Mass General Brigham

Institutional Review Board
Intervention/Interaction Detailed Protocol

Principal Investigator: Felipe Fregni

Project Title: Understanding the effects of non-invasive transauricular vagus nerve stimulation (taVNS) on neural networks and autonomic nervous system: a randomized double-blind sham-control mechanistic trial in healthy participants

Version Date: 08/01/2023

Version Name/Number: 2022P0023200

---

1. Background and Significance

Transauricular vagus nerve stimulation (taVNS) is a newer delivery system, using a non-invasive stimulation device placed at the concha or tragus of the ear, which does not require surgery; therefore it is more accessible and naturally less risky to patients(1). The taVNS field is growing exponentially in the last few years, and has been predominantly focused on clinical trials showing potential therapeutic benefits for various pathologic conditions, such as epilepsy, schizophrenia, migraine, Parkinson's disease, tinnitus, impaired glucose tolerance, and atrial fibrillation(2).

There is much to discuss about taVNS and its effects on biological processes in the central and peripheral systems. To study how taVNS impact physiological activity, ideally, a biological marker could provide information that the auricular nerve was truly stimulated, causing an effect on intracranial structures. However, without a reliable biomarker, we may not thoroughly learn from the taVNS results and may not have the best interpretation of its effects.

Electroencephalography (EEG) has proven to be a useful tool in understanding the mechanisms behind non-invasive brain stimulation and its effect on brain connectivity. EEG measurements are based on electrical potential differences between electrodes on the scalp. A potential difference is caused by the propagation of the current flow induced by synchronized postsynaptic potentials in pyramidal neuron cell membranes. These measurements allow for the evaluation of effective connectivity between different cortical and subcortical regions and provide high temporal and spatial resolution for the analysis of brain stimulation effects, such as those of Transcranial Magnetic Stimulation (TMS) and transcranial direct current stimulation (tDCS)(3, 4).

Based on that, EEG is seen as a promising imaging tool to provide biomarkers for taVNS. It is a reliable, non-invasive, and inexpensive method to measure and quantify cortical activity. Following through this thought process, our team of researchers has performed a systematic review, demonstrating preliminary information that taVNS could influence cortical activity, mainly increasing EEG power spectrum activity in lower frequencies (delta and theta). However, divergent results in higher frequencies (alpha), and in changes on early ERP components related to inhibitory tasks were also detected. A high heterogeneity between the studies was found; therefore future more homogeneous,

bigger, and well-designed studies are needed to make stronger conclusions about the effects of taVNS in brain activity measured by EEG(5).

Alpha asymmetry, which assesses the relative alpha band activity between the brain hemispheres, particularly at frontal electrodes, is a frequently researched EEG biomarker. Previous literature has used this biomarker to analyze the approach-withdraw hypothesis(6, 7), which holds that the right frontal side of the brain is associated with withdrawal behaviors while the left frontal side is associated with approach behaviors. Since alpha is associated with low brain activity, an increase in alpha on the left side of the brain suggests lower activity and may therefore suggest a lack of approach behavior. This concept helps to explain some results in the alpha band at the left side hemisphere in depressed patients(8).

Additionally, EEG electrodes record the activity of neuronal populations(9). Using this data, it is possible to examine the relationships between several brain regions and the brain's network structure and activity. Functional Connectivity (FC) is a general term used to describe connections between brain regions. It can be assessed in a variety of methods, such as utilizing coherence to denote synchronized activity between brain regions(10). Studies have shown that changes in the normality of large-scale brain networks, such as the default mode network (DMN) (11, 12) and affective network (AN)(13), or the dysconnectivity of some brain regions, such as corticolimbic pathways(14), are related with the psychiatric conditions, for example depression. So, FC has proven to be effective to investigate network dysfunction (15). Based on that, quantitative electroencephalography can be used to assess the effects of taVNS on brain activity; however, more studies are needed to systematically establish the specific effects and metrics that would reflect the non-invasive stimulation through the auricular branch of the vagus nerve.

Peripherally, the vagus nerve and sympathetic nervous system mediate the heart’s contractile and electrical function(16). This regulation promotes the heart’s pacemaker ability and controls it through physiological manifestations of the body, known as sinus depolarizations. Heart rate variability (HRV) records the changes per beat of sinus depolarization, thus, non-invasively describing vagal influences on the sinus node(17). Given this function, HRV has been seen and studied as a prominent biomarker for neurocardiac function, being associated with an individual’s well-being and the likelihood of morbidity, mortality, and stress(18, 19).

In the context of taVNS, HRV has been recorded in many trials, and stimulation has been thought to modulate HRV(16, 20, 21). Considering that a decreased HRV has been related to morbidity and mortality of different diseases through mechanisms such as over-activity of the sympathetic nervous system, inflammatory response, and oxidative stress, the vagal nerve modulation of taVNS is thought to directly impact HRV and its sinus regulation(22, 23). In fact, some trials have already conveyed that taVNS can increase HRV in healthy subjects, thus sustaining this hypothesis(20). However, there is still a lack of understanding on whether different taVNS parameters can affect HRV differently.

According to some studies(24, 25), taVNS may also influence nociception and pain perception, and that can lead to potential applications to a variety of painful illnesses, including headache, trigeminal allodynia, chronic pelvic pain, and fibromyalgia(26). The overlapping anatomical pathways between the nociceptive system and the central projections of the vagal afferents may be the cause of the potential mechanisms by which VNS modifies pain perception(27).

Regarding the vagal projections, some studies have demonstrated the vagal role in modulating pain pathways(25, 28-36), the inflammatory system and cortisol levels (37-40), and the gut microbiota (41-43). Several markers such as quantitative sensory testing (QST) (44-46), salivary cortisol (37, 47, 48), and salivary microbiota (49) have been used to measure pain and inflammatory systems, and the gut microbiome respectively. Although, there is still a need for more data to understand the mechanisms of taVNS on these systems.

Indeed, taVNS have been shown to be a safe, portable, and feasible tool (50, 51). Our group has demonstrated its safety through a systematic review and meta-analysis on the topic (Safety of transcutaneous auricular vagus nerve stimulation(taVNS): A systematic review and meta-analysis – peer review). Moreover, several studies have shown promising results of taVNS to treat various disorders such as depression, anxiety, Alzheimer's disease, headache, obesity, and diabetes(2, 52-55). However, no mechanistic studies have investigated the taVNS neural network and autonomic nervous system's effects of this technique. Therefore, we aim to assess how taVNS can affect EEG metrics and HRV and assess its safety. Also, we aim to evaluate predictors that can influence the response to taVNS, so understanding the variables associated with response to taVNS can help the design of future clinical trials to maximize the effects of this intervention.

2. Specific Aims and Objectives

Our goal is to perform an exploratory, mechanistic randomized double-blind sham-control trial in healthy participants, to assess the physiologic effects of a single session of bilateral taVNS, on neural networks and autonomic function.

The specific aims of this experiment are as follows:

Aim 1: We aim to compare the neurophysiologic effects of a single session of active taVNS versus sham taVNS in Frontal Alpha Asymmetry, Functional Connectivity and Phase Synchrony of DMN using EEG on a processing emotional facial expressions and a Go/NoGo task. We expect to find differences in face-related frontal alpha asymmetry between active taVNS versus sham taVNS in healthy controls. Also, regarding the DMN, we expect that taVNS decreases FC between DMN and other areas.

Aim 2: As a second aim, we will measure the effects of one active taVNS session versus sham in heart rate variability total power, very low frequencies (VLF), low frequencies (LF), and high frequencies (HF), and LF/HF ratio. We expect the taVNS to increase HF and decrease LF/HF ratio due to parasympathetic activation. Furthermore, the standard deviation of the R-R intervals (SDRR), the count of R-R intervals that differed more than 50 ms (NN50), the percentage of NN50 (pNN50), and the root mean square of the difference between successive R-R intervals (RMSSD) will be measured and we expect an increase on those metrics as well. We will also explore a non-linear method to calculate the short-term detrended fluctuation exponent (DFA-alpha1).

Additionally, we aim to assess the effects of taVNS in healthy subjects after one single session in some exploratory related outcomes.

3. General Description of Study Design

We will perform a randomized double-blind sham-control trial. We will randomize 44 healthy subjects, who will receive one single session of active taVNS or sham taVNS at the Neuromodulation Center/ Cambridge Spaulding Hospital.

	Consent and Screening	Baseline	Intervention	Post intervention
	Visit 1 (online)	Visit 2	Visit 2	Visit 2
Demographics	X			
Medical History	X			
Consent form	X			
Beck depression inventory	X			
EHI/SF	X			
Pregnancy test	X			
BMIS		X		X
VAS-F		X	X	X
EEG		X	X	X
HRV		X	X	X
QST		X		X
Side Effects Questionnaire for taVNS				X
Success of blinding questionnaire				X
Active taVNS			X	
Approximate visit time	60 min	60 min	60 min	30 min

Table 1: Visits Schema

4. Subject Selection

In this study, we will randomize 44 healthy subjects, and they will need to meet the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria:
1. Able to provide informed consent to participate in the study.
2. Subject is older than 18 years.
3. Subjects should be naive to the stimulation (taVNS)

Exclusion Criteria:
1. Pregnancy.
2. Subjects who have had a neuropsychiatric or a cardiac disorder diagnosis and have received treatment and chronic medication in the past six months, or who have functional deficits as a result.
3. History of alcohol or drug abuse within the past 6 months as self-reported.
4. Presence of the following contraindication to transauricular vagus nerve stimulation
    a. Ferromagnetic metal in the head and in the cranium (e.g., plates or pins, bullets, shrapnel)
    b. Implanted cranial electronic medical devices (e.g., cochlear implants)
    c. Implanted cardiac devices (e.g., pacemaker)
5. Unstable medical conditions (e.g. uncontrolled diabetes, uncompensated cardiac issues, heart failure or chronic obstructive pulmonary disease).
6. Uncontrolled epilepsy, as defined by previous clinical seizure in the past 3 months in patients with treatment for epilepsy.
7. Suffering from severe depression (as defined by a score of >30 in the Beck Depression Inventory).*

*If a subject screens out due to severe depression they will be provided with a local resource list (as recommended by Cheatle, 2014) (56). If the subject is presenting with suicidal ideation, the medical monitor will be contacted to provide further evaluation and instruction. Study participant will be escorted to the emergency room for assessment and triage if: spontaneous expression of suicidal ideation and/or BDI question 9 answers of 2 or 3.

The safety of taVNS in the pregnant population (and children) has not been assessed and therefore pregnant women (and children) will be excluded. Women of child-bearing potential will be required to take a urine pregnancy test during the screening process.

Potential subjects will be identified by the following sources:
1. Flyers posted in public areas across the Boston-land region.
2. Internet and newspaper advertisements.
3. Advertisements posted in public transportation (The T)
4. Via the Rally platform by Mass General Brigham Research.
5. Potential subjects might also be identified though their medical records (epic LMR, etc.)
6. Via the Partners Healthcare Research Patient Data Registry (RPDR) and Patient Gateway (Research Invitations allow for direct communication with any eligible subjects who have not opted out of receiving Research Invitations.)

Eligible subjects will contact, or give permission to be contacted, by a co-investigator to obtain more information about the study. At the first point of contact (usually a phone call or Zoom Enterprise call), a

study co-investigator will administer an online-prescreening questionnaire. Once the online pre-screening process is complete, the information gathered by the co-investigator will be taken to the PI of the study for further review to confirm eligibility. Data obtained from the pre-screening will be stored in an encrypted web-based platform (REDCap).

When contacting subjects by email, we will communicate through the Massachusetts General Brigham network as per the institutional policy regarding secure email communication using encryption by Mass General Brigham / Partners Healthcare "Send Secure" function. If the first method of contact with the subject is through email, we will explain that they will receive encrypted emails going forward. This initial unencrypted email will have instructions on how to open encrypted emails. We will also explain that they can opt out of secure emails after informing them of the possible security risks. If they choose this option, this preference will be noted in our contact log.

The online informed consent will be obtained by the study PI and/or a co-investigator using the REDCap platform approved by Massachusetts General Brigham network (MGB REDCap eConsent). The PI will be available if the subject wants to discuss the trial with them or have any questions. The study procedures will be described, and the equipment will be shown to the subject. Study co-investigators will clearly explain all the procedures and risks of the testing outlined in the consent form. The subject will be given the time needed to consider their decision and will be encouraged to ask questions, both during the initial online interview (phone or Zoom Enterprise call) and throughout the study. The PI or a co-investigator will answer any questions regarding the study at the time consent is given. Once enrolled, the subject may pause or terminate his/her participation at any time during the study.

5. Subject Enrollment

We will enroll up to 60 subjects to find about 44 subjects, that will be randomized (by web-based program randomization.com) into 2 Groups (allocation ratio 1:1): active taVNS group (active taVNS) or sham taVNS group (sham taVNS). Randomization order will be placed into sequentially numbered sealed envelopes by an otherwise uninvolved staff member. Allocation concealment will be maintained throughout the study. Blinding will be maintained for participants and co-investigators not performing taVNS, including outcome assessors.

All study procedures will be done at the Spaulding Neuromodulation Center/Spaulding Cambridge Hospital.

In case of non-English speakers, a translated version of a 'short form' consent document will be used to document the informed consent when a non-English speaking individual is unexpectedly encountered and a written translation of the Mass General Brigham IRB-approved consent form is not available. In that case the consent process will follow these procedures:

- The subject will be given a written translation of the 'short form' consent document in the language understandable to them to read and must have the opportunity to ask and receive answers to questions.
- The entire consent process will be witnessed by an individual who is fluent in both English and the language understandable to the subject. The interpreter may serve as the witness, if they are willing to do so, to the consent process (presentation of the information in the consent form in

the language understandable to the subject and the opportunity to ask and receive answers to questions)
- The Mass General Brigham IRB-approved English version of the consent form will be signed by the investigator obtaining informed consent and the witness to the consent process.
- The written translation of the 'short form' must be signed by the subject and the witness to the consent process.
- The translator present for the consent process will be from the MGB Interpreter Services

6. STUDY PROCEDURES

Study Outline

Pre-screening Procedures:
During the pre-screening process, the subject can contact a co-investigator, usually via encrypted Zoom Enterprise call, and REDCap form. During the pre-screening process, the co-investigator will discuss the details of the study, explain the study procedures, and encourage the subject to ask questions. In the privacy of the encrypted Zoom Enterprise call for healthcare, the co-investigator will ask the subject questions from the following:

1) Online Screening questionnaire
2) Phone Pre-screening questionnaire

Once this information is collected, the co-investigator will consult with the PI regarding the eligibility of the subject, who will then give the approval for the subject to come to our laboratory for the screening procedure.

Visit 1
Online or in-person Screening and Consent Visit – (Approximate Time: 1 hour)

If in-person, the visit 1 can be performed together with the visit 2 - (Approximate Time: 3 hours and 30 min).
If done remotely; the documentation would be signed electronically, and the visit would be done by telephone or using Zoom Healthcare)

During the Screening, the PI and/or a co-investigator will conduct once more a review of inclusion/exclusion criteria to determine the subject's eligibility for enrollment. If the consent is conducted by the co-investigator, the PI will review and sign off. Study procedures will be reviewed with the subject, and documentation of informed consent will be obtained. We will use an encrypted web-based platform (REDCap e-consent module) via Zoom call (Enterprise plan). Both services are already validated and in use by the MGB network.

- Discuss study-specific procedures with the subject.
- Review inclusion and exclusion criteria.
- Obtain a signed and dated consent form.
- Conduct a Demographics Survey, Brief Medical History, Beck depression inventory (BDI), and the Edinburg Handness Inventory Short form (EHI/SF).

Visit 2
Baseline Visit and First taVNS session – (Approximate Time: 2 hours and 30 min).

Urine pregnancy exam (if applicable).

Baseline assessments:

- EEG
- HRV
- QST
- The Brief Mood Introspection Scale (BMIS)
- Visual Analogue Scale to Evaluate Fatigue Severity (VAS-F) adaptation using tiredness item.

After the Baseline assessments:
Subjects will receive one session of either active taVNS or sham taVNS for 60 min. During the stimulation both groups will also be assessed with EEG, HRV, and VAS-F.

After the session, subjects will complete the following assessments:

- Side Effects Questionnaire for taVNS.
- Questionnaire to assess the success of blinding in clinical trials.
- EEG
- HRV
- QST
- BMIS
- VAS-F

Upon arrival and prior to the application, taVNS intensity will be measured for each participant separately, as above the detection threshold and below the pain threshold. The stimulation electrodes will be applied bilaterally to the ears, and in order to individually adjust the stimulation intensity, participants will receive increasing and decreasing series of 10-s stimulation trials and will rate the subjective sensation of the stimulation in a 10-point scale, ranging from nothing (0), light tingling (3), strong tingling (6), to painful (10). The increasing series of trials will start from an intensity of 0 mA and increase by 0.3 mA on a trial-by-trial basis until participants report it is uncomfortable to continue. This procedure will be repeated a second time. The final stimulation intensity for the experimental procedure will be calculated based on the average of the two intensities just below pain threshold.

We will use a Healaon Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) device (Neurive Inc., Gimhae, South Korea), which consists of an earset, with conductive eartips placed on the auricular concha of the ears. Electrodes are connected to a stimulator, and during active stimulation, both the cymba conchae of the auricular are stimulated at 30Hz, 200-250 us, with adjustable intensity for 60 min. The sham intervention will be done at the same location bilaterally to the cymba conchae of the auricular, however the device will be off during the 60 min session.

Data for this study will be collected from subjects using an electronic format capture system (REDCap), and each subject will be assigned a de-identified code to collect the data. The data entered will be securely transferred to the Mass General Brigham REDCap database, to which only IRB-trained and approved study staff will have access. The following assessments will be performed:

Demographic data: This survey will ask about demographic characteristics, including age, gender, sex, race, ethnicity, educational level, hand dominance, weight, height, and body mass index.

Medical history form: We will ask the history of medical conditions, a list of medications currently used, allergies, history of hospitalizations or visits to an emergency room in the last year, tobacco and alcohol consumption, and emergency and primary care physician contact.

BDI: This self-report inventory consists of 21 multiple-choice questions and is a widely used method to classify depression severity. It assesses the presence of several symptoms related to depression, such as irritability, hopelessness, and decreased cognitive performance. Physical symptoms such as weight loss and fatigue are also included.

EHI/SF: This short-form, self-report measure will be used to assess handedness in participants, asking their hand preference for 4 everyday tasks: writing, throwing, using a toothbrush, and using a spoon.

Eletroencephalogram: We will record the EEG using a 64-channel EGI system (Electrical Geodesics, Inc) (EGI, Eugene, USA) in resting and continuously during visual task stimulus presentation.
EEG phase synchronization of the DMN will be assessed in resting state with particular interest in the connectivity between mPFC to other brain regions in this network. EEG will be recorded using a high-density electrode array from two resting state conditions: eyes-open and eyes- closed. Oscillatory activity will be measured in the regional sources of interest that comprises nodes from the DMN, and phase synchrony between DMN regions of interest with reference to the medial prefrontal regions using phase-locking value (PLV). A data-driven statistical approach will be used to analyze the effects of taVNS on PLV. PLV, a measure of phase synchrony (i.e., the average of phase angle difference between two time series), can examine the synchronization in oscillatory activity between brain sources representative of the DMN (57). PLV can range from 0 to 1, with values close to 1 indicating strong synchronicity (i.e., perfect phase locking), while values close to 0 indicate substantial phase variation between the two signals, and thus low synchronicity between the two regions (i.e., no phase synchrony and randomly dispersed phases). To transform EEG data from scalp electrode space into source space, we will use a Matlab to model resting state activity representative of the DMN.
The DMN resting state montage comprised six regional sources of interest: medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), left and right Angular Gyru (AG), and left and right lateral temporal cortices (LTC). As the multiple discrete sources approach is applied to source montages (58), activity from other brain areas can affect source waveforms of the DMN sources if only these DMN sources of interest are included in the source montage. Therefore, six additional noise sources were also included in the montage to measure brain activity that were not part of the networks associated with resting state activity in the DMN and to increase the sensitivity of the sources of interest. These noise sources comprised left and right occipital sources, left, right, and midline frontal sources, and a middle parietal source. To measure PLV, a complex demodulation method with 0.5 Hz wide frequency bins and 100 ms time resolution in the range of 1 to 50 Hz is used for decomposing the single-trial EEG data into 2-second time–frequency representations of temporal spectral evolution (TSE, an equivalent measure of oscillatory power) normalized to the power spectrum per participant. PLV will be measured from the radial component of each source of interest relative to the mPFC source as the seed (i.e., between mPFC and each of the sources at the PCC, left/right LTC, and left/right AG).

Functional connectivity: Fourier transformation (STFT) will be applied to filter the data into five typically analyzed frequency bands, delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and gamma (30–80 Hz) bands, then we will apply the phase lag index (PLI) method to calculate the connectivity matrices and graph theory-based methods to measure the topology of brain networks across different frequency bands.

In this study, phase synchronization will be measured between all the pairs of channels by the Phase Length Index method, which is an asymmetry index that measures the distribution of phase differences (59, 60). Due to the instantaneous spread of current, the same sources collected by two electrodes are considered to cause a zero-lag phase difference, which is rejected by PLI. Therefore, PLI is less sensitive to the volume conduction effect, and it can reveal the true coupling strength between pairs of channels.
The value of PLI index varies between 0 and 1. A value of 0 indicates no coupling or coupling with a phase difference centered around 0 mod p, and a value of 1 indicates perfect phase synchronization between two signals at a constant lag except 0 or p.

Frontal alpha asymmetry (FAA): After transforming to average reference, the continuous EEG signal will be segmented (1000 ms after stimulus onset), Fast-Fourier will transform after the data had been weighted with a Hamming window that tapered the distal 5% of each epoch and averaged without overlap. As the result of the weighting functions in the windowing process, the ends of any 1000 ms-segment will be less weighted.
Power for theta (4-7 Hz), alpha-1 (8-10 Hz), and alpha-2 (11-13 Hz) frequency bands will be extracted separately for happy and sad face stimuli for the left (mean of F3, F5, and F7 electrodes) and right (mean of F4, F6, and F8 electrodes) frontal hemisphere for further statistical analysis.

Tasks and Stimuli

1) The task will consist of alternating trials showing a consecutive series of either sad or happy facial expressions randomized by sex. One trial consists of a series of five to eight faces of one emotion with a varied number of faces per emotion to maintain subjects' attention on the task. To further enhance attention, Participants will be instructed to press a button as they noticed a switch from one emotional expression to the other, i.e., switch from the happy to the sad or from the sad to the happy trial. After six trials, the participants will be asked, "To what extent were you able to emphasize with the faces?" followed by a short break of about 10 seconds until the next six trials are presented. Subjects will need to self-report to what extent they fell empathy towards the face stimuli on a Likert scale ranging from 1 (very much) to 4 (very little). There will be two long breaks of about 20 seconds. During this break and at the end of the task, participants will be also asked, "How do you feel?". Subjects will self-report how they themselves feel on a Likert scale ranging from 1 (very good) to 4 (very bad). Altogether, there will be 24 trials of sad and 24 trials of happy facial expressions, and 6 short and two long breaks (duration of face stimuli presentation: 1000 ms). We will use happy and sad emotional face stimuli from "The Karolinska Directed Emotional Faces" database (61), which have been evaluated on emotional content, intensity, and arousal.

2)The go/no-go task consists of randomly displayed visually cued tasks, including 150 go trials and 50 no-go trials. Each trial started with a white light presented centrally on a gray background for 1,500 ms, followed by a color cue that subtended 20°. Go trials are cued with a "long green" light presented centrally until the response button is pressed or for 300 ms if there is no response. No-go trials are cued with "short green (50 ms) + short red (100 ms)" lights which are displayed for 150 ms. Trials order is pseudo-random and permuted such that on 15% of trials, a no-go cue is shown after three, five, and seven go trials, and on 10% of trials, a no-go cue is displayed after two, four, and six go trials. Participants are instructed to focus on the white light and press the response button with their right hand as quickly as they could every time the go cue appeared and not to press the button when the no-go cue is shown (62).

Heart Rate Variability (HRV): HRV will be collected with a CorSense (EliteHRV, USA) device (an FDA cleared and validated device) from the third finger of the non-dominant hand following a standardized protocol. The measurements will be taken at baseline, during, and after stimulation. As both short- (5 min) and long- (24 hours) duration ECG are valid options for measuring HRV, the data will be stored in a web-based secure system. The recording will be performed for 5 min minutes as recommended in previous trials (63, 64). The HRV metrics will be: (1) total power ≤ 0.04 Hz; (2) very low frequencies ≤0.04 Hz (VLF), (3) low frequencies 0.04–0.15 Hz (LF), (4) high frequencies 0.15–0.4 Hz (HF), and (5) LF/HF ratio, as we performed in previous studies from our group (65, 66); (6) the standard deviation of the R-R intervals (SDRR); (7) the count of R-R intervals that differed more than 50 ms (NN50); (8) the percentage of NN50 (pNN50); and (9) the root mean square of the difference between successive R-R intervals (RMSSD) will be measured. Finally, since the HRV random fluctuations and fractal components, we will perform a non-linear method to calculate the short-term detrended fluctuation analysis (DFA-alpha1) (66-69).

Qualitative sensory testing (QST)
Conditioned Pain Modulation Test (CPM-test): We will follow the adapted protocol by Granot et al., 2008 and Nir et al., 2011(70, 71). We will first determine the pain-60 temperature by applying a thermode on the right forearm and delivering a short heat stimuli generated by a TSA-II Stimulator (Medoc Ltd) using the FDA approved safety temperature range of the device that goes up 48°C (118,4 degrees Fahrenheit), each lasting 7s. Subjects will be asked to rate the pain intensity with a numerical pain scale (NPS). Once determined, we will administer the test stimulus for 30s at that temperature and will be asked to rate their levels of pain at 10, 20 and 30s. For the conditioned stimulus, the left hand of the subject will be immersed in water at 10 to 12°C for 30s. Then, the same temperature will be applied on the right forearm (left hand will still immersed) at 10, 20 and 30s. CPM response will be calculated as the difference between the average of pain ratings from the test stimulus minus the conditioned stimulus.

Temporal Slow Pain Summation (TSPS): Heat pulses will be delivered to the right dominant proximal volar forearm using an appropriate size embedded HP-thermode. We will follow the adapted protocol suggested by Staud et al, 2014, in which the HP-thermode was programmed to deliver pulses rising/fall of 1-2-s, depending on subject's heat-evoked pain threshold, from adapting temperatures to peak temperatures, with a plateau of .7-s(72). Subjects will be trained to determine the temperature necessary to elicit pain-60 (see CPM protocol below). Subsequently, they will receive one train of 15 repetitive heat stimuli at 0.4 Hz to the same area, in which by being suitable for eliciting TSPS in most subjects, it allows the rating of individual pain stimuli and is unlikely to induce peripheral sensitization. TSPS will be calculated as the difference between heat pain rating after the 15th stimulus minus the 1st stimulus

Brief Mood Introspection Scale (BMIS): The BMIS scale is a mood scale consisting of 16 mood-adjectives to which a person responds (e.g., Are you "happy"?). The scale can yield measures of overall pleasant-unpleasant mood, arousal-calm mood, and it also can be scored according to positive-tired and negative-calm mood.Demographic data, medical comorbidities and adverse effects will be assessed using a standardized questionnaire.

Visual Analogue Scale to Evaluate Fatigue Severity (VAS-F): We will use an adaptation of the VAS-F assessing only the first question related to tiredness. The scale consists of 18 items relating to the subjective experience of fatigue. Each item asks respondents to place an "X," representing how they currently feel, along a visual analogue line that extends between two extremes (e.g., from "not at all tired" to "extremely tired"). In contrast to discrete, Likert-type scales, the VAS-F places fewer restrictions on the range of responses available to individuals.

Side Effects Questionnaire for taVNS: After the stimulation session, subjects will complete a questionnaire to evaluate potential adverse effects of taVNS on a 4-point scale (None, mild, moderate, and severe). They will be asked whether they have experienced any side effects in an open-ended manner and they will then be specifically asked about ear pain, headache, tingling, dizziness, skin redness, fatigue, prickling, pressure, itching, unpleasant feelings. If any side effects are reported, the degree of relatedness to the intervention will be assessed on a 5-point scale. This type of adverse events questionnaire has been suggested in our recent systematic review and meta-analysis on safety of taVNS and has been used similarly in all the tDCS studies of the group.

Questionnaire to assess the success of blinding in clinical trials: After the stimulation session, subjects will be asked to guess their treatment assignment, and they may be allowed to express treatment guess or uncertainty, i.e. subjects will be asked to guess their treatment assignment among active, sham or do not know. After that they will be asked how confident they are from 0 to 5 on their choice.

7. Risks and Discomforts

Transauricular vagus nerve stimulation (taVNS): it is a technique that poses a non-significant risk to subjects. The safety of taVNS has been addressed and tested by multiple researchers who have concluded that taVNS, as applied in a manner similar to our proposed protocol, induces only temporary mild effects. A review study in 2018 was the first to systematically report transcutaneous vagus nerve stimulation treatment safety(73). The author concluded that transcutaneous vagus nerve stimulation is well tolerated and safe, with only mild side effects(73). Recently, we have just conducted a systematic review and meta-analysis with 167 clinical trials involving 6,322 subjects, and we concluded that active taVNS and controls were not different in the risk of developing an AE and in the intensity of the events. In general, the incidence of AE was low, being ear pain, headache, and tingling the most frequent ones. No severe adverse event was shown to be caused by taVNS. We also found that more than half of the studies did not mention the presence or absence of AEs, and these studies did not differ from those that reported no AE or the presence of at least one AE regarding sex, age, study design, number of sessions, or parameters, such as stimulation intensity, pulse width, and frequency of the stimulation. Thus, we postulate that taVNS is a safe and feasible treatment option.

Electroencephalography (EEG): The EEG procedures are performed to measure the electrical activity in the brain and to examine the dynamic changes. It also allows for better understanding of the effects of electrical activity generated in different areas of the brain. EEG only measures brain activity and does not induce electrical current. These procedures are non-invasive and have been used extensively in clinical practice for diagnosis of neurological conditions such as epilepsy. Therefore, EEG poses no significant risk or anticipated discomfort.

Heart rate variability (HRV): As the EEG, the electrocardiogram (ECG) device is used to measure the electrical activity in the heart and its variability. ECG only records heart activity and does not deliver any electrical current. The procedure is non-invasive and has been used extensively in clinical practice for diagnosis of heart conditions. Therefore, ECG and the HRV recording poses no significant risk or discomfort to the subjects.

Quantitative sensory testing (QST)

Temporal slow pain summation (TSPS) and Conditioned Pain Modulation (CPM): Temporal slow pain summation (TSPS) is thought to represent summation of C fiber mediated pain and is being used to probe pain processing abnormalities in several chronic pain disorders(74, 75). In this protocol short duration noxious heat stimuli are delivered at 0.4 Hz with the destination temperature set to 44°C, 46°C, and 48°C.

Conditioned pain modulation (CPM) is a laboratory method to evaluate the individual capabilities to inhibit pain. This phenomenon is based on the fact that when a pain test stimulus is given together with a conditioning pain stimulus, the test stimulus is perceived as less painful than when it was given alone. In this protocol the conditioning pain stimulus will be performed by using a cold bath of water set to 12°C (76).

TSPS and CPM have no potential risks and are non-invasive. However, these tests assess pain, so a transitory level of discomfort is expected momentarily. These effects are brief and have no health repercussions. The subject can choose to stop this test at any time if they do not feel comfortable continuing.

Behavioral assessments/Questionnaires: All questionnaires and clinical evaluation scales will be administered in the privacy of a closed room. There are no potential risks posed to participants with respect to behavioral tasks and questionnaires. If participants become fatigued during testing or are uncomfortable answering any personal questions, they will be informed that they are allowed to take a break at any point during the experiment and that they may end their participation at any time.

8. Benefits

The results of this study will provide a ground for future studies and may benefit subsequent future subjects to the application of taVNS therapy.

9. Statistical Analysis

Sample size calculation:

We planned a sample size using the available preliminary data from previous mechanistic studies in taVNS and HRV, and transcranial direct current stimulation (tDCS) and EEG metrics. From our knowledge, there is no previous data on the effects of taVNS in FAA, synchrony of DMN, or functional connectivity. For this reason, we calculated the sample size for these outcomes based on an analogous intervention, the tDCS in the left dorsolateral prefrontal cortex. Based on that, we considered some scenarios based on our aims. We could detect a variety of scenarios and different magnitudes of sample sizes, and we decided to choose a more conservative approach taking in consideration the main outcome domains in EEG and HRV that we aim to test in our study with some the smallest effect sizes calculated (See Table 1). We assumed a type I error of 5% (alpha), a type II error of 20% (beta), and a power of 80%. Therefore, taking in consideration the most conservative scenario, we estimated a sample of 36 subjects. However, we expanded the sample in 20% to account for a conservative attrition rate and to increase power for secondary outcome analysis. Then, we will include a total sample size of 44 subjects, this will lead in 22 subjects each group.

Table 1. Sensitivity analysis for sample size calculation

Questions	Intervention	Previous studies	Study effect size (Cohen's d, 95% CI)	Needed sample size (power 80% and alpha 5%)	Final sample increasing by 20%
Aim 1: One active taVNS session effects on frontal alpha asymmetry (FAA), functional connectivity and phase synchrony of DMN using EEG on a processing emotional facial expressions and a Go/NoGo task.					
Effects in alpha asymmetry modulation	Left DLPFC tDCS	Liu 2022 (77)	F4-3: 1.03 (0.31 to 1.73)	30 subjects	36 subjects (18 each arm)
			F6-5: 0.94 (0.23 to 1.65)	36 subjects	44 subjects (22 each group)
Effects in gama band FC between the posterior cingulate cortex and r-IPL	Left DLPFC tDCS	Koizumi 2020 (78)	Pos-pre: 57.71 (43.16 to 72.24)	2 subjects	4 subjects (2 each group)
Aim 2: One active taVNS session effects in heart rate variability					
Effects in LF/HF ratio	taVNS	Vosseler 2020 (cross-over) (79)	-2.22 (-3.13 to -1.29)	8 subjects	10 subjects (5 each group)
Effects in HF	taVNS	Geng 2022 (cross-over) (20)	13.72 (9.93 to 17.49)	2 subjects	4 subjects (2 each group)
Effects in RMSSD	taVNS	Vosseler 2020 (cross-over) (79)	-1.54 (-2.35 to -0.71)	14 subjects	18 subjects (9 each group)
		Geng 2022 (cross-over) (20)	2.35 (1.36 to 3.32)	6 subjects	8 subjects (4 each group)
Effects in pRR50	taVNS	Geng 2022 (cross-over) (20)	1.53 (0.67 to 2.37)	14 subjects	18 subjects (9 each group)
Effects in SDRR	taVNS	Geng 2022 (cross-over) (20)	2.79 (1.72 to 3.83)	6 subjects	8 subjects (4 each group)

Effects in total power	taVNS	Geng 2022 (cross-over) (20)	1.01 (0.22 to 1.80)	32 subjects	38 subjects (19 each group)

AIM 1: Data forms and questionnaires will be coded in a standardized manner, and double-entered into our database. Digital measures/recordings will be similarly tracked in our database and regularly backed up. Analyses will be conducted using standard statistical software such as STATA and MATLAB toolboxes. All analyses will be performed on an intention-to-treat basis (using the method of multiple imputations for missing data). We will also perform an additional sensitivity analysis using completers only. The primary outcomes for this aim will be the EEG biomarkers (alpha asymmetry, functional connectivity, and phase synchrony of the default mode network). We will obtain all the measurements at baseline and during the stimulation period. Linear mixed models will be used to test for differences between the active and sham group, adjusting for covariates when necessary. Further exploratory analysis to detect predictors of response to taVNS will be performed taking in consideration the covariates assessed. Statistical significance will be considered if p-values < 0.05.

The between subjects' factor and measures on hemisphere (left and right), and emotion (happy and sad) will be performed for alpha-1, alpha-2, and theta. All the power will be ln-transformed. The significant main effects will be further analyzed with Cohen's d effect sizes and the interactions will be divided in simple effect and graphical interpretations.

In order to analyze phase synchrony of the default mode network, a non-parametric cluster-based permutation testing will be performed. A software identifies clusters in specific locations that are different regarding oscillatory amplitude and phase-locking within a frequency range between the groups. Power spectra and phase synchrony data-driven tests are also allowed by the software. The phase-locking value (PLV) from eyes open and close will be assessed between treatment groups. Then, we will perform a permutation testing with a Monte-Carlo resampling approach to localize the clusters higher than 95% of all clusters from random permutation of the data. For cluster building we will set an alpha of 0.01 and a number of permutations at 3000.

Functional connectivity will be analyzed by a software that can determine significant differences between the treatment groups. The network-based-statistic (NBS) identifies significant brain networks built by suprathreshold links, but not individual links. We will compute each test for each pairwise network using this threshold. The statistical analysis between treatment groups will be performed after taking the average of the matrices through the time windows. We will set a significant p-value < 0.05 and non-parametric permutation test in 5000.

AIM 2: We will have a similar statistical analysis plan as in Aim 1. The differences between the metrics during the after, during stimulation and baseline will be calculated. The HRV metrics will be summarized as means and standard deviations will be used to represent the variance. Linear mixed models will be used to test for differences between the active and sham group, adjusting for covariates when necessary. Further exploratory analysis to detect predictors of response to taVNS will be performed taking in consideration the covariates assessed. Statistical significance will be considered if p-values < 0.05.

[redacted]

[redacted]

[redacted]

12. References

1. Ben-Menachem E, Revesz D, Simon BJ, Silberstein S. Surgically implanted and non-invasive vagus nerve stimulation: a review of efficacy, safety and tolerability. Eur J Neurol. 2015;22(9):1260-8.
2. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. J Inflamm Res. 2018;11:203-13.
3. Fidalgo TM, Morales-Quezada JL, Muzy GS, Chiavetta NM, Mendonca ME, Santana MV, et al. Biological markers in noninvasive brain stimulation trials in major depressive disorder: a systematic review. J ect. 2014;30(1):47-61.
4. Leite J, Morales-Quezada L, Carvalho S, Thibaut A, Doruk D, Chen CF, et al. Surface EEG-Transcranial Direct Current Stimulation (tDCS) Closed-Loop System. Int J Neural Syst. 2017;27(6):1750026.
5. Gianlorenco ACL, de Melo PS, Marduy A, Kim AY, Kim CK, Choi H, et al. Electroencephalographic Patterns in taVNS: A Systematic Review. Biomedicines. 2022;10(9):2208.
6. Coan JA, Allen JJ. Frontal EEG asymmetry as a moderator and mediator of emotion. Biol Psychol. 2004;67(1-2):7-49.
7. Davidson RJ, editor Cerebral asymmetry, emotion, and affective style1995.
8. de Aguiar Neto FS, Rosa JLG. Depression biomarkers using non-invasive EEG: A review. Neuroscience & Biobehavioral Reviews. 2019;105:83-93.
9. Rao RPN. Brain-Computer Interfacing: An Introduction. Cambridge: Cambridge University Press; 2013.
10. Orgo L, Bachmann M, Kalev K, Jarvelaid M, Raik J, Hinrikus H. Resting EEG functional connectivity and graph theoretical measures for discrimination of depression. 2017 IEEE EMBS International Conference on Biomedical & Health Informatics (BHI). 2017:389-92.
11. Wu J, Zhang J, Liu C, Liu D, Ding X, Zhou C. Graph theoretical analysis of EEG functional connectivity during music perception. Brain Res. 2012;1483:71-81.
12. Zhu X, Wang X, Xiao J, Liao J, Zhong M, Wang W, et al. Evidence of a dissociation pattern in resting-state default mode network connectivity in first-episode, treatment-naive major depression patients. Biol Psychiatry. 2012;71(7):611-7.
13. Avery JA, Drevets WC, Moseman SE, Bodurka J, Barcalow JC, Simmons WK. Major depressive disorder is associated with abnormal interoceptive activity and functional connectivity in the insula. Biol Psychiatry. 2014;76(3):258-66.
14. Nugent AC, Farmer C, Evans JW, Snider SL, Banerjee D, Zarate CA, Jr. Multimodal imaging reveals a complex pattern of dysfunction in corticolimbic pathways in major depressive disorder. Hum Brain Mapp. 2019;40(13):3940-50.
15. Liu W, Zhang C, Wang X, Xu J, Chang Y, Ristaniemi T, et al. Functional connectivity of major depression disorder using ongoing EEG during music perception. Clinical Neurophysiology. 2020;131(10):2413-22.
16. Machetanz K, Berelidze L, Guggenberger R, Gharabaghi A. Brain-Heart Interaction During Transcutaneous Auricular Vagus Nerve Stimulation. Front Neurosci. 2021;15:632697.
17. Spyer KM. Annual review prize lecture. Central nervous mechanisms contributing to cardiovascular control. J Physiol. 1994;474(1):1-19.
18. Jarczok MN, Kleber ME, Koenig J, Loerbroks A, Herr RM, Hoffmann K, et al. Investigating the associations of self-rated health: heart rate variability is more strongly associated than inflammatory and other frequently used biomarkers in a cross sectional occupational sample. PLoS One. 2015;10(2):e0117196.
19. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health. 2017;5:258.
20. Geng D, Liu X, Wang Y, Wang J. The effect of transcutaneous auricular vagus nerve stimulation on HRV in healthy young people. PLoS One. 2022;17(2):e0263833.

[redacted]

21. Wolf V, Kühnel A, Teckentrup V, Koenig J, Kroemer NB. Does transcutaneous auricular vagus nerve stimulation affect vagally mediated heart rate variability? A living and interactive Bayesian meta-analysis. Psychophysiology. 2021;58(11):e13933.
22. Haensel A, Mills PJ, Nelesen RA, Ziegler MG, Dimsdale JE. The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. Psychoneuroendocrinology. 2008;33(10):1305-12.
23. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol. 2010;141(2):122-31.
24. Chakravarthy K, Chaudhry H, Williams K, Christo PJ. Review of the Uses of Vagal Nerve Stimulation in Chronic Pain Management. Curr Pain Headache Rep. 2015;19(12):54.
25. Randich A, Gebhart GF. Vagal afferent modulation of nociception. Brain Res Brain Res Rev. 1992;17(2):77-99.
26. Lange G, Janal MN, Maniker A, FitzGibbons J, Fobler M, Cook D, et al. Safety and Efficacy of Vagus Nerve Stimulation in Fibromyalgia: A Phase I/II Proof of Concept Trial. Pain Medicine. 2011;12(9):1406-13.
27. Dumoulin M, Liberati G, Mouraux A, Santos SF, El Tahry R. Transcutaneous auricular VNS applied to experimental pain: A paired behavioral and EEG study using thermonociceptive CO2 laser. PLoS One. 2021;16(7):e0254480.
28. Chien CH, Shieh JY, Ling EA, Tan CK, Wen CY. The composition and central projections of the internal auricular nerves of the dog. J Anat. 1996;189 (Pt 2)(Pt 2):349-62.
29. Manta S, El Mansari M, Debonnel G, Blier P. Electrophysiological and neurochemical effects of long-term vagus nerve stimulation on the rat monoaminergic systems. Int J Neuropsychopharmacol. 2013;16(2):459-70.
30. Napadow V, Sclocco R, Henderson LA. Brainstem neuroimaging of nociception and pain circuitries. Pain Rep. 2019;4(4):e745.
31. Nishikawa Y, Koyama N, Yoshida Y, Yokota T. Activation of ascending antinociceptive system by vagal afferent input as revealed in the nucleus ventralis posteromedialis. Brain Res. 1999;833(1):108-11.
32. Nomura S, Mizuno N. Central distribution of primary afferent fibers in the Arnold's nerve (the auricular branch of the vagus nerve): a transganglionic HRP study in the cat. Brain Res. 1984;292(2):199-205.
33. Raedt R, Clinckers R, Mollet L, Vonck K, El Tahry R, Wyckhuys T, et al. Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. J Neurochem. 2011;117(3):461-9.
34. Ren K, Randich A, Gebhart GF. Vagal afferent modulation of a nociceptive reflex in rats: involvement of spinal opioid and monoamine receptors. Brain Res. 1988;446(2):285-94.
35. Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res. 2006;1119(1):124-32.
36. Takeda M, Tanimoto T, Ojima K, Matsumoto S. Suppressive effect of vagal afferents on the activity of the trigeminal spinal neurons related to the jaw-opening reflex in rats: involvement of the endogenous opioid system. Brain Res Bull. 1998;47(1):49-56.
37. D'Agostini M, Burger AM, Franssen M, Claes N, Weymar M, von Leupoldt A, et al. Effects of transcutaneous auricular vagus nerve stimulation on reversal learning, tonic pupil size, salivary alpha-amylase, and cortisol. Psychophysiology. 2021;58(10):e13885.
38. De Herdt V, Puimege L, De Waele J, Raedt R, Wyckhuys T, El Tahry R, et al. Increased rat serum corticosterone suggests immunomodulation by stimulation of the vagal nerve. J Neuroimmunol. 2009;212(1-2):102-5.
39. Hosoi T, Okuma Y, Nomura Y. Electrical stimulation of afferent vagus nerve induces IL-1beta expression in the brain and activates HPA axis. Am J Physiol Regul Integr Comp Physiol. 2000;279(1):R141-7.
40. Lerman I, Hauger R, Sorkin L, Proudfoot J, Davis B, Huang A, et al. Noninvasive Transcutaneous Vagus Nerve Stimulation Decreases Whole Blood Culture-Derived Cytokines and Chemokines: A Randomized, Blinded, Healthy Control Pilot Trial. Neuromodulation. 2016;19(3):283-90.
41. Costantini TW, Bansal V, Peterson CY, Loomis WH, Putnam JG, Rankin F, et al. Efferent vagal nerve stimulation attenuates gut barrier injury after burn: modulation of intestinal occludin expression. J Trauma. 2010;68(6):1349-54; discussion 54-6.

42. Goehler LE, Gaykema RP, Opitz N, Reddaway R, Badr N, Lyte M. Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with Campylobacter jejuni. Brain Behav Immun. 2005;19(4):334-44.
43. Wu J, Yin Y, Qin M, Li K, Liu F, Zhou X, et al. Vagus Nerve Stimulation Protects Enterocyte Glycocalyx After Hemorrhagic Shock Via the Cholinergic Anti-Inflammatory Pathway. Shock. 2021;56(5):832-9.
44. Pavlaković G, Petzke F. The role of quantitative sensory testing in the evaluation of musculoskeletal pain conditions. Curr Rheumatol Rep. 2010;12(6):455-61.
45. Uddin Z, MacDermid JC. Quantitative Sensory Testing in Chronic Musculoskeletal Pain. Pain Medicine. 2016;17(9):1694-703.
46. Weaver KR, Griffioen MA, Klinedinst NJ, Galik E, Duarte AC, Colloca L, et al. Quantitative Sensory Testing Across Chronic Pain Conditions and Use in Special Populations. Front Pain Res (Lausanne). 2021;2:779068.
47. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34(2):163-71.
48. Tammayan M, Jantaratnotai N, Pachimsawat P. Differential responses of salivary cortisol, amylase, and chromogranin A to academic stress. PLOS ONE. 2021;16(8):e0256172.
49. Dinan TG, Cryan JF. The Microbiome-Gut-Brain Axis in Health and Disease. Gastroenterol Clin North Am. 2017;46(1):77-89.
50. Noé E, Ferri J, Colomer C, Moliner B, O'Valle M, Ugart P, et al. Feasibility, safety and efficacy of transauricular vagus nerve stimulation in a cohort of patients with disorders of consciousness. Brain Stimul. 2020;13(2):427-9.
51. Redgrave JN, Moore L, Oyekunle T, Ebrahim M, Falidas K, Snowdon N, et al. Transcutaneous Auricular Vagus Nerve Stimulation with Concurrent Upper Limb Repetitive Task Practice for Poststroke Motor Recovery: A Pilot Study. J Stroke Cerebrovasc Dis. 2018;27(7):1998-2005.
52. Rong P, Liu J, Wang L, Liu R, Fang J, Zhao J, et al. Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study. J Affect Disord. 2016;195:172-9.
53. Ylikoski J, Markkanen M, Pirvola U, Lehtimäki JA, Ylikoski M, Jing Z, et al. Stress and Tinnitus; Transcutaneous Auricular Vagal Nerve Stimulation Attenuates Tinnitus-Triggered Stress Reaction. Front Psychol. 2020;11:570196.
54. Zhang Y, Huang Y, Li H, Yan Z, Zhang Y, Liu X, et al. Transcutaneous auricular vagus nerve stimulation (taVNS) for migraine: an fMRI study. Reg Anesth Pain Med. 2021;46(2):145-50.
55. Huang F, Dong J, Kong J, Wang H, Meng H, Spaeth RB, et al. Erratum to: Effect of transcutaneous auricular vagus nerve stimulation on impaired glucose tolerance: a pilot randomized study. BMC Complement Altern Med. 2016;16(1):218.
56. Cheatle MD. Depression, chronic pain, and suicide by overdose: on the edge. Pain Med. 2011;12 Suppl 2(Suppl 2):S43-8.
57. Lachaux JP, Rodriguez E, Martinerie J, Varela FJ. Measuring phase synchrony in brain signals. Hum Brain Mapp. 1999;8(4):194-208.
58. Scherg M, Berg P, Nakasato N, Beniczky S. Taking the EEG Back Into the Brain: The Power of Multiple Discrete Sources. Front Neurol. 2019;10:855.
59. Stam CJ, Nolte G, Daffertshofer A. Phase lag index: assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources. Hum Brain Mapp. 2007;28(11):1178-93.
60. Vinck M, Oostenveld R, van Wingerden M, Battaglia F, Pennartz CM. An improved index of phase-synchronization for electrophysiological data in the presence of volume-conduction, noise and sample-size bias. Neuroimage. 2011;55(4):1548-65.
61. Garrido MV, Prada M. KDEF-PT: Valence, Emotional Intensity, Familiarity and Attractiveness Ratings of Angry, Neutral, and Happy Faces. Front Psychol. 2017;8:2181.
62. Han YL, Dai ZP, Ridwan MC, Lin PH, Zhou HL, Wang HF, et al. Connectivity of the Frontal Cortical Oscillatory Dynamics Underlying Inhibitory Control During a Go/No-Go Task as a Predictive Biomarker in Major Depression. Front Psychiatry. 2020;11:707.
63. Brunoni AR, Kemp AH, Dantas EM, Goulart AC, Nunes MA, Boggio PS, et al. Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs. electric current therapy to treat depression clinical study. Int J Neuropsychopharmacol. 2013;16(9):1937-49.

64. Nikolin S, Boonstra TW, Loo CK, Martin D. Combined effect of prefrontal transcranial direct current stimulation and a working memory task on heart rate variability. PLOS ONE. 2017;12(8):e0181833.
65. Morales-Quezada L, Cosmo C, Carvalho S, Leite J, Castillo-Saavedra L, Rozisky JR, et al. Cognitive effects and autonomic responses to transcranial pulsed current stimulation. Exp Brain Res. 2015;233(3):701-9.
66. Pacheco-Barrios K, Cardenas-Rojas A, de Melo PS, Marduy A, Gonzalez-Mego P, Castelo-Branco L, et al. Home-based transcranial direct current stimulation (tDCS) and motor imagery for phantom limb pain using statistical learning to predict treatment response: an open-label study protocol. Princ Pract Clin Res. 2021;7(4):8-22.
67. Costa MD, Davis RB, Goldberger AL. Heart Rate Fragmentation: A New Approach to the Analysis of Cardiac Interbeat Interval Dynamics. Front Physiol. 2017;8:255.
68. Goldberger AL, Amaral LA, Hausdorff JM, Ivanov P, Peng CK, Stanley HE. Fractal dynamics in physiology: alterations with disease and aging. Proc Natl Acad Sci U S A. 2002;99 Suppl 1(Suppl 1):2466-72.
69. Gronwald T, Rogers B, Hoos O. Fractal Correlation Properties of Heart Rate Variability: A New Biomarker for Intensity Distribution in Endurance Exercise and Training Prescription? Front Physiol. 2020;11:550572.
70. Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? Pain. 2008;136(1-2):142-9.
71. Nir RR, Granovsky Y, Yarnitsky D, Sprecher E, Granot M. A psychophysical study of endogenous analgesia: the role of the conditioning pain in the induction and magnitude of conditioned pain modulation. Eur J Pain. 2011;15(5):491-7.
72. Staud R, Weyl EE, Riley JL, 3rd, Fillingim RB. Slow temporal summation of pain for assessment of central pain sensitivity and clinical pain of fibromyalgia patients. PLoS One. 2014;9(2):e89086.
73. Redgrave J, Day D, Leung H, Laud PJ, Ali A, Lindert R, et al. Safety and tolerability of Transcutaneous Vagus Nerve stimulation in humans; a systematic review. Brain Stimul. 2018;11(6):1225-38.
74. Bosma RL, Mojarad EA, Leung L, Pukall C, Staud R, Stroman PW. FMRI of spinal and supra-spinal correlates of temporal pain summation in fibromyalgia patients. Human Brain Mapping. 2016;37(4):1349-60.
75. Craggs JG, Staud R, Robinson ME, Perlstein WM, Price DD. Effective connectivity among brain regions associated with slow temporal summation of C-fiber-evoked pain in fibromyalgia patients and healthy controls. J Pain. 2012;13(4):390-400.
76. Potvin S, Marchand S. Pain facilitation and pain inhibition during conditioned pain modulation in fibromyalgia and in healthy controls. Pain. 2016;157(8):1704-10.
77. Liu S, Zhai S, Guo D, Chen S, He Y, Ke Y, et al. Transcranial Direct Current Stimulation Over the Left Dorsolateral Prefrontal Cortex Reduced Attention Bias Toward Negative Facial Expression: A Pilot Study in Healthy Subjects. Front Neurosci. 2022;16:894798.
78. Koizumi K, Ueda K, Li Z, Nakao M. Effects of Transcranial Direct Current Stimulation on Brain Networks Related to Creative Thinking. Front Hum Neurosci. 2020;14:541052.
79. Vosseler A, Zhao D, Fritsche L, Lehmann R, Kantartzis K, Small DM, et al. No modulation of postprandial metabolism by transcutaneous auricular vagus nerve stimulation: a cross-over study in 15 healthy men. Sci Rep [Internet]. 2020 2020/11//; 10(1):[20466 p.]. Available from: https://europepmc.org/article/pmc/7686306.

[redacted]