**Non-invasive stimulation of vagal afferents reduces gastric frequency**

Supplementary Information

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# Methods

## 1.1 Participants and procedure

Both experimental days were scheduled back to back and participants arrived between 7:00 and 12:30 am for a 30-minute stimulation session (either taVNS or sham) on each day. Participants were instructed not to consume any calories for at least 4 hours and not to drink anything for 2 hours prior to the experiment. Moreover, they were not allowed to engage in strenuous physical activity 24 hours prior to the experiment. After providing written informed consent, participants were asked regarding their last meal and drink as well as their physical activity on the day of the measurement. We furthermore measured weight, height and waist and hip circumference. Participants were then asked to lie down on a bed. To measure the electrogastrogram (EGG), the skin above the abdomen was cleaned and six (four EGG electrodes, reference and ground electrode) standard electrocardiogram electrodes with solid gel (3M red dot) were applied according to the placement described in [[1]](https://paperpile.com/c/vm5tNh/agWm). The electrodes were connected to a BrainAmp DC (BrainProducts, Gilching, Germany) EEG recording system and electrodes were left in place for the second session. EGG was acquired at a sampling rate of 250 Hz with a low-pass filter of 80 Hz. An EGG non-invasively measures electrical activity reflecting the rhythmic contractions of the stomach [2]. This gastric rhythm is initiated by pacemaker cells (interstitial cells of Cajal) seated in the mid to upper corpus of the stomach [3] which receive inhibitory efferent vagal inputs via the NTS [2] and are connected to afferent vagal sensory neurons [2]. The pacemaker currents propagate to the rest of the stomach, ensuring that muscle cells in the stomach contract in an orchestrated manner for digestion [1]. This propagating wave is captured by electrodes that are placed over the epigastrium.

To assess resting energy expenditure (REE), we used the Vmax (CareFusion, San Diego, CA, USA) ventilated hood system for indirect calorimetry which measures the ratios of O2 and CO2 flowing in vs. out of the ventilated hood. During measurement, participants were asked to lie still on the bed and breathe normally.

For administering taVNS, we used Cerbomed NEMOS (Erlangen, Germany) following the protocol of [[4]](https://paperpile.com/c/vm5tNh/LxiX). The electrode was placed at the left cymba conchae (taVNS) or was turned upside down and placed at the earlobe (sham). Prior to each measurement, the stimulus intensity was individually adjusted based on subjective pain thresholds using concurrent VAS ratings [[5]](https://paperpile.com/c/vm5tNh/YQ4n). As recommended by the manufacturer, the intensity was increased from 0.1 mA in 0.1 mA increments until participants reported a “tingling” sensation (which was supposed to not be painful). Given this matching procedure, participants do not guess better than chance which stimulation condition they had received ([6], total of recorded guesses: 148, correct guesses: 79, accuracy: 53.4%, *p*binom = .18). The stimulation protocol of NEMOS is preset with a biphasic impulse frequency of 25 Hz with alternating intervals of 30 seconds stimulation on and 30 seconds off.

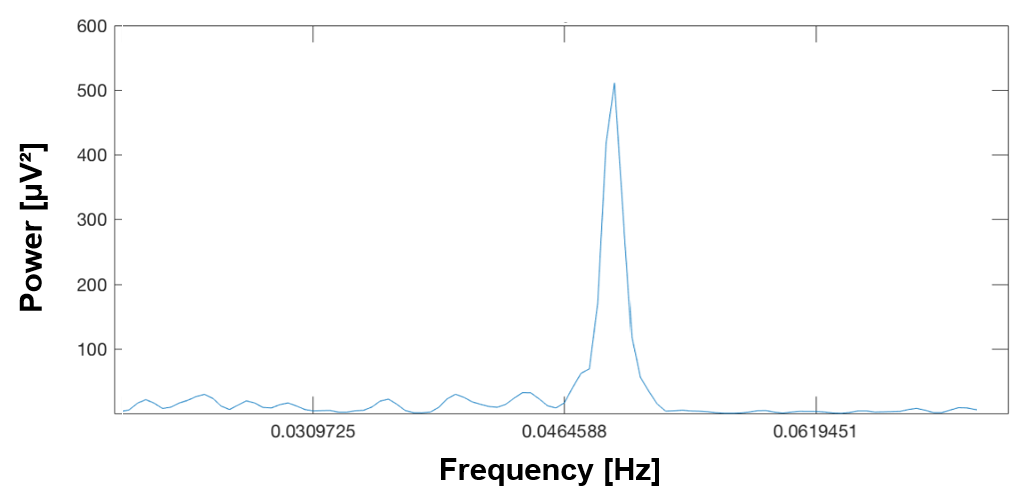
During the entire recording, we played an audiobook for the participants in order to keep them from falling asleep. After a minimum resting period of 15 minutes during which the indirect calorimetry device was calibrated, we started with a 15-minute baseline measurement for both EGG and calorimetry. Next, we placed the taVNS device on the participants’ left ear according to the randomization protocol and adjusted the stimulus intensity as described above. We then recorded at least 30 minutes of EGG and indirect calorimetry with active stimulation before the participant was debriefed. Markers for EGG were set manually and coded for the removal and respective re-application of the calorimetry hood during placement of the NEMOS earpiece, the start of the stimulation phase and any disturbances during the recording (such as loss of contact of the NEMOS earpiece, calorimetry device malfunction or excessive movement of the subject).

## 1.2 Data preprocessing and statistical analysis

Data from EGG was read into MATLAB R2017a (The Mathworks Inc., Natick, MA, USA) and processed using fieldtrip [[7]](https://paperpile.com/c/vm5tNh/wdYg) based on scripts released by Ref. [[1]](https://paperpile.com/c/vm5tNh/agWm)[[1]](#footnote-1). We cut the data according to the markers set during measurement to get two EGG time series, one for the baseline phase and one for the stimulation phase (taVNS or sham). Next, we low-pass filtered the data at 5 Hz to prevent aliasing, downsampled to 10 Hz and the mean was removed from the time series. For quality control, the time series were visually inspected for muscle artifacts. Affected segments were marked and only clean, continuous segments were kept for further analyses. To identify the gastric peak frequency for baseline, taVNS and sham, respectively, we used the fast fourier transform with a Hanning taper to calculate spectral density for each EGG channel. The peak and associated EGG channel were then identified via visual inspection based on sharpness and power within the frequency range of interest (0.033 - 0.066 Hz, *Table S1, see Figure S1 for a representative power spectrum of a single participants’ EGG recording*). For calorimetry data, we excluded the first 3 minutes after placing the hood to allow the system to reach equilibrium and extracted the measure of mean REE (in kcal/day) for statistical analysis (*Table S1*). For one participant, the REE baseline measurement for the first session (sham condition) was not available due to a technical error. This was compensated for by using the respective participants’ REE baseline measurement from the second session.

**Table S1.** Mean gastric peak frequency (in Hz) as well as mean resting energy expenditure (in kcal/day) and associated standard deviations for the two baseline measurements, taVNS and sham.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Baseline taVNS | Baseline sham | Stimulation taVNS | Stimulation sham |
| Gastric frequency [Hz ± SD] | 0.0484  ± 0.004 | 0.0488  ± 0.006 | 0.0471 ± 0.003 | 0.0498 ± 0.005 |
| Resting energy expenditure [kcal/day ± SD] | 1491.187 ± 367.087 | 1489.06 ± 339.95 | 1519.009 ± 312.488 | 1520.555 ± 333.533 |

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**Figure S1.** Exemplary power spectrum of an EGG recording in a single participant showing the typical gastric spectral signature in the frequency range between 0.03 and 0.06 Hz.

*Effects of order of the condition*

To test for effects of order of the condition on gastric frequency with this protocol, we computed a linear mixed effects model. The model was set up to predict delta gastric frequency (stimulation - baseline, in mHz) based on the stimulation condition (taVNS, sham), the order (taVNS first, sham first) and the interaction between stimulation condition and order. On the second level we modeled a random intercept and slope for the stimulation condition for each participant. We then tested for the Order × Stimulation condition interaction. We did not observe an effect of order of the stimulation condition on gastric frequency (mean [95% CI] Order × Stimulation condition: -1.95 mHz [-5.61, 1.71], t = -1.08, *p* = .287).

*Effects of stimulation intensity*

To test for effects of stimulation intensity on gastric frequency, we computed a separate linear mixed effects model. The model was set up to predict delta gastric frequency (stimulation - baseline, in mHz) based on the stimulation condition (taVNS, sham), the stimulation intensity (in mA) and the interaction between stimulation condition and stimulation intensity. On the second level we modeled a random intercept and slope for the stimulation condition for each participant. We then tested for the Stimulation intensity × Stimulation condition interaction. However, there was no significant effect of stimulation intensity on gastric frequency (mean [95% CI] Intensity × Stimulation condition: -2.59 mHz [-6.09, 0.9], t = -1.50, *p* = .141).

# Discussion

*Targets of taVNS: stimulation of vagal afferents versus efferents*

Currently, two possible routes are discussed that could mediate the taVNS-induced effects on efferent targets [18]. First, taVNS might directly affect efferent fibers eliciting downstream effects. The second option is an afferent-efferent feedback loop that is mediated by the brainstem. It is well documented that afferent fibers originating from internal organs make up 80% of the vagus nerve [8]. In particular, the auricular branch of the vagus nerve that is targeted by taVNS consists of somatosensory afferent fibers whose cell bodies are located in the jugular ganglion [8,9]. In contrast to afferents, the 20% efferent fibers originate from the brainstem and provide control over viscera and the heart [9]. Moreover, in line with neuroimaging results showing consistent activation of afferent vagal targets such as the nucleus tractus solitarii during transcutaneous vagal stimulation [4,10-12], works in humans on the physiological basis of VNS concluded that effective transcutaneous stimulation is primarily mediated by afferent Aβ axons [13,14]. Additionally, work in humans demonstrated far-field vagal somatosensory evoked potentials after transcutaneous vagal stimulation that are attributed to the activation of vagal sensory afferents [15,16]. Thus, the question of what stimulation protocol modulates which specific set of fibers is far from resolved. Nevertheless, to date, there is more evidence in favor of an afferent-efferent (vago-vagal) feedback loop mediated via the dorsal vagal complex [17,18].

*Effects of chronic versus acute stimulation on measures of energy homeostasis*

If taVNS is used as a therapy, study results point to an increasing effectiveness over time. This is hypothesized to be due to neuromodulatory long-term effects of taVNS. Such an increase with repeated administration has been reported for all pathologies where non-invasive VNS has been frequently applied such as depression [19,20], chronic migraine [21] and epilepsy [22,23]. Notably, regarding homeostatic control, extensive work in animals has shown a reduction in food intake and weight loss after chronic VNS [24–30]. The direction of these chronic VNS effects is in line with acute VNS effects on digestion as suggested by a closed-loop study in rodents [24]. Nevertheless, to better understand the effects of acute versus chronic stimulation on gastric motility, studies investigating changes in EGG parameters over longer periods of vagal stimulation are needed.

# Acknowledgements

Visualization for the graphical abstract was generated using R (https://www.r-project.org/) and gganatogram [31].

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