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

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8

9

10 **Abstract**

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

20

21 Introduction

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

31 So far, the mechanisms of action of tVNS are not fully understood, and an improved understanding of
32 these mechanisms will be highly relevant and necessary for future research, highlighting how patients
33 can benefit from tVNS as well as for therapy development and improvement. It is consistently found
34 that the locus coeruleus-norepinephrine (LC-NE) system is activated through both iVNS and tVNS.
35 This activation is mediated by the nucleus of the solitary tract (NTS), the principal brain projection
36 area of the afferent branches of the vagus nerve (Ruffoli et al., 2011). LC activation is considered the
37 core mechanism of tVNS (Assenza et al., 2017; Badran et al., 2018; Raedt et al., 2011; Ventura-Bort
38 et al., 2018; Warren et al., 2019). One of several other candidate mechanisms of action is an increase
39 in γ -aminobutyric acid (GABA) transmission in the brain (Ruffoli et al., 2011; Walker, Easton, &
40 Gale, 1999; Woodbury & Woodbury, 1991), mediated through activation of the NTS and LC
41 (Berridge & Waterhouse, 2003; Toussay, Basu, Lacoste, & Hamel, 2013). The research literature on
42 GABAergic neuromodulation by tVNS is sparse, compared to the amount of studies investigating
43 effects of tVNS on LC-NE activity. Given that GABA transmission has a role in the pathophysiology
44 of epilepsy (Baulac et al., 2001), depression (Möhler, 2012), tinnitus (Brozoski, Spires, & Bauer,
45 2007), and other neurological and psychiatric conditions, it is of high relevance to better understand
46 GABAergic actions of tVNS in order to predict and understand its therapeutic effects.

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

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

65 Brain oscillations as measured by EEG or magnetoencephalography (MEG) often have specific
66 relationships to local GABA concentrations and can therefore be used as biomarkers:
67 Pharmacological increases of systemic GABA levels are consistently associated to increases in beta
68 power at rest (Greenblatt et al., 1989; Hall, Barnes, Furlong, Seri, & Hillebrand, 2010; Nutt et al.,
69 2015; van Lier, Drinkenburg, van Eeten, & Coenen, 2004). Furthermore, GABA concentration in the
70 motor cortex is related to peri-movement beta and gamma power modulations (Gaetz, Edgar, Wang,
71 & Roberts, 2011; Muthukumaraswamy et al., 2013), and GABA concentration in the visual cortex is

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

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

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

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

88 H₁: global resting-state beta power is increased during tVNS compared to sham.

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

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

93 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.

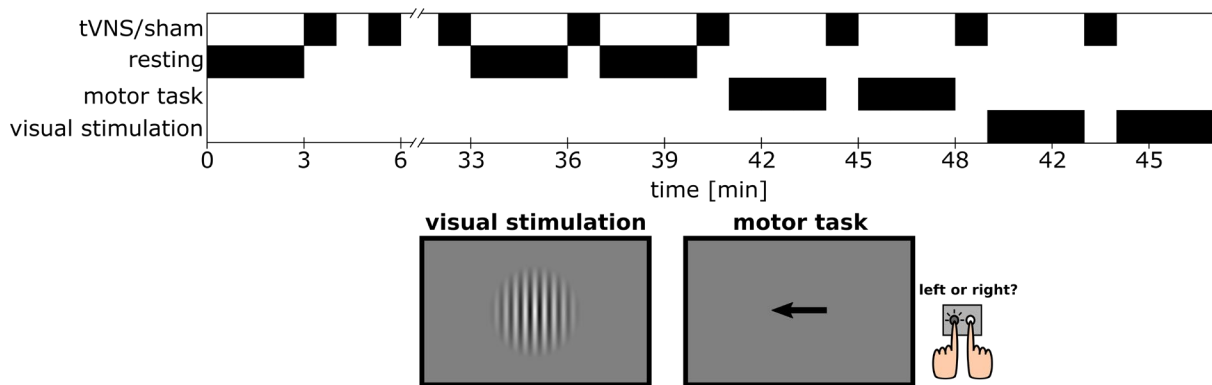
100 H_{5B}: The tVNS effect on PMBR will be stronger in the right (contralateral) hemisphere for left-hand
101 responses compared to PMBR in the left motor cortex for right-hand responses.

102

103 **Methods**

104 *General procedure*

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



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

125 Panels below: *Illustration of experimental stimuli (not true to scale).*

126 *Participants*

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

135 *Motor task*

136 Peri-movement beta power was assessed using a cued finger movement task. Participants were
 137 instructed to press a button with their left or right index finger, according to the direction of an arrow
 138 displayed centrally on the screen (displayed in black on a grey background, width 1 degree, height 0.5
 139 degree of visual angle). During each 180 s block, 24 left-pointing and 24 right-pointing arrows were
 140 presented in pseudo-randomized order, with stimulus durations of 200 ms and a randomly jittered
 141 inter-stimulus interval between 3 and 3.5 s. A red fixation point was visible on the center of the screen
 142 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 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

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

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

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

192 motor task, pre-movement beta desynchronization (PMBD) and post-movement beta rebound
193 (PMBR) were assessed by subtracting \log_{10} -transformed source-space power in the contralateral
194 motor cortex (virtual sensor at MNI coordinates [-48,-8,50] and [48,-8,50]³ for left and right primary
195 motor cortex, respectively) across the beta band (15-30 Hz) and over a time window between -1.25 –
196 0.5 s relative to the button press (for PMBD) or between 1 – 1.75 s (for PMBR) from time-averaged
197 log-power over the entire trial (-1.25 – 1.75 s). For the visual stimulation, we used a baseline of -1 – 0
198 s relative to stimulus onset and compared it to the presentation time of the stimuli (0 – 1 s). For
199 analysis of visual stimulation data, we created virtual sensors at MNI coordinates [-2,-80,34], [-28,-
200 96,-6] and [28,-96,-6] for central, left, and right primary visual cortex, respectively, and analyzed
201 gamma power averaged across the three virtual sensors. For the analysis of resting and movement-
202 related beta power, we averaged the baseline-corrected log-power values over beta frequencies (15 –
203 30 Hz), for the analysis of gamma power, we averaged over gamma frequencies (30 – 60 Hz). For
204 event-related data from the motor task and visual stimulation, we additionally averaged over time
205 bins and trials. To test for lateralization of tVNS effects, we computed lateralization indices as
206 differences between resting beta log-power in the left and right hemisphere, and between PMBD and
207 PMBR to left- and right-hand movements in the contralateral motor cortex, respectively. We
208 calculated all lateralization indices such that hypotheses H₄, H_{5A} and H_{5B} predict higher values for
209 tVNS compared to sham (i.e., subtracting right hemisphere values from left hemisphere values for
210 PMBD and PMBR, and vice versa for resting beta power)⁴.

211 Resulting session-wise values for resting beta power, PMBD, PMBR, visual gamma response, and
212 lateralization indices were compared between sham and tVNS sessions by means of paired-sample
213 one tailed Bayesian t-tests using R and the BayesFactor package (Morey, Rouder, & Jamil, 2015).
214 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 H₁-H₃ 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.

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

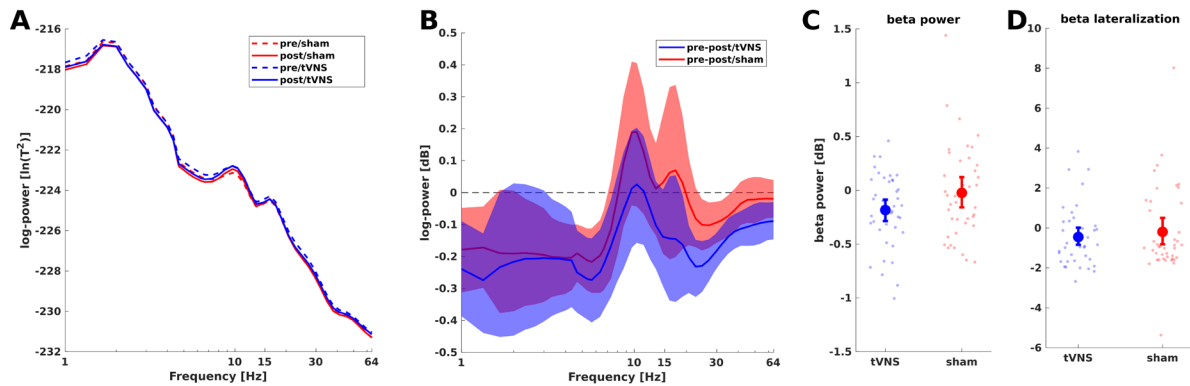
	Hypothesis	Variable of interest
H1	global resting-state beta power is increased during tVNS compared to sham.	Global beta power
H2A	peri-movement beta desynchronization (PMBD) in the motor cortex is stronger during tVNS compared to sham.	PMBD (averaged over left- and right-hand responses, from the contralateral 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 H₄-H₅ 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.

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>).

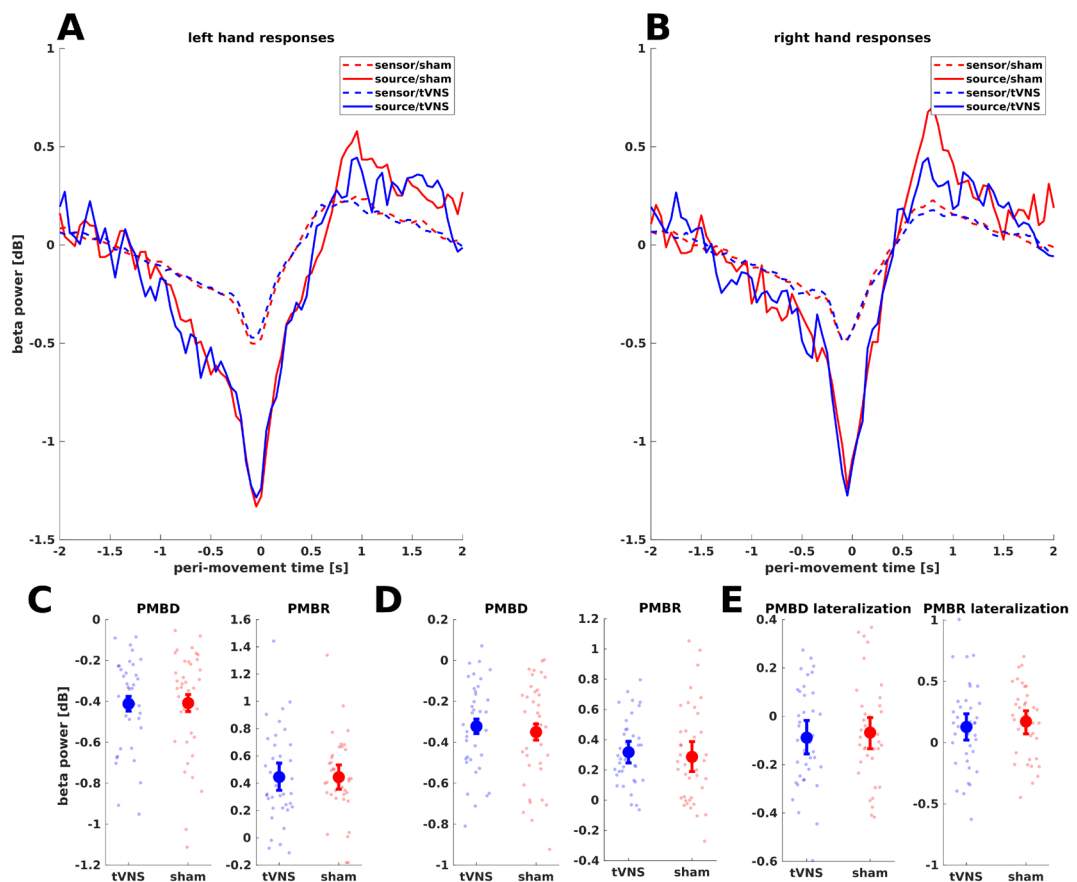
251 **Results**

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

256 Resting spectral power in the theta band (~ 8 Hz) and in the high beta band (~ 25 Hz) was reduced
 257 pre-to-post-stimulation, across sham and tVNS sessions (Confidence interval does not overlap zero,
 258 see Figure 2B). Mean beta power was numerically lower in tVNS compared to sham sessions,
 259 contrary to our hypothesis. Accordingly, we found substantial evidence against H_1 ($t_{40} = -1.98$, $BF_{01} =$
 260 16.4). Furthermore, lateralization of beta power, i.e., power difference between left- and right-
 261 hemisphere sensors, was numerically lower in tVNS sessions, therefore, we found substantial
 262 evidence against H_4 ($t_{40} = -0.60$, $BF_{01} = 8.6$).

263

264



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

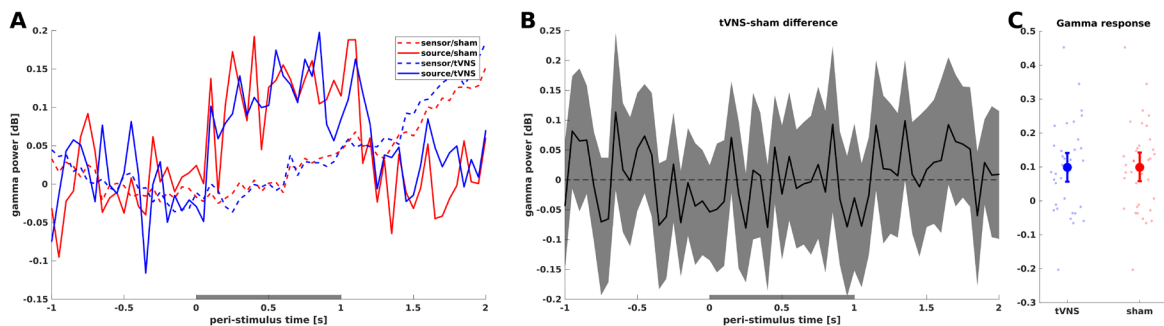
272 Mean PMBD across response hands was -0.37 dB in tVNS as well as sham sessions. We found
 273 substantial evidence against H_{2A} ($t_{37} = 0.24$, $BF_{01} = 6.8$). Furthermore, we found no effect of tVNS on
 274 PMBD lateralization, i.e., substantial evidence against H_{5A} ($t_{37} = -0.53$, $BF_{01}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

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

278

279

280



281 *Figure 4. A: Time course of gamma power around visual stimulation. Dashed lines: Power averaged*
 282 *across all sensors; solid lines: Power from virtual sensors in the primary visual cortex. Grey*
 283 *horizontal bar indicates time of stimulus presentation. B: tVNS-sham difference with bootstrapped*
 284 *95% CI. C: Subject-wise mean gamma response during stimulus presentation.*

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

287

288 Discussion

289 In this study, our goal was to better understand the cortical dynamics induced by tVNS. Even though
290 the neuromodulatory effects of VNS have been shown by a range of animal studies, especially with
291 respect to the locus coeruleus and NE transmission, and, to a lesser extent, inhibitory GABAergic
292 transmission, the human VNS literature has remained rather inconsistent. For instance, no robust
293 effect of tVNS on noninvasive markers of NEergic neuromodulation (e.g., pupil dilation; Keute et al.,
294 2019; Warren et al., 2019; Burger et al., 2020b; Sharon et al., 2021) and peripheral vagus-associated
295 activation (e.g., heart rate variability; Clancy et al., 2014; De Couck et al., 2017; Borges et al., 2019)
296 has been shown, even though the anatomical and physiological underpinnings of VNS would predict
297 such effects. In our study, we tested for effects of tVNS on oscillatory markers for cortical
298 GABAergic activity. We hypothesized that tVNS would impact resting beta power, movement-related
299 beta power deflections, and visual gamma responses. Furthermore, based on tentative evidence from
300 previous studies, we predicted the beta effects to be lateralized, i.e., stronger in the contralateral
301 hemisphere relative to the stimulated ear. Our data provide substantial evidence against all
302 hypotheses: we found that tVNS did not modulate the beta and gamma power markers, nor was there
303 a lateralized effect of tVNS.

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

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

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

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

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

341 It is currently one of the central challenges in VNS research to understand why treatment responses
342 are so variable between studies, subjects, and within subjects, and to identify short-term biomarkers
343 that allow for a reliable prediction of long-term treatment response and titration of stimulation
344 parameters. GABA-associated brain oscillations appeared to be a promising marker, especially
345 because of the GABAergic mediation of anti-epileptic VNS effects (Ben-Menachem et al., 1995;
346 Marrosu et al., 2003), but this prediction did not hold true. This is not to say, however, that readouts
347 from ongoing MEG or EEG are altogether unsuitable as VNS biomarkers. A growing number of
348 studies have shown behavioral, cognitive and neurological VNS effects, and it appears likely that
349 these effects are systematically reflected in altered brain activity patterns. This might require using
350 more involved methods, e.g., connectivity or network metrics, as some first studies have done to
351 predict long-term clinical outcomes of invasive VNS (Babajani-Feremi et al., 2018; Mithani et al.,
352 2019). It is important to note that in order to qualify as a predictive biomarker, a physiological readout
353 would not only have to be systematically changed by the stimulation, but the readout (or its change)
354 would also need to be reliably correlated to a clinical, physiological, or behavioral outcome of the
355 stimulation (Burger et al., 2020a; Keute et al., 2021). Furthermore, specific patterns of brain
356 oscillations in clinical populations will have to be taken into account, as they might interact with
357 oscillatory VNS markers (cf. Marrosu et al., 2005). Overall, we are confident that predictive markers
358 will also be identifiable for short-term tVNS, and we encourage the use of our data, which will be
359 made available for download, for further exploration.

360 Conflict of interest / Acknowledgements

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