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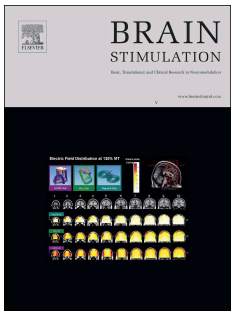

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Non-invasive vagus nerve stimulation acutely improves spontaneous cardiac baroreflex sensitivity in healthy young men: A randomized placebo-controlled trial

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ABSTRACT

Background: Despite positive outcomes of transcutaneous vagus nerve stimulation (tVNS) via the auricular branch of the vagus nerve (ABVN), the mechanisms underlying these outcomes remain unclear. Additionally, previous studies have not been controlled the possible placebo effects of tVNS.

Objective: To test the hypothesis that tVNS acutely improves spontaneous cardiac baroreflex sensitivity (cBRS) and autonomic modulation, and that these effects are specific to stimulation of ABVN.

Methods: Thirteen healthy men (23±1yrs) were randomized across three experimental visits. In active tVNS, electrodes were placed on the tragus of the ear and electrical current was applied by using a Transcutaneous Electrical Nerve Stimulation device. A time-control visit was performed with the electrodes placed on tragus, but no current was applied (sham-T). Additionally, to avoid a placebo effect, another sham protocol was performed with same electrical current of the active visit, but the electrodes were placed on the ear lobe (an area without cutaneous nerve endings from the vagus – tLS). Beat-to-beat heart rate (HR) and blood pressure (BP) were monitored at rest, during stimulation (active, sham-T and tLS) and recovery. cBRS was measured via sequence technique. Both HR (HRV) and BP variability (BPV) were also measured.

Results: Arterial BP and BPV were not affected by any active or sham protocols (P>0.05). Resting HR and LF/HF ratio of HRV decreased (Δ–3.4±1% and Δ–15±12%, P<0.05, respectively) and cBRS increased (Δ24±8%, P<0.05) during active tVNS, but were unchanged during both sham protocols.

Conclusion: tVNS acutely improves cBRS and autonomic modulation in healthy young men.

Key-words: baroreflex; autonomic nervous system; sympathetic nervous system; neuromodulation.

INTRODUCTION

It is well established that some diseases are accompanied by severe cardiac autonomic dysfunction characterized by sustained excessive sympathetic outflow and parasympathetic withdrawal (e.g. hypertension, diabetes, heart failure, coronary artery disease, obesity) [1-4]. Given the significant financial costs associated with the development of novel pharmaceutical drugs, there is increasing interest in non-pharmacological alternatives.

Electrical vagus nerve stimulation (VNS) has been approved for use in treatment-resistant patients with epilepsy and major depressive disorder and has been further suggested as a potential treatment for a broad range of conditions including Alzheimer's disease, heart failure, inflammation, chronic pain, diabetes, tinnitus and obesity [5-12]. However, VNS requires an invasive surgical procedure for electrode implantation and has been associated with adverse side-effects such as dysphonia, vocal hoarseness and dyspnea [13, 14]. As such, this limits the application of VNS to patients who are treatment-resistant to all existing pharmacological approaches. Given these considerations, non-invasive transcutaneous vagus nerve stimulation (tVNS) via the auricular branch of the vagus nerve (ABVN) has been developed, with similar efficacy to the invasive technique [15] and beneficial outcomes for patients with epilepsy [16, 17], coronary artery disease [18] and major depressive disorder [19].

The physiological mechanisms behind VNS-induced changes in cardiovascular function have been investigated over the past few years. An underlying mechanism that may explain the improvements in autonomic function by VNS is the increase in arterial baroreflex sensitivity. In this context, a recent study demonstrated that invasive VNS resets the baroreflex function and induces sympathoinhibition in rats [20]. However, the effect of VNS on arterial baroreflex sensitivity in humans requires further investigation.

Clancy et al. [21] demonstrated in humans that tVNS acutely improved cardiac autonomic modulation assessed by heart rate (HR) variability (HRV), likely to be at least in part due to a reduction in sympathetic nerve activity measured with microneurography. More recently, De Couck et al. [22] verified that short (10-min) and long term (60-min) tVNS slightly improves HRV in healthy subjects. Unfortunately, these previous studies did not measure spontaneous cardiac baroreflex sensitivity (cBRS).

Additionally, both Clancy et al. [21] and De Couck et al. [22] compared active tVNS (electrical current applied on ABVN) with control protocol (electrodes placed on the ABVN but no current was applied). Such approach can possibly bias the interpretation about the tVNS effect, because tVNS induces nuisance, which not occurs during the control protocol. In this sense, the subject's expectancy about the intervention becomes unpredictable, such that subjects may think the tVNS is either beneficial (i.e., placebo) or harmful (i.e., nocebo). Placebo and nocebo effects, in turn, could *per se* influence autonomic regulation [23]. In this context, a possible approach to avoid the placebo effects of tVNS is to mimic the same electrical stimulation, but applying the current on an auricular site that is not innervated by the ABVN, such as the ear lobe [24].

On the basis of these considerations, the present study was designed to test the hypothesis that acute non-invasive VNS through the ABVN would improve spontaneous cBRS, HRV and blood pressure (BP) variability (BPV) in healthy young male subjects. It further tested whether these effects were specific to stimulation of auricular regions innervated by the ABVN or is partially placebo-mediated.

METHODS

Subjects

Young male subjects (n = 13; age = 23 ± 1 yrs) participated in the present study with mean weight and height of, 80 ± 3 kg; 177 ± 2 cm (mean ± SEM), respectively. The rationale for including only men are based on the fact the cardiovascular control is markedly different between men and women [25]. In addition, the cyclical variations in female sex hormones across the menstrual cycle (i.e., estrogen and progesterone) could be a confound factor [26, 27]. All subjects were healthy, normotensive, non-smokers, and were recreationally active (self-reported habitual physical activity for at least 6 consecutive months with a minimum frequency of 3 days per week in ≥ 30-min sessions). Subjects had no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease as determined from a detailed medical health history questionnaire. No subjects were using prescribed or over-the-counter medications. Participants were recruited through posters placed at the University of Brasília, Brazil. Written informed consent was obtained from all subjects and all study procedures were approved by the University of Brasília institutional research committee (CAAE: 54104216.0.0000.0030) in accordance with the Declaration of Helsinki.

Study Protocol

The study was randomized, placebo-controlled, and crossed-over. Subjects performed four visits to the laboratory. In the visit one, the participants' body weight and height were assessed, and a familiarization session of tVNS was performed. Then, on visits two, three and four the subjects were exposed in a random order to : 1) active tVNS 2) sham, where electrodes were placed on the tragus of the ear but no current was

applied (sham tragus – sham-T), and 3) electrodes were placed on the ear lobe and current applied according with active tVNS (transcutaneous lobe stimulation – tLS). Participants were seated (90° of knee angle) while HR, finger arterial BP and respiration were monitored continuously. Data recordings were obtained at baseline (10 minutes), during test period (15 minutes; active tVNS, sham-T or tLS), and during recovery (10 minutes).

HR and arterial BP were measured on a continuous beat-to-beat basis using finger photoplethysmography (Human NIBP Controller, AD instruments, NSW, Australia). Brachial arterial BP was also measured with an automated digital sphygmomanometer (Omron, HEM-7200, Japan) for absolute measures of BP and to confirm finger measurements. Respiratory frequency was visually monitored in order to avoid the potential confounding influence of large respiratory excursions on cardiovascular measurements.

The subjects were asked to refrain from consuming caffeine/alcohol and from engaging in physical exercise for 6 and 24 h, respectively, prior to the tests. To avoid potential diurnal variations, subjects were always tested at the same time of day for each subject and in the same quiet, temperature-controlled room (~21°C).

Transcutaneous vagus nerve stimulation (tVNS)

Figure 1 shows the experimental design of the present study. The ABVN innervates the skin of parts of the ear (i.e. concha, tragus and cymba concha) and the ear lobe has no nerve endings of the vagus nerve (Fig. 1A). The transcutaneous electrical nerve stimulation (TENS) device consisted of a small stimulation unit (V-TENS Plus, Body Clock Health Care Ltd, UK) and modified surface electrodes bilaterally placed on

the inner and outer surface of the tragus of the ear during active and sham-T protocols (Fig. 1B), and on the ear lobe during tLS protocol (Fig. 1C). The active tVNS protocol was performed according with previous reports [15, 21, 28]. Briefly, the electrical current was applied continuously for 15 min with a pulse width of 200 μs and pulse frequency of 30 Hz. The stimulation amplitude was adjusted between 10 and 50 mA, at level of each participant's sensory threshold. In the sham-T protocol, the electrode was attached to the tragus and the amplitude was increased until the participant reported sensation. Participants were then told that stimulation interventions would be equivalent types of "nerve stimulation", though they might perceive them differently but the electrode leads were disconnected from the TENS machine without the participants' knowledge. During the tLS protocol, the electrode was placed on the ear lobe and the electrical current was applied with the same parameters as the active tVNS. The tLS protocol was performed to exclude the possibility of any confounding sensory effect of tVNS due to electrodes being sited at the ABVN dermatome, and to determine if the effects of stimulation were specific to the tragus and not due to the sensation of stimulus.

Spontaneous cardiac baroreflex sensitivity (cBRS)

Beat-to-beat time series of systolic BP and RR intervals were analyzed using the sequence technique for estimating spontaneous cBRS (CardioSeries v2.4, Brazil). The sequence technique is based on the identification of sequences of consecutive beats in which progressive increases in systolic BP are followed by a progressive lengthening in RR interval or vice versa; progressive decreases in systolic BP are followed by a progressive shortening in RR interval [29]. Briefly, sequences of three or more

consecutive beats with corresponding increases or decreases in systolic BP and RR interval were identified as arterial baroreflex sequences ($GAIN_{all}$). Sequences were detected only when the variation in RR interval was greater than 1.0 ms, systolic BP changes were greater than 1 mmHg, and ≥3 consecutive cardiac cycles. A linear regression was applied to each individual sequence and only those sequences in which R^2 was >0.85 were accepted. The slopes of the systolic BP and RR interval relationships were then calculated and averaged for a measure of spontaneous cBRS. Overall results were similar when HR was used as the dependent variable for these cBRS measures and therefore only RR interval measures are presented. In addition, the gains for up ($GAIN_{up}$) and down ($GAIN_{down}$) sequences and the total number of sequences detected were also calculated.

Heart rate variability (HRV)

HRV was determined in accordance with the guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [30]. A continuous recording of a single lead ECG, usually CC5 or CM5, was obtained continuously during the test. Variables were sampled at 1000 Hz and stored for offline analysis (CardioSeries v2.4, Brazil). Only segments without signal noise were analyzed. All ectopic beats on the ECG trace were identified both automatically and manually before exclusion from the analysis. A fast Fourier transformation (512 points) was used for spectral analysis of HRV. The power spectra were quantified by measuring the area under the following frequency bands: very low frequency power (VLF) (< 0.04Hz), low-frequency power (LF) (0.04–0.15 Hz) and

high-frequency power (HF) (0.15–0.4 Hz). As no firm evidence has yet been presented for the physiological meaning of the VLF band, only the power densities of LF and HF band were investigated. Total power and normalized units of LF and HF were also calculated. Normalized units were calculated by dividing each spectral band by the total power minus the VLF power and were multiplied by 100. The ratio of LF to HF (LF/HF) was also calculated as a measure of autonomic balance.

Blood pressure variability (BPV)

Spectral analysis of BPV was performed employing the software CardioSeries v2.4, which uses Fourier transformation to calculate spectral power of HRV and systolic BPV. BPV was calculated after appropriate spline interpolation and equidistant representation of systolic BP data. The VLF band was defined in the range of 0.02–0.07 Hz, the LF band in the area of 0.07–0.15 Hz, and the HF band between 0.15 and 0.40 Hz [31]. The LF component of BPV was used as a proxy of the sympathetic modulation of the vascular tone [31].

Statistical analysis

Shapiro-Wilk normality test was used to verify the normal distribution of the data. As the majority of the data presents a non-normal distribution, non-parametric statistical tests were applied. To analyze the effects of active, control and sham interventions, the baseline, tVNS and recovery recordings were compared using Friedman's analysis of variance (ANOVA), followed by Wilcoxon signed-rank paired test with Bonferroni correction to detect the difference in pairwise comparisons. All

data are presented as mean ± SEM. The figures are expressed as percentage of delta. The level of significance accepted for main effects was $P < 0.05$ and for post-hoc pairwise comparisons was $P < 0.017$. Statistical analyses were conducted using the software STATISTICA (Statsoft, USA).

RESULTS

Baseline characteristics of the subjects are present in Table 1. No significant differences are found in any resting physiological variables between active, sham-T and tLS protocols ($P > 0.05$). During both active tVNS and tLS, the range of the stimulation amplitude was between 40-50 mA, with an average of 45 ± 1 mA.

Active tVNS significantly increased cBRS $GAIN_{all}$ (baseline: 12.7 ± 1 ms/mmHg; tVNS: 15.1 ± 1 ms/mmHg; recove/ry: 13.1 ± 1 ms/mmHg; Interaction P = 0.04, Fig. 2A black square) (Δ24 ± 8%, active tVNS vs. baseline; P = 0.0159). In striking contrast, no changes were observed during sham-T (baseline: 13.1 ± 1 ms/mmHg; sham-T tVNS: 13.7 ± 1 ms/mmHg; recovery: 13.4 ± 1 ms/mmHg; Interaction P = 0.73, Fig. 2A white circles) (Δ6 ± 5%, sham-T vs. baseline) or tLS (baseline: 13.6 ± 1 ms/mmHg; tLS: 13.9 ± 1 ms/mmHg; recovery: 13.8 ± 1 ms/mmHg; Interaction P = 0.23, Fig. 2A gray triangles) (Δ3 ± 2%, tLS vs. baseline) protocols. Similarly, the gains for up (Fig. 2B) and down (Fig. 2C) sequences significantly increased during active tVNS ($GAIN_{up}$: Δ24 ± 11% vs. rest, P = 0.014; $GAIN_{down}$: Δ26 ± 7% vs. rest, P = 0.003), but was unchanged during either sham-T ($GAIN_{up}$: Δ8 ± 6% vs. rest, P = 0.202; $GAIN_{down}$: Δ4 ± 5% vs. rest, P = 0.257) and tLS ($GAIN_{up}$: Δ-2 ± 3% vs. rest, P = 0.336; $GAIN_{down}$: Δ7 ± 4% vs. rest, P = 0.072). The total number of sequences was not significantly different between active tVNS, sham-T or tLS (125 ± 12 vs. 125 ± 11 vs. 122 ± 14 respectively, P = 0.93).

Active tVNS slightly but significantly reduces HR before return to baseline values during recovery period (baseline: 72 ± 3 bpm; active tVNS: 69 ± 2 bpm; recovery: 71 ± 2 bpm; Interaction P = 0.02, Fig. 3A black squares) (Δ–3.4 ± 1% active

tVNS vs. baseline; P = 0.004). However, HR was unchanged during sham-T (baseline: 73 ± 2 bpm; sham-T: 71 ± 1 bpm; recovery: 73 ± 1 bpm; Interaction P = 0.07, Fig. 3A white circles) (Δ–1.5± 1% sham-T vs. baseline) or tLS (baseline: 73 ± 2 bpm; tLS: 73 ± 2 bpm; recovery: 73 ± 3 bpm; Interaction P = 0.38, Fig. 3A gray triangles) (Δ–1.2 ± 1% tLS vs. baseline). Systolic BP was unchanged by either active, sham-T or tLS protocols (P > 0.05, Fig. 3B).

Consistent with previous publications, active tVNS significantly reduced LF/HF ratio, an index of sympathovagally-mediated oscillations in HR variability, and returned to baseline values during recovery (baseline: 2.0 ± 0.3; active tVNS: 1.53 ± 0.3; recovery: 2.43 ± 0.5; Interaction P = 0.02, Fig. 3C black squares) (Δ–15 ± 12%, active tVNS vs. baseline; P = 0.014). On the other hand, no changes in LF/HF ratio were observed in either sham-T (baseline: 2.3 ± 0.4; sham-T: 2.65 ± 0.5; recovery: 2.87 ± 0.4; Interaction P = 0.50, Fig. 3C white circles) (Δ23 ± 16%, sham-T vs. baseline) or tLS (baseline: 1.72 ± 0.2; tLS: 1.65 ± 0.2; recovery: 2.1 ± 0.3; Interaction P = 0.23, Fig. 3C gray triangles) (Δ4 ± 10%, tLS vs. baseline) protocols. Interesting, both absolute (ms^2) and normalized (nu) values for LF and HF components of HRV were unchanged during any conditions with exception of LF (ms^2) which significantly increased during sham-T (Δ48 ± 15%, vs. rest, P = 0.002) and trend to increase during tLS (Δ29 ± 13%, vs. rest, P = 0.023), but was unchanged during active tVNS (Δ4 ± 11%, vs. rest). Importantly, the respiration rate was unchanged during any of the conditions.

No differences were found in LF power of SBP variability, during any active (Δ–8 ± 10% active tVNS vs. baseline; Interaction P = 0.56, Fig. 3D black squares), sham-T (Δ37 ± 13%, sham-T vs. baseline; Interaction P = 0.80, Fig. 3D white circles)

and tLS (Δ29 ± 16%, tLS vs. baseline; Interaction P = 0.79, Fig. 3D grey triangles) protocols

DISCUSSION

In accordance with our initial hypothesis, the present study shows that: 1) active tVNS acutely improves spontaneous cBRS; 2) LF/HF ratio is decreased by tVNS in healthy young men; 3) tVNS evokes slight decrease in HR; and 4) the aforementioned effects are specific to stimulation of ABVN.

A major finding of this study is that spontaneous cBRS increased in response to tVNS of the ABVN. The baroreflex is a closed-loop, negative feedback control system that constantly senses arterial pressure by baroreceptors in a beat-to-beat fashion and quickly regulates systemic arterial pressure physiologically to attenuate perturbations in arterial pressure [32]. Previous studies demonstrate that afferent VNS resets the baroreflex operating point and induces sympathoinhibition in animal models [20, 33]. A striking outcome of the current study was that increases in BRS were evident even with non-invasive VNS. Further these effects were observable even in healthy young men, who would be expected to have a strong baseline cBRS. Since BRS decreases with disease [34] and age [35], this suggests that tVNS could provide a significant opportunity to improve BRS in an inexpensive and non-invasive approach that could be generally applicable.

This current study provides evidence supporting the view that the autonomic effects of auricular stimulation are mediate by the ABVN. Circumventing the possible sensory effects with stimulation at the earlobe, a non-ABVN innervated region, has no effect on reflex control of BP. This is consistent with the functional magnetic resonance imaging study by Frangos et al. [36] that electrical stimulation of the ear lobe did not activate the nucleus tractus solitarius (NTS) in the brainstem, whereas that of the ABVN-innervated cymba concha did. Therefore, we can attribute that the positive effects on reflex control of BP are due the active stimulation of ABVN per se. The

precise mechanisms underlying the main findings of the present study are not fully understood, but some relevant points should be considered.

The central circuitry associated with tVNS autonomic effects might involve activation of the NTS by ABVN afferents. Since the baroreceptor afferent fibers join their respective glossopharyngeal nerve and also project to the NTS, tVNS might potentiate the effectiveness of cBRS at the NTS level [15], again consistent with activation of NTS by tVNS [36]. In addition, this could activate the caudal ventrolateral medulla to inhibit the rostral ventrolateral medulla and thus reduce sympathetic output to both the heart and peripheral vasculature [15, 37]. Furthermore, the NTS could also activate the dorsal motor nucleus of the vagus and the nucleus ambiguus to increase cardiac parasympathetic activity [38]. In support of this idea, Clancy et al. [21] demonstrated a significant attenuation in muscle sympathetic nerve activity evoked by tVNS, which may attenuate α-adrenergic receptors constrictor function in blood vessels and thus decrease peripheral vascular resistance. Considering our findings, the aforementioned physiological responses can be responsible for the small decreases in HR, the increases in cardiac autonomic modulation and spontaneous cBRS observed during active tVNS.

The decreases in HR and LF/HF ratio during active tVNS are in accordance with the results of Clancy et al. [21], demonstrating that tVNS improves the sympathovagal balance, but extends it to show that tVNS was effective even in this sample of young males. Interestingly, the baseline LF/HF ratio was higher in our subjects when compared to their study [21], which may be attributed to the different sample sizes. Our study was performed only in young men and Clancy et al. [21] studied both sexes and previous studies have demonstrated that female sex hormones, more specifically estrogen [39], have an effect on cardiac autonomic modulation [40].

In this sense, men tend to have a higher baseline LF/HF than women [41]. The reasons of these discrepancies are unclear, but some aspects should be considered. For example, it has been shown that body posture may change the autonomic and hemodynamic control [42, 43], and our subjects were seated while Clancy et al. [21] performed tests in a semi-supine position. We decided to perform experiments in a seated position due the fact that it is likely possible that people will conduct tVNS whilst watching TV or other such daily activity (i.e., external validity). In addition, our subjects were healthy, young and male, and the sample of Clancy et al. [21] was composed by both male and female subjects with a range of age between 20 to 62 years. Furthermore, the present study added a sham protocol that minimizes the sensory effects of tVNS (i.e., tLS) which was not performed by the Clancy study.

Limitations

The present study has several limitations. First, the small sample size increases the risk of type II error. Second, since we tested only healthy young male subjects, it is not possible to extrapolate the results for other populations such as female, older and/or diseased subjects. Future studies are necessary to examine the impact of tVNS on neural control of BP in these populations. Third, we used a non-perturbational spontaneous method to assess the arterial baroreflex sensitivity. Perturbational methods, such as vasoactive drugs infusion (i.e. modified Oxford), allow the examination of a prevailing range of pressure, while non-perturbational spontaneous method assesses a limited range of pressure for the stimulus-response baroreflex relationship. The results of the present study, however, show that sequence method was able to confirm our initial hypothesis. In addition, several clinical studies have used the sequence technique and previous authors have reported high reproducibility of spontaneous baroreflex

sensitivity using the sequence technique at rest and during perturbations [44-46], and the sequence method has been shown to correlate with the Oxford technique [47].

Perspectives

The improvements in cBRS and sympathovagal balance caused by tVNS have clinical implications. Several cardiovascular disease states are accompanied by autonomic dysfunction, characterized by an impairment in cardiac baroreflex sensitivity, increased sympathetic nerve activity and parasympathetic withdrawal (e.g. hypertension, coronary artery disease, heart failure, diabetes and obesity) [1, 3, 4, 20]. In addition, cardiac autonomic dysfunction is a powerful predictor of mortality in patients with cardiac disease [48, 49], and in older people with low cardiovascular risk [50].

In this regard, non-pharmacological approaches (i. e., exercise and diet regimens) have been consistently shown to improve cBRS and autonomic modulation [51-53]. More recently, a promising strategy to promote beneficial outcomes for a range of conditions is the electrical stimulation of vagus nerve. Although VNS has been suggested for potential complementary treatment of a broad range of conditions including epilepsy, depression, Alzheimer's disease, heart failure, inflammation, chronic pain, diabetes, tinnitus and obesity [5-12], the mechanisms involved in the efficacy of this technique remain unclear.

Conclusions

The results of the present study provide evidence that non-invasive VNS through the ABVN improves cBRS and autonomic modulation in healthy young male subjects. These findings allow us to suggest that spontaneous cBRS and cardiac

sympathovagal balance could play a role in the mechanisms involved in previously reported beneficial outcomes caused by tVNS. Future studies are needed to confirm these findings in older and diseased populations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure Legends

Figure 1. Experimental protocol of the study. A. The shaded area shows the distribution of the auricular branch of the vagus nerve to the external ear. B. Position of the electrodes which were placed on the tragus of the ear during active and sham-T protocols. C. Position of the electrodes which were placed on the ear lobe during tLS protocol.

Figure 2. Response of cardiac baroreflex sensitivity for all ($GAIN_{all}$, panel A), up ($GAIN_{up}$, panel B) and down ($GAIN_{down}$, panel C) sequences during the baseline, tVNS and recovery in the active (black squares), sham-T (white circles) and tLS (grey triangles) protocols. All values are mean ± SE. **P* < 0.05 tVNS vs. baseline in the active protocol.

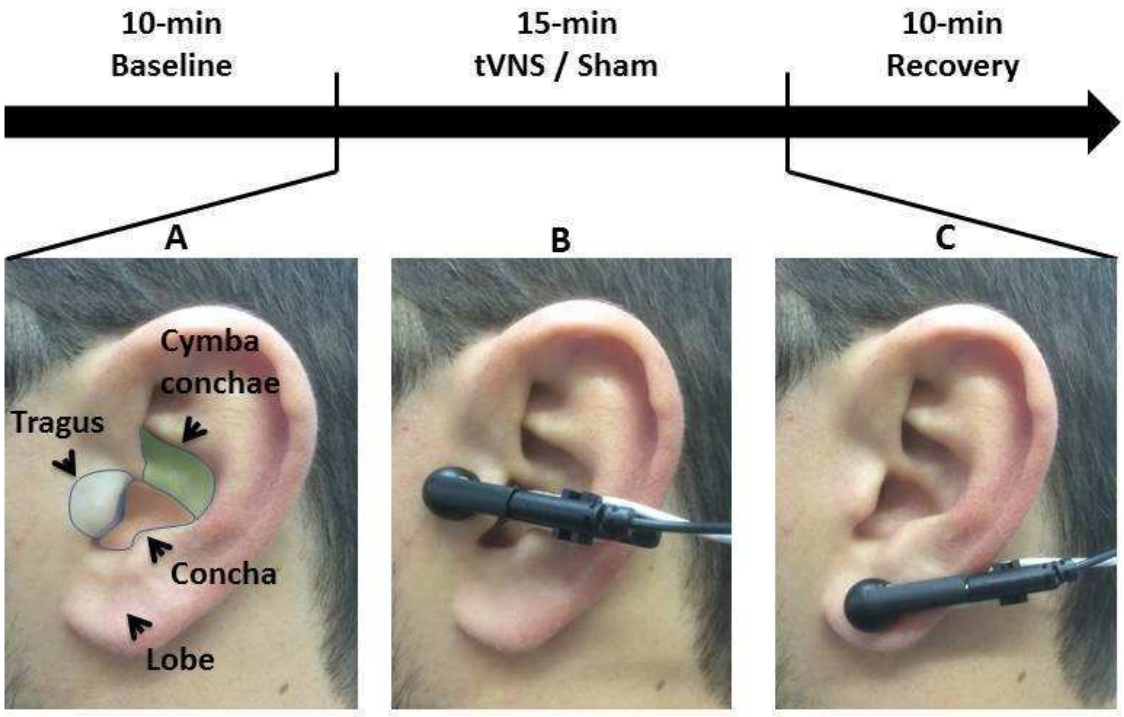
Figure 3. Response of heart rate (HR, panel A), systolic blood pressure (SBP, panel B), sympathovagal balance, represented by the ratio between the low and high frequency components of heart rate variability (LF/HF, panel C), and low frequency component of systolic blood pressure variability (LF_{SBP}, panel D) during the baseline, tVNS and recovery in the active (black squares), sham-T (white circles) and tLS (grey triangles) protocols. Values are mean ± SE. **P* < 0.05 tVNS vs. baseline in the active protocol. †*P* < 0.05 tVNS vs. recovery in active protocol. ‡*P* < 0.05 tVNS vs. recovery in tLS protocol.

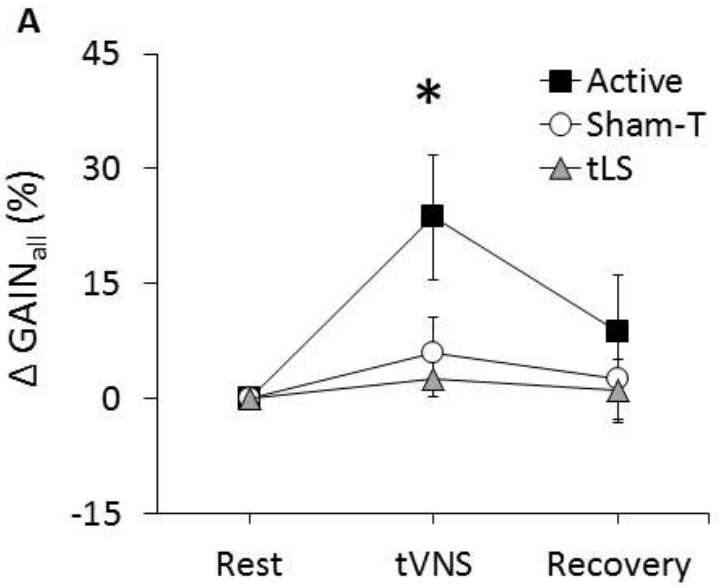
Table

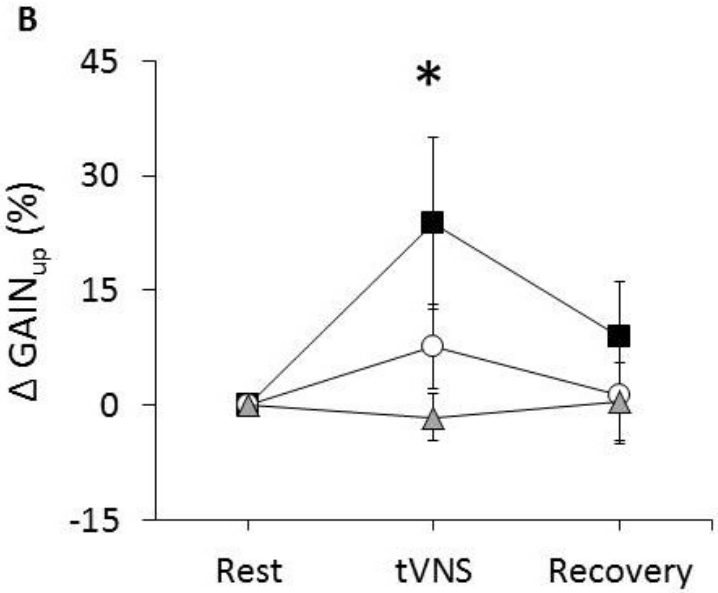
Table 1. Baseline characteristics.

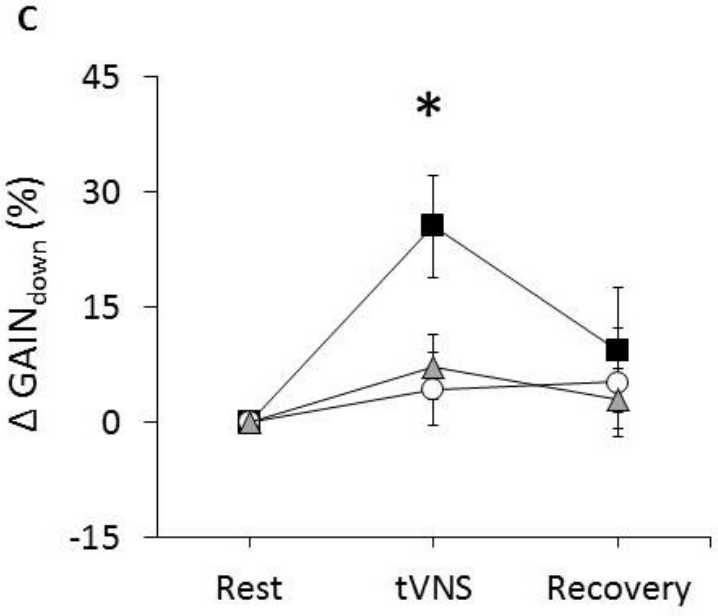
	Active	Sham-T	tLS	*P*
Anthropometrics				
Age, years	22.6 ± 1	-	-	-
Weight, kg	79.6 ± 3	-	-	-
Height, cm	177 ± 2	-	-	-
BMI, kg/m²	25.4 ± 1	-	-	-
Hemodynamics				
SBP, mmHg	111 ± 1	113 ± 2	112 ± 2	0.15
DBP, mmHg	63 ± 1	63 ± 1	63 ± 2	0.71
MAP, mmHg	78 ± 1	79 ± 1	79 ± 2	0.49
HR, beats/min	72 ± 3	73 ± 2	73 ± 2	0.27
Cardiac baroreflex function				
$GAIN_{all}$, ms/mmHg	12.7 ± 1	13.1 ± 1	13.6 ± 1	0.37
$GAIN_{up}$, ms/mmHg	12.7 ± 1	13.4 ± 1	14.1 ± 1	0.37
$GAIN_{down}$, ms/mmHg	12.5 ± 1	13.1 ± 1	13.1 ± 1	0.37
HR variability				
LF, ms^2	1320.6 ± 132.0	1360.1 ± 206.8	1035.9 ± 124.9	0.23
HF, ms^2	1038.8 ± 156.2	916.9 ± 161.7	752.4 ± 78.9	0.37
LF, nu	52.8 ± 4.5	57.7 ± 3.7	52.5 ± 3.0	0.29
HF, nu	47.2 ± 4.5	42.3 ± 3.7	47.5 ± 3.0	0.29
LF/HF	2.0 ± 0.3	2.3 ± 0.4	1.7 ± 0.2	0.12
BP variability				
LF_{SBP}, $mmHg^2$	8.2 ± 1	7.8 ± 1	6.4 ± 1	0.07

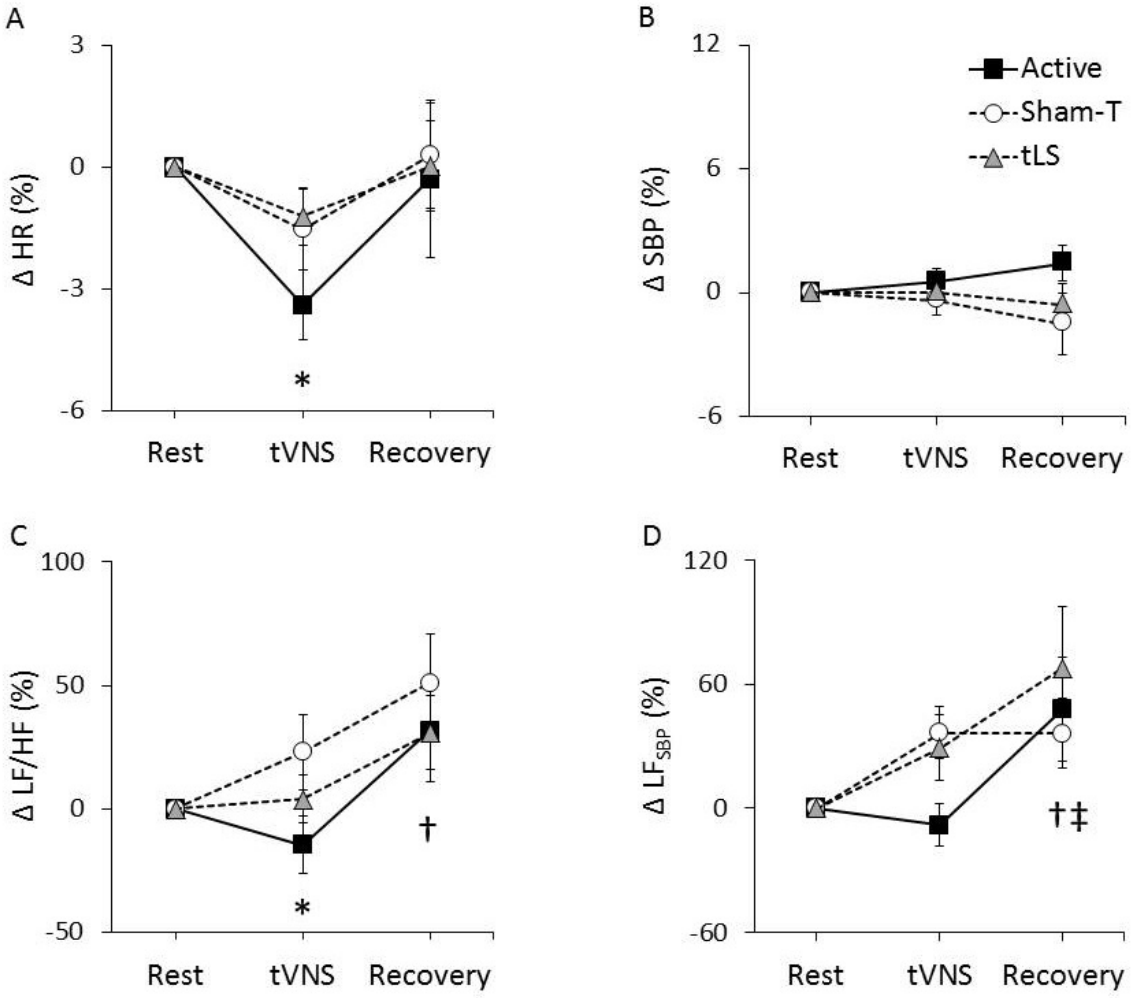
Values are means ± SE. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; LF/HF, ratio between low and high frequency powers of heart rate variability; BP, blood pressure; LF_{SBP}, low frequency component of systolic blood pressure variability; *P*, level of significance ($P < 0.05$).




A
45
30
15
0
-15
Δ GAIN$_{all}$ (%)
*
Active
Sham-T
tLS
Rest
tVNS
Recovery


B
45
30
15
0
-15
Δ GAIN$_{up}$ (%)
*
Rest
tVNS
Recovery


C
45
30
15
0
-15
Δ GAIN$_{down}$ (%)
*
Rest
tVNS
Recovery


A
3
0
-3
-6
Δ HR (%)
*
Rest
tVNS
Recovery
B
12
6
0
-6
Δ SBP (%)
Active
Sham-T
tLS
Rest
tVNS
Recovery
C
100
50
0
-50
Δ LF/HF (%)
*
†
Rest
tVNS
Recovery
D
120
60
0
-60
Δ LF$_{SBP}$ (%)
†‡
Rest
tVNS
Recovery

Highlights

- Non-invasive vagus nerve stimulation (tVNS) is investigated as a therapy for a range of conditions.
- The mechanisms involved in positive outcomes of tVNS are not fully understood.
- We show that tVNS improves spontaneous cardiac baroreflex sensitivity in healthy men.
- These effects are specific to stimulation of auricular branch of the vagus nerve.