Medicine
Neuroscience

The effect of transcutaneous auricular vagus nerve stimulation on cardiovascular function in subarachnoid hemorrhage patients: a safety study

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Gansheng Tan, Anna L Huguenard, Kara M Donovan, Phillip Demarest, Xiaoxuan Liu, Ziwei Li, Markus Adamek, Kory Lavine, Ananth K Vellimana, Terrance T Kummer, Joshua W Osbun, Gregory J Zipfel, Peter Brunner, Eric C Leuthardt

Department of Neurosurgery, Washington University School of Medicine, St. Louis, USA • Department of Biomedical Engineering, Washington University in St. Louis, USA • Department of Neuroscience, Washington University in St. Louis, USA • Department of Neurology, Washington University in St. Louis, USA

Abstract

Introduction

Subarachnoid hemorrhage (SAH) is characterized by intense central inflammation, leading to substantial post-hemorrhagic complications such as vasospasm and delayed cerebral ischemia. Given the anti-inflammatory effect of transcutaneous auricular vagus nerve stimulation (taVNS) and its ability to promote brain plasticity, taVNS has emerged as a promising therapeutic option for SAH patients.[3,10,13] However, the effects of taVNS on cardiovascular dynamics in critically ill patients like those with SAH have not yet been investigated. Given the association between cardiac complications and elevated risk of poor clinical outcomes after SAH, it is essential to characterize the cardiovascular effects of taVNS to ensure this approach is safe in this fragile population[5]. Therefore, we assessed the impact of both acute taVNS and repetitive taVNS on cardiovascular function in this study.

Methods

In this randomized clinical trial, 24 SAH patients were assigned to either a taVNS treatment or a Sham treatment group. During their stay in the intensive care unit, we monitored patient electrocardiogram (ECG) readings and vital signs. We compared long-term changes in heart rate, heart rate variability, QT interval, and blood pressure between the two groups. Additionally, we assessed the effects of acute taVNS by comparing cardiovascular metrics before, during, and after the intervention. We also explored rapidly responsive cardiovascular biomarkers in patients exhibiting clinical improvement.

Results

We found that repetitive taVNS did not significantly alter heart rate, corrected QT interval, blood pressure, or intracranial pressure. However, taVNS increased overall heart rate variability and parasympathetic activity from 5–10 days after initial treatment, as compared to the sham treatment. Acutely, taVNS increased heart rate, blood pressure, and peripheral perfusion index without affecting the corrected QT interval, intracranial pressure, or heart rate variability. The acute post-treatment elevation in heart rate was more pronounced in patients who experienced a decrease of more than 1 point in their Modified Rankin Score at the time of discharge.

Conclusions

Our study found that taVNS treatment did not induce adverse cardiovascular effects, such as bradycardia or QT prolongation, supporting its development as a safe immunomodulatory treatment approach for SAH patients. The observed acute increase in heart rate after taVNS treatment may serve as a biomarker for SAH patients who could derive greater benefit from this treatment.

Trial registration: NCT04557618

eLife assessment

This study evaluated the role of transcutaneous auricular vagal nerve stimulation (taVNS) in patients with subarachnoid hemorrhage (SAH) randomized to taVNS vs sham, finding that those with active taVNS exhibited increased parasympathetic activity. The findings are **important** and cross-disciplinary, while the level of evidence is **solid**.

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Introduction

Subarachnoid hemorrhage (SAH) is a devastating subtype of stroke that represents a significant global health burden and causes permanent disability in approximately 30% of survivors.[1,6,40] Early brain injury can occur within the first 24 to 48 hours after ictus, which involves a cascade of elevated intracranial pressure and a subsequent drop of cerebral perfusion.[4] Systemic and local inflammation, cerebral edema, blood-brain barrier (BBB) disruption, sympathetic nervous system activation, autoregulatory failure, microthrombosis, spreading depolarizations (SDs), and inflammation have all been observed during this period.[11,12] These biological processes result in the inability of cerebral perfusion to match metabolic demands, leading to secondary brain injury and delayed cerebral ischemia that typically occurs between 3 and 14 days after the SAH.[2,7,8,9] Delayed cerebral ischemia and deleterious inflammation are major predictors of poor outcomes and morbidity. The autonomic nervous system (ANS), comprising the sympathetic nervous system and the parasympathetic nervous system, plays a critical role in maintaining physiological homeostasis.

SAH is believed to cause sympathetic predominance which plays a key role in the development of cerebral vasospasm, renders patients more susceptible to non-neurological complications, and exacerbates deleterious inflammatory processes.

Numerous interventions have been explored to address the complex pathologies of subarachnoid hemorrhage (SAH) that contribute to secondary brain injury, aiming to improve patient outcomes.[14] Transcutaneous auricular vagus nerve stimulation (taVNS) is one of the most promising therapeutic options, as recent studies have demonstrated its efficacy in reducing inflammation, improving autonomic balance and enhancing brain plasticity.[3,10,13,14] The auricular branch of the vagus nerve is a sensory nerve that innervates the external ear, including the cymba concha. Stimulating the auricular branch of the vagus nerve has been shown to activate the same brain regions as cervical vagus nerve stimulation[16]. Specifically, taVNS mediates cholinergic signaling and regulates proinflammatory responses via the inflammatory reflex (**Figure 1A**).[17,18] In this reflex, inflammatory mediators such as cytokines trigger afferent vagus nerve signaling. This afferent signal then prompts an efferent response from the vagus nerve that acts to reduce the production of pro-inflammatory cytokines.[19]

The vagus nerve also mediates cardiovascular function by regulating the autonomic system and metabolic homeostasis (**Figure 1A**).[21] In theory, taVNS increase parasympathetic activity, which can be measured as increased heart rate variability (HRV). However, some animal studies have reported a potential risk of bradycardia and decreased blood pressure associated with vagus nerve stimulation[20]. Given that critically ill patients, such as those with SAH, are extremely vulnerable to cardiovascular complications, it is essential to thoroughly examine the cardiovascular implications of taVNS to ensure its safety in this fragile population. This is particularly notable as cardiovascular abnormalities following SAH such as prolonged elevated heart rate and QT prolongation are associated with an increased risk of poor outcomes[5,22,23]. However, our limited understanding of these effects constitutes a significant barrier, preventing the advancement of taVNS from a promising therapeutic approach to an established clinical treatment for SAH. This study aimed to assess the effects of taVNS on cardiovascular function based on electrocardiogram (ECG) and other monitored vital signs from SAH patients receiving treatment in the intensive care unit (ICU). The primary outcome metrics examined in this study include heart rate, QT interval, HRV, and blood pressure. We aim to analyze change in these cardiovascular metrics at the phase of early brain injury (within 72 hours) and at the phase when delayed cerebral ischemia develops (after day 4) between patients receiving taVNS treatment and sham treatment. Root mean square of successive differences (RMSSD) and the standard deviation of normal RR intervals (SDNN) are two commonly used HRV metrics among many others. RMSSD is indicative of parasympathetic activity, while lower SDNN is associated with increased cardiac risk.[30,31] Providing effective taVNS treatment modulates the autonomic system, we expect that heart rate or HRV following acute taVNS could inform which SAH patients may experience the most clinical benefit from this therapy in the future. To this aim, we correlated the changes in heart rate and HRV following acute taVNS treatment and changes of the modified Rankin Score (mRS), which measures the degree of disability or dependence in the daily activities of people suffering from neurological disability.

Results

24 Participants were randomized to receive the taVNS (N = 11) or Sham (N = 13) treatment. The participants, the medical team who dictated all management decisions for the patient's subarachnoid hemorrhage, and the outcomes assessors who assigned modified Rankin Scores (mRS) at admission and discharge were blinded to the treatment. The structure of this study is shown in **Figure 1B**. Following randomization, enrolled patients underwent 20 minutes of either taVNS or sham stimulation twice daily during their stay in the ICU. During treatment periods, a portable transcutaneous electrical nerve stimulation (TENS) device (TENS 7000 Digital

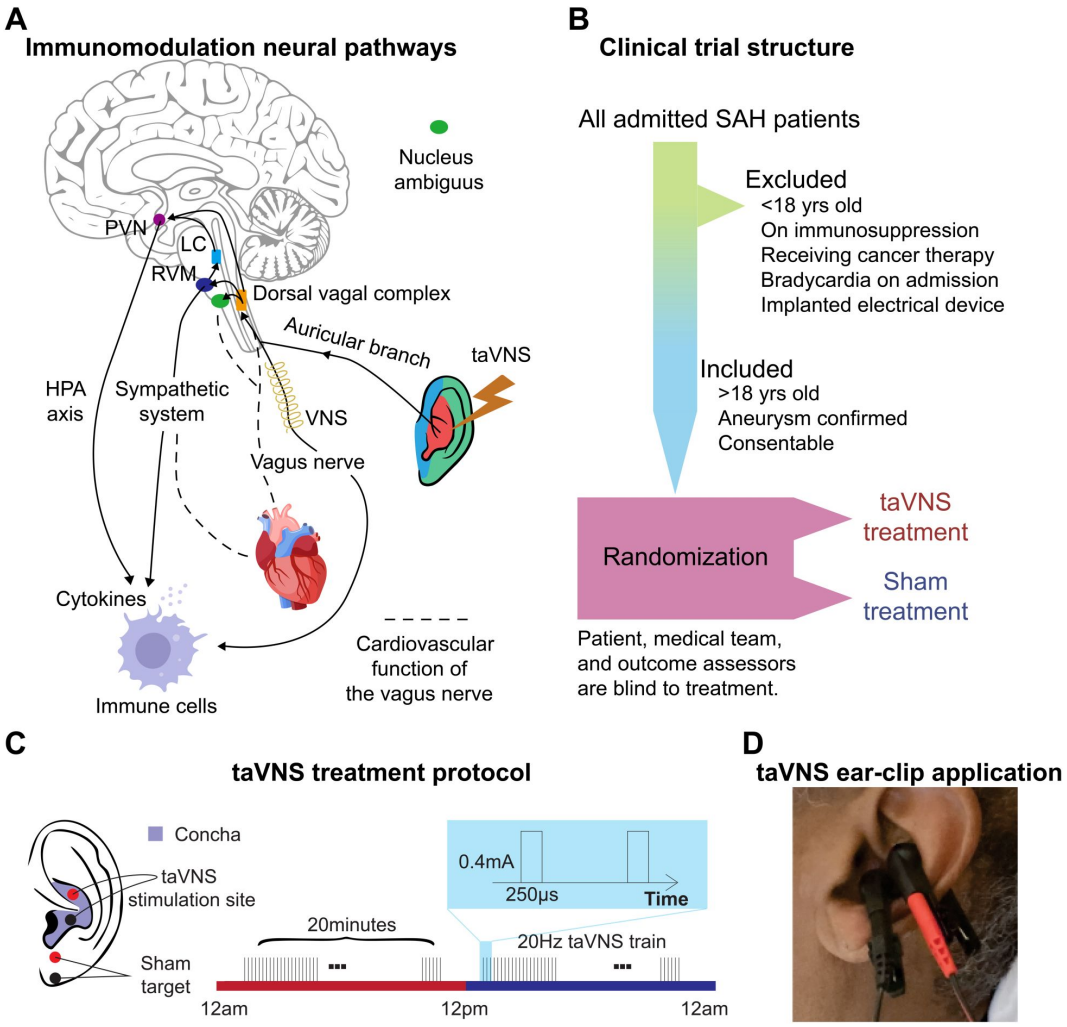

Figure 1.

Study rationale and clinical trial design.

A. Immunomodulation neural pathways associated with vagus nerve stimulation include cholinergic anti-inflammatory pathway, sympathetic nervous system, and hypothalamic-pituitary-adrenal (HPA) axis. Immunogenic stimuli activate vagal afferents terminating primarily in the dorsal vagal complex. Ascending projections from the dorsal vagal complex reach the paraventricular nucleus (PVN) and rostral ventromedial medulla (RVM), activating the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic system, respectively, to regulate the immune response. taVNS can affect cardiovascular function through the sympathetic system or efferent vagus nerve. **B**-**C**. Clinical trial structure and treatment protocol. Patients in the taVNS group received electrical stimulation (0.4 mA, 250 μs pulse width, 20 Hz) for 20 minutes, twice daily. Sham group patients wore the ear clip on the earlobe for the same duration. **D.** Ear clip application for taVNS treatment.

TENS Unit, Compass Health Brands, OH, USA) was connected to the patient's left ear using two ear clips (**Figure 1C and D**). For taVNS treatments, these ear clips were placed along the concha of the ear, while for sham treatments, the clips were placed along the earlobe to avoid stimulation of the auricular vagus nerve from tactile pressure (**Figure 1** **Figure *1C***). For the taVNS group, stimulation parameters were selected based on values reported in prior studies that sought to maximize vagus somatosensory evoked potentials while avoiding the perception of pain: 20 Hz frequency, 250 μs pulse width, and 0.4 mA intensity[24] The stimulation parameters were designed to be imperceptible to the patients, and there were no reports of detection of taVNS, suggesting the success of the blinding. No electrical current was delivered during sham treatments. Please see [26] for a detailed protocol of this study.

Effects of cumulative taVNS on cardiac function

We first investigated whether taVNS treatment induced bradycardia or QT prolongation, both potential adverse effects of vagus nerve stimulation. This analysis showed no significant differences in heart rate calculated from 24-hour ECG recording between groups (Mann–Whitney U test, N(taVNS) = 94, N(Sham)=95, p-value = 0.69, Cohen's d =-0.01, W-statistics = 4317, power = 0.93). Changes in heart rate from Day 1 was equivalent between groups (Two-tailed equivalence tests, *p*[*lower threshold*] = 0.006, test statistics[*lower threshold*] = 2.53; *p*[*lower threshold*] = 0.004, test statistics[*lower threshold*] = -2.72, N(VNS)=94, N(VNS)=95). We further confirmed that changes in heart rate were similar between treatment groups following SAH (**Figure 3A** |Cohen's d| < 0.2 for Day 2-4, Day 5-8, Day 8-10, and Day 11-13). Moreover, changes in corrected QT interval from Day 1 was higher in the Sham group compared to the taVNS group (**Figure 3B**, Mann–Whitney U test, N(taVNS) = 94, N(Sham)=95, p-value < 0.001, Cohen's d = - 0.57). After the phase of early brain injury, the mean and median corrected QT interval were lower in the taVNS group with large effect sizes (**Figure 3B**, |Cohen's d| > 0.5).

Subsequently, we investigated the effect of taVNS treatment on RMSSD and SDNN. We found that changes in SDNN using Day 1 as baseline were not significantly different between the treatment groups (T-test, N(taVNS) = 94, N(Sham) = 95, p = 0.479, Cohen's d =0.10, t statistics = 0.71, **Figure 2A**). Changes in RMSSD using Day 1 as baseline were significantly higher in the taVNS treatment group (T-test, N(taVNS) = 94, N(Sham) = 95, Bonferroni-corrected p = 0.025, Cohen's d =0.42, t statistics = 2.91, **Figure 2B**). We further studied the effects of taVNS at the phase of early brain injury and later phase. **Figure 2A-B** show the changes in SDNN and RMSSD in bins of three days for the two treatment groups. The taVNS treatment increased RMSSD over the course of the treatment, with a smaller effect size (Cohen's d = 0.29) observed between days 2-4, corresponding to the early brain injury phase, and large effect sizes at the later phases (Cohen's d = 0.41 for Days 5–7, Cohen's d = 0.54 for Days 8–10, Cohen's d = 0.66 for Days 11–13). We further tested if the RMSSD reduction rate was greater in the Sham treatment group with a linear regression model: RMSSD change ~ Day * Treatment. The results show that the RMSSD reduced slower in the taVNS treatment group when compared to the sham treatment, but this trend did not reach significance (coefficient of taVNS * Day interaction effect = 2.00, p = 0.21, Supplementary Figure 1).

RMSSD and SDNN are two of the most commonly used methods for quantifying heart rate variability. Bartlett's test indicated that there are significant correlation among these measures (p < 0.01, **Figure 3C**). To analyze the effect of taVNS treatment on the autonomic system, we factor analysis to identify the underlying factors. We focused on the two factors with the greatest eigenvalue. **Figure 3D** shows the factor loading, that is, the variance explained by heart rate variability metrics on the two factors. The first factor correlates positively with metrics representing variability, including RMSSD, SDNN, pNNI_50, and total power, and therefore has been termed Overall Heart Rate Variability. The second factor correlates positively with RMSSD and normalized high-frequency power that represents parasympathetic activity, and correlates negatively with the cardiac sympathetic index. Hence, it is termed Parasympathetic Activity. The effects of taVNS on Overall Heart Rate Variability and Parasympathetic Activity aligned with the observed effects on SDNN and RMSSD. Overall, Heart Rate Variability change from Day 1 was

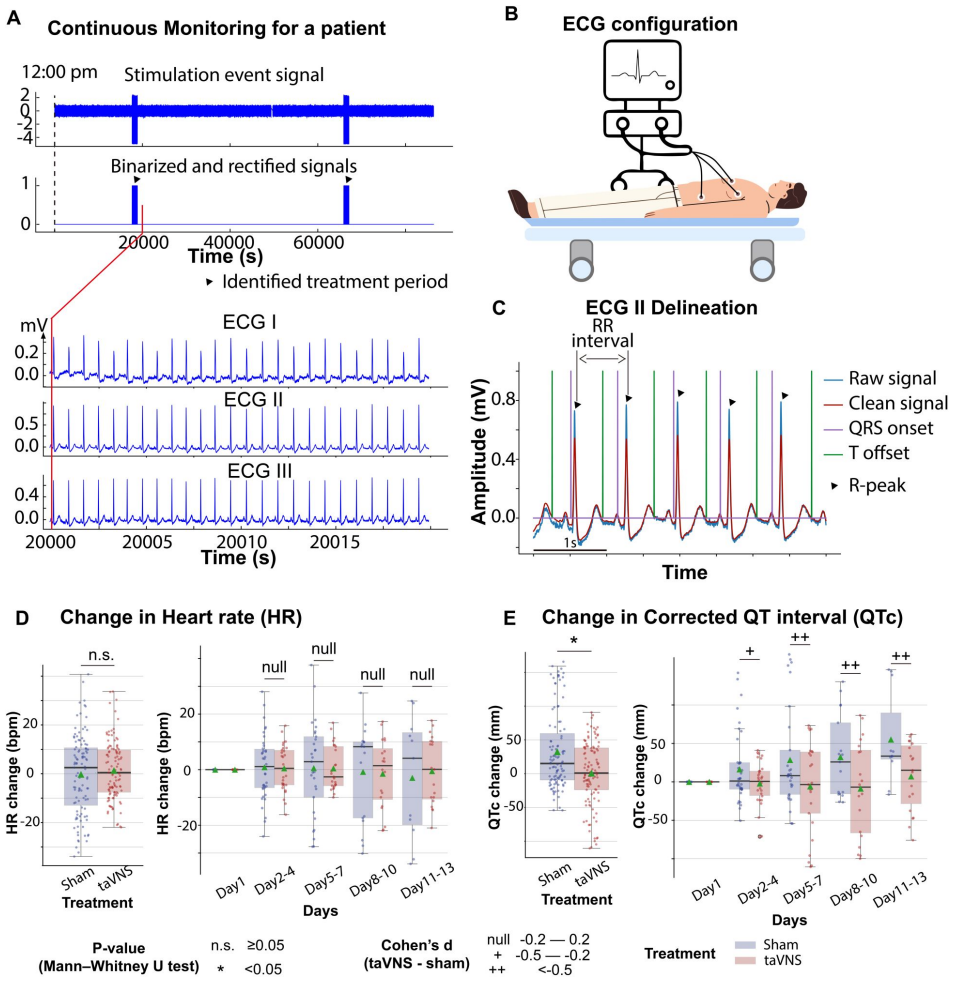

Figure 2.

The effects of taVNS on cardiac function.

A. Signals encoding treatment period and ECG signals in a representative patient. **B**. 3-lead ECG configuration in the intensive care unit. **C**. P wave, T wave, and QRS complex are delineated from clean ECG II signals. **D and E**. Heart rate and corrected QT interval changes from the first hospitalized day in the two treatment groups

significantly higher in the VNS group (**Figure 3E**, Mann–Whitney U test, N(taVNS) = 94, N(Sham)=95, p-value = 0.04, Cohen's d =0.37). The effect size was trivial between Day 2-4 and increased over the course of treatment. The parasympathetic activity was also significantly higher in the VNS treatment group, and we observed the largest effect size between Day 2-4 (**Figure 3F**).

We also investigated the potential association between clinical outcomes, as measured by changes in the mRS from admission to discharge, and heart rate variability metrics. We found that heart rate was lower in patients with improved mRS (i.e., <0) (Mann–Whitney U test, N(mRS < 0) = 122, N(mRS > 0)=98, p-value < 0.01, Cohen's d =- 0.54). Parasympathetic Activity, Overall Heart Rate Variability, and corrected QT interval did not differ significantly between patients with improved mRS and patients with worsened mRS (Supplementary Figure 3).

Cumulative effects of taVNS on vascular function

Elevated blood pressure is a common occurrence in SAH and is linked with a higher risk of re-rupture of cerebral aneurysms and vasospasm.[33,34] In this study, patients in both treatment groups received medical treatment determined by the medical team, including vasopressors and medication for blood pressure management. We investigated whether taVNS induced any additional blood pressure changes beyond those managed by the medical team. We found that the median and mean blood pressure change from the first hospitalized day were greater than 0 for both treatment groups (**Figure *4*B**). No significant differences were detected in changes in blood pressure and intracranial pressure (ICP) between the treatment groups (**Figure 4B and C**). Equivalence testing confirmed that the ICP changes from the first hospitalization day were not significantly different between treatment groups, with a 2mmHg equivalence margin (two one-sided t-tests, p[*lower threshold*] = 3.66 x 10^{-13}, t[*lower threshold*] = 8.07; p[*upper threshold*] = 3.33 x 10^{-10}, t[*upper threshold*] = -6.73). Equivalence testing also indicated that there were no significant different changes in blood pressure between treatment groups (two one-sided t-tests, p[*lower threshold*] = 0.07, t[*lower threshold*] = 1.51; p[*upper threshold*] = 0.002, t[upper threshold] = -3.00). We further verified that there were no significant changes in arterial line blood pressure obtained via continuous invasive monitoring between treatment groups (Supplementary Figure 4). We subsequently compared the Plethysmography Peripheral Perfusion Index (PPI) between the treatment groups as it is a proxy metric for cardiac stroke volume and vascular tone.[35,38] We found that PPI change was significantly lower (Mann–Whitney U test, N(taVNS) = 83, N(Sham)=95, Bonferroni corrected p < 0.01, Cohen's d =-0.49). In addition, respiration rate change was significantly higher (Mann–Whitney U test, N(taVNS) = 94, N(Sham)=95, Bonferroni corrected p = 0.02, Cohen's d =0.37) in the taVNS group, as compared to the Sham group (**Figure 4D and E**). We hypothesized that the increase in respiratory rate was a compensatory mechanism to ensure similar oxygen delivery. We found a significant negative correlation between changes in PPI and changes in respiration rate only for the taVNS treatment group (Pearson correlation coefficient = -0.37, p < 0.001, t-test, Supplementary Figure 4D). The Pearson correlation coefficient for the sham treatment group is -0.08 (p = 0.36).

Acute effects of taVNS on cardiovascular function

Understanding the acute effect of taVNS on cardiovascular function is an essential step to translating it toward clinical usage. We compared the acute change of heart rate, corrected QT interval, and heart rate variability between treatment groups as these metrics indicate cardiovascular complications and predict clinical outcomes.[36] The change in heart rate from treatment onset is shown in **Figure *5*B**. We subsequently tested whether taVNS affects changes in heart rate between post-treatment and pre-treatment. We found that the changes in heart rate were not significantly different between treatment groups although heart rate increased in the taVNS group (Wilcoxon rank-sum test, N = 188, Bonferroni corrected p = 0.03, Cohen's d =0.11) but not in the Sham group (Wilcoxon signed ranked test, N = 199, Bonferroni corrected p = 0.72,

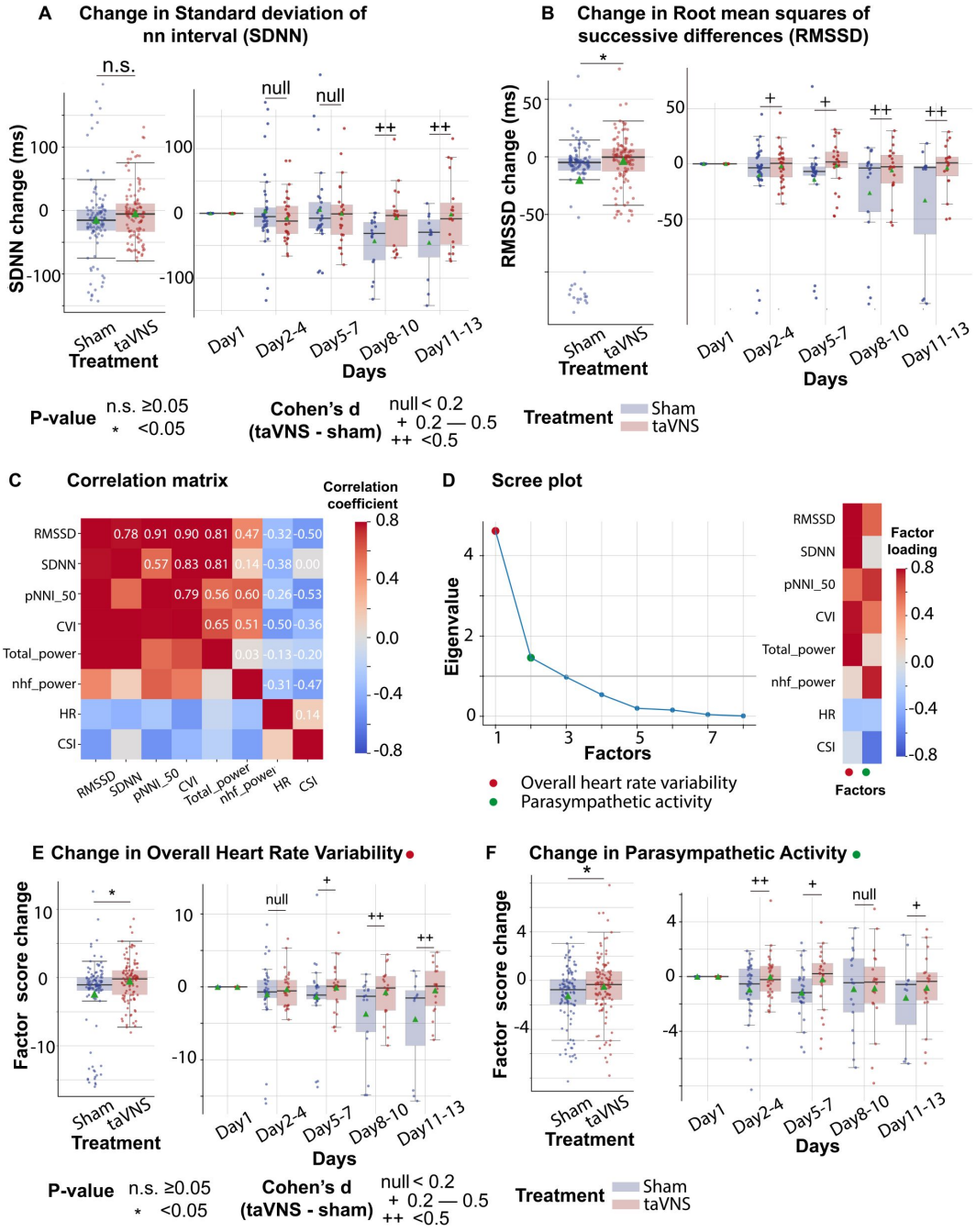

Figure 3.

The effects of taVNS on overall heart rate variability and parasympathetic activity.

A-B. Changes in standard deviation of NN interval (SDNN) changes and Root mean squares of successive differences over time for the two treatment groups. The color represents the treatment group. Green triangles represent the mean. **C**. Correlation between standard ECG features underlying autonomic activities. **D**. Factor analysis showed that there are two factors underlying the standard ECG features. The first factor is referred to as overall heart rate variability. The second factor is referred to as parasympathetic activity. **E-F**. The effect of taVNS on the two factors. pNNI_50: Percentage of Number of successive NN Intervals that differ by more than 50 ms. CVI: cardiac vagal index. Total power: total power below 0.4Hz of normal RR interval. nhf_power: relative power of the high-frequency band (0.15–0.4 Hz). CSI: cardiac sympathetic index.

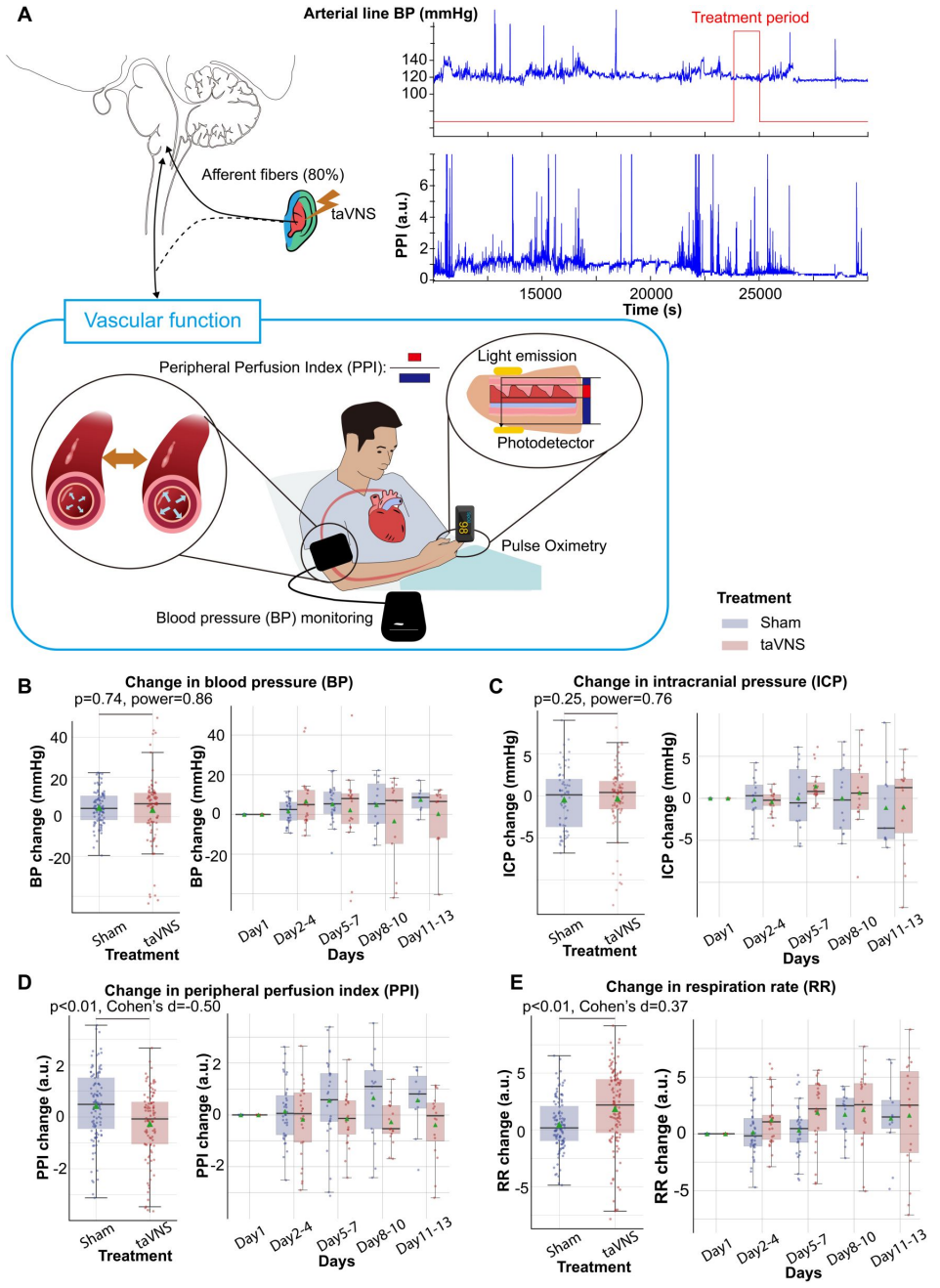

Figure 4.

The cumulative effects of taVNS on vascular function.

A. Representative vital signs and their physiology. Arterial line blood pressure (see supplementary Figure 4), intracranial pressure, and mean blood pressure measured regularly by nurses (BP) were recorded. Blood pressure is an index of vasodilation. PPI is the ratio between the pulsatile and the non-pulsatile blood flow, reflecting the cardiac output. **B-C.** Mean BP and ICP changes from the first hospitalization day did not differ significantly between the treatment groups. **D-E**. PPI change from the first hospitalized day was lower in the VNS treatment group, while RR change was higher.

Cohen's d =0.00) (**Figure 5C**). However, the increase in heart rate after taVNS was within 0.5 standard deviations of daily heart rate. There were no significant differences in changes in corrected QT interval or heart rate variability, as measured by RMSSD and SDNN, between treatment groups (**Figure 5D and E** and Supplementary Figure 5). We further asked whether heart rate can serve as a biomarker that indicates which SAH patients would receive the greatest benefit from continuing taVNS treatment. We investigated the relationship between changes in heart rate from pre- to post-taVNS treatment and changes in mRS between admission and discharge using a linear mixed-effects model. In this model, the treatment group, mRS change, and their interaction were included as fixed effects, while subject was included as a random effect. Our analysis revealed that the slope between changes in heart rate and changes in mRS is significantly more negative for the taVNS treatment group (**Table 2**). This finding suggests that an increase in heart rate following acute taVNS treatment is associated with improved clinical outcomes (**Figure 5F**). Post-hoc analysis showed that patients in the taVNS treatment group who had an improvement in mRS of - 2 or greater compared to other patients had significantly greater increases in heart rate (Mann-Whitney U test, p=0.02, N(mRS change <-1) = 53, N(mRS change >= -1) = 135, Cohen's d=0.34, Supplementary Figure 5C). Conversely, HRV change, represented by RMSSD, was not significantly different based on mRS in SAH patients (Supplementary Figure 5D).

Subsequently, we compared changes in blood pressure, PPI, ICP, and respiration rate from pre- to post-treatment periods between treatment groups. We found that changes in PPI and blood pressure were significantly higher in the taVNS group, as compared to the Sham group (Mann-Whitney U test, blood pressure: p = 0.03, Cohen's d = 0.22, N = 180 for Sham and 159 for taVNS; PPI: p < 0.01, Cohen's d = 0.19, N = 227 for Sham and 186 for taVNS, Supplementary Figure 6). Only PPI remained significantly different between treatment groups after Bonferroni correction. The acute changes in PPI and blood pressure remained within the daily standard deviation. No significant differences in post-treatment changes in ICP or respiration rate were observed between treatment groups.

Discussion

This study examined the effects of transcutaneous auricular vagus nerve stimulation (taVNS) on cardiovascular function in patients with subarachnoid hemorrhage (SAH). We investigated both the cumulative and acute impacts of taVNS. The findings in our study indicates that repetitive taVNS is not associated with previously suggested risks, such as bradycardia and QT prolongation. Furthermore, cumulative taVNS treatment increased overall heart rate variability and parasympathetic activity, which are indicators of a healthy cardiovascular system. When looking at the acute effects, taVNS only significantly increased the peripheral perfusion index but not heart rate, heart rate variability, corrected QT interval, blood pressure, or intracranial pressure. The findings are summarized in **Table 3**. Interestingly, we discovered that heart rate as a biomarker for identifying SAH patients who are most likely to benefit from taVNS treatment. Collectively, this study substantiates the safety of treating SAH patients with taVNS and provides foundational data for future efforts to optimize and translate taVNS therapy toward clinical use.

taVNS and autonomic system

The autonomic nervous system (ANS), comprising the sympathetic nervous system and the parasympathetic nervous system, plays a critical role in maintaining physiological homeostasis. These systems work synergistically to mediate interactions between the nervous and immune systems, which is thought to be the underlying mechanism for the immunomodulatory effect of taVNS. Our study is aligned with the finding that the autonomic balance is shifted toward

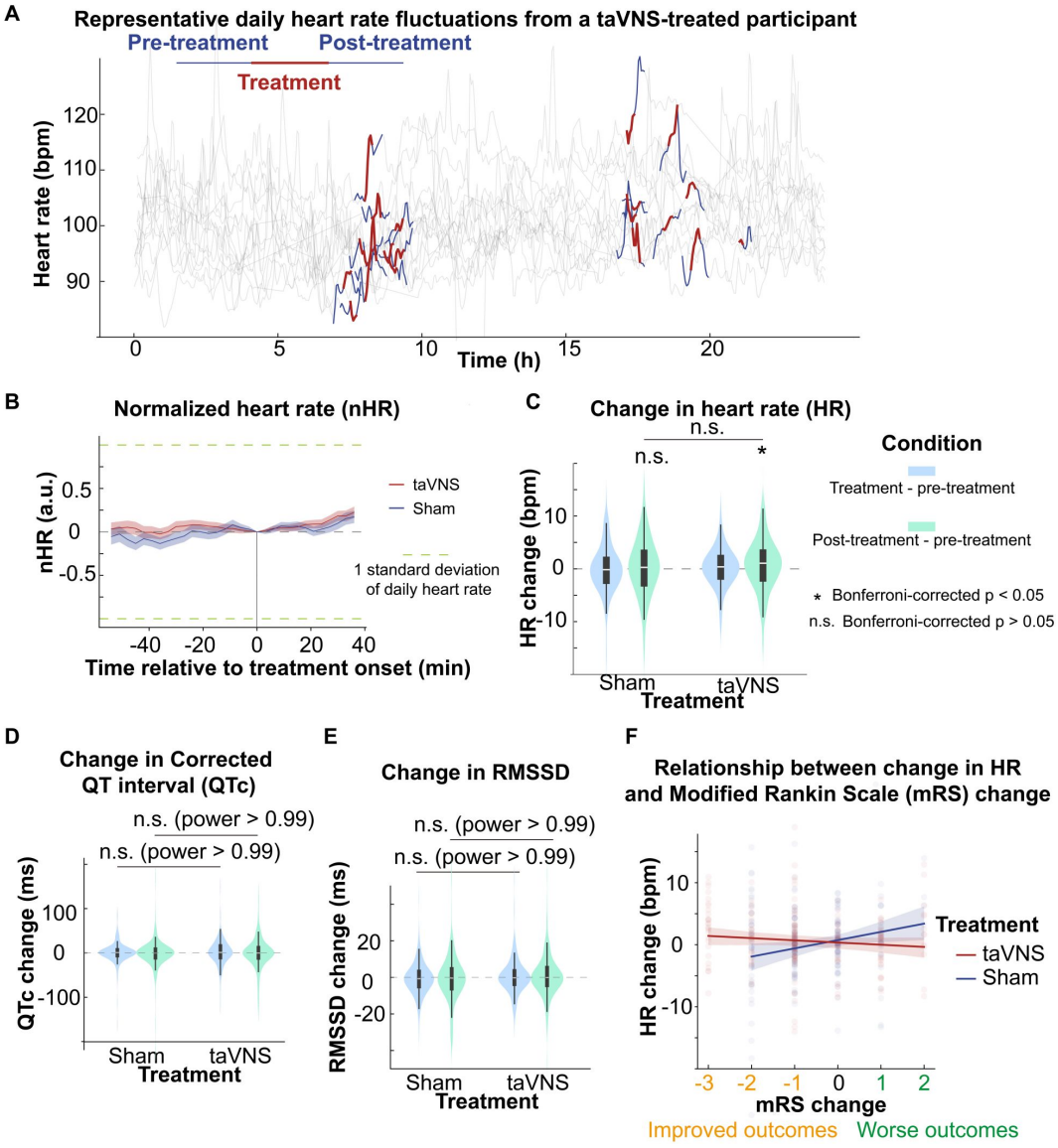

Figure 5.

The acute effects of taVNS on cardiac function.

A. Daily fluctuation of heart rate of a subject receiving VNS treatment. The treatment period, a 20-minute period before and after treatment, is highlighted. Note that a small proportion of ECG signals to derive heart rate was missing due to the expected cyclical restarting of the monitoring system. **B**. Normalized heart rate aligned at the treatment onset over time for the two treatment groups. The heart rate is normalized based on the mean and standard error of heart rate for each day. **C-E**. The difference of HR, QTc, and RMSSD between the treatment period, post-treatment period, and pre-treatment period for the two groups. Wilcoxon signed ranked test was used to test if the HR difference is statistically different from 0 in the VNS treatment group. Bonferroni-corrected p-value for HR difference between post-treatment and treatment period is 0.03 (N=188, Cohen's d = 0.1). Mann– Whitney U tests were used to compare cardiac function metric differences between the two treatment groups. **F**. The relationship between heart rate changes following acute taVNS and functional outcome.

Patient	Age	Gender	Race	HH	mRS (admission)	mRS (discharge)	Vasospasm treated by blood pressure goal augmentation	Indwelling arterial lines	ICP monitoring
1	71 – 75	M	White	3	5	5	Y	Y	Y
2	51 – 55	M	Black/African American	3	4	3	N	Y	Y
3	51 – 55	F	White	2	2	3	N	N	N
4	61 – 65	M	White	2	3	4	Y	N	N
5	66 – 70	F	White	2	2	3	Y	N	N
6	71 – 75	F	White	2	3	2	N	Y	N
7	66 – 70	M	White	4	5	4	N	Y	Y
8	61 – 65	F	White	4	5	2	N	Y	Y
9	71 – 75	F	White	2	3	0	Y	N	N
10	71 – 75	F	White	2	3	3	Y	Y	N
11	41 – 45	M	Black/African American	4	5	4	Y	Y	Y
12	46 – 50	F	White	2	4	3	N	N	N
13	51 – 55	F	Black/African American	2	2	1	Y	N	N
14	81 – 85	F	White	1	3	3	Y	N	N
15	71 – 75	F	Black/African American	4	5	3	N	N	Y
16	46 – 50	F	Black/African American	2	2	3	N	N	N
17	66 – 70	F	White	2	1	0	N	N	N
18	36 – 40	F	White	3	4	2	N	N	Y
19	61 – 65	F	White	3	4	2	N	N	Y
20	81 - 85	F	White	2	2	3	N	Y	Y
21	51 - 55	F	White	3	4	4	N	Y	Y
N22	71 - 75	F	White	1	2	4	N	N	N
23	41 – 45	F	Black/African American	2	2	4	N	Y	N
24	66 - 70	M	White	4	5	5	Y	Y	Y

Table 1

Patient demography.

HH: Hunt & Hess classification. mRS: modified Rankin Scale. Y: yes. N: no.

Table 2

Relationship between HR changes following acute taVNS and clinical outcomes.

Model	$HR_{subject,treatment}(mRS) = \beta_0 + \beta_{taVNS} \times 1_{treatment=taVNS} + (\beta_{mRS} + \beta_{taVNS,slope} \times 1_{treatment=taVNS}) \times mRS + u_{subject}$		
	Coefficient	P value	95% Confidence interval
β_0	0.73	0.211	-0.41 – 1.87
β_{taVNS}	-0.29	0.737	-1.95 – 1.38
β_{mRS}	1.47	**0.006**	0.43 – 2.51
$\beta_{taVNS,slope}$	-1.85	**0.005**	-3.12 - 0.57

Table 3.

Summary of effects of acute and repetitive taVNS on cardiovascular function in SAH patients.

Metrics for cardiovascular function include heart rate variability, heart rate, QT interval, blood pressure, intracranial pressure, peripheral perfusion index, and respiration rate.

		Positive findings	Null findings	Implications
Repetitive	Cardiac function	Increased HRV	QT interval Heart rate	Increased parasympathetic activity
	Vascular function	Reduced peripheral perfusion index Increased respiration rate	Blood pressure Intracranial pressure	Compensatory mechanisms to maintain autonomic balance
Acute	Cardiac function		Heart rate Heart rate variability QT interval	No to small acute effect
	Vascular function	Increased peripheral perfusion index	Intracranial pressure Respiration rate	

sympathetic dominance following SAH (**Figure 3**, Supplementary Figure 2). In this study, we found that dysregulation of sympathovagal balance toward sympathetic dominance could be restored by taVNS treatment.[37,42]

A key metric that reflects this restored sympathovagal balance is the increase in heart rate variability. Additionally, taVNS led to a decreased QTc without a significant change in heart rate, miming the effects observed with propranolol administration, a beta-blocker that reduces sympathetic activity[47]. This finding suggests that cumulative taVNS reduces sympathetic overactivation and influences ventricular repolarization processes. PPI is primarily influenced by cardiac output and vascular tone. Elevated PPI is associated with vasodilation and/or increased stroke volume. In the Sham group, increases in both PPI and blood pressure were observed when compared to Day 1 values (**Figure 3**). This effect may be due to higher stroke volume resulting from sympathetic activation following SAH. Alternatively, this could represent the heightened need for vasopressor interventions aiming to improve cerebral perfusion due to more robust sympathetically driven cerebral vasospasm. the PPI increase was less for the taVNS treatment group (**Figure *4***), suggesting a restored autonomic balance in the taVNS treatment group, However, the effects of taVNS on blood pressure require further investigation as more than half of the patients were on vasopressor and ionotropic drugs. Intuitively, sympathetic activation is associated with increases in both PPI and blood pressure. The blood pressure management might lead to similar blood pressure changes between the two treatment groups, Additional considerations are the differences between repetitive and acute changes associated with taVNS. Although repetitive taVNS increases heart rate variability days after initiation of the treatment, this effect is not seen acutely. Also, while repetitive taVNS was associated with a reduced PPI and no change in heart rate and blood pressure, there were small acute increases in PPI, heart rate, and blood pressure. These results indicate that taVNS might increase arousal in the acute period.[45,46] Ultimately, these speculative mechanisms warrant further validation through animal or pharmacological studies directly investigating the effects of taVNS on autonomic function and vascular tone.

Considerations for applying taVNS on SAH patients

Blood pressure management and cardiac function monitoring are crucial in patients following SAH.[33] This study shows that blood pressure, QT interval, and heart rate over days were not significantly different between taVNS and sham treatment groups. This suggests that adding taVNS in treatments for SAH patients is unlikely to cause adverse blood pressure alterations or cardiac complications. Our findings suggest that repetitive taVNS could rescue the sympathetic excitation and parasympathetic withdrawal following SAH. This effect could lead to favorable clinical outcomes as lower HRV was found to be associated with Neurocardiogenic Injury.[42,43] Additionally, reduced PPI was associated with increased respiration rate only for the taVNS treatment group, suggesting that the autonomic system self-regulates to maintain cardiovascular homeostasis. Thus, it is important to consider autonomic system self-regulation when studying the therapeutic effects of taVNS.[39] Also, it is important to note that while acute cardiovascular changes were noted after taVNS, these changes were within normal daily variations in this study, making them unlikely to pose a risk to the patient. That said, the observed acute increases in PPI following taVNS necessitate caution when considering taVNS treatment for patients to whom peripheral vasodilatation is not desired.

Limitations and outlook

While this study supports the safety of taVNS treatment in SAH patients, the findings may not be universally applicable across different patient populations. For instance, a decrease in heart rate variability was noted in the Sham group, highlighting the influence of SAH pathology on cardiovascular metrics. Importantly, additional care should be paid when interpreting the results of blood pressure, as hypertension was intentionally induced for some patients being treated for vasospasm. Patient medical histories are summarized in **Table 1**.

We note that heart rate was lower in patients with improved mRS, while other cardiovascular metrics were not different between patients with improved mRS and patients with worsened mRS (Supplementary Figure 3 and Supplementary Figure 7). Future studies should aim to delineate the relationship between cardiovascular function, the autonomic system, and SAH complications, such as vasospasm, to rigorously assess the efficacy of taVNS treatment.

Conclusions

Utilizing taVNS as a neuromodulation technique in SAH patients appears to be safe without inducing bradycardia or QT prolongation. Repetitive taVNS treatment increased parasympathetic activity. Acute taVNS elevated heart rate, which might be an acute biomarker to identify SAH patients who are likely to respond favorably to taVNS treatment.

Methods details

Study Participants

Participants in this study were recruited from adult patients who were admitted to the ICU at Barnes Jewish Hospital, St. Louis, MO, following an acute, spontaneous, aneurysmal SAH. Inclusion criteria were: **(1)** Patients with SAH confirmed by CT scan; **(2)** Age > 18; **(3)** Patients or their legally authorized representative are able to give consent. Exclusion criteria were: **(1)** Age < 18; **(2)** Use of immunosuppressive medications; **(3)** Receiving ongoing cancer therapy; **(4)** Implanted electrical device; **(5)** Bradycardia on admission; **(6)** Considered moribund/at risk of imminent death. Participants were randomized to receive either the taVNS (N = 11) or Sham (N = 13) treatment. Patients were enrolled prior to randomization by a member of the research team who went through the informed consent process with the patient or their legally authorized representative. Treatment group assignment was via a computer-generated randomization sequence, with the next number obscured until patient enrollment. Research team members who applied the ear clips and set stimulation parameters were not blinded to the treatment. The participants, the medical team who dictated all management decisions for the patient's subarachnoid hemorrhage, and the outcomes assessors who assigned modified Rankin Scores (mRS) at admission and discharge were blinded to the treatment. The structure of this study is shown in **Figure 1B**. This study was approved by the Washington University School of Medicine Review Board and was conducted in accordance with institutional and national ethics guidelines and the Declaration of Helsinki (Clinical trial number: NCT04557618).

taVNS protocol

Following randomization, enrolled patients underwent 20 minutes of either taVNS or sham stimulation twice daily during their stay in the ICU. During treatment periods, a portable transcutaneous electrical nerve stimulation (TENS) device (TENS 7000 Digital TENS Unit, Compass Health Brands, OH, USA) was connected to the patient's left ear using two ear clips (**Figure 1C and D**). For taVNS treatments, these ear clips were placed along the concha of the ear, while for sham treatments, the clips were placed along the earlobe to avoid stimulation of the auricular vagus nerve from tactile pressure (**Figure 1** **Figure *1C***). For the taVNS group, stimulation parameters were selected based on values reported in prior studies that sought to maximize vagus somatosensory evoked potentials while avoiding the perception of pain: 20 Hz frequency, 250 μs pulse width, and 0.4 mA intensity[24]. The stimulation was not perceptible for the patients. No electrical current was delivered during sham treatments. For both groups, the TENS device was connected to the patient and a bedside recording computer. The computer recorded continuous ECG and vital signs, including blood pressure, temperature, respiration rate, peripheral perfusion index, intracranial pressure, and arterial blood pressure. The collection of intracranial pressure

and arterial blood pressure data varied, being dependent on the treatment protocol assigned by the clinical team, and thus was not uniformly available for all patients throughout the study. Please see [26] for a detailed protocol of this study.

Data processing

A 3-lead system was used for electrocardiograms (ECG). ECG signals, sampled at 500 Hz, and other vital signs, such as blood pressure, sampled at 1 Hz, were recorded from the Intellivue patient monitor (Philips®, Netherlands) using vitalDB software.[27]

To calculate cardiac metrics, we first applied a 0.5 Hz fifth-order high-pass Butterworth filter and a 50 Hz powerline filter on ECG data to reduce artifacts. We detected QRS complexes based on the steepness of the absolute gradient of the ECG signal using Neurokit2 software package.[28] R-peaks were detected as local maxima in the QRS complexes. P-waves, T-waves, and QRS waves were delineated based on the wavelet transform (**Figure 2A-C**).[29] RR intervals were preprocessed to exclude outliers, defined as RR intervals greater than 2 s or less than 300 ms. RR intervals with > 20% relative difference to the previous interval were considered ectopic beats and excluded from analyses. After preprocessing, RR intervals were used to calculate heart rate, heart rate variability, and corrected QT based on Bazett's formula.[41] Heart rate variability measures included the root mean square of successive difference of normal RR intervals (RMSSD), which indicates parasympathetic activity, and the standard deviation of normal RR intervals (SDNN), which is a clinical measure of cardiac risk.[30,31] Heart rate variability calculations are detailed in Supplementary Materials.

To investigate the effect of repetitive taVNS on cardiovascular function, we compared heart rate variability, heart rate, corrected QT intervals, blood pressure, and intracranial pressure calculated over 24 hours between patients receiving taVNS and sham treatment. In addition, we compared the mean peripheral perfusion index and respiration rate over 24 hours between treatment groups to determine the effects of repetitive taVNS on the autonomic system. Data collection commenced on the first day of each patient's ICU admission. The average duration of continuous data recording was 11.1 days, with a standard deviation of 6.8 days. To analyze the effects of taVNS treatment more granularly, we segmented the changes in these metrics from the initial day at three-day intervals, facilitating comparison between the taVNS and sham treatment groups over the course of their ICU stay.

To study the effects of acute treatment over time, we focused on blood pressure, heart rate variability, heart rate, and corrected QT intervals 20 minutes before treatment (pre-treatment), during the 20-minute treatment (during-treatment), and 20 minutes after treatment (post-treatment). The treatment event signals were rectified and binarized based on their half-maximum value to identify the treatment onset and offset (). We calculated metrics using 6-minute sliding windows over ECG data starting from treatment onset/offset and moving bi-directionally with a 3-minute step. To correct daily and between-subject variation, we applied the same sliding window strategy to calculate the mean and standard deviation of these cardiac metrics for each patient for each day as a reference. Subsequently, heart rate variability, heart rate, and corrected QT interval around treatment onset/offset were normalized based on the reference. In addition, we calculated the difference in blood pressure, heart rate variability, heart rate, and corrected QT intervals between during-treatment and pre-treatment, and the difference between post-treatment and pre-treatment for each patient and for each treatment. To study the effects of acute taVNS, we compared the two differences between the treatment groups.

Factor Analysis

We performed an exploratory factor analysis to identify the factors underlying autonomic system activity. Besides RMSSD and SDNN, variables derived from preprocessed RR intervals and used to perform factor analysis included the percentage of successive normal-to-normal (NN) Intervals

that differ by more than 50 ms (pNNI_50), total power (below 0.4 Hz), normalized high-frequency power (0.15-0.4Hz), cardiac vagal index, and cardiac sympathetic index. The total power is thought to represent the overall heart rate variability, while normalized high-frequency power primarily reflects parasympathetic activity[30]. These variables were normalized using a z-score method based on individual daily means and standard deviations before factor analysis. Factor analysis was performed using the factor_analyzer Python package. The number of factors was set to 2 based on the Scree plot. The factor loadings were calculated using the Minimum Residual Method. After factor extraction, a Varimax rotation was applied for better interpretability, so that each factor has high loadings for a smaller number of variables and low loadings for the remaining variables.

Statistical Analyses

To investigate the effect of taVNS at the phase of early brain injury and later phases, we grouped the change of heart rate variability, heart rate, and corrected QT interval from the first hospitalized day in bins of three days. The change in blood pressure, intracranial pressure, respiration rate, and peripheral perfusion index from the first hospitalization day were also compared between treatment groups. We used t-tests for comparisons between treatment groups when the data were normally distributed, as determined by the Shapiro-Wilk test. We employed Mann–Whitney U tests for non-normally distributed data. We used Wilcoxon signed-rank tests to compare the difference in heart rate between post-treatment and during-treatment against 0. To control the familywise error rate, we applied Bonferroni correction. Specifically, when investigating the cardiac effects of taVNS, we compared six metrics between treatment groups, including heart rate, corrected QT interval, RMSSD, SDNN, and two factors representing heart rate variability. Consequently, the p-values were corrected by a factor of six. Power analysis was performed based on the t-test, assuming the medium effect size (Cohen's d = 0.5). Two one-sided tests were used to confirm that taVNS did not induce long-term changes in heart rate, corrected QT interval, or blood pressure, with equivalency test margins set to 5 bpm for heart rate, 50 ms for QT interval, and 2 mmHg for blood pressure. A summary of statistical tests is provided in Supplementary Table 1.

Data Availability

All data produced in the present study are available upon reasonable request to the authors. *https://github.com/GanshengT/taVNS_SAH*

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Authors' contributions

Gansheng Tan: Conceptualization, Methodology, Investigation, Formal analysis, Software, Writing – original draft, Writing – review & editing. **Anna L. Huguenard**: Conceptualization, Funding acquisition, Methodology, Data curation, Writing - original draft, Writing – review & editing. **Kara M. Donovan**: Writing – review & editing. **Philip Demarest**: Writing – review & editing. **Xiaoxuan Liu**: Methodology, Writing – review & editing. **Ziwei Li**: Methodology, Writing – review & editing.

Markus Adamek: Methodology, Software. **Kory Lavine**: Writing – review & editing. **Ananth K. Vellimana**: Data curation, Writing – review & editing. **Terrance T. Kummer:** Data curation, Writing – review & editing. **Joshua W. Osbun**: Data curation, Writing – review & editing. **Gregory J. Zipfel**: Funding acquisition, Resources, Writing - review & editing. **Peter Brunner**: Funding acquisition, Resources, Supervision, Writing - review & editing. **Eric C. Leuthardt**: Conceptualization, Supervision, Funding acquisition, Writing – review & editing, Writing – original draft.

Declaration of competing interest

Eric Leuthardt has stock ownership in Neurolutions, Face to Face Biometrics, Caeli Vascular, Acera, Sora Neuroscience, Inner Cosmos, Kinetrix, NeuroDev, Inflexion Vascular, Aurenar, Cordance Medical, Silent Surgical, and Petal Surgical. He is a consultant for E15, Neurolutions, Inc., Petal Surgical. Washington University owns equity in Neurolutions.

Anna Huguenard has stock ownership in Aurenar.

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Editors

Reviewing Editor
Olujimi Ajijola
University of California, Los Angeles, Los Angeles, United States of America

Senior Editor
Olujimi Ajijola
University of California, Los Angeles, Los Angeles, United States of America

Reviewer #1 (Public Review):

The authors report the results of a randomized clinical trial of taVNS as a neuromodulation technique in SAH patients. They found that taVNS appears to be safe without inducing bradycardia or QT prolongation. taVNS also increased parasympathetic activity, as assessed by heart rate variability measures. Acute elevation in heart rate might be a biomarker to identify SAH patients who are likely to respond favorably to taVNS treatment. The latter is very important in light of the need for acute biomarkers of response to neuromodulation treatments.

Comments:

(1) Frequency domain heart rate variability measures should be analyzed and reported. Given the short duration of the ECG recording, the frequency domain may more accurately reflect autonomic tone.

(2) How was the "dose" chosen (20 minutes twice daily)?

(3) The use of an acute biomarker of response is very important. A bimodal response to taVNS has been previously shown in patients with atrial fibrillation (Kulkarni et al. JAHA 2021).

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Reviewer #2 (Public Review):

Summary:

This study investigated the effects of transcutaneous auricular vagus nerve stimulation (taVNS) on cardiovascular dynamics in subarachnoid hemorrhage (SAH) patients. The researchers conducted a randomized clinical trial with 24 SAH patients, comparing taVNS treatment to a Sham treatment group (20 minutes per day twice a day during the ICU stay). They monitored electrocardiogram (ECG) readings and vital signs to assess acute as well as middle-term changes in heart rate, heart rate variability, QT interval, and blood pressure between the two groups. The results showed that repetitive taVNS did not significantly alter heart rate, corrected QT interval, blood pressure, or intracranial pressure. However, it

increased overall heart rate variability and parasympathetic activity after 5-10 days of treatment compared to the sham treatment. Acute taVNS led to an increase in heart rate, blood pressure, and peripheral perfusion index without affecting corrected QT interval, intracranial pressure, or heart rate variability. The acute post-treatment elevation in heart rate was more pronounced in patients who showed clinical improvement. In conclusion, the study found that taVNS treatment did not cause adverse cardiovascular effects, suggesting it is a safe immunomodulatory treatment for SAH patients. The mild acute increase in heart rate post-treatment could potentially serve as a biomarker for identifying SAH patients who may benefit more from taVNS therapy.

Strengths:

The paper is overall well written, and the topic is of great interest. The methods are solid and the presented data are convincing.

Weaknesses:

(1) It should be clearly pointed out that the current paper is part of the NAVSaH trial (NCT04557618) and presents one of the secondary outcomes of that study while the declared first outcomes (change in the inflammatory cytokine TNF-α in plasma and cerebrospinal fluid between day 1 and day 13, rate of radiographic vasospasm, and rate of requirement for long-term CSF diversion via a ventricular shunt) are available as a pre-print and currently under review (doi: 10.1101/2024.04.29.24306598.). The authors should better stress this point as well as the potential association of the primary with the secondary outcomes.

(2) The references should be implemented particularly concerning other relevant papers (including reviews and meta-analysis) of taVNS safety, particularly from a cardiovascular standpoint, such as doi: 10.1038/s41598-022-25864-1 and doi: 10.3389/fnins.2023.1227858).

(3) The dose-response issue that affects both VNS and taVNS applications in different settings should be mentioned (doi: 10.1093/eurheartjsupp/suac036.) as well as the need for more dose-finding preclinical as well as clinical studies in different settings (the best stimulation protocol is likely to be disease-specific).

Overall, the present work has the important potential to further promote the usage of taVNS even on critically ill patients and might set the basis for future randomized studies in this setting.

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Reviewer #3 (Public Review):

Summary:

The authors aimed to characterize the cardiovascular effects of acute and repetitive taVNS as an index of safety. The authors concluded that taVNS treatment did not induce adverse cardiovascular effects, such as bradycardia or QT prolongation.

Strengths:

This study has the potential to contribute important information about the clinical utility of taVNS as a safe immunomodulatory treatment approach for SAH patients.

Weaknesses:

A number of limitations were identified:

(1) A primary hypothesis should be clearly stated. Even though the authors state the design is a randomized clinical trial, several aspects of the study appear to be exploratory. The method of randomization was not stated. I am assuming it is a forced randomization given the small sample size and approximately equal numbers in each arm.

(2) The authors "first investigated whether taVNS treatment induced bradycardia or QT prolongation, both potential adverse effects of vagus nerve stimulation. This analysis showed no significant differences in heart rate calculated from 24-hour ECG recording between groups." A justification should be provided for why a difference is expected from 20 minutes of taVNS over a period of 24 hours. Acute ECG changes are a concern for increasing arrhythmic risk, for example, due to cardiac electrical restitution properties.

(3) More rigorous evaluation is necessary to support the conclusion that taVNS did not change heart rate, HRV, QTc, etc. For example, shifts in peak frequencies of the high-frequency vs. low-frequency power may be effective at distinguishing the effects of taVNS. Further, compensatory sympathetic responses due to taVNS should be explored by quantifying the changes in the trajectory of these metrics during and following taVNS.

(4) The authors do not state how the QT was corrected and at what range of heart rates. Because all forms of corrections are approximations, the actual QT data should be reported along with the corrected QT.

(5) The QT extraction method needs to be more robust. For example, in Figure 2C, the baseline voltage of the ECG is shifting while the threshold appears to be fixed. If indeed the threshold is not dynamic and does not account for baseline fluctuations (e.g., due to impedance changes from respiration), then the measures of the QT intervals were likely inaccurate.

(6) More statistical rigor is needed. For example, in Figure 2D, the change in heart rate for days 5-7, 8-10, and 11-13 is clearly a bimodal distribution and as such, should not be analyzed as a single distribution. Similarly, Figure 2E also shows a bimodal distribution. Without the QT data, it is unclear whether this is due to the application of the heart rate correction method.

(7) Figure 3A shows a number of outliers. A SDNN range of 200 msec should raise concern for a non-sinus rhythm such as arrhythmia or artifact, instead of sinus arrhythmia. Moreover, Figure 3B shows that the Sham RMSSD data distribution is substantially skewed by the presence of at least 3 outliers, resulting in lower RMSSD values compared to taVNS. What types of artifact or arrhythmia discrimination did the authors employ to ensure the reported analysis is on sinus rhythm? The overall results seem to be driven by outliers.

(8) The above concern will also affect the power analysis, which was reported by authors to have been performed based on the t-test assuming the medium effect size, but the details of sample size calculations were not reported, e.g., X% power, t-test assumed Bonferroni correction in the power analysis, etc.

(9) If the study was designed to show a cardiovascular effect, I am surprised that N=10 per group was considered to be sufficiently powered given the extensive reports in the literature on how HRV measures (except when pathologically low) vary within individuals. Moreover, HRV measures are especially susceptible to noise, artifacts, and outliers.

If the study was designed to show a lack of cardiovascular effect (as the conclusions and introduction seem to suggest), then a several-fold larger sample size is warranted.

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