Effects of transcutaneous vagus nerve stimulation (tVNS) on beta and gamma brain oscillations

Marius Keute[1,3], Christian Wienke[1,2], Philipp Ruhnau[1,2], Tino Zaehle[1,2]

[1]Department of Neurology, Otto-von Guericke-University, Magdeburg, Germany

[2]Center for Behavioral Brain Sciences, Otto-von-Guericke University, Magdeburg

[3]Institute for Neuromodulation and Neurotechnology, University of Tübingen, Tübingen, Germany

Abstract

Physiological and behavioral effects induced through transcutaneous vagus nerve stimulation (tVNS) are under scrutiny in a growing number of studies, yet its mechanisms of action remain poorly understood. One candidate mechanism is a modulation of γ-aminobutyric acid (GABA) transmission through tVNS. Two recent behavioral studies suggest that such a GABAergic effect might occur in a lateralized fashion, i.e., the GABA modulation might be stronger in the left than in the right brain hemisphere after tVNS applied to the left ear. Using magnetoencephalography (MEG), we tested for GABA-associated modulations in resting and event-related brain oscillations and for a lateralization of those effects in a sample of 41 healthy young adults. Our data provide substantial evidence against all hypotheses, i.e., we neither find effects of tVNS on oscillatory power nor a lateralization of effects.

Introduction

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive brain stimulation technique that has received increasing attention in recent years. It has been introduced as a non-invasive alternative to direct or invasive vagus nerve stimulation (iVNS) (Ventureyra, 2000). Clinically, it is effective as an adjunct therapy for pharmacoresistant epilepsy (Bauer et al., 2016; He et al., 2013; Stefan et al., 2012) and depression (Fang et al., 2016; Trevizol et al., 2015). Furthermore, it has been suggested as a prospective treatment for a variety of conditions, including chronic headache (Barbanti et al., 2015; Magis, Gérard, & Schoenen, 2013), tinnitus (Lehtimäki et al., 2013), post-operative cognitive dysfunction (Xiong et al., 2009), cerebral ischemia (Lu et al., 2017), and Alzheimer's disease (Kaczmarczyk, Tejera, Simon, & Heneka, 2018).

So far, the mechanisms of action of tVNS are not fully understood, and an improved understanding of these mechanisms will be highly relevant and necessary for future research, highlighting how patients can benefit from tVNS as well as for therapy development and improvement. It is consistently found that the locus coeruleus-norepinephrine (LC-NE) system is activated through both iVNS and tVNS. This activation is mediated by the nucleus of the solitary tract (NTS), the principal brain projection area of the afferent branches of the vagus nerve (Ruffoli et al., 2011). LC activation is considered the core mechanism of tVNS (Assenza et al., 2017; Badran et al., 2018; Raedt et al., 2011; Ventura-Bort et al., 2018; Warren et al., 2019). One of several other candidate mechanisms of action is an increase in γ-aminobutyric acid (GABA) transmission in the brain (Ruffoli et al., 2011; Walker, Easton, & Gale, 1999; Woodbury & Woodbury, 1991), mediated through activation of the NTS and LC (Berridge & Waterhouse, 2003; Toussay, Basu, Lacoste, & Hamel, 2013). The research literature on GABAergic neuromodulation by tVNS is sparse, compared to the amount of studies investigating effects of tVNS on LC-NE activity. Given that GABA transmission has a role in the pathophysiology of epilepsy (Baulac et al., 2001), depression (Möhler, 2012), tinnitus (Brozoski, Spires, & Bauer, 2007), and other neurological and psychiatric conditions, it is of high relevance to better understand GABAergic actions of tVNS in order to predict and understand its therapeutic effects.

In support of a GABAergic mechanism of tVNS, it has been found that $GABA_A$ receptor density was increased in patients after receiving long-term iVNS (Marrosu et al., 2003). Moreover, GABA concentration in the cerebrospinal fluid of patients receiving iVNS was increased (Ben-Menachem et al., 1995; Carpenter et al., 2004). The number of studies specifically investigating the relationship between tVNS and GABA transmission, however, is limited. Short-term (~1h) tVNS in healthy subjects modulated cortical excitability (Capone et al., 2015) as well as automatic motor inhibition (Keute, Ruhnau, Heinze, & Zaehle, 2018), both of which are highly correlated to GABA concentration in the motor cortex as measured by magnetic resonance spectroscopy (Boy et al., 2010; Stagg et al., 2011).

Interestingly, both studies (Capone et al., 2015; Keute et al., 2018) suggest a possible lateralization of the tVNS effect, in that GABA-associated parameters were modulated in the right, but not in the left brain hemisphere. Similarly, effects of iVNS on the electroencephalogram (EEG) spectrum have been found that were stronger in the right hemisphere (Marrosu et al., 2005). Since both iVNS and tVNS are almost exclusively administered to the left ear / vagus nerve, these findings are compatible with a selective or stronger GABAergic effect of t-/iVNS in the contralateral hemisphere. Even though we are not aware of any anatomical or physiological evidence that could account for a lateralization of tVNS effects, the potential occurrence of such a lateralization in three independent studies warrants further investigation.

Brain oscillations as measured by EEG or magnetoencephalography (MEG) often have specific relationships to local GABA concentrations and can therefore be used as biomarkers: Pharmacological increases of systemic GABA levels are consistently associated to increases in beta power at rest (Greenblatt et al., 1989; Hall, Barnes, Furlong, Seri, & Hillebrand, 2010; Nutt et al., 2015; van Lier, Drinkenburg, van Eeten, & Coenen, 2004). Furthermore, GABA concentration in the motor cortex is related to peri-movement beta and gamma power modulations (Gaetz, Edgar, Wang, & Roberts, 2011; Muthukumaraswamy et al., 2013), and GABA concentration in the visual cortex is

related to gamma power responses to visual stimulation (R. A. E. Edden, Muthukumaraswamy, Freeman, & Singh, 2009; Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009).

This study will use MEG to capture brain oscillations associated to GABA transmission. Using brain oscillations as a marker for GABA has several advantages: the combination of resting and event-related oscillations outlined above has a very specific relationship to GABA. MEG allows to record from the whole brain simultaneously at a good temporal resolution, and to spatially reconstruct sources of specific signals in the brain, which will be helpful to capture a possible lateralization of tVNS effects.

In fact, a recent study found that cervical tVNS increased beta and gamma power and decreased theta and alpha power (Lewine, Paulson, Bangera, & Simon, 2018). Moreover, invasive stimulation of the nucleus of the solitary tract (NTS) in cats increased beta power (Martínez-Vargas, Valdés-Cruz, Magdaleno-Madrigal, Fernández-Mas, & Almazán-Alvarado, 2017). The NTS is one of the neural targets of vagus nerve stimulation (Clancy, Deuchars, & Deuchars, 2013).

We hypothesize that tVNS will increase GABA concentration, leading to GABA-associated MEG alterations. Specifically, our first set of hypotheses relate to overall GABAergic modulation through tVNS:

H_1: global resting-state beta power is increased during tVNS compared to sham.

H_{2A}: peri-movement beta desynchronization (PMBD) in the motor cortex is stronger during tVNS compared to sham.

H_{2B}: post-movement beta rebound (PMBR) in the motor cortex is weaker during tVNS compared to sham.

H_3: gamma power response to visual stimulation in the visual cortex is stronger during tVNS.

Furthermore, we hypothesize that the effects from H_1 and H_2 are lateralized, i.e., stronger in the brain hemisphere contralateral to the stimulation.

H_4: The tVNS effect on resting-state beta power will be stronger in the right (contralateral) hemisphere.

H_{5A}: The tVNS effect on PMBD will be stronger in the right (contralateral) hemisphere for left-hand responses compared to PMBD in the left motor cortex for right-hand responses.

H_{5B}: The tVNS effect on PMBR will be stronger in the right (contralateral) hemisphere for left-hand responses compared to PMBR in the left motor cortex for right-hand responses.

Methods

General procedure

Upon arrival, written informed consent was obtained from each participant. Participants were reimbursed with money (8 €/hr) or course credit. Head landmarks and head shape were digitized using a Polhemus Fastrak digitizer (Polhemus, VT, USA). The stimulation electrodes were attached (see below), and the participant was seated inside the MEG device. The following procedure is sketched in Figure 1: A 3-minute baseline MEG measurement was carried out, with the instruction for the participant to relax, not to think about anything in particular, keep the eyes open and blink, cough, and move only during stimulation, as far as possible. Subsequently, electrical stimulation was administered for 30 minutes with a 60s ON / 60s OFF cycle, during which the participant had no specific instruction. After pre-stimulation, two blocks of resting MEG were obtained, each with a duration of 3 minutes, with one minute of stimulation between both blocks. All resting and on-task MEG recordings were carried out while the electrical stimulation is turned off to avoid contamination of the data with stimulation artifacts. After the resting blocks, two blocks (180s each) of the motor task and two blocks (180 s each) of visual stimulation were carried out, with 60s of stimulation between all blocks. The order of the tasks was counterbalanced across participants, but kept constant within each participant (i.e., in the sham and tVNS session). The procedure was identical for sham and tVNS sessions, with the only difference being the stimulation site (cymba conchae / tVNS vs. scapha / sham). All experimental procedures were carried out in accordance with the declaration of Helsinki and have been approved by the ethics committee of the medical faculty at the University of Magdeburg.

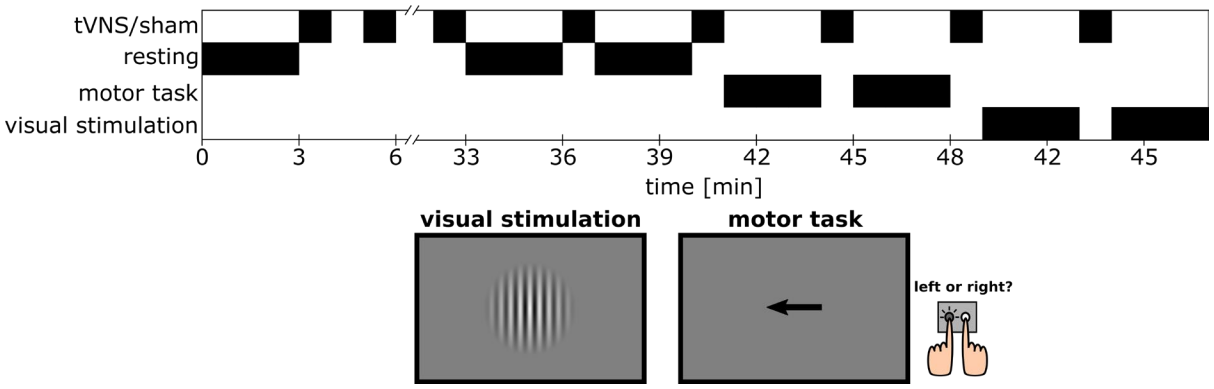

Fig. 1: Experimental procedure. The order of the motor task and visual stimulation were counterbalanced across participants. Panels below: Illustration of experimental stimuli (not true to scale).

Participants

The experiment was carried out with 41 healthy young participants (29 females). Mean age was 23.8 years (SD 3.4, range 19-30). Each participant underwent sham and tVNS stimulation in pseudo-randomized order on separate days. Sham and tVNS measurements for each participant were scheduled at least 48 hours apart and at the same daytime (± 1h). All participants were free from any current or past neurological or psychiatric diseases and regular drug intake (both medical and recreational, except for oral contraceptives), They had normal or corrected-to-normal vision and were eligible for tVNS, MEG and MRI (in particular, no cardiac pacemakers or metal implants in or close to the head).

Motor task

Peri-movement beta power was assessed using a cued finger movement task. Participants were instructed to press a button with their left or right index finger, according to the direction of an arrow displayed centrally on the screen (displayed in black on a grey background, width 1 degree, height 0.5 degree of visual angle). During each 180 s block, 24 left-pointing and 24 right-pointing arrows were presented in pseudo-randomized order, with stimulus durations of 200 ms and a randomly jittered inter-stimulus interval between 3 and 3.5 s. A red fixation point was visible on the center of the screen throughout the task to prevent eye movements.

143 *Visual stimulation*

144 Visual stimuli were stationary, vertical circular gratings with a spatial frequency of 3 cycles per
145 degree and maximum contrast. Throughout the experiment, a central fixation dot was visible. The
146 screen background had the average luminance of the gratings. Stimuli were presented centrally on the
147 screen and subtended 2 degrees of visual angle. In each 180 s block, 48 gratings were presented for 1
148 s, followed by a jittered inter-stimulus interval between 2 and 2.5 s. This stimulus design is similar to
149 the one used by Muthukumaraswamy et al. (2009).

150 *Electrical stimulation*

151 TVNS was administered to the cymba conchae, sham stimulation to the scapha of the left ear. Two
152 medical Ag/AgCl stimulation electrodes (4×4 mm) were mounted on a piece of silicone at a center-to-
153 center distance of 1 cm. The electrodes were attached to the ear using a small amount of adhesive
154 electrode cream (Natus Neurology, www.natus.com) and medical adhesive tape, if necessary. Direct
155 current pulses were delivered using a medical stimulation device (Digitimer DS7,
156 www.digitimer.com). Current intensity was set to 1 mA, delivered in 200 µs pulses at 25 Hz.
157 Stimulation was administered in blocks of 60 s, each followed by a 30 s break (during pre-task
158 stimulation) or by a 180 s MEG recording block. These parameters are within the range of standard
159 parameters used in other tVNS studies (Badran et al., 2018; Frangos, Ellrich, & Komisaruk, 2015).

160 *MEG measurement and analysis*

161 MEG was recorded from 306 sensors (102 magnetometers and 204 planar gradiometers) from 102
162 head positions using a Neuromag Triux device (Elekta AB[1]) at a sampling rate of 1000 Hz and an
163 online band-pass filter (0.01 - 330 Hz). Offline data analysis was carried out using the FieldTrip
164 toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011) in Matlab 2018 (MathWorks[2]). Bad sensors
165 (high noise level or flat) were identified by visual inspection, removed from the data and, for data

[1] www.elekta.com
[2] www.mathworks.com

visualization only, reconstructed using spline interpolation. Severely artifact-laden epochs were excluded from further analysis, based on visual inspection. Ocular and heart beat related artifacts were removed by means of independent component analysis (ICA). Data were visually inspected again, and segments with remaining gross artifacts were excluded. Participants were excluded from further analyses if more than half of the epochs in the motor task or more than half of the visual stimulation epochs or half of the resting-state recording time have to be excluded, or if they have no clear PMBD, PMBR, or visual gamma response, based on visual inspection and running t-tests against baseline, in one or both sessions. We exluded three participants from analysis of the motor task data, and five participants from analysis of the visual stimulation data.

Subsequently, MEG data were transformed to source space using linearly constrained minimum variance (LCMV) beamforming, resulting in source level epochs (Lithari, Sánchez-García, Ruhnau, & Weisz, 2016; Neuling et al., 2015). Briefly, individual structural magnetic resonance images where obtainable were aligned to the MEG space with the information from the head shapes. In case the individual MRI was not available we used the template MRI available in the Fieldtrip toolbox and morphed it to the individual head shapes using affine transformation. Then an equally spaced 1 cm grid in MNI space was warped to the individual brain volume. Using this MNI space grid (~3000 voxels) allowed for direct statistical comparisons of activity across participants. The aligned brain volumes were further used to create single-sphere head models and lead field matrices (Nolte, 2003). Together with the head model, the lead field matrix and the average covariance matrix beamformer filters for each grid point were calculated. These filters were subsequently multiplied with the sensor level epochs resulting in source level epochs.

A time-frequency analysis of source level data was carried out using Morlet wavelets. Center frequencies were logarithmically spaced between 1 and 64 Hz in steps of 0.125 octaves at a frequency resolution $f/\sigma_f = 6$, moving along the signal in steps of 50 ms. Resulting power estimates were baseline-normalized and converted to dB [$10*\log_{10}(\text{Power} / \text{Power}_{\text{baseline}})$]. For the resting-state measurement, the 3 minutes measurement prior to electrical stimulation served as baseline. For the

motor task, pre-movement beta desynchronization (PMBD) and post-movement beta rebound (PMBR) were assessed by subtracting $\log_{10}$-transformed source-space power in the contralateral motor cortex (virtual sensor at MNI coordinates [-48,-8,50] and [48,-8,50][3] for left and right primary motor cortex, respectively) across the beta band (15-30 Hz) and over a time window between -1.25 – 0.5 s relative to the button press (for PMBD) or between 1 – 1.75 s (for PMBR) from time-averaged log-power over the entire trial (-1.25 – 1.75 s). For the visual stimulation, we used a baseline of -1 – 0 s relative to stimulus onset and compared it to the presentation time of the stimuli (0 – 1 s). For analysis of visual stimulation data, we created virtual sensors at MNI coordinates [-2,-80,34], [-28,-96,-6] and [28,-96,-6] for central, left, and right primary visual cortex, respectively, and analyzed gamma power averaged across the three virtual sensors. For the analysis of resting and movement-related beta power, we averaged the baseline-corrected log-power values over beta frequencies (15 – 30 Hz), for the analysis of gamma power, we averaged over gamma frequencies (30 – 60 Hz). For event-related data from the motor task and visual stimulation, we additionally averaged over time bins and trials. To test for lateralization of tVNS effects, we computed lateralization indices as differences between resting beta log-power in the left and right hemisphere, and between PMBD and PMBR to left- and right-hand movements in the contralateral motor cortex, respectively. We calculated all lateralization indices such that hypotheses H_4, H_{5A} and H_{5B} predict higher values for tVNS compared to sham (i.e., subtracting right hemisphere values from left hemisphere values for PMBD and PMBR, and vice versa for resting beta power)4.

Resulting session-wise values for resting beta power, PMBD, PMBR, visual gamma response, and lateralization indices were compared between sham and tVNS sessions by means of paired-sample one tailed Bayesian t-tests using R and the BayesFactor package (Morey, Rouder, & Jamil, 2015). Based on previous literature, we expected $\log_{10}$-transformed spectral power values to have

[3]The MNI coordinates for the virtual sensors were not included in the stage 1 protocol. They were specified for increased transparency.
[4]We further specified calculation of lat. indices compared to the stage 1 protocol.

approximately normal distributions (Kiebel, Tallon-Baudry, & Friston, 2005), rendering the use of t-tests appropriate[5].

Design analysis and interpretation plan

A recent study, though in a small sample, found that cervical tVNS increased beta and gamma power and decreased theta and alpha power (Lewine et al., 2018). This study reports, for the comparison between baseline-normalized beta power in the tVNS vs. sham condition, a t-value of 2.64, which, given a sample size of 8 subjects in a within-subjects design, corresponds to an effect size of $d_z \sim 0.93$. Effects of similar magnitude have been found for peri-movement beta oscillations 3h after administration of 15mg tiagabine ($d_z \sim 0.81$, Muthukumaraswamy et al., 2013), and for alpha power following transcranial alternating current stimulation ($d_z \sim 0.86$, Zaehle, Rach, & Herrmann, 2010). Given a possible publication bias, we had a more conservative expectation to find effect sizes $d_z \sim 0.5$ for all our hypotheses. A simulation-based Bayes factor design analysis (Schönbrodt & Wagenmakers, 2018) found that given $d_z = 0.5$ and $n = 40$, Bayes factors conclusively favored the working hypothesis ($BF > 6$) 76.5% of the time for the simulated data. If necessary, sample size would have been increased until Bayes factors clearly favor either the null or working hypothesis for all hypotheses, up to a total sample size of 60 participants (120 experimental sessions), which we consider the maximum number of participants that is technically and economically feasible.

All hypotheses were tested by paired-sample Bayesian t-tests, as described above. The specific variables of interest for each hypothesis can be found in Table 1. If all of hypotheses H_1-H_3 were confirmed, we would interpret this as a confirmation for an overall increase in GABAergic activity induced through tVNS. Conversely, if all respective null hypotheses were confirmed, we would conclude that tVNS has no effect on GABAergic activity in healthy individuals. If only some of the hypotheses were confirmed, we would conclude that tVNS has regionally or functionally selective

[5]In the stage 1 protocol, we had stated that we would use Gaussian priors for the t-tests. We were unaware, however, that the Bayesian t-test method has pre-defined (Jeffreys / Cauchy) priors, so that we were not at liberty to define our own. We have corrected this error.

238 effects on GABAergic activity. The strength of this conclusion would depend on whether or not tests
239 for the non-confirmed hypotheses would have conclusive results (in favor of the respective null
240 hypotheses).

241

	Hypothesis	Variable of interest
H1	global resting-state beta power is increased during tVNS compared to sham.	Global beta power
H2A	peri-movement beta desynchronization (PMBD) in the motor cortex is stronger during tVNS compared to sham.	PMBD (averaged over left- and right-hand responses, from the contralateral motor cortices)
H2B	post-movement beta rebound (PMBR) in the motor cortex is weaker during tVNS compared to sham.	PMBR (averaged over left- and right-hand responses, from the contralateral motor cortices)
H3	gamma power response to visual stimulation in the visual cortex is stronger during tVNS.	Gamma power response from the visual cortex
H4	The tVNS effect on resting-state beta power will be stronger in the right (contralateral) hemisphere.	Lateralization index for global beta power
H5A	The tVNS effect on PMBD will be stronger in the right (contralateral) hemisphere for left-hand responses compared to PMBD in the left motor cortex for right-hand responses.	Lateralization index for PMBD
H5B	The tVNS effect on PMBR will be stronger in the right (contralateral) hemisphere for left-hand responses compared to PMBR in the left motor cortex for right-hand responses.	Lateralization index for PMBR

242 Table 1. Overview of variables to be tested for each hypothesis.

243 Likewise, confirmation of hypotheses H_4-H_5 would lead us to the conclusion that GABAergic
244 modulation through tVNS occurs in a lateralized fashion, and a partial confirmation to the conclusion
245 that lateralization is functionally specific.

This study was pre-registered with the Open Science Framework. The original proposal, including a design analysis and pilot data, can be found at https://osf.io/xn47t/.

The Matlab and R code used for data analysis will be made available on Github (https://github.com/mkeute/tVNS-oscillations). MEG data will be made available on Harvard Dataverse (*https://doi.org/10.7910/DVN/OD0SU0*).

Results

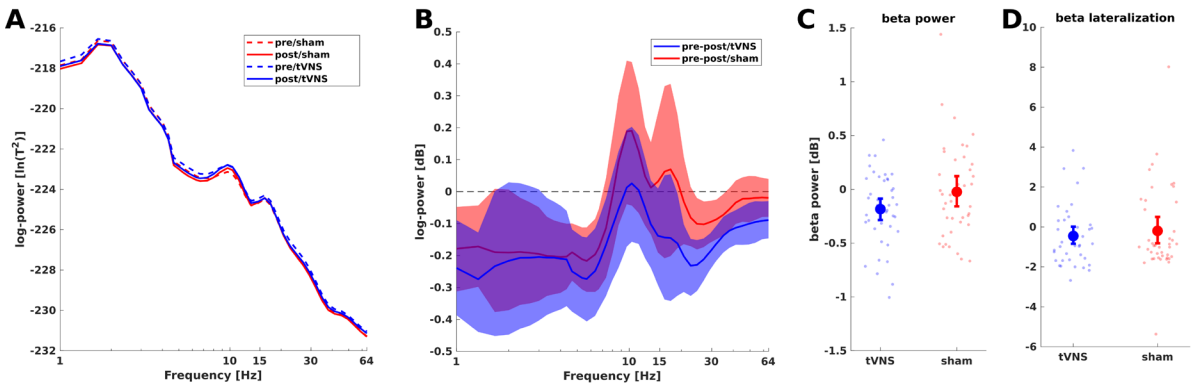

Figure 2. A: Log-transformed mean resting spectra pre- and post- sham/tVNS stimulation. Spectra were calculated for each sensor and averaged across sensors and subjects. B: Difference between pre- and post-stimulation spectra with bootstrapped 95% CI. C: Subject-wise pre-post beta (15-30 Hz) power difference. D: Beta power lateralization.

Resting spectral power in the theta band (~ 8 Hz) and in the high beta band (~ 25 Hz) was reduced pre-to-post-stimulation, across sham and tVNS sessions (Confidence interval does not overlap zero, see Figure 2B). Mean beta power was numerically lower in tVNS compared to sham sessions, contrary to our hypothesis. Accordingly, we found substantial evidence against H_1 (t_{40} = -1.98, BF_{01} = 16.4). Furthermore, lateralization of beta power, i.e., power difference between left- and right-hemisphere sensors, was numerically lower in tVNS sessions, therefore, we found substantial evidence against H_4 (t_{40} = -0.60, BF_{01} = 8.6).

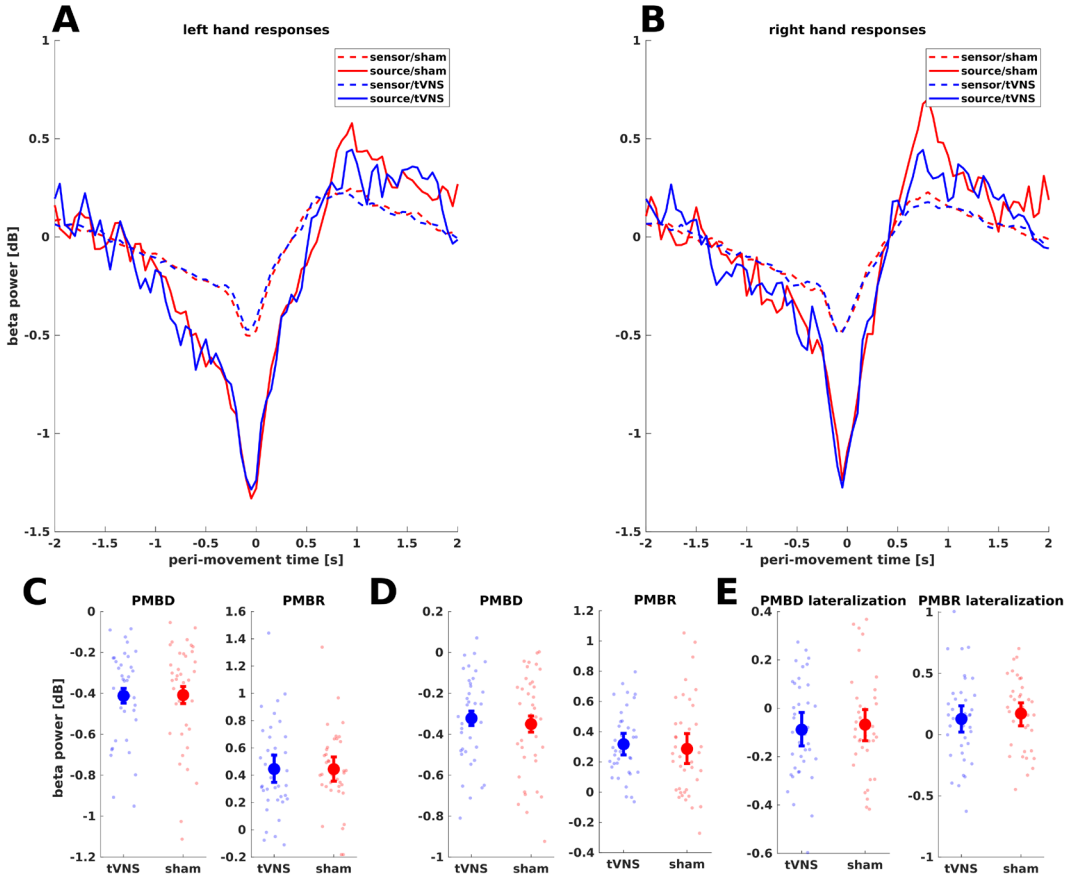

Fig. 3. A: Time course of beta power around left-hand responses in the motor task. Dashed lines: Power averaged across all sensors; solid lines: Power from virtual sensor in the contralateral primary motor cortex. For visualization, data were baseline corrected to a period from -2 to -1 s. B: Same for right-hand responses. C: Subject-wise extracted PMBD and PMBR values for left-hand responses, baseline-corrected for the time windows specified in the Methods section, and bootstrapped 95% CI. D: Same for right-hand responses. E: PMBD and PMBR lateralization with bootstrapped 95% CI.

Mean PMBD across response hands was -0.37 dB in tVNS as well as sham sessions. We found substantial evidence against H_{2A} ($t_{37} = 0.24$, $BF_{01} = 6.8$). Furthermore, we found no effect of tVNS on PMBD lateralization, i.e., substantial evidence against H_{5A} ($t_{37} = -0.53$, $BF_{01} = 8.2$).

Mean PMBR across response hands was 0.38 dB in tVNS and 0.36 dB in sham sessions. We found substantial evidence against H_{2B} (t_{37} = 0.24, BF_{01} = 8.7). Furthermore, we found no effect of tVNS on PMBR lateralization, i.e., substantial evidence against H_{5B} (t_{37} = -0.68, BF_{01} = 8.9).

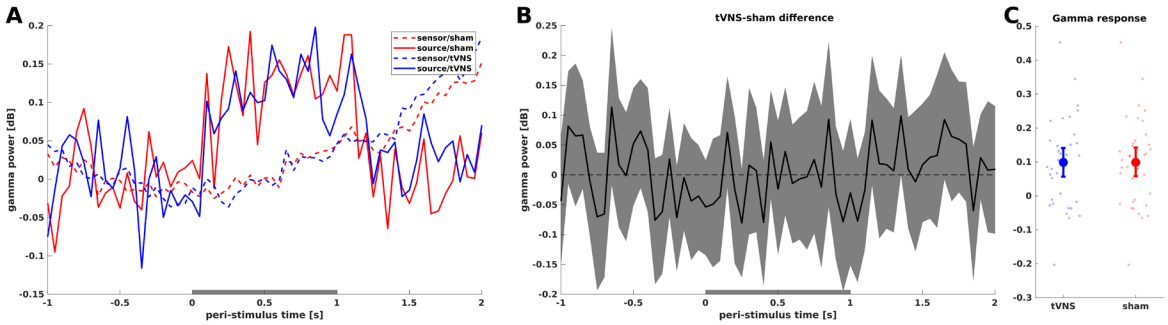

Figure 4. A. Time course of gamma power around visual stimulation. Dashed lines: Power averaged across all sensors; solid lines: Power from virtual sensors in the primary visual cortex. Grey horizontal bar indicates time of stimulus presentation. B: tVNS-sham difference with bootstrapped 95% CI. C: Subject-wise mean gamma response during stimulus presentation.

Mean gamma response was 0.1 dB in tVNS as well as sham sessions. We found substantial evidence against H_3 (t_{35} = -0.42, BF_{01} = 7.6).

Discussion

In this study, our goal was to better understand the cortical dynamics induced by tVNS. Even though the neuromodulatory effects of VNS have been shown by a range of animal studies, especially with respect to the locus coeruleus and NE transmission, and, to a lesser extent, inhibitory GABAergic transmission, the human VNS literature has remained rather inconsistent. For instance, no robust effect of tVNS on noninvasive markers of NEergic neuromodulation (e.g., pupil dilation; Keute et al., 2019; Warren et al., 2019; Burger et al., 2020b; Sharon et al., 2021) and peripheral vagus-associated activation (e.g., heart rate variability; Clancy et al., 2014; De Couck et al, 2017; Borges et al., 2019) has been shown, even though the anatomical and physiological underpinnings of VNS would predict such effects. In our study, we tested for effects of tVNS on oscillatory markers for cortical GABAergic activity. We hypothesized that tVNS would impact resting beta power, movement-related beta power deflections, and visual gamma responses. Furthermore, based on tentative evidence from previous studies, we predicted the beta effects to be lateralized, i.e., stronger in the contralateral hemisphere relative to the stimulated ear. Our data provide substantial evidence against all hypotheses: we found that tVNS did not modulate the beta and gamma power markers, nor was there a lateralized effect of tVNS.

To the best of our knowledge, only one previous study has examined effects of non-invasive (cervical) VNS on spectral power of brain oscillations at rest across several frequency bands (Lewine et al., 2018). This study reported diminished theta and alpha power as well as increased beta and gamma power at selected EEG electrodes, both compared to sham and baseline. With respect to the theta band, our data show some compatibility with these findings in that we found resting theta power to be diminished pre-to-post-stimulation, albeit not between tVNS and sham. However, none of the other findings are in line with our data, which may be partially accounted for by methodical differences between both studies (cervical vs. auricular stimulation; EEG vs. MEG; resting power from single electrodes vs. global resting power).

Besides oscillatory power at rest, we investigated characteristic oscillations of the active primary motor and primary visual cortex at source level. We predicted specific, GABA-associated changes in beta and gamma power deflections by tVNS, respectively, but did not find any.

Overall, our findings do not support any short-term effect of tVNS on GABAergic cortical activity in healthy subjects. Previous studies had reported increases in extrasynaptic GABA concentration and GABA receptor density following invasive VNS in epilepsy patients (Ben-Menachem et al., 1995; Marrosu et al., 2003). Our findings suggest that these changes probably reflect a neuroplastic adaptation triggered by long-term VNS rather than a fast upregulation of cortical GABA levels following VNS treatment onset. Furthermore, the role of GABA transmission in epileptogenesis is more complex than could be described in terms of 'too much' or 'not enough': the postsynaptic effect of GABAergic interneurons is partially reversed in epileptic brains, i.e., excitatory rather than inhibitory, so that an increase in GABA transmission, without further synaptic reorganization, could even promote, rather than alleviate, seizures (Kaila et al., 2014). In light of this, it appears plausible that VNS helps the epileptic brain initiate a specific, plastic process to revert pathological GABA signaling, rather than just acting by a global GABA increase.

On the other hand, two previous studies (Capone et al., 2015; Keute et al; 2018) reported behavioral and electrophysiological effects of tVNS that could be accounted for by a modulation in GABA transmission in the motor cortex. Both studies also provided tentative evidence for a lateralized tVNS effect, but did not formally test for such an effect. Neither the GABAergic mechanism nor the lateralized effect was confirmed by the present study. Importantly, the assumed GABAergic mechanisms of both studies had opposite signs (Keute et al., 2018 was more compatible with a GABA decrease; Capone et al., 2015 was more compatible with a GABA increase), so it appears likely that other, possibly GABA-unrelated mechanisms underlie the findings of both studies. Furthermore, our findings do not confirm any lateralization of effects. Of note, stimulation parameters in both previous studies differed from those in the present study. Specifically, in the previous studies, a higher stimulation intensity (8 mA) was used, and stimulation was intermittent rather than continuous.

Therefore, comparability between the studies might be limited, even though there is no apparent reason to expect a systematic bias with respect to GABAergic neuromodulatory effects.

It is currently one of the central challenges in VNS research to understand why treatment responses are so variable between studies, subjects, and within subjects, and to identify short-term biomarkers that allow for a reliable prediction of long-term treatment response and titration of stimulation parameters. GABA-associated brain oscillations appeared to be a promising marker, especially because of the GABAergic mediation of anti-epileptic VNS effects (Ben-Menachem et al., 1995; Marrosu et al., 2003), but this prediction did not hold true. This is not to say, however, that readouts from ongoing MEG or EEG are altogether unsuitable as VNS biomarkers. A growing number of studies have shown behavioral, cognitive and neurological VNS effects, and it appears likely that these effects are systematically reflected in altered brain activity patterns. This might require using more involved methods, e.g., connectivity or network metrics, as some first studies have done to predict long-term clinical outcomes of invasive VNS (Babajani-Feremi et al., 2018; Mithani et al., 2019). It is important to note that in order to qualify as a predictive biomarker, a physiological readout would not only have to be systematically changed by the stimulation, but the readout (or its change) would also need to be reliably correlated to a clinical, physiological, or behavioral outcome of the stimulation (Burger et al., 2020a; Keute et al., 2021). Furthermore, specific patterns of brain oscillations in clinical populations will have to be taken into account, as they might interact with oscillatory VNS markers (cf. Marrosu et al., 2005). Overall, we are confident that predictive markers will also be identifiable for short-term tVNS, and we encourage the use of our data, which will be made available for download, for further exploration.

360 **Conflict of interest / Acknowledgements**

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