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Title: Assessing Primary Motor Cortex Excitability and Excitability Modulation by Pairing Transcranial Magnetic Stimulation with Electromyography

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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, 11 done (2 remaining, will be Shot by the videographer)**

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: 26
Number of Shots: 55 (13 SC)

Introduction

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

1.1. **Daniel Rodrigues da Silva:** Our research investigates how standardized TMS-EMG protocols can provide stable and clinically meaningful markers of neuroplasticity, ultimately supporting their potential use as reliable biomarkers in neuropsychiatric research and clinical practice.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **André Delgado:** The main challenges are methodological inconsistencies in the literature, including heterogeneous stimulation parameters, limited standardization of EMG protocols, and small sample sizes, reducing reliability and reproducibility of experimental results.

1.2.1. 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?

1.3. **Carolina Seybert:** We demonstrated that a structured and comprehensive protocol offers a replicable framework for investigating neuroplasticity-like phenomena in the human brain, providing a strong foundation for translational research and potential future clinical applications.

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

What advantage does your protocol offer compared to other techniques?

1.4. **Francisco Faro Viana:** Our protocol was built by combining neuronavigation, validated EMG procedures, and optimized stimulation parameters, ensuring precise coil placement, stable recordings, and reliable results across participants and sessions.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2*

What questions will future research focus on?

1.5. **Albino J. Oliveira-Maia:** Future research should test whether excitability modulation reliably distinguishes healthy individuals from those with neuropsychiatric conditions, such as major depressive disorder, exploring group-specific neuroplasticity differences, diagnostic sensitivity and prognostic value.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

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. **Albino J. Oliveira-Maia, Director of Neuropsychiatry at the Champalimaud Foundation**: (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: 2.3.1*

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. **Albino J. Oliveira-Maia, Director of Neuropsychiatry at the Champalimaud Foundation**: (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: 3.2.1*

Ethics Title Card

This research has been approved by the Ethics Committees at the Champalimaud Foundation

Protocol

2. Setup Requirements and Preparation

Demonstrators: Daniel Rodrigues da Silva, Francisco Faro Viana, André Delgado

2.1. To begin, plug the medical-grade silver or silver chloride electrodes into the corresponding contacts to prepare three electrodes **[1]**. Connect the cables with the electrodes to the EMG board **[2-TXT]**. Connect a cable from the TMS machine to the EMG board to output the trigger signal for each TMS pulse **[3-TXT]**.

2.1.1. WIDE: Talent plugging in three silver/silver chloride electrodes into the designated electrode ports.

2.1.2. Talent connecting the cables with the electrodes to the EMG board. **TXT: EMG: Electromyography**

2.1.3. Talent connecting a cable from the TMS machine to the EMG board. **TXT: TMS: Transcranial Magnetic Stimulation**

2.2. On one of the computer monitors, open the BONSAI *(bon-zai)* software and load the EMG data acquisition script **[1]**. Click the **Start** button to start data acquisition only when ready **[2]**.

2.2.1. SCREEN: 68897_screenshot_1 00:03 – 00:10

2.2.2. SCREEN: 68897_screenshot_1. 00:11 – 00:18.

2.3. Attach paper strips over all areas of the TMS machine screen where the maximum stimulation output percentage is displayed, to blind the technician during resting motor threshold and active motor threshold assessment **[1]**.

2.3.1. Talent taping paper strips over multiple sections of the TMS machine screen, covering the maximum stimulation output display.

2.4. Position a tripod-mounted camera to maintain a clear view of all neuronavigation components throughout the entire session **[1]**. Then, place the coil calibration tracker and the calibration board on top of the TMS coil **[2]**. Now, launch the neuro-navigation system software, input the participant identification number, and proceed to calibrate all neuronavigation components **[3]**.

2.4.1. Talent adjusting the tripod and angling the camera to capture a wide field of view including all neuronavigation components.

2.4.2. Talent placing the calibration tracker and board securely onto the TMS coil.

2.4.3. SCREEN: 68897_screenshot_1 00:15-00:20; 00:39-00:43.

3. Preparing the Participant for the Procedure

3.1. After positioning the participant, use water sandpaper to gently scrub the skin over the first dorsal interosseous to improve the electrode-skin interface **[1]**. Rinse the area with gauze embedded in alcohol and repeat the same procedure over the contralateral elbow **[2]**. Once the skin is dry, place the recording electrode on the contralateral hand, aligning the center approximately 2.5-centimeters along the direction of the muscle tendon over the first dorsal interosseous. **[3]**. Then, place the ground electrode over the contralateral elbow to serve as a zero-voltage reference point **[4]**.

3.1.1. Talent gently scrubbing the FDI region with water sandpaper.

3.1.2. Talent wiping the area using alcohol-embedded gauze.

3.1.3. Talent placing the recording electrode over the first dorsal interosseous on the contralateral hand.

3.1.4. Talent placing the ground electrode on the cleaned elbow.

3.2. Place a swimming cap on the participant's head in an anterior-posterior orientation **[1]**. Align the cap with the eyebrow line at the front and the root of the helix of each ear, leaving the ears exposed **[2]**. Offer the participant ear plugs and explain how to use them **[3].**

3.2.1. Talent placing the cap over the participant's scalp with the correct orientation.

3.2.2. Talent adjusting the cap alignment to match the anatomical landmarks and ensuring ears are left uncovered.
AUTHOR'S NOTE: Shots 3.2.1-3.2.2 were filmed together

3.2.3. Talent giving the participant ear plugs and showing how to us3e them.

3.3. Draw the medial sagittal line and the intertragus line on the cap to identify their intersection point at the vertex **[1]**. From the vertex point, measure 5 centimeters to the left along the intertragus line and 5 centimeters forward along the sagittal line to define two new points **[2]**.

3.3.1. Talent using a marker to draw both the medial sagittal and intertragus lines, marking their point of intersection.

3.3.2. Talent using a measuring tape to mark two separate points — one leftward on the intertragus line and one forward on the sagittal line — from the vertex.
AUTHOR'S NOTE: In the last part of the shot, a line was drawn in the direction of the right hemisphere. Please, do not consider it.

3.4. Next, draw a diagonal line connecting the lateral point to the medial anterior point **[1]**. From each lateral point, measure 2.5 centimeters in the antero-medial direction along the diagonal lines to mark the initial estimated motor hotspot **[2]**.

3.4.1. Talent drawing a single diagonal line between the two previously marked points on the cap.

3.4.2. Talent measuring and marking 2.5 centimeters antero-medially from the lateral point to indicate the estimated motor hotspot.

3.5. Around the initial motor hotspot estimate, mark four additional points spaced within a 0.5-centimeter radius to serve as alternative testing sites **[1]**. ~~Highlight the overall region being considered for the motor hotspot using a red marker line **[2].**~~

3.5.1. Talent marking four surrounding points around the initial estimate using a marker and a ruler.

3.5.2. ~~Talent drawing a red outline over the broader hotspot region~~.
AUTHOR'S NOTE: Shot moved as 4.2.4

3.6. Next, place the elastic headband onto the participant's head, making sure that the marker strip is facing the camera and that the band fits comfortably **[1]**. Using the pointer tool, define key anatomical landmarks on the participant's head, including the nasion, left and right tragus, and head shape points **[2]**. This allows the system to align and track stimulation targets based on head and brain anatomy **[3]**.

3.6.1. Talent placing and adjusting the elastic headband, ensuring the marker strip is camera-facing and properly aligned.

3.6.2. Talent using the pointer to tap and mark anatomical landmarks on the participant's head.

3.6.3. SCREEN: 68897_screenshot_3. 00:01-00:10.

4. Measuring the Motor Hotspot and Establishing the Resting Motor Threshold (rMT)

4.1. Position the TMS coil tangentially on the participant's scalp with the handle angled posteriorly at 45 degrees to the midsagittal line **[1-TXT]**.

4.1.1. Talent placing the TMS coil on the scalp with correct angle and alignment. ~~**TXT: Place the coil's center directly over the previously marked location**~~

4.2. Deliver pulses at 30 percent of the maximum stimulation output over the initial motor hotspot estimate **[1]**. Then, increase stimulation intensity in 5 to 10 percent increments of maximum stimulation output until a consistent motor response is observed **[2]**. Deliver single pulses at the initial estimate and surrounding marked locations to determine which site most consistently produces high-amplitude motor evoked potentials and isolated first dorsal interosseous contractions **[3]**. Highlight the overall region being considered for the motor hotspot using a red marker line **[4]**.
NOTE: VO for moved shot has been added

4.2.1. Talent pressing the trigger to apply stimulation while holding the coil over the initial hotspot.

4.2.2. Talent adjusting the device settings.

4.2.3. Talent applying single pulses at multiple marked sites while observing participant's hand and the monitor.

Moved shot: "3.5.2" Talent drawing a red outline over the broader hotspot region.

4.3. In the neuronavigation software, select the pulse that produced the most reliable response to define the motor hotspot **[1]**.

4.3.1. SCREEN: 68897_screenshot_3. 00:40 – 00:48.

4.4. To establish the resting motor threshold or rMT *(R-M-T)*, use the neuronavigation system to guide the coil back to the identified motor hotspot **[1]**. Deliver 10 single-pulse stimulations, spaced 5 seconds apart, and identify the minimum percentage of maximum stimulation output that produces at least 5 motor evoked potentials exceeding 50 microvolts in the contralateral first dorsal interosseous **[2]**.

4.4.1. Shot of the coil being aligned to the previously identified hotspot.

4.4.2. Talent delivering single pulses and monitor showing visible motor evoked potential thresholds.

4.5. Starting from the previously defined motor hotspot intensity, reduce the stimulation level in 2 percent increments until fewer than 5 out of 10 pulses result in a motor evoked potential of 50 microvolts or more in the contralateral first dorsal interosseous **[1]**.

4.5.1. Talent decreasing intensity on the device.

4.6. Next, increase the maximum stimulation output in 1 percent increments until 5 or more out of 10 pulses generate a 50 microvolt or higher response in the contralateral first dorsal interosseous **[1]**. Record the percentage of maximum stimulation output that defines the resting motor threshold **[2]**.

4.6.1. Shot of the monitor showing adjustments in the stimulation intensity.

4.6.2. Shot of pointing to the final resting motor threshold percentage on the screen.

5. Maximum Voluntary Muscle Activation and Establishing the Active Motor Threshold (aMT)

5.1. Instruct the participant to generate maximum voluntary activation of the first dorsal interosseous muscle by forcefully pressing the index fingernail against the base of the thumb to form a tight circle shape **[1]**. Approximately 2 seconds after the muscle contraction begins, press the space bar on the computer running the BONSAI script **[2]** to record the time point while avoiding the initial movement artifact **[3]**.

5.1.1. Participant forcefully pressing the index fingernail against the base of the thumb.
AUTHOR'S NOTE: Move as 5.2.1

5.1.2. Talent pressing the space bar.

5.1.3. SCREEN: 68897_screenshot_4.mkv 00:05-00:15

5.2. Now, ask the participant to maintain a submaximal muscle contraction between 10 and 20 percent of their maximum voluntary isometric contraction **[1]**. Use EMG feedback to monitor and guide the participant to maintain the correct contraction level **[2]**.

5.2.1. Talent instructing the participant on how to perform a submaximal contraction and begin holding it.
AUTHOR'S NOTE: Move as 5.1.1

5.2.2. SCREEN: 68897_screenshot_4.mkv 00:18-00:30

5.3. For active motor threshold, use the neuronavigation system to guide the coil to the previously identified motor hotspot **[1]**. Determine the active motor threshold as the minimum percentage of maximum stimulation output that elicits motor evoked potentials of at least 200 microvolts in 5 out of 10 pulses during slight voluntary contraction **[2]**.

5.3.1. Talent guiding the coil position using the neuronavigation interface.

5.3.2. Talent applying pulses while observing for motor evoked potentials during slight contraction.

5.4. From the resting motor threshold intensity, reduce the stimulation level by 2 percent decrements until fewer than 5 out of 10 pulses produce a 200-microvolt response **[1]**. Then, increase the intensity in 1 percent steps until at least 5 out of 10 pulses result in motor evoked potentials of 200 microvolts or more **[2]**. Record the percentage of maximum stimulation output that defines the active motor threshold **[3]**.
AUTHOR'S NOTE: All shots filmed together

5.4.1. Talent lowering stimulation intensity by 2 percent.

5.4.2. Talent adjusting the intensity settings.

5.4.3. Talent observing the readings on the monitor.

6. Defining Baseline Cortical Excitability and Assessing Cortical Excitability Modulation

6.1. Accurately position the coil over the defined motor hotspot using the neuronavigation system **[1]**. Check that surface EMG electrodes are correctly positioned over the first dorsal interosseous and verify that the signal quality shows minimal noise **[2]**.

6.1.1. Talent making fine adjustments to the coil based on neuronavigation system feedback to align with the hotspot.

6.1.2. SCREEN: 68897_screenshot_5 00:10 – 00:23

6.2. Load the single-pulse stimulation protocol on the TMS device **[1]** and enter the resting motor threshold value previously recorded. Now, begin running the stimulation protocol **[2-TXT]**.
AUTHOR'S NOTE: All shots filmed together

6.2.1. Talent navigating the device interface and loading the appropriate single-pulse protocol.

6.2.2. Talent inputting the resting motor threshold and pressing **Start** to initiate the stimulation sequence. **TXT: Assess the baseline cortical excitability; Average the peak-to-peak amplitudes of 40 motor evoked potentials**

6.3. Then, load the intermittent theta burst stimulation protocol onto the TMS device **[1]**. Enter the previously recorded active motor threshold and start the intermittent theta burst stimulation protocol **[2]**.
AUTHOR'S NOTE: All shots filmed together

6.3.1. Talent navigating through the TMS device interface and selecting the intermittent theta burst stimulation protocol for loading.

6.3.2. Talent inputting the active motor threshold value and pressing **Start** to initiate the protocol.

6.4. Immediately after the intermittent theta burst stimulation protocol ends, start a stopwatch to monitor post-stimulation intervals **[1]**. At time point zero, immediately after the end of the cortical excitability modulation protocol, reapply the single-pulse stimulation protocol using 120 percent of the resting motor threshold **[2-TXT]**.

6.4.1. Talent pressing a stopwatch or digital timer.

6.4.2. Talent initiating the single-pulse protocol again at 120 percent resting motor threshold immediately following stimulation. **TXT: Do the same at 10, 20 , 30 min after end of cortical excitability modulation protocol**
AUTHOR'S NOTE: Same as 6.2.1 and 6.2.2

6.5. Validate the neuronavigation accuracy by placing the pointer on the nasion and checking alignment in the neuronavigation system software **[1]**. Ensure that the spatial deviation is less than 5 millimeters and record the measured value **[2-TXT]**.

6.5.1. Talent placing the pointer on the nasion.

6.5.2. SCREEN: 68897_screenshot_6. 00:02 – 00:09. **TXT: Repeat single-pulse stimulation after 10, 20, and 30 min**

6.6. Finally, after completing the final stimulation block, stop the BONSAI script and finalize the neuronavigation session in the software **[1]**. Confirm that a binary data file is generated from the BONSAI acquisition session **[2]**.

6.6.1. SCREEN: 68897_screenshot_7. 00:07 – 00:11 and 68897_screenshot_8 00:02 – 00:10 *Video editor: Please use split screen to show both these recordings*

6.6.2. SCREEN: 68897_screenshot_7. 00:12 – 00:19.

Results

7. Results

7.1. Significant increases in motor evoked potential amplitude were observed at T-0 *(T-zero)* **[1]**, T-10 **[2]**, and T-20 following intermittent theta burst stimulation during the baseline session **[3]**, but not at T-30 **[4].**

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7.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the group of green dots above the dashed line under the "T0" label corresponding to baseline M1* .

7.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the group of green dots above the dashed line under the "T10" corresponding to baseline M1*.

7.1.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the group of green dots above the dashed line under the "T20" corresponding to baseline M1*.

7.1.4. LAB MEDIA: Figure 4A. *Video editor: Highlight the cluster of green dots near the dashed line under the "T30" corresponding to baseline M1*.

7.2. At follow-up, significant increases in motor evoked potential amplitude were again observed at T0 **[1]**, T10 **[2]**, T20 **[3]**, and T30 **[4]**.

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7.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the green dots above the dashed line under the "T0" corresponding to Follow-up M1*.

7.2.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the green dots above the dashed line under the "T10" corresponding to Follow-up M1*.

7.2.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the green dots above the dashed line under the "T20" corresponding to Follow-up M1*.

7.2.4. LAB MEDIA: Figure 4A. *Video editor: Highlight the green dots above the dashed line under the "T30" corresponding to Follow-up M1*.

7.3. Test-retest reliability of motor evoked potential changes at T0 showed moderate to good consistency **[1]**, with an intraclass correlation coefficient of 0.67 **[2].**

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7.3.1. LAB MEDIA: Figure 4B.

7.3.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the text "ICC = .67" near the graph*.

Pronunciation Guide:

1. **Champalimaud**
   Pronunciation link: https://www.howtopronounce.com/champalimaud
   IPA: /ʃɑːmpəˈloʊ/
   Phonetic Spelling: shahm-puh-loh
2. **transcranial**
   Pronunciation link: https://www.merriam-webster.com/dictionary/transcranial
   IPA: /ˌtrænsˈkreɪniəl/
   Phonetic Spelling: trans-kray-nee-uhl
3. **electromyography**
   Pronunciation link: https://www.merriam-webster.com/dictionary/electromyography
   IPA: /ɪˌlɛktroʊmaɪˈɑːgrəfi/
   Phonetic Spelling: ih-lek-troh-my-AH-gruh-fee
4. **neuronavigation**
   Pronunciation link: https://www.howtopronounce.com/neuronavigation
   IPA: /ˌnʊroʊˌnævɪˈgeɪʃən/
   Phonetic Spelling: noo-roh-nav-i-GAY-shun
5. **neuroplasticity**
   Pronunciation link: https://www.merriam-webster.com/dictionary/neuroplasticity
   IPA: /ˌnjʊroʊplæˈstɪsɪti/
   Phonetic Spelling: nyoo-roh-plas-TISS-ih-tee
6. **intermittent**
   Pronunciation link: https://www.merriam-webster.com/dictionary/intermittent
   IPA: /ˌɪntərˈmɪtənt/
   Phonetic Spelling: in-ter-MIT-ent
7. **theta**
   Pronunciation link: https://www.merriam-webster.com/dictionary/theta
   IPA: /ˈθeɪtə/
   Phonetic Spelling: THAY-tuh
8. **burst**
   Pronunciation link: https://www.merriam-webster.com/dictionary/burst
   IPA: /bɜrst/
   Phonetic Spelling: burst
9. **magnetic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/magnetic
   IPA: /mægˈnɛtɪk/
   Phonetic Spelling: mag-NET-ik
10. **excitability**

Pronunciation link: https://www.merriam-webster.com/dictionary/excitability
IPA: /ɪkˌsaɪtəˈbɪlɪti/
Phonetic Spelling: ik-SY-tuh-BIL-i-tee

11. **modulation**
    Pronunciation link: https://www.merriam-webster.com/dictionary/modulation
    IPA: /ˌmɑːdʒʊˈleɪʃən/
    Phonetic Spelling: mod-yuh-LAY-shun
12. **psychiatric**
    Pronunciation link: https://www.merriam-webster.com/dictionary/psychiatric
    IPA: /ˌsaɪkiˈætrɪk/
    Phonetic Spelling: sy-kee-AT-rik
13. **intraclass**
    Pronunciation link: https://www.howtopronounce.com/intraclass
    IPA: /ˌɪntrəˈklæs/
    Phonetic Spelling: in-truh-CLASS
14. **coronal**, **sagittal**
    - **coronal**
      Pronunciation link: https://www.merriam-webster.com/dictionary/coronal
      IPA: /ˈkɔːrəˌnəl/
      Phonetic Spelling: KOR-uh-nuhl
    - **sagittal**
      Pronunciation link: https://www.merriam-webster.com/dictionary/sagittal
      IPA: /ˈsædʒɪtəl/
      Phonetic Spelling: SAJ-i-tuhl
15. **anterior-posterior**
    Pronunciation link: https://www.merriam-webster.com/dictionary/anterior
    and https://www.merriam-webster.com/dictionary/posterior
    IPA: /ænˈtɪriər/ + /pɒˈstɪriər/
    Phonetic Spelling: an-TEER-ee-ər / pos-TIR-ee-ər