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A two-week course of transcutaneous vagal nerve stimulation improves global sleep: Findings from a randomised trial in community-dwelling adults☆

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ABSTRACT

Short sleep duration and poor sleep quality are common in the general population. This study tested if a 2-week course of daily transcutaneous vagal nerve stimulation (tVNS) improves sleep in community-dwelling adults. Participants were n = 68 men and women aged 18–75 years randomised into four groups: early and sham tVNS and late and sham tVNS. Early groups underwent daily 4 h stimulation between Day 0 and 13, while late groups underwent daily 4 h stimulation between Day 14 and 28. tVNS was performed with transcutaneous electrical nerve stimulation (TENS) on the left tragus, and sham tVNS (control conditions) was applied on the left earlobe. Sleep was measured with the Pittsburgh Sleep Quality Index. Analysis of prespecified contrasts (C), based on linear mixed modelling, revealed that for tVNS there were significant improvements in global sleep scores over time between Day 0 and Day 13 in the early stimulation phase (C = −1.90; 95% CI = −2.87 to −0.94), and between Day 14 and Day 28 in the late phase (C = −0.87; 95% CI = −1.41 to −0.32). No such differences were found under sham tVNS (applied early or late). However, global sleep scores showed no significant improvement under tVNS when compared against control groups during both the early ($\chi 2 = 0.83$, $p = 0.36$), or late stimulation phase ($\chi 2 = 0.24$, $p = 0.63$). We showed that two weeks of tVNS improves global sleep scores, but the change in sleep was not significantly different to control groups. Further studies are warranted to test the utility of tVNS in alleviating sleep complains in community-dwelling adults.

1. Introduction

Poor sleep quality and too short sleep duration, typically defined as ≤5–6 h per night, are common in the general population (Hisler et al., 2019; van de Straat and Bracke, 2015). For example, data from over 80.000 British men and women showed that as many as 43.3% slept for <6 h (Zhu et al., 2019). A study based on over 40.000 men and women from 9 countries reported a 9.2% prevalence rate of poor sleep quality (defined as a combination of difficulties with falling asleep, waking up frequently in the night and waking up too early in the morning), with countries like Poland and India reporting a 17% and 15% prevalence, respectively (Koyanagi et al., 2014). This is noteworthy given the well-established links between too short sleep and poor sleep quality and heighten risk of many non-communicable diseases such as cardiovascular outcomes and mortality (Cappuccio et al., 2011; Sofi et al., 2014), type 2 diabetes, obesity, hypertension (Itani et al., 2017; Schmid et al., 2015), and cancer (Chen et al., 2018). Reduced cognitive performance (Jackowska and Cadar, 2020) and cognitive decline (Lo et al., 2014), as well as depression (Zhai et al., 2015), are also predicted by unhealthy sleep patterns including both too short sleep and low sleep quality.

Given the deleterious consequences of poor sleep on physical and mental health in community-dwelling adults, efforts are warranted to improve people's sleep. For mild to moderate sleep difficulties, improvements in sleep hygiene (e.g., timing of exercise, alcohol use, napping, regular bedtime) are often recommended, yet empirical support for their effectiveness in the general population have been criticised and

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remain mainly restricted to experimental or clinical data (Irish et al., 2015). Severe and chronic sleep disturbances, in particular insomnia disorder, can be treated with pharmacotherapy (e.g., benzodiazepines, hypnotics) and cognitive behavioural therapy for insomnia (CBT-I) (e.g., see Miller et al. (2014) for a review). There is also growing evidence that CBT-I delivered online can be efficacious (Zachariae et al., 2016). However, pharmacotherapy is limited to short-term improvements, and is associated with side effects and potentially addiction (Miller et al., 2014). There is also a shortage of therapists specialising in CBT-I, and many individuals struggle to complete a full course of CBT-I (Matthews et al., 2013).

Interestingly, a recent review reported preliminary but promising effects of two non-invasive brain stimulation techniques on sleep difficulties. Briefly, Herrero Babiloni et al. (2021) found evidence for repetitive transcranial magnetic stimulation (rTMS), which utilises magnetic pulses, to be particularly useful for ameliorating sleep disturbances in conditions including major depressive disorder, Parkinson's disease, and chronic pain. One way through which rTMS may improve sleep is by decreasing cortical hyperarousal, achieved by lowering hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-thyroid (HPT) axis activity. The review also found transcranial direct current stimulation (tDCS), which uses electrical currents, to be effective in insomnia, and it has been proposed that in this technique sleep improves by altering electroencephalographic (EEG) activity (see Herrero Babiloni et al., 2021 for more details). However, findings of this review are limited to individuals with neurological and neuropsychiatric conditions, or individuals with insomnia disorder, thus do not generalize to the general population.

Another non-invasive technique that could potentially improve sleep is transcutaneous vagal nerve stimulation (tVNS). In tVNS, stimulation is applied to the ear where the auricular branch of the vagus nerve is located (see Yap et al., 2020 for a detailed review). In healthy sleepers, the transition from wake to sleep states is linked to a decrease of sympathetic activity and an increase of parasympathetic modulation of the heart, which is facilitated by the vagus nerve (Meerlo et al., 2008; Stein and Pu, 2012). Therefore, stimulating the vagus nerve may potentially help to maintain a flexible balance of the autonomic nervous system, needed to facilitate wake and sleep states. Increasing vagal nerve activity may also help to improve sleep via a reduction of inflammation and cortisol levels, both of which are implicated in poor sleep (Abell et al., 2016; Irwin, 2019). Briefly, stimulation of the vagal nerve activates neurons in the nucleus of the solitary tract (NST), and efferent vagal nerves that project from the NST to various organs in the body (e.g. the heart or lungs) release acetylcholine, which offsets pro-inflammatory cytokines (Thayer and Sternberg, 2010).

So far, tVNS has been mainly used to treat depression, epilepsy, tinnitus, pain and migraine (Yap et al., 2020). To date, very few studies have tested the effects of tVNS on sleep, and the therapeutic impact of non-invasive vagal nerve stimulation on this key health behaviour is yet to be recognised. Luo et al. (2017) showed that stimulation of the auricular concha led to improvements in sleep in patients with insomnia disorder, however since this study lacked a control group its findings are challenging to interpret. Another study, also based on patients with insomnia disorder, found that tVNS was associated with improvements in sleep, but this effect was not superior to sleep changes seen in a control group (Jiao et al., 2020). Importantly, this experiment as well as the study by Luo et al. (2017), were based on clinical populations, limiting a generalization to community-dwelling adults, who nonetheless often report a high prevalence of short sleep of low quality. Bretherton et al. (2019) showed that in community-dwelling adults, who were free of insomnia disorder, tVNS applied daily over a two-week period led to improvements in sleep quality. Notably, this trial was based on n = 26 participants (of whom n = 9 responded to the stimulation) and lacked a control group, while sleep was measured with an unvalidated questionnaire. This leaves the utility of tVNS in alleviating non-clinical sleep complaints in the general population uncertain. Therefore, given the growing interest in non-invasive and portable devices stimulating vagus nerve (Gidron et al., 2018), and the need to test novel techniques that could improve sleep in community-dwelling adults, this study tested the hypothesis that a two-week course of tVNS would lead to improved global sleep ratings, when compared with placebo stimulation.

2. Method

2.1. Design

This study was a single-blinded randomised control clinical trial (NCT04070547) that tested the effect of a 2-week course of tVNS on cognitive function and health-related variables including global sleep scores (focus of the present manuscript). Participants were randomised into early or late (waitlist) group (first step randomisation). In the early group, participants were further randomised into tVNS (active/actual intervention) and a control condition (sham tVNS) that started immediately after baseline measures (Day 0 to 13); two weeks after tVNS and control stimulation ended participants further provided follow-up assessments (Day 14 to 28). In the late (waitlist) group, participants randomised into active and sham tVNS groups began their conditions two weeks after baseline assessments (Day 14 to 28). In total, participants in the 4 arms detailed above remained in the study for 4 weeks.

Our rationale to randomise participants into early and late groups was to enable us to test changes in variables of interest between the pre- and postintervention phase in active intervention and placebo groups, as well as to test changes in variables of interest after active and sham stimulation ceased (Day 14 to 28) in the early groups, and whether any change occurred during waiting time in the late groups (Day 0 to 13).

2.2. Participants

Participants were n = 78 men and women aged 18–75 years recruited through advertisement from Ostrava University, Czech Republic, and neighbouring institutions. The exclusion criteria were as follows: cardiovascular disease (e.g., arrhythmia, history of coronary heart disease, history of stroke), severe mental condition (e.g., clinical depression, schizophrenia, anxiety disorder), severe neurological condition (e.g., epilepsy, brain tumours, significant migraine, traumatic brain injury), brain surgery, and pregnancy. Sample size was not determined based on formal a-priori power analysis but in line with existing research (e.g., Bretherton et al., 2019). The study was approved by the Ethnical Committee of University of Ostrava. Participants providing written informed consent and who completed the study received a small honorarium of 1000 CZK.

2.3. Procedure

Following eligibility screening, participants were allocated into early (n = 38) and late groups (n = 40). Written consent was obtained during the first visit to the research laboratory. Sociodemographic data and income information were measured via questionnaire during screening, at which stage participants were also asked in detail about their health and prescribed medication. Blood pressure, heart rate variability (HRV) (not described here), and height and weight measures were taken, followed by a battery of cognitive and emotion testing (not described here). Following these assessments, a second step randomisation allocation was applied whereby participants were allocated into active (actual) tVNS or active sham (placebo) tVNS conditions. Participants were explained how to fit and use the tVNS stimulator, and were given a manual. Information on adherence to tVNS device was submitted daily as well as after the stimulation ended (see Section 2.4 Transcutaneous vagus nerve stimulation below, for full details). Online questionnaires (e.g., sleep scale or depressive symptoms described below) were completed at home on the day of the laboratory measurement (in most cases prior the lab session) (Day 0). Late group had an identical lab

session 2 weeks later (Day 14). Online questionnaires, including the sleep scale, were repeated at Day 14 (postintervention assessment for active/sham early groups, beginning of active/sham stimulation for late groups), and at Day 28 (follow-up assessment for early groups, post-intervention assessment for late groups).

2.4. Measures

2.4.1. Background measures

As mentioned, sociodemographic data were collected at screening stage. In our study, we included age and sex, education and employment status (see Table 1) as measures of sociodemographic and economic information. Height and weight were used to calculate body mass index (BMI, kg/m^2). We also included information on whether participants were taking prescribed medication including antihypertensives, diabetes, depression and sleep medication (see statistical analysis and Table 1). Depressive symptoms were measured with a shortened version of the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), which has been deemed valid and reliable (Andresen et al., 1994). In this study, we used a double back translation method as to obtain the scale in Czech language. In this questionnaire, participants are asked to give information on negative affect and somatic symptoms, and items are rated on a scale ranging from 0 to 3. Higher scores are indicative of greater depressive symptoms. In our sample, at baseline, the Cronbach alpha was 0.73, reflecting an acceptable internal consistency.

2.4.2. Sleep

Global sleep was measured with the self-reported Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), and a Czech version of the validated English version was obtained by a double back translation method into Czech language. Briefly, the PSQI consists of 19 items which measure 7 components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction; the sum of these components yields a global sleep score. In this study the scale was answered with regards to the past two weeks. Higher scores are indicative of greater sleep disturbances, and at baseline scores ranged from 1 to 13. Cronbach alpha was 0.71 at baseline. Each participant was asked to complete the PSQI at 3 time points: the early groups at preintervention (baseline), postintervention and at follow-up; the late group filled in the scale at beginning of the 2-week waiting period, then at preintervention and postintervention stage. For comparison purposes with other studies, we also present baseline sleep duration and efficiency, both derived from PSQI data; mean sleep duration was 6.9 (SD 1.1), and mean sleep efficiency was 90.5% (SD 9.4). These data suggest that our participants were, on average, good sleepers.

2.4.3. Transcutaneous vagus nerve stimulation

tVNS was delivered via modified transcutaneous electrical nerve stimulation (TENS) devices (Parasym, Parasym Ltd., London, UK) with 2 electrodes on a clip. The active stimulation group underwent 14 days of auricular tVNS placed on the left tragus as this ear location was shown to be 45% innervated via auricular vagus nerve (Yakunina et al., 2017). The sham (control) group underwent 14 days of sham tVNS placed on the left earlobe, which is not innervated via the vagus nerve (Yakunina et al., 2017), and therefore it is thought to be an appropriate ear location that may be used as an active placebo location (Farmer et al., 2021; Burger et al., 2020). Every participant was instructed to use the stimulator for 4 h (240 min) a day, in several time segments, for a period of 14 days in their own home. Participants were asked to set the intensity of stimulation with a constant current, based on an individual level of sensitivity, with a pulse width of 200–300 μs at a frequency of 25 Hz with no on/off cycles. Before placement of the clip on the ear, participants were instructed to spray a conductive liquid with electrolytes on the electrodes. After 14 days of tVNS intervention (active or sham) participants were asked to demonstrate how they set the tVNS device every day, and where on the ear they placed the electrodes. Only in 1 case the setting was incorrect therefore the participant was excluded from the study. To monitor protocol adherence, we used an online daily diaries, in which participants were asked to indicate duration of usage of the tVNS stimulator, and in how many separate time segments it was worn. Additionally, participants were asked to write on a piece of paper (white for active tVNS and yellow for sham tVNS) how many days of the intervention they used the tVNS device, and how many hours on average they used the stimulator every day. Then they were asked to drop the paper into a cardboard box, which was sealed until the end of the study. The settings of the tVNS device were designed in accordance with existing studies and recommendations of the recently established tVNS consensus group (see Farmer et al., 2021).

2.5. Statistical analysis

Of the n = 78 participants that were enrolled, n = 68 were included into our analytical sample. We had to exclude participants due to their personal circumstances (n = 1), and an incorrect wear of the tVNS

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

	Early group		Late group		p-Value
	Active tVNS (n = 15) Means (95% CI/frequency (%))	Sham tVNS (n = 16) Means (95% CI/frequency (%))	Active tVNS (n = 22) Means (95% CI/frequency (%))	Sham tVNS (n = 15) Means (95% CI/frequency (%))	
Age	47.3 (37.3 to 57.3)	51.8 (42 to 61.5)	50 (43.1 to 56.9)	42 (30.6 to 53.4)	0.46
Sex (men)	7 (46.7)	7 (43.8)	9 (39.1)	5 (35.7)	0.93
High educational level (degree)	5 (33.3)	10 (62.5)	14 (60.9)	8 (57.1)	0.32
Employment status					0.39
Student	3 (20)	2 (18.2)	2(10.5)	4 (33.3)	
Employed	8 (53.3)	7 (63.6)	16(84.2)	6 (50.0)	
Unemployed	4 (26.7)	2 (18.2)	1 (5.26)	2 (16.7)	
Body mass index	25.3 (22.5 to 28.1)	26.9 (24.5 to 29.3)	25.7 (23.8 to 27.6)	26.3 (23.6 to 28.9)	0.96
CESD-10 depressive symptoms	6.7 (4.2 to 9.3)	5.1 (3.6 to 6.5)	6.2 (4.6 to 7.8)	7.1 (4.3 to 10.03)	0.50
Prescribed medication use	5 (33.3)	6 (37.5)	9 (39.1)	4 (28.6)	0.92
Antihypertensives	1 (6.7)	5 (31.3)	5 (21.7)	1 (7.1)	0.20
Diabetes medication	1 (6.7)	1 (6.3)	1 (4.4)	0 (0)	0.81
Global sleep score[a]	5.9 (4.3 to 7.4)	4.7 (3.3 to 6.1)	5.3 (3.6 to 7.0)	4.9 (3.6 to 6.3)	0.71
Sleep duration[b]	7.0 (6.5 to 7.6)	7.1 (6.5 to 7.6)	6.9 (6.4 to 7.3)	6.8 (6.2 to 7.4)	0.89
Sleep efficiency (%)[b]	89.3 (84.4 to 94.2)	90.6 (85.8 to 95.3)	92.3 (88.1 to 96.4)	88.9 (83.4 to 94.4)	0.73

[a] Baseline difference in PSQI between active tVNS and sham tVN in early and late groups was tested separately.
[b] Derived from PSQI data.

stimulator (n = 1). Given that this study focuses on sleep and its longitudinal change, we also excluded participants who were taking hypnotics (n = 1), antidepressant medication (n = 3), and those who completed the sleep questionnaire only at 1 time point (n = 4). Of n = 68 participants (our analytical sample), n = 57 provided their global sleep ratings at all 3 time points, and n = 11 rated their sleep at 2 time points.

Participants characteristics were described as frequencies or means and 95% confidence intervals (95% CI), as appropriate. Differences in sociodemographic and economic characteristics, depressive symptoms and baseline sleep scores between the active and sham tVNS groups were tested using *t*-tests, univariate ANOVAS and chi-square tests, as appropriate. Mixed linear regression models were computed to examine main effects of the fixed effect of time (T0, T1, T2), group (early, late) and condition (active tVNS, sham tVNS), as well as their interaction. Mixed linear models use all available data over the follow-up period, and take into account the fact that repeated measures on the same individual are dependent. In these analyses, both the intercept and the slope were fitted as random effects, allowing individuals to have different sleep scores at baseline, and different rates of change in sleep over the follow-up period. The basic models included the following terms: sleep measure, time (0, 1 and 2), group (active tVNS and sham tVNS), phase (early and late), sex, age and two-way interaction terms between TIME × GROUP, TIME × PHASE and GROUP × PHASE; and a three-way interaction term between TIME × GROUP × PHASE (STATA syntax used to perform this analysis can be found in the supplementary materials). In our analyses, first, 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. Using contrasts has been suggested as an effective way to test a priori expectations based on statistical models such as those implemented here. Planned comparisons between specific conditions (groups) or clusters of conditions are recommended to be implemented as contrasts (see Hays, 1973; Schad et al., 2020). More specifically, in experimental designs including ours, tests such as analysis of variance (ANOVA) F-statistics offer limited information about the source of effect or interaction with regards to a factor of interest. Furthermore, in situations when ANOVA yields a significant main effect the magnitude and source of effect remains vague without proper post-hoc tests. It is common that in experiments including ours, researchers have a priori expectation about the pattern of means. We could have implemented subsequent individual *t*-tests, but given the complexity of our design and the number of study groups, this could have resulted in a loss of power and would not have considered all observations in our data set. Therefore, based on the recommendations by Schad et al. (2020), instead of performing multiple comparisons that are not recommended for complex models including linear mixed-effects models we used contrasts. In the case of significant differences for one condition between adjacent time periods per expectation in the group (i.e., when actual stimulation was applied), superiority of the effect was tested against the other active stimulation condition. Men and women were combined in the analyses as the interaction term between time (0, 1 and 2), group (active tVNS and sham tVNS), phase (early and late) and sex (male and female) suggested no significant differences in sleep scores over time between men and women (p = 0.131).

All analyses were conducted using STATA 14. Results are presented as contrasts (C), 95% confidence intervals (CI) and p-values.

3. Results

3.1. Descriptive statistics

Table 1 shows baseline characteristics of the study participants in active tVNS and sham tVNS groups. Participants did not differ across the four groups in any of the variables that may impact vagal nerve activity or sleep, such as age, sex, BMI, or depressive symptoms.

In terms of adherence to wearing the tVNS device, data from the anonymous box showed that all participants used the tVNS device for a minimum of 12 out of 14 days of intervention. There were 95% of participants in active tVNS and 92% in sham tVNS groups who reported using the tVNS device every day. The average daily mean time of using the tVNS device was 3.8 h for active tVNS groups, and 3.9 h for sham tVNS groups. (Interestingly there were 67% and 72% of participants in active and sham tVNS groups, respectively, who reported anonymously using the tVNS device daily for the full 4 h; usually in 2 to 3 sessions).

3.2. Main analysis

On average, results from the mixed models indicated a significant main effect of TIME in global sleep scores, specifically, on average PSQI scores decreased over time (p = 0.0006).

Analysis of prespecified contrasts revealed that for the active tVNS group there was a statistical difference in the estimated means between time 0 and time 1 in participants who underwent stimulation "early" (see Fig. 1, blue line) (C = −1.90; 95% CI = −2.87 to −0.94), and between time 1 and time 2 in participants who received stimulation "late" (see Fig. 1, red line) (C = −0.87; 95% CI = −1.41 to −0.32) (see Table 2). Furthermore, as shown in Fig. 1 (see blue line), the improvement in global sleep scores achieved in the early tVNS group between time 0 and time 1 reduced over the subsequent follow-up period between time 1 and time 2, but the reduction was not statistically significant (C = 0.6; 95% CI = −0.21 to 1.41) (see Table 2). Similarly, in the late active tVNS group (see Fig. 1, red line), global sleep scores did not change over the first 14 days of waiting between time 0 and time 1 (C = 0.37; 95% CI = −0.63 to 1.36) (see Table 2). For the sham tVNS (control) group, the difference in the estimated means between time 0 and time 1 in participants who underwent stimulation "early", and between time 1 and time 2 in participants who underwent sham stimulation "late" was not significant (see Table 2). In the sham early (Fig. 1, green line) group, global sleep scores did not change significantly in the follow-up period between time 1 and time 2; and participants in the sham late tVNS group (Fig. 1, yellow line) did not report significant changes in global sleep scores in the waiting period between time 0 and time 1 (see Table 2). In supplementary materials we further present results of our linear mixed model with random intercepts for the effect of

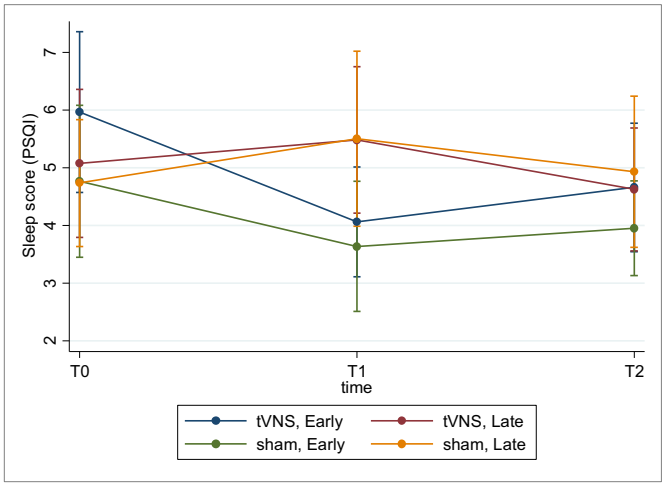

Fig. 1. Change in global sleep scores in 4 intervention groups over the course of the study.
Predicted change in global sleep scores (PSQI) with 95% confidence intervals in 68 men and women aged 18–75 years who underwent 14 days of daily active tVNS (blue and red lines) or daily sham tVNS (green and orange lines). Estimates for each timepoint for each group were predictions from mixed model including global sleep scores, time, group, phase, age, gender and the following interaction terms: time × group; time × phase; group × phase; time × group × phase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Analysis of contrasts between active tVNS and sham tVNS groups.

Time I group × phase	Contrast	Std. error	95% CI	p-Value
(1 vs 0) active tVNS early	−1.90	0.49	−2.87 to −0.94	<0.001
(1 vs 0) sham tVNS early	−1.13	0.70	−2.50 to 0.25	0.11
(2 vs 1) active tVNS early	0.6	0.41	−0.21 to 1.41	0.15
(2 vs 1) sham tVNS early	0.31	0.47	−0.61 to 1.23	0.51
(1 vs 0) active tVNS late	0.37	0.51	−0.63 to 1.36	0.47
(1 vs 0) sham tVNS late	0.77	0.59	−0.38 to 1.92	0.19
(2 vs 1) active tVNS late	−0.87	0.28	−1.41 to −0.32	0.02
(2 vs 1) sham tVNS late	−0.54	0.60	−1.72 to 0.64	0.31

time, group and phase on global sleep score in 68 participants (see Supplementary Table 1).

In the main analysis, tVNS was not superior to sham in both early ($\chi^2 = 0.83$, $p = 0.36$) and late stimulation phases ($\chi^2 = 0.24$, $p = 0.63$).

3.3. Sensitivity analyses

As illustrated in the Supplementary Table 2, analyses restricted to participants with complete global sleep scores at all 3 time points (T0, T1, T2) showed that these participants did not differ at baseline in terms of any variables except antihypertensive medication. The effects of time seen in global sleep scores in active tVNS and sham tVNS groups were replicated. Specifically, analysis of prespecified contrasts revealed that for the active tVNS group there was a statistical difference in the estimated means between time 0 and time 1 in participants who underwent stimulation "early" (C = −1.93; 95% CI = −2.90 to −0.96), and between time 1 and time 2 in participants who received stimulation "late" (C = −0.94, 95% CI = −1.58 to −0.30). In line with the main results, no significant changes in global sleep scores were found during the follow-up period (time 1 to time 2) for the early active tVNS group, or in the waiting period (time 0 to time 2) for the late active tVNS group (data not shown). For both sham groups, changes in global sleep scores were not statistically significant in any phase of the study (data not shown), in line with our analyses based on n = 68 participants.

4. Discussion

This study tested whether a two-week course of tVNS would lead to improved global sleep scores, when compared to respective control groups. We found that sleep improved significantly in participants who received active tVNS (early and late phase of the trial), while global sleep scores did not improve in participants who underwent sham stimulation (early or late phase). However, our study's hypothesis was not supported since we did not find tVNS to be superior to sham; participants in the active tVNS arms of the intervention (early and late phase) did not have their sleep improved over and above sleep ratings of participants in the control group (early and late sham).

Our findings support data from a recent study by Bretherton et al. (2019), where, like in our trial, participants who received tVNS over a 2-week period also reported improvements in self-reported sleep. Unlike in the study of Bretherton et al. (2019) that did not have any control group, we showed that the change in global sleep scores in participants who received active tVNS was not significant in comparison with placebo groups. To the best of our knowledge no other (published) study has demonstrated this effect so far in the general population, but some preliminary data exist in clinical populations including patients with insomnia disorder (Jiao et al., 2020; Luo et al., 2017) or sleep disordered breathing (Marzec et al., 2003).

Stimulation of vagal never may improve sleep via a number of plausible biological pathways. One possibility includes reducing arousal by restoring a more flexible balance of the autonomic nervous system whereby activity of the parasympathetic branch, which promotes restorative processes including sleep, is increased and activity of the sympathetic branch that is linked to arousal is decreased (Stein and Pu, 2012; Yap et al., 2020). Another possibility is that an increase in vagal nerve activity reduces circulating levels of inflammatory markers and cortisol, both of which impede sleep, via its activation of neurons in the nucleus of the solitary tract (Thayer and Sternberg, 2010). Clearly, these biological pathways need to be confirmed with studies using techniques such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG) (Yap et al., 2020).

It is notable that tVNS improved global sleep ratings, but our study cannot yet offer evidence that tVNS can improve sleep when compared with appropriate control conditions. The most obvious explanation could be that a 2-week course of tVNS is not sufficient to trigger substantial changes in sleep, especially if participants' sleep difficulties are of chronic rather than acute nature. Another explanation could be that our participants had too modest sleep difficulties (PSQI mean at baseline was 5.5, SD 2.8), and more severe sleep problems could have offered more room for improvement, and responded more strongly to stimulation. Finally, our selection of the stimulation site, namely the tragus, could have also impacted on our findings; anatomical studies show that tragus is rather not exclusively innervated by the auricular branch of the vagus nerve (Farmer et al., 2021). There is currently no consensus which site should be used for external vagus nerve stimulation (Yap et al., 2020). However, we followed current recommendations with regards to current strength (see Farmer et al., 2021; Burger et al., 2020), and used current strength matched to each participant's individual level.

Our findings need to be interpreted in light of our study limitations. Sleep was measured with a well described sleep questionnaire, but sleep ratings are affected by range of biases (Jackowska et al., 2011), and are only modestly correlated with objective assessments (Lauderdale et al., 2008). Therefore, this study should be replicated with the use of objective sleep monitoring such as actigraphy. Our participants' age ranged from 18 to 75 years, and it is well established that sleep vary by age (Ohayon et al., 2004). To address this issue all analyses were adjusted for age, and participants randomised into four groups did not differ on age (see Table 1). Sample size was determined in line with existing studies on sleep and tVNS (e.g. Bretherton et al., 2019) as well as by asking experts in the field, but we did not conduct a formal a priori power analysis. We had sleep data from n = 68 participants who provided sleep reports at two time points, and n = 57 participants rated their sleep at all three sleep assessments. Importantly, findings were broadly similar across these two groups of participants, as shown in sensitivity analyses. Albeit participants provided longitudinal sleep data spanning over at least two weeks apart (Day 0, Day 14, Day 28), it is still a relatively short period of time. While participants were not invited to take part in this study if they had cardiovascular disease, severe mental or neurological condition or reported pregnancy (see method section for full details), we did not consider sleep disorders as an exclusion criteria. Finally, adherence to the tVNS protocol was assessed via self-reports, which makes it prone to a social desirability bias. Although we took great care to ensure participants were not aware whether they were undergoing active or sham tVNS, an objective monitoring of adherence would have been a more valid tool to indicate whether the tVNS device was worn as requested. Our study has a number of strengths. We included an active sham control group in order to control for effects of participants' expectations, and our strong study design enabled us to test both the within-subject and between-subject effects. Our analyses were controlled for important confounding factors relevant to sleep and vagal nerve function, in particular, age, sex, sleep medication and depression medication, and individuals with health conditions, potentially impacting vagus nerve activity, have not been invited to participate. To model within-individual changes over the 4 weeks of participating in the study, we used mixed-effects models which are considered largely robust.

In conclusion, the benefits and application of non-invasive vagal nerve stimulation, such as tVNS, go beyond treating clinical populations, and could be scaled-up to a population level to prevent global burden of non-communicable diseases (see Gidron et al., 2018). Poor sleep habits

are a significant contribution to a number of non-communicable diseases including cardiovascular disease, diabetes or cancer. We showed that two weeks of active tVNS led to a significant improvement of global sleep ratings, while 2 weeks of sham tVNS had no effect on sleep. However, the change in sleep pattern was not significantly different when directly comparing the groups. Further well-powered studies are urgently needed to test the utility of tVNS in alleviating sleep complains in community-dwelling adults.

Declaration of competing interest

All authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autneu.2022.102972.

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