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Effects of transcutaneous vagus nerve stimulation (tVNS) on beta and gamma brain oscillationsMarius Keute^{1,3}, Christian Wienke^{1,2}, Philipp Ruhnau^{1,2}, Tino Zaehle^{1,2}¹Department of Neurology, Otto-von Guericke-University, Magdeburg, Germany²Center for Behavioral Brain Sciences, Otto-von-Guericke University, Magdeburg³Institute 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:

H₁: 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.

H₃: gamma power response to visual stimulation in the visual cortex is stronger during tVNS.

94 Furthermore, we hypothesize that the effects from H₁ and H₂ are lateralized, i.e., stronger in the brain
95 hemisphere contralateral to the stimulation.

96 H₄: The tVNS effect on resting-state beta power will be stronger in the right (contralateral)
97 hemisphere.

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

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

102

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.

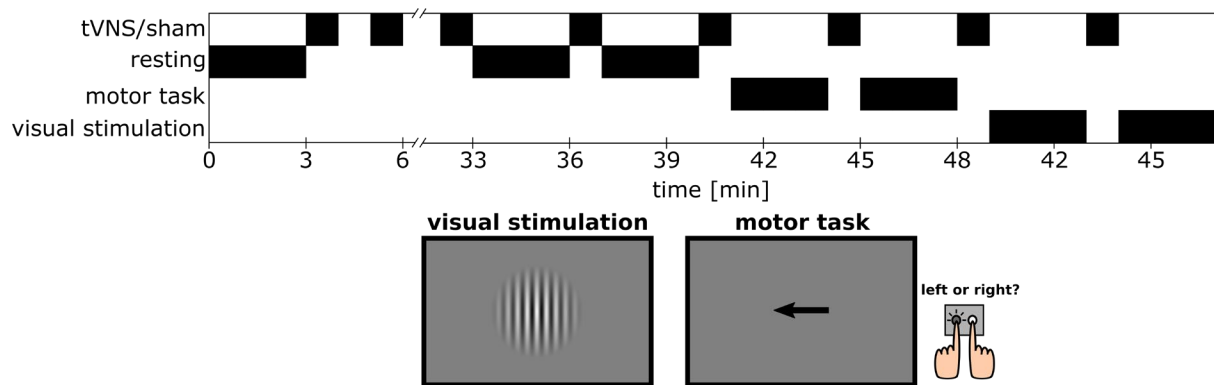


Fig. 1: Experimental procedure. The order of the motor task and visual stimulation were counterbalanced across participants.

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 (± 1 h). 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 μ s 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 AB¹) 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 (MathWorks²). 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 excluded 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 [$10 \cdot \log_{10}(\text{Power} / \text{Power}_{\text{baseline}})$]. 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

³The MNI coordinates for the virtual sensors were not included in the stage 1 protocol. They were specified for increased transparency.

⁴We further specified calculation of lat. indices compared to the stage 1 protocol.

215 approximately normal distributions (Kiebel, Tallon-Baudry, & Friston, 2005), rendering the use of t-
216 tests appropriate⁵.

217 *Design analysis and interpretation plan*

218 A recent study, though in a small sample, found that cervical tVNS increased beta and gamma power
219 and decreased theta and alpha power (Lewine et al., 2018). This study reports, for the comparison
220 between baseline-normalized beta power in the tVNS vs. sham condition, a t-value of 2.64, which,
221 given a sample size of 8 subjects in a within-subjects design, corresponds to an effect size of $d_z \sim$
222 0.93. Effects of similar magnitude have been found for peri-movement beta oscillations 3h after
223 administration of 15mg tiagabine ($d_z \sim 0.81$, Muthukumaraswamy et al., 2013), and for alpha power
224 following transcranial alternating current stimulation ($d_z \sim 0.86$, Zaehle, Rach, & Herrmann, 2010).
225 Given a possible publication bias, we had a more conservative expectation to find effect sizes $d_z \sim 0.5$
226 for all our hypotheses. A simulation-based Bayes factor design analysis (Schönbrodt &
227 Wagenmakers, 2018) found that given $d_z = 0.5$ and $n = 40$, Bayes factors conclusively favored the
228 working hypothesis ($BF > 6$) 76.5% of the time for the simulated data. If necessary, sample size
229 would have been increased until Bayes factors clearly favor either the null or working hypothesis for
230 all hypotheses, up to a total sample size of 60 participants (120 experimental sessions), which we
231 consider the maximum number of participants that is technically and economically feasible.

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

⁵In 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.

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

	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

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

Likewise, confirmation of hypotheses H₄-H₅ would lead us to the conclusion that GABAergic modulation through tVNS occurs in a lateralized fashion, and a partial confirmation to the conclusion that lateralization is functionally specific.

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

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

Results

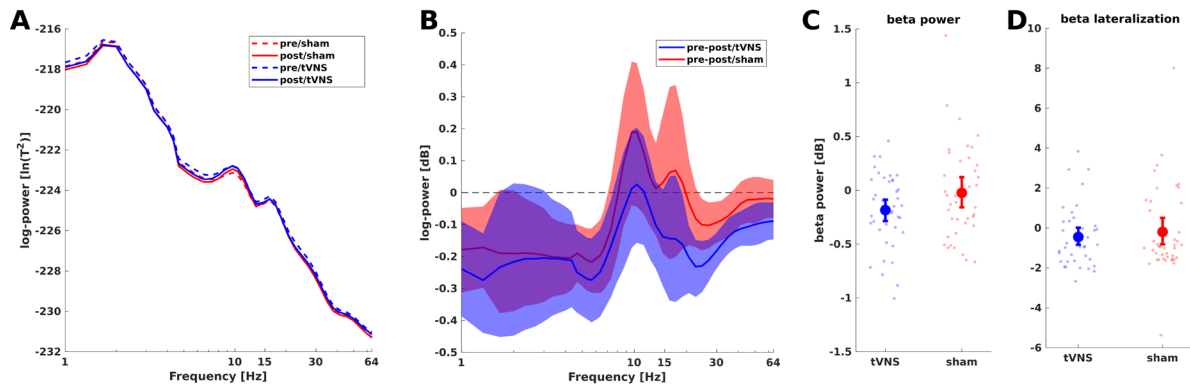


Figure 2. *A: Log-transformed mean resting spectra pre- and post- sham/tVNS stimulation. Spectra were calculated for each sensor and averaged across sensors and subjects. B: Difference between pre- and post-stimulation spectra with bootstrapped 95% CI. C: Subject-wise pre-post beta (15-30 Hz) power difference. 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 ($t_{40} = -0.60$, $BF_{01} = 8.6$).

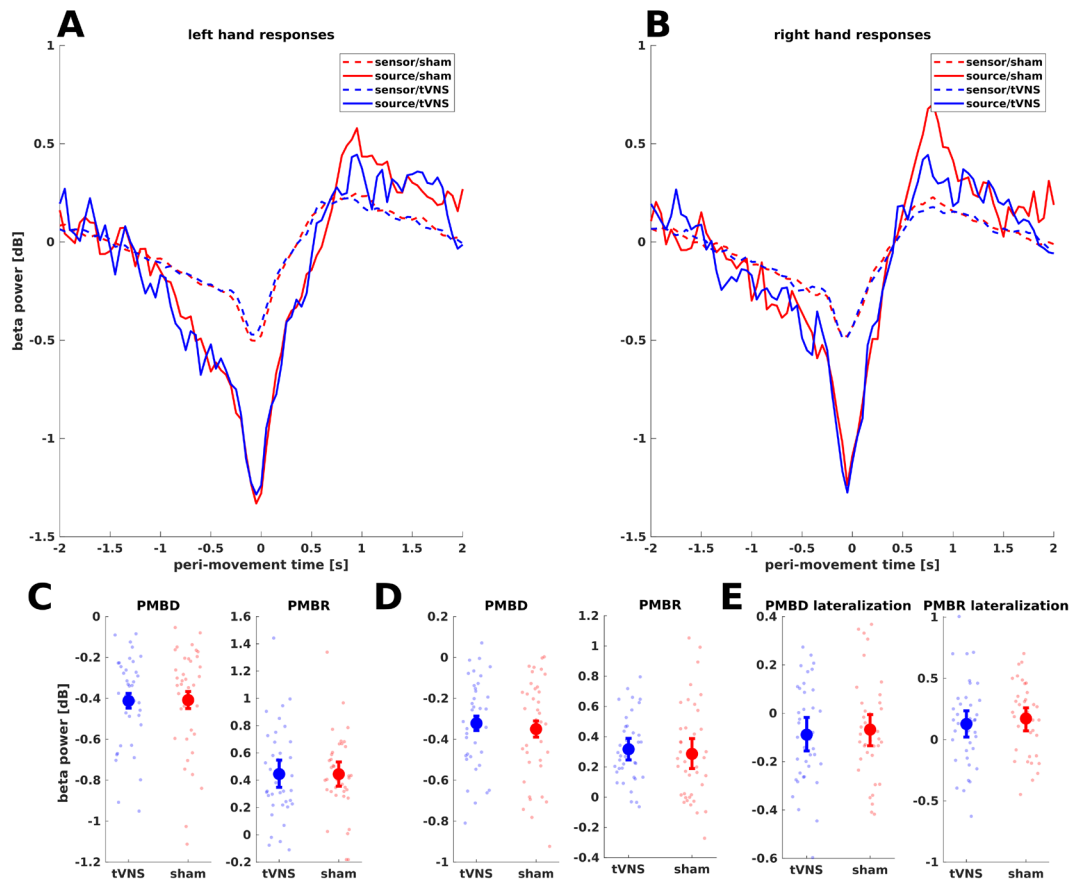


Fig. 3. A: Time course of beta power around left-hand responses in the motor task. Dashed lines: Power averaged across all sensors; solid lines: Power from virtual sensor in the contralateral primary motor cortex. For visualization, data were baseline corrected to a period from -2 to -1 s. B: Same for 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} ($t_{37} = -0.68$, $BF_{01} = 8.9$).

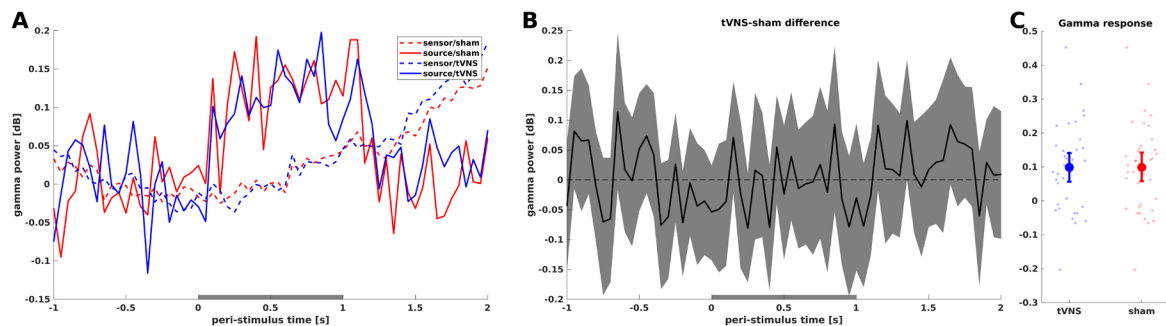


Figure 4. A: Time course of gamma power around visual stimulation. Dashed lines: Power averaged across all sensors; solid lines: Power from virtual sensors in the primary visual cortex. Grey horizontal bar indicates time of stimulus presentation. B: tVNS-sham difference with bootstrapped 95% CI. C: Subject-wise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

- Assenza, G., Campana, C., Colicchio, G., Tombini, M., Assenza, F., Di Pino, G., & Di Lazzaro, V. (2017, July 1). Transcutaneous and invasive vagal nerve stimulations engage the same neural pathways: In-vivo human evidence. *Brain Stimulation*, Vol. 10, pp. 853–854. <https://doi.org/10.1016/j.brs.2017.03.005>
- Babajani-Feremi, A., Noorizadeh, N., Mudigoudar, B., & Wheless, J. W. (2018). Predicting seizure outcome of vagus nerve stimulation using MEG-based network topology. *NeuroImage: Clinical*, 19, 990-999.
- Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., ... George, M. S. (2018). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review. *Brain Stimulation*. <https://doi.org/10.1016/j.brs.2017.12.009>
- Barbanti, P., Grazzi, L., Egeo, G., Padovan, A. M., Liebler, E., & Bussone, G. (2015). Non-invasive vagus nerve stimulation for acute treatment of high-frequency and chronic migraine: an open-label study. *The Journal of Headache and Pain*, 16(1), 61.
- Bauer, S., Baier, H., Baumgartner, C., Bohlmann, K., Fauser, S., Graf, W., ... Lerche, H. (2016). Transcutaneous Vagus Nerve Stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). *Brain Stimulation*, 9(3), 356–363.
- Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., Beranger, A., Prud'homme, J.-F., ... LeGuern, E. (2001). First genetic evidence of GABA A receptor dysfunction in epilepsy: a mutation in the $\gamma 2$ -subunit gene. *Nature Genetics*, 28(1), 46.
- Ben-Menachem, E., Hamberger, A., Hedner, T., Hammond, E. J., Uthman, B. M., Slater, J., ... Wilder, B. J. (1995). Effects of vagus nerve stimulation on amino acids and other metabolites in the

- 387 CSF of patients with partial seizures. *Epilepsy Research*, 20(3), 221–227.
388 [https://doi.org/10.1016/0920-1211\(94\)00083-9](https://doi.org/10.1016/0920-1211(94)00083-9)
- 389 Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: Modulation
390 of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*, 42(1), 33–84.
391 [https://doi.org/10.1016/S0165-0173\(03\)00143-7](https://doi.org/10.1016/S0165-0173(03)00143-7)
- 392 Borges, U., Laborde, S., & Raab, M. (2019). Influence of transcutaneous vagus nerve stimulation on
393 cardiac vagal activity: not different from sham stimulation and no effect of stimulation intensity. *PloS*
394 *one*, 14(10), e0223848.
- 395 Boy, F., Evans, C. J., Edden, R. A. E., Singh, K. D., Husain, M., & Sumner, P. (2010). Individual
396 differences in subconscious motor control predicted by GABA concentration in SMA. *Current*
397 *Biology*, 20(19), 1779–1785. <https://doi.org/10.1016/j.cub.2010.09.003>
- 398 Brozoski, T. J., Spires, T. J. D., & Bauer, C. A. (2007). Vigabatrin, a GABA transaminase inhibitor,
399 reversibly eliminates tinnitus in an animal model. *Journal of the Association for Research in*
400 *Otolaryngology*, 8(1), 105–118.
- 401 Burger, A. M., D’Agostini, M., Verkuil, B., & Van Diest, I. (2020). Moving beyond belief: A
402 narrative review of potential biomarkers for transcutaneous vagus nerve stimulation.
403 *Psychophysiology*, 57(6), e13571.
- 404 Burger, A. M., Van der Does, W., Brosschot, J. F., & Verkuil, B. (2020). From ear to eye? No effect
405 of transcutaneous vagus nerve stimulation on human pupil dilation: a report of three studies.
406 *Biological psychology*, 152, 107863.
- 407 Capone, F., Assenza, G., Di Pino, G., Musumeci, G., Ranieri, F., Florio, L., ... Di Lazzaro, V. (2015).
408 The effect of transcutaneous vagus nerve stimulation on cortical excitability. *Journal of Neural*
409 *Transmission*, 122(5), 679–685. <https://doi.org/10.1007/s00702-014-1299-7>

- 410 Carpenter, L. L., Moreno, F. A., Kling, M. A., Anderson, G. M., Regenold, W. T., Labiner, D. M., &
411 Price, L. H. (2004). Effect of vagus nerve stimulation on cerebrospinal fluid monoamine metabolites,
412 norepinephrine, and gamma-aminobutyric acid concentrations in depressed patients. *Biological*
413 *Psychiatry*, 56(6), 418–426. <https://doi.org/10.1016/j.biopsych.2004.06.025>
- 414 Clancy, J. A., Deuchars, S. A., & Deuchars, J. (2013). The wonders of the Wanderer. *Experimental*
415 *Physiology*, 98(1), 38–45. <https://doi.org/10.1113/expphysiol.2012.064543>
- 416 Clancy, J. A., Mary, D. A., Witte, K. K., Greenwood, J. P., Deuchars, S. A., & Deuchars, J. (2014).
417 Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. *Brain*
418 *stimulation*, 7(6), 871-877.
- 419 De Couck, M., Cserjesi, R., Caers, R., Zijlstra, W. P., Widjaja, D., Wolf, N., ... & Gidron, Y. (2017).
420 Effects of short and prolonged transcutaneous vagus nerve stimulation on heart rate variability in
421 healthy subjects. *Autonomic Neuroscience*, 203, 88-96.
- 422 Edden, R. A., Crocetti, D., Zhu, H., Gilbert, D. L., & Mostofsky, S. H. (2012). Reduced GABA
423 concentration in attention-deficit/hyperactivity disorder. *Archives of General Psychiatry*, 69(7), 750–
424 753.
- 425 Edden, R. A. E., Muthukumaraswamy, S. D., Freeman, T. C. A., & Singh, K. D. (2009). Orientation
426 Discrimination Performance Is Predicted by GABA Concentration and Gamma Oscillation Frequency
427 in Human Primary Visual Cortex. *Journal of Neuroscience*, 29(50), 15721–15726.
428 <https://doi.org/10.1523/JNEUROSCI.4426-09.2009>
- 429 Fang, J., Rong, P., Hong, Y., Fan, Y., Liu, J., Wang, H., ... Kong, J. (2016). Transcutaneous vagus
430 nerve stimulation modulates default mode network in major depressive disorder. *Biological*
431 *Psychiatry*, 79(4), 266–273. <https://doi.org/10.1016/j.biopsych.2015.03.025>

- 432 Frangos, E., Ellrich, J., & Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central
433 projections via electrical stimulation of the external ear: FMRI evidence in humans. *Brain*
434 *Stimulation*, 8(3). <https://doi.org/10.1016/j.brs.2014.11.018>
- 435 Gaetz, W., Edgar, J. C., Wang, D. J., & Roberts, T. P. L. (2011). Relating MEG measured motor
436 cortical oscillations to resting γ -aminobutyric acid (GABA) concentration. *Neuroimage*, 55(2), 616–
437 621.
- 438 Greenblatt, D. J., Ehrenberg, B. L., Gunderman, J., Locniskar, A., Scavone, J. M., Harmatz, J. S., &
439 Shader, R. I. (1989). Pharmacokinetic and electroencephalographic study of intravenous diazepam,
440 midazolam, and placebo. *Clinical Pharmacology & Therapeutics*, 45(4), 356–365.
- 441 Hall, S. D., Barnes, G. R., Furlong, P. L., Seri, S., & Hillebrand, A. (2010). Neuronal Network
442 Pharmacodynamics of GABAergic Modulation in the Human Cortex Determined Using Pharmaco-
443 Magnetoencephalography. 594(December 2008), 581–594. <https://doi.org/10.1002/hbm.20889>
- 444 He, W., Jing, X.-H., Zhu, B., Zhu, X.-L., Li, L., Bai, W.-Z., & Ben, H. (2013). The auriculo-vagal
445 afferent pathway and its role in seizure suppression in rats. *BMC Neuroscience*, 14, 1.
446 <https://doi.org/10.1186/1471-2202-14-85>
- 447 Kaczmarczyk, R., Tejera, D., Simon, B. J., & Heneka, M. T. (2018). Microglia modulation through
448 external vagus nerve stimulation in a murine model of Alzheimer's disease. *Journal of*
449 *Neurochemistry*, 146(1), 76–85.
- 450 Kaila, K., Ruusuvuori, E., Seja, P., Voipio, J., & Puskarjov, M. (2014). GABA actions and ionic
451 plasticity in epilepsy. *Current opinion in neurobiology*, 26, 34-41.
- 452 Keute, M., Ruhnau, P., Heinze, H.-J., & Zaehle, T. (2018). Behavioral and electrophysiological
453 evidence for GABAergic modulation through transcutaneous vagus nerve stimulation. *Clinical*
454 *Neurophysiology*.

- 455 Keute, M., Demirezen, M., Graf, A., Mueller, N. G., & Zaehle, T. (2019). No modulation of pupil size
456 and event-related pupil response by transcutaneous auricular vagus nerve stimulation (taVNS).
457 Scientific reports, 9(1), 1-10.
- 458 Keute, M., Machetanz, K., Berelidze, L., Guggenberger, R., & Gharabaghi, A. (2021). Neuro-cardiac
459 coupling predicts transcutaneous auricular vagus nerve stimulation effects. Brain stimulation, 14(2),
460 209-216.
- 461 Kiebel, S. J., Tallon-Baudry, C., & Friston, K. J. (2005). Parametric analysis of oscillatory activity as
462 measured with EEG/MEG. Human Brain Mapping, 26(3), 170–177.
- 463 Lehtimäki, J., Hyvärinen, P., Ylikoski, M., Bergholm, M., Mäkelä, J. P., Aarnisalo, A., ... Ylikoski, J.
464 (2013). Transcutaneous vagus nerve stimulation in tinnitus: a pilot study. Acta Oto-Laryngologica,
465 133(February), 378–382. <https://doi.org/10.3109/00016489.2012.750736>
- 466 Lewine, J. D., Paulson, K., Bangera, N., & Simon, B. J. (2018). Exploration of the Impact of Brief
467 Noninvasive Vagal Nerve Stimulation on EEG and Event-Related Potentials. Neuromodulation:
468 Technology at the Neural Interface.
- 469 Lewis, D. A., Pierri, J. N., Volk, D. W., Melchitzky, D. S., & Woo, T.-U. W. (1999). Altered GABA
470 neurotransmission and prefrontal cortical dysfunction in schizophrenia. Biological Psychiatry, 46(5),
471 616–626.
- 472 Lithari, C., Sánchez-García, C., Ruhnau, P., & Weisz, N. (2016). Large-scale network-level processes
473 during entrainment. Brain Research, 1635, 143–152.
- 474 Lu, X., Hong, Z., Tan, Z., Sui, M., Zhuang, Z., Liu, H., ... Jin, D. (2017). Nicotinic acetylcholine
475 receptor alpha7 subunit mediates vagus nerve stimulation-induced neuroprotection in acute permanent
476 cerebral ischemia by $\alpha 7$ nAChR/JAK2 pathway. Medical Science Monitor: International Medical
477 Journal of Experimental and Clinical Research, 23, 6072.

- 478 Magis, D., Gérard, P., & Schoenen, J. (2013). Transcutaneous Vagus Nerve Stimulation (tVNS) for
479 headache prophylaxis: initial experience. *The Journal of Headache and Pain*, 14(S1), P198.
- 480 Marrosu, F., Santoni, F., Puligheddu, M., Barberini, L., Maleci, A., Ennas, F., ... Biggio, G. (2005).
481 Increase in 20–50 Hz (gamma frequencies) power spectrum and synchronization after chronic vagal
482 nerve stimulation. *Clinical Neurophysiology*, 116(9), 2026–2036.
- 483 Marrosu, F., Serra, A., Maleci, A., Puligheddu, M., Biggio, G., & Piga, M. (2003). Correlation
484 between GABAA receptor density and vagus nerve stimulation in individuals with drug-resistant
485 partial epilepsy. *Epilepsy Research*, 55(1–2), 59–70. [https://doi.org/10.1016/S0920-1211\(03\)00107-4](https://doi.org/10.1016/S0920-1211(03)00107-4)
- 486 Martínez-Vargas, D., Valdés-Cruz, A., Magdaleno-Madrigal, V., Fernández-Mas, R., & Almazán-
487 Alvarado, S. (2017). Effect of Electrical Stimulation of the Nucleus of the Solitary Tract on
488 Electroencephalographic Spectral Power and the Sleep–Wake Cycle in Freely Moving Cats. *Brain*
489 *Stimulation*, 10(1), 116–125.
- 490 Mithani, K., Mikhail, M., Morgan, B. R., Wong, S., Weil, A. G., Deschenes, S., ... & Ibrahim, G. M.
491 (2019). Connectomic profiling identifies responders to vagus nerve stimulation. *Annals of neurology*,
492 86(5), 743–753.
- 493 Möhler, H. (2012). The GABA system in anxiety and depression and its therapeutic potential.
494 *Neuropharmacology*, 62(1), 42–53.
- 495 Morey, R. D., Rouder, J. N., & Jamil, T. (2015). BayesFactor: Computation of Bayes factors for
496 common designs. R Package Version 0.9, 9, 2014.
- 497 Muthukumaraswamy, S. D., Edden, R. A. E., Jones, D. K., Swettenham, J. B., & Singh, K. D. (2009).
498 Resting GABA concentration predicts peak gamma frequency and fMRI amplitude in response to
499 visual stimulation in humans. *Proceedings of the National Academy of Sciences*, 106(20), 8356–8361.

- 500 Muthukumaraswamy, S. D., Myers, J. F. M., Wilson, S. J., Nutt, D. J., Lingford-Hughes, A., Singh,
501 K. D., & Hamandi, K. (2013). The effects of elevated endogenous GABA levels on movement-related
502 network oscillations. *Neuroimage*, 66, 36–41.
- 503 Neuling, T., Ruhnau, P., Fuscà, M., Demarchi, G., Herrmann, C. S., & Weisz, N. (2015). Shed light
504 on the black box: Using MEG to recover brain activity during tACS. *Brain Stimulation: Basic,*
505 *Translational, and Clinical Research in Neuromodulation*, 8(2), 381–382.
- 506 Nolte, G. (2003). The magnetic lead field theorem in the quasi-static approximation and its use for
507 magnetoencephalography forward calculation in realistic volume conductors. *Physics in Medicine &*
508 *Biology*, 48(22), 3637.
- 509 Nutt, D., Wilson, S., Lingford-Hughes, A., Myers, J., Papadopoulos, A., & Muthukumaraswamy, S.
510 (2015). Differences between magnetoencephalographic (MEG) spectral profiles of drugs acting on
511 GABA at synaptic and extrasynaptic sites: a study in healthy volunteers. *Neuropharmacology*, 88,
512 155–163.
- 513 Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2011). FieldTrip: open source software for
514 advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence*
515 *and Neuroscience*, 2011, 1.
- 516 Raedt, R., Clinckers, R., Mollet, L., Vonck, K., El Tahry, R., Wyckhuys, T., ... Meurs, A. (2011).
517 Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a
518 limbic seizure model. *Journal of Neurochemistry*, 117(3), 461–469. [https://doi.org/10.1111/j.1471-](https://doi.org/10.1111/j.1471-4159.2011.07214.x)
519 [4159.2011.07214.x](https://doi.org/10.1111/j.1471-4159.2011.07214.x)
- 520 Ruffoli, R., Giorgi, F. S., Pizzanelli, C., Murri, L., Paparelli, A., & Fornai, F. (2011). The chemical
521 neuroanatomy of vagus nerve stimulation. *Journal of Chemical Neuroanatomy*, 42(4), 288–296.
522 <https://doi.org/10.1016/j.jchemneu.2010.12.002>

- 523 Schönbrodt, F. D., & Wagenmakers, E.-J. (2018). Bayes factor design analysis: Planning for
524 compelling evidence. *Psychonomic Bulletin & Review*, 25(1), 128–142.
- 525 Sharon, O., Fahoum, F., & Nir, Y. (2021). Transcutaneous vagus nerve stimulation in humans induces
526 pupil dilation and attenuates alpha oscillations. *Journal of Neuroscience*, 41(2), 320–330.
- 527 Stagg, C. J., Bestmann, S., Constantinescu, A. O., Moreno Moreno, L., Allman, C., Mekle, R., ...
528 Rothwell, J. C. (2011). Relationship between physiological measures of excitability and levels of
529 glutamate and GABA in the human motor cortex. *The Journal of Physiology*, 589(23), 5845–5855.
530 <https://doi.org/10.1113/jphysiol.2011.216978>
- 531 Stefan, H., Kreiselmeier, G., Kerling, F., Kurzbuch, K., Rauch, C., Heers, M., ... Pauli, E. (2012).
532 Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: A proof of concept
533 trial. *Epilepsia*, 53(7), e115–e118.
- 534 Toussay, X., Basu, K., Lacoste, B., & Hamel, E. (2013). Locus coeruleus stimulation recruits a broad
535 cortical neuronal network and increases cortical perfusion. *Journal of Neuroscience*, 33(8), 3390–
536 3401.
- 537 Trevizol, A. P., Taiar, I., Barros, M. D., Liquidatto, B., Cordeiro, Q., & Shiozawa, P. (2015).
538 Transcutaneous vagus nerve stimulation (tVNS) protocol for the treatment of major depressive
539 disorder: A case study assessing the auricular branch of the vagus nerve. *Epilepsy & Behavior*, 53,
540 166–167.
- 541 van Lier, H., Drinkenburg, W. H. I. M., van Eeten, Y. J. W., & Coenen, A. M. L. (2004). Effects of
542 diazepam and zolpidem on EEG beta frequencies are behavior-specific in rats. *Neuropharmacology*,
543 47(2), 163–174.
- 544 Ventura-Bort, C., Wirkner, J., Genheimer, H., Wendt, J., Hamm, A. O., & Weymar, M. (2018).
545 Effects of Transcutaneous Vagus Nerve Stimulation (tVNS) on the P300 and Alpha-Amylase Level:
546 A Pilot Study. *Frontiers in Human Neuroscience*, 12. <https://doi.org/10.3389/fnhum.2018.00202>

- 547 Ventureyra, E. C. (2000). Transcutaneous vagus nerve stimulation for partial onset seizure therapy. A
548 new concept. *Child's Nervous System: ChNS: Official Journal of the International Society for*
549 *Pediatric Neurosurgery*, 16(2), 101–102. <https://doi.org/10.1007/s003810050021>
- 550 Walker, B. R., Easton, A., & Gale, K. (1999). Regulation of limbic motor seizures by GABA and
551 glutamate transmission in nucleus tractus solitarius. *Epilepsia*, 40(8), 1051–1057.
- 552 Warren, C. M., Tona, K. D., Ouwerkerk, L., Van Paridon, J., Poletiek, F., van Steenbergen, H., ...
553 Nieuwenhuis, S. (2019). The neuromodulatory and hormonal effects of transcutaneous vagus nerve
554 stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3
555 event-related potential. *Brain Stimulation*, 12(3), 635–642.
- 556 Woodbury, J. W., & Woodbury, D. M. (1991). Vagal stimulation reduces the severity of maximal
557 electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. *Pacing and*
558 *Clinical Electrophysiology*, 14(1), 94–107.
- 559 Xiong, J., Xue, F. S., Liu, J. H., Xu, Y. C., Liao, X., Zhang, Y. M., ... Li, S. (2009). Transcutaneous
560 vagus nerve stimulation may attenuate postoperative cognitive dysfunction in elderly patients.
561 *Medical Hypotheses*, 73(6), 938–941.
- 562 Zaehle, T., Rach, S., & Herrmann, C. S. (2010). Transcranial Alternating Current Stimulation
563 Enhances Individual Alpha Activity in Human EEG. *PLOS ONE*, 5(11), e13766.
564 <https://doi.org/10.1371/journal.pone.0013766>
565

