Submission ID #: 68062
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20758958

Title: Transauricular Vagus Nerve Stimulation and Electroencephalographic Assessment in Disorders of Consciousness

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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

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

Current Protocol Length
Number of Steps: 10
Number of Shots: 16 (5 SC)

Introduction

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

1.1. **Alejandro Galvao Carmona:** We aim to evaluate the clinical and neurophysiological effects of transauricular vagus nerve stimulation in patients with disorders of consciousness, using standardized EEG and behavioral protocols **[1].**

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

What technologies are currently used to advance research in your field?

1.2. **~~Myrtha O´Valle Rodríguez~~ Marta Gómez Herranz:** We use wearable taVNS stimulators, EEG system for monitoring neural responses, and quantitative behavioral scales like CRS-R to assess clinical improvements in disorders of consciousness **[1].**

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

What are the current experimental challenges?

1.3. **Samuel López Rodríguez:** We face challenges standardizing stimulation protocols, separating genuine neural recovery from placebo effects, and ensuring reliable longitudinal EEG-behavior correlations across heterogeneous patient populations **[1].**

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1*

What significant findings have you established in your field?

1.4. **Samuel López Rodríguez:** We found that taVNS can improve CRS-R scores and modulate EEG markers like alpha power and P300, supporting its potential as a therapeutic tool in disorders of consciousness **[1].**

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What research questions will your laboratory focus on in the future?

1.5. **Samuel López Rodríguez:** We will explore how taVNS modulates brain network dynamics and investigate whether EEG-based predictors can guide personalized neuromodulation strategies in patients with disorders of consciousness **[1].**

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.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Alejandro Galvao Carona, PhD. Associate Professor of Psychobiology, Universidad Loyola Andalucía; Neuropsychologist and researcher at IRENEA-Vithas. Principal Investigator, NEUROCAP Research Group**: (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. *Suggested B-roll: 3.2.2*

(Publishing with JoVE will allow our protocol to reach a wider scientific and clinical audience through visual demonstration, making techniques like taVNS and EEG more accessible and reproducible worldwide).

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.7. **Samuel López Rodríguez, Predoctoral researcher, Neurobiologist**: (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. *Suggested B-roll: 2.3.1*

We expect JoVE to help reduce training time for our clinical partners and collaborators, making it easier to standardize taVNS and EEG protocols across multicenter studies in disorders of consciousness.)

Ethics Title Card

This research has been approved by the Regional Ethics Committee (Comité Ético de Investigación Clínica Sevilla Sur) at Hospital Universitario Virgen de Valme

Protocol

2. Treatment of Patients with Disorders of Consciousness

Demonstrator: Samuel López Rodríguez

2.1. To begin, position the patient comfortably in a chair or on a bed **[1]**. Use additional straps or cushions to maintain a stable posture and prevent body movement during stimulation **[2]**.

2.1.1. WIDE: Talent assisting the patient to sit or lie down comfortably on a bed or chair.

2.1.2. Talent securing the patient's posture using cushions or adjustable straps.

2.2. Select the appropriate region on the ear to stimulate the auricular branch of the vagus nerve, such as the tragus **[1]**. Clean the electrodes before placing them on the tragus for the experimental group **[2-TXT]**.

2.2.1. Talent inspecting the ear and pointing to the ~~cymba region~~ tragus.

2.2.2. Talent cleaning the anode. **TXT: Control group: No electrical stimulation**

2.3. Using an alcohol-impregnated wipe, clean the targeted ear skin to remove oils or debris **[1-TXT]**.

2.3.1. Talent wiping the ear area with an alcohol wipe. **TXT: Ensure the ear is free from makeup or earrings before proceeding** NOTE: VO of the deleted shot is placed here as a text overlay

2.3.2. ~~Talent removing earrings~~. NOTE: Shot not filmed

2.4. Now, apply a thin layer of conductive gel or paste to the targeted area **[1]**.

2.4.1. Talent squeezing conductive gel onto a gloved fingertip and spreading it on the designated ear area.

NOTE: Step 2.5 was removed since the device that was used had only one electrode without a cathode.

~~2.5. Then, connect the electrodes to the stimulation device, ensuring the red wire is attached to the anode and the black wire to the cathode **[1]**.~~

2.5.1. ~~Talent connecting the red and black wires to the respective ports on the stimulation device~~.

2.6. Place the anode electrode on the selected ear region, such as the tragus **[1-TXT]**. NOTE: VO is modified for the deleted shot.

2.6.1. Talent placing the anode on the ~~cymba or designated stimulation area~~ tragus. **TXT: Other regions could be tragus or cavum conchae**

2.6.2. ~~Talent attaching the cathode electrode onto the earlobe~~. NOTE: Shot removed since the device that was used had no cathode

2.7. On the stimulation device, select the appropriate parametrization for transcutaneous auricular vagus nerve stimulation **[1]**.

2.7.1. SCREEN: Show the settings menu of the taVNS device and select the correct stimulation protocol from the list. NOTE: Steps 2.8 and 2.9 have been filmed together with 2.7 (Frequency, time, and amplitude)

Videographer: Please record the computer screen for the shots labeled as SCREEN

2.8. Set the session duration to 30 minutes **[1]** and choose a duty cycle of 30 seconds on and 30 seconds off **[2]**.

2.8.1. SCREEN: Show the timer interface and set the duration to 30 minutes.

2.8.2. SCREEN: Adjust the duty cycle settings to 30 seconds on and 30 seconds off.

2.9. Start with a current intensity of 3 milliamperes **[1]**. Select a current intensity of 1.5 milliamperes to avoid any disconformity, without adjusting with the de Nociception Coma Scale. If the Nociception Coma Scale-Revised score increases by three points, blood oxygenation drops to 95%, or heart rate increases by 20%, reduce the current intensity **[2]**. NOTE: VO is added for the added shot.

2.9.1. SCREEN: Adjust the current intensity dial or input field to 3 milliamperes.

Added shot: A current intensity of 1,5 mA (Level 20) has been selected to avoid any disconformity, without adjusting with de Nociception Coma Scale.

2.9.2. SCREEN: Highlight monitoring of patient vitals and show adjustment of current in response to changes.

2.10. After the stimulation session, remove the electrodes from the ear **[1-TXT]**.

2.10.1. Talent gently detaching the electrodes from the ear. **TXT: Conduct electroencephalographic and behavioral assessments**

Results

3. Results

3.1. This study included six unresponsive wakefulness syndrome patients and eight minimally conscious state patients who underwent the treatment, with Coma Recovery Scale-Revised or CRS-R assessments **[1]**.

3.1.1. LAB MEDIA: Figure 5

3.2. The mean CRS-R score in unresponsive wakefulness syndrome patients remained unchanged at 7 across baseline, after 4 weeks of treatment, and at the 4-week follow-up **[1]**.

3.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the three blue bars*

3.3. The mean CRS-R score in minimally conscious state patients was 11 at baseline **[1]**, did not significantly change after 4 weeks of transcutaneous auricular vagus nerve stimulation treatment **[2]**, but showed a significant increase to 12 at the 4-week follow-up **[3]**.

3.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the orange bar labeled "Baseline", which is around the value of 11*.

3.3.2. LAB MEDIA: Figure 5. *Video editor: Highlight the orange bar labeled "Four-week treatment", which is similar in height to the baseline bar*.

3.3.3. LAB MEDIA: Figure 5. *Video editor: Highlight the orange bar labeled "Four-week follow-up", which is visibly taller than the previous two and marked with an asterisk*.

Pronunciation Guide:

1. Tragus
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/tragus
- **IPA:** /ˈtrægəs/
- **Phonetic Spelling:** trag-uhs

2. Conchae (plural of concha, as in "cymba conchae")
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/conchae
- **IPA:** /ˈkɒŋki/ (American)
- **Phonetic Spelling:** kon-kee

3. Auricular
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/auricular
- **IPA:** /ɔːˈrɪkjələr/ (in American, often /əˈrɪkjələr/)
- **Phonetic Spelling:** uh-RICK-yuh-ler

4. Vagus (as in vagus nerve)
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/vagus
- **IPA:** /ˈveɪgəs/
- **Phonetic Spelling:** vay-guhs

5. Nociception
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/nociception
- **IPA:** /ˌnoʊsɪˈsɛpʃən/
- **Phonetic Spelling:** noh-sih-SEP-shun

6. Conductive
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/conductive
- **IPA:** /kənˈdʌktɪv/
- **Phonetic Spelling:** kun-DUCK-tiv

7. Electrode
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/electrode
- **IPA:** /ɪˈlɛkˌtroʊd/
- **Phonetic Spelling:** ih-LEK-trohd

8. Parameterization (or Parameterisation)
- **Pronunciation link:** https://www.merriam-webster.com/dictionary/parameterization
- **IPA:** /pəˌræmətəˌraɪˈzeɪʃən/
- **Phonetic Spelling:** puh-ram-uh-tuh-rye-ZAY-shun