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Title: Functional Transcranial Doppler Ultrasound for Monitoring Cerebral Blood Flow

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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**

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: 19
Number of Shots: 38

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Greg Bashford**: This protocol forms the basis for a functional TCD experiment, because almost all functional TCD experiments require placement of a fixation device to record a stable signal over an extended period of time.

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

1.2. **Greg Bashford:** The main advantage of functional TCD is its high temporal resolution measurement of changes in cerebral blood flow.

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

OPTIONAL:

1.3. **Ed Truemper:** Finding the middle cerebral artery using TCD takes practice. It is crucial to hold the transducer steady and move very slowly. The fine motor control needed for making small adjustments in transducer position and direction takes time to develop. Practice on as many volunteers as you can find!

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

1.4. **Ed Truemper:** Visual demonstration is important for two reasons: first, it is helpful to see exactly where to place the transducer. Second, a crucial part of learning fTCD is learning the sounds associated with the different arteries.

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

Ethics Title Card

1.5. Procedures involving human subjects have been approved by the Institutional Review Board (IRB) at the University of Nebraska-Lincoln.

Protocol

Video Editor: Some of the screen captures should be played with sound, please see specific notes below.

2. Locating the Middle Cerebral Artery (MCA) Signal by Freehand TCD

2.1. Begin by setting the parameters for transcranial Doppler ultrasound, or TCD **[1]**. Keep the power at a reasonably high value during the initial search for the middle cerebral artery, or MCA. Once the MCA signal is located, reduce the power as much as possible while still maintaining a good signal **[2]**.

2.1.1. Establishing shot of talent at the ultrasound machine.

2.1.2. SCREEN: 62048_screenshot_1. 0:12 – 0:28.

2.2. Set the sample volume to 8 to 12 millimeters during the initial search for the MCA signal **[1]**. If the signal is difficult to find, increase the gate size to increase the intensity of the signal. Set the gain at a medium level, with the goal of keeping background noise at a minimum **[2]**.

2.2.1. SCREEN: 62048_screenshot_1. 0:32 – 0:48. Talent setting the sample volume and gain.

2.2.2. SCREEN: 62048_screenshot_1. 1:00 – 1:25. *Video Editor: keep sound from approximately 01:08 – 01:20, so the increase in audio volume as the gain is set can be heard*

2.3. Set the high-pass filter cutoff between 50 and 150 Hertz **[1]**. If the subject is an adult, set the depth to 50 millimeters, which is the average mid-point depth of the M1 segment of the MCA **[2]**.

2.3.1. SCREEN: 62048_screenshot_1. 1:25 – 1:35. *Video Editor: filter is already set at proper level (150 Hertz) (top right of screen)*

2.3.2. SCREEN: 62048_screenshot_1. 1:36 – 1:55. Talent setting the high-pass filter cutoff and the depth. *Video Editor: Shorten this interval to match VO, keep sound*

2.4. Apply enough ultrasound gel to cover the surface of the transducer **[1]**. Alert the subject that the gel may feel cold, then place the transducer on the temporal window **[2]**.

2.4.1. Talent applying gel.

2.4.2. Talent placing the transducer on the temporal window.

2.5. After placing the transducer on the scalp, search for the MCA signal, which will generally be located slightly anterior and rostral from the location of the initial transducer placement **[1]**. *Videographer: This step is difficult and important!*

2.5.1. Talent searching for the MCA signal.

2.6. If the TCD spectral signal is not immediately obvious, adjust the angle of the transducer while keeping it in the same location relative to the scalp. Slowly angle the probe from rostral to caudal *(kaw-dul)* and posterior to anterior **[1]**. *Videographer: This step is difficult and important!*

2.6.1. Talent adjusting the angle.

2.7. If a signal is still absent, check the color M-mode display for flow in the MCA, which is indicated by red coloring **[1]**. Increment or decrement the signal depth in 5-millimeter steps and continue searching **[2]**.

2.7.1. SCREEN: 62048_screenshot_3. 0:10 – 0:20. *Video Editor: Emphasize M-mode display, which is at the bottom half of the screen*

2.7.2. SCREEN: 62048_screenshot_3. 0:20 – 0:35. *Video Editor: Keep sound from 0:25 – 0:30*

2.8. If flow is visible in M-mode but not in the Doppler spectrum, increase or decrease the depth until the flow signal is visible in the Doppler spectrum **[1]**. If a satisfactory signal is still not obtained, move the transducer to a nearby, slightly more anterior position on the scalp and repeat the search **[2]**.

2.8.1. SCREEN: 62048_screenshot_3. 0:35 – 1:00. *Video Editor: Keep sound*

2.8.2. Talent repositioning the transducer.

2.9. When an optimal MCA signal is obtained, note the depth and maximum velocity **[1]**. Using a washable makeup pen, place a mark on the scalp where the optimal signal was found **[2]**. *Videographer: This step is important!*

2.9.1. SCREEN: 62048_screenshot_3. 1:00 – 1:10.

2.9.2. Talent placing a mark on the scalp.

2.10. Next, search for the bifurcation. Increase the depth until the signal from the bifurcation of the internal carotid artery into the middle cerebral artery and anterior cerebral artery is noted, typically at a depth of 51 to 65 millimeters **[1]**.

2.10.1. SCREEN: 62048_screenshot_4(1). 0:10 – 0:40. *Video Editor: Keep sound*

2.11. Search for the optimum bifurcation spectral signal, striving for the highest-velocity spectral signal possible **[1]**. When an optimal bifurcation signal is obtained, note the depth of the bifurcation **[2]**.

2.11.1. Talent searching for the best bifurcation signal.

2.11.2. SCREEN: 62048_screenshot_4(1). 0:40 – 0:50. *Video Editor: Emphasize the depth at the top left-hand of the screen*

2.12. Adjust the fixation device to the subject's approximate head size **[1]**. Alert the subject before placing the headset on his or her head. After placing the headset **[2]**, adjust the fixation device's fit and ask the subject if the device is too tight **[3]**.

2.12.1. Talent adjusting the fixation device.

2.12.2. Talent placing the headset. NOTE: You can also see the talent talking to the subject in this shot, alerting them that the headband will be placed.

2.12.3. Talent adjusting the device's fit.

2.13. Loosen the mechanism of the fixation device so that the transducer can move freely **[1]**. Apply enough ultrasound gel to the transducer to cover the face of the transducer **[2]**.

2.13.1. Talent loosening the fixation device.

2.13.2. Talent applying ultrasound gel.

2.14. Adjust the fixation device so that the transducer is located over the top of the previously made mark **[1]**. Search for the optimal MCA spectral signal, striving for the highest-velocity spectral signal possible **[2]**. *Videographer: This step is important!*

2.14.1. Talent adjusting the transducer over the mark.

2.14.2. Talent searching for the MCA signal. NOTE: This step involves talent moving the transducer and checking the computer screen

2.15. When the optimal MCA spectral signal is found, tighten the mechanism of the fixation device to lock the transducer in place **[1]**. Note the depth and all other settings **[2]**. Decrease the power as much as possible while still maintaining a spectral envelope that traces the maximal velocity accurately **[3]**.

2.15.1. Talent tightening the fixation device.

2.15.2. SCREEN: 62048_screenshot_5. 0:05 – 0:15. *Video Editor: Emphasize the depth at the top left-hand of the screen*

2.15.3. SCREEN: 62048_screenshot_5. 0:20 – 0:32.

3. Breath-hold Maneuver

3.1. Begin recording on the TCD software **[1]**. Instruct the subject to breathe normally for 3 minutes **[2]** to achieve a good baseline recording and allow cerebral blood flow velocity to stabilize from any previous experiments or stimuli **[3]**.

3.1.1. SCREEN: 62048_screenshot_6. 0:15 – 0:25.

3.1.2. Subject breathing normally.

3.1.3. SCREEN: 62048_screenshot_6. 0:26 – 0:40. *Video Editor: Keep sound*

3.2. Count down slowly from three **[1]**. On the count of one, ask the subject to begin breath-holding after a normal inspiration **[2]**.

3.2.1. Talent counting down. NOTE: The countdown from three to one occurs immediately after the talent, Ed Truemper, holds up three fingers in the video.

3.2.2. Subject holding their breath.

3.3. Place a marker in the TCD recording to signify the start of breath-holding **[1]**. Have the subject hold their breath for 30 seconds **[2]**, or until they are no longer comfortable holding their breath **[3]**.

3.3.1. WIDE: Talent placing the marker on the TCD (pressing button on keyboard).

3.3.1.b. CU: Talent pressing button on keyboard (added shot)

3.3.2. SCREEN: 62048_screenshot_6. 2:35 – 3:11. *Video Editor: Skip from 2:45 – 3:00, keep sound*

NOTE: at 2:38 in 62048_screenshot_6, the words "Begin Breath Holding" appear in the bottom right of the screen. This occurs when the talent presses the button in 3.3.1/3.3.1.b and is the actual start of the breath-hold.

NOTE: This shot is the heart of the experiment. If possible, show 2:35-3:11 entirely without skipping.

3.3.3. WIDE: Subject releasing their breath.

3.3.3.b. CU: Subject releasing their breath (added shot)

3.4. When the subject inhales, place a marker in the TCD recording to signify the end of breath-holding **[1]**. Continue monitoring cerebral blood flow velocity using TCD for at least 30 seconds following the end of breath-holding to ensure that the velocity returns to baseline values **[2]**.

3.4.1. WIDE: Talent placing a marker in the TCD recording (pressing button on keyboard).

3.4.1.b. CU: Talent pressing button on keyboard (added shot)

3.4.2. SCREEN: 62048_screenshot_6. 3:12 – 4:00. *Video Editor: Keep sound. If clip is too long, skip 3:30 – 3:40*

NOTE: at 3:09 in "62048_screenshot_6", the words "Stop Holding Breath" appear in the bottom right of the screen. This occurs when the talent presses the button in 3.4.1/3.4.1.b and is the end of the breath-hold, i.e. when the subject releases their breath.

Results

4. **Results: Doppler Spectra and M-Mode Images from the MCA during Different Stages of the Breath-Hold Maneuver**

4.1. Doppler spectra and color M-modes from the midpoint of the M1 segment of the middle cerebral artery, or MCA, are shown here. The spectra were taken at the same position on the scalp, but at different angles **[1]**. It is important to note that a very small change in angle can greatly improve Doppler signal strength **[2]**.

4.1.1. LAB MEDIA: Figure 3.

4.1.2. LAB MEDIA: Figure 3 B. *Video Editor: Emphasize the high-intensity yellow color.*

4.2. A sample Doppler spectrum and M-mode from the bifurcation of the ICA into the ACA and MCA is shown here. The overlapping red- and blue-shaded regions in the M-mode image denote the MCA and ACA, respectively **[1-TXT]**.

4.2.1. LAB MEDIA: Figure 4. **TEXT: ICA - internal carotid artery; ACA – anterior cerebral artery**

4.3. Doppler spectra and M-mode images were taken at different time points in the breath-hold maneuver **[1]**. The mean blood flow velocity at baseline was 56 centimeters per second **[2]**, increasing to 70 centimeters per second by the end of breath-holding **[3]**, and undershooting to 47 centimeters per second after breath-holding ended **[4]**.

4.3.1. LAB MEDIA: Figure 6.

4.3.2. LAB MEDIA: Figure 6. *Video Editor: Emphasize the Mean in A.*

4.3.3. LAB MEDIA: Figure 6. *Video Editor: Emphasize the Mean in B*.

4.3.4. LAB MEDIA: Figure 6. *Video Editor: Emphasize the Mean in C.*

4.4. The entire breath-holding experiment is shown here **[1]**. The envelope, shown by the white line, increases gradually during breath-holding **[2]**, remains elevated for approximately 15 seconds after breath-holding ends **[3]**, undershoots for approximately 20 seconds, and then recovers to baseline values **[4]**.

4.4.1. LAB MEDIA: Figure 7.

4.4.2. LAB MEDIA: Figure 7. *Video Editor: Emphasize the Breath-holding section.*

4.4.3. LAB MEDIA: Figure 7. *Video Editor: Emphasize the Post-breathhold section.*

4.4.4. LAB MEDIA: Figure 7. *Video Editor: Emphasize the Undershoot section.*

4.5. Several examples of bilateral TCD spectra and M-modes suitable for bilateral fTCD are shown here **[1]**.

4.5.1. LAB MEDIA: Figure 8.

Conclusion

5. Conclusion Interview Statements

5.1. **Ed Truemper:** It is important to remember that finding the MCA signal involves very controlled, fine-motor movements. The only way to become proficient at finding the MCA is to practice on as many different people as possible.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1, 2.6.1, 2.14.2.*
NOTE: Maybe slated as 5.4.1

5.2. **Greg Bashford:** This technique paved the way for researchers to measure brain activity in environments that were previously inaccessible. For example, unlike in an MRI, subjects wearing fTCD fixation devices can move freely, so brain lateralization during active tasks can be studied.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
NOTE: Maybe slated as 5.5.1