Effects of transcutaneous vagus nerve stimulation on subthreshold affective symptoms and perceived stress: Findings from a single-blinded randomized trial in community-dwelling adults

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ARTICLE INFO

ABSTRACT

Transcutaneous vagus nerve stimulation (tVNS) lowers depression and anxiety in clinical populations, but its preventive utility in alleviating subthreshold depression and anxiety symptoms or perceived stress in the general population is uncertain. In this single-blinded randomized controlled trial 70 participants (28 men; M_{age} 49,33 years, 18–75 age range) were allocated to four groups: early active or sham tVNS and late active or sham tVNS to explore outcome changes between the preintervention and postintervention in active and sham groups, changes after active and sham stimulation ended in the early groups, or outcomes during waiting time in the late groups. Early intervention and sham groups received daily 4 h tVNS between Day 0 and 13, while late intervention and sham groups received tVNS between day 14 and 28. Active tVNS was delivered via transcutaneous electrical stimulation on the left tragus and sham tVNS was applied on the left earlobe. Affective symptoms and stress were measured with questionnaires. Effects of active tVNS stimulation were superior to sham stimulation in early phase groups, but not in late phase groups, for anxiety symptoms and perceived stress, with no superior effects of tVNS against sham detected for depressive symptoms. Our study tentatively indicates that tVNS application could be scaled-up to a population level to potentially mitigate stress vulnerability and higher anxiety, which are often prevalent in older adults and increased in the ageing process.

1. Introduction

Depression and anxiety are the most prevalent mental health disorders that are a major contribution towards disability (Kyu et al., 2018), reduced quality of life (Olatunji et al., 2007), as well as cardiovascular outcomes and mortality (Silverman et al., 2019). Much progress has been made in enhancing treatment by means of psychological and pharmacological interventions of these disorders, but for some people treatment still remains challenging. Cognitive behavioural therapy is recommended as the first line of treatment for depression and anxiety, but treatment effects are moderate at best, with less than half of patients responding to a therapy in case of anxiety disorder (Hunot et al., 2007). Moreover, analysis of WHO World Mental Health survey found that people suffering from major depressive disorder (MDD) need to see up to

9 different specialists to perceive their depression treatment as helpful (Harris et al., 2020). On the other hand, pharmacological treatment is associated with less than optimal uptake for an initial course of antidepressant medication (Aznar-Lou et al., 2018), and those taking antidepressant medication often struggle with adherence that is reduced markedly at 6 months (Ereshefsky et al., 2010). This is important as longer duration of untreated mental health conditions/disorders has negative implications for treatment outcomes (Bukh et al., 2013; Ghio et al., 2014).

Given the substantial difficulties with treatment of mental ill health, it is vital for those struggling with affective symptoms and society at large to address avenues for prevention, alleviating sub-threshold symptoms, thereby altering the trajectories of affective disorders and disease progression over time. Meta-analytic evidence suggests that

physical activity is moderately effective (effect sizes range from 0.10 to 0.81) in preventing depressive symptoms (but not depression onset) in the general population, with benefits noted in all age groups (Hu et al., 2020). Similarly, psychological interventions (e.g. resilience building programs) delivered at schools, workplaces and online are also deemed as effective preventive strategies (Hoare et al., 2021). However, given the heterogeneity of affective disorders and of populations they affect, a search for novel therapeutic interventions is warranted. In particular, recent technological advances and growing knowledge of neural underpinnings of affective disorders offer a possibility to focus on interventions that are easily implementable, relatively inexpensive, and thus potentially scalable.

One such a possibility is the therapeutic strategy targeting the vagal pathway including vagus nerve stimulation (VNS). This is based on evidence that diminished peripheral (cardiac) vagal modulation is present in stress conditions, depressive symptoms or anxiety, and is also an indicator of stress vulnerability and low capacity for parasympathetic inhibition of autonomic arousal in emotion regulation (Thayer and Sternberg, 2010). However, while conventional VNS has been approved as treatment for drug-resistant depression in the US in 2005 (Cristancho et al., 2011), it requires a surgical procedure, thus it can lead to complications or side effects such as vocal cord dysfunction. Moreover, VNS is only available to patients fulfilling strict eligibility criteria, and it is associated with a considerable cost that may be prohibitive for many (Yap et al., 2020).

Importantly, vagus nerve can also be stimulated transcutaneously (tVNS) with stimulation applied to the ear, where the auricular branch of the vagus nerve is located (Yap et al., 2020). The auricular branch of the vagus nerve projects to the nucleus tractus solitarii (NTS) that is both directly and indirectly connected to brain areas involved in affective regulation, as well as stress responses including, among others, the locus coeruleus, parabrachial nucleus, hypothalamus, thalamus, amygdala, hippocampus, anterior cingulate cortex, anterior insula, and lateral prefrontal cortex (Austelle et al., 2022; Kong et al., 2018; Yap et al., 2020). The precise mechanisms via which tVNS reduces mood disturbances or the experience of stress are still uncertain, but increased vagal activity boosts parasympathetic nervous system activity, which is involved in emotion regulation (Stellar et al., 2015). Activity in frontal brain circuits, essential for emotion regulation, responds to vagus stimulation, and it is thought that mood may improve through modulating brain activity, which is altered in those with depression (Biermann et al., 2011). Furthermore, studies in animals and humans suggest that stimulating the vagus nerve alters monoamine metabolism subsequently increasing levels of neurotransmitters including serotonin, dopamine, norepinephrine and GABA (Breit et al., 2018; Yap et al., 2020).

To date, a number of studies addressed the effects of tVNS on depression. Since the first study published by Hein et al. (2013, n=37), who found that in patients with MDD, relative to a control group, 2-weeks of tVNS led to a reduction of depressive symptoms, two meta-analytic reviews concluded that tVNS can ameliorate depressive symptoms (Tan et al., 2023; Wu et al., 2018). There is also evidence that tVNS leads to improvements among patients with milder depressive symptoms suggesting therapeutic benefits of tVNS may extend beyond those with diagnosed severe depression (Rong et al., 2016, n = 69). However, while existing results are encouraging, caution is warranted given methodological shortcomings of existing trials (Tan et al., 2023).

Studies on tVNS in depression showed secondary improvements in anxiety. Fang et al. (2016, n = 49) found improvements in symptoms of anxiety in the tVNS group, when compared to sham. More recently, Li et al. (2022, n = 107) reported a reduction of anxiety symptoms in depressed patients receiving tVNS, albeit these were evident as simple time effects rather than as significant group-by-time interactions. Beneficial effects of tVNS on symptoms of anxiety have also been reported in n = 30 patients with Parkinson's disease (Zhang et al., 2024). There is also emerging evidence from non-clinical studies that tVNS may

offer some promise in reducing symptoms of anxiety. A recent randomized control trial in n = 42 university students with elevated anxious symptoms demonstrated that 3 sessions of tVNS, applied over one week, led to significant reductions in anxiety, in comparison with the sham group (Ferreira at al., 2024). A 4-week course of tVNS led to a reduction of anxiety, when compared to relaxation, in n = 42 older healthcare workers during COVID-19 pandemic (Srinivasan et al., 2023), as well as in n = 60 retired teachers (Srinivasan et al., 2024). Relatedly, a lab-based study showed that acute tVNS, relative to sham stimulation, was associated with less negative thought intrusion among n = 97 participants who were habitual worriers (Burger et al., 2019).

To date, virtually no research has been published showing tVNS effects on the experience of stress in a non-clinical population. However, there is experimental evidence showing that tVNS, compared to sham, may inhibit behavioral and inflammatory responses to a stress paradigm in those with posttraumatic stress disorder (PTSD) (Bremner et al., 2020, n = 30), or lower neurobiological stress response (indexed with pituitary adenylate cyclase-activating peptide) in people with prior exposure to psychological trauma (Gurel et al., 2020, n = 30).

2. The current study

Taken together, there is emerging evidence that in addition to depressive symptoms tVNS may also reduce anxiety and modulate stress responses, but very few studies tested the effects of tVNS on these psychological measures in the general population. Therefore, given the growing interest in non-invasive devices stimulating vagus nerve, and the need to test novel techniques that could improve mental health difficulties in community-dwelling adults, this study tested the hypothesis that a two-week course of tVNS would lead to a reduction of depressive and anxiety symptoms when compared with placebo stimulation. We also hypothesized that two weeks of tVNS, relative to sham, would be associated with lower perceived stress.

3. Method

3.1. Participants

Data for the present analyses come from a larger single-blinded randomized control clinical trial (registered at https:// clinicaltrials. gov; NCT04070547) designed to address the effect of a 2-week course of tVNS on cognitive function, health-related variables (for more details see Cibulcova et al., 2024; Jackowska et al., 2022), as well as psychological outcomes described here. n = 78 participants (n = 31 men) aged 18–75 years were recruited via advertisement from University of Ostrava in Czech Republic and other places of work in the vicinity. The study was advertised as a project exploring the role of the autonomic nervous system in cognitive and emotional functioning, and whether transcutaneous stimulation of part of the autonomic nervous system, i. e., the vagus nerve, could modulate these functions. No therapeutic intent was communicated to participants. The inclusion criteria included: (1) self-declared healthy status, (2) age between 18 and 75 years, and (3) speaking Czech. Participants were excluded if they endorsed any cardiovascular disease (e.g., arrhythmia, history of coronary heart disease, history of stroke), severe mental illness (e.g., clinical depression, schizophrenia, anxiety), severe neurological condition (e.g., epilepsy, significant migraine, brain tumors, traumatic brain injury), severe inflammation, taking medication that may affect autonomic pathways, brain surgery, and pregnancy (for more details visit https:// clinicaltrials.gov).

Given the complexity of our design, comprising four groups with two different intervention periods (active vs. sham × early vs. late) and the use of linear mixed-effects models, it was not feasible to conduct a reliable sample size estimation. Thus, sample size was not determined based on formal a priori power analysis, but in line with existing tVNS research in non-clinical populations (e.g., Bretherton et al., 2019

(n = 29); Burger et al., 2016 (n = 31); Jacobs et al., 2015 (n = 30); De Smet et al., 2021 (n = 83). We aimed to enroll between n = 15 to n = 20 participants to each of the four study arms resulting in n = 78 participants that completed the study. The trial was approved by the Ethnical Committee of University of Ostrava. All participants provided written informed consent and were given 1000 CZK upon study completion.

3.2. Transcutaneous vagus nerve stimulation

A Parasym® PK01 transcutaneous neurostimulation device was used (Parasym Ltd., London, UK) with 2 electrodes on a clip. Both active and sham stimulation lasted 14 days and was performed at home. All participants were requested to use the device for 4 h (240 min) a day, and the number of daily time segments was decided by participants. The left tragus was targeted for active stimulation since it is innervated by the auricular branch of the vagus nerve (Yakunina et al., 2017). Participants in the sham group were instructed to wear the electrode clip on the left earlobe, not innervated by the vagus nerve (Yakunina et al., 2017), which has been proposed as a suitable location for sham stimulation (Farmer et al., 2021). Participants were instructed to use the device with constant stimulation (no on/off cycles), and set the intensity of stimulation based on their individual sensory threshold of intensity, with a pulse width of 250 µs at a frequency of 25 Hz. Before putting the device on, participants were asked to spray a conductive liquid on the electrodes. Importantly, reporting of settings of the tVNS device was set in line with the recommendations of the recently established tVNS consensus group (Farmer et al., 2021; Kamboj et al., 2023). (Refer to the supplementary material for a table detailing stimulation parameters in accordance with minimum reporting standards.) After completing the active or sham stimulation period, all participants were required to demonstrate to a member of a research team how they set the device each day, and on which precise ear location the electrodes were applied. This procedure indicated that one participant wore their device incorrectly, hence they were not invited to continue in the study. As briefly outlined in the procedure section, over the 14-day period of the active and sham stimulation, participants completed daily, online diaries as the measure of adherence to the study protocol. In these diaries participants entered information on a daily length of stimulation, and the number of separate stimulation sessions. As an additional measure of adherence, at the end of the stimulation (active or sham) period, participants provided information on how many days of the trial they wore the tVNS device, and over how many hours on average stimulation was applied on each day. A cardboard box was used to collect this information, which remained sealed until the end of the trial.

3.3. Randomization

A two-step randomization process was used and allocation of participants into discrete study groups was undisclosed until their inclusion in the study was confirmed. To determine group assignments, a simple randomization, using a shuffled deck of cards, was employed. In the first step randomization, participants were randomized into early or late (waitlist) group (with a 1:1 allocation ratio). In the second step randomization, using a 1:1 allocation ratio, participants in the early group were randomized into tVNS (active/actual intervention) and a control condition (sham tVNS), with stimulation initiating immediately after baseline (day 0–13); two weeks later stimulation ended and participants provided follow-up assessments (day 14–28). In the late (waitlist) group, participants randomized into active and sham tVNS groups (with a 1:1 allocation ratio), initiated stimulation two weeks after baseline (day 14–28). Participants were unaware of their stimulation group (active or sham). To achieve this, each tVNS stimulator had hidden its name and identifying details, as to prevent participants from searching information about the stimulator on the internet or elsewhere. Double-blinding was not conducted because the laboratory staff, four researchers with thorough knowledge of tVNS and prior stimulation

protocols, were responsible for instructing participants on stimulator use and providing written guidance. After the intervention, they verified correct application by having participants demonstrate device placement and settings. However, statistical analyses were performed independently by two team members who were not, or only minimally, involved in data collection.

3.4. Measures

3.4.1. Background information

Sex, age, education and employment status were collected among sociodemographic data (see Table 1). Information on prescribed medication use (including antihypertensives, antidepressants, anxiolytics) was assessed. Self-reported height and objectively measured weight were used to calculate body mass index (BMI, kg/m^2).

3.4.2. Depressive symptoms, anxiety symptoms and perceived stress measures

All scales were answered with reference to the presence of symptoms in the last two weeks. For the purpose of our trial a double back translation of all scales into Czech language was performed.

3.4.2.1. Depressive symptoms. The Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) was used to assess depressive symptoms. A shortened, 10-item scale that has been reported as valid

Table 1
Baseline characteristics of participants in the four experimental conditions.

	Early group		Late group	
	Active tVNS (n = 15) Means (95 % C.I./ frequency (%)	**Sham tVNS (n = 17)** Means (95 % C.I./ frequency (%)	**Active tVNS (n = 23)** Means (95 % C.I./ frequency (%)	**Sham tVNS (n = 15)** Means (95 % C.I./ frequency (%)
Age	48.4 (37.7–59.1)	52.8 (43.4–62.1)	51.6 (44.9–58.2)	44.5 (33.4–55.5)
Sex (men)	8 (53.3)	7 (41.2)	8 (34.8)	5 (33.3)
High educational level (university degree)	6 (40)	11 (64.7)	14 (60.9)	9 (60.0)
Employment status Student Employed Unemployed, retied, self-employed	3 (20) 7 (46.7) 5 (33.3)	2 (16.7) 8 (66.7) 2 (16.7)	2 (10.5) 16 (84.2) 1 (5.3)	4 (30.8) 7 (53.9) 2 (15.4)
Body Mass Index	25.0 (22.3–27.6)	26.7 (24.4–29.0)	26.0 (24.2–27.9)	26.0 (23.6–28.5)
Prescribed medication use Antihypertensives	5 (33.3) 1 (6.7)	7 (41.2) 5 (29.4)	10 (43.5) 5 (21.7)	4 (26.7) 1 (6.7)
Diabetes medication	1 (6.7)	1 (5.9)	1 (4.4)	0 (0.0)
Depressive symptoms, CESD-10 (0−7)[a]	3.9 (1.6–6.2) (0−6)[b]	4.2 (2.2–6.1) (2−6)	6.2 (4.3–8.1) (0−7)	8.4 (5.2–11.6) (1−7)
Depressive symptoms ≥ 10	1 (6.7)	2 (11.8)	5 (22.7)	5 (38.5)
Anxiety symptoms, GAD-7 (0−8)[a]	3.5 (1.8–5.3) (0−5)	1.9 (0.7–3.0) (0−3)	3.5 (2.1–5.0) (0−7)	4.4 (1.2–7.5) (0−8)
Anxiety symptoms ≥ 10	1 (6.7)	0 (0.0)	1 (4.6)	3 (23.1)
Perceived stress, PSS-10 (2−29)[a]	12.2 (8.4–16.0) (7−28)	7.9 (5.7–10.1) (3−18)	11.6 (8.9–14.3) (4−24)	13.3 (8.2–18.3) (2−29)

[a] Continuously distributed data including ranges;
[b] range of scores.

and reliable in previous research, was selected (Andresen et al., 1994). The 10-item CES-D (CESD-10) asks participants about their mood and somatic symptoms that are rated on a 4-point rating scale ranging from 0 to 3. Items were summed with higher scores indicating greater depressive symptoms. A cut-off point of ≥ 10 was used as a marker of elevated depressive symptoms in line with prior research (Fu et al., 2022). At baseline the Cronbach alpha was 0.79.

3.4.2.2. Anxiety symptoms. Anxiety symptoms were indexed with the brief measure of generalized anxiety disorder (GAD-7) (Spitzer et al., 2006). In this questionnaire, items are rated on a 4-point rating scale ranging from 0 to 3 with higher (summed) scores being indicative of greater symptoms of anxiety. The cut-off point of ≥ 10 was used as a marker of elevated anxiety symptoms in line with prior research (Spitzer

et al., 2006). At baseline the Cronbach alpha was 0.85.

3.4.2.3. Perceived stress. Perceived stress was measured with the 10-item perceived stress scale (PSS-10) (Cohen & Williamson,1988), asking participants to what extent they feel events in their life are overloading, uncontrollable or unpredictable. Items are scored on a 5-point rating scale anchored from 0 to 4 with higher (calculated as a sum) scores indicating greater perceived stress. At baseline the Cronbach alpha was 0.86.

3.5. Assessment protocol

In total, based on a combination of ethical, practical, and methodological considerations, participants in the four groups detailed above
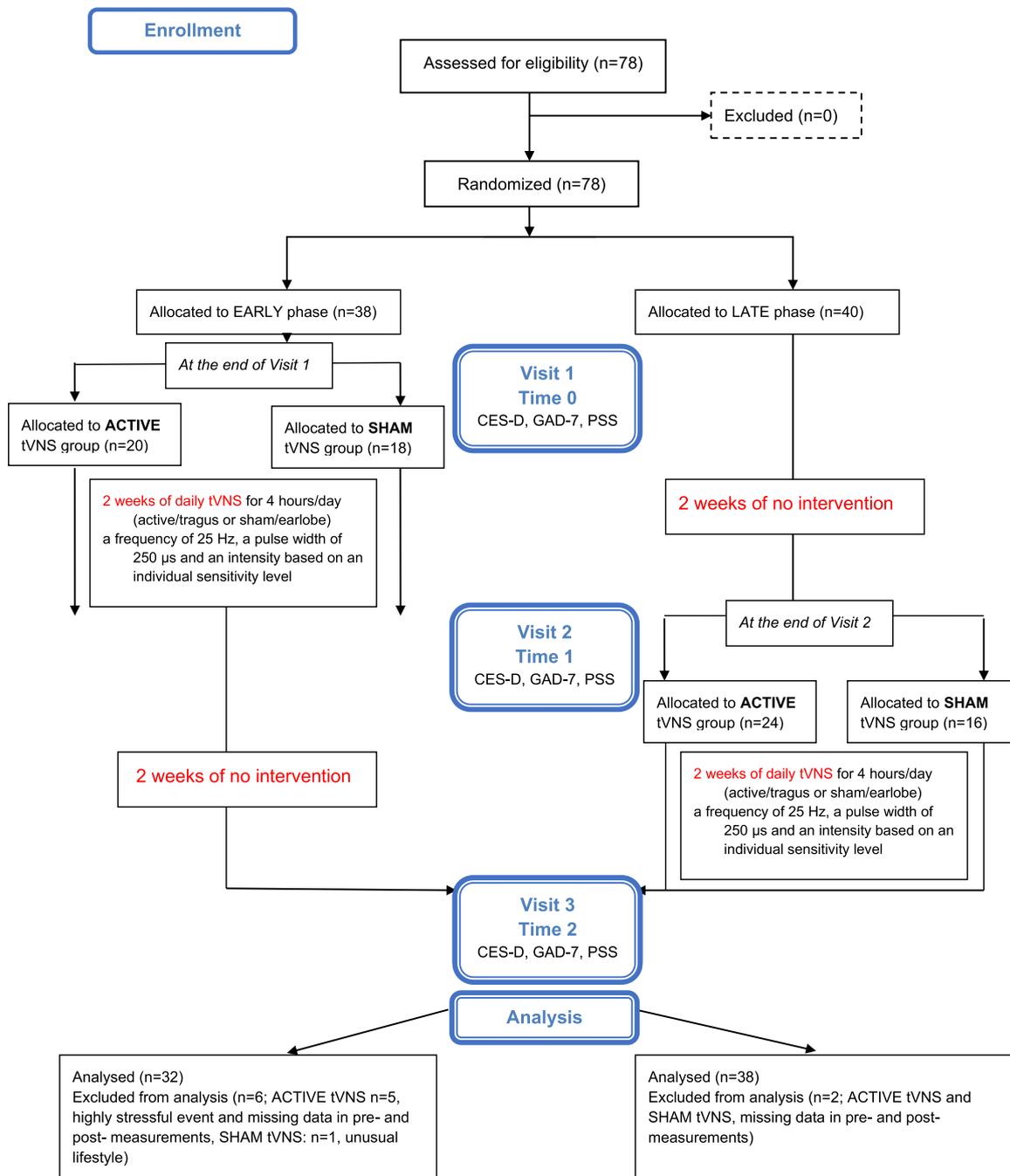

Fig. 1. Consort Flow diagram depicting the study's design.

remained in the study for four weeks. The intervention period lasted two weeks and was chosen based on relevant tVNS literature (e.g., Hein et al., 2013; Trevizol et al., 2016; Wu et al., 2018), and intended to balance expected efficacy with participant burden and safety in a non-clinical sample. Participants were randomized into early and late groups to take advantage of within- and between-subject design, and to test both between-subject and within-subject effects on outcomes of interest, and within-individual changes in outcomes either as a result of stimulation per se, no-stimulation follow-up for early (tVNS and sham) groups, or waiting time before the stimulation period in late (tVNS and sham) groups. Specifically, in addition to our hypotheses testing the superiority of active tVNS against sham stimulation, this design allowed us to explore potential carry over effects after active and sham stimulation ended between day 14 and 28 in the early tVNS and early sham groups, and to evaluate whether any changes occurred during the waiting period without stimulation (days 0–13) in the late tVNS and late sham groups. This created a unique waitlist-like control and included a follow-up phase, both designed to enhance the internal validity of the study while keeping the procedure practical and acceptable for participants. The trial is reported in accordance with the Consolidated Standards of Reporting Trials (i.e., CONSORT 2010 Statement by Moher et al., 2010) (see Fig. 1).

3.6. Procedure

Following screening, prior to the first laboratory visit, participants were randomly assigned by the study's principal investigator to either the early group (n = 38) or the late group (n = 40). During screening sociodemographic data and information on health and medication were obtained. At the initial laboratory visit, after providing written consent, recordings of blood pressure, heart rate variability (HRV) (not described here), height and weight were taken, as well as cognitive testing was performed (not described here). Next, participants were randomized into active (actual) tVNS or sham (placebo) tVNS conditions (at the end of pre-intervention lab visit). Participants were instructed on the use of the tVNS device, and provided with a written manual. All participants filled in a daily online questionnaire including questions on duration and intensity of stimulation, number of tVNS sessions per day, or reports of pain. Participants were also requested to provide anonymous information on adherence after the trial's end (see section on randomization). Psychological measures were collected via online questionnaires (e.g., measures of affect symptoms) filled in on the same day of the initial research lab visit (day 0), repeated on day 14 (postintervention assessment for active/sham early groups, baseline for active/sham stimulation in late groups), and on day 28 (follow-up assessment for early groups, postintervention for late groups). See Fig. 1 for Consort Flow diagram depicting the study's design.

3.7. Statistical analysis

In total n = 78 participants were enrolled into our study, but n = 2 were excluded: one because of a stressful event and another with an unusual lifestyle. Additionally, we had to exclude participants who did not provide data on depressive symptoms, symptoms of anxiety, perceived stress pre- and poststimulation (n = 6). Of the n = 70 subjected to statistical analyses (early groups: active tVNS (n = 15), sham tVNS (n = 17); late groups: active tVNS (n = 23), sham tVNS (n = 15), n = 66 had complete data on all three outcomes of interest. Participants' characteristics are provided in Table 1.

Our hypotheses were tested with contrasts based on mixed linear regression models that were carried out to explore the main effect of time (T0 (day 0), T1 (day 13), T2 (day 28), within each group (early, late) and condition (active tVNS, sham tVNS); this was tested by a three-way interaction term and contrasts were derived. (T0 and T1 and T1 and T2 were 14 days apart). All analyses were adjusted for age, sex and prescribed medication use. Mixed linear models were used since they

consider all available data from the entire (or pre-specified) study duration, taking into consideration the notion that repeated measures of the same participant are dependent. In our statistical approach, the intercept was fitted as a random effect based on participant IDs, allowing the model to account for individual differences in baseline levels of depressive symptoms, anxiety, or stress. The focus of the model is on within-person change between the three timepoints, and on whether these changes differ mainly between active tVNS and sham tVNS.

The basic models included the following terms: CESD-10, GAD-7, or PSS-10 as outcome, time (T0,T1 and T2), group (active tVNS and sham tVNS), phase (early and late), sex, age, prescribed medication, and a two-way interaction term between Time x Group, Time x Phase and Group x Phase; as well as a three-way interaction term between Time x Group x Phase. In the first step of our analyses, contrasts were derived a priori from the respective model, investigating significant changes between adjacent time points (T0 vs. T1, T1 vs. T2) for each interaction of group and condition. In statistical models such as the one fitted in our analysis, performing contrasts is recommended to test a priori expectations. Indeed, planned comparisons between specific conditions (groups) or clusters of conditions have been suggested to be used as contrasts in the literature (Hays, 1973; Schad et al., 2020).

Hypotheses were tested with two priori pre-specified primary superiority contrasts defined as linear combinations of within-group time contrasts for early phase (T0 vs. T1) and late phase (T1 vs. T2) (each phase tested separately). To control for multiple comparisons in the six main tests (two main contrasts by three outcomes), p-values were adjusted using Holm step-down correction. These contrasts are the between-subjects test of the hypotheses. We recognize that testing early and late phases separately may reduce statistical power due to smaller sample sizes. Therefore, as additional analyses, we also calculated two combined contrasts for each outcome; first contrast across both phases (i.e. early and late) to increase precision and strengthen inference, and second combined contrast testing the change from T0 to T1 vs. the change from T1 to T2 within the active/late group, to the change from T0 to T1 vs. the change from T1 to T2 within the sham/late group. Additionally, we calculated exploratory contrasts within each superiority contrast of the main analyses presenting simple within-group time comparisons between T0 and T1 in early tVNS and early sham tVNS groups, and between T1 and T2 in late tVNS and late sham tVNS groups. For transparency these results are presented with nominal p-values, and are not used to draw primary conclusions. Hedge's g (preferred when sample sizes are smaller) were calculated as measures of effect size (Hedges, 1981).

In order to graphically visualize differences between pre- and post-intervention values, we calculated changes in depression and anxiety symptoms and perceived stress scores from T0 to T1 for early phases, and from T1 to T2 for late phases (for the early phase this meant subtracting T1 from baseline/T0 values and for the late phase it meant subtracting T2 from T1 values). Linear regression and relevant contrasts, controlled for age, sex and prescribed medication, tested the difference in the calculated changes in outcome of interest between active and sham in early and late phases. The model, which is used to visualize the differences in change over the 2 weeks of intervention between active and sham groups in each phase, included the following terms: pre- and postintervention change in depression and anxiety symptoms and perceived stress as outcome, group (active tVNS and sham tVNS), phase (early and late), sex, age, prescribed medication and a two-way interaction term between Group x Phase. These data are shown in Fig. 5.

Sensitivity analyses were performed to test the robustness of our findings and minimize the possibility of regression to the mean; analyses we repeated controlling for three variables: baseline values, Baseline x Change between T0 and T1, and Baseline x Change between T1 and T2. For these models we have deleted the random intercept.

All analyses were conducted using STATA 15.1. Results are presented as Contrasts (C), 95 % Confidence Intervals (CI) and p-values (nominal and corrected with Holm step-down correction, as appropriate).

4. Results

4.1. Background characteristics

Table 1 indicates that at baseline participants did not differ across the study variables except that depressive symptoms were higher in the late active and late sham tVNS groups. However, there were no significant differences in depressive symptoms at baseline (T0) between early tVNS and early sham tVNS groups (p = .86), which served as a pre-intervention measurement, and also there were no significant differences between late tVNS and late sham tVNS groups (p = .08) at T1, which served as a pre-intervention timepoint for late groups in our study. In the tVNS groups, the length of average daily use of the device was 3.8 h, and it was 3.9 h in the sham groups. Data from daily diaries used to gather additional information on adherence confirmed that in the active and sham groups daily use of the device was over 90 %. Participants did not report adverse side effects except light pain localized at the site of electrode contact due to wearing the simulator.

4.2. Hypotheses testing: superiority of active against sham tVNS

Analysis concerning depressive symptoms showed no superiority of tVNS against sham in the early stimulation phase (C = 0.83, p = .44, Hedges̍g = 0.26), and the late stimulation phase (C = 2.14, p = .10, Hedges̍g = 0.67) (see Table 2). For symptoms of anxiety, tVNS was superior to sham in the early stimulation phase (C = −1.67, p = .03, Hedges̍g = −0.85), but not in the late stimulation phase (C = 0.63, p = .42, Hedges̍g = 0.24) (see Table 3). For perceived stress, tVNS was superior to sham in the early stimulation phase (C = −3.24, p = .02, Hedges̍g = −0.89), but not in the late stimulation phase (C = 2.45, p = .12, Hedges̍g = 0.49) (see Table 4). See also Figs. 2–4 that show the means for each outcome at each timepoint for each group.

4.3. Additional analyses: combined contrasts Main 1 and Main 2

Overall combined effect of Main 1 and Main 2 contrasts were not significant for depressive and anxiety symptoms or perceived stress (shown as Combined 1 results in Tables 2–4, respectively). Likewise, the combined contrast for late phase groups (shown as Combined 2 results in Tables 2–4, respectively) testing the change from T0 to T1 vs. the change from T1 to T2 within the active/late group, to the change from T0 to T1 vs. the change from T1 to T2 within the sham/late group, was not significant for any of the three outcome measures.

4.4. Additional analyses: simple within-group time comparisons

Tables 2–4 additionally show simple effects of the prespecified contrasts of interest. Simple effects were observed mainly for active tVNS (as shown for depressive and anxiety symptoms, and for perceived stress), but also for sham tVNS time comparisons of interest. There was also tentative evidence that the effect remained stable in the followed-up period from T1 to T2 for perceived stress (C= −1.48, 95 % CI = −2.87 to −0.09) (data not shown in table but see Fig. 4, solid blue line).

4.5. Visualization of differences in calculated change from pre- to postintervention session between active and sham tVNS

Fig. 5 demonstrates the differences between active and sham tVNS groups in either the early or the late phase of calculated changes between pre- and postintervention periods. Fig. 5A shows that there is no difference in change in depressive symptoms from pre- to post-intervention period between active tVNS and sham tVNS in both early and late phases (early: p = .22 and late: p = .24). Fig. 5B demonstrates that anxiety symptoms from pre- to postintervention period differed between active and sham in the early but not the late phase (early: p = .02 and late: p = .56). Fig. 5C shows that the change in perceived stress from pre- to postintervention period is much greater in the early active tVNS group than in the early sham tVNS group (early: p = .01), while in the late phase there is no difference (p = .09).

4.6. Sensitivity analyses

Our main results were confirmed in the sensitivity analyses where we controlled for baseline levels, interaction between baseline and change between T0 and T1, and interaction between baseline and change between T1 and T2. In particular, following Holm step-down correction, the superiority of tVNS over sham tVNS remained significant for anxiety symptoms (C = −1.67, p = .01) and perceived stress (C = −3.64,

Table 2
Main and exploratory contrasts for depressive symptoms (CESD-10).

Contrast	Description	Contrast	95 % CI	P-value (Nominal)	Adjusted P-value (Holm)	Hedge̍s effect size
Hypotheses testing: superiority of active against sham tVNS						
Main 1: Superiority of active against sham tVNS in early groups	Difference of change from T0 vs. T1[a] between active tVNS early and sham tVNS early	0.83	-1.28 to 2.93	.44	.44	0.26
Main 2: Superiority of active against sham tVNS in late groups	Difference of change from T1 vs. T2[b] between active tVNS late and sham tVNS late	2.14	0.08 to 4.21	.04	.10	0.67
Additional contrast analyses						
Combined 1: Superiority of active against sham tVNS in early and late groups combined	Overall combined difference of change from T0 vs. T1 between active tVNS early and sham tVNS early and difference of change from T1 vs. T2 between active tVNS late and sham tVNS late	1.20	-2.09 to 4.49	.47	–	0.17
Combined 2: Waiting period against stimulation period - superiority of active against sham tVNS in late groups	Combined difference of change from T0 vs. T1 and change from T1 vs. T2 between active tVNS late and sham tVNS late	-2.88	-7.67 to 1.92	.24	–	-0.28
Simple 1	T0 vs. T1 active tVNS early	0.53	-0.70 to 1.77	.40	–	0.83
Simple 2	T0 vs. T1 sham tVNS early	-0.29	-2.00 to 1.41	.74	–	-0.33
Simple 3	T1 vs. T2 active tVNS late	-1.57	-2.55 to -0.58	**.002**[c]	–	-3.14
Simple 4	T1 vs. T2 sham tVNS late	-3.67	-5.49 to -1.85	**< .001**	–	-3.95

[a] T0 vs. T1 = change between day 0 vs. day 13;

[b] T1 vs. T2 = change between day 14 vs. day 28;

[c] For easier interpretation significant effects are marked in bold.

Table 3
Main and exploratory contrasts for anxiety symptoms (GAD-7).

Contrast	Description	Contrast	95 % CI	P-value (Nominal)	Adjusted P-value (Holm)	Hedge's g effect size
Hypotheses testing: superiority of active against sham tVNS						
Main 1: Superiority of active against sham tVNS in early groups	Difference of change from T0 vs. T1[a] between active tVNS early and sham tVNS early	-1.67	-2.99 to -0.34	.01	**.03**[c]	-0.85
Main 2: Superiority of active against sham tVNS in late groups	Difference of change from T1 vs. T2[b] between active tVNS late and sham tVNS late	0.63	-0.10 to 2.25	.45	.42	0.24
Additional contrast analyses						
Combined 1: Superiority of active against sham tVNS in early and late groups combined	Overall combined difference of change from T0 vs. T1 between active tVNS early and sham tVNS early and difference of change from T1 vs. T2 between active tVNS late and sham tVNS late	2.31	-0.30 to 4.94	.08	–	0.41
Combined 2: Waiting period against stimulation period - superiority of active against sham tVNS in late groups	Combined difference of change from T0 vs. T1 and change from T1 vs. T2 between active tVNS late and sham tVNS late	1.19	-2.28 to 4.65	.50	–	0.16
Simple 1	T0 vs. T1 active tVNS early	-1.67	-2.63 to -0.71	**.001**	–	-3.37
Simple 2	T0 vs. T1 sham tVNS early	1.11	-0.91 to 0.91	1.00	–	2.33
Simple 3	T1 vs. T2 active tVNS late	-1.13	-1.84 to -0.42	**.002**	–	-3.10
Simple 4	T1 vs. T2 sham tVNS late	-1.8	-3.26 to -0.34	**.02**	–	-2.41

[a] T0 vs. T1 = change between day 0 vs. day 13;
[b] T1 vs. T2 = change between day 14 vs. day 28;
[c] For easier interpretation significant effects are marked in bold.

Table 4
Main and exploratory contrasts for perceived stress (PSS-10).

Contrast	Description	Contrast	95 % CI	P-value (Nominal)	Adjusted P-value (Holm)	Hedge's effect size
Hypotheses testing: superiority of active against sham tVNS						
Main 1: Superiority of active against sham tVNS in early groups	Difference of change from T0 vs. T1[a] between active tVNS early and sham tVNS early	-3.24	-5.71 to -0.77	.01	**.02**[c]	-0.89
Main 2: Superiority of active against sham tVNS in late groups	Difference of change from T1 vs. T2[b] between active tVNS late and sham tVNS late	2.45	-0.64 to 5.55	.12	.12	0.49
Additional contrast analyses						
Combined 1: Superiority of active against sham tVNS in early and late groups combined	Overall combined difference of change from T0 vs. T1 between active tVNS early and sham tVNS early and difference of change from T1 vs. T2 between active tVNS late and sham tVNS late	2.78	-1.64 to 7.20	.22	–	0.29
Combined 2: Waiting period against stimulation period - superiority of active against sham tVNS in late groups	Combined difference of change from T0 vs. T1 and change from T1 vs. T2 between active tVNS late and sham tVNS late	-1.52	-2.28 to 4.65	.44	–	–0.18
Simple 1	T0 vs. T1 active tVNS early	-2.07	-3.67 to -0.46	**.01**	–	-2.50
Simple 2	T0 vs. T1 sham tVNS early	1.18	-0.70 to 3.05	.22	–	1.22
Simple 3	T1 vs. T2 active tVNS late	-1.52	-3.01 to -0.04	**.04**	–	-1.98
Simple 4	T1 vs. T2 sham tVNS late	-3.93	-6.63 to -1.24	**.004**	–	-2.84

[a] T0 vs. T1 = change between day 0 vs. day 13;
[b] T1 vs. T2 = change between day 14 vs. day 28;
[c] For easier interpretation significant effects are marked in bold.

p = .004) in early phase groups, with no effects seen for late groups (anxiety symptoms C = 1.03, p = .54, perceived stress symptoms C = 2.67, p = .38). There was no indication of superiority effects for depressive symptoms either in early (C = 0.96, p = .54) or late groups (C = 2.16, p = .13). The combined contrasts remained non-significant (data not shown) with the exception of anxiety symptoms, which became significant (p = .045) for the superiority of active against sham tVNS in early and late groups combined contrast. Overall, these findings indicate that our results are not due to regression to the mean.

5. Discussion

The study tested if a two-week course of tVNS would lead to a reduction of affective symptoms (depression and anxiety) and stress, when compared with sham stimulation. Analyses showed that the effects of active tVNS stimulation were superior to sham stimulation in early phase groups, but not in late phase groups, for anxiety symptoms and perceived stress, with a large effect size. No superior effects of tVNS against sham was detected for depressive symptoms. These results were also confirmed in sensitivity analyses controlling for regression to the mean, where for anxiety symptoms the combined overall effect of early
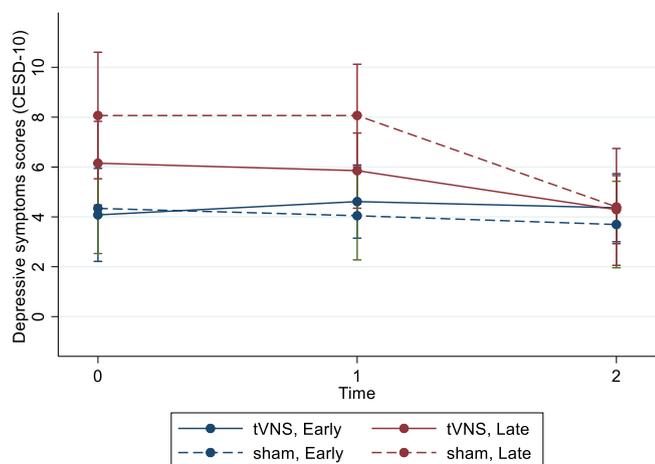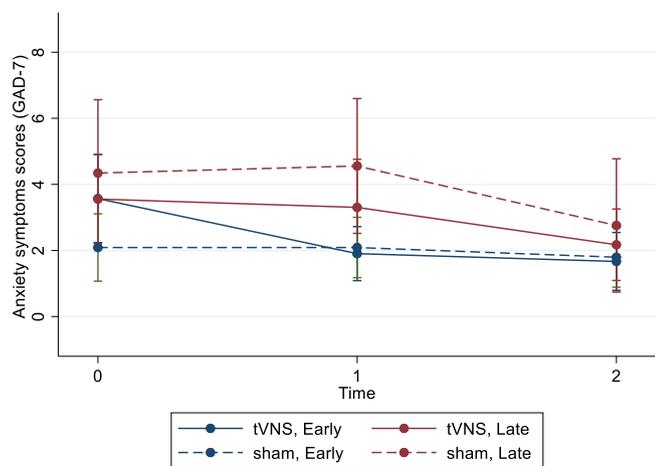
Fig. 2. Change in depressive symptoms scores (CESD-10) in 4 intervention groups over the course of the study. Predicted change in depressive symptoms scores (CESD-10) with 95 % confidence intervals in n = 70 men and women aged 18–75 years who underwent 14 days of daily active tVNS (solid blue and red lines) or daily sham tVNS (dashed blue and red lines). Estimates for each timepoint for each group were predictions from mixed model including depressive symptoms, time, group, phase, age, gender, prescribed medication and the following interaction terms: Time x Group; Time x Phase; Group x Phase; Time x Group x Phase.

Fig. 3. Change in anxiety symptoms scores (GAD-7) in 4 intervention groups over the course of the study. Predicted change in anxiety symptoms scores (GAD-7) with 95 % confidence intervals in n = 70 men and women aged 18–75 years who underwent 14 days of daily active tVNS (solid blue and red lines) or daily sham tVNS (dashed blue and red lines). Estimates for each timepoint for each group were predictions from mixed model including global sleep scores, time, group, phase, age, gender, prescribed medication, and the following interaction terms: Time x Group; Time x Phase; Group x Phase; Time x Group x Phase.

and late active tVNS versus sham was found superior, rather than only in the early group in the main analysis. Our subsequent explanatory simple within-group time comparisons tests showed that depressive symptoms, anxiety symptoms and perceived stress were mainly reduced following tVNS, but improvements were also seen in placebo groups. These analyses also provided a tentative support that the effects of active tVNS on perceived stress in the early group persisted over the follow-up period. Overall, our research hypotheses were partially supported by the data, and our findings suggest that 2 weeks of active tVNS may be effective in improving anxiety and perceived stress, but not depressive symptoms.

The finding that tVNS can lower symptoms of anxiety in non-clinical adults tentatively supports existing data in clinical populations (e.g.
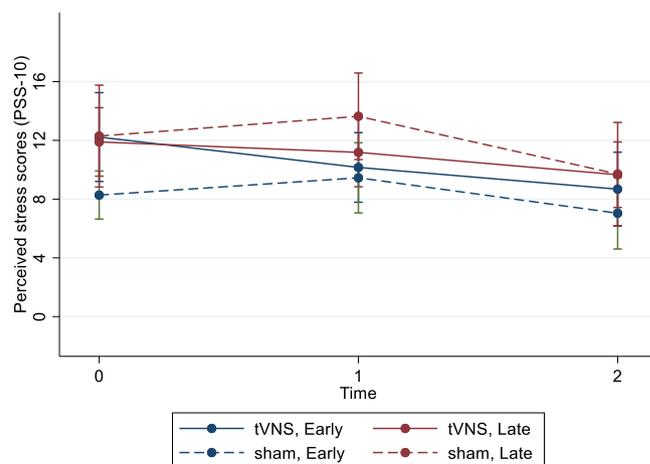

Fig. 4. Change in perceived stress scores (PSS-10) in 4 intervention groups over the course of the study. Predicted change in perceived stress scores with 95 % confidence intervals in n = 70 men and women aged 18–75 years who underwent 14 days of daily active tVNS (solid blue and red lines) or daily sham tVNS (dashed blue and red lines). Estimates for each timepoint for each group were predictions from mixed model including global sleep scores, time, group, phase, age, gender, prescribed medication, and the following interaction terms: Time x Group; Time x Phase; Group x phase; Time x Group x Phase.

Fang et al., 2016; Li et al., 2022; Zhang et al., 2024). Our findings are in line with recent non-clinical studies conducted in university students with elevated anxiety levels (Ferreira et al., 2024), or retired teachers (Srinivasan et al., 2024). With regards to the effectiveness of tVNS in lowering perceived stress levels, to the best of our knowledge, this is a first published study to demonstrate such an effect, especially in a long-term setting. However, in an experimental study conducted in PTSD patients, Bremner and colleagues (2020) found that following an exposure to personalized traumatic scripts, tVNS lowered inflammatory responses (i.e., interleukin-6 (IL-6) and interferon-γ (IFN-γ), and ratings of subjective anger, when compared with sham tVNS. Relatedly, beneficial effects of tVNS were also confirmed for neurobiological stress response, whereby tVNS lowered levels of pituitary adenylate cyclase-activating peptide in people with prior exposure to psychological trauma and PTSD diagnosis (Gurel et al., 2020). Another experimental study in healthy adults, albeit not directly related to the experience of stress, showed that acute tVNS, relative to sham, led to differences in cognitive reappraisals whereby those receiving an active stimulation rated their response to emotion-eliciting pictures as less intense (De Smet et al., 2021). Clearly, more naturalistic studies are warranted to confirm the utility of tVNS in modulating the stress experience.

In contrast to the effects seen for anxiety and perceived stress, we failed to demonstrate that a 2-week course of tVNS decreases depressive symptoms. In fact, as shown in simple contrasts analysis, in late groups the effect of sham was slightly larger than the simple effect of active tVNS, but the superiority effect was not statistically significant. One possible explanation for the lack of superiority of active tVNS is due to higher (though not statistically significant) baseline levels of depressive symptoms in the late sham group (see Table 1), compared to the late active tVNS group, suggesting that those presenting with greater depressive symptoms responded to a larger degree to (sham) stimulation preventing detection of stronger effect of active tVNS, compared to sham. Likewise, in the early groups, the average depressive symptoms scores were approximately 4, which is below average for community-dwelling young or middle-aged populations (Vilagut et al., 2016), suggesting that floor effects of low symptoms may have prevented the scores to improve (i.e., decrease) following tVNS stimulation. Lastly, the reason we did not observe a superior effect of active tVNS compared to sham stimulation on depressive symptoms may be that depressive
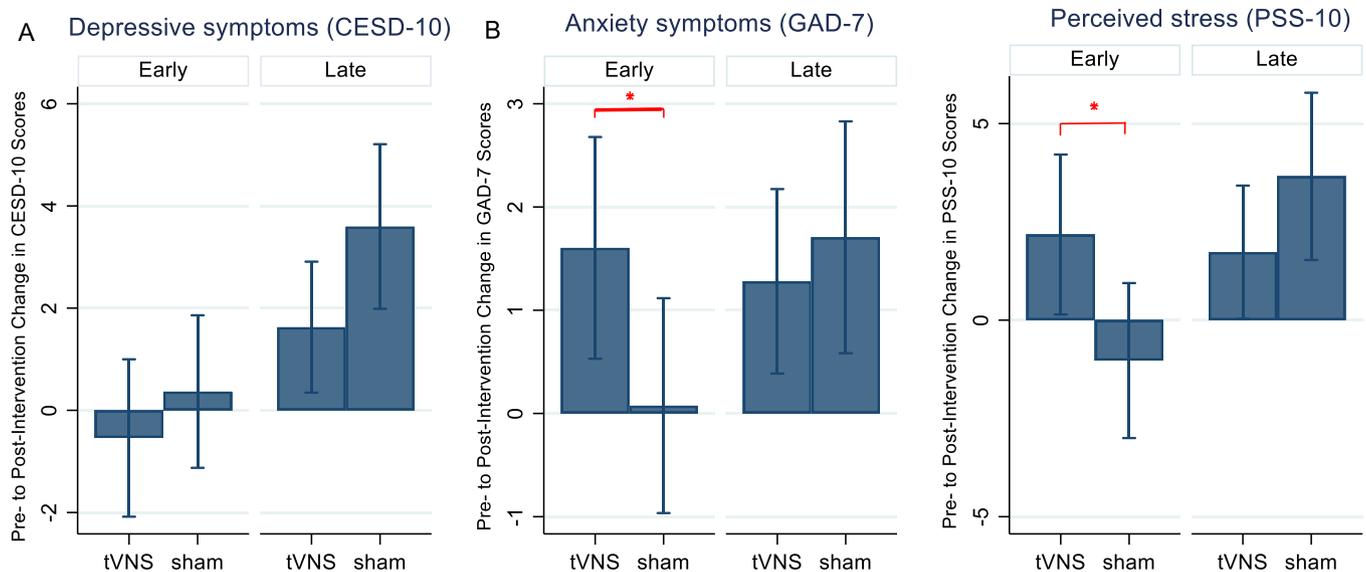
Fig. 5. Differences between the pre- and post-intervention values of depressive symptoms score (CESD-10) (A), anxiety symptoms score (GAD-7) (B) and perceived stress score (PSS-10) (C) between T1 and T0 (baseline) for early groups and between T2 and T1 for late groups. * $p \leq 0.05$.

symptoms are generally more stable and less reactive in everyday life, whereas anxiety symptoms and perceived stress are more dynamic, state-like, and easier to modulate (Hammen, 2005).

There is still no agreement between experts in the field relating potential mechanisms responsible for improvements in affective wellbeing following VNS or tVNS (Austelle et al., 2022). Moreover, while stimulating vagus nerve has been found to reduce depression and (to some extent) anxiety in clinical populations, it is still unclear which fibers of the vagus nerve are responsible for these improvements, and how to establish effective stimulus parameters to excite with precision these and no other fibers (see Karemaker, 2022, for a review). Nonetheless, based on the anatomical literature relating tVNS and its effects on the brain, it may be speculated that the pathways through which tVNS may reduce symptoms of anxiety likely include stimulation of the brain areas of the central autonomic network implicated in both autonomic regulation and anxiety, including the prefrontal cortex and the anterior cingulate (Yakunina et al., 2017), as well as an enhanced connectivity between the amygdala and the prefrontal cortex (Liu et al., 2015). The pathways through which tVNS modulates stress are less understood, but likely overlap with those involved in anxiety (as well as in PTSD) and include the locus coeruleus, hypothalamus, the amygdala as well as the medial prefrontal cortex and the anterior cingulate (Hardy, 1995). While anxiety symptoms and stress experience involve emotional regulation, anxiety is driven by fear and worry, and stress is more related to the body's acute responses to danger. tVNS may thus lower anxiety and stress symptoms by influencing autonomic regulation and dampening overactive responses in the amygdala and prefrontal cortex (Austelle et al., 2022; Kong et al., 2018).

This study has limitations. A priori power analysis was not performed due to the complexity of the study's design comprising four groups with two different intervention periods (active vs. sham × early vs. late) carried out in a non-clinical population; the use of linear mixed-effects models additionally prevented us from conducting a reliable power estimate that was based on prior evidence relating expected effect sizes. Instead, the sample size was aligned with previous experiments in non-clinical samples (e.g., Bretherton et al., 2019; Burger et al., 2016; Jacobs et al., 2015; De Smet et al., 2021), and additionally for all contrasts we report Hedge's g statistics as measures of effect sizes that is a preferred approach when sample sizes are smaller. While in our main contrast analysis we found superiority of active against sham tVNS for anxiety symptoms and perceived stress scores in early groups, the combined

contrast was not significant except for anxiety symptoms, which in the sensitivity analysis became significant for the superiority of active against sham tVNS in early and late groups combined contrast. The lack of blinding of laboratory staff is a potential limitation. While this was common practice at the time, current research standards increasingly emphasize double-blinding to reduce bias. Using the earlobe for sham stimulation, although generally considered anatomically free of vagal innervation (Peuker & Filler, 2002), can be seen as another shortcoming. This choice has been questioned (Rangon, 2018), as the earlobe is also used in other non-invasive brain stimulation methods, such as cranial electrotherapy stimulation (Gianlorenco et al., 2022). Therefore, it may not be entirely physiologically neutral and could produce effects similar to auricular tVNS, potentially confounding results (Butt et al., 2020; Yakunina et al., 2017), and explaining some of the effects in sham groups. Despite this, the earlobe remains the standard sham site in auricular stimulation studies (Urbin et al., 2021). It produces sensations similar to active stimulation without activating vagal brainstem pathways (Colzato & Beste, 2020), which helps reduce bias due to unblinding (Wolf et al., 2021). tVNS lasted 2 weeks, which is a relatively brief period, and other protocols comprised stimulation over 4 weeks (e.g., Fang et al., 2016; Srinivasan et al., 2024). On the other hand, studies on long-term tVNS in non-clinical populations are scarce, and most evidence stems from acute single sessions of tVNS. Data on adherence were obtained, but relied on self-reports. Finally, as there is no generally agreed and accepted valid evidence-based biomarker of vagal activation, our research was limited in ability to confirm whether all participants responded to tVNS or not. This limitation made it difficult to account for individual differences in our treatment response, which may have influenced the observed outcomes.

Notwithstanding, the soundness of our results is enhanced by the study's strengths. In contrast to many studies that used tVNS, we employed an active sham control. The design used allowed to explore within-subject as well as between-subject effects. Additionally, in the early stimulation group, we were able to monitor the persistence of the stimulation effects over a 2-week follow-up period. Furthermore, to model within-individual changes over the study's duration, we employed mixed-effects models that are robust. Importantly, the study was conducted in a non-clinical sample tested in a naturalistic setting (4 h of daily home-based stimulation), which remains scarce in the tVNS research. Given that subclinical stress and mood disturbances are widespread in the general population, the use of a non-clinical sample

enabled us to assess the preventive potential of tVNS in everyday contexts, as well as its potential in mitigating emotion regulation difficulties often present in the aging process, thereby enhancing ecological validity. At the same time, this population allowed us to reduce potential confounding associated with psychiatric diagnoses, pharmacological treatment, or therapy history, which are often difficult to control in clinical samples. However, we acknowledge that our participants were likely healthier than the general population, which is a common feature of studies involving non-clinical volunteers. Finally, our statistical models were adjusted for factors relevant to vagal nerve function, in particular, age, sex, and prescribed medication, and subjects endorsing health conditions potentially impacting vagus nerve activity were not recruited.

Taken together, our findings suggest that a two-week course of tVNS may lower anxiety symptoms in a non-clinical sample. We additionally demonstrate, for the very first time, that long-term tVNS may modulate perceived stress, pointing to its potential as a low-risk, accessible method to reduced stress perception and support psychological wellbeing. Importantly, the significant effects seen on reported levels of anxiety and perceived stress were superior to sham stimulation, but only in early groups and not in late groups, suggesting the findings require further replication. Our study failed to demonstrate that tVNS, relative to sham stimulation, may reduce reported levels of subthreshold depressive symptoms in a non-clinical setting. Notwithstanding, our study adds to the growing evidence that tVNS application could be scaled-up to a population level to improve mental health outcomes and potentially mitigate stress vulnerability and higher anxiety, which are often prevalent in older adults and increased in the ageing process. To validate and extend these findings, future research should include larger samples, apply longer than 2 weeks intervention periods, but similarly as in our study, future studies should administer a 4 h daily stimulation, include older participants and incorporate follow-up assessments to evaluate the durability of effects. The use of stimulation devices with adherence-monitoring capabilities will be valuable, particularly in naturalistic settings. Although our study focused on a non-clinical sample, these results may inform future trials in clinical populations experiencing elevated level of perceived stress or anxiety. If replicated, such findings could support the integration of tVNS into broader non-pharmacological strategies for mental health promotion in both community and clinical contexts.

CRediT authorship contribution statement

Marta Jackowska: Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. **Julian Koenig:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Veronika Cibulcova:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Vera K. Jandackova:** Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. Marta Jackowska and Vera K. Jandackova contributed equally to this work. Vera K. Jandackova also served as a principal investigator and project lead.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use generative AI technologies for preparation of this work.

Declaration of Competing Interest

All authors of this article have no conflicting interest and nothing to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopsycho.2025.109169.

Data availability

Data will be made available on request.

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