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REVIEW ARTICLE

A systematic review of the effects of transcutaneous auricular vagus nerve stimulation on baroreflex sensitivity and heart rate variability in healthy subjects

Danesh Soltani[1] · Bayan Azizi[1] · Sepehr Sima[2] · Kiarash Tavakoli[1,3] · Negin Sadat Hosseini Mohammadi[1,3] · Abdol-Hossein Vahabie[4,5] · Kaveh Akbarzadeh-Sherbaf[6] · Ali Vasheghani-Farahani[1]

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Abstract

Purpose This systematic review aimed to evaluate the effect of transcutaneous auricular vagus nerve stimulation on heart rate variability and baroreflex sensitivity in healthy populations.

Method PubMed, Scopus, the Cochrane Library, Embase, and Web of Science were systematically searched for controlled trials that examined the effects of transcutaneous auricular vagus nerve stimulation on heart rate variability parameters and baroreflex sensitivity in apparently healthy individuals. Two independent researchers screened the search results, extracted the data, and evaluated the quality of the included studies.

Results From 2458 screened studies, 21 were included. Compared with baseline measures or the comparison group, significant changes in the standard deviation of NN intervals, the root mean square of successive RR intervals, the proportion of consecutive RR intervals that differ by more than 50 ms, high-frequency power, low-frequency to high-frequency ratio, and low-frequency power were found in 86%, 75%, 69%, 47%, 36%, and 25% of the studies evaluating the effects of transcutaneous auricular vagus nerve stimulation on these indices, respectively. Baroreflex sensitivity was evaluated in six studies, of which a significant change was detected in only one. Some studies have shown that the worse the basic autonomic function, the better the response to transcutaneous auricular vagus nerve stimulation.

Conclusion The results were mixed, which may be mainly attributable to the heterogeneity of the study designs and stimulation delivery dosages. Thus, future studies with comparable designs are required to determine the optimal stimulation parameters and clarify the significance of autonomic indices as a reliable marker of neuromodulation responsiveness.

Bayan Azizi and Sepehr Sima contributed equally to this work.

✉ Ali Vasheghani-Farahani
avasheghani@tums.ac.ir

Danesh Soltani
soltani.danesh93@gmail.com

Bayan Azizi
bayan.azizi@ymail.com

[1] Cardiac Primary Prevention Research Center (CPPRC), Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Psychology, University of Tehran, Tehran, Iran

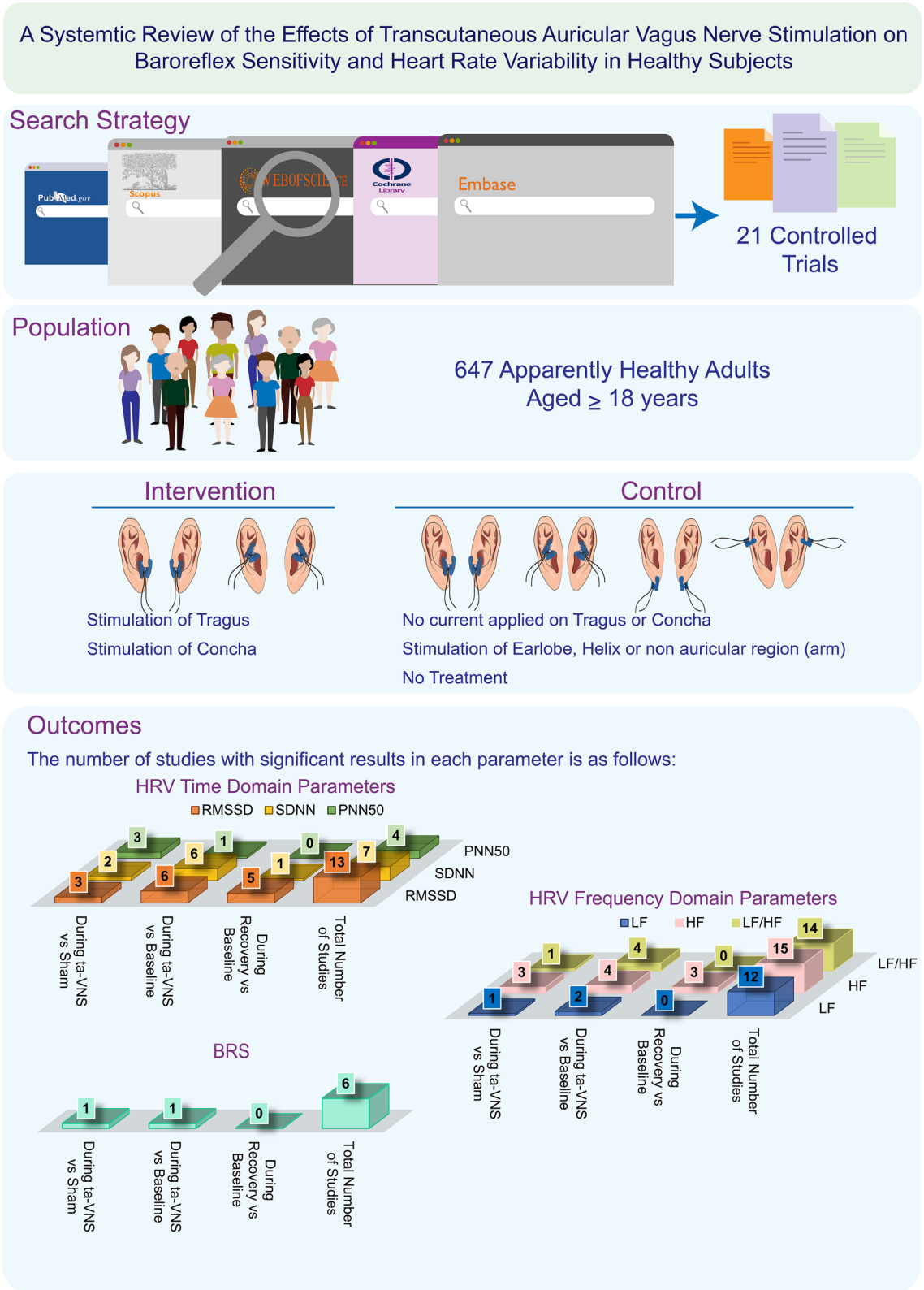
[3] Students' Scientific Research Center (SSRC), Tehran University of Medical Sciences, Tehran, Iran

[4] Control and Intelligent Processing Center of Excellence (CIPCE), Cognitive Systems Laboratory, School of Electrical and Computer Engineering, College of Engineering, University of Tehran, Tehran, Iran

[5] Department of Psychology, Faculty of Psychology and Education, University of Tehran, Tehran, Iran

[6] Department of Computer Engineering and Information Technology, Imam Reza International University, Mashhad, Iran

Graphical abstract



Keywords Vagus nerve stimulation · Heart rate variability · Baroreflex · Healthy volunteers

Introduction

Despite significant breakthroughs in preventive and therapeutic strategies, cardiovascular disease (CVD) continues to be the leading cause of morbidity and mortality worldwide [1, 2]. Prior to a cardiovascular event, risk factors appear in apparently healthy subjects, at which point preventive actions can be effective [3]. The autonomic nervous system (ANS) is responsible for controlling visceral functions to keep up with environmental stimuli and maintain homeostasis. Imbalance in ANS, when persists, is known as a preceding factor for many disorders [4–6]. This is also true for CVDs as many studies have shown the association between an imbalance in cardiovascular ANS function and developing hypertension, heart failure, arrhythmias, and acute myocardial infarction [7–10]. Therefore, regulating cardiovascular autonomic function in apparently healthy subjects seems to be a promising strategy for preventing future CVDs.

Cardiovascular ANS can be modulated by pharmacological and nonpharmacological methods. In recent years, nonpharmacologic approaches to treat CVDs draw more interest because of the limited efficacy, fewer adverse side effects, and the significant costs of pharmacological agents [8, 10, 11]. Nonpharmacological methods are comprised of invasive and noninvasive therapies. Invasive techniques consist of low-level vagus nerve stimulation (VNS) [12], low-level baroreceptor activation therapy [13], spinal cord stimulation [14], ganglionated plexi ablation [15], renal sympathetic denervation [16], and cardiac sympathetic nerve denervation [17]. Noninvasive approaches generally use electrical pulses, electromagnetic field, ultrasound energy, and optogenetics to transcutaneously target cardiovascular ANS [10, 18].

As a safe and noninvasive method to regulate cardiovascular ANS, transcutaneous auricular VNS (ta-VNS) has attracted much attention in recent years. Many clinical trials speculated the effects of ta-VNS on cardiovascular ANS indices in apparently healthy populations [8, 9, 19–21]. However, the results are mixed, and there are still many uncertainties on how ta-VNS can effectively modulate the autonomic function and which individuals might benefit the most from this intervention. In this systematic review, we aim to summarize the current evidence of the effect of ta-VNS on two of the most commonly used indicators of cardiovascular autonomic function, heart rate variability (HRV) and baroreflex sensitivity (BRS) in apparently healthy subjects. We also discuss the individual specific determinants of response to ta-VNS and the challenges associated with selecting the optimal stimulation dosage in these subjects.

Methods

This study was undertaken and reported in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards [22]. The study protocol has been registered in PROSPERO (CRD42022334252).

Search strategy and eligibility criteria

We performed a systematic search in PubMed, Scopus, Web of Science, the Cochrane Library, and Embase databases from inception to February 2023. The search strategy included combinations of keywords related to or describing VNS and cardiac autonomic function indices including HRV and BRS. The details of search strategy are presented in Supplementary Table 1. In addition, reference lists of retrieved studies were searched for additional relevant reports. The search was limited to published English-language studies. Original studies were included in this review if they met the following criteria: (1) Studies included apparently healthy adults; (2) the intervention group received ta-VNS on tragus or concha areas; (3) the comparison group received "no treatment," "stimulation OFF" on either the tragus or concha, or "stimulation ON" on areas presumed to have no vagal innervation, such as the earlobe and helix; (4) studies whose primary or secondary outcomes were an endpoint measure of HRV or BRS; and (5) double-arm, controlled clinical trials with a parallel or crossover design. Exclusion criteria were: (1) Observational, single-arm noncontrolled interventional studies, case reports, case series, letters, conference paper, and review articles; (2) studies included nonhealthy population.

Study selection and data extraction

The study selection and data extraction were carried out independently by two investigators, with a third being consulted in case of discrepancies. First, titles and abstracts of the selected studies were screened according to eligibility criteria, and then, the full texts of those primarily considered relevant were evaluated in detail. Data on study design, sample size, gender distribution, age, and intervention characteristics in active and sham groups, including stimulation technical parameters, duration of exposure, site of stimulation, and outcome measures were extracted from final included studies.

Quality assessment

Two independent researchers assessed the quality of included studies using two different tools, based on the design of the

studies: Cochrane Risk of Bias Tool version 2.0 (RoB 2.0) for crossover trials [23] and Cochrane RoB 2.0 for parallel trials [24]. Cochrane RoB 2.0 for crossover studies consists of six domains that assess bias in various methodological steps of studies, such as randomization process, washout period and carryover effects, deviations from the intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result [23]. Cochrane RoB 2.0 for parallel trials is identical to that for crossover studies, with the exception that this tool lacks a domain for evaluating washout period and carryover effects [24]. Cochrane RoB 2.0 classifies studies as follows, based on their overall quality: (1) low risk of bias, which includes studies rated low risk in all domains; (2) some concerns, which includes studies rated of some concern in at least one domain but no high-risk judgement; and (3) high risk of bias, which includes studies rated high risk in at least one domain or have some concerns for multiple domains in a way that significantly reduces confidence in the results [23, 24].

Data synthesis and visualization

We deemed it inappropriate to perform a meta-analysis of study results due to heterogeneity in study design, stimulation delivery protocols, and outcome reporting across studies. Instead, we provided a detailed narrative synthesis of findings in the main text and structured tables, as well as several 3D scatter plots illustrating the distribution of ta-VNS stimulation parameters used in included studies and the effect size of ta-VNS on various HRV indices and BRS in individual studies.

The mean and standard deviation (SD) of HRV indices and BRS measured at three time points—baseline, during stimulation, and recovery—were extracted for the sham and active groups. In the case of reporting other than SD, including standard error (SE) and confidence intervals (CI), they were converted to SD using standard formulas [25]. Due to differences in outcome measures among the included studies, we estimated the effect size of the included studies by calculating the standardized mean difference (SMD) and 95% confidence interval for each within-group and between-group comparison, wherever possible based on the available data. If not, we described the direct findings from the study. An SMD (Cohen's d) < 0.5 is generally interpreted as a small effect size, 0.5–0.8 as a moderate effect size, and > 0.8 as a large effect size.

Results

Identification and selection of the studies

Figure 1 depicts the study selection process. A total of 2458 papers were retrieved from five databases. After eliminating duplicates and irrelevant research by title and abstract screening, 40 studies were examined for full-text screening. At this step, 19 studies were discarded based on the exclusion criteria, leaving 21 relevant controlled trials for inclusion in this systematic review.

Study characteristics

The characteristics of the included studies are summarized in Table 1. Except for four studies that focused only on men [8, 26–28], the majority of studies included participants of both sexes. The age ranges of the participants included in studies were as follows: young adults (18–39 years) in 17 studies [8, 9, 19, 21, 26–38], middle-aged adults (40–59 years) in four studies [34, 39–41], and older adults (≥ 60 years) in one study [42]. All studies evaluated the efficacy of ta-VNS, administered either to the tragus [8, 9, 27, 32, 37, 42], cymba concha [21, 26, 28–31, 34–36, 38–41], or both [19, 33] on HRV parameters [8, 9, 19, 21, 26–41] and/or BRS [8, 27, 34, 40–42]. As shown in Figs. 3, 4, 5, 6, 7, 8, and 9, the most used frequency in included studies was 25 Hz in 12 studies [19, 21, 28–31, 33, 35–38, 40] followed by 10 Hz in four studies [34, 35, 39, 41], and 20 Hz [26, 27, 32] and 30 Hz [8, 9, 42] each in three studies. The frequencies used in four experiments—2 [35], 5 [26], 100 [35], and 500 Hz [36]—were vastly different from those in other studies. Except for one study that employed a set stimulation of 2 mA [33], the majority of experiments relied on the sensory thresholds of the participants to determine the stimulation intensity. This has been determined using one of two methods: up-titration, which begins at a very low amplitude and gradually rises to the sensory threshold (13 studies) [9, 19, 26, 28, 29, 31, 32, 34, 36, 37, 39–41] or down-titration, in which the pain threshold is first detected and then the amplitude is set just below that at the sensory level (two studies) [30, 42]. In addition, the specific procedure for selecting the stimulation intensity was not described in four of the trials [8, 27, 35, 38]. As shown in Figs. 3, 4, 5, 6, 7, 8, and 9, the most frequently employed pulse width in the included studies was 250 μs (five studies) [19, 29, 30, 32, 40], followed by 200 μs [8, 9, 26, 42] and 300 μs [34, 35, 39, 41] each in four studies, 500 μs in two studies [36, 38], and 100 μs [33] and 1000 μs [27] in one study each. Instead of utilizing a fixed number, two investigations reported the pulse width as a range between 200 and 300 μs [21, 31]. Only eight investigations detailed the waveform of the electrical pulses: four used rectangular pulses [27, 29, 35, 36], three of which were monophasic [27, 35, 36]; two used square pulses, one of which was monophasic [30] and the other biphasic [40]; one used a combination of rectangular and square pulses [33]; and one simply stated "biphasic" to describe the waveform [31]. Fifteen trials utilized a continuous stimulation pattern [8, 9, 19, 26, 27, 32, 34–37, 39–42] while six studies used an

on–off cycle stimulation pattern [21, 28–31, 38]. As shown in Fig. 2, the following sham or comparison strategies were employed in the included studies: (1) "stimulation ON" on the earlobe (nine trials) [8, 19, 21, 26, 28, 30, 32, 37, 40], (2) "stimulation ON" on the helix (one study) [31], (3) "stimulation OFF" on the tragus (four trials) [8, 27, 33, 42], (4) "stimulation OFF" on the concha (five studies) [29, 33–36], and (5) "stimulation ON" on the nonauricular region (arm) (one study) [38]. Two studies compared the active groups with "no treatment" groups [39, 41].

ta-VNS and cardiac autonomic nervous system indices

This section evaluates the influence of ta-VNS on HRV frequency-domain (LF, HF, and LF/HF ratio) and time-domain (RMSSD, SDNN, PNN50) parameters, as well as BRS, in the included studies.

Heart rate variability frequency-domain parameters

The effect of ta-VNS on LF-HRV was assessed in a total of 17 experiments from 12 studies including 323 healthy subjects [8, 9, 26, 29, 31–34, 36, 38, 40, 41]. The majority of studies found no substantial change in LF-HRV with ta-VNS compared with the sham group or baseline levels. Forte et al. [31] observed a substantial increase in LF during ta-VNS compared with baseline levels. Shen et al. [36] identified responders to ta-VNS based on a 20% decrease in the LF/HF ratio; in this group of subjects, there was a substantial decrease in LF during burst stimulation relative to the baseline level. Only Zhu et al. [38] observed a significant

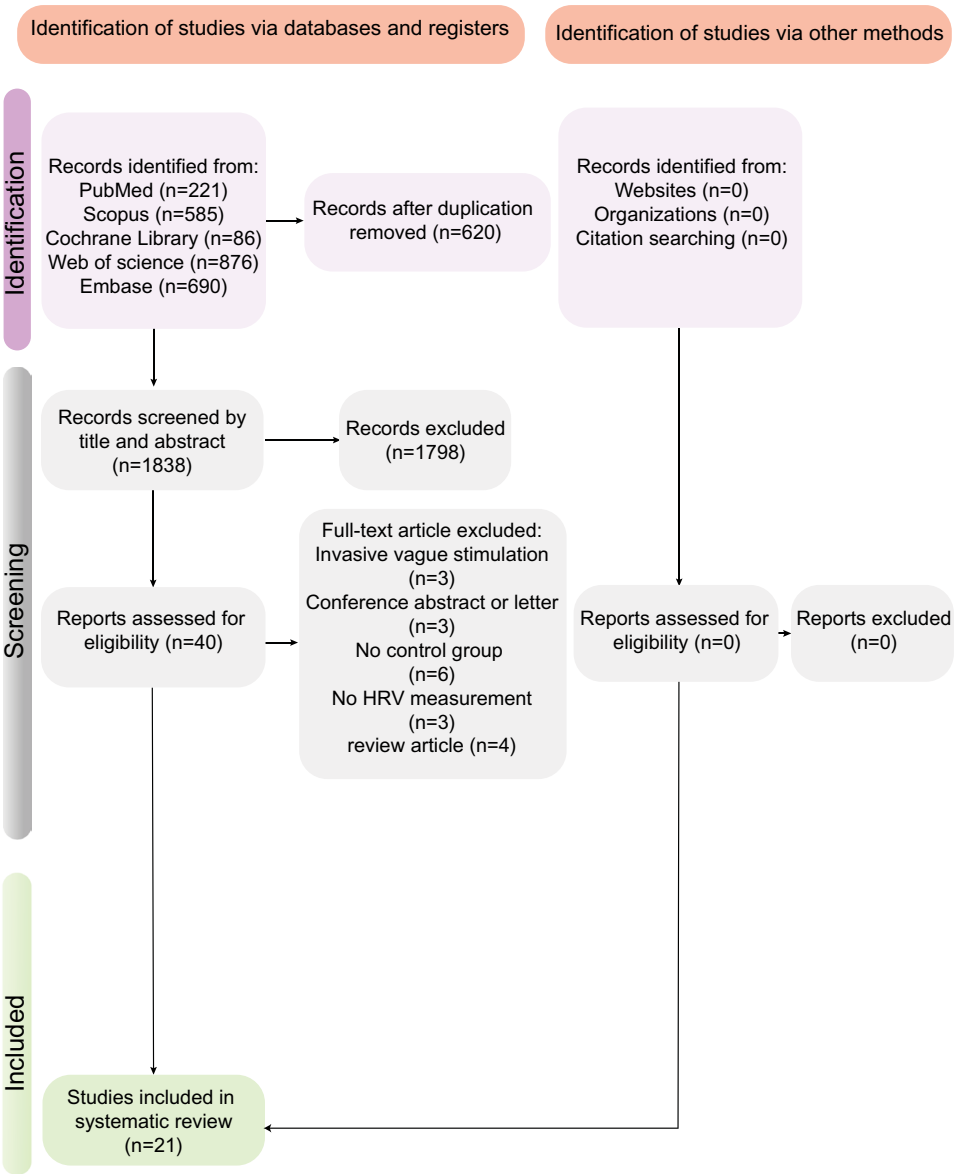


Fig. 1 PRISMA chart depicting the manuscript selection process

Table 1 Characteristics of the included studies

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Antonino et al. 2017 [1]	22.6 ± 3.6	100	Within-subjects crossover design	13	ta-VNS (tragus)	30	Adjusted between 10 and 50 mA at the level of individuals sensory threshold (45 ± 1)	200	Continuously for 15 min	#1: Sham tragus[a] #2: Sham earlobe[b]	During stimulation: sig ↑ of BRS and ↓ of LH/HF during ta-VNS compared with baseline levels Recovery: LF/HF in the ta-VNS group returned to baseline levels Nonsig change in LF and HF during stimulation or in recovery time compared with sham or baseline levels
Borges et al. 2019, [2]	23.62	48.3	Within-subject crossover design	60	ta-VNS (left cymba concha)	25	The highest comfortable intensity freely chosen by each participants (active: 2.5 ± 0.93, sham: 2.76 ± 1.01)	200–300	30 s on, 30 s off for 10 min	Sham earlobe	Nonsig change in cardiac vagal activity between active and sham group Sig ↑ in RMSSD during the second half of the stimulation in both active and sham group compared with baseline

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Borges et al. 2021 [3]	23.2 ± 3.1	42.8	Within-subject crossover design	42	ta-VNS (#1: left cymba concha #2: left tragus)	25	Adjusted between the individually detectable intensity and the discomfort threshold (#1: active: 0.94 ± 0.57, sham: 2.19 ± 0.71 #2: active: 2.18 ± 0.69, sham: 2.19 ± 0.71)	250	Continuously for 4 min	Sham earlobe	No significant different in HF and RMSSD between the active and sham groups Sig ↑ in RMSSD and HF during stimulation and recovery compared with baseline levels
Bretherton et al. 2019 [4]	69.14 ± 6.8	64.2	Within-subject crossover design	14	ta-VNS (tragus)	30	Adjusted between 2 and 4 mA at the level of individuals sensory threshold (NM)	200	Continuously for 15 min	Sham tragus	Sig ↑ in BRS during stimulation compared with sham
Clancy et al. 2014 [5]	35.16 ± 13.28	50	Between-subject parallel design	48	ta-VNS (tragus)	30	Adjusted between 10 and 50 mA at the level of individuals sensory threshold (NM)	200	Continuously for 15 min	Sham tragus	Sig ↓ in LF/HF during ta-VNS compared with baseline Nonsig change during sham compared with baseline Nonsig change in LF and HF in active group compared with baseline or sham

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Dalgleish et al. 2021 [6]	56.94 ± 18.02	31.5	Between-subject parallel design	19	ta-VNS (left cymba concha)	10	Turn down the intensity from the sensory threshold to a level of mild tingling or no feeling (NM)	300	Continuously for 15 min per day: 3 days	No treatment	Sig ↑ in RMSSD during recovery compared with baseline levels in the active group Nonsig change in RMSSD in the no treatment group
Decouck et al. 2017 [7]	37.00	50	Within-subject crossover design	30	ta-VNS (#1: left, #2: right cymba concha)	25	Adjusted to a level of clear tingling without any pain (0.7)	250	30 s on, 30 s off for 10 min	Sham concha[c]	Sig ↑ in SDNN during left ta-VNS stimulation compared with baseline Near to significant ↑ in SDNN during right ta-VNS stimulation compared with baseline Nonsig change in LF, HF, LF/HF, and RMSSD during stimulation compared with baseline No significant change in HRV parameters in the sham group

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
De Smet et al. 2023 [8]	21.1 ± 3.11	21	Between-subject parallel design	83	ta-VNS (left cymba concha)	25	Set above the individual detection level and below the pain level (sham: 1.89 ± 0.89, active: 1.37 ± 0.81)	250	30 s on, 30 s off for 20 min	Sham earlobe	During stimulation: marginal ↑ in RMSSD compared with baseline in active group During recovery: sig ↑ in RMSSD compared with baseline in active group
Forte et al. 2022 [9]	23.15 ± 3.16	17.8	Within-subject crossover design	28	ta-VNS (left cymba concha)	25	Adjusted to a level of clear tingling without any pain (1.2 ± 0.4)	200–300	30 s on, 30 s off for 10 min	Sham helix[d]	Sig ↑ in SDNN, RMSSD, and HF during ta-VNS in the active group compared with the sham group and baseline levels Sig ↑ in LF during stimulation in the active group compared with the baseline levels Sig ↑ in LF/HF in the sham group compared with the active group and baseline levels

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Gancheva et al. 2017 [10]	51.10 ± 6.00	80	Within-subject crossover design	10	ta-VNS (left cymba concha)	25	Adjusted to the level of sensory threshold (sham: 0.8 ± 0.1, active: 0.9 ± 0.1)	250	Continuously for 14 min	Sham earlobe	Nonsig change in LF, HF, LF/HF, and BRS during stimulation or recovery compared with the baseline levels and the sham group
Gauthey et al. 2020 [11]	27.00 ± 4.00	100	Within-subject crossover design	28	ta-VNS (right cymba concha)	#1 = 5 #2 = 20	Adjusted to the level of sensory perception (#1: active:1.2 ± 0.5, sham: 1.5 ± 0.6) (#2: active: 5.5 ± 1.6, sham: 1.5 ± 0.6)	200	Continuously for 10 min	Sham earlobe	There is no overall effect of ta-VNS on HRV parameters except for LF/HF in both the 5 and 20 Hz groups Sig ↑ in LF/HF in the 5 Hz-group compared with the baseline

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Geng et al. 2022 [12]	23.42 ± 1.29	57.14	Within-subject crossover design	14	ta-VNS (left tragus)	20	Adjusted to the level of sensory perception (active:14.79 ± 6.02, sham: 13.91 ± 5.28)	250	Continuously for 5 min	Sham earlobe	Sig ↑ in HF, RMSSD, SDNN, and PNN50 during stimulation compared with the sham group Sig ↑ in PNN50 during stimulation compared with baseline Nonsig difference in HRV parameters comparing recovery with baseline Nonsig change in LF and LF/HF during stimulation or recovery compared with baseline or the sham group

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Kania et al. 2021 [13]	55 ± 17.99	26.3%	Between-subject parallel design	19	ta-VNS (cymba concha)	10	Adjusted to the level of sensory threshold (NM)	300	Continuously for 15 min, 4 days	No treatment	Sig ↑ in RMSSD and HF during recovery in the active group compared with the baseline levels Nonsig change in BRS and LF in the active group compared with the baseline or no treatment group
Keute et al. 2021 [14]	28.2 ± 5.6	50	Within-subject crossover design	44	ta-VNS (tragus–concha)	25	Set intensity 2	100	Burst of five pulses: 5 min	Sham tragus–concha	During stimulation versus baseline: sig ↑ in RMSSD, SDNN, PNN50, and HF in the active group During stimulation versus sham: sig ↑ in RMSSD, PNN50, and SD1/SD2

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Kozorosky et al. 2022 [15]	#1: 33 ± 16 #2: 44 ± 26.45	18.75	Within-subject crossover design	#1: 16 #2: 10	ta-VNS (left cymba concha)	10	Adjusted to the level of individual sensory threshold or a maximum current of 2.5 (NM)	300	Continuously for 30 min	Sham concha	Nonsig change in LF, HF, LF/HF, RMSSD, SDNN, and BRS in the ta-VNS group compared with the sham group in both protocols Sig ↑ in SDNN (1) and LF/HF (2) in the sham group compared with the baseline levels
Sclocco et al. 2020 [16]	29.0 ± 9.8	43.3	Within-subject crossover design	30	ta-VNS (left cymba concha)	#1: 2 #2: 10 #3: 25 #4: 100	Perception match across subjects, targeting 4–5 on a 0–10 numeric rating scale (NM)	300	Continuously for 8.5 min	Sham concha	Nonsig difference in HF in the ta-VNS group compared with the sham group

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Shen et al. 2021 [17]	21.67 ± 0.34	47.6	Within-subject crossover design	42	ta-VNS (left cymba concha)	#1: 5 pulses per burst at 500 Hz #2: 25 Hz	Adjusted to 150% of the current value at the perception threshold (NM)	500	15 min on, 15 min off for continuous and burst stimulation	Sham concha	Post burst and tonic stimulation versus baseline: sig ↑ in SDNN Post burst stimulation versus baseline: Sig ↑ in RMSSD During burst stimulation versus sham: sig ↑ in PNN50 Responders During and post burst and tonic stimulation versus baseline: sig ↑ in RMSSD, PNN50, and HF During burst versus baseline: sig ↓ in LF/HF
Sinkovec et al. 2021 [18]	23	100	Within-subject crossover design	15	ta-VNS (right tragus)	20	Adjusted to the level of being barely felt and was typically less than 150 μA. (NM)	1000	Continuously for 60 min	Sham tragus	Nonsig change in LF/HF and BRS in the ta-VNS group compared with the sham group

Table 1 (continued)

Author, year	Age, year (mean ± SD)	Men, %	Study design	Sample number	Intervention					Comparison/ sham strategy	Outcomes
					Intervention type (site)	Frequency (Hz)	Intensity (mean intensity mA)	Pulse width (μs)	Stimulation duration (minutes)		
Villani et al. 2019 [19]	21.2 ± 3.1	30.4	Within-subject crossover design	46	ta-VNS (tragus)	25	Adjusted to a level just above the participant's perceptual threshold (NM)	250	Continuously for 37 min	Sham earlobe	Nonsig change in HF and LF/HF between the ta-VNS and sham groups
Vosseler et al. 2020 [20]	24 ± 3.0	100	Within-subject crossover design	15	ta-VNS (left cymba concha)	25	Adjusted to the level of individual sensory threshold (active: 2.5 ± 0.9, sham: 3.2 ± 1.5)	NM	30 s on, 30 s off for 150 min	Sham earlobe	Nonsig change in RMSSD and LF/HF between the ta-VNS and sham groups
Zhu et al. 2022 [21]	28.2 ± 1.8	61.9	Within-subject crossover design	21	ta-VNS (bilateral cymba concha)	25	Ranging from 0.5 to 1.5 based on the participant's tolerance	500	2 s on, 3 s off for 30 min	Sham arm[e]	The cold stress-induced impairment (↓HF, ↑LF, and ↑LF/HF) was significantly improved in the ta-VNS group (↑HF, ↓LF/HF, ↓LF) but not in the sham group

[a]No current applied on tragus

[b]Stimulation of earlobe

[c]No current applied on concha

[d]Stimulation of helix

[e]Stimulation of the arm

Sig significant, *Nonsig* nonsignificant, *ta-VNS* transcutaneous auricular vagus nerve stimulation, *NM* not mentioned, *LF* low-frequency power, *HF* high-frequency power, *RMSSD* root mean square of successive RR interval differences, *SDNN* standard deviation of NN intervals, *pNN50* proportion of consecutive RR intervals that differ by more than 50 ms, *BRS* baroreflex sensitivity, *TP* total power

decrease in LF-HRV during ta-VNS compared with the sham group.

HF-HRV has been assessed in 21 experiments from 15 studies involving 441 healthy volunteers [8, 9, 19, 26, 29, 31–38, 40, 41]. The results of eight studies did not demonstrate a statistically significant change with ta-VNS when compared with sham or baseline levels [8, 9, 26, 29, 34, 35, 37, 40]. Three studies have revealed a significant rise in HF-HRV during ta-VNS compared with the sham group [31, 32, 38]. In addition, Keute et al. [33] and Forte et al. [31] and Borges et al. [19] reported a significant increase in HF-HRV during stimulation relative to baseline levels. Furthermore, Borges et al. [19] and Kania et al. [41] showed a significant increase in HF during recovery relative to baseline levels. Similarly, Shen et al. [36] observed a significant increase in this parameter among responders to both stimulation patterns (burst and continuous) during stimulation and recovery relative to baseline levels.

Nineteen experiments from 15 studies assessed the influence of ta-VNS on the LF/HF ratio in 380 healthy participants [8, 9, 26–29, 31–34, 36–38, 40]. Seven studies did not find a substantial difference between the ta-VNS group and the sham or baseline levels [27–29, 32, 33, 37, 40]. Compared with baseline values, Antonino et al. [8] and Clancy et al. [9] observed a substantial decrease in the LF/HF ratio during ta-VNS; Shen et al. [36] observed a significant decrease in this parameter during stimulation in responders to burst ta-VNS; and Gauthey et al. [26] observed a significant increase in the LF/HF ratio during ta-VNS with a frequency of 5 Hz. compared with the sham group. Only Zhu et al.[38] detected a substantial reduction in the LF/HF ratio during stimulation. In addition, two investigations found a substantial rise in the LF/HF ratio in the sham group compared with the baseline values [31, 34].

Heart rate variability time-domain parameters

Eighteen experiments from 13 studies involving 450 healthy adults assessed the effect of ta-VNS on RMSSD [19, 21, 26, 28–34, 36, 39, 41]. The results of four trials did not indicate a significant change in the ta-VNS group compared with sham or baseline levels [26, 28, 29, 34]. Three studies demonstrated a statistically significant rise in RMSSD during ta-VNS versus the sham group [31, 32, 36]. In three investigations [30, 39, 41], although there was no significant difference between the active and sham groups, there was a substantial rise in RMSSD during recovery relative to baseline values. In addition, Borges et al. [21] separated the stimulation session into first and second halves and compared them with sham and baseline levels; although, there was no significant difference between the active and sham groups, a significant increase was identified in the second

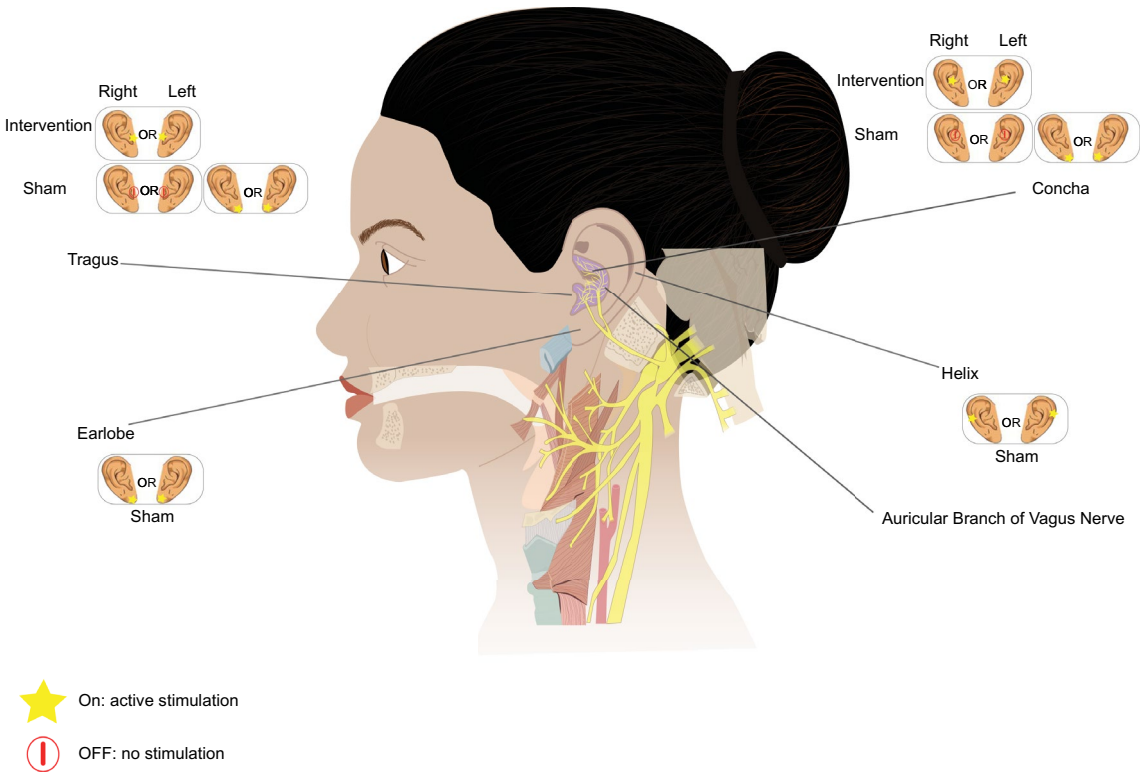


Fig. 2 A summary of all stimulation sites and auricular sham techniques used in transcutaneous auricular vagus nerve stimulation

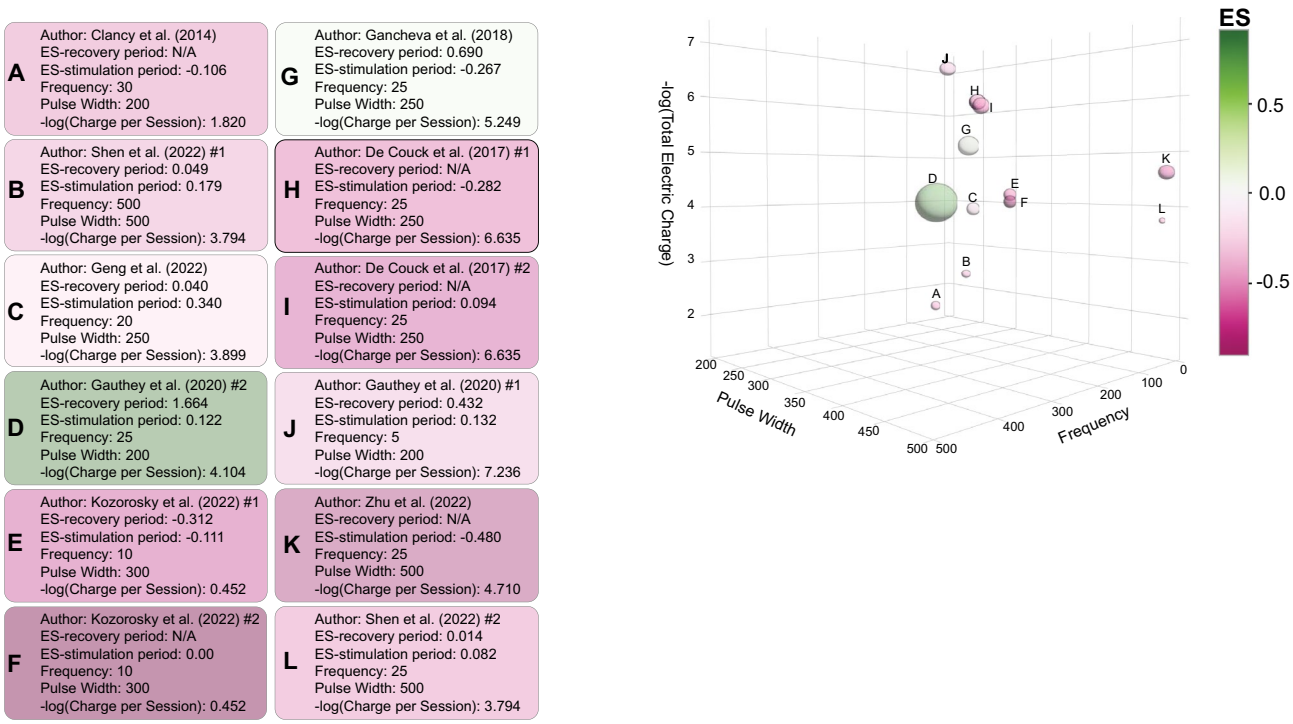


Fig. 3 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on LF-HRV in each study. ES: effect size

half of the stimulation compared with baseline. In another study, Borges et al. [19] also found a significant increase in RMSSD during stimulation and recovery compared with baseline levels. In addition, Shen et al. [36] showed a substantial rise in RMSSD during stimulation with both patterns relative to baseline; moreover, a significant increase in this parameter was found during recovery in the burst stimulation responders relative to baseline.

The effects of ta-VNS on SDNN have been investigated in 11 experiments from 7 studies including 212 healthy participants [26, 29, 31–34, 36]. Gauthey et al. [26] found no significant change in SDNN in the active group compared with the sham group or baseline values. Two studies detected a significant increase in SDNN in during stimulation compared with the sham group [31, 32]. Also, De Couck et al. [29] and Keute et al. [33] did not identify a significant difference between the ta-VNS group and the sham group, but they did note a substantial rise in SDNN during stimulation relative to their baseline levels. In addition, Shen et al. [36] observed a significant increase in SDNN during recovery compared with baseline values for both stimulation patterns, as well as a significant increase during stimulation among responders to both stimulation patterns. Kozorosky et al. [34] found no significant difference between the active group and the sham group or baseline values in any of their experiments; however, the sham group in their first experiment demonstrated a substantial rise in SDNN from baseline.

The effect of ta-VNS on PNN50 has been evaluated in six experiments from four studies [32–34, 36].The results of the experiments conducted by Geng et al.[32], Shen et al. [36], and Keute et al. [33] demonstrated a substantial rise in this parameter during ta-VNS stimulation compared with the sham group. Kozorosky et al. [34] did not observe any significant change in PNN50 in the ta-VNS group compared with the sham group or baseline values.

Baroreflex sensitivity

Eight experiments from six studies [8, 27, 34, 40–42] including a total of 97 healthy subjects, investigated the effect of ta-VNS on BRS. Only Bretherton et al. [42] detected a significant rise in BRS during ta-VNS stimulation compared with the sham group. Antonino et al. [8] found no statistically significant difference between the active and sham groups, however, a significant rise in BRS during ta-VNS stimulation relative to baseline values was observed.

Quality assessment and publication bias

The results of the quality assessment are shown in Supplementary Fig. 1A and B. As previously stated, a total of 17 crossover trials and 4 parallel trials were included in this study. For crossover trials, the risk of bias assessment using

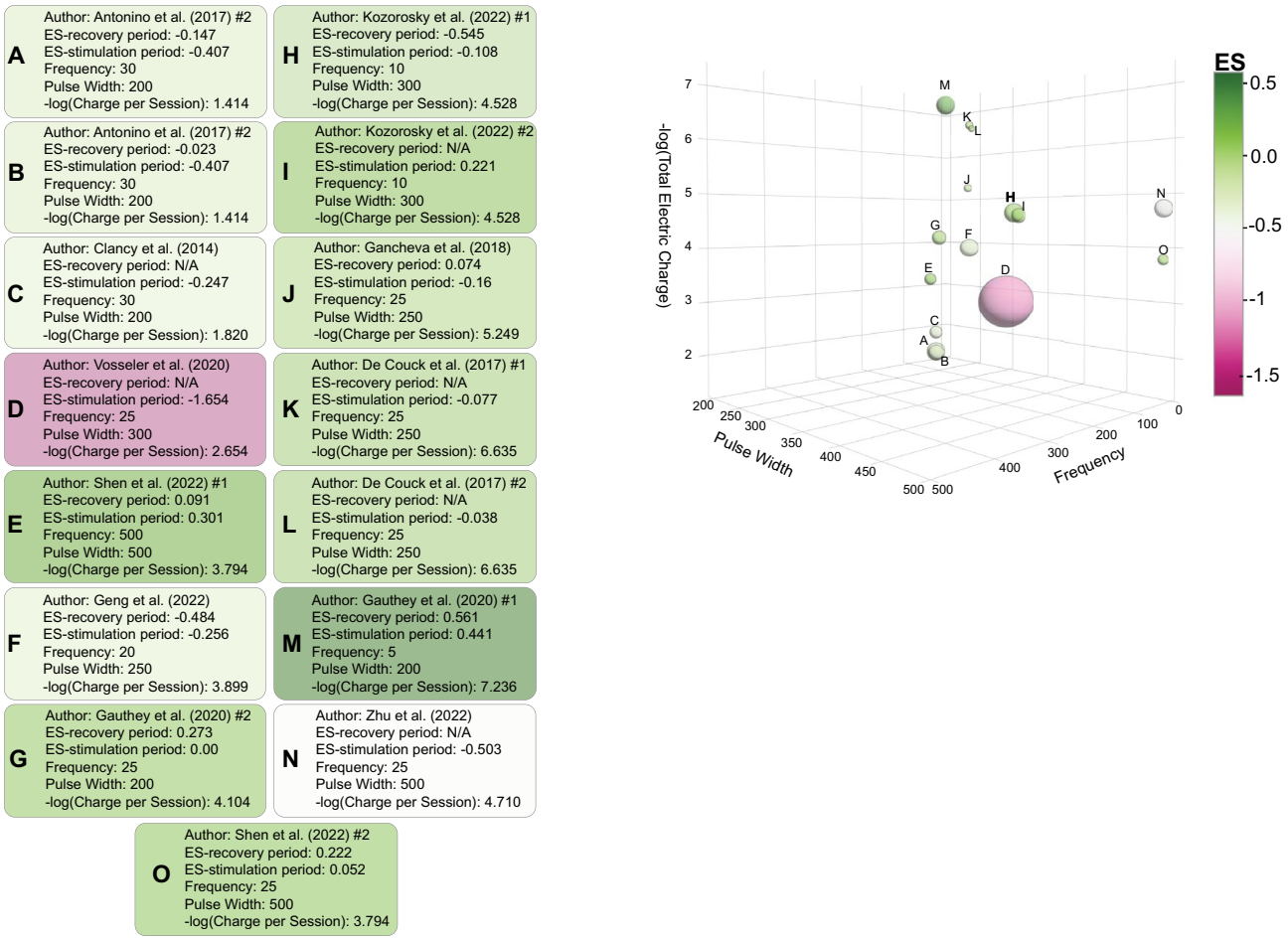


Fig.4 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on HF-HRV in each study. ES: effect size

Cochrane RoB 2.0 revealed that all of the included studies have some concerns in at least one domain, mainly in those related to the lack of a prespecified analysis plan (14 studies), insufficient washout duration and carryover effect (six studies), and inappropriate randomization process (five studies). Therefore, none of these crossover studies had a low risk of bias, and four of them have been determined to have a high risk. Cochrane RoB 2.0 for parallel randomized controlled trials was utilized to evaluate the four trials conducted using this design. The lack of a prespecified analysis plan was a concern for all of these trials, and two of them also had concerns with randomization.

Discussion

The purpose of this systematic review was to determine whether ta-VNS might significantly influence HRV parameters and BRS, and serve as a preventative strategy to enhance overall health in apparently healthy individuals. ANS imbalance, as revealed by HRV and BRS disturbances, is not only a potent and independent predictor of poor prognosis in patients with CVDs [43–46], but also a risk factor for death in healthy subjects [47, 48]. The potential of ta-VNS to improve autonomic function in a healthy population is substantial, and it might be used by many individuals where the cardiovascular autonomic balance is changed toward sympathetic predominance [49, 50].

Discussion on main findings

The results generally indicate conflicting conclusions about the effectiveness of ta-VNS on HRV or BRS. As stated previously and illustrated in the graphical abstract, 25%, 47%, and 36% of the studies evaluating LF, HF, and LF/HF, respectively, observed a significant effect of ta-VNS in changing these indices compared with the sham group, the pre-stimulation baseline levels, or both. Regarding time-domain indices, ta-VNS in 69%, 86%, and 75% of the studies has caused a significant change in RMSSD, SDNN, and

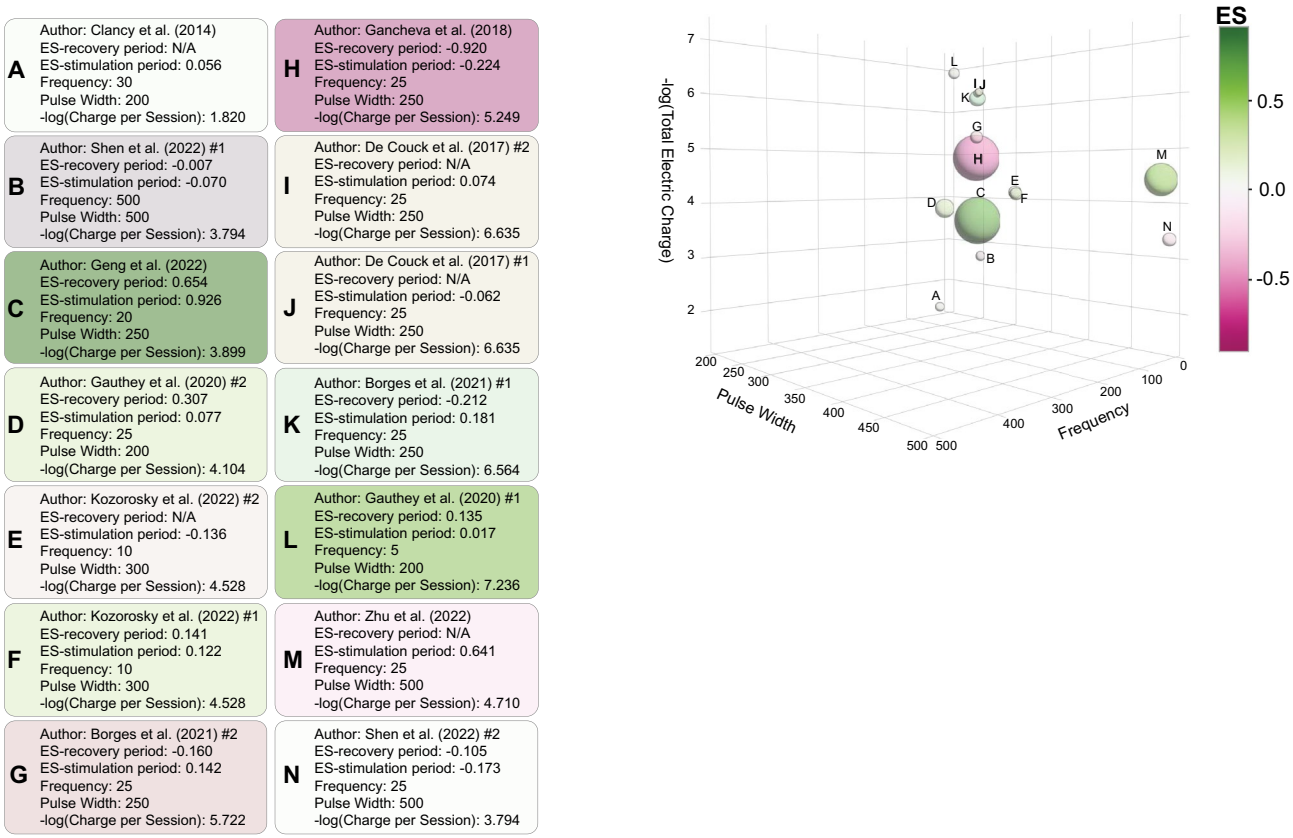


Fig. 5 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on HF-HRV in each study. ES: effect size

PNN50, respectively, compared with pre-stimulation baseline levels or comparison groups. Some studies continued the measurement of vagally-mediated indices such as HF, SDNN, and RMSSD in a short period after the cessation of stimulation, and have shown that the level of these indices remains higher than the baseline level during the recovery period [19, 32, 39]. This implies a "carry-over" effect, which is corroborated by earlier research showing greater HF power compared with baseline for at least 1 hour after ceasing auricular VNS with acupuncture (66). Regarding the BRS, only one study (17%) found the benefit of ta-VNS in improving this index compared with the baseline level and comparison group.

LF-HRV power may be generated by both the parasympathetic and sympathetic nervous systems, and slow breathing (i.e., < 8.5 breaths per minute) may influence its measurement [51]. A minimum of 2 min is required for recording LF power [52]. HRV recordings in included studies ranged from 15 to 150 min, which is sufficient for LF analysis, and most studies controlled the respiratory rate of their participants and exclude abnormal respiratory rate from their analysis. The LF/HF ratio is generally considered to be associated with sympathovagal balance, with a high ratio indicating sympathetic dominance and a low ratio indicating parasympathetic dominance [51, 53]. As illustrated in Figs. 3 and 4, exposure to ta-VNS has been associated with a decrease in LF compared with the comparison group in five and nine experiments, but the effect size was only significant in the Zhu et al. study [38] with a near-to-moderate effect size (−0.480) for LF and a moderate effect size for LF/HF (−0.503). The study by Zhu et al. has characteristics that distinguish its design from other studies and that partially explain the significant effect size observed for ta-VNS. This study exposed individuals to cold stress to induce autonomic dysfunction and then investigated the efficacy of ta-VNS on HRV parameters. Some studies have shown that the worse the basic autonomic function, indicated by higher LF/HF, the better the response to ta-VNS [9, 32]. This may partially explain how exposure to ta-VNS led to a higher effect size in the Zhu et al. study, in comparison with other studies that exposed subjects with physiologic baseline autonomic function to intervention. In addition, the study by Zhu et al. was the only one to use bilateral stimulation, which may be more helpful than the unilateral stimulation used in other studies; however, there is no study that compares the two, and this must be investigated in future research. Another

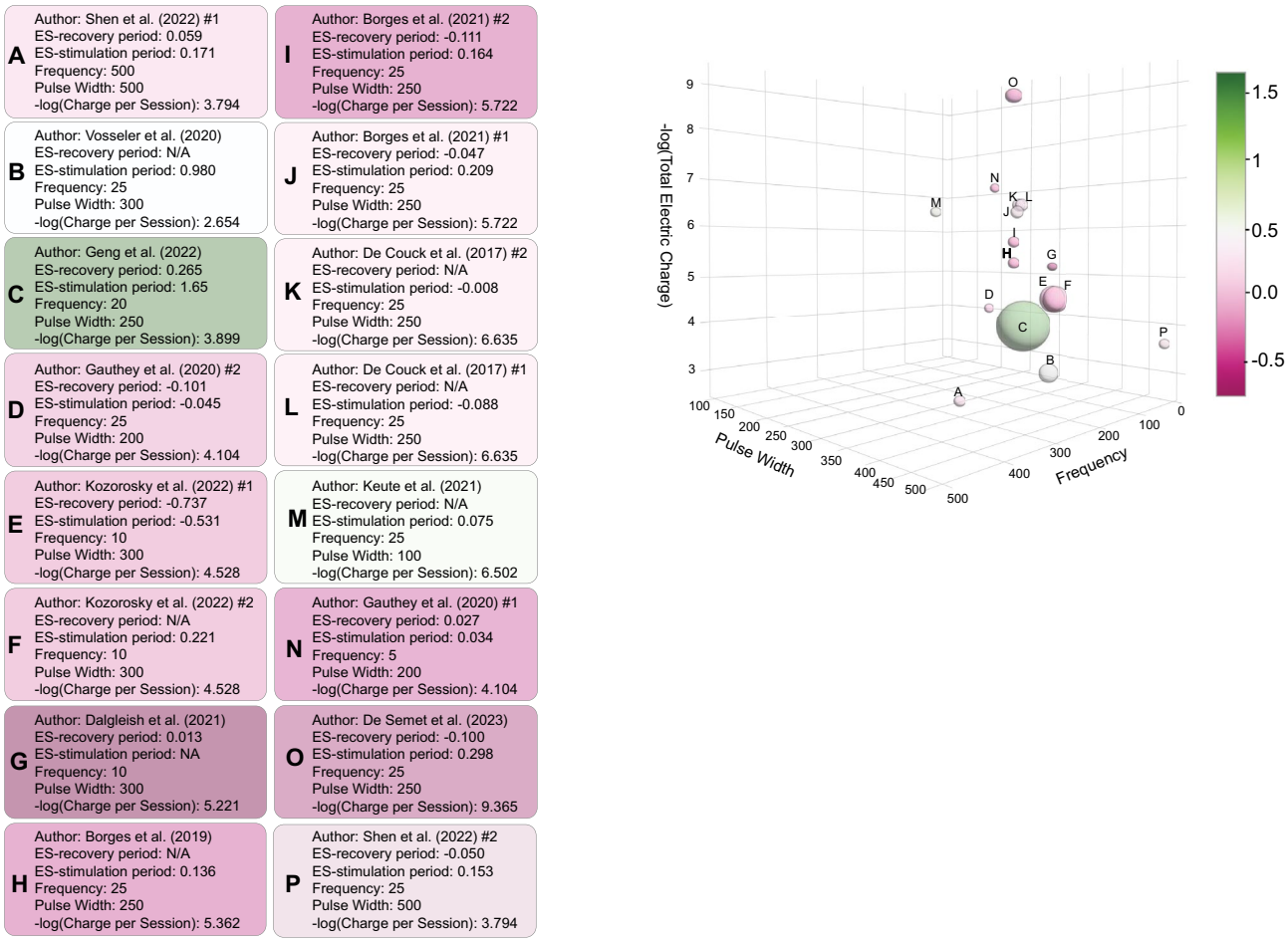


Fig. 6 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on RMSSD in each study. ES: effect size

difference between the Zhu et al. study and other studies is in their sham groups. The former used arm stimulation as a sham group, which can be superior to a "stimulation OFF" approach on either the tragus or conecha and a "stimulation ON" approach on the earlobe due to proper blinding and a lack of definitive stimulation of the vagus nerve, respectively.

HF, another frequency-domain index, represents parasympathetic activity and could be significantly influenced by respiration [9]. As depicted in Fig. 5, exposure to ta-VNS was associated with an increase in HF compared with the sham group in nine experiments; however, the effect size was only statistically significant in the studies by Geng et al. [32] and Zhu et al. [38], with high (+0.926) and moderate (+0.641) effect sizes, respectively. Despite being adjusted to the same sensory level as other investigations, the mean current intensity in the study by Geng et al. was significantly (up to two to three times) higher than in other studies. According to a recent study, greater ta-VNS intensities may be needed to provide meaningful neuromodulatory effects [54]. Furthermore, while the evidence is conflicting [21], there is some research indicating a positive linear association between ta-VNS intensity and several HRV parameters [55]. This could explain why the Geng et al. study had a considerably larger effect size than other studies evaluating HF-HRV; however, this should be clarified in future studies. Furthermore, the characteristics of the study by Zhu et al. that were described above as possible explanations for the observed substantial impacts on LF and LF/HF indices may also be true for HF.

SDNN is believed to indicate both sympathetic and parasympathetic inputs, whereas RMSSD is supposed to reflect vagally-mediated HRV, and both are less affected by variations in respiratory parameters than frequency-domain indices [43, 56]. Another time-domain HRV parameter is pNN50, which is indicative of parasympathetic nervous system activity and is correlated with HF and RMSSD [56]. As shown in Figs. 6, 7, and 8, Geng et al.'s study [32] found significant increases in all three indices compared with

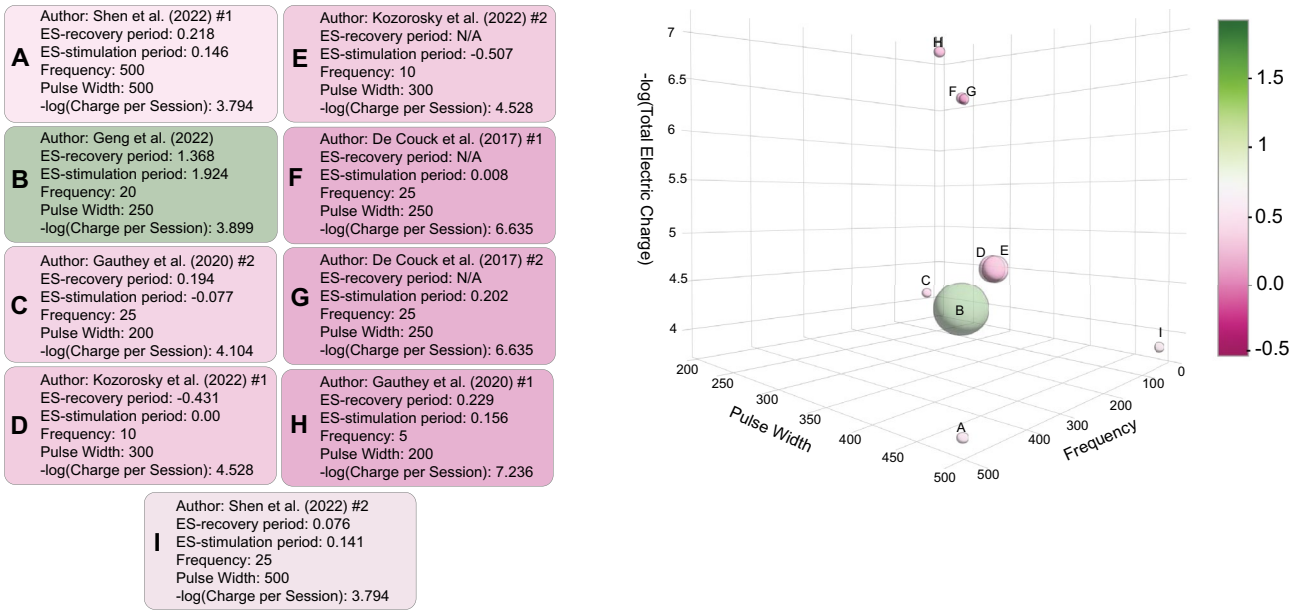


Fig. 7 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on SDNN in each study. ES: effect size

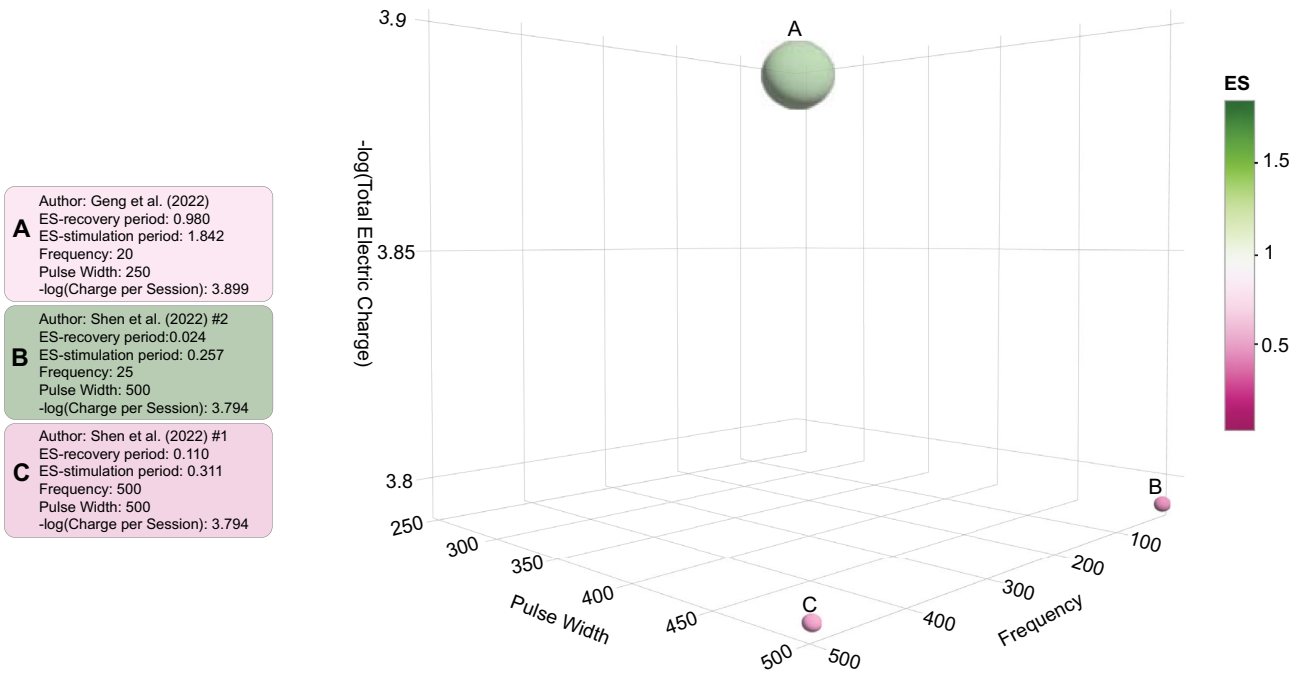


Fig. 8 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on PNN50 in each study. ES: effect size

the comparison group, with a high effect size for RMSSD (+1.65), SDNN (+1.924), and pNN50 (+1.842). Above, we described the characteristics of this study, which can be the possible reasons for the higher effect size in this study as compared with other studies.

HRV: a potential biomarkers for ta-VNS responsiveness

HRV is regarded as a noninvasive tool for assessing autonomic function and may be utilized to assess efferent vagus

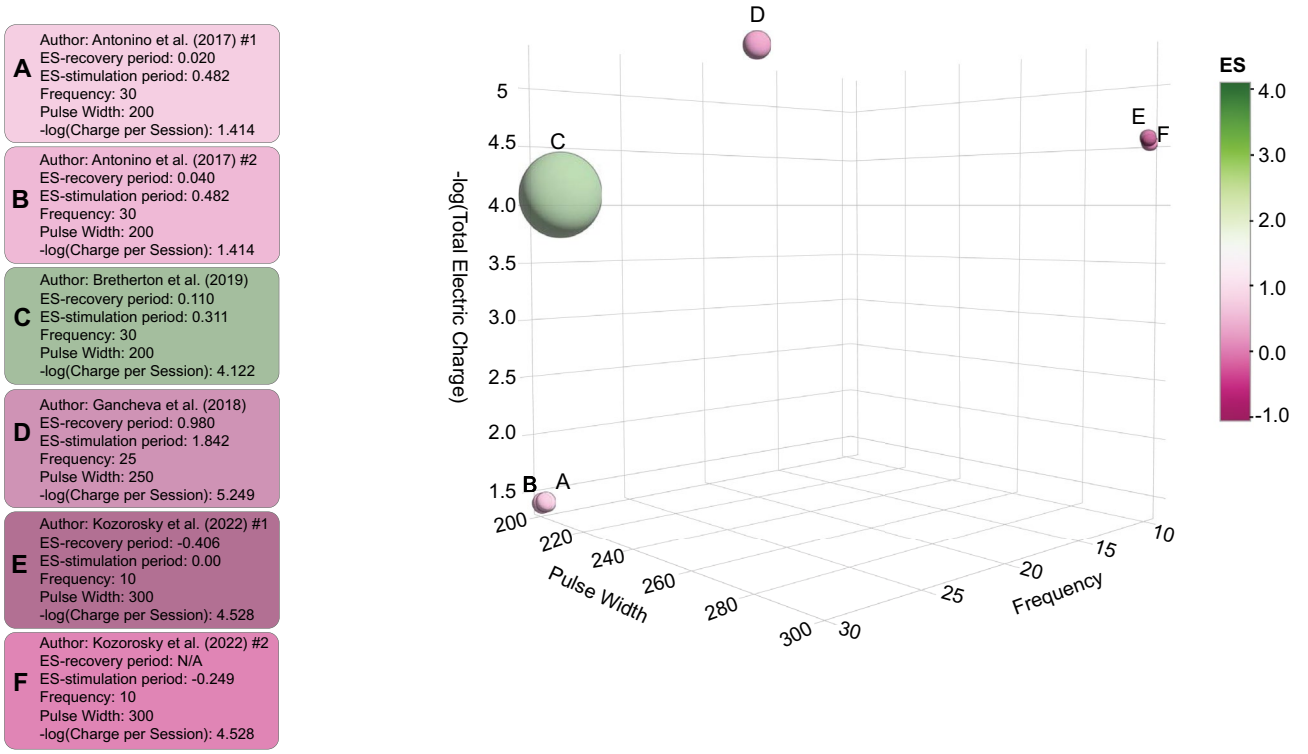


Fig. 9 3D scatter plot illustrating the distribution of stimulation parameters utilized in included studies and the effect size of ta-VNS on BRS in each study. ES: effect size

nerve activity [57]. According to the literature, sympathetic overactivity, the inflammatory response, and oxidative stress are three physiological pathways that contribute autonomic function imbalance to the development of many diseases [58, 59]. Moreover, vagally-mediated indices of HRV are inversely linked to the surrogate markers of these pathways [60–62]. Therefore, it is essential to systematically examine how ta-VNS affects HRV. This can not only aid us in finding out the impact of this intervention on autonomic function, but also determine whether HRV could be utilized as a predictive biomarker of ta-VNS responsiveness since it can help in selecting the right individuals, stimulation sites, and stimulation dosage to further optimize neuromodulation therapies. Variations in response to ta-VNS in the aforementioned studies could be explained by differences in study design, stimulation dosage, and individual characteristics. Future research using the same design and stimulation protocol will help to clarify the significance of HRV in predicting treatment response. Using this marker as a response evaluation tool in a ta-VNS closed-loop system can show the response to treatment in real time and aid in optimizing patient selection and stimulation dosage. Furthermore, HRV could be compared with other trustworthy indicators of ta-VNS efficiency, such as somatosensory evoked potentials [63] and skin sympathetic nerve activity [64].

Patient-specific baseline determinants of response to ta-VNS

The heterogeneity of results among studies appears to be influenced by variations in patient-specific baseline factors. Evidence shows that baseline HRV could significantly predict response to ta-VNS, where higher resting LF/HF ratio was associated with greater decreases during ta-VNS [9, 42, 65]. This finding implies that the LF/HF ratio can be utilized to screen individuals who are more likely to benefit from ta-VNS in terms of improved autonomic function. This may make it possible to select ideal individuals for ta-VNS, which is especially important because of the wide range of disorders associated with autonomic imbalance. Bretherton et al. [42] evaluated what baseline HRV threshold can predict response to ta-VNS and found that values greater than 1.5 had a better response to therapy. This issue should be more precisely investigated in future investigations. Furthermore, baseline HRV declines with age [66, 67], and because ta-VNS responds better in individuals with lower baseline HRV [9, 42, 65], ta-VNS may be more effective in older adults than in younger individuals. Importantly, there is a U-shaped link between age and various time-domain indices such RMSSD and pNN50, with a decrease in middle-aged adults and an increase in older ages [68]. Moreover, baseline autonomic function differs significantly between men

and women; males exhibited higher LF/HF than women, indicating a higher sympathetic tone in men [69]. Future research should explore the influence of gender on the ta-VNS responsiveness rate.

Considerations in selecting stimulation protocol

The inconsistency of the results reported in the literature may also be due to variation in ta-VNS parameters including intensity, frequency, pulse width, stimulation site (tragus, concha, etc.), and side (right, left, or bilateral). Different stimulation intensity in the same nerve tissue have been shown to yield various clinical results [70]. It has been revealed that ta-VNS can produce vagus somatosensory evoked potentials in brain stem nuclei at stimulation levels lower than those that cause pain perception [71]. In addition, peripheral stimulation with a current adjusted below the pain threshold where Aβ fibers are stimulated provides therapeutic effects [72]. The intensity level in the most studies examined in this review was consistently lower than the pain threshold at the level of sensory perception. Future research should determine whether ta-VNS intensity and HRV variations are linearly related. As shown in Figs. 3, 4, 5, 6, 7, 8, and 9, we calculated another parameter, namely total electrical charge, defined by the mean intensity multiplied by the effective stimulation time, for each individual study and found no linear association between this parameter and calculated effect sizes. Another set of stimulation parameters, such as frequency and pulse width, varied between studies; hence, future dose–response studies are required to find the optimal value for these stimulation parameters. HRV and the other predictive biomarkers of response to ta-VNS can assist in identifying the most effective stimulation parameters once their function as surrogates for neuronal engagement following stimulation is precisely determined.

Conclusions

The results of the included studies were mixed, which may be mainly attributable to the heterogeneity of their study design and stimulation delivery dosage. Thus, future studies with comparable designs are required to determine the optimal stimulation parameters and clarify the significance of autonomic indices as a reliable marker of neuromodulation responsiveness. In addition, it has been shown that the worse the basic autonomic function, the better the response to transcutaneous auricular vagus nerve stimulation, suggesting the importance of patient-specific baseline factors in optimizing neuromodulation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10286-023-00938-w.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The manuscript does not contain clinical studies or patient data.

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