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Title: Functional Near-Infrared Spectroscopy-Guided Frequency Mapping of Auricular Vagus Nerve Stimulation in Depression

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **no**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

Videographer: Please record the computer screen for the shots labeled as SCREEN. The screen should be in focus and the fonts/traces should be clearly visible

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 18

Number of Shots: 45 (15 SC)

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

We have chosen the best 5 answers.

INTRODUCTION:

NOTE: Authors changed all interview answers on spot, their answers are given in red font

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Felix Bernhard:** We investigate how different taVNS frequencies modulate brain networks in individuals with major depressive disorder.

Our research aims to understand how non-invasive vagus nerve stimulation influences brain network function in depression. By combining taVNS with functional near-infrared spectroscopy, we seek to identify objective neurophysiological and neuro-anatomical biomarkers that allow individualized optimization of stimulation parameters. ~~In the long term, we hope this work will contribute to precision neuromodulation, enabling treatments to be tailored to each patient's unique brain network profile.~~

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Felix Bernhard:** Existing taVNS studies use heterogeneous stimulation protocols by using the same frequency in multiple approaches of medical intervention: e.g. epilepsy and depression are commonly stimulated by 25 Hertz- disease independent. Our protocol enables systematic within-subject frequency comparison to explore stimulation effects by different stimulation frequencies dependent on disease and individual to optimize treatment effects .

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Felix Bernhard** This protocol measures frequency-specific brain network responses to taVNS using concurrent fNIRS recordings.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Felix Bernhard:** Reliable stimulation calibration and rigorous fNIRS quality control are critical for meaningful network analysis. **Therefore well established protocols , especially for row-signal-analysis need to be established to address neuronal networks and their changes due to neurostimulation.**
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Felix Bernhard:** **In our approach, we aim to see psychiatric pathologies as neuronal-network disorders, whose dysfunction needs to be addressed and “re-wired”. Future studies can evaluate whether these short term effects of “re-wiring” these dysfunctional networks – frequency- based improves symptoms as well and have to verify long-term clinical and neurophysiological outcomes – as well as network dependent, as symptom-dependent.**
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. **Felix Bernhard, Principal Investigator, University of Marburg**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Felix Bernhard, Principal Investigator, University of Marburg**: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Authors: Could you please also deliver the above statements in German?

Videographer: Please film the testimonials in both English and German.

Ethics Title Card

This research has been approved by the Human Research Ethics Committee of Philipps University Marburg

Authors: We need to keep the ethics statement same as that in the manuscript.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Participant Screening and Experimental Setup

Demonstrator: Felix Bernhard

2.1. Begin by recruiting adult participants with major depressive disorder and healthy controls according to the study criteria. After obtaining written informed consent [1], perform the baseline clinical assessment by administering the standardized questionnaires and recording the total score for each instrument [2-TXT].

2.1.1. WIDE: Talent explaining the procedure to the participant with some gesturing. ***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Talent handing over the questionnaire notepad to the participant and participant writing the responses. **TXT: Hamilton Depression Rating Scale (HAMD-24); Beck Depression Inventory–II (BDI-II)**

2.2. Seat the participant in a quiet, dimly lit room [1]. Position a monitor with a fixation cross approximately 1 meter in front of the participant [2]. Instruct the participant to remain awake and to fixate on the cross throughout all recordings [3].

2.2.1. Talent seating the participant in a quiet, dimly lit room.

2.2.2. Talent positioning the monitor with a fixation cross approximately 1 meter in front of the participant.

2.2.3. Talent instructing the participant to remain awake and fixate on the cross by pointing to it.

3. taVNS Preparation and Configuration

3.1. Prepare a transcutaneous auricular vagus nerve stimulation or taVNS device with a titanium-iridium stimulation electrode [1]. Place the electrode on the left cymba conchae of the ear [2]. Adjust the electrode to ensure stability and adequate skin contact [3].

3.1.1. Talent preparing the taVNS device with the titanium–iridium stimulation electrode.

3.1.2. Talent placing the electrode on the left cymba conchae of the ear.

3.1.3. Talent adjusting the electrode to ensure stability and adequate skin contact.

3.2. For the sham configuration, adjust the device settings to not deliver any electrical current [1].

3.2.1. SCREEN: Configuring the device to deliver no electrical current for the sham condition or setting the current parameter to 0.

3.3. For current amplitude calibration, set the pulse width to 300 microseconds [1]. Enable continuous stimulation without duty cycling [2]. Gradually increase the stimulation current [3].

3.3.1. SCREEN: the pulse width setting being set to 300 microseconds. ***Videographer: Please record the computer screen for the shots labeled as SCREEN as back up. The screen should be in focus and the fonts should be clearly visible***

3.3.2. SCREEN: continuous stimulation being enabled without duty cycling.

3.3.3. SCREEN: Inputting settings for gradual increase in the stimulation current.

3.4. Calibrate the stimulation current separately for 2, 10, 25, and 40 hertz [1]. Record the selected current amplitude for each setting, and repeat the calibration before every stimulation block [2]. Insert a 2-minute inter-condition interval without stimulation between consecutive blocks [3]. Configure the device to apply taVNS once per condition within the session [4].

3.4.1. Talent adjusting the settings for next calibration.

3.4.2. Talent recording the final current amplitude selected for each frequency in a book or on the computer.

3.4.3. SCREEN: Setting the 2-minute inter-condition interval without stimulation.

3.4.4. SCREEN: Configuring the taVNS settings and then **showing the complete panel of settings.**

4. taVNS Stimulation Procedure

4.1. Remind the participant to maintain gaze on the centrally presented fixation cross throughout all resting-state recordings and inform them that no response is required

during stimulation or resting-state periods [1].

4.1.1. Talent instructing the participant to fixate on the centrally presented fixation cross by gesturing and signaling not to give any response.

4.2. After placing the headcap on the participant [1], begin the stimulation block procedure and conduct the experiment in a single session [2].

Added shot 4.1.2: Talent puts the cap on participants' heads.

4.2.1. Talent starting the single experimental session.

4.3. After the initial resting-state baseline and the 2-minute inter-condition interval [1], apply the sham condition for 6 minutes [2]. Inform the participant that stimulation is being applied [3]. Match all tactile, visual, and auditory cues to active stimulation conditions [4].

4.3.1. SCREEN: recording during the resting state. **Videographer's NOTE: 4.3.1 file number is 9597. (The audio slate is false)**

4.3.2. SCREEN: show the acquisition data during the sham condition.

4.3.3. Talent informs the participant that stimulation is being applied.

4.3.4. Talent giving some visual and auditory cues to the participant.

4.4. Perform perceptual checks between blocks to ensure that sham and active conditions remain indistinguishable to the participant [1]. Then, apply the four active stimulation frequencies of 2, 10, 25, and 40 hertz for 6 minutes each according to the single-blind randomization sequence. Avoid extended washout periods [2].

4.4.1. Talent questioning the participant and reviewing the data on the monitor.

4.4.2. SCREEN: applying the active stimulation frequencies according to the single-blind randomization sequence.

4.5. Monitor stimulation delivery and electrode contact continuously during each block [1].

4.5.1. Talent looking at the monitor and pointing to the electrode contact during a block.

4.6. Stop the increase at the highest tolerable level without pain. Target a subjective intensity of 7 to 8 on a 10-point visual analog scale [1].

4.6.1. Talent stopping the stimulation current increase after the participant gestures

pain.

5. fNIRS System Setup

Demonstrator: Svenja Francke

5.1. Prepare two synchronized functional near-infrared spectroscopy or fNIRS (*F-N-I-R-S*) systems [1]. Ensure that each system is equipped with 16 light sources and 16 detectors [2]. Use an optical layout planning tool to define the optode montage for default mode network regions based on a standardized anatomical atlas [3-TXT].

5.1.1. Talent preparing two synchronized fNIRS systems.

5.1.2. Talent pointing to the 16 light sources and 16 detectors.

5.1.3. SCREEN: the optode montage being defined in the optical layout planning tool.
TXT: fNIRS Optodes' Location Decider (fOLD) toolbox

5.2. Position optodes to cover the bilateral precuneus and posterior cingulate cortex, medial prefrontal cortex, bilateral angular gyri, and medial temporal regions [1]. Configure long-separation channels using a 3-centimeter source-detector distance [2]. Add short-separation channels of approximately 8 millimeters for extracerebral signal regression [3].

Videographer's NOTE: 5.2.1, 5.2.2 and 5.2.3 are in one clip. the file number is 9580
(The audio slate is false)

5.2.1. Talent positioning optodes to cover the specified cortical regions.

5.2.2. Talent configuring long-separation channels with a 3-centimeter source–detector distance.

5.2.3. Talent adding short-separation channels for extracerebral signal regression.

5.3. Configure the systems to acquire dual-wavelength optical signals at 760 and 850 nanometers and set the sampling frequency to 5.1 hertz [1] ~~and synchronize the two fNIRS systems before each recording run [3].~~

5.3.1. SCREEN: adjusting settings to acquire dual-wavelength optical signals at 760 nanometers and 850 nanometers. **Videographer's NOTE:** Use 5.1.3 for 5.3.1 as well

~~5.3.2. SCREEN: the sampling frequency being set to 5.1 hertz.~~

~~5.3.3. SCREEN: synchronizing the two fNIRS systems before a recording run.~~

Videographer's NOTE: Delete the shot 5.3.2 and 5.3.3

6. fNIRS Data Acquisition

6.1. Begin acquiring the fNIRS data continuously throughout the session [1]. Record an initial resting-state baseline for 6 minutes without stimulation [2].

6.1.1. Talent starting the fNIRS data acquisition and data appearing on the monitor.

6.1.2. SCREEN: Show the initial resting-state baseline without stimulation.

6.2. During the experimental blocks, use the control software synchronized with the fNIRS acquisition system to manage block timing and randomization [1 and 2]. Continue fNIRS acquisition throughout the sham condition, active stimulation blocks, and all inter-condition intervals [3].

6.2.1. SCREEN: Show block timing and randomization settings.

6.2.2. LAB MEDIA: Figure 1A *Video editor: Please show 6.2.1 and 6.2.2 using split screen. Can split horizontally if that fits better. Please highlight the 4 orange stimulation boxes*

6.2.3. SCREEN: fNIRS live traces during the experimental blocks. **Videographer's NOTE:** Use 6.1.2 for 6.2.3 as well

6.3. After the final stimulation block, record a post-stimulation resting state for 6 minutes without stimulation [1].

6.3.1. SCREEN: recording the post-stimulation resting state without stimulation.

Results

7. Results

- 7.1. After preprocessing and network analysis of the fNIRS recordings, condition-dependent variations in default mode network organization were observed in a representative participant with major depressive disorder [1]. These variations were evaluated relative to the healthy control reference template across all stimulation conditions [2].
 - 7.1.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the 6 connectivity maps labeled D11: RSO0, D11 Sham, D11 2HZ, D11 10HZ, D11 25 HZ, D11 40HZ that are present at the outer circle of the grid.*
 - 7.1.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the HC template map.*
- 7.2. Distance measures quantified differences between each condition and the healthy control template across connectivity [1], eigenvalue [2], eigenvector [3], and average controllability metrics [4].
 - 7.2.1. LAB MEDIA: Figure 5B. *Video editor: Show the connectivity distance graph.*
 - 7.2.2. LAB MEDIA: Figure 5B. *Video editor: show the eigenvalue graph*
 - 7.2.3. LAB MEDIA: Figure 5B. *Video editor: show the Eigenvector Distance graph*
 - 7.2.4. LAB MEDIA: Figure 5B. *Video editor: show the Average Controllability Distance graph*
- 7.3. Conditions were ranked according to their proximity to the healthy control template, with lower distance values indicating closer similarity [1].
 - 7.3.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the rank labels displayed above the bars.*
- 7.4. The ranking of conditions differed across the distance metrics, indicating that each metric captured complementary aspects of the network representation [1].
 - 7.4.1. LAB MEDIA: Figure 5B. *Video editor: highlight the “rank: 1” labeled bars in different panels.*

7.5. Scores from the four metrics were aggregated within each participant to generate a consensus ranking across stimulation conditions [1]. The condition with the lowest combined score was selected as the representative stimulation frequency [2].

7.5.1. LAB MEDIA: Figure 5C.

7.5.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the bar enclosed by the red box and labeled as F opt.*

1. Major depressive disorder
Pronunciation link: <https://www.merriam-webster.com/dictionary/major%20depressive%20disorder>
IPA: /'meɪdʒər dɪ'presɪv dɪs'ɔrdər/
Phonetic Spelling: may·jer dih·press·iv dih·sor·der
2. Hamilton Depression Rating Scale
Pronunciation link: <https://www.merriam-webster.com/dictionary/Hamilton>
IPA: /'hæməltən dɪ'preʃən 'reɪtɪŋ skeɪl/
Phonetic Spelling: ham·uhl·tuhn dih·presh·uhn ray·ting skayl
3. Beck Depression Inventory
Pronunciation link: <https://www.merriam-webster.com/dictionary/inventory>
IPA: /bɛk dɪ'preʃən 'ɪnvən'tɔri/
Phonetic Spelling: bek dih·presh·uhn in·vuhn·tor·ee
4. Fixation
Pronunciation link: <https://www.merriam-webster.com/dictionary/fixation>
IPA: /fɪk'seɪʃən/
Phonetic Spelling: fik·say·shuhn
5. Transcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcutaneous>
IPA: /,trænz'kju:'teɪniəs/
Phonetic Spelling: tranz·kyoo·tay·nee·uhs
6. Auricular
Pronunciation link: <https://www.merriam-webster.com/dictionary/auricular>
IPA: /ɔ' rɪkjələr/
Phonetic Spelling: aw·rik·yuh·ler
7. Vagus
Pronunciation link: <https://www.merriam-webster.com/dictionary/vagus>
IPA: /'veɪɡəs/
Phonetic Spelling: vay·guhs
8. Stimulation
Pronunciation link: <https://www.merriam-webster.com/dictionary/stimulation>
IPA: /,stɪmjə'leɪʃən/
Phonetic Spelling: stim·yuh·lay·shuhn

9. Titanium
Pronunciation link: <https://www.merriam-webster.com/dictionary/titanium>
IPA: /taɪˈteɪniəm/
Phonetic Spelling: tie·tay·nee·uhm
10. Iridium
Pronunciation link: <https://www.merriam-webster.com/dictionary/iridium>
IPA: /ɪˈrɪdiəm/
Phonetic Spelling: ih·rid·ee·uhm
11. Electrode
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrode>
IPA: /ɪˈlekˌtroʊd/
Phonetic Spelling: ih·lek·trohd
12. Cymba conchae
Pronunciation link: No confirmed link found
IPA: /ˈsɪmbə ˈkɒŋki/
Phonetic Spelling: sim·buh kong·kee
13. Microseconds
Pronunciation link: <https://www.merriam-webster.com/dictionary/microsecond>
IPA: /ˈmaɪkroʊˌsekənd/
Phonetic Spelling: my·kroh·sek·uhnd
14. Calibration
Pronunciation link: <https://www.merriam-webster.com/dictionary/calibration>
IPA: /ˌkæləˈbreɪʃən/
Phonetic Spelling: kal·uh·bray·shuhn
15. Hertz
Pronunciation link: <https://www.merriam-webster.com/dictionary/hertz>
IPA: /hɜːts/
Phonetic Spelling: hurts
16. Visual analog scale
Pronunciation link: <https://www.merriam-webster.com/dictionary/analog>
IPA: /ˈvɪzuəl ˈænəlɔːɡ skel/
Phonetic Spelling: vɪzh·oo·uhl an·uh·log skayl
17. Functional near-infrared spectroscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectroscopy>
IPA: /ˈfʌŋkʃənəl nɪr ˌɪnfrəˈred spɛkˈtrɔːskəpi/
Phonetic Spelling: funk·shuh·nuhl neer in·fruh·red spek·tros·kuh·pee
18. Synchronized
Pronunciation link: <https://www.merriam-webster.com/dictionary/synchronize>
IPA: /ˈsɪŋkrəˌnaɪzd/
Phonetic Spelling: sing·kruh·nyzd
19. Optode
Pronunciation link: No confirmed link found
IPA: /ˈɑpˌtoʊd/
Phonetic Spelling: op·tohd

20. Montage
Pronunciation link: <https://www.merriam-webster.com/dictionary/montage>
IPA: /mənˈtɑːʒ/
Phonetic Spelling: mon·tazh
21. Precuneus
Pronunciation link: <https://www.howtopronounce.com/precuneus>
IPA: /priːˈkjuːniəs/
Phonetic Spelling: pree·kyoo·nee·uhs
22. Posterior cingulate cortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/posterior>
IPA: /pɑːˈstɪriər ˈsɪŋɡjələt ˈkɔːtɛks/
Phonetic Spelling: pos·teer·ee·er sing·gyuh·luht kor·teks
23. Medial prefrontal cortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/medial>
IPA: /ˈmiːdiəl priˈfrʌntəl ˈkɔːtɛks/
Phonetic Spelling: mee·dee·uhl pree·frun·tl kor·teks
24. Angular gyri
Pronunciation link: <https://www.merriam-webster.com/dictionary/angular>
IPA: /ˈæŋɡjələr ˈdʒaɪrɪ/
Phonetic Spelling: ang·gyuh·ler jy·rye
25. Extracerebral
Pronunciation link: No confirmed link found
IPA: /ˌɛkstrə səˈriːbrəl/
Phonetic Spelling: eks·truh suh·ree·bruhl
26. Regression
Pronunciation link: <https://www.merriam-webster.com/dictionary/regression>
IPA: /rɪˈɡrɛʃən/
Phonetic Spelling: ruh·gresh·uhn
27. Nanometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
IPA: /ˈnænəˌmiːtər/
Phonetic Spelling: nan·uh·mee·ter
28. Preprocessing
Pronunciation link: <https://www.merriam-webster.com/dictionary/preprocess>
IPA: /ˌpriːˈprɑːsɛsɪŋ/
Phonetic Spelling: pree·prah·sess·ing
29. Eigenvalue
Pronunciation link: <https://www.howtopronounce.com/eigenvalue>
IPA: /ˈaɪɡənˌvæljuː/
Phonetic Spelling: eye·guhn·val·yoo
30. Eigenvector
Pronunciation link: <https://www.howtopronounce.com/eigenvector>
IPA: /ˈaɪɡənˌvektər/
Phonetic Spelling: eye·guhn·vek·ter

31. Controllability

Pronunciation link: <https://www.merriam-webster.com/dictionary/controllability>

IPA: /kənˌtroʊləˈbɪləti/

Phonetic Spelling: kuhn·troh·luh·bil·uh·tee

32. Consensus

Pronunciation link: <https://www.merriam-webster.com/dictionary/consensus>

IPA: /kənˈsənsəs/

Phonetic Spelling: kuhn·sen·suhs