FINAL SCRIPT: APPROVED FOR FILMING

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Title: A Novel Scalp Acupuncture-Based Method to Target the Hand Motor Hotspot for Non-Invasive Brain Stimulation

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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**

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: 24
Number of Shots: 40 (8 SC)

Introduction

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

INTRODUCTION:

1.1. **Hao Meng:** We study non-invasive brain stimulation for stroke motor recovery, with a focus on improving neuromodulatory effectiveness when targeting the primary motor cortex.

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

1.2. **Hao Meng:** A major challenge is achieving a quick and accurate setup for high-definition stimulation targeting the upper-limb primary motor cortex.

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

CONCLUSION:

1.3. **Hao Meng:** We are exploring how to quickly and reliably localize the M1 hotspot for forearm muscles when neuroimaging tools are not available.

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

1.4. **Hao Meng:** Our method is based on empirical evidence, quick to perform, requires minimal equipment, and has strong potential for clinical use.

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

1.5. **Hao Meng:** Our future research will examine whether the protocol can be translated into stroke motor-recovery applications.

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**.

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

1.6. **Hao Meng, Postdoctoral Research Fellow**: (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.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Hao Meng, Postdoctoral Research Fellow**: (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 Chinese?
Videographer: Please film the testimonials in both English and Chinese

Ethics Title Card

This research has been approved by the Committee for the Protection of Human Subjects (CPHS) at The University of Texas Health Science Center at Houston

Protocol

2. Localizing the Upper and the Lower Motor Points

Demonstrator: Hao Meng

2.1. To begin, ensure that the participant is seated upright in a comfortable position with both arms resting naturally **[1]**. Prepare a tape measure and a marker for the M1 flexor digitorum superficialis hotspot localization **[2]**.

2.1.1. WIDE: Talent ensuring the participant is seated upright with arms relaxed on the armrest.

2.1.2. Talent placing a tape measure and marker on the table, ready for use.

2.2. Using a tape measure, align the zero mark with the glabella, which is the midpoint between the eyebrows **[1]**. Ensure that the tape is held straight and level across the scalp **[2]**.

2.2.1. Talent aligning the tape measure's zero mark precisely at the glabella.

2.2.2. Close-up of the tape being adjusted to lie level across the participant's scalp.

2.3. Now, stretch the tape measure to reach the inion, which is the tip of the external occipital protuberance **[1]**. Confirm that the tape is held firmly and straight between these two points **[2]**.

2.3.1. Talent extending the tape from the glabella to the inion along the scalp.

2.3.2. Close-up showing the tape stretched tightly.

2.4. Identify the midpoint between the glabella and the inion along the longitudinal line **[1]**. From this midpoint, measure 0.5 centimeters posteriorly and mark the location with a visible dot using the marker **[2]**.

2.4.1. Talent pointing the midpoint on the longitudinal line.

2.4.2. Talent marking the point 0.5 centimeters posterior to the midpoint with a visible dot.

2.5. Next, align the tape measure between the left and right preauricular points or the apex of the ears **[1]**. Ensure the tape is level and mark the midpoint of this transverse line with a visible dot **[2]**.

2.5.1. Talent positioning the tape across both preauricular points.

2.5.2. Close-up of the midpoint being marked with a visible dot on the scalp.

2.6. Using the marked midpoint as a reference, adjust the point identified earlier laterally if required, to ensure that the final upper motor point is centered on the transverse midpoint **[1]**.

2.6.1. Talent adjusting the previously marked point slightly to align with the transverse midpoint.

2.7. For the lower motor point, align the zero mark of the tape measure to the lateral end of the eyebrow on the left side of the face **[1]**. Stretch the tape from the lateral end of the eyebrow to the inion, maintaining a straight diagonal line across the scalp **[2]**.

2.7.1. Talent placing the tape's zero mark precisely at the lateral end of the left eyebrow.

2.7.2. Talent extending the tape diagonally across the participant's head from the eyebrow to the inion.

2.8. Then, identify the point where the diagonal line intersects the anterior hairline **[1]** and mark this intersection clearly with a visible dot, defining it as the lower motor point **[2]**.

2.8.1. Close-up of pointing to the intersection point along the anterior hairline.

2.8.2. Talent marking the lower motor point with a visible dot on the participant's scalp.

3. Localization and Validation of the M1 FDS Hotspot

3.1. Align the zero mark of the tape measure with the upper motor point **[1]** and extend the tape measure in a straight line to reach the lower motor point **[2]**.

3.1.1. Talent aligning the tape measure's zero mark at the upper motor point on the participant's scalp.

3.1.2. Talent extending the tape down to the lower motor point, ensuring a straight and firm alignment.

3.2. Record the total distance between the upper motor point and the lower motor point **[1]**.

3.2.1. Shot of distance being noted down in a logbook.

3.3. Calculate the point located at two-fifths of the measured distance from the upper motor point **[1]** and mark this point clearly with a visible dot, defining it as the AC hotspot **[2-TXT]**.

3.3.1. Talent measuring two-fifths of the total distance from the upper motor point using the tape.

3.3.2. Close-up of the marker creating a visible dot at the calculated AC hotspot. **TXT: AC: Acupuncture**

3.4. For validation, place bipolar surface electromyography electrodes over the belly of the right flexor digitorum superficialis muscle **[1]** and set the sampling frequency to two kilohertz on the recording system **[2]**.

3.4.1. Talent attaching two bipolar electrodes to the belly of the right flexor digitorum superficialis muscle.

3.4.2. SCREEN: Show the electromyography interface where the sampling frequency is set to 2 kHz. ***Videographer: Please record the computer screen for the shots labeled as SCREEN***

3.5. Identify and mark the C3 site according to the International 10-20 electroencephalography system **[1]**.

3.5.1. Talent locating the C3 position on the scalp using measurement landmarks and marking it with a visible dot.

3.6. After marking the FDS hotspot, position the transcranial magnetic stimulation coil tangentially over the scalp at the AC site, with the handle directed backward at a 45-degree angle **[1]**.

3.6.1. Talent positioning the transcranial magnetic stimulation coil correctly over the AC hotspot with the handle angled backward at 45 degrees.

3.7. Set the device to begin at 30 percent of the maximum transcranial magnetic stimulation output and deliver a single pulse of stimulation **[1]**.

3.7.1. SCREEN: Show the transcranial magnetic stimulation interface set to 30% output.

3.8. Now, gradually increase the stimulation intensity to determine the resting motor threshold **[1]**.

3.8.1. SCREEN: Show the electromyography signal traces used to identify the resting motor threshold.

3.9. Then, set the final stimulation intensity to 120% of the resting motor threshold **[1]** and record a representative motor-evoked potential at the AC site **[2]**.

3.9.1. SCREEN: Show the stimulation intensity being adjusted to 120% RMT.

3.9.2. SCREEN: Display the electromyography traces capturing motor-evoked potential recording at the AC site.

4. High-Definition Transcranial Electrical Stimulation (HD-tES) Intervention

4.1. Fit the electroencephalography cap according to the standard protocol, ensuring that it is properly aligned with the scalp landmarks **[1-TXT]**.

4.1.1. Talent fitting the electroencephalography cap snugly on the participant's head, aligning with the nasion and inion. **TXT: Landmarks: Nasion at the forehead and the inion at the back of the head**

4.2. Position one electrode directly over the target site, either the AC or C3 location **[1]**. Place four reference electrodes evenly spaced around the target site to ensure stable current distribution **[2]** and apply conductive gel to all electrode sites to minimize impedance **[3]**.

4.2.1. Talent positioning the active electrode over the AC site on the electroencephalography cap.

4.2.2. Talent placing four reference electrodes evenly around the target area.

4.2.3. Close-up of the talent applying conductive gel to each electrode site using a syringe applicator.

4.3. Now, check the impedance of all electrodes using the stimulator's built-in impedance meter, ensuring that all values are below two kilo-ohms before proceeding **[1]**.

4.3.1. SCREEN: Show the impedance check screen on the stimulator interface displaying electrode impedance values.

4.4. Set the stimulation parameters on the stimulator to two milliamperes intensity and twenty-one hertz frequency for a total duration of twenty minutes **[1]**.

4.4.1. SCREEN: Show the parameter setup panel with **2 mA**, **21 Hz**, and **20 min** entered and confirmed.

4.5. Then, turn on the high-definition transcranial electrical stimulation device **[1]** and gradually ramp the current intensity to two milliamperes over a period of thirty seconds **[2]**.

4.5.1. SCREEN: Show the device being switched on and the current ramp-up progression on the display.

4.5.2. Shot of the participant and the monitor as the device ramps up to the target intensity.

4.6. Continuously monitor the participant's reactions during the twenty-minute stimulation session **[1]** and check for any signs of discomfort, skin irritation, or unusual sensations **[2]**.

4.6.1. Talent observing the participant during the stimulation session and pointing to the monitor.

4.6.2. Close-up of the participant's face and scalp being inspected by the talent for any signs of irritation.

4.7. Finally, after twenty minutes, turn off the stimulation device **[1]**. Carefully remove all electrodes from the scalp and clean the electrode sites thoroughly **[2]**.

4.7.1. Shot of the device being turned off at the end of the session.

4.7.2. Talent gently removing the electrodes from the participant's scalp.

Results

5. Results

5.1. The motor evoked potential waveforms from the AC and C3 sites were obtained at baseline and after stimulation **[1]**. At baseline, the peak-to-peak amplitude at the AC site was greater than that at the C3 site **[2]**.

5.1.1. LAB MEDIA: Figure 6

5.1.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the red waveform labelled "AC*" .

5.2. Following high-definition transcranial electrical stimulation, a significant increase in motor evoked potential amplitude was observed at the AC site **[1]**, whereas no change was detected at the C3 site **[2]**.

5.2.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the red waveform labelled "AC*" .

5.2.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the black waveform labelled "C3*"

- **Glabella** – *gluh-BELL-uh* | /ɡləˈbɛlə/
- **Inion** – *IN-ee-on* | /ˈɪniˌɑn/
- **Preauricular** – *pree-aw-RIK-yuh-ler* | /ˌpriːɔːˈrɪkjələr/
- **Occipital** – *ok-SIP-uh-tl* | /ɑkˈsɪpɪt̬l/
- **Protuberance** – *pro-TOO-ber-uhns* | /proʊˈtuːbərəns/
- **Occipital protuberance** – *ok-SIP-uh-tl pro-TOO-ber-uhns* | /ɑkˈsɪpɪt̬l proʊˈtuːbərəns/
- **Longitudinal** – *lon-juh-TOO-dn-uhl* | /ˌlɑndʒəˈtuːdənəl/
- **Transverse** – *TRANZ-vers* | /ˈtrænzvɝːs/
- **Lateral** – *LAT-er-uhl* | /ˈlætərəl/
- **Anterior** – *an-TEER-ee-er* | /ænˈtɪriər/

- **Electromyography** – *ee-LEK-troh-my-OG-ruh-fee* | /ɪˌlɛktroʊˌmaɪˈɑgrəfi/
- **Bipolar** – *bye-POH-ler* | /baɪˈpoʊlər/
- **Flexor digitorum superficialis** – *FLEK-ser dij-ih-TOR-um soo-per-fish-ee-AL-iss* | /ˈflɛksərˌdɪdʒɪˈtɔːrəm ˌsuːpərˌfɪʃiˈælɪs/
- **Electroencephalography** – *ee-LEK-troh-en-SEF-uh-LOG-ruh-fee* | /ɪˌlɛktroʊɛnˌsɛfəˈlɑgrəfi/
- **Transcranial** – *trans-KRAY-nee-uhl* | /trænzˈkreɪniəl/
- **Acupuncture** – *AK-yoo-punk-cher* | /ˈækjəˌpʌŋktʃər/
- **Impedance** – *im-PEE-dns* | /ɪmˈpiːdəns/
- **Milliamperes** – *MIL-ee-am-peers* | /ˈmɪliˌæmˌpɪrz/
- **Kilohertz** – *KILL-oh-herts* | /ˈkɪloʊˌhɝːts/
- **Nasion** – *NAY-zhun* | /ˈneɪʒən/
- **Motor evoked potential** – *MOH-ter ih-VOKT puh-TEN-shul* | /ˈmoʊtər ɪˈvoʊkt pəˈtɛnʃəl/