FINAL SCRIPT: APPROVED FOR FILMING

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Title: Standardized Induction and Assessment of Long-term Potentiation-like Cortical Plasticity Using Transcranial Magnetic 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, all done**

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: 49

Introduction

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

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Wenjun Dai:** We study cortical plasticity to understand and measure synaptic changes in health and neurological disorders.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B.roll:4.1*~~

~~What are the current experimental challenges?~~

1.2. **Wenjun Dai:** Variability in stimulation, coil positioning, and measurement timing makes reproducible iTBS plasticity studies challenging.

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

CONCLUSION:

~~What research gap are you addressing with your protocol?~~

1.3. **Wenjun Dai:** We offer a standardized neuronavigation-guided iTBS protocol for reliable and reproducible plasticity assessment.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B.roll:3.11*~~

~~What advantage does your protocol offer compared to other techniques?~~

1.4. **Wenjun Dai:** Our protocol uses neuronavigation, ensuring precise, reliable iTBS delivery and robust LTP-like plasticity with better consistency.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B.roll:3.4*~~

~~How will your findings advance research in your field?~~

1.5. **Wenjun Dai:** They enable reproducible studies of synaptic plasticity, supporting therapeutic development and neurorehabilitation strategies.

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 Ethics Committee at the First Affiliated Hospital with Nanjing Medical University. Written informed consent was obtained from the participants

Protocol

2. Preparation of the Head Model using a Neuronavigation System

Demonstrator: Jianjian Ding

2.1. To begin, explain the aim of the assessment, the main experimental procedures, and any potential risk factors associated with the study **[1-TXT]**. Request an acknowledgment of the consent process and obtain a signature on the informed consent form **[2]**.

2.1.1. WIDE: Talent speaking with the participant and explaining the assessment goals and experimental procedures using visual aids. **TXT: Respond to any questions or concerns from the participant**

2.1.2. Talent handing over the informed consent form and pointing out the signature area as the participant signs the document.

2.2. To identify the functional motor hotspot in the primary motor cortex corresponding to the contralateral target muscle, use the system to obtain real-time visual feedback of the TMS coil position and its alignment with anatomical landmarks **[1]**. Confirm accurate targeting using the visual feedback from the neuronavigation software **[2]**.

2.2.1. Talent positioning the transcranial magnetic stimulation coil near the participant's scalp.

2.2.2. SCREEN: 69425_screenshot_1_2.2.2.mp4 00:00-00:15 .

2.3. Now, generate a three-dimensional head model using the individual MRI *(M-R-I)* scans **[1]**. Select and register anatomical landmarks across the axial, sagittal, and coronal planes **[2]**. Perform scalp surface segmentation to complete the generation of the three-dimensional head model **[3]**.

2.3.1. SCREEN: 69425_screenshot_2_2.3.1.mp4 00:00-00:08 .

2.3.2. SCREEN: 69425_screenshot_2_2.3.2.mp4 00:00-00:17.

2.3.3. SCREEN: 69425_screenshot_4_2.3.3.mp4 00:02-00:14,00:19-00:22 .

2.4. After completing head model registration, perform coil calibration to ensure precise real-time tracking within the neuronavigation system **[1]**. In the software, navigate to **Settings**, click on **Coil**, and select **Calibrate Tool Faces [2]**. Place the TMS coil with reflective markers on the designated calibration block **[3]**. Calibrate fiducial points on the physical coil with the matching points on the coil face[4].

2.4.1. Talent in front of the computer screen, navigating through the software.

2.4.2. SCREEN: 69425_screenshot_5_2.4.2revised.mp4 00:00-00:06 .

2.4.3. .Talent placing the TMS coil with reflective markers on the calibration block

2.4.4. SCREEN: 69425_screenshot_6_2.4.4 revised.mp4 00:00-00:05 .
AUTHOR'S NOTE: Move 2.4.4 before 2.4.3

2.5. After initial calibration, follow the system prompt to place the coil over the calibration block and confirm its position for accuracy **[1]**. Then, click on **Neuronavigation** to validate single coil**[2]**. Accept only those calibrations with a spatial error less than 3 millimeters. Otherwise, repeat the calibration process **[3]**.

2.5.1. Talent positioning the TMS coil on the calibration block following system instructions.

2.5.2. SCREEN: 69425_screenshot_7_2.5.2 revised.mp4 00:00-00:12

2.5.3. SCREEN: 69425_screenshot_8_2.5.3revised.mp4 00:00-00:03
Video Editor: Please freeze frame

3. Motor Hotspot Identification

3.1. Ask the participant to sit in a comfortable chair with both back and arm support to minimize head and body movement **[1]**. Instruct the participant to keep both hands completely relaxed and still throughout the procedure **[2-TXT]**.

3.1.1. WIDE: Participant sitting in a supportive chair with arm and back support.

3.1.2. Talent instructing the participant to relax and keep both hands still during the procedure. **TXT: Avoid movements of the non-targeted hand**

3.2. Attach infrared reflective markers to the participant's forehead to enable real-time head tracking during the session **[1]** and secure the markers using adhesive patches **[2]**. Confirm that the markers are recognized by the neuronavigation system, indicated by their green appearance on screen **[3]**.

3.2.1. Talent applying infrared reflective markers to the participant's forehead.

3.2.2. Talent pressing adhesive patches firmly to secure the markers in place.

3.2.3. SCREEN: 69425_screenshot_9_3.2.3+-3.3.2.mp4 00:00-00:02
Video Editor: Please freeze frame here.
AUTHOR'S NOTE: Move 3.2.3 after 3.3.1

3.3. Using a tracked pointer, mark three anatomical landmarks on the participant's scalp: the nasion, the left supratragic notch, and the right supratragic notch **[1-TXT]**.

3.3.1. Talent using a tracked pointer to touch the nasion, left supratragic notch, and right supratragic notch on the participant's head. **TXT: Allow the system to register the participant's actual head position with the 3D model**

3.4. Using the same pointer, collect approximately 200 to 300 additional points on the scalp to improve registration accuracy **[1]**. Allow the neuronavigation software to automatically generate an individualized head shape model from these points to optimize alignment between the MRI scans and the actual coordinate system **[2]**.

3.4.1. Talent moving the pointer across the scalp while collecting multiple points.

3.4.2. SCREEN: 69425_screenshot_10_3.4.2.mp4 00:00-00:30 .

3.5. Next, place surface electrodes 2 centimeters apart on the abductor pollicis brevis muscle in a belly-tendon montage **[1]**. Position the reference electrode distally near the interphalangeal joint of the thumb **[2]**.

3.5.1. Talent placing surface electrodes 2 centimeters apart on the abductor pollicis brevis muscle.

3.5.2. Talent positioning the reference electrode near the thumb's interphalangeal joint.

3.6. Next, administer single-pulse TMS *(T-M-S)* approximately 5 centimeters lateral and 0 to 1 centimeters anterior to the vertex, contralateral to the target muscle **[1]**. Use this to determine the primary motor cortex coordinates that elicit the maximal motor evoked potential amplitude for motor hotspot localization **[2]**. Instruct the participant to keep their hand completely relaxed to prevent voluntary muscle contractions that may affect measurements **[3]**.

3.6.1. Talent positioning the TMS coil 5 centimeters lateral and 1 centimeter anterior to the vertex.

3.6.2. SCREEN: 69425_screenshot_11_3.6.2.mp4 00:10-00:33 .

3.6.3. Talent instructing the participant to keep the hand relaxed and still.

3.7. Orient the handle of the coil 45 degrees posterior to the midline to direct the electromagnetic current perpendicularly to the central sulcus **[1]**. Systematically move the coil in 1-centimeter steps around the motor cortex region in all directions, anterior, posterior, medial, and lateral, at 5-second intervals **[2]**. Identify the motor hotspot as the site that produces the largest motor evoked potential amplitude and the shortest latency in the relaxed abductor pollicis brevis muscle **[3]**.

3.7.1. Talent holding the coil at a 45-degree posterior angle to the midline.

3.7.2. Talent moving the coil around the marked region in 1-centimeter steps, pausing at each position for 5 seconds.

3.7.3. SCREEN: 69425_screenshot_12_3.7.3.mp4 00:02-00:03
Video Editor: Please freeze frame here

3.8. Allow the system to automatically record key spatial parameters, including the distance between the coil and the target point, the tilt deviation, and the rotation deviation **[1]**. Use these values to ensure accurate and reproducible coil placement during stimulation **[2]**.

3.8.1. SCREEN: 69425_screenshot_13_3.8.1.mp4 00:00-00:26 .

3.8.2. Talent reviewing the recorded metrics to confirm alignment for consistent coil positioning.

3.9. Using the same surface electromyography setup and parameters, measure the peak-to-peak amplitude of the motor evoked potential **[1]**. Define the resting motor threshold or RMT *(R-M-T)* as the minimum TMS *(T-M-S)* stimulus intensity that produces motor evoked potentials over 50 microvolts in at least 5 of 10 single-pulse trials **[2]**.
AUTHOR'S NOTE: Please blur patient information on the left side of the video

3.9.1. SCREEN: 69425_screenshot_14_3.9.1.+3.9.2.mp4. 00:00-00:20

3.9.2. SCREEN: 69425_screenshot_14_3.9.1.+3.9.2.mp4. 00:46-01:10

3.10. Record 20 consecutive motor evoked potentials at 5-second intervals from the motor hotspot as a baseline measurement. Set the stimulation intensity to 120 percent of the RMT *(R-M-T)* to evoke approximately 1 millivolt motor evoked potentials **[1]**.

3.10.1. SCREEN: 69425_screenshot_15_3.10.1.mp4 00:00-00:40 .
AUTHOR'S NOTE: Please blur patient information on the left side of the video

3.11. Deliver stimulation using a TMS device **[1]**. Set the stimulation intensity to 80 percent of the RMT *(R-M-T)* . Apply bursts of three pulses at 50 hertz, repeated at 5 hertz **[2-TXT]**.

3.11.1. Talent holding the coil over the motor hotspot.

3.11.2. SCREEN: 69425_screenshot_16_3.11.2.+3.11.3.mp4. 00:00-00:12

TXT: Repeat a 2 s train/10 s; Total of 200 s (600 pulses)

AUTHOR'S NOTE: Please blur patient information on the left side of the video

3.12. At 5, 10, 15, and 30 minutes after the iTBS *(i-T-B-S)*, record 20 motor evoked potentials using the same stimulation intensity to assess plasticity **[1]**.

3.12.1. SCREEN: 69425_screenshot_17_3.12.1.mp4 00:00-00:30 .
AUTHOR'S NOTE: Please blur patient information on the left side of the video

3.13. Calculate the mean peak-to-peak amplitude of the 20 motor evoked potentials recorded at baseline and at each post-stimulation time point to quantify cortical excitability **[1]**. Express the post-stimulation amplitudes as normalized ratios relative to baseline to standardize measurements across sessions and individuals **[2]**.

3.13.1. LAB MEDIA : 3.13.1.tif
Video Editor: Please highlight the blue part of the schematic (labeled 1)

3.13.2. LAB MEDIA : 3.13.1.tif
Video Editor: Please highlight the yellow part of the schematic (labeled 2)

Results

4. Results

4.1. Following iTBS *(i-T-B-S)* stimulation, motor evoked potential amplitudes were recorded at multiple time points to assess cortical excitability over time **[1].**

4.1.1. LAB MEDIA: Figure 6. *Video editor: Show all four panels (A, B, C, D) representing the sequential MEP traces at post-stimulation time points.*

4.2. Mean raw motor evoked potential amplitude increased after stimulation, peaking at 15 minutes and declining by 30 minutes **[1].**

4.2.1. LAB MEDIA: Figure 7. *Video editor: Highlight the rising line from baseline to 15 minutes, then the drop at 30 minutes on the graph*.

4.3. Normalized motor evoked potential amplitude showed a similar trend, increasing above baseline and gradually declining by 30 minutes **[1].** The mean normalized motor evoked potential amplitude across all post-stimulation time points exceeded 1.1, classifying the participant as facilitated **[2].**

4.3.1. LAB MEDIA: Figure 8. *Video editor: Highlight the upward slope in the red line from baseline to 15 minutes, followed by the decrease at 30 minutes.*

4.3.2. LAB MEDIA: Figure 8. *Video editor: Emphasize that all data points from 5 to 30 minutes lie above the 1.1 value line on the vertical axis.*

Pronunciation Guide:

- Potentiation
  Pronunciation link: https://www.merriam-webster.com/dictionary/potentiation
  IPA: /pəˌtɛnʃiˈeɪʃən/
  Phonetic Spelling: puh·ten·shee·ay·shun
- Cortical
  Pronunciation link: https://www.merriam-webster.com/dictionary/cortical
  IPA: /ˈkɔːr.tɪ.kəl/
  Phonetic Spelling: kor·tih·kuhl
- Transcranial
  Pronunciation link: https://www.merriam-webster.com/dictionary/transcranial
  IPA: /trænsˈkreɪniəl/
  Phonetic Spelling: tranz·kray·nee·uhl
- Neuronavigation
  Pronunciation link: No confirmed link found
  IPA: /ˌnʊroʊˌnævɪˈgeɪʃən/
  Phonetic Spelling: nyoo·roh·nav·ih·gay·shun
- Electromyography
  Pronunciation link: https://www.merriam-webster.com/dictionary/electromyography
  IPA: /iˌlɛk.troʊˌmaɪˈɑːgrəfi/
  Phonetic Spelling: ee·lek·troh·my·ah·gruh·fee
- Interphalangeal
  Pronunciation link: No confirmed link found
  IPA: /ˌɪn.tər.fəˈlæn.dʒi.əl/
  Phonetic Spelling: in·ter·fuh·lan·jee·uhl
- Excitability
  Pronunciation link: https://www.merriam-webster.com/dictionary/excitability
  IPA: /ɪkˌsaɪ.təˈbɪl.ə.ti/
  Phonetic Spelling: ik·sai·tuh·bil·ih·tee