BRAIN
STIMULATION

Transcutaneous auricular vagus nerve stimulation influences gastric motility: A randomized, double-blind trial in healthy individuals

Kenan Steidel [a], Kristina Krause [a, b, d], Katja Menzler [a, b], Adam Strzelczyk [c, d, a],
Ilka Immisch [a], Sven Fuest [a], Iris Gorny [a], Peter Mross [a], Lukas Hakel [a], Laura Schmidt [a],
Lars Timmermann [a, b], Felix Rosenow [a, c, d], Sebastian Bauer [a, c, d, 1], Susanne Knake [a, b, d, *, 1]

[a] Epilepsy Center Hessen and Department of Neurology, Philipps-University Marburg, Marburg, Germany
[b] Center for Mind, Brain and Behavior, CMBB, Philipps-University Marburg, Marburg, Germany
[c] Epilepsy Center Frankfurt Rhine-Main and Department of Neurology, Goethe-University Frankfurt, Frankfurt am Main, Germany
[d] Center for Personalized Translational Epilepsy Research (CePTER), Goethe-University Frankfurt, Germany

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ABSTRACT

Background: Transcutaneous auricular vagus nerve stimulation (taVNS) has been investigated regarding its therapeutic properties in several several conditions such as epilepsy, migraine and major depressive disorder and was shown to access similar neural pathways as invasive vagus nerve stimulation. While the vagus nerve's role in gut motility is physiologically established, the effect of taVNS has scarcely been investigated in humans and yielded conflicting results. Real-time gastric magnetic resonance imaging (rtMRI) is an established reproducible method to investigate gastric motility non-invasively.
Objective: To investigate the influence of taVNS on gastric motility of healthy participants using rtMRI.
Methods: We conducted a randomized, double-blind study using high-frequency (HF) stimulation at 25Hz or low-frequency (LF) taVNS at 1Hz after ingestions of a standardized meal in 57 healthy participants. The gastric motility index (GMI) was determined by measuring the amplitude and velocity of the peristaltic waves using rtMRI.
Results: After HF taVNS, GMI was significantly higher than after LF stimulation (p = 0.005), which was mainly attributable to a higher amplitude of the peristaltic waves (p = 0.003).
Conclusion: We provide evidence that 4-h of taVNS influences gastric motility in healthy human participants for the first time using rtMRI. HF stimulation is associated with higher amplitudes of peristaltic waves in the gastric antrum compared to LF stimulation. Further studies are needed to investigate the effect of different frequencies of taVNS and its therapeutic properties in conditions with impaired gastric motility.

Introduction

Invasive electrical stimulation of the vagus nerve (iVNS) was established for epilepsy treatment in 1988 and has been investigated for several other therapeutic targets over the years. IVNS has been described to show mainly anticonvulsive [1] and antidepressant [2] properties as well as a positive effect on global cognition [3]. In recent years, transcutaneous auricular vagus nerve stimulation (taVNS) has been developed as a safe and non-invasive alternative [4]. The cymba conchae is known to be exclusively innervated by the auricular branch of the vagus nerve (ABVN) [5,6]. Furthermore, ABVN includes thick myelinated afferent fibres [7] that were specified as a necessity for the clinical effectiveness of this neuromodulatory approach [8] and that were shown to project to the nucleus tractus solitarii (NTS) in animal studies [9]. In a functional magnetic resonance (fMRI) imaging study on humans, Frangos et al. [10] demonstrated activation of the same neural pathway in taVNS as in iVNS. Although smooth muscle function in the gastrointestinal tract (GIT) is intrinsically regulated by pacemaker cells of the enteric nervous system (ENS) [11], a strong extrinsic influence via autonomous nerve fibres is generally accepted in the literature [12–14]. The lower third of the

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* Corresponding author. University Hospital Marburg, Philipps-Universität Marburg Dept. of Neurology Baldingerstr, D-35033, Marburg, Germany.
E-mail address: knake@med.uni-marburg.de (S. Knake).
[1] authors contributed equally.

oesophagus and stomach especially dispose strong dependency from centrally originated pathways [15]. These pathways comprise two individual circuits between the NTS and dorsal nucleus of the vagus nerve (DMV) in the lower brainstem that are mediated by the vagus nerve (VN) and exercise both an inhibitory (gastric inhibitory vagal motor circuit) and an excitatory (gastric excitatory vagal circuit) influence [16—18]. Furthermore, the VN's importance for gastric motility (GM) is underlined by its delay after vagotomy [19,20]. In a recent study with iVNS on rats, stimulation of the vagus nerve was shown to improve gastric emptying by relaxing the sphincter while simultaneously stimulating contraction in the gastric antrum [21]. Another animal study demonstrated normalisation of impaired GM after intraoperative iVNS [22]. Due to its safety, feasibility, and non-invasive nature, taVNS is an ideal device to be tested for its propulsive properties on the GIT.

Evidence in humans was provided in the transVaGa study by demonstrating the stimulating effect of taVNS on the gastric action potential amplitude as a potential treatment for postoperative ileus, assessed via intraoperative electromyography (EMG) [23]. Recent trials of taVNS in healthy human participants indicated an increased GM measured via ultrasonography [24] but also found reduced myoelectric frequency via electrogastrography (EGG) [25]. However, a study on patients with chronic pancreatitis could not find any influence of taVNS on GM [26].

In a study on patients with gastroparesis, Ajaj et al. [27] were able to demonstrate that real-time MRI (rtMRI) is a valid and reproducible method for he measurement of GM in healthy individuals and patients with gastroparesis. Moreover, in a successor study, it was shown that rtMRI reliably detects pharmacologically induced changes in GM [28]. Consequently, rtMRI emerged as a well reproducible method [29,30] that offered good congruence with gastric emptying scintigraphy as the gold standard [31]. The procedure was further utilised to detect gastropareses in diabetes mellitus [32,33] as well as in Parkinson's disease (PD) compared to healthy controls [34].

Evidence for the influence of taVNS on GM in humans was scarcely investigated until now, with studies generally containing small sample sizes and demonstrating conflicting results across different methods [24—26,35]. To our best knowledge, this subject has not been evaluated via rtMRI. We conducted a randomised, double-blind trial to investiagte the effect of high-frequency taVNS (HF, 25 Hz) versus low-frequency taVNS (LF, 1 Hz) on GM in healthy individuals using rtMRI.

Material and methods

Participants and study design

Participants between 18 and 65 years of age gave written informed consent before entering the study. They underwent a medical screening including electrocardiography (ECG) and exclusion of pregnancy. Exclusion criteria encompassed pregnancy, active implants, such as cochlea implants, pacemakers, iVNS, contraindications for MRI, anamnestic substance abuse, abnormal findings on routine ECG, chronic or acute disease of the nervous, cardiovascular, or gastrointestinal system, and impaired ability to consent. The trial was reviewed and approved by the internal review board of the Medical Faculty of Philipps-University Marburg, Germany and by the German regulatory authority (BfArM; EUDAMED CIV-16-03-015,044). The study was registered at ClinicalTrials.gov (NCT02359188). Participants underwent a randomised and double-blind stimulation protocol comparable to the one used by Bauer et al. [36] with either LF stimulation with 1 Hz (250 μs pulse width, biphasic with 30 s on/30 s off), assuming a subtherapeutic stimulation effect, or HF stimulation with 25 Hz (250 μs

pulse width, biphasic with 30 s on/30 s off) as an active treatment condition. The stimulation site was the left cymba conchae in both cases. The stimulated area was approximately 2 cm^2 using two titan electrodes with a length of 4.6 mm and a diameter of 2,34 mm seated on a gel frame, which was connected to a neurostimulation device via a wire. Stimulation was implemented using Nemos® taVNS stimulators (cerbomed, Erlangen, Germany), which was preprogrammed by the manufacturer to generate either of the aforementioned conditions, whereas every participant was stimulated with a single distinct device, which was prepared and tested by the manufacturer. Active control with LF taVNS was necessary to ensure blinding as it produces a tingling sensation on the participant's skin. All subjects and the study team were completely blinded. Unblinding was performed after the data acquisition was complete. According to clinical recommendations, total stimulation time was 4 h and all stimulations were initiated at 08:30 a.m. The stimulation current (SC) was calibrated in mA in three consecutive trials, using the ascending and descending method of limits [37]. The mean between pain and detection thresholds was implemented as SC. Participants were instructed to request adjustments in stimulation currents if the subjective stimulation intensity decreased or became painful during the study. The mean of all applied SCs was used for analysis.

Participants were instructed to fast overnight and received a standardised semisolid meal consisting of one scrambled egg, one bread roll with 10 g margarine, and 250 ml of water, wich was to be consumed within a period of 15 min. A second standardised meal was given 15 min prior to the MRI assessment, consisting of 10 g wheat bran suspended in 400 ml of orange juice [34]. After the first test meal at 08:30 a.m., the 4-h stimulation period with taVNS was performed in the Department of Neurology with subsequent transportation to the Department of Psychiatry, where the MRI measurements were conducted.

Magnetic resonance imaging

All participants were examined in supine position on the same 3T MRI scanner (Magnetom Trio; Siemens Medical Systems, Erlangen, Germany) with a gradient performance of 40 mT/m and slew rate of 200 T/m/s, after a mean period of 35 min post-ingestion of the test meal. A body-phased array coil with eight elements was wrapped around the participants' abdomen to ensure good gastric coverage. After acquiring localiser images to display the stomach, True FISP sequences (TR/TE/FA: 4.3 ms/2.15 ms/408) were obtained in transversal oblique and sagittal oblique planes orthogonal to the short axis of the stomach. The images were respiration-triggered by placing a navigator sequence on the right diaphragm. Each repetition was acquired one breathing cycle after the previous image. A total of 25 repetitions allowed imaging over a period ranging from 2 to 5 min, depending on the breathing frequency of the participant [34]. The gastric motility sequence was initiated approximately 1.5 h (mean = 97.5min; SD = 13.3min) after the end of the stimulation period.

Postprocessing and analysis

Qualitative and quantitative analysis was implemented using the picture archiving and communication system (PACS) IMPAX1 (Agfa HealthCare, Cologne, Germany). GMI was calculated for each participant as a measure of gastric motor function [34,38], using the following formula:

$$GMI[mm^2 \big/ s] = \frac{\Lambda X * \ \Lambda d}{\Lambda t}$$

ΛX describes the distance each peristaltic wave propagated during the time interval Λt. Adjustment of the time interval was used instead of a fixed time interval. Time intervals were calculated from onset to end for each peristaltic wave, utilising the entire time interval for which the peristaltic wave could be observed. The distance between the nadir of the peristaltic wave and a line connecting the gastric wall at the two endpoints of the peristaltic wave served as the baseline, from which the maximum inward deflection of the peristaltic wave (i.e., its amplitude), Λd, was calculated. Separate GMIs were obtained in the transversal oblique and sagittal oblique planes, which were subsequently used to calculate a mean GMI from three separately measured peristaltic waves. A wave frequency index was calculated by dividing the total number of observed waves by the time between the occurrence of the first and the final observable wave during the respective period of measurement. As the GMI did not include the frequency of peristaltic waves in this technique, we additionally measured this frequency as the mean number of waves generated per second on the gastric antrum.

Statistical analysis

Statistical analyses were performed using R [R-project (R Core Team, 2018)]. Normality assumptions were tested using the Shapiro-Wilks test [39]. Comparisons between participants in the HF and LF groups were performed using the Welch two sample *t*-test or the Mann-Whitney-U-test. Bonferroni correction [40] for multiple testing was applied the 4 MRI parameters: GMI, amplitude, velocity, and frequency. For correlation analyses, Pearson's r and Kendall's τ rank correlation coefficients were calculated [41]. Effect sizes were determined using Cohen's d [42]. Consolidated Standards of Reporting Trials (CONSORT) guidelines were followed.

Results

The final sample included 52 participants. 6 of the 63 screened participants dropped out due to scheduling problems, and 4 participants were excluded from analysis due to the absence of interpretable waveforms in the abdominal scans (see Fig. 1).

There was no significant difference in age between the LF (Mdn = 24 years) and HF (Mdn = 24 years) groups (p = 0.88). There was additionally no significant difference in BMI between the LF (Mdn = 22.3) and HF (Mdn = 23.1) groups (p = 0.79). The results of the Shapiro-Wilks test indicated normal distribution for all tested variables aside from SC (p = 0.001) (see Table 1).

Parametric and non-parametric testing revealed significantly higher means for amplitude (t = 3.09, p = 0.003) and GMI (t = 2.9, p = 0.005) and higher mean ranks for SC (W = 497.5, p = 0.003) in the HF condition (see Table 2 and Fig. 2). There were no significant group differences or non-significant trends towards altered velocity or frequency of peristaltic waves. Additionally, there was no difference between latency, which was defined as time passed between the end of stimulation and the beginning of MRI measurements. The results for difference in mean for amplitude (p = 0.013) and GMI (p = 0.022) remained significant after correction for multiple testing using Bonferroni correction. The values for Cohen's d resembled strong (GMI) and small to moderate (amplitude) effect sizes [43].

Correlation analysis for the entire sample displayed significant positive correlations for mean amplitude (r = 0.87, p < 0.001) and mean velocity (r = 0.42, p = 0.002), as the GMI was calculated from these values. Aside from this no other correlations were found. After correction for multiple testing using the Bonferroni-Holm method, only the correlation with mean amplitude remained significant (p < 0.001). Notably, significant correlations between SC

and GMI or amplitude were found in neither the entire sample (GMI, τ = −0.070, p = 0.476, uncorrected) (Amplitude, τ = −0.027, p = 0.780, uncorrected) nor in HF (GMI, τ = −0.133, p = 0.379, uncorrected) (Amplitude, τ = −0.107, p = 0.480, uncorrected) or LF (GMI, τ = −0.236, p = 0.087, uncorrected) (Amplitude, τ = −0.2, p = 0.146, uncorrected) subgroups.

Discussion

This study aimed to demonstrate the effect of taVNS of the ABVN on the GM of healthy participants by comparing clinically used HF stimulation at 25 Hz with LF stimulation at 1 Hz using rtMRI. We found a significantly higher GMI in the therapeutic HF group in comparison to the presumed subtherapeutic LF group. This effect on GMI was mainly caused by a significantly higher amplitude of peristaltic waves, whereas there was no difference in mean velocity. Interestingly, there was also no difference in the mean frequency of peristaltic waves.

Activation of efferent VN fibres via taVNS

The afferent activation of central vagal structures, such as NTS, is well established in the literature [4]. To alter GM, activation of the efferent brain stem nuclei and their fibres within the VN must be presumed, which a growing body of evidence suggests. Firstly, there are complex connections between NTS and DMV [44], whose interplay enables the adaptation of the DMV's output on sensory feedback, e.g., via vagovagal reflexes [18]. Indirect connections between the substantia nigra (SN), which is a part of the central vagal activation pattern [10,45], and the DMV via the hypothalamus [46], as well as a regulatory function of the SN via indirect dopaminergic signalling on the DMV and GM [47], have been demonstrated. Secondly, efferent vagal activity after taVNS in the form of altered gene expression [23] and altered GM [21] was observed in animal studies. Lastly, Hong et al. [47] found direct activation of the DMV via immunohistochemical detection of c-fos after taVNS and presumed indirect access within the framework of a cholinergic anti-inflammatory pathway. In summary, indirect activation of the DMV via the NTS with concomitant stimulation of efferent fibres within the VN as a physiological basis of altered GM after taVNS can be presumed.

Effect on gastric motility

As the main cause of a higher GMI in HF taVNS, we identified significantly higher amplitudes of peristaltic waves using rtMRI without any differences in velocity or frequency, therefore showing related modulatory effects of taVNS on GM as iVNS in an animal study [21]. Accordingly, Lu et al. [21] found an increased amplitude and velocity of peristaltic waves in the gastric antrum after iVNS with 10 Hz on rats using an extensive rtMRI protocol, while the frequency remained unchanged; therefore, they hypothesised that iVNS with these settings mainly activates excitatory branches. Concerning studies in humans, Frøkjaer et al. [24] found a positive trend towards deeper antral contractions after taVNS using sonography, which was also shown in a transVaGa study using an intraoperative gastral EMG [23] and elevated gastrin levels as a surrogate lab parameter. Accordingly, this effect seems to be reproducible among various studies and measurement techniques. The frequency of gastric contractions as a basic electric rhythm of gut smooth muscle activity within the GIT is primarily set by intrinsic pacemaker cells of the ENS, whereas contraction and propagation are mainly thought to be influenced by vagal efferents [48,49]; in light of the established circumstances, a missing influence on frequency seems plausible, although Frøkjaer et al. also
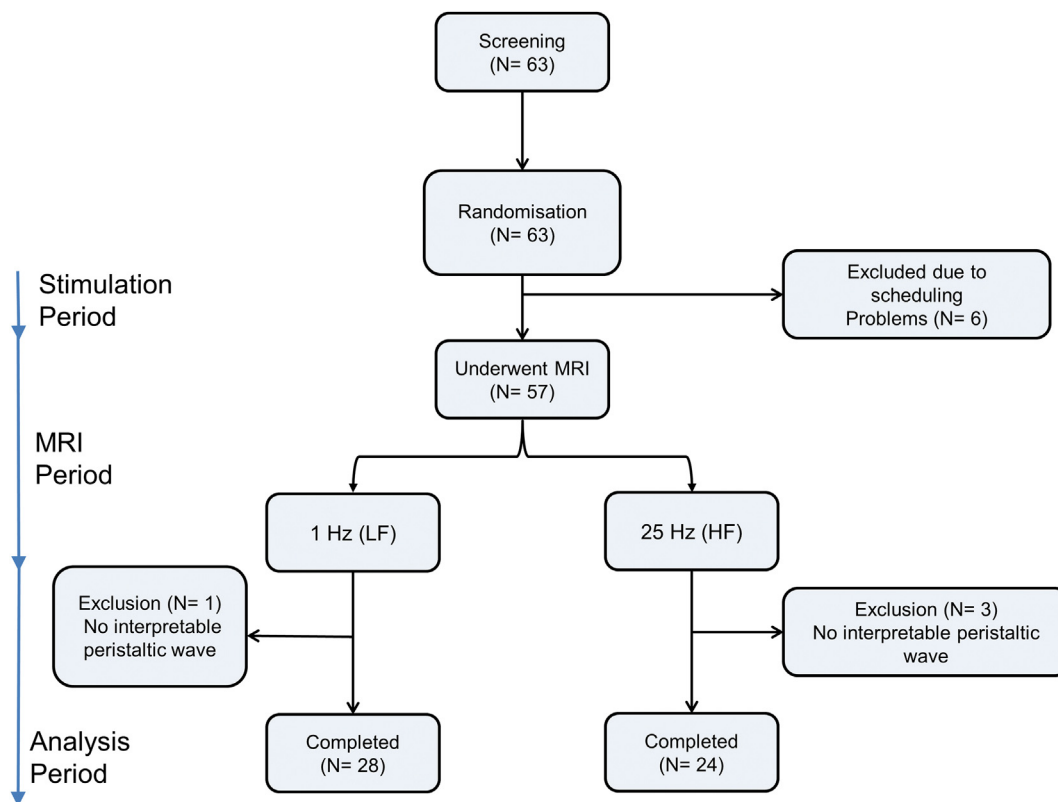
Fig. 1. The study diagram shows participants that underwent stimulation and magnetic resonance imaging (MRI) period, exclusions, and participants that were subsequently included in analysis.

observed a significant increase in antral contraction frequency [24]. The necessity of synchronisation and desynchronisation of the activity of pacemaker cells to provide proper smooth muscle contractability within the GIT was granted more attention in recent work [50,51], and the possible influence of taVNS on this synchronicity has been discussed previously [23]. Other recent taVNS studies, however, found a reduced myoelectric frequency using electrogastrography after taVNS [35] and no effect on patients with chronic pancreatitis [26]. There are several explanations for these conflicting results on GM. Firstly, different stimulation protocols were used, where the frequency varies from 10 Hz [23] to 25 [35] and 30 Hz [24,26], as well as different durations of stimulation that varied from 10 min [23] to two days [35], while measurements were performed mostly during, but also directly after, stimulation. Secondly, different methods of GM assessment with different parameters were used. In contrast to rtMRI, GMI assessment via ultrasonography does not involve velocity but frequency [52], whereas EGG was used to describe myoelectric frequency without direct visualisation of the actual contraction amplitude [35]. Notably, Teckentrup et al., who used the same stimulation frequency and biphasic intervals as in our HF group, did not assess the amplitude and velocity of peristaltic waves, which could have been altered despite the lower myoelectric frequency in the EGG. RTMRI, however, allows the visualisation of gastric amplitudes. Compared with ultrasonography, rtMRI is described as being less operator-dependent and more reliable in obese individuals [53]. While there are methods like scintigraphy that measure endpoints of GM, such as gastric emptying, these studies use methods that rely on single or multiple parameters, which limits their direct comparability [54]. Juel et al. already discussed probable causes of the missing effect on GM, such as concomitant analgesic treatment and chronic neural changes in patients with chronic pancreatitis [26]. Lastly, our study had a larger sample size than the aforementioned studies, in which the number of participants ranged from N = 18 to N = 22.

Aside from the fact that there were no differences in latency between the groups, correlation analysis showed no relationship between latency and GMI. Since there was a strong effect size for GMI more than 90 min after the end of the stimulation, we presumed a tonic effect on gastric motility, which was also found in the transVaGa study [23]. This further emphasizes the possibility of therapeutic use of taVNS, since wearing the device for a short

**Table 1**
Demographic variables.

	Total (mean ± SD)	1 Hz condition (mean ± SD)	25 Hz condition (mean ± SD)
Number of participants	N = 52	N = 28	N = 24
Age (in years)	25.5 ± 5.2	25.4 ± 4.8	25.6 ± 5.7
Gender	female: 33, male: 19	female: 18, male: 10	female: 15, male: 9
BMI	23.5 ± 3.1	23.4 ± 5.5	23.6 ± 6.0

Demographic characteristics (mean ± standard deviation) are summarised in the study population and stimulation groups. Abbreviations: BMI = body mass index.

Table 2
Gastric motility index and stimulation-related parameters.

	Stimulation (Hz)	Mean ± SD	Absolute difference (95%-CI)	p	Cohen's d
Amplitude [mm]	1	4.66 ± 2.21	2.0 (0.7−3.3)	**0.013***	0.41
	25	6.66 ± 2.42			
Velocity [$\frac{mm}{s}$]	1	2.18 ± 0.41	0.08 (−0.34 to −0.18)	1.0*	
	25	2.26 ± 0.51			
GMI [$\frac{mm^2}{s}$]	1	10.18 ± 5.46	4.68 (1.45−7.91)	**0.022***	0.82
	25	14.86 ± 6.03			
Frequency x10^{-2}[$\frac{1}{s}$]	1	5.58 ± 0.97	0.34 (−7.97 to 1.15)	0.558*	
	25	5.92 ± 0.66			
Stimulation current [mA]	1	0.66 ± 0.53	0,25 (0.1−0.45)	**0.003**	
	25	0.91 ± 0.43			
Latency [min]	1	95.18 ± 15.43	4.57 (−2.5 to 11.6)	0.202	
	25	99.75 ± 9.74			

Welch's *t*-test and Mann-Whitney-U-test results with 95% confidence intervals (95%-CI) and p-values of stimulation-related variables. Effect sizes of the significantly altered stimulation parameters were calculated by Cohen's d. Significant p values are indicated by bold formatting. * p-values after Bonferroni Correction. Abbreviations: GMI = gastric motility index.
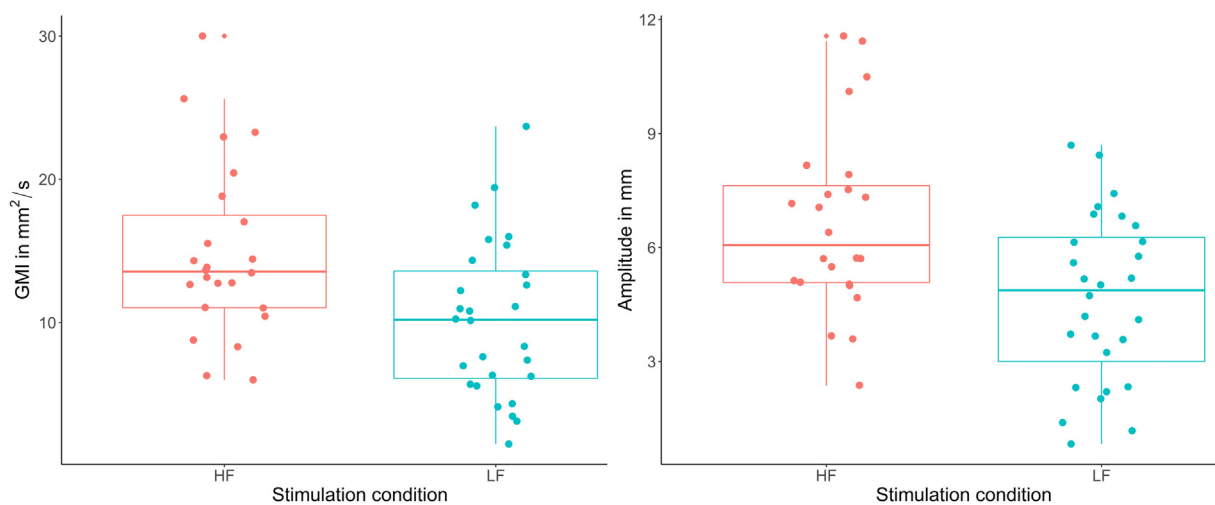
Fig. 2. Boxplots with median and quantiles and dots indicating individual measurements for mean GMI (p = 0.022, corrected) and mean amplitude of peristaltic contractions (p = 0.013, corrected).

period of time might induce a sustained effect. Although there was a rather small yet significant difference between groups, SC did not correlate with GMI or amplitude. Therefore, there is no indication that the intensity of the applied current influenced the measured MRI-parameters or was responsible for the higher GMI and mean amplitude in the HF group.

This study has a few limitations. We compared GMI after presumed therapeutic HF taVNS versus GMI in presumed subtherapeutic LF taVNS comparable to the protocol used by Bauer et al. [36]. The 25 Hz stimulation was considered as therapeutic based on its antiseizure properties. Accordingly, the efficacy of 1 Hz stimulation for treating chronic migraines was previously demonstrated [55]. Consequently, considering a missing baseline measure in our study, LF stimulation might have negatively altered GM and cannot be ruled out. Activation of distinct vagal efferent fibres at different stimulation frequencies has been discussed for iVNS [21]. Our assessment was limited to antric motility, whereas concomitantly reduced pyloric pressure was identified as an additional factor of improved GM after iVNS [21].

To our best knowledge this is the first study to use rtMRI to visualise the effects of taVNS on GM in healthy participants.

Additional studies are needed to further investigate the clinical relevance of taVNS treatment in patients with disturbed GM. This study demonstrated an effect of taVNS on the motility of the human gastric antrum, which resulted in a higher GMI for HF in comparison to LF stimulation.

Conclusions

TaVNS via the ABVN exercises an effect on GM in healthy human participants visualised by rt-MRI. We demonstrated a higher GMI, as a measure of antric motility, in HF taVNS with 25 Hz than in LF stimulation with 1 Hz as a presumed active control. While evidence regarding the effect on GM in humans is still controversial and varies from positive and negative to no effect, our study provides evidence that GM can be influenced by taVNS mainly via the strength of contractions in the gastric antrum, resulting in an altered amplitude of peristaltic waves. Altered GM was observed 90 min, on average, after stimulation, suggesting a sustained tonic effect. Further randomised trials in healthy participants, as well as studies in patients with conditions that impair GM, are needed to

precisely investigate the impact of different frequencies and potential therapeutic properties of chronic taVNS.

CRediT authorship contribution statement

Kenan Steidel: Investigation, Writing – original draft, Formal analysis. **Kristina Krause:** Formal analysis, Conceptualization. **Katja Menzler:** Writing – review & editing, Conceptualization. **Adam Strzelczyk:** Writing – review & editing. **Ilka Immisch:** Investigation. **Sven Fuest:** Writing – review & editing. **Iris Gorny:** Conceptualization. **Peter Mross:** Formal analysis, Writing – review & editing. **Lukas Hakel:** Investigation. **Laura Schmidt:** Investigation. **Lars Timmermann:** Writing – review & editing, Supervision. **Felix Rosenow:** Writing – review & editing. **Sebastian Bauer:** Conceptualization, Supervision, Writing – review & editing. **Susanne Knake:** Conceptualization, Supervision, Writing – review & editing, Project administration.

Declaration of competing interest

Declarations of interest: none concerning this topic.

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