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Effects of transcutaneous auricular vagus nerve stimulation paired with tones on electrophysiological markers of auditory perception

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ABSTRACT

Background: Transcutaneous auricular vagus nerve stimulation (taVNS) has been introduced as a non-invasive alternative to invasive vagus nerve stimulation (iVNS). While iVNS paired with tones has been highlighted as a potential effective therapy for the treatment of auditory disorders such as tinnitus, there is still scarce data available confirming the efficacy of non-invasive taVNS. Here, we assessed the effect of taVNS paired with acoustic stimuli on sensory-related electrophysiological responses.

Methods: A total of 22 healthy participants were investigated with a taVNS tone-pairing paradigm using a within-subjects design. In a single session pure tones paired with either active taVNS or sham taVNS were repeatedly presented. Novel tones without electrical stimulation served as control condition. Auditory event related potentials and auditory cortex oscillations were compared before and after the tone pairing procedure between stimulation conditions.

Results: From pre to post pairing, we observed a decrease in the N1 amplitude and in theta power to tones paired with sham taVNS while these electrophysiological measures remained stable for tones paired with active taVNS a pattern mirroring auditory sensory processing of novel, unpaired control tones.

Conclusion: Our results demonstrate the efficacy of a short-term application of non-invasive taVNS to modulate auditory processing in healthy individuals and, thereby, have potential implications for interventions in auditory processing deficits.

1. Introduction

Electrical stimulation of the vagus nerve applied in pharmaco-resistant epilepsy and depression has been demonstrated to reduce the disease-related symptoms [1]. Although the precise neurophysiological mechanisms underlying these therapeutic effects are still not fully understood, it is suggested that vagus nerve stimulation (VNS) acts via afferent projections to the nucleus of the solitary tract from which there are projections to subcortical structures including the nucleus basalis, the locus coeruleus, and the dorsal raphe nuclei [2]. Thereby, VNS affects excitatory neurotransmitter systems related to these subcortical structures and, finally, the release of mainly norepinephrine and serotonin to various brain structures including the thalamus, the cerebellum, and the neocortex [3,4]. Additionally, VNS may also activate inhibitory mechanisms via gamma-aminobutyric acid (GABA) release [5,6]. Since the availability of neurotransmitters in the neocortex is critical in sensory perception and cognition VNS has also been shown to affect behavior in the animal model [7] and in human patients [8–11]. The main drawback of VNS is, however, its inherently invasive nature as well as the risks and costs associated with the necessary surgical implantation [12,13].

Transcutaneous auricular vagus nerve stimulation (taVNS) has been introduced as a non-invasive alternative to VNS [14,15]. TaVNS applies electrical pulses to the aurical branch of the vagus nerve via electrodes placed at the outer ear, i.e. the cymba concha or the tragus [4,16]. Functional magnetic resonance imaging (fMRI) has demonstrated that

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taVNS can stimulate the "classical" central vagal projections in humans [15] and that taVNS-evoked effects on perception and cognition are most likely caused by increased norepinephrine-availability in the neocortex [17]. However, despite its sparse, easy, and straight forward characteristic there is only scarce evidence on the efficacy of taVNS to modulate sensory perception in human subjects, and, accordingly, its efficacy is still under debate.

An established paradigm to assess the impact of a neuromodulator on functional properties of sensory cortex areas relies on the temporal coupling of a sensory stimulus and a neuromodulator [18]. In rodents, pairing acoustic stimuli with VNS resulted in structural and functional changes in the auditory system and, on the behavioral level, in related sensory perception [12,13,19–22]. In order to shed more light on the efficacy of taVNS in healthy human participants we here investigated its effect on objective electrophysiological markers of auditory sensory perception, i.e. the N1 amplitude. In concrete, we adapted a VNS tone-pairing paradigm established in the animal research [19] but using non-invasive taVNS and examined the changes in auditory event-related potentials from pre tone-pairing to post tone-pairing with active taVNS or sham taVNS. In addition, we assessed taVNS-induced changes in oscillatory activity. Since previous work reported a decrease in the auditory N1 amplitude after repetitive stimulus presentation [23,24] we hypothesized that the N1 amplitude and the power in oscillatory activity in the auditory cortex will decrease to tones paired with sham taVNS from pre tone-pairing to post tone-pairing. Based on previous work performed in the animal model demonstrating that pairing a pure tone with VNS is sufficient to generate frequency-specific changes in cortical map organization in the auditory cortex [19–21] we furthermore hypothesized that pairing tones with active taVNS would differentially modulate the N1 amplitude and oscillatory activity as compared to sham taVNS.

2. Material and methods

2.1. Participants

For this study 22 healthy participants (10 female) at the age of 19–30 years (*M:* 23.91, *SD:* 3.04) were recruited via advertisements at the Otto-von-Guericke University Magdeburg. Sample size was determined based on previous studies assessing the effect of transauricular vagus nerve stimulation in humans [25–27]. All participants were native German speakers with normal or corrected-to-normal vision. Further inclusion criteria were: age 18–30 years, normal hearing performance. Exclusion criteria were: a history of neurologic and psychiatric disorders, brain surgery, CNS-influencing medication, pregnancy, history of migraine or epilepsy, metal pieces in the body (e.g., shunts, pacemaker), and active implants in the ear (e.g., cochlear implant). After information about the study procedure, participants gave their written informed consent. Participants received a monetary reimbursement (€ 8 per hour.). The study was approved by the local ethics committee of the Medical Faculty, University of Magdeburg and was conducted in accordance with the Declaration of Helsinki.

2.2. Experimental procedure

The current study represents a single-blinded, sham-controlled within-subject design. The order in which sham taVNS and active taVNS was applied was randomized between participants. Fig. 1 gives an overview of the experimental procedure. The experiment consisted of a baseline block followed by a stimulation block and an evaluation block. All participants completed all three blocks consecutively.

In the baseline block (pre tone-pairing) brain responses to pure tones were measured. We presented 60 tones with a frequency of 500 Hz, 60 tones with a frequency of 5000 Hz, and 60 tones with random frequencies chosen between 1000 and 4000 Hz (*control tones*) in randomized order at an interstimulus interval (ISI) of 2500 ± 75 ms. In order to assure that participants remained focused and actively listened to the

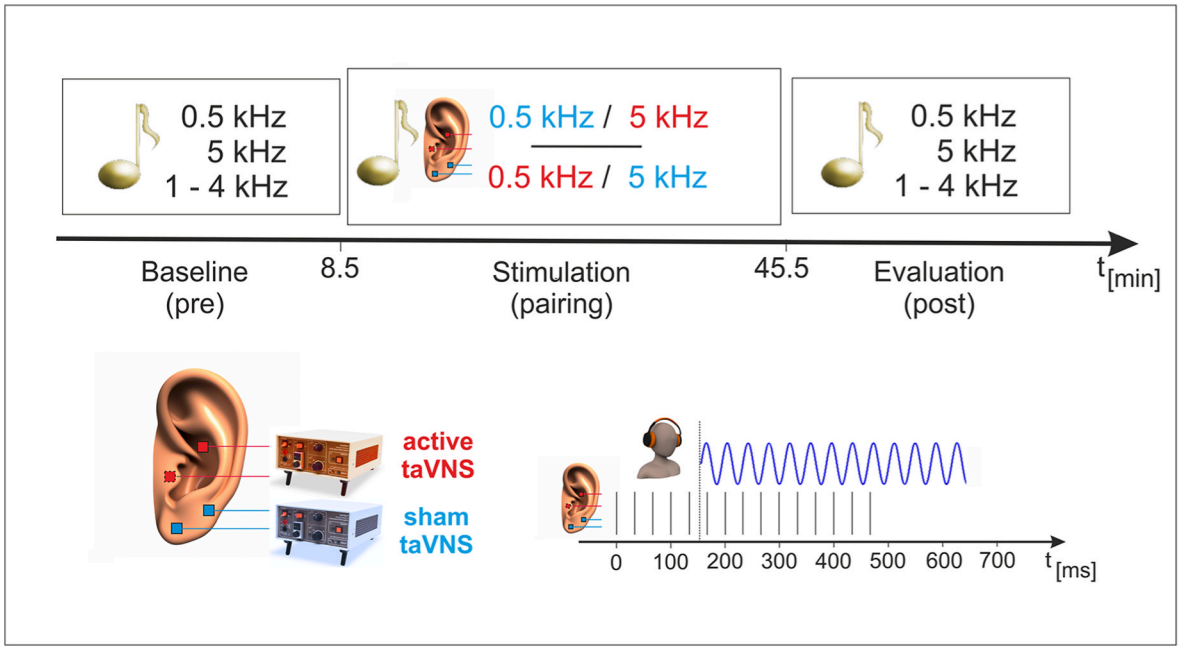

Fig. 1. Experimental procedure. Upper row: Initial brain responses to pure tones were assessed in a baseline measurement (pre). Subsequently, these pure tones were paired with either active taVNS or sham taVNS. In the evaluation block (post) brain responses were again measured to quantify stimulation specific effects of the pairing procedure. Lower row, left: Electrode positions used for taVNS (red rectangles) and sham stimulation (blue rectangles). Note that each participant received both the taVNS-pairing and the sham-pairing procedure within a single session. Lower row, right: The electrical stimulation consisted of 15 pulses (200 μs) delivered at 30 Hz. The pulse series started 150 ms prior to the onset of the to-be paired pure tone (dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tones, they had to respond to intermittently presented white noise target tones (n = 24) by pressing a button with their right index finger (auditory vigilance task). The baseline block took about 8.5 min.

In the stimulation block, 500 Hz and 5000 Hz pure tones were paired with active taVNS and sham taVNS. The assignment of the tones to the stimulation condition was counterbalanced between participants so that in 50% of participants the 500 Hz tones were paired with active taVNS while the 5000 Hz tones were paired with sham taVNS, and vice versa. 222 tone pairings were performed (111 tones paired with active taVNS, 111 tones paired with sham taVNS). Note that tones paired with sham taVNS and tones paired with active taVNS were presented in randomized order (i.e. not in a block design that would result in carry over effects) with an ISI of 8000 ms ± 125 ms while the participants watched a silent movie. The stimulation block took about 37 min. The subsequent evaluation block (post tone-pairing) was identical to the baseline block.

All acoustic stimuli had a duration of 500 ms including a 5 ms rise/fall and were presented at 65 dB SPL binaurally via headphones (Sennheiser HD 65 TV). Stimulus presentation as well as control of the electrical stimulation (see below) was achieved using the Presentation software, Version 18.1 (Neurobehavioral Systems, Inc., Berkeley, CA, https://www.neurobs.com/). The total duration of the experiment was about 60 min.

2.3. taVNS parameters and tone pairing procedure

We used two Digitimer Constant Current Stimulators DS7A (Welwyn Garden City, UK) to apply active taVNS and sham taVNS. For active taVNS, two 0.5 cm^2 Ag/AgCl-electrodes (Neuroline 700) were fixated at the cymba conchae region and medial of the tragus at the entry of the meatus of the left ear (Fig. 1) using a small amount of conductive and adhesive paste (Grass EC2). Sham taVNS was achieved by placing two additional electrodes at the left ear lobe, which is free of vagal fibers [3,20] and verified not to activate cortical and brain stem regions [21]. We used monophasic square wave pulses with a duration of 200 μs. The frequency was set to 30 Hz as it allowed us to apply an integer number of pulses (i.e. 15) in a 500 ms time window. The stimulation intensity was set to 4 mA. The electrical stimulation in the active taVNS and the sham taVNS condition started 150 ms before tone onset [21,28] (Fig. 1, bottom). Participants were blinded to the hypothesized differential mechanisms of the electrodes. Debriefing after performing the last block revealed that participants were not able to correctly indicate which electrode served as active taVNS and sham taVNS.

2.4. Electroencephalogram (EEG)

During the baseline and the evaluation blocks, we continuously recorded EEG data with a sampling rate of 1000 Hz using 25 Ag/AgCl-electrodes equally distributed over the scalp. We used a BrainAmp DC-amplifier (BrainVision Recorder 1.21, Brainproducts, Munich, Germany). The reference electrode was positioned on the right mastoid, the ground electrode at position AFz. To monitor eye movements, one electrode was positioned lateral and one below the right eye. The further electrodes were placed at Fp1, Fp2, F7, F3, Fz, F4, F8, FC5, FC6, T7, C3, Cz, C4, T8, Tp9, CP5, CP6, Tp10, P7, P3, Pz, P4, P8, O1, O2 and at the left mastoid according to the international 10–20 system of electrode placement. The impedance of all electrodes was kept below 10 kΩ.

2.5. Data analysis

Data preprocessing and analyses were performed using Matlab R2018b (MATLAB and Statistics Toolbox release, 2018, The Mathworks Inc., Natick, Massachusetts, US) and custom made scripts using the Fieldtrip toolbox [29]. Offline, continuous EEG data were high pass filtered at 1 Hz and low pass filtered at 60 Hz. A band stop filter between 48.5 and 51.5 Hz was used to remove line noise. Bidirectional IIR Butterworth filters were used. The filtered EEG time series were then cut into epochs from ± 2 s relative to sound onset. Epochs were visually inspected and trials containing gross artifacts (e.g., electrode saturation, extensive muscle contractions) were removed before applying an independent component analysis (logistic infomax algorithm). For each subject 4–7 components reflecting eye blinks, eye movements, cardiac artifacts or stimulation artifacts were removed. Epochs were then re-referenced to the linked mastoids. A second visual inspection was performed in which trials exceeding ± 100 μV as well as remaining artifacts were removed.

Subsequently, in order to investigate taVNS-induced effects on electrophysiological brain response patterns, event related potentials (ERPs) at electrode Cz were computed for each of the three stimulation conditions (active taVNS, sham taVNS, control condition) and for both the baseline and the evaluation block, separately. ERPs were baseline corrected relative to the 100 ms before tone onset. We extracted the mean component amplitudes as they are less susceptible to noise and latency variability [30]. We therefore computed the individual ERP averaged across all six conditions (active taVNS, sham taVNS, control condition, separate for baseline and evaluation block). Based on this waveform, we determined the local peak for the P50 (maximum positive amplitude value between 0 and 100 ms), the N1 (maximum negative amplitude value between 50 and 150 ms), and the P2 (maximum positive amplitude value between 150 and 250 ms). Peak latencies were then used to define an individual time window for each component (peak latency ± 5 ms for the P50; peak latency ± 25 ms for the N1 and the P2). Individual P50, N1 and P2 amplitudes were then quantified as mean amplitude in the respective time windows for each of the six conditions. Additionally, time-frequency representations (TFRs) of the six conditions were obtained using complex Morlet wavelets between 2 and 45 Hz in 1 Hz steps in 25 ms bins from −2 to 2s. Wavelet cycles increased linearly from 3 cycles at 2 Hz to a maximum of 7 cycles at 40 Hz. Wavelet analysis was applied to the single trials before averaging to obtain total power. For the TFR data, a baseline correction using decibel transformation was performed for the time window from −500 ms to −200 ms before tone onset, since the time-frequency decomposition can leak trial related activity into the pre-trial period due to temporal smoothing [31]. Finally, source reconstruction of the N1 ERP was performed using linearly constrained minimum variance (LCMV) beamforming [25]. Beamforming was performed using the standard boundary element forward model and the MNI brain template [32], both implemented in Fieldtrip (see Ref. [31] for a detailed description of forward model construction). EEG electrodes were aligned with the head model before the lead field matrix was computed for each grid point (resolution: 1 cm). The regularization factor lambda was set to 15%. The covariance matrix was computed from −500 to 500 ms. For each stimulation condition (active taVNS, sham taVNS, control condition) a common spatial filter was computed using the combined data from pre tone-pairing and post tone-pairing. This filter was then applied to the pre tone-pairing and post tone-pairing ERPs, separately. Dipole moments around individual N1 peaks ± 25 ms were averaged [28] before relative activity changes of brain activity was computed by subtracting the post tone-pairing activation from the pre tone-pairing activation and dividing by the post tone-pairing activation: $\frac{source(pre)-source(post)}{source(post)}$.

We used the Automated Anatomical Labeling (AAL) atlas [33] to define left and right Heschl's Gyrus, i.e. the primary auditory cortex (A1) as region of interest (ROI). Average values of the relative activity difference (pre-to-post) were extracted from this ROI for each subject for further analysis.

2.6. Statistical analyses

Statistical analyses were conducted using R 4.1.1 (R Core Team 2021) and R Studio 2021.9.0.351 (Rstudio Team 2021). To ensure that participants paid adequate attention to the acoustic stimuli, we employed non-parametric Wilcoxon test to compare accuracy in the

auditory vigilance task between the baseline block and the evaluation block.

In order to assess effects of taVNS tone-pairing on brain responses, linear mixed models (LMMs) were used to analyze ERPs and source amplitudes. LMMs were fitted using *the lme4*-package [34]. Statistical significance of predictors and their interaction was determined using the ANOVA-function from the *car*-package [35], which calculates the Wald chi-square statistic for each predictor. *Stimulation* (active taVNS, sham taVNS, control) and *block* (baseline, evaluation) and their interaction were treated as fixed effects. The random effect structures contained random intercepts and slopes for *stimulation* and *block* across subjects. In case of a significant interaction we performed pre-to-post comparisons for each stimulation condition (active taVNS, sham taVNS, control tones) separately using Bonferroni corrected Wilcoxon tests. Additionally, subject-wise differences between baseline (pre tone-pairing) and evaluation block (post tone-pairing) were computed for each stimulation condition separately. These diffferences were then compared between the stimulation conditions. Finally, we controlled for potential differences in the baseline block using separate LMMs.

For the TFR analysis, pre-to-post tone-pairing differences were assessed using non-parametric cluster based permutation testing as implemented in Fieldtrip [29]. Across all electrodes, power values from pre tone-pairing and post tone-pairing were compared using a two-tailed dependent sample *t*-test for each of the three stimulation conditions (active taVNS, sham taVNS, control condition) separately. The relevant time window was set from −200 to 500 ms relative to tone onset. A Monte Carlo approach using cluster-based statistics was used to determine statistical significance and control for multiple comparisons. Test statistic was the maximal sum per cluster, 5000 randomizations were used and the significance level was set to $\alpha = 5\%$. In case the cluster based permutation testing revealed differences between pre tone-pairing and post tone-pairing for one condition, average pre-to-post tone-pairing power differences for each stimulation condition from this time-frequency-electrode constellation were extracted for each subject for further analysis.

3. Results

3.1. Results of the auditory vigilance task

Participants paid sufficient and stable attention to the stimuli throughout the experiment: Response accuracy for detecting the white noise stimuli was 99.05% in the baseline block and 99.62% in the evaluation block ($V = 8.0$, $p = 0.3$).

3.2. Results of the scalp N1 amplitude

The N1 amplitudes significantly decreased from the baseline block to the evaluation block ($\chi^2_{(1)} = 10.745$, $p = 0.001$). Furthermore, N1 amplitudes were significantly modulated by the factor *stimulation* ($\chi^2_{(2)} = 15.768$, $p < 0.001$) driven by a significant *stimulation* x *block* interaction ($\chi^2_{(2)} = 13.110$, $p = 0.001$). Analyzing the N1 amplitudes for each stimulation condition separately revealed a significant reduction of the N1 amplitude to tones paired with sham taVNS from baseline (M: −7.95 μV, SD: 3.72) to evaluation (M: −5.41 μV, SD: 2.93, V = 26, $p_{corr} = 0.002$, Fig. 2A, blue), while N1 amplitudes to tones paired with active taVNS showed no significant difference from baseline (M: −7.49 μV, SD: 3.94) to evaluation (M: −6.48 μV, SD: 3.51, V = 92.0, $p_{corr} = 0.827$, Fig. 2A, red). Similarly, no significant pre-to-post change was found for control tones (baseline: M: −8.14 μV, SD: 3.10; evaluation M: −7.51μV, SD: 2.84, V = 103.0, $p_{corr} = 1$, Fig. 2A, green).

Furthermore, the decrease in the N1 amplitude from pre tone-pairing to post tone-pairing was stronger in the sham taVNS condition (M: −2.54, SD: 2.94, Fig. 3A, blue) compared to the active taVNS condition (M: −1.01, SD: 2.49; V = 46.0, $p_{corr} = 0.022$, Fig. 3A, red) and compared to the control tones (M: −0.62, SD: 1.97; V = 208.0, $p_{corr} = 0.02$, Fig. 3A, green). No significant difference was observed between N1 amplitude differences to tones paired with active taVNS and to control tones (V = 132.0, $p_{corr} = 1$). Thus, the differences from baseline to evaluation (i.e. from pre tone-pairing to post tone-pairing) were significantly modulated by the factor *stimulation*. Finally, no difference in the baseline N1 amplitudes were found between the stimulation conditions ($\chi^2_{(2)} = 0.72$, $p = 0.7$). In sum, pairing tones with active taVNS systematically affected the electrophysiological responses of the auditory system: The reduction of the N1 amplitude in response to tones paired with active taVNS was

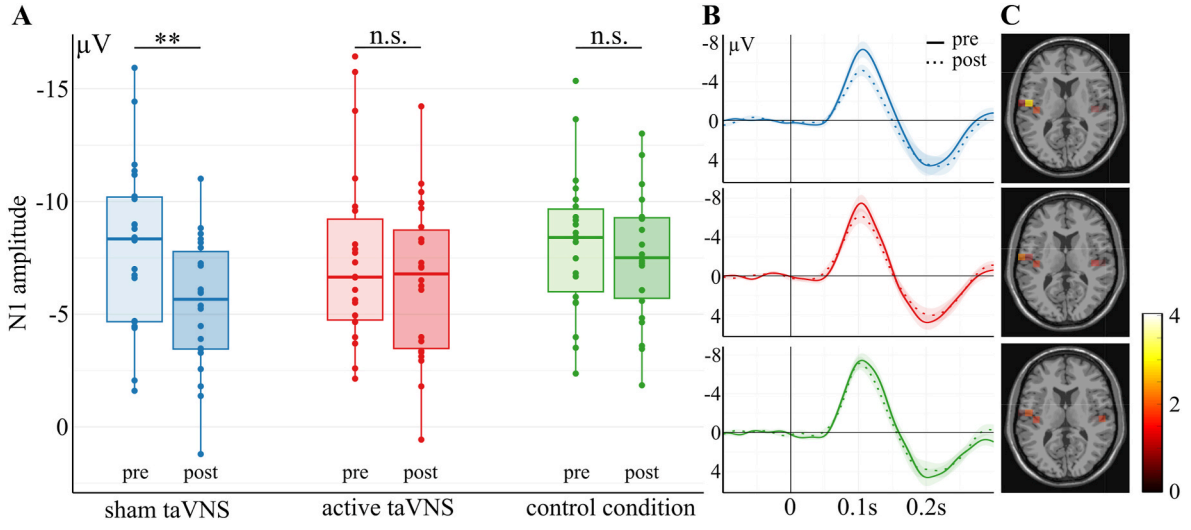

Fig. 2. Stimulation-specific changes on the N1 amplitude. A) Boxplots showing the observed changes in N1 amplitude from pre tone-pairing (light colors) to post tone-pairing procedure (darker colors) for the three stimulation conditions, separately. **B)** Event related potentials (ERP) in the pre (solid lines) and the post block (dashed lines) depicted separately for the sham taVNS- condition (blue lines), the active taVNS condition (red lines), and the control condition (green lines). **C)** Relative changes in A1-activity from pre to post tone-pairing for sham taVNS (upper panel), active taVNS (middle panel), and the control condition (lower panel). Dots represent the subjects individual data. Error bars indicate standard deviation, Asterisks indicate statistically significant differences. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

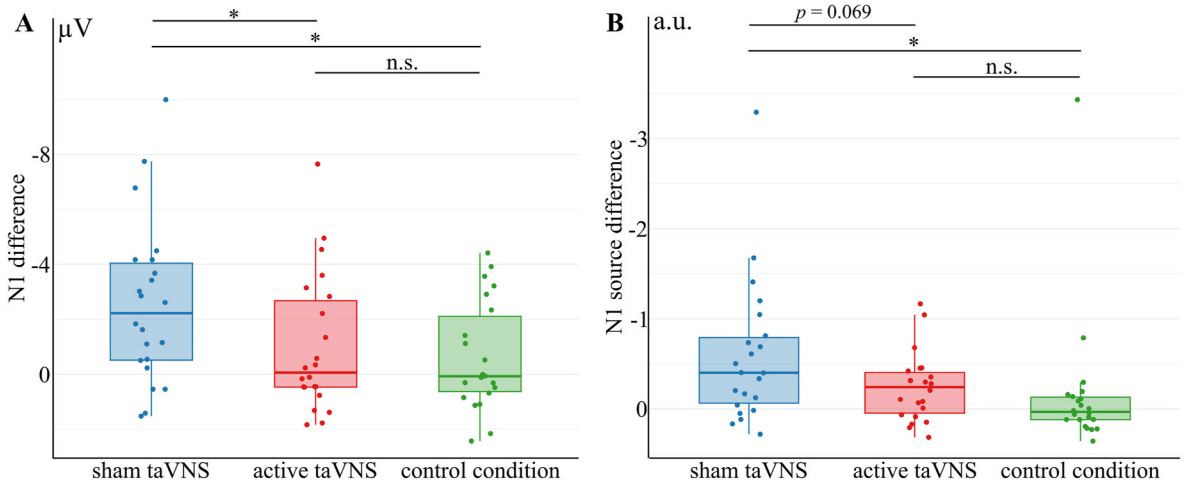

Fig. 3. N1 amplitude differences from baseline to evaluation. Pre-to-post pairing changes on the N1 amplitude recorded at the Cz-electrode (A) and in the primary auditory cortex ROI (B). Blue boxplots represent data from the sham taVNS condition, red boxplots from the active taVNS condition, and green boxplots from the control condition without any electrical stimulation. Dots represent the subjects individual data. Error bars indicate standard deviation. Asterisks indicate statistically significant differences, numbers represent the corrected p-values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

markedly attenuated compared to tones paired with sham taVNS and this attenuated pre-to-post reduction was comparable to the N1 amplitude reduction in response to novel control tones.

3.3. Results of the scalp P50 and P2 amplitude

Neither the LMM for the P50 amplitude nor for the P2 amplitude revealed a significant main effect or interaction (all $p > 0.1$).

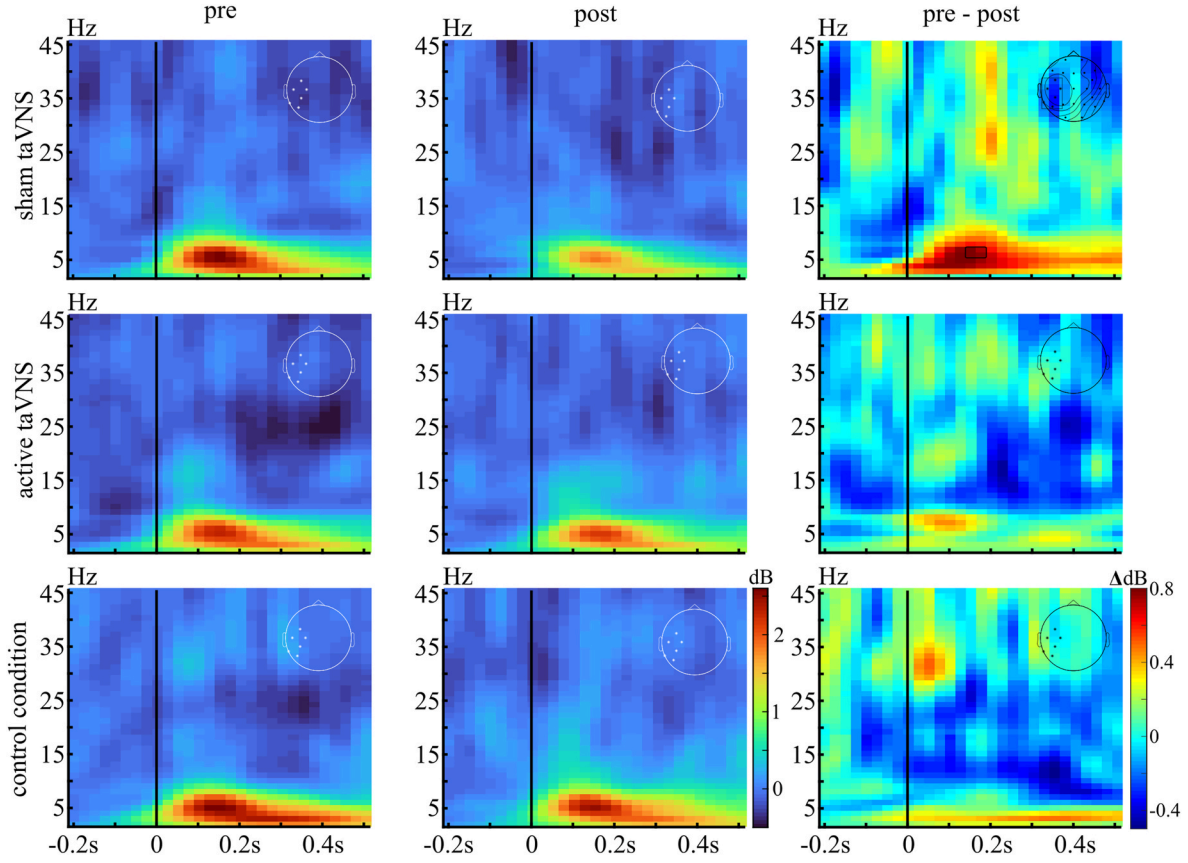

Fig. 4. taVNS-induced changes on TFR-data. In the sham taVNS condition (upper row), a significant electrodes cluster located over temporo-parietal areas was found in the theta range (6–7 Hz) peaking at about 150–200 ms after tone onset: Here, power changes from pre-to-post pairing were significantly stronger in the sham taVNS condition compared to the control condition (lower row). A numerical difference was evident between sham taVNS and active taVNS (middle row).

3.4. Results of the N1 in the auditory cortex ROI

Relative changes in the N1 amplitude from baseline to evaluation revealed activity peaks in bilateral A1-regions (Fig. 3C). The ROI analysis on N1-source activity revealed no significant main effect of the factors *stimulation* ($\chi^2_{(2)} = 0.041$, $p = 0.98$) or *block* ($\chi^2_{(2)} = 3.33$, $p = 0.07$) but a significant *stimulation* x *block* interaction ($\chi^2_{(2)} = 11.25$, p = 0.004). Analogues to the N1 amplitudes, analysis of N1-source data for each stimulation condition separately showed a statistical trend towards a reduction of the N1-source activity to tones paired with sham taVNS from baseline (M: 0.66, SD: 0.27) to evaluation (M: 0.56, SD: 0.29, V = 196.0, $p_{corr} = 0.069$), while the N1-source activity to tones paired with active taVNS showed no significant difference from baseline (M: 0.65, SD: 0.33) to evaluation (M: 0.58, SD: 0.24, V = 186.0, $p_{corr} = 0.16$). Similarly, no significant pre-to-post change was found to control tones (baseline: M: 0.61, SD: 0.28; evaluation: M: 0.63, SD: 0.29, V = 109.0, $p_{corr} = 1$). Furthermore, the pre-to-post pairing decrease in the N1-source activity to tones paired with sham taVNS (M: 0.59, SD: 0.79, Fig. 3B, blue) was stronger compared to tones paired with active taVNS (M: 0.24, SD: 0.38; V = 197.0, $p_{corr} = 0.069$, Fig. 3B, red) as well as compared to the control tones (M: 0.15, SD: 0.77; V = 38.0, $p_{corr} = 0.013$, Fig. 3B, green). No significant difference was observed between pre-to-post chances in N1-source activity to tones paired with active taVNS and to control tones (V = 59.0, $p_{corr} = 0.089$). Finally, no difference in the baseline N1-source activity was evident between the stimulation conditions ($\chi^2_{(2)} = 2.01$, $p = 0.37$).

3.5. Results of the TFR-data

Statistical analysis of the TFR-data revealed a significant electrode cluster ($p_{cluster} = 0.018$) of six temporo-parietal electrodes over the left hemisphere (Fig. 4, upper right panel, black rectangle). Pairing tones with sham taVNS led to a significant power reduction specifically in the theta band (6–7 Hz) peaking around 150–200 ms after onset of the pure tones. This change in the theta power was significantly stronger in the sham taVNS condition (M: 0.76, SD: 1.02, Fig. 4, upper row) compared to the control condition (M: 0.15, SD: 0.89; $t_{(21)} = -2.3$, $p_{corr} = 0.048$, Fig. 4, lower row). A numerical difference was evident between sham taVNS and active taVNS (M: 0.18, SD: 0.79). Here, however, statistical analysis failed to reach significance after correcting for multiple comparisons ($t_{(21)} = -2.0$, $p_{corr} = 0.087$; p uncorrected = 0.029). No significant difference in theta power was found between active taVNS and the control condition ($t_{(21)} = 0.16$, $p_{corr} = 1$). Results from the TFR-analysis thus mirror the findings from the N1-data.

4. Discussion

Studies in the animal model have shown that pairing (invasive) VNS with acoustic stimuli over extended time periods can elicit plasticity in sound coding of the primary auditory cortex [19,20,36,37]. Building on this, we investigated short-term effects of non-invasive taVNS on electrophysiological markers of auditory sensory processing. We applied a tone-pairing paradigm in which healthy adult participants were presented with acoustic stimuli paired with either active taVNS or sham taVNS and compared stimulation-specific changes. As expected, we found that the N1 amplitude and theta power decreased after repeatedly pairing tones with sham taVNS. Importantly, these electrophysiological measures remained stable for tones paired with active taVNS, a finding that mirrored the auditory sensory processing of the novel, unpaired control tones. These results indicate that also a short-term application of non-invasive taVNS can have significant effects on auditory processing in healthy individuals.

In the present study we observed that N1 amplitudes to tones paired with sham taVNS decreased after repetitive presentation, a typical finding generally interpreted as sensory adaptation [23,24]. Importantly, the N1 amplitude to tones paired with active taVNS remained stable after repetitive stimulus presentation. The auditory N1-component, which is generated in the primary and secondary auditory cortex region [38–40], is probably one of the most often investigated auditory ERPs in humans. Typically, a decrease in the N1 amplitude is found when physically identical stimuli are repeatedly presented. This pattern has been discussed as the consequence of sensory adaptation due to refractoriness and/or latent inhibition [24,41,42]. Interestingly, after presenting tones repetitively at high frequency and short interstimulus intervals, increased N1 amplitudes were found and interpreted as a consequence of long-term potentiation (LTP)-driven learning and cortical plasticity [43,44]. Besides the repeated occurrence of external sensory stimuli, LTP-driven cortical plasticity requires activation of the norepinephrine (NE) system [45–48]. In the rodent auditory system, disruption of NE supply caused a lack of plasticity [21–23] whereas relative short periods of increased NE supply improved long lasting neuroplastic changes [49]. A similar pattern as to the N1 was found in oscillatory brain activity: Theta power to tones paired with active taVNS remained stable from baseline to post-pairing while it decreased to tones paired with sham taVNS. Theta oscillations are typically observed during learning [50–52] and are associated with LTP and experience-dependent neural plasticity as well [53–55].

There is compelling evidence that direct neuromodulation of the noradrenergic and cholinergic path can drive plasticity in the auditory cortex. In their seminal work on auditory plasticity, Kilgard et al. (2002) [56] demonstrated that electrical stimulation of the cholinergic nucleus basalis paired with sensory stimulation drives plasticity in the auditory cortex that mimics neuroplastic changes induced by perceptual learning. In the animal model, tone-pairing with invasive stimulation of the nucleus basalis resulted in cortical map reorganization of the auditory cortex that was specific to the frequencies of the paired tones [57]. Also in the animal model, the pairing of sounds with invasive VNS has been demonstrated to reverse tinnitus-related cortical maladaptation [19]. In humans, invasive VNS paired with tones adjusted to the individual tinnitus frequency improved tinnitus-related symptoms [12,58]. For non-invasive taVNS, however, there is yet no data available on its efficacy in combination with a tone pairing procedure in tinnitus patients (see Refs. [59,60]).

Results of the present study extend these previous findings in that we report taVNS-effects already after a single tone-pairing session and, most importantly, via non-invasively stimulating vagus nerve projections. Previous research in humans has shown that taVNS activates cerebral afferents of the vagal pathway [14,61]. In addition, behavioral and electrophysiological effects on auditory sensory processing have been measured as a consequence of taVNS [62,63]. Since taVNS-induced effects on the availability of neurotransmitters is not limited to the auditory cortex but occur in the entire neocortex [3,7,64–66] our findings have implications for sensory processing in other cortical areas and modalities. Awaiting data from studies pairing e.g. visual or tactile stimuli with taVNS, we suggest in the meantime that results of the present study can be replicated in other modalities and cortical sites.

In order to verify that our effects were actually caused by taVNS our study design included two control conditions: The co-occurrence of pure tones and sham taVNS applied to the ear lobe as well as pure tones of varying frequencies without any electrical stimulation. While the aim of the former condition was to control for potential sensory effects of the electrical stimulation, the latter targeted on mere habituation effects due to the repetitive exposure to the identical stimulus. Because the stimulation intensity was above the subject's perceptual threshold, a sham taVNS (control) condition in which identical electrical pulses as in the verum taVNS condition were applied but to an area without any vagal projects was inevitable to allow for a successful blinding of the participants. Moreover, in that we used such an active sham taVNS condition, effects caused by e.g. anticipating the pulse trains or potential unspecific modulations of attention due to sensory perception of the electrical stimulation can be excluded.

A limitation of our work is that no conclusions on the temporal

stability of the observed effects can be drawn because our study design did not include a follow-up measurement. In the animal model, however, using invasive stimulation of the vagus nerve together with a tone pairing procedure over 20 days led to long-term effects up to three weeks [19]. Since we found stimulation specific electrophysiological changes already after a single session, it seems likely that the repetitive administration of (non-invasive) taVNS and a tone pairing paradigm can also evoke longer lasting effects. However, future studies will shed more light on this important aspect.

Furthermore, our study design did not include measures such as e.g. accuracy in the detection or discrimination of acoustic stimuli that would allow drawing conclusions on the behavioral consequences of a taVNS tone-pairing procedure. Thus, although the results of the present work emphasize that taVNS can influence sensory processing in the auditory cortex, future studies are needed to investigate the behavioral impact of this intervention to, ultimately, pave the way on the applicability of taVNS in the clinical setting.

Finally, we used the same stimulation intensity for active taVNS and sham taVNS to avoid potential confounding effects. Therefore, we cannot fully rule out the fact that the stimulation conditions were differently perceived by the participants and that this might have contributed to the reported stimulation-specific effects.

5. Conclusion

Together, our results demonstrate that short-term periods of non-invasive taVNS can have significant effects on auditory processing in healthy individuals: Pairing acoustic stimuli with taVNS systematically affected electrophysiological markers of auditory processing. Thereby, we provide first evidence on the efficacy of taVNS to modulate sensory perception in the *in vivo* human auditory cortex. Our findings provide evidence that non-invasive, peripheral neuromodulation may be a useful tool to enhance and restore sensory processing. Perspectively, taVNS paired with sensory stimulation may allow to compensate for sensory processing deficits by modulating neuroplasticity and related changes in cortical structures.

Author contributions

KSR: planned the study, analyzed the data, wrote the original draft. CW: analyzed the data, wrote the original draft. AS: collected the data, analyzed the data. AH: provided materials/reagents. TZ: planned the study, wrote the original draft, and provided materials/reagents. All authors participated in interpreting the results, writing the manuscript and approved the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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