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**Scriptwriter Name: Sulakshana Karkala**
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# Title: The Combination of Transcranial Alternating Current Stimulation and Electroencephalogram

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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**

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. **No**

**Current Protocol Length**
Number of Steps: 18
Number of Shots: 40

# Introduction

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

**INTRODUCTION:**

1.1. **Qinghua He:** The current protocol aims to demonstrate how to combine high-definition transcranial alternating current stimulation with EEG.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.11*

What are the current experimental challenges?

1.2. **Qinghua He**: By recording EEG before and after stimulation, this protocol enables researchers to assess neurophysiological effects of stimulation.

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

**CONCLUSION:**

What significant findings have you established in your field?

1.3. **Qinghua He:** This protocol presents a practical method for integrating HD-tACS with EEG.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.16*

How will your findings advance research in your field?

1.4. **Qinghua He:** We hope this protocol will facilitate broader applications of tACS and EEG integration and promote more rigorous exploration of neural entrainment dynamics.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What questions will future research focus on?

1.5. **Qinghua He:** This protocol can be extended to larger sample sizes with tailored stimulation parameters to investigate a broader range of cognitive and affective functions.

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.***

**Ethics Title Card**
This research has been approved by the Institutional Review Board (IRB) at Southwest University

# Protocol

## 2. Pre- and Post-Stimulation EEG Recording and Transcranial Electrical Stimulation (tES) Setup Using a 64-Channel EEG Braincap

**Demonstrators:** Jiayue Zou, Yawei Qi, Jingzhen He

2.1. To begin, clean the participant's scalp before the experiment **[1]**. Place the 64-channel EEG brain cap with electrodes arranged according to the 10–20 *(Ten-Twenty)* system on the participant's head **[2]**.

2.1.1. WIDE: Talent cleaning the participant's scalp using standard cleaning materials.

2.1.2. Talent placing the EEG brain cap on the participant's head.

2.2. Monitor electrode impedance by selecting **Impedance** in the software interface **[1]**.

2.2.1. SCREEN: 68743_screenshot_1.mp4: 00:00-00:12

2.3. Set the impedance display range to 0 to 5 kilo-ohms to ensure high recording quality **[1]**. Interpret orange or green signals on the display as below 5 kilo-ohms **[2]**.

2.3.1. SCREEN: 68743_screenshot_1.mp4: 00:13-00:23

2.3.2. SCREEN: 68743_screenshot_1.mp4: 00:36-00:46

2.4. Use the syringe tip to part the hair through the electrode openings **[1]**, then inject the gel to ensure direct contact between each electrode and the scalp **[2]**. Gently rub the scalp with the syringe tip until the impedance of each electrode drops below 5 kilo-ohms **[3]**.

2.4.1. Talent parting the participant's hair through an electrode opening with the syringe tip.

2.4.2. Talent injecting gel into the parted hair area to contact the scalp.

2.4.3. Talent rubbing the scalp gently with the syringe tip to reduce impedance.
*Videographer: Please capture the screen of the instrument for this shot*

2.5. Once impedance is confirmed for all electrodes, initiate EEG recording by pressing **Start** in the software as the participant performs the 2-back task **[1]**. Visualize EEG signals during the task **[2]**.

2.5.1. SCREEN: 68743_screenshot_2.mp4: 00:03-000:07, 00:10-00:21

2.5.2. SCREEN: 68743_screenshot_2.mp4: 00:21-00:31

2.6. Clean the participant's scalp again before stimulation **[1]**. Prepare all necessary

materials in advance **[2]**. Install batteries and confirm they are fully charged **[3]**. Use the input cable to connect the 2-channel transcranial electrical stimulation stimulator to the 4 by 1 stimulation interface **[4]**.

2.6.1. Talent cleaning the scalp once more.

2.6.2. Talent organizing and laying out all required equipment. Videographer's notes: 2.6.2 The video segment corresponding to this step is C9527.mp4, but it was incorrectly labeled as 2.6.1 on the slate.

2.6.3. Talent inserting and verifying fully charged batteries.

2.6.4. Talent connecting the input cable from the stimulator to the 4×1 interface.

2.7. Connect the output cable to the 4 by 1 interface **[1]** and attach five silver or silver chloride electrodes to the output cable **[2]**. Power on the 2-channel stimulator and the 4 by 1 system after completing all connections **[3]**.

2.7.1. Talent attaching the output cable to the 4×1 interface.

2.7.2. Talent connecting five silver/silver chloride electrodes to the output cable.

2.7.3. Talent powering on both the stimulator and interface system.

2.8. Now, embed five plastic high-definition casings into designated sites in the EEG brain cap at the sites P3, CP3, P1, PO3, and P5 **[1]**. Then place the EEG cap on the participant's head **[2]**.

2.8.1. Talent inserting HD casings into the EEG cap at the specified sites.

2.8.2. Talent placing the EEG braincap on the participant's head.

2.9. Apply conductive gel to the scalp through each HD casing opening using a syringe tip **[1]**. Part the hair to expose the scalp and apply gel directly **[2]**. Insert five electrodes into the casings, with electrode 5 at P3 and the rest surrounding it **[3]**.

2.9.1. Talent inserting syringe tip into each casing and dispensing gel.

2.9.2. Talent parting hair and exposing the scalp.

2.9.3. Talent placing electrodes in HD casings, aligning positions as specified.

2.10. Fill all EEG electrodes with conductive gel again **[1]** and confirm impedance values below 5 kilo-ohms **[2]**.

2.10.1. Talent injecting gel into EEG electrodes.

2.10.2. SCREEN: 68743_screenshot_3.mp4: 00:21-00:26

2.11. Confirm that the default mode on the stimulator is set to **SCAN** **[1]**. Then, view impedance values of each electrode individually by pressing the numbered buttons on the 4 by 1 interface **[2]**.

2.11.1. Talent pressing the default mode set to **SCAN** on the stimulator.

*Videographer: Please capture the screen of the instrument for this shot*

2.11.2. Talent pressing individual numbered buttons to view impedance readings.

*Videographer: Please capture the screen of the instrument for this shot*

2.12. If impedance exceeds 1.5, open the cap and scrub the scalp with a syringe tip to reduce impedance **[1]**.

2.12.1. Talent lifting the cap and scrubbing the exposed scalp with syringe tip.

2.13. Set stimulation parameters on the 2-channel stimulator, including waveform, duration, and intensity using four knobs **[1]**. Use the joystick to toggle the sham condition **[2]**.

2.13.1. Talent setting the waveform, duration and intensity simulation parameters.

2.13.2. Talent toggling sham mode using the joystick.

2.14. Set parameters to alternating current waveform, 2 milliamperes, and 4 hertz for 10 minutes **[1]**. Switch the **RELAX** level to full current **[2]**.

2.14.1. Talent parameter settings being entered: AC, 2 mA, 4 Hz, 10 minutes.

2.14.2. Talent toggling **RELAX** level to full current.

2.15. Next, change the stimulator mode from **SCAN** to **PASS** using the **MODE SELECT** button **[1]**.

2.15.1. Talent pressing **MODE SELECT** to activate **PASS** mode.

2.16. Start stimulation by pressing the **START** button on the stimulator **[1]**. Observe current ramp-up on the screen until target intensity is reached **[2]**. If discomfort occurs, reduce intensity with the **RELAX** lever and resume once the participant is comfortable **[3]**. Terminate stimulation if necessary, by pressing the **ABORT** button **[4]**.

2.16.1. Talent pressing **START** on the stimulator.

2.16.2. Live ramp-up of current displayed on device.

*Videographer: Please capture the screen of the instrument for this shot*

2.16.3. Talent adjusting the **RELAX** lever in response to participant feedback.

2.16.4. Talent pressing **ABORT** to stop stimulation.

2.17. Remove the HD casings and stimulation electrodes from the EEG cap **[1]**. Reinsert the original EEG electrodes at sites P3, CP3, P1, PO3, and P5 **[2]**.

2.17.1. Talent removing HD casings and stimulation electrodes.

2.17.2. Talent reinserting EEG electrodes into designated cap openings.

2.18. Use the syringe tip to part the hair and apply conductive gel directly to each reinserted electrode **[1]**. Confirm impedance values are below 5 kilo-ohms before starting post-stimulation EEG recording **[2]**.

2.18.1. Talent parting the hair and applying gel using syringe tip.

2.18.2. SCREEN: 68743_screenshot_3.mp4: 00:21-00:26

# Results

## 3. Results

3.1. Ocular artifacts were successfully removed during EEG preprocessing, as illustrated by the absence of typical eye movement signals in the post-artifact rejection traces **[1]**.

3.1.1. LAB MEDIA: Figure 8. *Video editor: Highlight the red rectangle in panel A, then show the same the same boxed region in panel B*

3.2. Time-frequency spectrograms revealed a reduction in theta-band power following stimulation compared to pre-stimulation **[1]**.

3.2.1. LAB MEDIA: Figure 9A. *Video editor: Highlight the boxed region in the "Pre" panel, then show the same boxed region in the "Post" panel*

1. **electroencephalography (EEG)**
   Pronunciation link: https://www.merriam-webster.com/dictionary/electroencephalography
   IPA: /ɪˌlɛktroʊɛnˌsɛfəˈlɑgrəfi/
   Phonetic Spelling: ih-LEK-troh-en-SEF-uh-LAL-uh-gree
2. **impedance**
   Pronunciation link: https://www.merriam-webster.com/dictionary/impedance
   IPA: /ɪmˈpiːdəns/
   Phonetic Spelling: im-PEE-duns
3. **kilohm(s)**
   Pronunciation link: https://www.merriam-webster.com/dictionary/kilohm
   IPA: /ˈkɪloʊm/
   Phonetic Spelling: KIL-ohm
4. **syringe**
   Pronunciation link: https://www.merriam-webster.com/dictionary/syringe
   IPA: /səˈrɪndʒ/
   Phonetic Spelling: suh-RINJ
5. **transcranial**
   Pronunciation link: https://www.merriam-webster.com/dictionary/transcranial
   IPA: /ˌtrænsˈkreɪniəl/
   Phonetic Spelling: trans-KRAY-nee-uhl
6. **stimulation**
   Pronunciation link: https://www.merriam-webster.com/dictionary/stimulation
   IPA: /ˌstɪmjəˈleɪʃən/
   Phonetic Spelling: stim-yuh-LAY-shun
7. **silver chloride (Ag/AgCl)**
   – **silver**: /ˈsɪlvər/ (SIL-vur)
   – **chloride**: /ˈklɔrɪd/ (KLOR-ide)
   Full term phonetic: SIL-vur KLOR-ide
8. **waveform**
   Pronunciation link: https://www.merriam-webster.com/dictionary/waveform
   IPA: /ˈweɪvˌfɔrm/
   Phonetic Spelling: WAVE-form
9. **alternating current (AC)**
   – **alternating**: /ˈɔltərˌneɪtɪŋ/ (AWL-tur-NAY-ting)
   – **current**: /ˈkɜrənt/ (KUR-unt)
   Phonetic Spelling: AWL-tur-NAY-ting KUR-unt
10. **frequency (Hz)**
    Pronunciation link: https://www.merriam-webster.com/dictionary/frequency
    IPA: /ˈfriːkwənsi/
    Phonetic Spelling: FREE-kwuhn-see
11. **spectrogram**
    Pronunciation link: https://www.merriam-webster.com/dictionary/spectrogram

IPA: /ˈspɛktrəˌgræm/
Phonetic Spelling: SPEK-truh-gram

12. **theta-band**
– **theta**: Pronunciation link: https://www.merriam-webster.com/dictionary/theta
IPA: /ˈθiːtə/
Phonetic Spelling: THEE-tuh
– **band**: /bænd/ (BAND)
Full term: THEE-tuh BAND
13. **ocular**
Pronunciation link: https://www.merriam-webster.com/dictionary/ocular
IPA: /ˈɑkjələr/
Phonetic Spelling: OK-yuh-lur