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Original Articles

Non-invasive Vagus Nerve Stimulation in Healthy Humans Reduces Sympathetic Nerve Activity

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ABSTRACT

Background: Vagus nerve stimulation (VNS) is currently used to treat refractory epilepsy and is being investigated as a potential therapy for a range of conditions, including heart failure, tinnitus, obesity and Alzheimer's disease. However, the invasive nature and expense limits the use of VNS in patient populations and hinders the exploration of the mechanisms involved.
Objective: We investigated a non-invasive method of VNS through electrical stimulation of the auricular branch of the vagus nerve distributed to the skin of the ear – transcutaneous VNS (tVNS) and measured the autonomic effects.
Methods: The effects of tVNS parameters on autonomic function in 48 healthy participants were investigated using heart rate variability (HRV) and microneurography. tVNS was performed using a transcutaneous electrical nerve stimulation (TENS) machine and modified surface electrodes. Participants visited the laboratory once and received either active (200 μs, 30 Hz; $n = 34$) or sham ($n = 14$) stimulation.
Results: Active tVNS significantly increased HRV in healthy participants ($P = 0.026$) indicating a shift in cardiac autonomic function toward parasympathetic predominance. Microneurographic recordings revealed a significant decrease in frequency ($P = 0.0001$) and incidence ($P = 0.0002$) of muscle sympathetic nerve activity during tVNS.
Conclusion: tVNS can increase HRV and reduce sympathetic nerve outflow, which is desirable in conditions characterized by enhanced sympathetic nerve activity, such as heart failure. tVNS can therefore influence human physiology and provide a simple and inexpensive alternative to invasive VNS.

Introduction

Electrical stimulation of the cervical vagus nerve has been approved for treatment resistant epilepsy in Europe and the USA for over 15 years and has been used to treat over 50,000 epilepsy patients [1]. VNS is also an approved therapy for treatment resistant depression in the USA [2] and has been investigated as a potential therapy for a wide range of conditions including heart failure [3], inflammation [4], Alzheimer's disease [5], obesity [6], chronic pain [7] and tinnitus [8]. However, despite positive indications from pilot studies, larger scale trials are rarer. For example, even though the cognitive function of 70% of patients with Alzheimer's disease improved or did not decline during a 1 year pilot study [5], no larger scale trials have been reported.

One factor that may hinder larger trials is the invasive nature of VNS. VNS requires surgical implantation of a bipolar electrode around the cervical vagus nerve and implantation of a generator subcutaneously in the thoracic wall. This is associated with technical and surgical complications including wound infection, cardiac arrhythmia under test stimulation and electrode malfunction [9]. In addition, side effects include hoarseness, dysphagia, cough and pain [10].

Given the number of conditions that VNS has the potential to benefit, a simpler, less invasive approach would enable treatment of significantly larger numbers. A potential non-invasive route for VNS is electrical stimulation of the auricular branch of the vagus nerve (ABVN), which is distributed to the external ear (Fig. 1) [11]. This stimulation can be performed transcutaneously by applying surface electrodes or acupuncture needles to the external ear (tVNS). Such

Abbreviations: ABVN, auricular branch of the vagus nerve; HF, high frequency; HRV, heart rate variability; LF, low frequency; MSNA, muscle sympathetic nerve activity; TENS, transcutaneous electrical nerve stimulation; tVNS, transcutaneous vagus nerve stimulation; VNS, vagus nerve stimulation.

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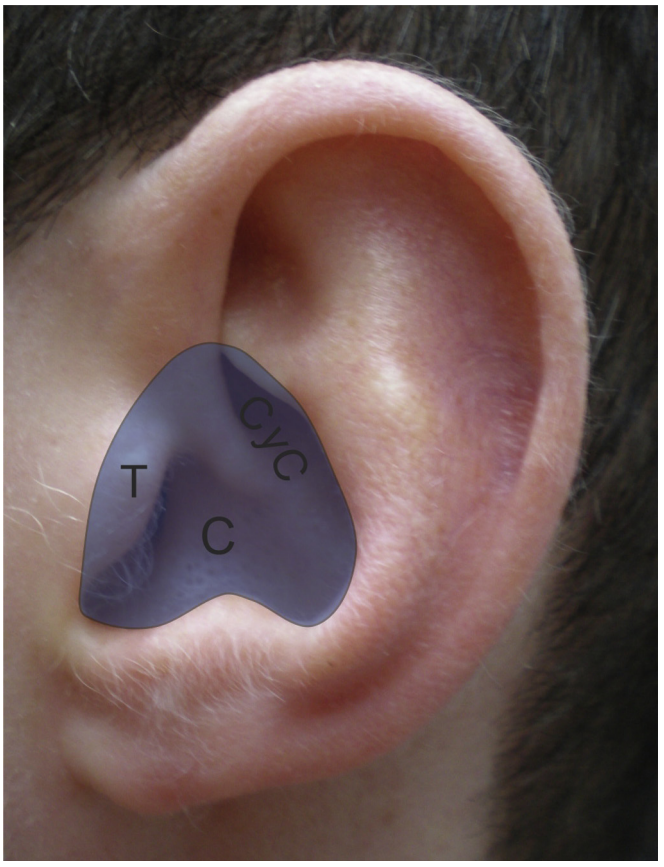

Figure 1. The distribution of the auricular branch of the vagus nerve to the external ear (shaded area, extrapolated from Peuker and Filler [11]). C, concha; T, tragus; CyC, cymba concha.

tVNS has been trialed in patients with coronary artery disease [12,13], epilepsy [14] and chronic pain [15], however, the outcomes investigated are varied and mostly subjective. In addition, the stimulation parameters used differ widely, therefore, little is known about the optimal parameters for tVNS.

VNS has proven effective in pilot studies for the treatment of heart failure [16], for which a multi-center trial is on-going (ClinicalTrials.gov Identifier: NCT01303718). Approximately 5.7 million people in the US have heart failure [17] costing the US economy $34.4 billion every year [18]. Heart failure is a leading cause of mortality and it is estimated that 50% of people die within 5 years of diagnosis. Heart failure is characterized by decreased parasympathetic and increased sympathetic nerve activity [19]. Therefore, if tVNS can be shown to influence this autonomic balance toward parasympathetic predominance it could provide a method to correct imbalance in heart failure patients.

In this study we investigated the effects of tVNS on cardiovascular autonomic function in healthy participants by measuring heart rate variability. We then applied microneurography to record muscle sympathetic nerve activity directly during tVNS.

Methods

General protocol

The study was approved by the University of Leeds Ethics Committee and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants. 48 healthy participants (24 female, 24 male; 20–62 years old) were recruited for the study. Inclusion criteria were male or female over the age of 18 years. Exclusion criteria were a history of cardiovascular disease, diabetes or hypertension. The study began between 8 and 10am in a dedicated study room at 21 ± 2 °C. All participants were asked to avoid alcohol and intense exercise 12 h prior to attendance. They were also asked to avoid caffeine and nicotine on the morning of the study and to void their bladder before the study commenced. Participants were asked to lie on a couch in a semi-supine position while heart rate, blood pressure and respiration were monitored continuously. Data were recorded at baseline, during tVNS and during recovery and each recording period lasted 15 min. All participants visited the laboratory once and received either active or sham transcutaneous vagus nerve stimulation (tVNS).

Transcutaneous vagus nerve stimulation (tVNS)

tVNS was performed using a Transcutaneous Electrical Nerve Stimulation (TENS) device (V-TENS Plus, Body Clock Health Care Ltd, UK) with modified surface electrodes. Electrodes were placed on the inner and outer surface of the tragus of the ear. Active tVNS ($n = 34$) was applied continuously for 15 min with a pulse width of 200 μs and pulse frequency of 30 Hz. Amplitude was adjusted to the level of sensory threshold (10–50 mA). Sham tVNS ($n = 14$) was performed by placing the electrodes on the tragus and increasing amplitude until the participant reported feeling sensation. Participants were then told that the amplitude would be reduced slightly to prevent discomfort but the electrode leads were disconnected from the TENS machine without the participants' knowledge.

Heart rate variability (HRV)

A three lead ECG was used to monitor and record heart rate. Electrodes (Ambu, UK) were placed on left and right clavicles and costal margins. This arrangement enabled changing electrode polarities to select the lead that detected the most prominent *R* peak for subsequent HRV analysis (normally lead II). HRV was analyzed offline using LabVIEW software (National Instruments, USA). A threshold was set to detect *R* peaks from an 8 min ECG recording and R–R intervals used to produce a tachogram. The ECG was inspected to ensure all *R* peaks were detected and there were no abnormalities in the ECG such as ectopic beats (e.g. premature ventricular complexes). Ectopic beats could be corrected using a linear spline to average the R–R interval prior to and following the ectopic. If more than 2 ectopics were detected the recording was excluded. The resulting tachogram underwent 512 point Fast Fourier Transform with a Hanning window to calculate the power spectrum of HRV with the low frequency (LF) component at 0.04–0.15 Hz and the high frequency (HF) component at 0.15–0.40 Hz. LF and HF power were also converted to normalized units as a percentage of the total power to determine LF/HF ratio. The HF component reflects parasympathetic modulation of heart rate [20] and the LF component reflects both sympathetic and parasympathetic modulation of heart rate [21]. The ratio of low frequency (LF) and high frequency (HF) oscillations of heart rate variability can be used as an index of cardiac autonomic balance such that a decrease in LF/HF ratio indicates a shift in cardiac autonomic balance toward parasympathetic predominance and thus an improvement in HRV [20,22]. It is important to note that this may be due to either a decrease in sympathetic activity or an increase in parasympathetic activity.

Respiration

A piezo-electric transducer (Pneumotrace, UFI, USA) was placed round the thorax to monitor and record respiration rate. A

Table 1
Baseline characteristics. There was no significant difference in baseline characteristics between active and sham tVNS groups. Sham tVNS Active tVNS.

	Sham	Active
Number	14 (6 ♀; 8 ♂)	34 (18 ♀; 16 ♂)
Age (years)	38 ± 3.48	34 ± 2.3
BMI (kg/m^2)	23.9 ± 0.67	24.9 ± 0.71
Heart rate (bpm)	64 ± 2.49	64 ± 1.31
Mean BP (mm Hg)	79 ± 3.59	83 ± 1.99

respiration rate <10 breaths/min was unacceptable for HRV analysis as the HF component is respiration dependent. At slow respiration rates the HF peak of the HRV spectrum can merge with the LF peak [23]. In this case the subjects were asked to use a breathing metronome set at 16 breaths/min ($n = 3$).

Microneurography

Muscle sympathetic nerve activity (MSNA) was recorded as previously described [24–26] in 10 volunteers receiving active tVNS (8 male, 2 female; 29–59 years). Two tungsten microelectrodes were inserted percutaneously below the knee. One electrode was inserted into the peroneal nerve (recording electrode) and the second was inserted into subcutaneous tissue 1–2 cm away (reference electrode). The raw nerve signal was amplified (×50 k), filtered (0.7–2 kHz; Neurolog) and digitized (16 kHz; Power 1401, CED). The data were displayed in real time and recorded on a PC (Dell laptop) using Spike2 (version 7; CED). This allowed inspection of the nerve signal during the experiment. The recording microelectrode was manipulated until a single unit could be visualized. To confirm that this was a sympathetic vasoconstrictor unit the following conditions were met; 1) the unit occurred in diastole, 2) there was no increase in activity in response to brushing the skin of the leg, 3) activity increased in response to cold pressor test or isometric handgrip test (Fig. 4A). Cold pressor test comprised placing one hand in ice water (approximately 4 °C) for 1 min. Isometric handgrip test involved squeezing a handgrip at 50% maximal voluntary contraction for 2 min. Further confirmation was obtained during offline analysis by superimposing all putative MSNA units to ensure the amplitude and shape remained constant indicating that these were recorded from the same axon (Fig. 4B,C). MSNA bursts were also inspected by rectifying and integrating (time constant 0.1 s) the neurogram. MSNA single unit frequency (per min) was calculated. MSNA single unit incidence (per 100 heart beats) was also calculated to limit the effect of any changes in heart rate. Data were normalized to baseline due to a high degree of inter-individual variation.

Data acquisition

ECG, MSNA, blood pressure and respiration data were split into two channels and fed into two data amplification systems (Coulbourn Lab Sinc V, Coulbourn Ltd, USA and Neurolog, CED, UK). Channels were independently calibrated before digitization and storage on PCs. Data channels were then displayed on monitors using LabVIEW (National Instruments, USA) and Spike2 (CED, UK) software. The data were sampled at 12–16 kHz and stored on hard drives.

Statistical analysis

All statistical analyzes were carried out using SPSS (version 18). Independent *t*-tests or Mann–Whitney *U* test were used to compare group characteristics. Repeated measures ANOVA was used to analyze time effect (baseline, stimulation, recovery) in each group alone with post hoc Bonferroni correction. To analyze the effects of active and sham tVNS, a mixed mode ANOVA with group (active or sham stimulation) and time (baseline, stimulation, recovery) was used. Where interactions were revealed, post hoc analyzes were undertaken using repeated measures ANOVA on each group separately. The Greenhouse–Geisser correction was used where data did not meet sphericity. Linear regression was used to explore the relationship between variables. Data are presented as group mean ± standard error of the mean (S.E.M.) unless stated otherwise. A 2-tailed probability value <0.05 was considered statistically significant.

Results

tVNS significantly alters heart rate variability

Baseline characteristics of the active and sham tVNS groups were not significantly different (Mann–Whitney *U* test, $P > 0.05$; Table 1). Repeated measures ANOVA revealed a significant decrease in LF/HF ratio during active tVNS (time effect, $P = 0.026$; Table 2; Fig. 2). There was no significant change in LF/HF ratio in the sham group ($P > 0.05$). There was a modest but significant decrease in heart rate ($P < 0.005$) during active and sham tVNS. There was also a significant increase in mean BP ($P < 0.005$) during active and sham tVNS that did not recover. This finding is likely to be due to the method of measurement (Finometer) as there was no significant change in BP measurements taken using an arm sphygmomanometer (see Supplementary Figure 2) whereas the increase in BP measured using the Finometer persisted into the recovery period suggesting that the increase may be due to constriction and edema in the finger [27].

Response to tVNS is correlated with baseline LF/HF ratio

Linear regression revealed a relationship between baseline LF/HF ratio and the change in LF/HF ratio during tVNS such that a higher LF/HF ratio predicts a greater response to tVNS ($R^2 = 0.58$; $P < 0.0005$; Fig. 3A). Higher baseline LF/HF values were also observed with increasing age ($R^2 = 0.19$; $P = 0.013$; Fig. 3B).

tVNS reduces muscle sympathetic nerve activity

A significant decrease in MSNA frequency (time effect, $P = 0.001$) and incidence (time effect, $P = 0.002$; Fig. 4) was detected during tVNS ($n = 10$) using microneurography to directly record sympathetic vasoconstrictor nerve activity. Eight of these 10

Table 2
Heart rate variability values for sham and active tVNS groups There was a significant decrease in LF/HF ratio during active tVNS ($P = 0.026$). There was no significant (n.s.) change in total power, low power or high power during active tVNS. There was no significant change in any HRV values in the sham tVNS group.

	Base	Stimulation	*P*	Base	Stimulation	*P*
Total power (ms^2)	2463.75±732.50	2789.22±843.02	n.s.	2735.31±422.23	3212.17±497.45	n.s.
LF Power (ms^2)	615.52±168.65	664.63±160.85	n.s.	906.39±133.74	821.49±177.62	n.s.
HF Power (ms^2)	1109.41±395.90	1286.40±469.05	n.s.	972.67±208.32	1043.02±178.65	n.s.
LF/HF	1.16±0.30	1.19±0.32	n.s.	1.26±0.15	1.04±0.14	**0.026**

Significant *P* values are highlighted in bold.

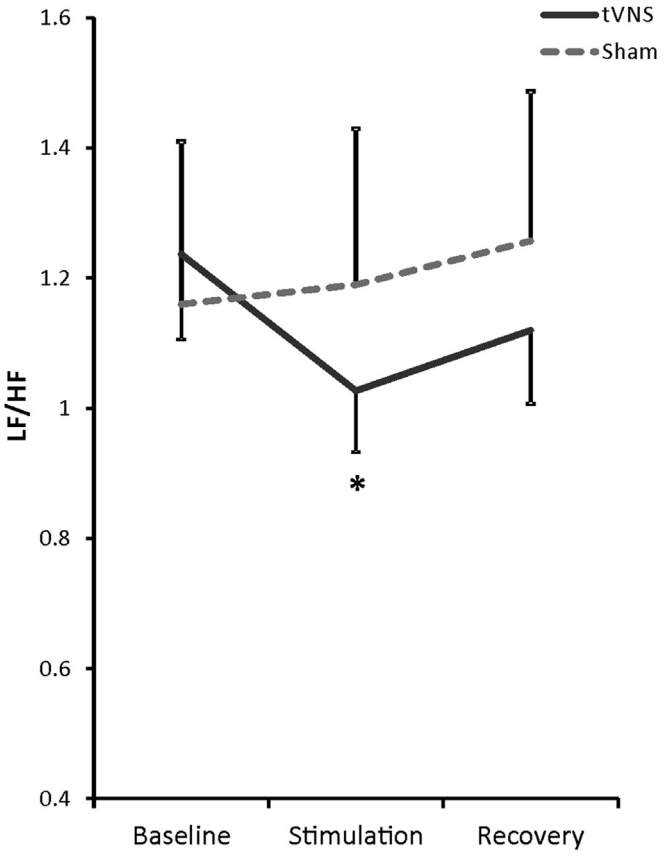

Figure 2. There is a significant decrease in LF/HF ratio during active tVNS ($P = 0.026$) whereas there is no significant change during sham tVNS.

participants responded to tVNS with a decrease in MSNA, whilst the remaining 2 showed no change, which corresponded with the effects of tVNS on HRV on these individuals (Supplementary Figure 3). These participants did not differ in baseline characteristics (age, BMI, heart rate, blood pressure etc) from the rest of the tVNS group or sham groups (Kruskal–Wallis test, $P > 0.05$).

Discussion

This study shows that transcutaneous vagus nerve stimulation (tVNS) can alter cardiovascular autonomic control in healthy humans and highlights the role of the sympathetic nervous system in mediating tVNS effects. tVNS significantly decreased LF/HF ratio, indicating improved heart rate variability with a shift in cardiac autonomic balance toward parasympathetic/vagal dominance. This shift occurred alongside a decrease in MSNA, revealed by microneurography during tVNS.

tVNS effects on cardiovascular autonomic function

Increased sympathetic activity and/or reduced parasympathetic nerve activity as indicated by HRV is not only a powerful and independent predictor of poor prognosis in patients with cardiovascular disease [28,29], but also a risk factor for mortality in healthy populations [30]. Similarly, increased MSNA is associated with poor prognosis in heart failure and is also elevated in hypertension, obstructive sleep apnea and obesity [31]. The ability to favorably alter HRV and MSNA through tVNS in a healthy population is significant and could be applied to many populations where cardiovascular autonomic balance is shifted toward sympathetic predominance e.g. older or sedentary [32] populations or in conditions with sympathoexcitation such as heart failure [33]. Indeed, the significant correlation between baseline LF/HF ratio and the change in LF/HF ratio during stimulation implies that tVNS may be even more effective in these populations compared to the healthy population used in this study.

Of particular interest in this study is the finding that the LF/HF ratio and MSNA remain lower than baseline levels during the recovery period after tVNS has ceased. The stimulation and recovery period lasted 15 min therefore the long term effects of tVNS on cardiovascular autonomic control require further investigation, however, HRV effects of tVNS performed using acupuncture increased HF power (indicating increased parasympathetic activity) for at least an hour after stimulation had ceased [34]. The increase in HF power reported by this study [34] is contrary to our findings. This may be due to the smaller sample size used ($n = 12$), the different tVNS technique used or the limitation of HRV analysis as an indirect measure of cardiac autonomic

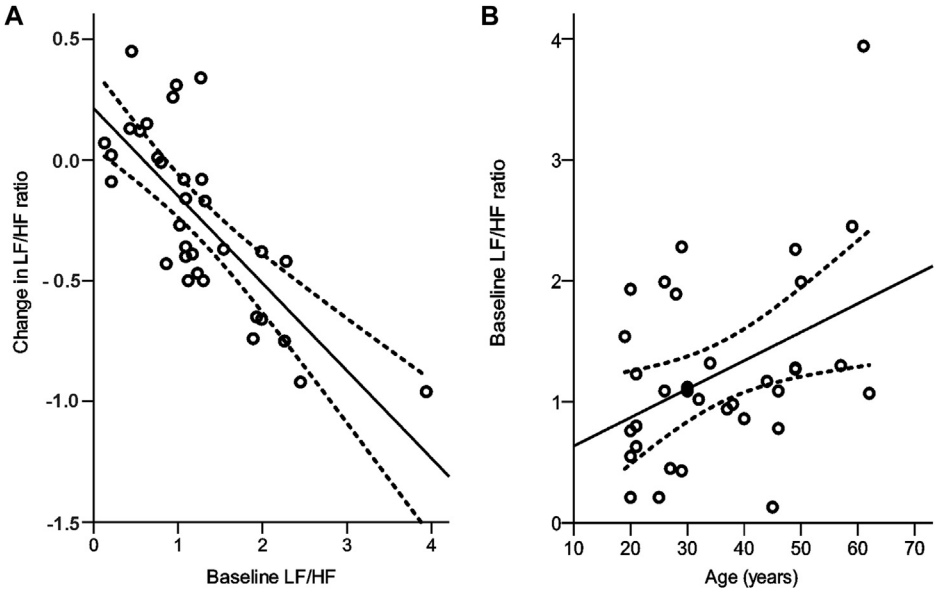

Figure 3. There is a relationship between baseline LF/HF ratio and change in LF/HF ratio during tVNS indicating that higher LF/HF ratios predict a greater decrease in LF/HF during tVNS ($R^2 = 0.58$; $P < 0.0005$; A). There is a relationship between age and baseline LF/HF ratio ($R^2 = 0.19$; $P = 0.013$; B).

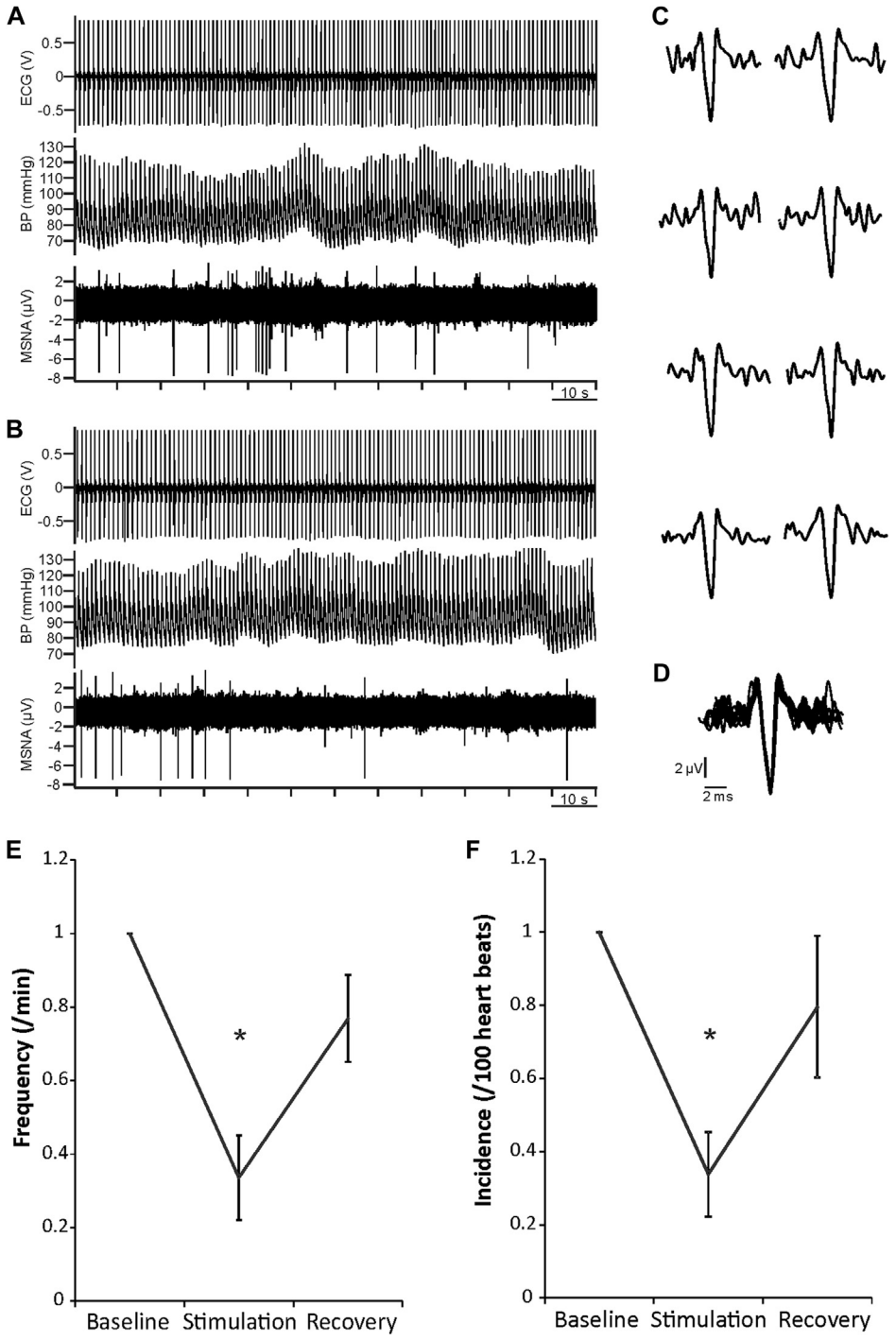

Figure 4. Example microneurography recording during baseline (A) and during tVNS (B) indicating electrocardiogram (ECG), blood pressure (BP) and MSNA. MSNA units in more detail (C) and overlaid (D). tVNS significantly reduces MSNA frequency (E; $^{*}P = 0.0001$) and incidence (F; $^{*}P = 0.0002$; normalized data; $n = 10$).

activity. La Marca et al. [35] demonstrated that auricular electroacupuncture increases respiratory sinus arrhythmia (RSA, mediated by the vagus nerve) in healthy participants ($n = 14$) suggesting increased vagal activity, however, this is also an indirect measure of parasympathetic activity. One of the limitations of these studies, including ours, is that they have all used healthy participants. The extent of the tVNS effects that might be observed in some patient groups, sedentary or older populations which characteristically have reduced parasympathetic activity therefore seems likely to be underestimated.

The first clinical study of tVNS found that electroacupuncture of both ears was beneficial for patients with coronary artery disease [12,13]. Patients ($n = 10$) received tVNS for 15 min/day for 10 consecutive days. After 4 treatments, angina symptoms at rest were abolished and patients no longer required vasodilators. After 7 treatments, patients had improved exercise tolerance and were able to climb 5–7 flights of stairs without developing angina symptoms. These studies also reported that the improvement in angina symptoms persisted after the cessation of tVNS treatment for up to 3 weeks. Recently, tVNS using surface electrodes has been

investigated as a possible analgesic [15,36] and has also been trialed as an alternative to invasive cervical VNS in patients with refractory epilepsy [14]. These studies also monitored heart rate and blood pressure and reported no significant changes. Napadow et al. [15] also analyzed ECG data for HRV and found tVNS had no significant effect ($n = 10$). These findings are contrary to the results of our study, however this seems likely to be due to the smaller sample sizes and the different stimulation parameters used.

Potential pathways of tVNS cardiovascular autonomic effects

The neurocircuitry underlying tVNS autonomic effects requires further elucidation. The auricular branch of the vagus nerve has previously received little attention and hence there is a dearth of information regarding its central projections and its peripheral distribution. Only one study in the literature investigates the distribution of the ABVN to the external ear [11]. This reports that the ABVN is distributed to the tragus, concha, cymba concha and anti-helix of the ear. EEG studies have shown that tVNS of the tragus elicits vagus somatosensory evoked potentials (VSEP) [37,38]. Interestingly, stimulation at other sites in the ear not supplied by the ABVN (helix, anti-helix, scapha and lobe) did not elicit VSEPs supporting evidence that the tragus is innervated by the ABVN. Functional MRI of tVNS of the tragus in humans revealed a similar activation pattern to conventional cervical VNS, further supporting the potential of tVNS at the tragus as a non-invasive alternative [39].

The central projections of the ABVN have been investigated in cats and dogs and were found to project to the nucleus tractus solitarius (NTS), which plays an integral role in relaying vagal afferent visceral information [40,41]. In addition, neuronal tracing from the junction of the concha and external auditory meatus in rats revealed sensory afferent terminations in the NTS and dorsomedial spinal trigeminal tract [42]. Indeed, the ABVN is thought to be involved in some peculiar somatovisceral reflexes. These include the ear-cough reflex (Arnold's reflex), estimated to be present in approximately 4% of the general population [43], whereby stimulation of the external auditory meatus (e.g. syringing) mimics the cough response mediated by vagal afferent innervation of the trachea. Other examples include the ear-gag reflex, ear-lacrimal reflex and auricular syncope [43,44] although these are rare. Another interesting phenomenon involving the ABVN is pain referred to the external ear from viscera supplied by the vagus nerve in conditions such as lung cancer [45–47], gastroeosophageal reflux [48] and myocardial infarction [49,50]. Furthermore, cervical vagus nerve stimulation has also been reported to cause ear pain [16,51].

Based on the results of this study, the proposed pathway of tVNS autonomic effects could involve activation of the NTS by ABVN afferents. This could activate the caudal ventrolateral medulla to inhibit the rostral ventrolateral medulla and thus reduce sympathetic output [52]. In addition, the NTS could also activate the dorsal motor nucleus of the vagus and the nucleus ambiguus to increase parasympathetic activity [53]. However, the effects of tVNS on parasympathetic activity are unclear.

Scope of tVNS therapy for cardiovascular diseases

VNS is already being trialed as a potential heart failure therapy and has resulted in positive clinical outcomes [3]. Our findings support the use of tVNS as a non-invasive method of VNS for cardiovascular diseases. Of particular interest is the finding that tVNS reduces sympathetic outflow. Sympathoexcitation is the hallmark of many conditions including heart failure, hypertension and obstructive sleep apnea [31]. Further, auricular electroacupuncture has beneficial effects in coronary artery disease [12,13]. The tVNS approach described here may therefore offer a simple, non-invasive and economical alternative that could make vagus nerve stimulation a widely available therapy and potentially improve quality of life for patients with a broad range of cardiovascular diseases.

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Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.brs.2014.07.031

References

[1] Shahwan A, Bailey C, Maxiner W, Harvey AS. Vagus nerve stimulation for refractory epilepsy in children: more to VNS than seizure frequency reduction. Epilepsia 2009;50(5):1220–8.
[2] Milby AH, Halpern CH, Baltuch GH. Vagus nerve stimulation for epilepsy and depression. Neurotherapeutics 2008;5(1):75–85.
[3] De Ferrari GM, Crijns HJGM, Borggrefe M, et al. Chronic vagus nerve stimulation: a new and promising therapeutic approach for chronic heart failure. Eur Heart J 2011;32(7):847–55.
[4] De Herdt V, Bogaert S, Bracke KR, et al. Effects of vagus nerve stimulation on pro- and anti-inflammatory cytokine induction in patients with refractory epilepsy. J Neuroimmunol 2009;214(1–2):104–8.
[5] Merrill CA, Jonsson MAG, Minthon L, et al. Vagus nerve stimulation in patients with Alzheimer's disease: additional follow-up results of a pilot study through 1 year. J Clin Psychiatry 2006;67(8):1171–8.
[6] Val-Laillet D, Biraben A, Randuineau G, Malbert CH. Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs. Appetite 2010;55(2):245–52.
[7] Kirchner ABF, Stephan H, Handwerker HO. Left vagus nerve stimulation suppresses experimentally induced pain. Neurology 2000;55(8):1167–71.
[8] Engineer ND, Riley JR, Seale JD, et al. Reversing pathological neural activity using targeted plasticity. Nature 2011;470(7332):101–4.
[9] Spuck S, Tronnier V, Orosz I, et al. Operative and technical complications of vagus nerve stimulator implantation. Neurosurgery 2010;67(2 Suppl. Operative):489–94.
[10] Elliott RE, Morsi A, Kalhorn SP, et al. Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: long-term outcomes and predictors of response. Epilepsy Behav 2011;20(1):57–63.
[11] Peuker ET, Filler TJ. The nerve supply of the human auricle. Clin Anat 2002;15(1):35–7.
[12] Zamotrinsky A, Afanasiev S, Karpov RS, Cherniavsky A. Effects of electrostimulation of the vagus afferent endings in patients with coronary artery disease. Coron Artery Dis 1997;8(8–9):551–8.
[13] Zamotrinsky AV, Kondratiev B, de Jong JW. Vagal neurostimulation in patients with coronary artery disease. Auton Neurosci 2001;88(1–2):109.
[14] Stefan H, Kreiselmeyer G, Kerling F, et al. Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: a proof of concept trial. Epilepsia 2012;53(7):e115–8.
[15] Napadow V, Edwards RR, Cahalan CM, et al. Evoked pain analgesia in chronic pelvic pain patients using respiratory-gated auricular vagal afferent nerve stimulation. Pain Med 2012;13(6):777–89.
[16] Schwartz PJ, De Ferrari GM, Sanzo A, et al. Long term vagal stimulation in patients with advanced heart failure first experience in man. Eur J Heart Fail 2008;10(9):884–91.
[17] Members WG, Roger VL, Go AS, et al. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 2012;125(1):e2–220.
[18] Heidenreich PA, Trogdon JG, Khavjou OA, et al. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation 2011;123(8):933–44.
[19] Bibevski S, Dunlap M. Evidence for impaired vagus nerve activity in heart failure. Heart Fail Rev 2011;16(2):129–35.
[20] Chapleau M, Sabharwal R. Methods of assessing vagus nerve activity and reflexes. Heart Fail Rev 2011;16(2):109–27.
[21] Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 1981;213(4504):220–2.
[22] Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology. Heart rate variability : standards of measurement, physiological interpretation, and clinical use. Circulation 1996;93(5):1043–65.
[23] Malliani A. Heart rate variability: from bench to bedside. Eur J Intern Med 2005;16(1):12.
[24] Macefield VG, Wallin BG, Vallbo AB. The discharge behaviour of single vasoconstrictor motoneurones in human muscle nerves. J Physiol 1994;481(Pt 3):799–809.

[25] Greenwood JP, Stoker JB, Mary DASG. Single-unit sympathetic discharge. Circulation 1999;100(12):1305–10.
[26] Clancy JA, Johnson R, Raw R, Deuchars SA, Deuchars J. Anodal Transcranial Direct Current Stimulation (tDCS) over the motor cortex increases sympathetic nerve activity. Brain Stimul 2014;7(1):97–104.
[27] Ristuccia HL, Grossman P, Watkins LL, Lown B. Incremental bias in Finapres estimation of baseline blood pressure levels over time. Hypertension 1997;29(4):1039–43.
[28] Kleiger RE, Miller JP, Bigger Jr JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987;59(4):256–62.
[29] Nolan J, Batin PD, Andrews R, et al. Prospective study of heart rate variability and mortality in chronic heart failure : results of the United Kingdom Heart Failure Evaluation and Assessment of Risk Trial (UK-Heart). Circulation 1998;98(15):1510–6.
[30] Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D, Ducimetiere P. Heart-rate profile during exercise as a predictor of sudden death. N Engl J Med 2005;352(19):1951–8.
[31] Charkoudian N, Rabbitts JA. Sympathetic neural mechanisms in human cardiovascular health and disease. Mayo Clin Proc 2009;84(9):822–30.
[32] Mischel NA, Mueller PJ. (In)activity-dependent alterations in resting and reflex control of splanchnic sympathetic nerve activity. J Appl Physiol 2011;111(6):1854–62.
[33] Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. The sympathetic nervous system in heart failure: physiology, pathophysiology, and clinical implications. J Am Coll Cardiol 2009;54(19):1747.
[34] Haker E, Egekvist H, Bjerring P. Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. J Auton Nerv Syst 2000;79(1):52.
[35] La Marca R, Nedeljkovic M, Yuan L, Maercker A, Ehlert U. Effects of auricular electrical stimulation on vagal activity in healthy men: evidence from a three-armed randomized trial. Clin Sci 2010;118(8):537–46.
[36] Busch V, Zeman F, Heckel A, Menne F, Ellrich J, Eichhammer P. The effect of transcutaneous vagus nerve stimulation on pain perception – an experimental study. Brain Stimul 2013;6(2):202–9.
[37] Fallgatter AJ, Neuhauser B, Herrmann MJ, et al. Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. J Neural Transm 2003;110(12):1437–43.
[38] Polak T, Markulin F, Ehlis A-C, Langer J, Ringel T, Fallgatter A. Far field potentials from brain stem after transcutaneous Vagus nerve stimulation: optimization of stimulation and recording parameters. J Neural Transm 2009;116(10):1237–42.
[39] Kraus T, Hosl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. J Neural Transm 2007;114:1485–93.
[40] Chien C, Shieh J, Ling E, Tan C, Wen C. The composition and central projections of the internal auriculae nerves of the dog. J Anat 1996;189(2):349–62.
[41] Nomura S, Mizuno N. Central distribution of primary afferent fibers in the Arnold's nerve (the auricular branch of the vagus nerve): a transganglionic HRP study in the cat. Brain Res 1984;292(2):199–205.
[42] He W, Jing XH, Zhu B, et al. The auriculo-vagal afferent pathway and its role in seizure suppression in rats. BMC Neurosci 2013;14(1):85.
[43] Gupta D, Verma S, Vishwakarma S. Anatomic basis of Arnold's ear-cough reflex. Surg Radiol Anat 1986;8(4):217–20.
[44] Thaker A, Deepak KK, Kumar SS. Auricular syncope. J Laryngol Otol 2008;122(10):1115–7.
[45] Abraham PJ, Capobianco DJ, Cheshire WP. Facial pain as the presenting symptom of lung carcinoma with normal chest radiograph. Headache 2003;43(5):499–504.
[46] Palmieri A. Lung Cancer presenting with unilateral facial pain: remission after laryngeal nerve palsy. Headache 2006;46(5):813–5.
[47] Bindoff L, Heseltine D. Unilateral facial pain in patients with lung cancer: a referred pain via the vagus? Lancet 1988;331(8589):812–5.
[48] Blau JN. Ear pain referred by the vagus. BMJ 1989;299(6715):1569–70.
[49] Amirhaeri S, Spencer D. Myocardial infarction with unusual presentation of otalgia: a case report. Int J Emerg Med 2010;3(4):459–60.
[50] Rothwell PM. Angina and mycardial infarction presenting with pain confined to the ear. Postgrad Med J 1993;69(810):300–1.
[51] Myers DE. Vagus nerve pain referred to the craniofacial region. A case report and literature review with implications for referred cardiac pain. Br Dent J 2008;204(4):187–9.
[52] Guyenet PG. The sympathetic control of blood pressure. Nat Rev Neurosci 2006;7(5):335–46.
[53] Izzo PN, Deuchars J, Spyer KM. Localization of cardiac vagal preganglionic motoneurones in the rat: Immunocytochemical evidence of synaptic inputs containing 5-hydroxytryptamine. J Comp Neurol 1993;327(4):572–83.