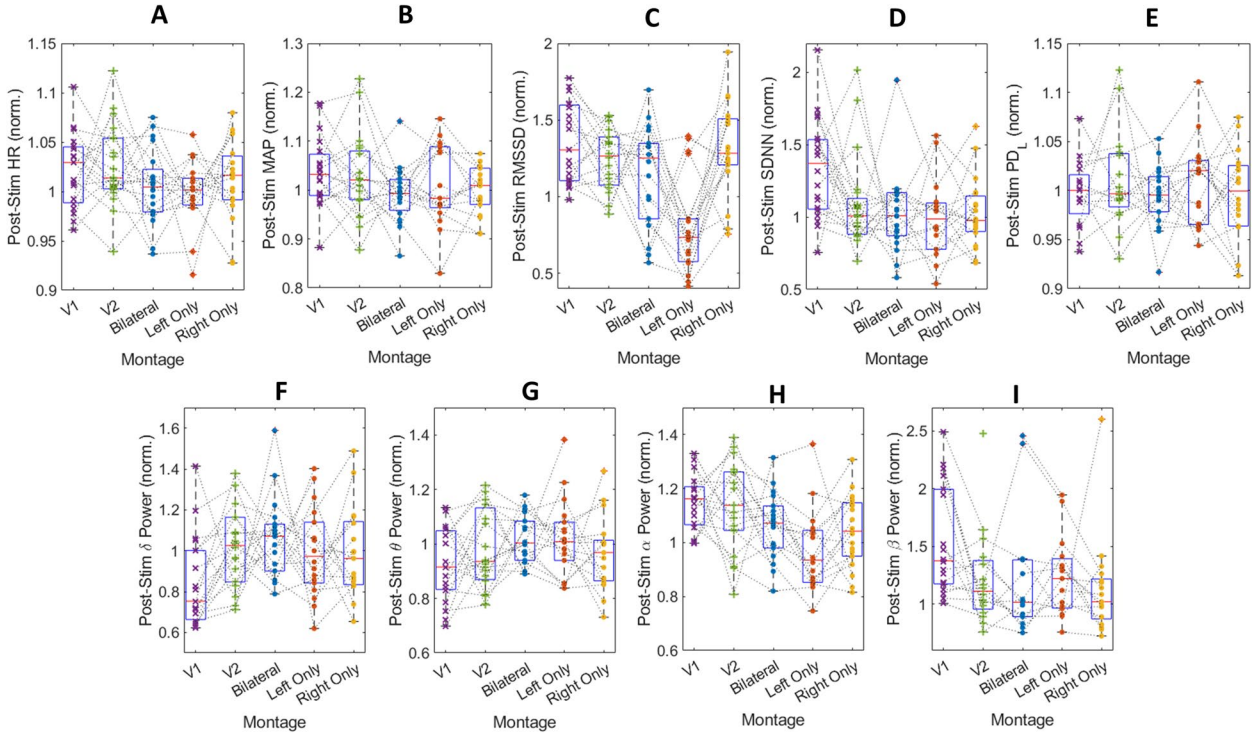

Fig. 6 Normalized post-stimulation responses to varying montages, visit 3. Boxplots show normalized post-stimulation responses for **A** HR, **B** MAP, **C** RMSSD, **D** SDNN, **E** PD_L, **F** delta, **G** theta, **H** alpha, and **I** beta band power. Visit 1 (V1) and Visit 2 (V2) have matched parameters with Visit 3's Bilateral stimulation. For each boxplot, the red horizontal line denotes the median, while the box is determined by the 25th to 75th percentiles. Whiskers extended to the maximum and minimum values that are less than 1.5 times the interquartile range away from the bottom or top of the box; outliers are beyond this limit. Individual responses are displayed by the colored points and connected by dotted lines

used on all subjects (Fig. 9C). The personalized RMSSD increase exceeded the expected gain from selecting the maximum of four independent measurements from the distribution of all recorded responses (null expectation: 4.79% ± 0.2735, $p = 0.036$). Neural responses were also compared for the favorite frequency with visit 1 results (Fig. 8, bottom row); the average delta, theta, and alpha band power responses were higher in visit 4, with a lowered power response in the beta band. Table 1 shows the favored frequencies for each participant measured in visits 1 and 4, which was different in 75% of participants; agreement between visits 1 and 4 is denoted by green values. Over both visit 1 and visit 4, totaling 40 visits, the most responsive stimulation frequency was the same for both RMSSD and alpha band power in 25% of visits, denoted by asterisks (*) in Table 1.

Duration analysis

The effects of stimulation duration on autonomic vitals and neural responses are shown in Fig. 10, showing the normalized post-stimulation responses for duration in visit 4. Both durations had other stimulation parameters constant, with the favored frequency chosen in visit 4, high current intensity, and right-sided only stimulation. In general, the 4-min stimulation modulated post-stimulation vitals more; with 20-min stimulation, all vitals increased with lower magnitude, while median HR decreased. Among EEG power bands, delta, theta, and beta band power effects remained steady, while there was a drop in alpha band power (Fig. 10G) for the longer stimulation duration. However, these data, for both autonomic and neural signals, do not represent dynamics during the stimulation period.

In visit 4, finger PPG was added to the recorded vitals; while ECG is overwhelmed by stimulation artifact, the PPG signal remained intact. These detected blood volume changes can be used to extract heart rate and, therefore, calculate a substitute heart rate variability measure, pulse rate variability (PRV), which is a PPG-derived RMSSD. To ensure that this information was valid, RR-intervals were calculated from both simultaneous PPG and ECG in non-stimulation periods and compared (Fig. 11A). Overall, the RR-intervals match well ($p < 0.005$), with a correlation coefficient of 0.7439 and a line of best fit of $y = 1.0639x - 0.0539$. There is less variability in the PRV measures, as the signal can be filtered more effectively with additional electrical noise.

Using a rolling 1-min window, a continuous PRV can be calculated for the 20-min stimulation block to observe dynamic changes in the parasympathetic nervous system.

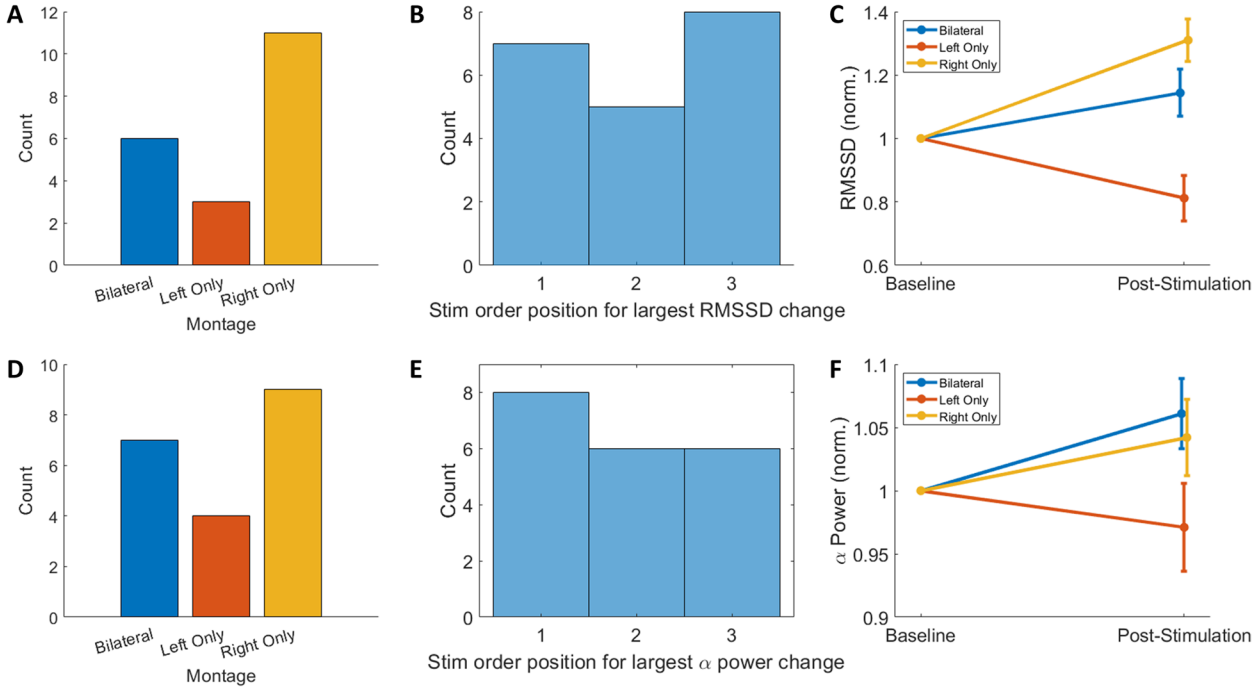

Fig. 7 Comparing electrode montages. **A** Histogram of montage laterality with the largest RMSSD increase. **B** This RMSSD response occurred at random during the testing visit. **C** Shown are the average RMSSD responses at each montage (colored lines) at each individual's favored frequency and high stimulation intensity. **D** Histogram of montage laterality with the largest alpha band power increase. **E** This alpha band power response occurred at random during the testing visit. **F** Shown are the average alpha band power responses at each montage (colored lines) at each individual's favored frequency and high stimulation intensity

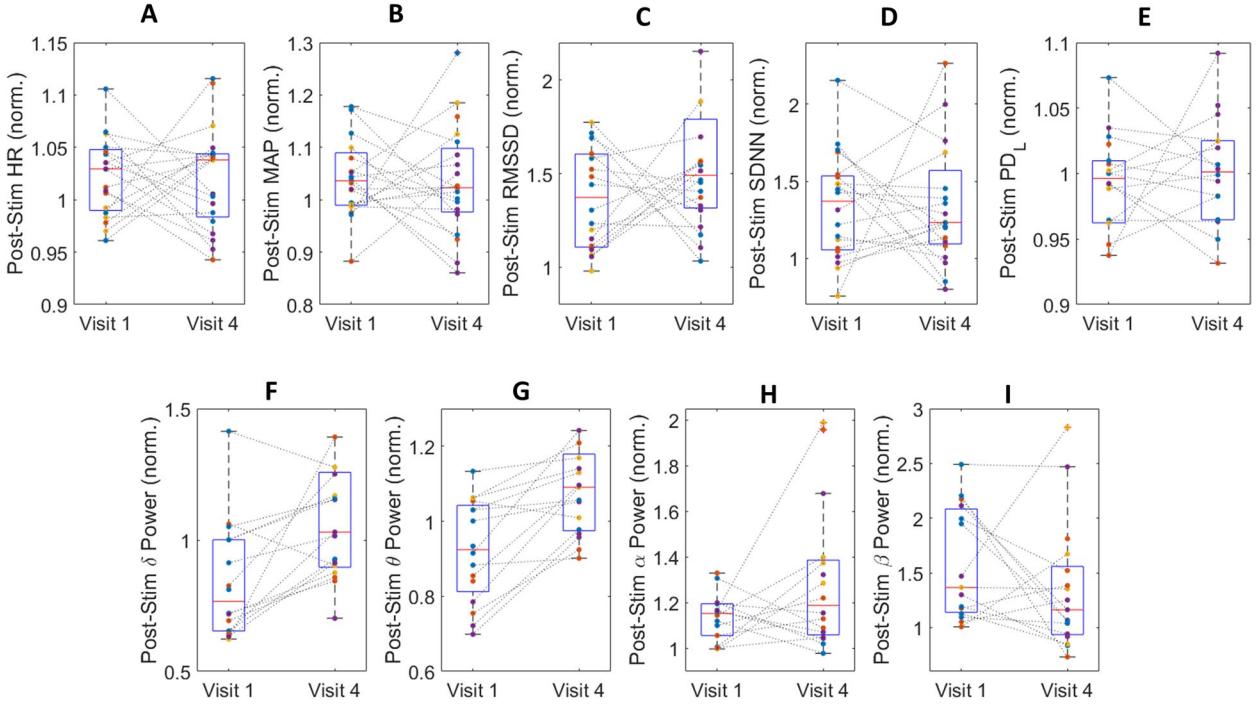

Fig. 8 Normalized post-stimulation responses to varying frequencies, comparing visits 1 and 4. Boxplots show normalized post-stimulation responses for **A** HR, **B** MAP, **C** RMSSD, **D** SDNN, **E** PD_L, **F** delta, **G** theta, **H** alpha, and **I** beta band power. For each boxplot, the red horizontal line denotes the median, while the box is determined by the 25th to 75th percentiles. Whiskers extended to the maximum and minimum values that are less than 1.5 times the interquartile range away from the bottom or top of the box; outliers are beyond this limit. Individual responses are displayed by the colored points and connected by dotted lines

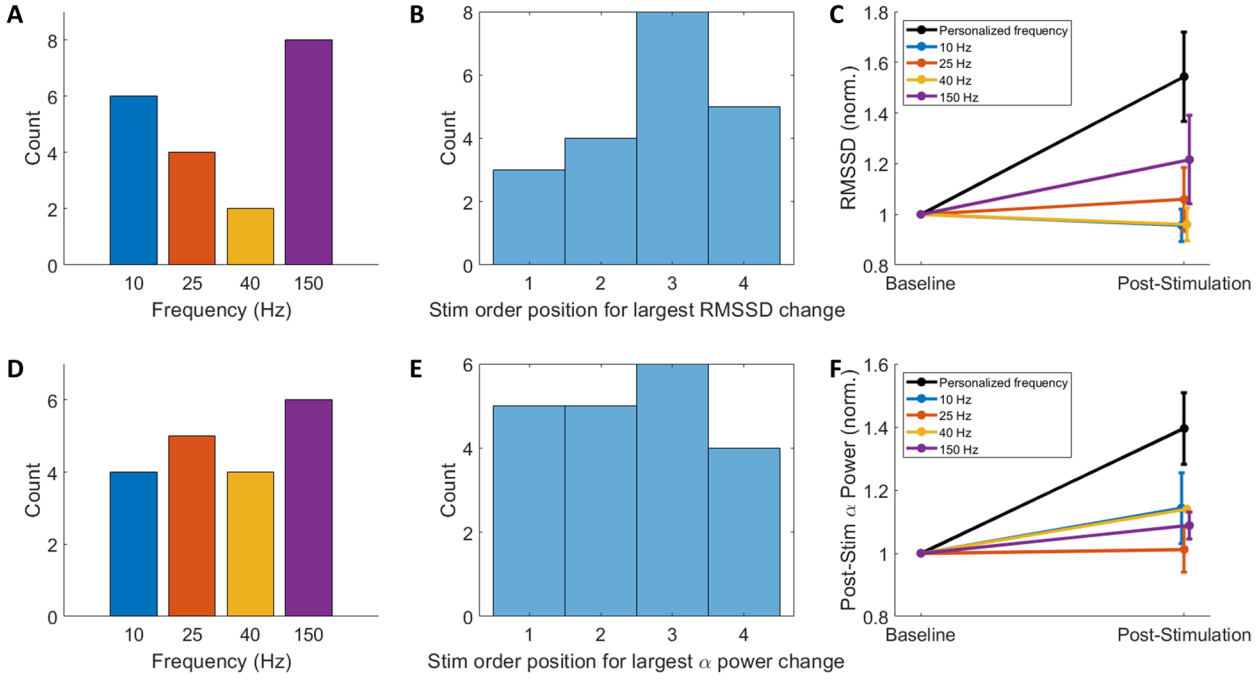

Fig. 9 Personalizing stimulation frequency, visit 4. **A** Histogram of preferred frequencies based on the largest RMSSD increase. **B** This favored frequency occurred at random during the testing visit, though it most often was the third stimulation block. **C** Shown are the average RMSSD responses at each frequency (colored lines) compared to the average RMSSD response to the favored frequency for each individual (black line), with high stimulation intensity and right-sided stimulation. **D** Histogram of preferred frequencies based on largest alpha band power increase. **E** This favored frequency occurred at random during the testing visit, though it most often was the third or last stimulation block. **F** Shown are the average alpha band power responses at each frequency (colored lines) compared to the average alpha band power response to the favored frequency for each individual (black line), with high stimulation intensity and right-sided stimulation

The average trendline, with each participant's favored frequency, is shown by the black line in Fig. 11B. These trends can also be separated by stimulation frequency to observe any dynamics specific to this parameter. Overall, the response oscillates approximately every 2 min, especially in the first 8 min. There is a peak in PRV response around 4 min, with a larger favored frequency response at around 11 min before returning to baseline.

Discussion

This work shows the effect of varying stimulation parameters of a novel TCES approach on autonomic and brain function. Non-invasive surrogate measures of autonomic nervous system activity were collected, including ECG, NIBP, pupillometry, and electroencephalography (EEG). Derived metrics included HR, RMSSD, SDNN, PRV, MAP, corrected pupil diameter, and global mean EEG power across five frequency bands. Four frequencies, three current intensities, three electrode montages, and two durations were administered over four testing sessions, resulting in observed changes in cardiovascular and pupillary activity, parasympathetic tone, and brain activity.

In most previously published studies that have attempted to noninvasively stimulate the VN, either via the auricular branch (taVNS) or cervical level (tcVNS or ncVNS), there is no certainty of specific VN engagement nor to what extent (Yap et al. 2020; Verma et al. 2021; Yang et al. 2026). In this work as well, there remains no confirmation of VN engagement, and, as mentioned earlier, TCES refers to the stimulation protocol used in this study. Due to similarities in location, parameters, and purpose, these results can be compared with results from other noninvasive stimulation studies referring to targeting the VN.

Stimulating the parasympathetic nervous system offers several potential clinical applications. By driving reductions in heart rate, dilation of blood vessels, increased gastrointestinal motility, and lowered stress, arousal, and reactivity, increased parasympathetic tone holds promise for managing various conditions. Furthermore, because the vagus nerve regulates immune function and inhibits pro-inflammatory cytokines, modulating the inflammatory reflex could be valuable in treating inflammation-related disorders, including autoimmune, metabolic, cardiovascular, and neurological conditions. To this end, increased RMSSD and alpha band power were goal measures in determining the optimal stimulation parameters.

No single frequency had a significantly better average response in the RMSSD calculation. Most clinical trials

Table 1 Frequencies that elicited the largest RMSSD (left) and alpha-band power responses (right) at visit 1 and visit 4. Green numbers denote agreement between visit 1 and visit 4, while asterisk (*) denote agreement between autonomic and neural preference

	Frequency based on RMSSD response		Frequency based on alpha-band power response	
Participant	Visit 1	Visit 4	Visit 1	Visit 4
1	40*	25	**40***	**40**
2	10*	150*	10*	150*
3	25*	150	25*	40
4	10	150	150	10
5	**10**	**10**	**25**	**25**
6	25	40	10	25
7	**25**	**25**	10	40
8	**10***	**10**	10*	150
9	**10**	**10**	150	40
10	10*	150*	10*	150*
11	**150**	**150***	10	150*
12	40	25	25	10
13	25	10	40	25
14	10	150*	25	150*
15	40	10*	**10**	**10***
16	40	10	**150**	**150**
17	40	150	10	25
18	150	40	**25**	**25**
19	150	25	40	10
20	25	150*	40	150*

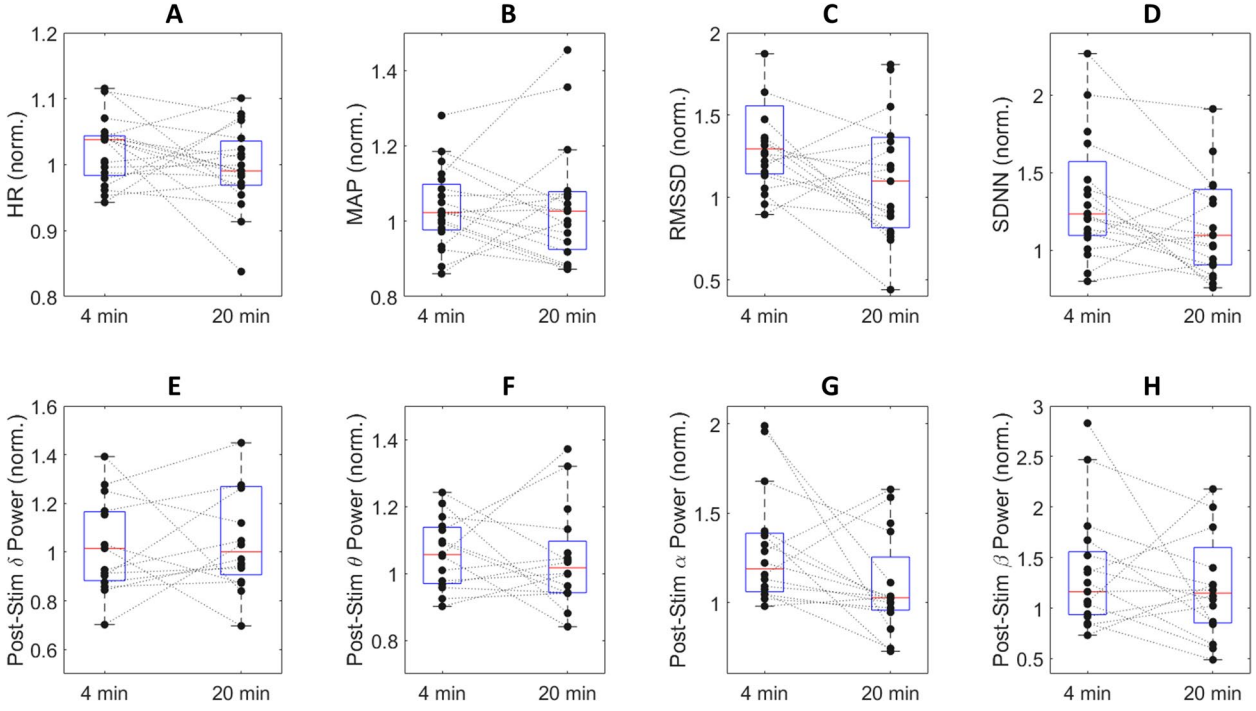

Fig. 10 Normalized post-stimulation responses to varying duration, visit 4. Boxplots show normalized post-stimulation responses for **A** HR, **B** MAP, **C** RMSSD, **D** SDNN, **E** delta, **F** theta, **G** alpha, and **H** beta band power. For each boxplot, the red horizontal line denotes the median, while the box is determined by the 25th to 75th percentiles. Whiskers extended to the maximum and minimum values that are less than 1.5 times the interquartile range away from the bottom or top of the box; outliers are beyond this limit. Individual responses are displayed by the colored points and connected by dotted lines

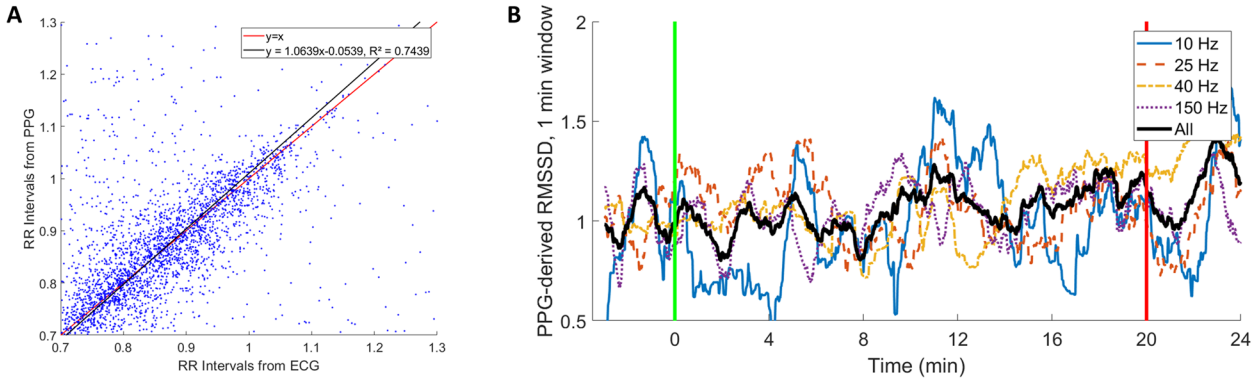

Fig. 11 Validity of PPG-derived RR-intervals and PRV response to stimulation. **A** Shown is a scatter plot of all extracted RR interval values from PPG vs. those extracted from ECG in non-stimulation periods, along with a linear regression trendline (black, y = 1.0639x-0.0539, $R.^2 = 0.7439$). The unity line (y = x) is shown in red. **B** Using RR intervals extracted from PPG, RMSSD as a pulse rate variability measure can be calculated during the 20-min stimulation period. The average over all participants is shown in black, while other colors denote the average of all participants with the same preferred frequency, determined by the largest RMSSD (from RR intervals extracted from ECG) from the 4-min stimulation in visit 4

applying noninvasive VNS, either targeting auricular or cervical branches, use frequencies within 20 to 30 Hz (Thompson et al. 2021) based on early research that tested a range of parameters in dogs and rodents (Lockard et al. 1990; Zabara 1992), often at current amplitudes over 1 mA (compared to a max value of 400 μA in this study). These parameters were tested to decrease seizure frequency (George et al. 1995; Handforth et al. 1998; Ghani et al. 2015) or mood changes (Elger et al. 2000; Mu et al. 2004). In this study, neither 25 Hz nor 40 Hz (close to previously established ranges) modulated a parasympathetic response with more efficacy than others; DeGiorgio et al. (2001) also reported no statistically significant association between stimulation frequency and treatment effect. Based on results from visit 1, each participant had a favored frequency that modulated their RMSSD the most; half of participants did prefer 25 or 40 Hz. However, matching frequency and other stimulation parameters (high intensity, bilateral) through visits 2 and 3 resulted in a decreased RMSSD response over time, from 41 to 31% to 14%. While this habituation or regression could be interpreted as a confound, this observation motivates the need to ongoing parameter recalibration in any clinical protocol. For visit 4, with electrode montage reduced to only the right side, the favored frequency was recalibrated and increased average RMSSD by 54%. This finding is consistent with previous research, as Ylikoski et al. (2020) reported an average 53.2% increase in RMSSD lasting up to 60 min following taVNS, while Constantinescu et al. (2019) observed increased RMSSD during tcVNS in a patient with epilepsy. In future studies, it may be crucial to check the personalized stimulation frequency intermittently.

Supra-sensation threshold stimulation had the most efficacious response in RMSSD. In particular, the stimulation amplitude was increased as high as possible while remaining tolerable and without pain; 60% of patients tolerated the stimulation intensity at the ceiling value of 400 μA at their favored frequency. This result matches those in many noninvasive VNS studies that apply supra-sensation threshold intensities (Thompson et al. 2021). The autonomic effects were tested at sub-sensation threshold and sensation threshold to aid with patient comfort and device battery life in future studies. Sub-sensation threshold intensities, often paired with high frequency stimulation, have shown some improvement in major depressive disorders, but larger sham-controlled studies are necessary to confirm the findings (Deligiannidis et al., 2022).

Contrary to clinical practice and previous work, RMSSD showed a modulated response nearly equally to bilateral and right-sided stimulation in this study; there was a mean increase of 9% for right-sided TCES, compared to 14% increase for bilateral stimulation and a 19% decrease for left only stimulation. Historically, left-sided invasively implanted VNS is standard for conditions like epilepsy to avoid any cardiac complications that might occur from electrical stimulation of the right side (Sharma et al. 2025). Indeed, the right VN travels near the sinoatrial node and could induce bradyarrhythmia with stimulation (Olshansky et al. 2008; Hammer et al. 2015; Ottaviani et al. 2022). However, recent studies suggest that right-sided VNS may be a viable, safe, and effective approach (Navas et al. 2010; Brougher et al. 2021). In this study, right-sided TCES almost matched that of bilateral stimulation in increasing RMSSD; for efficiency, future studies focusing on driving parasympathetic activity could apply right-sided TCES only, while clinical applications that need to reduce parasympathetic activity could apply left-sided TCES only. The relationship between stimulation laterality and autonomic output may be more context-dependent that previously studied and could be

crucial insight for both non-invasive and implanted ANS stimulation approaches.

Effects of stimulation duration are mixed. After stimulation frequency calibration in 4-min stimulation blocks during visit 4, a 20-min stimulation block was applied. However, all autonomic vitals did not modulate as much or more in the normalized post-stimulation values. In visit 1 to 3, HRV during stimulation could not be investigated further due to stimulation artifact. In visit 4, a finger PPG sensor was added, providing a clean, continuous blood flow signal that could be used to extract heartbeat timestamps, and therefore R-R intervals and a surrogate for HRV. Using PRV, the dynamics of the parasympathetic nervous system could be tracked during the 20-min stimulation block. There was an autonomic balancing effect that pulsed approximately every 2 min, but some peaks around 4 min; there was an additional peak after 10 min and a slightly higher baseline after this. These oscillations could be related to parasympathetic withdrawal and reactivation cycle that have been observed in very low frequency (VLF) HRV analysis when attempting to increase vagal outflow (Tripathi, 2011; Michael et al. 2017). VNS in most other studies was not continuously delivered but described as an ON/OFF protocol to prevent uncomfortable prolonged symptoms during stimulation, like voice alterations or cough. Other commercial devices (Truvaga Plus, Truvaga, Rockaway, New Jersey, USA) suggest stimulation periods as low as 2 min per session, but this could be repeated up to 24 sessions per day. The PRV results indicate that up to 4 min of stimulation may be enough to promote an RMSSD increase, and further stimulation may have a plateauing or decreasing effect.

TCES parameter effects on RMSSD were mirrored in EEG alpha band power, in that no single frequency was more efficacious than others, high intensity stimulation was overall most effective, and bilateral and right-sided stimulation generated largest increases. This is consistent with another pilot study, in which alpha activity correlated positively with RMSSD in a refractory epilepsy cohort (Melo et al. 2022); there was also higher HEP amplitudes in the epilepsy group, supporting brain–heart coupling. This suggests coordinated brain-autonomic modulation, as has been observed during cognitive tasks and pain experiences (Alba et al. 2019; Pascalis & Vecchio 2022). Transcranial direct current stimulation (tDCS) has been reported to reduce heart rate and increase HRV during fear exposure, as well as influence long-term parasympathetic dominance (Cobb et al. 2025), while preliminary transcranial magnetic stimulation (TMS) studies have modulated heart-brain coupling indices related to interbeat interval and TMS cycle times (Dijkstra et al. 2023); there remains inconsistent results and heterogeneity in tDCS, TMS, and other neuromodulation protocols measuring HRV (Miron et al. 2021; Souza et al., 2026). Noninvasive VNS studies have shown significant differences in neuro-cardiac coupling indices calculated using delta-band power and heartbeat length (Keute et al. 2021), along with parasympathetic HRV indices, alpha-band power, and delta-band power (Kang et al. 2024). Studies with invasive VNS show more mixed results across all EEG frequency bands; successful therapy from VNS might be an effect of repetitive desynchronization effects over time to promote neuroplasticity in the brain (Biggio et al. 2009; Hays et al., 2013; Olsen et al. 2022; Ahmed et al. 2026). In this study, many stimulation parameters were varied in acute sessions, and long-term EEG analysis may be required to find correlations with TCES clinical benefits.

Each participant had a preferred frequency that modulated alpha band power most, but this response decreased in subsequent visits, from a mean increase of 24% to 14% to 9%, with all other stimulation parameters matched. With stimulation frequency re-personalized in visit 4, the mean increase based on preferred stimulation frequency to modulate alpha band power was 39.5%, indicating recalibration necessary for productive cardiac vagal index modulation. Increases in alpha band power have been observed from 20-min auricular VNS in healthy individuals, with larger increases from right-sided and bilateral protocols (Konakoglu et al., 2023), as well as more pronounced alpha wave activity in acute bilateral cervical VNS compared to unilateral auricular VNS (Ahmed et al. 2026). In general, these responses may demonstrate a more relaxed and parasympathetic-driven state during cervical VNS; relaxation has been associated with increased alpha power and parasympathetic HRV (Kawashima et al. 2024). While alpha-band activity can track vagal HRV markers, association is clearly context dependent and stronger in resting conditions.

Autonomic dynamics during stimulation could not be studied here, as stimulation artifact was rampant in EEG recordings during therapy, similar to that of ECG. Additionally, band power was averaged over the entire brain, giving information about overall brain states, but these results are less precise about how cortical areas are related to VN activity and stimulation. Reporting region-specific alpha responses, or other frequency bands, across all parameter condition would have inflated multiple comparisons beyond the limits of this pilot study. Nevertheless, alpha activity is the strongest electrophysiological signal from the human brain since it inhibits other task-irrelevant regions and is crucial in understanding functional brain architecture related to downstream stimulation and therapy (Jensen et al., 2010). In any future task or context-dependent TCES study, distinct functional measures should be included in a regionally resolved analysis of EEG data.

This study focused on a small and healthy cohort between only the ages of 22 and 41, providing a baseline that reflects autonomic changes in response to varying stimulation parameters. Significant responses were not always measured in this limited sample, and there was high inter-individual variability with no single parameter set effecting the RMSSD response universally. While calculating RMSSD, measurement variability is unlikely, as R-peak detection in clean ECG is well-established. While individuals respond differently in cardiac vagal output to stimulation, the inter-individual variability could be interpreted as a biological response due to metabolic or hemodynamic features (Pikkujämsä et al. 2001; Nunan et al. 2010). However, for the alpha-band power measurements, EEG measurement noise due to contact impedance or muscle artifacts could contribute to some high variance in responses recorded in this study (Lopez-Gordo et al. 2014). There may be more observable changes in a future, larger study utilizing specific patient populations with diagnosed dysautonomia. This will include steps to determining the gap between stimulation frequency recalibration to ensure efficacious therapy with clinical improvements. There was also no sham condition; however, comparisons were within participant and within visit to characterize relative responsiveness to cardiac vagal and cortical biomarkers to different stimulation parameters. Any future study that aims to make efficacy claims in a defined disease population will require a sham-controlled design.

Additional limitations to our sample size also could extend to recording modalities that could better characterize ANS function beyond only cardiac vagal modulation and hemodynamics; other possible biomarkers include electrodermal activity (EDA), for which impairments have been related to diabetic neuropathy (Petrofsky & McLellen, 2009; Khalfallah et al., 2012) and PTSD (Pole 2007), or PPG amplitude, a previously used biomarker to track tcVNS responses (Gazi et al. 2020). Blood biomarkers could also be used to track stimulation efficacy while tracking clinical symptoms in a long-term study (Gurel et al. 2020; Workewych et al. 2020; Manca et al. 2023; Mondal et al. 2024). Another confound could be visit order; the sequential design was selected to begin with the parameter with the most variable and least informative prior data and using these results to constrain further visits. While settings for each parameter type were randomly ordered in each session, visits were always in the same sequence and could have contributed to the declining RMSSD response. Further randomizing parameters would have required a much larger sample or fewer conditions, but these parameters in conjunction have not been systematically studied previously. There are also other stimulation parameters that could be examined in-depth, including pulse shape, pulse width, and electrode size and type, along with other frequencies and durations.

Conclusion

This method of TCES offers numerous therapeutic possibilities. Existing research demonstrates the therapeutic potential of VNS, but personalized, disease-specific non-invasive approaches are essential for advancing both clinical care and our understanding of the autonomic nervous system while offering advantages over surgical or pharmacological interventions, potentially reducing risks, costs, and side effects while improving efficacy. A critical next step is validating this personalized favored frequency approach in a disease population. It will be crucial to evaluate safety and feasibility of TCES in, for example, PTSD patients, while assessing efficacy by tracking PTSD symptoms, depression and anxiety symptoms, quality of sleep, autonomic dysfunction, and blood biomarkers for inflammation. The stimulation parameters can be optimized further based on anatomical targets and specific disorders, with the ultimate goal of modulating the inflammatory reflex and enhancing parasympathetic activity. Precise parameter selection to stimulate specific vagal nerve fibers and, consequently, targeted organs will be crucial for effective clinical application.

Abbreviations

ANS	Autonomic nervous system
ASR	Artifact space reconstruction
ECG	Electrocardiography
EDA	Electrodermal activity
EEG	Electroencephalography
HEP	Heart-evoke potential
HR	Heart rate
HRV	Heart rate variability
ICA	Independent component analysis
MAP	Mean arterial pressure
ncVNS	Noninvasive cervical vagus nerve stimulation
NIBP	Non-invasive blood pressure
PD_L	Pupil diameter, left
PPG	Photoplethysmography
PRV	Pulse rate variability
PTSD	Post-traumatic stress disorder
RMSSD	Root mean square of successive R-R interval differences
SDNN	Standard deviation of N–N intervals
tACS	Transcranial alternating current stimulation
taVNS	Transcutaneous auricular vagus nerve stimulation
TCES	Transcutaneous cervical electrical stimulation
tcVNS	Transcutaneous cervical vagus nerve stimulation
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
VLF	Very low frequency
VN	Vagus nerve
VNS	Vagus nerve stimulation

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Authors' contributions
SD, BTG, and TPZ conceptualized and designed the study. SD and FF acquired and analyzed all data. SD, FF, BTG, and TPZ contributed to interpretation and discussions at multiples stages of data acquisition and manuscript development. SD, BTG, and TPZ contributed to drafts of the manuscript. All authors critically revised and approved the final version of the manuscript and take responsibility for the integrity of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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

Declarations

Ethics approval and consent to participate
The study was approved by the Northwell Health Institutional Review Board, IRB #19–0461. All participants were given detailed information about the research study and completed a Consent for Participation in a Research Study form.

Consent for publication
Not applicable.

Competing interests
SD and TPZ are staff of The Feinstein Institutes for Medical Research and Northwell Health. BTG was the Chief Science Officer of Tivic Health Systems, Inc., at the time this study was conducted. TPZ is the associate editor for BEME.

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