FINAL SCRIPT: APPROVED FOR FILMING

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Title: Neuroimaging-Guided TMS-EEG for Real-Time Cortical Network Mapping

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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: 25
Number of Shots: 58 (14 SC, 5 Lab media)

Introduction

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

1.1. **Elena Ukharova:** We're developing reproducible, fully personalized neuromodulation protocols by integrating cortical morphology, structural and functional connectivity, and neurophysiology to enhance TMS-EEG specificity for clinical biomarker discovery.
 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Ida Granö:** Our protocol enables reliable access to early TMS-evoked potentials that reflect immediate cortical reactivity in the targeted area—signals typically obscured by artefacts and poor data quality in non-personalized approaches.
 1.2.1. 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?

1.3. **Joonas Laurinoja:** We aim to automate cortical mapping using real-time algorithms, combined with multilocus TMS and robotics, to enhance precision in neuromodulation research and improve TMS efficacy in clinical applications.
 1.3.1. 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):

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

1.4. **Elena Ukharova:** We aim to share best practices in TMS-EEG as interest in the methodology expands across research and clinical fields. Visual demonstration of the key procedures makes training more accessible and enables high-quality data acquisition without the need for in-person instruction and supervision.
    1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

Ethics Title Card

This research has been approved by the Hospital District of Helsinki and Uusimaa Ethics Committee

Protocol

2. Preparing the Patient and the Electroencephalography (EEG) Setup

Demonstrators: Elena Ukharova, Ida Granö

2.1. To begin, seat the subject in a chair positioned close enough for the cap cables to connect to the electroencephalography system **[1]**.

2.1.1. WIDE: Talent positioning the subject in a chair with cables visible, leaving open space around the chair.

2.2. Measure the subject's head circumference to select a matching electroencephalography cap **[1]**.

2.2.1. Talent wrapping a measuring tape around the subject's head.

2.3. Place the cap on starting from the forehead while keeping the hair under the cap **[1]**. Measure the distance from the nasion to the inion and from left to right tragus **[2],** then adjust the cap position to ensure Cz *(C-zee)* is centered halfway between these anatomical landmarks **[3]**.

2.3.1. Talent positioning the cap on the forehead and pulling it over the head.

2.3.2. Talent measuring the nasion-inion and inter-tragus distances using a measuring tape.

2.3.3. Talent adjusting the cap position to align Cz properly.

2.4. Using alcohol wipes and abrasive tape, clean the mastoid and zygomatic bone skin opposite to the stimulation side to improve conductivity **[1]**. Place the ground electrode on the zygomatic bone **[2]** and the reference electrode on the mastoid using ring electrode washers **[3]**.

2.4.1. Talent wiping the mastoid and zygomatic regions with alcohol and tape.

2.4.2. Talent attaching ground electrode on the zygomatic bone.

2.4.3. Talent attaching the reference electrode on mastoid.

2.5. Apply an abrasive paste to the ground electrode sites **[1-TXT]** and lightly scrub the skin with a blunt needle **[2]**. Then, fill each electrode with conductive gel **[3]**.

2.5.1. Talent applying the abrasive paste to the ground electrodes. **TXT: Repeat the procedure for the reference electrode** **NOTE**: 2.5.1, 2.5.2 and 2.5.3 may have been shot together

2.5.2. Shot of scrubbing skin using blunt needle.

2.5.3. Talent filling electrodes with conductive gel.

2.6. Confirm that the ear slits are correctly positioned for ear access during neuronavigation and earphone placement **[1]**. Now, secure the cap in place using hook-and-loop fasteners under the chin **[2]**.

2.6.1. Talent pointing to the alignment of ear slits to ensure accessibility.

2.6.2. Talent helping the subject to fasten the cap strap beneath the subject's chin.

2.7. To prepare the cap electrodes, apply abrasive gel and use a blunt needle to clear hair from under each electrode until the skin is visible **[1-TXT]**. Fill the electrode with conductive gel while gently pressing it down to ensure adequate but not excessive gel is applied **[2]**.

2.7.1. Talent applying gel and removing hair from beneath the cap electrode using a blunt needle, using a cross motion. **TXT: Move in an 'up-down-left-right' fashion**

2.7.2. Talent pressing and filling the cap electrode with gel.

2.8. If the impedance is high after initial preparation, use a blunt needle or cotton swab to scrub the skin inside the electrode again while avoiding hair displacement **[1]**. Then, refill the electrode with conductive gel **[2]** and check impedance levels, repeating until all are below 5 kiloohms **[3]**.

2.8.1. Talent swirling a swab or needle inside an electrode to rescrub the contact area.

2.8.2. Talent refilling the electrode.

2.8.3. Talent looking at the impedance on the screen.

3. Electromyography (EMG) and Neuronavigation Preparation

3.1. Clean each electrode site with alcohol wipes **[1]**, lightly scratch the skin with abrasive tape **[2]**, and wipe again with alcohol before letting it dry **[3]**.

3.1.1. Talent cleaning electrode with alcohol wipe.

3.1.2. Talent lightly scratching the skin with abrasive tape.

3.1.3. Talent wiping the electrode site with alcohol and keeping it aside.

3.2. Place the active electrode on the muscle belly, typically the right abductor pollicis brevis or first dorsal interosseous **[1]**. Place the reference electrode over the muscle tendon **[2]** and the ground electrode on the dorsum of the hand **[3]**.

3.2.1. Talent attaching the active electrode to the muscle belly. **NOTE**: 3.2.1, 3.2.2 and 3.2.3 was shot together

3.2.2. Talent placing the reference electrode over the muscle tendon.

3.2.3. Talent placing the ground electrode on the dorsum of the hand.

3.3. For neuronavigation preparation, position the subject comfortably in the chair, ensuring the neck, hands, and legs are relaxed **[1-TXT]**.

3.3.1. Talent adjusting the chair head rest and subject's neck position. **TXT: Adjust the chair height as needed**

3.4. Now, secure the head tracker and ensure that it is stable during the stimulation **[1]**.

3.4.1. Talent applying double-sided medical tape to the head tracker.

~~3.5. Identify the cardinal points, including the nasion and preauricular points, on the subject's magnetic resonance imaging []. **[1]**.~~

3.5.1. ~~LAB MEDIA: Display the MRI scan with highlighted nasion and preauricular points~~. **NOTE**: Not provided, VO merged with the next step

3.6. Identify the cardinal points, including the nasion and preauricular points, on the subject's magnetic resonance imaging **[1]** and using a digitizing pen, mark the corresponding cardinal points on the subject's head to view the ear **[2, 3]** and the nasion landmarks **[4, 5]**.

3.6.1. Talent marking anatomical landmarks on the subject's head with the digitizing pen.

3.6.2. Close-up shot of the preauricular points placed near the ear. **NOTE**: Use 3.6.2. take 1 *Video Editor: Use a split screen to show both 3.6.2 and 3.6.3 simultaneously*

3.6.3. LAB MEDIA: 67339_screenshot_2.png **NOTE**: Show the 2 MRI images on the top left only

3.6.4. Close-up shot of the preauricular points placed near the nose. **NOTE**: Use 3.6.2. take 2 here *Video Editor: Use a split screen to show both 3.6.4 and 3.6.5* *simultaneously*

3.6.5. LAB MEDIA: 67339_screenshot_1.png **NOTE**: Show the 2 MRI images on the top left only

3.7. Digitize additional points across the skull surface to reduce registration errors **[1]**.

3.7.1. Talent marking multiple points on the skull with the digitizing pen.

4. Noise Masking and Determination of Resting Motor Threshold (RMT)

4.1. Prepare the noise masking using a looped audio track of white noise mixed with recorded coil clicks from the specific coil used **[1]**.

4.1.1. SCREEN: Show the loading and playback of the looped noise file with waveform display. Authors, it is better if the videographer films this. Please perform the steps on the computer so that it is an 'in action' video.

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

4.2. Ensure that the subject places eartips of noise masking headphones correctly into the ear canals **[1]**. Then, place the coil approximately five centimeters above the subject's head **[2]**. ~~Explain to the subject that masking noise will gradually increase and ask the subject to indicate when coil clicks are no longer distinguishable from the noise masking~~. Deliver high-intensity pulses with a jittered interstimulus interval **[3]** and gradually increase the PC volume by 1 to 2 percent until the subject indicates that they no longer hear the click **[4]**. **NOTE**: VO edited

ADDED shot: talent giving the earphones to the subject and looking that they are placed correctly.

4.2.1. Talent placing the coil 5 cm above the subject's head.

~~4.2.2. Talent explaining the subject.~~**NOTE**: Not filmed

4.2.3. SCREEN: Display stimulation control panel with pulse delivery.

4.2.4. Talent increasing the volume slider incrementally.

4.3. Deliver 20 to 30 pulses while monitoring real-time transcranial evoked potential visualization **[1]**. If an auditory evoked potential is observed, increase the masking noise volume in 2 percent increments until the component disappears **[2]**.

4.3.1. SCREEN: Show waveform trace of TEPs in real time during pulse delivery.

4.3.2. LAB MEDIA: Show both 67339_screenshot_3.png and 67339_screenshot_4.png side by side *Video editor: Show only the right part with green waveforms*

4.4. To determine resting motor threshold, instruct the subject to keep their muscles relaxed with the palm facing upwards **[1]**.

4.4.1. Talent verbally instructing and subject relaxing hands with palms up.

4.5. Position the transcranial magnetic stimulation coil over the motor knob with the electric field perpendicular to the sulcus **[1]**. Begin with approximately 30 percent of maximum stimulator output **[2]** and increase intensity until localized muscle activation is seen **[3]**. Adjust coil location and orientation until a response specific to the abductor pollicis brevis is observed **[4]**.

4.5.1. SCREEN: Show the placement of the coil on the MRI in neuronavigation.

4.5.2. SCREEN: Beginning with 30 percent of maximum stimulator output.

4.5.3. SCREEN: Show EMG responses to several pulses with the stimulation intensity visible. **Author's NOTE:** 4.5.1, 4.5.2, 4.5.3 were taken in one shot, select the period with visible EMG responce on upper rigt side of the left screen.

4.5.4. Talent adjusting coil position for consistent muscle response in target.

4.6. Using the saved location, apply automated algorithms in the system to determine the resting motor threshold **[1]**.

4.6.1. A shot of the subject while the algorithm is running, so the hand movement in response to the pulses is visible.

5. Cortical Mapping and Transcranial Magnetic Stimulation (TMS)-EEG Data Recording

5.1. For cortical mapping, overlay the anatomical parcellations and functional connectivity maps from the MRI in the neuronavigation software **[1]**. Define at least 2 to 3 stimulation targets based on promising structural and functional connectivity **[2]**.

5.1.1. SCREEN: Show MRI overlays with brain maps and connectivity data. **NOTE**: 2 takes were filmed

5.1.2. SCREEN: Show real-time tractography for the approximate areas shown in the neuronavigation. **Author's NOTE:** It is a longer take, select a period where there are noticeably different streamlines.

5.2. Begin stimulation at 100 to 110 percent of resting motor threshold with noise masking active **[1]**. Average 20 pulses per set and monitor the resulting transcranial evoked potentials **[2]**. Adjust the intensity in 2 percent maximum stimulator output increments until the early response amplitude exceeds 6 microvolts **[3]**.

5.2.1. Show the subject so the position of the coil is visible as well as the neuronavigation screen with stimulation intensity.

5.2.2. SCREEN: Display averaged waveform across 20 pulses with overlaid intensity,

5.2.3. SCREEN: zoom into early latency range on TEPs to show the amplitude, zoom in to show that amplitude is less than 6 microvolts.

5.3. Monitor the signal for decay artifacts, recognizable by their exponential nature as well as bipolar muscle artifact **[1]**. If muscle artifacts last longer than 15 milliseconds, try to rotate the coil to minimize them **[2, 3]**. Proceed until a clean, early component above 6 microvolts is achieved **[4]**.

5.3.1. SCREEN: Display muscle artifact example on the TEP traces. **NOTE**: Show both the takes

5.3.2. Talent rotating the coil slightly to optimize signal.

5.3.3. SCREEN: Show reduced muscle artifact and click on later peaks to show latencies.

ADDED SHOT: shot of good TEP (F3, F1 electrodes).

5.4. If coil rotation doesn't improve signal quality, try to shift the stimulation location a few millimeters and repeat the process **[1-TXT]**, mapping the cortical responses across the cortical area **[2]**. If the artifacts persist, proceed to investigate the next predefined cortical target **[3]**.

5.4.1. Talent shifting the coil a bit to the side, the stimulation location movement is visible on the neuronavigation screen. **TXT: Repeat the process multiple times to obtain a distinct brain signal**

5.4.2. SCREEN: Show the points of where stimulation was delivered around the original target.

5.4.3. Talent shifting the coil to the next location with neuronavigation visible in the view.

5.5. When the stimulation target and parameters are optimized, measure electrode impedance, before starting TMS–EEG data recording, **[1]**. If impedance exceeds 5 kiloohms, add conductive gel to reduce it. If still too high, repeat preparation and add more gel only if required **[2-TXT]**.

5.5.1. LAB MEDIA: 67339_screenshot_5.png.

5.5.2. Talent applying additional gel to specific electrode sites. **TXT: Collect a minimum of 300 trials using the finalized parameters**

~~5.5.3. Talent re-prepping the electrode site with abrasive gel.~~ **NOTE**: Not filmed, VO merged with the previous shot

~~5.6. Finally, collect a minimum of 300 trials using the finalized stimulation location, orientation, and intensity with noise masking **[1]**.~~

5.6.1. ~~LAB MEDIA: Show averages of 25 to 30 pulses, 100 to 110, 200 to 210 and final 10. **Authors**, please provide a still screenshot for this~~ **NOTE**: Not filmed, VO added as on screen text to the previous shot

Results

6. Results

6.1. The initial stimulation site selected from the fMRI cluster showed anti-correlation with the subgenual anterior cingulate cortex and tractography, indicating primarily cross-callosal and ipsilateral connections to the contralateral frontal pole **[1]**.

6.1.1. LAB MEDIA: Figure 3A, B.

6.2. A potential TEP-like response is observed in the F3, F1, and FC3 electrodes **[1]**. The AF3 electrode exhibits a large-amplitude ringing artifact **[2]**, while F5 is affected by a small muscle artifact recognizable by the high-frequency high-amplitude peak right after the TMS pulse **[3]**.

6.2.1. LAB MEDIA: Figure 3C. *Video editor: Highlight F3, F1, FC3 graphs*.

6.2.2. LAB MEDIA: Figure 3C *Video editor: Highlight AF3*.

6.2.3. LAB MEDIA: Figure 3C *Video editor: Highlight F5 plot*.

6.3. Preprocessing confirmed the F3 response was unaffected by artifacts, preserving the amplitude of the early component **[1]**.

6.3.1. LAB MEDIA: Figure 3G. *Video editor: Emphasize the F3 plot* .

6.4. Switching to a posterior-anterior coil orientation increased muscle artifacts and discomfort, with strong artifacts visible in all channels **[1]**.

6.4.1. LAB MEDIA: Figure 4A and B.

6.5. Using a lateral-medial coil orientation produced a clean, large-amplitude TEP at the F3 electrode, peaking at 12 microvolts within 20 milliseconds **[1]**.

6.5.1. LAB MEDIA: Figure 4 E and F. *Video editor: Focus on F3*.

6.6. The final stimulation target with lateral-medial coil orientation, located near the initial one, exhibited broader structural connectivity than earlier targets **[1]**.

6.6.1. LAB MEDIA: Figure 6A and B.

6.7. After minimal preprocessing, a 9-microvolt early component was detected between 17 and 35 milliseconds in the F3 electrode, free of muscle artifact **[1]**. Full preprocessing of the 300-trial dataset preserved the waveform shape, showing a reduced 6-microvolt early response between 20 and 40 milliseconds **[2]**.

6.7.1. LAB MEDIA: Figure 6D.

6.7.2. LAB MEDIA: Figure 6F.

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1. Electroencephalography

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/electroencephalography
- **IPA:** /i-ˌlek-trō-in-ˌsef-ə-ˈlä-grə-fē/
- **Phonetic Spelling:** ee-lek-troh-in-sef-uh-LAW-gruh-fee(merriam-webster.com)

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2. Nasion

- **Pronunciation link:** https://www.merriam-webster.com/medical/nasion
- **IPA:** /ˈnā-zē-ˌän/
- **Phonetic Spelling:** NAY-zee-ahn(merriam-webster.com)

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3. Inion

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/inion
- **IPA:** /ˈin-ē-ən/
- **Phonetic Spelling:** IN-ee-uhn(merriam-webster.com, merriam-webster.com, merriam-webster.com)

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4. Tragus

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/tragus
- **IPA:** /ˈtrā-gəs/
- **Phonetic Spelling:** TRAY-gus(merriam-webster.com, merriam-webster.com)

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5. Zygomatic

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/zygomatic
- **IPA:** /ˌzī-gə-ˈmat-ik/
- **Phonetic Spelling:** ZYE-guh-MAT-ik(merriam-webster.com, merriam-webster.com)

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6. Mastoid

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/mastoid
- **IPA:** /ˈmas-ˌtȯid/
- **Phonetic Spelling:** MAS-toid(merriam-webster.com, merriam-webster.com)

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7. Abductor Pollicis Brevis

- **Pronunciation link:** https://www.merriam-webster.com/medical/abductor%20pollicis%20brevis
- **IPA:** /ab-ˌdək-tər ˈpäl-ə-səs ˈbrev-əs/
- **Phonetic Spelling:** ab-DUK-tor PAHL-uh-sis BREV-iss(merriam-webster.com)

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8. Interosseous

- **Pronunciation link:** https://www.merriam-webster.com/medical/interosseous
- **IPA:** /ˌin-tə-ˈräs-ē-əs/
- **Phonetic Spelling:** in-ter-OSS-ee-us(merriam-webster.com, merriam-webster.com)

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9. Dorsum

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/dorsum
- **IPA:** /ˈdȯr-səm/
- **Phonetic Spelling:** DOR-sum(merriam-webster.com, merriam-webster.com, merriam-webster.com)

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10. Preauricular

- **Pronunciation link:** https://www.merriam-webster.com/medical/preauricular
- **IPA:** /ˌprē-ȯ-ˈrik-yə-lər/
- **Phonetic Spelling:** pree-aw-RIK-yuh-lur(merriam-webster.com, merriam-webster.com)

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11. Tractography

- **Pronunciation link:** https://www.howtopronounce.com/tractography
- **IPA:** /træk-ˈtɒ-grə-fi/
- **Phonetic Spelling:** trak-TOG-ruh-fee(merriam-webster.com)

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12. Sulcus

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/sulcus
- **IPA:** /ˈsəl-kəs/
- **Phonetic Spelling:** SUL-kus(merriam-webster.com)

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13. Subgenual

- **Pronunciation link:** https://www.howtopronounce.com/subgenual
- **IPA:** /səb-ˈjen-yu-əl/
- **Phonetic Spelling:** sub-JEN-yoo-uhl

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14. Cingulate

- **Pronunciation link:** https://www.howtopronounce.com/cingulate
- **IPA:** /ˈsɪŋ-gyə-lət/
- **Phonetic Spelling:** SING-gyuh-lut

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15. Ipsilateral

- **Pronunciation link:** https://www.howtopronounce.com/ipsilateral
- **IPA:** /ˌɪp-sə-ˈlæt-ər-əl/
- **Phonetic Spelling:** IP-suh-LAT-uh-rul(merriam-webster.com)

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16. Contralateral

- **Pronunciation link:** https://www.howtopronounce.com/contralateral
- **IPA:** /ˌkɒn-trə-ˈlæt-ər-əl/
- **Phonetic Spelling:** KON-truh-LAT-uh-rul

17. Parcellation

- **Pronunciation link:** https://www.howtopronounce.com/parcellation
- **IPA:** /ˌpɑːr-sə-ˈleɪ-ʃən/
- **Phonetic Spelling:** PAR-suh-LAY-shun(merriam-webster.com)