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Title: Reliable Acquisition of Electroencephalography Data During Simultaneous Electroencephalography and Functional MRI

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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? **No**

3. Interview statements: Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 26
Number of Shots: 43

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Hui Ming Khoo:** Our protocol provides important steps that ensure data quality, particularly the quality of EEG data, which is key to the success of a simultaneous EEG-fMRI study but is rarely emphasized.

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

1.2. **Hui Ming Khoo:** This protocol is straightforward and uses only readily available medical products. Therefore, it is very easy to follow.

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

OPTIONAL:

1.3. **Hui Ming Khoo:** This protocol leads to better quality and readability of the EEG acquired simultaneously with fMRI, which is particularly useful for identifying subtle EEG changes in epilepsy studies.

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

1.4. **Yuya Fujita**: This protocol is also useful for identifying subtle EEG events in single-trial ERPs of neurocognitive studies.

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

Ethics Title Card

1.5. The research ethics committee of Osaka University Hospital and the safety committee of the Center for Information and Neural Networks (CiNET) approved the protocol.

2. Preparation of the Experimental Setup and Application of the EEG Cap and ECG Electrode.

2.1. To begin, connect the MRI-compatible EEG and bipolar amplifiers to the fully charged battery packs and to the recording computer **[1].** Ensure that the workspace of the recording software is set up correctly **[2]**.

2.1.1. Talent connecting the EEG and bipolar amplifiers to the battery and computer.
2.1.2. Talent setting up the recording software.

2.2. Load a 10-milliliter syringe with the abrasive conductive gel for application of the EEG cap **[1].**

2.2.1. Talent loading the syringe with conductive gel.

2.3. Measure the head circumference of the subject by wrapping a flexible non-stretchable measuring tape around the head over the supraorbital ridges and the occiput **[1].** Select an appropriately sized cap that is 1 centimeter larger than the head circumference and make sure that it is comfortable for the subject **[2].**

2.3.1. Talent measuring the head circumference of the subject using tape.

2.3.2. Talent giving appropriately sized cap to the subject.

2.4. After placing the cap at the approximate position over the subject's head, measure the lengths of the inion-nasion arc and mark the intersection of the inion-nasion arc and the peri-auricular arc **[1].**

2.4.1. Talent measuring the length of inion-nasion arc and marking the site of intersection of inion-nasion arc and peri-auricular arc.

2.5. Slide the cap over the head so that the position of electrode Cz *(C-Z)* is adjusted to this intersection. Ensure that the cap is not rotated horizontally by manually checking whether electrodes Fz, Pz, Oz, *(Spell out)* Reference, and Ground are positioned over the inion-nasion arc **[1]**.

2.5.1. Talent manually checking position of the electrodes.

2.6. Expose the skin underneath each electrode by displacing the hair to the side using the back of a cotton swab. Rub the skin beneath each electrode by quickly spinning a cotton swab containing 70% alcohol solution placed through the opening of the electrode **[1].** *Videographer: This step is important!*

2.6.1. Talent displacing the hair and rubbing the skin beneath each electrode with ethanol.

2.7. Apply a small amount of the abrasive conductive gel in the opening **[1]** and abrade the skin by quickly spinning a cotton swab **[2].** *Videographer: This step is difficult!*

2.7.1. Talent applying a small amount of abrasive gel into the opening.

2.7.2. Talent abrading the skin by spinning a cotton swab.

2.8. Monitor the impedance of the electrode **[1]** and repeat the abrasion until the impedance drops to at least below 20 kilo ohms **[2].**

2.8.1. Talent monitoring the impedance of the electrode.

2.8.2. Talent abrading the skin again with the gel.

2.9. Once the impedance is satisfactory, fill the opening with the same gel, making sure not to apply excessive gel in the opening to avoid bridging between electrodes **[1-TXT].** Before placing the ECG electrode at the back, ask the subject to sit upright without flexing the neck **[2].**

2.9.1. Talent filling the opening with the gel. **TEXT: Repeat for all the scalp EEG electrodes**

2.9.2. Subject sitting upright without flexing the neck.

2.10. Place the ECG electrode 2 to 3 centimeters left from the median furrow, making sure that the wire is straight at the back but with allowance along the curve of the neck to avoid displacement when the subject lays down on the MRI table **[1].** Rub the skin underneath the ECG electrode with an alcohol swab **[2].** *Videographer: This step is important!*

2.10.1. Talent placing the ECG electrodes.

2.10.2. Talent rubbing the skin beneath the electrodes with alcohol.

2.11. Attach the ECG electrode to the skin using a double-sided adhesive ring **[1]** and monitor the impedance by repeating the abrasion of skin with a cotton swab. **[2].**

2.11.1. Talent attaching the ECG electrode to the skin with adhesive tapes.

2.11.2. Talent monitoring the impedance while abrading the skin with gel and swab.

2.12. Fold the dry alcohol cotton swab into four and place it on the ECG electrode, then tape the swab to the skin using a surgical adhesive tape **[1]**. Tape the ECG electrode wire to the skin up to the shoulder **[2]**. *Videographer: This step is important!*

2.12.1. Talent placing the folded alcohol cotton swab on the ECG electrode and taping the swab to the skin.

2.12.2. Talent taping the electrode wire to the shoulder.

3. Applying the Carbon Wire Loop and Securing the Cap and the Carbon Wire Loops.

3.1. Place a set of pre-braided carbon wires consisting of six loops over the cap so that the wires are in parallel with the bundle of electrodes on top of the head **[1].**

3.1.1. Talent placing set of pre-braid carbon wire over the cap.

3.2. Use surgical tape to secure the loops around the electrodes, so that the loops cover the head with each loop covering almost an equal area **[1-TXT].** *Videographer: This step is important!*

3.2.1. Talent securing the loops using surgical tape. **TEXT: EEG electrodes must not form loops**

3.3. Wrap the subject's head with an elastic bandage over the EEG cap and the carbon loops, making sure that the bandage covers all the electrodes and is not too tight. Ask whether the subject feels uncomfortable pressure on the head while applying the bandage **[1].** *Videographer: This step is important!*

3.3.1. Talent wrapping the head with elastic bandage over the EEG cap.

4. Placing the Subject in the MR Scanner

4.1. For resting-state acquisition, instruct the subject to apply MRI-compatible earbuds. Use a headset or earphones for task-based acquisition, making sure that the subject can hear through both sides of the headset or earphones **[1].**

4.1.1. Subject wearing MRI-compatible earbuds. Videographer NOTE: Take 2 has no slate (filename: A005_02051204_C188.mov)

4.2. Place an MRI-compatible flat memory foam pillow in the lower half of the head coil before asking the subject to lie down and place the head in the coil **[1]**, then place the electrode and carbon wire bundles straight through the top opening of the head coil **[2].**

4.2.1. Talent placing flat memory foam pillow in the lower half of the head coil.

4.2.2. Talent placing the electrode and carbon wire bundles through top opening of head coil.

4.3. Add memory foam pillows to the top of the head, forehead, and temporal area, filling up all spaces left within the head coil while not compressing the subject's head too tightly **[1].** *Videographer: This step is important!*

4.3.1. Talent adding memory foam pillows to the head, forehead, and temporal area.

4.4. Place the upper half of the head coil and close the coil, making sure that the pillows are not squeezing the head **[1].**

4.4.1. Talent placing the upper half of the head coil and closing it.

4.5. Place a half cylinder-shape memory foam pillow at the back of the neck so that the ECG electrode wire is sandwiched well between the pillow and the neck **[1].** After placing all the memory foam pillows, test that the subject can still hear through both sides of the headsets or earphones **[2].**

4.5.1. Talent placing half cylinder-shape pillow at the back of the neck.

4.5.2. Talent testing the hearing of the subject.

4.6. Place the mirror and instruct the subject to adjust the mirror once the subject's head is at the isocenter of the MRI bore **[1].** Connect the amplifiers placed at the back of the MRI bore to the recording computer using optic fibers **[2].**

4.6.1. Talent placing and adjusting the mirror.

4.6.2. Talent connecting the amplifiers to the recording computer using optic fibers. Videographer NOTE: This shot also contains content for shot 4.7.1, recorded from an LCD monitor connected to a hard-wired camera in the MRI room

4.7. After connecting the EEG electrodes, ECG electrodes, and the carbon wire loops to the EEG and the bipolar amplifiers at the back of the MRI bore, switch on the amplifiers **[1]**. Check the impedance of all electrodes to make sure that it is still low **[2-TXT].**

4.7.1. Talent switching on the amplifiers. Videographer NOTE: recorded from an LCD monitor connected to a hard-wired camera in the MRI room

4.7.2. Talent checking the impedance of all the electrodes. **TEXT: < 20 kΩ.**

5. Configuration of Wires and Amplifiers with EEG-fMRI Data Acquisition

5.1. Arrange all wires between the outlet of the top opening of the head coil and the amplifiers so that they are placed straight and at the center of the MRI bore **[1].**

5.1.1. Talent arranging the wires. Videographer NOTE: 5.1.1 – 5.3.3 recorded from an LCD monitor connected to a hard-wired camera in the MRI room

5.2. Place one carbon wire loop around the ribbon cable going from the EEG or ECG electrodes connector box to the amplifier **[1]** and connect all the carbon wire loops to the input box of the bipolar amplifier **[2].**

5.2.1. Talent placing one carbon wire loop around the ribbon cable.

5.2.2. Talent connecting all the carbon wire loops to the input box of bipolar amplifier.

5.3. Immobilize the wires by sandwiching all of them with MR-safe and non-ferromagnetic sandbags between the outlet of the top opening of the head coil and the amplifiers **[1]**. Place sandbags on the amplifiers **[2]** and position the amplifiers outside the bore of the magnet using cables supplied by the manufacturer **[3].** *Videographer: This step is important!*

5.3.1. Talent sandwiching the wires with MR-safe and sandbags.

5.3.2. Talent placing sandbags on the amplifiers.

5.3.3. Talent positioning the amplifiers outside the bore of the magnet.

5.4. Communicate with the subject from the console room to confirm that the subject can hear the operator and instruct the subject not to move during data acquisition **[1]**. Start the EEG recording before starting fMRI acquisition **[2].**

5.4.1. Talent confirming the hearing of the subject.

5.4.2. Talent starting the EEG recording. Videographer NOTE: This shot was mis-slated as also containing 5.5.1 content, but does not contain 5.5.1 content (this is a separate shot).

5.5. Check whether markers from the scanner and volume trigger are periodically displayed in the online EEG recording **[1].**

5.5.1. Talent checking the EEG recording.

Results

6. EEG signals from subjects who participated in neurocognitive and epilepsy studies.

6.1. Representative EEG signals obtained from subjects who participated in the neurocognitive study and epilepsy study using this protocol are shown here. The EEG signals acquired from both studies were similar before processing **[1].**

6.1.1. LAB MEDIA: Figure 2.

6.2. The resultant EEG signals from both studies were of analyzable quality without visible contamination of BCG artifacts, as seen here **[1].** Epileptic activities were clearly seen on the EEG during the epilepsy study **[2].**

6.2.1. LAB MEDIA: Figure 3A.

6.2.2. LAB MEDIA: Figure 3B.

6.3. On the EEG acquired during the neurocognitive study, blinking, eye movement, and muscle artifacts were seen, especially in the frontal leads Fp1 and Fp2 after artifact removal **[1].**

6.3.1. LAB MEDIA: Figure 3B. *Video Editor: Emphasize the Fp1 and Fp2 in the middle panel (located at the top).*

6.4. No artifact originating from machinery vibrations was seen on post-processed EEG signals acquired during both studies **[1].**

6.4.1. LAB MEDIA: Figure 3B. *Video Editor: Please emphasize on comparing Figure 3B (post-processed EEG signals) with Figure 3C (EEG signals acquired outside the MRI).*

6.5. Furthermore, the EEG electrodes did not cause visible artifacts on the MR images acquired simultaneously **[1].**

6.5.1. LAB MEDIA: Figure 4.

Conclusion

7. Conclusion Interview Statements

7.1. **Hui Ming Khoo:** When attempting this protocol, immobilize the head of the subject, the wires and the cables to adequately diminish mechanical vibrations.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.12, 3.3, 4.2, and 5.3.*

7.2. **Yuya Fujita:** This protocol is currently used for data acquisition for our neuroscience project that requires ERP single-trial detection. It is paving the way to using simultaneous EEG-fMRI in non-invasive brain-computer interface research.

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