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Title: A Benchtop Approach to the Location Specific Blood Brain Barrier Opening Using Focused Ultrasound in a Rat Model

Authors and Affiliations:

Megan Rich[1], Quentin Whitsett[1], Farah Lubin[2], Mark Bolding[1]

[1]Department of Radiology, University of Alabama at Birmingham, Birmingham, AL
[2]Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL

Corresponding Authors:

Mark Bolding (mbolding@uab.edu)

Email Addresses for Co-authors:

mcrich@uab.edu
qwhitsit@uab.edu
flubin@uab.edu
mbolding@uab.edu

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps.

   Authors: Please upload screen capture videos for all shots labeled SCREEN to your project page: https://www.jove.com/account/file-uploader?src=18639513

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, all locations are within walking distance**

Videographer: Please film the screen for all SCREEN shots.

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Megan Rich:** Focused-ultrasound combined with circulating microbubbles can be used to provide temporary, millimeter-sized openings in the blood brain barrier. This enables noninvasive delivery of systemically circulating agents to target brain regions.

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

Ethics Title Card

1.2. Procedures involving animal subjects have been approved by the UAB Institutional Animal Care and Use Committee (IACUC)

Protocol

2. Intracranial Targeting Procedure

2.1. To begin, fill the catheter plug with saline **[1]** and warm the rat's tail with a lamp, taking care to not overheat the animal **[2]**. Insert a 24-gauge tail vein catheter that will be used to deliver microbubbles, Evans blue dye, gadobutrol MRI contrast if using MRI, and the experimental agent of interest **[3]**.

2.1.1. WIDE: Establishing shot of talent filling the catheter plug with saline.

2.1.2. Talent warming the vein with the lamp.

2.1.3. Talent inserting the catheter. Videographer NOTE: 2.1.3 – 2.3.1 combined

2.2. Blood will fill the sheath when the vein is hit. Slowly remove the inner needle while pushing the sheath further into the vein **[1]**.

2.2.1. Talent removing the inner sheath while pushing the sheath further into the vein.

2.3. Screw the catheter plug into the end of the catheter port as soon as the port has filled with blood **[1]**. Carefully wrap lab tape around the catheter and the tail to keep it in place, starting with a small piece at the top and working in the caudal direction. Leave the very end of the catheter plug exposed **[2]**.

2.3.1. Talent screwing the catheter plug into the end of the catheter port.

2.3.2. Talent wrapping lab tape around the catheter and leaving the end exposed.

2.4. Plug the anesthesia line onto the anesthesia connector on the stereotaxic frame **[1]**, then fix the animal's head into the frame by placing the mouth onto the bite bar and by guiding the ear bars into both ear canals **[2]** and tighten the set screws **[3]**. Move the animal to the MRI bed **[4]**. *Videographer: This step is difficult and important!*

2.4.1. Talent plugging the anesthesia line. Videographer NOTE: 2.4.1 – 2.4.3 combined

2.4.2. Talent fixing the animal's head into the frame.

2.4.3. Talent tightening the set screws.

2.4.4. Talent moving the animal into the MRI bed.

2.5. Using parameters described in the text manuscript, collect coronal and axial T2-weighted images that capture the whole brain as well as the MRI fiducial for coordinate measurements **[1]**.

2.5.1. Talent at the MRI collecting images.

2.6. Collect coordinate measurements from the above images by recording the distance from the MRI fiducial to the brain region that will be targeted with FUS *(pronounce like 'fuss')* **[1]**.

2.6.1. Talent at the computer making measurements. Videographer NOTE: 2.6.1 – 2.8.1 combined

2.7. On the coronal images, find the image in which the fiducial is the largest, indicating the center of the fiducial. Record the x and y coordinates for the top of the fiducial, then record the x and y coordinates of the brain region of interest. Calculate the distance from the top of the fiducial to the brain region of interest in both the medial-lateral direction and in the dorsal ventral direction **[1]**. NOTE to VO: Long one, please split in two.

2.7.1. SCREEN: Distance from the top of the fiducial to the brain region of interest being measured on the coronal image.

NOTE: Authors indicated that the screen was filmed by the videographer and may not be planning to submit screen capture videos

Videographer: Please film the screen for all SCREEN shots.

2.8. On the axial images, find the image that shows the very top of the fiducial and record the x and y coordinates for the center of the fiducial and the x and y coordinates of the brain region of interest. Calculate the distance from the center of the fiducial to the target brain region in both the rostral-caudal and medial-lateral directions. After gathering the coordinates, collect the pre-scan images **[1]**. NOTE to VO: Long one, please split in two.

2.8.1. SCREEN: Distance from the center of the fiducial to the target brain region being measured on the axial image.

2.9. Keeping the animal in the stereotaxic frame, quickly transport it from the MRI bed to the benchtop FUS setup **[1]**. Ensure that the animal remains asleep under the effect of anesthesia **[2]**.

2.9.1. Talent moving bringing the animal to the FUS setup. Videographer NOTE: 2.9.1 – 2.9.2 combined

2.9.2. Properly anesthetized animal.

3. Focused Ultrasound Procedure

3.1. Slide the frame into the frame holder and firmly snap it into place **[1]**. Use clippers to shave the animal's head **[2]**, then brush away excess hair and apply hair remover cream to the scalp **[3]**. Let it sit for 3 minutes and wipe it away with water and gauze **[4]**.

3.1.1. Talent sliding the frame into the holder and snapping it in place.

3.1.2. Talent shaving the animal's head with clippers. Videographer NOTE: 3.1.2 – 2.1.3 combined

3.1.3. Talent applying hair remover cream to the scalp.

3.1.4. Talent wiping away the cream.

3.2. If using MRI guidance, attach the pointer and move the pointer to the location of the MRI fiducial **[1]**. Position the pointer at the very top and center of the MRI fiducial, then, which is the point from which all distances in the MRI image were calculated **[2]**. *Videographer: This step is important!*

3.2.1. Talent attaching the pointer and moving it to the MRI fiducial location. Videographer NOTE: 3.2.1 – 2.2.2 combined

3.2.2. Pointer moving to the center of the MRI fiducial.

3.3. Remove the pointer and move the positioner **[1]** to the medial-lateral coordinates and the rostral-caudal coordinates. Click the null position button and raise the positioner up by pressing the **up 50** button to allow for the placement of the water bath and ultrasound gel **[2]**.

3.3.1. Talent removing pointer.

3.3.2. SCREEN: Positioner being moved and raised.

3.4. Apply ultrasound gel to the animal's scalp **[1]** and place the water bath over the animal with the polyimide tape window pressed onto the gel, making sure that there are no air bubbles in the gel **[2]**. Fill the water bath with degassed water **[3]**. *Videographer: This step is important!*

3.4.1. Talent applying ultrasound gel. Videographer NOTE: 3.4.1 – 3.4.3 combined

3.4.2. Talent placing the water bath over the animal.

3.4.3. Talent filling the water bath.

3.5. If using the high-power transducer, lower the positioner so that the magnet is just above the water **[1]**. Attach the transducer to the positioner by carefully lowering the transducer into the water at an angle and connecting the magnets **[2]**.

3.5.1. Talent lowering the positioner. Videographer NOTE: 3.5.1 – 3.5.2 combined

3.5.2. Talent attaching the high-power transducer.

3.6. Lower the positioner to the dorsal-ventral coordinate **[1]** and turn on the RF power amp **[2]**.

3.6.1. SCREEN: Positioner lowering to the dorsal-ventral coordinate.

3.6.2. Talent turning on the RF power amp. Videographer NOTE: 3.6.2 – 3.7.1 combined

3.7. Inject 1 milliliter per kilogram of 3% Evans blue dye by sticking a needle tip into the catheter plug and injecting. The whole animal should become blue within seconds, indicating the catheter is properly positioned in the tail vein **[1-TXT]**. Allow the dye to circulate for 5 minutes, then activate the microbubbles by shaking them violently with the bubble shaker **[2]**.

3.7.1. Talent sticking the needle into the catheter plug. **TEXT: NOTE: The animal was not injected for filming purposes** NOTE: Author didn't actually inject dye because the animal was borrowed, so they recommended adding the disclosure.

3.7.2. Talent shaking the bubbles.

3.8. Invert the syringe several times to get a uniform distribution of microbubbles **[1]**, then attach and fill the winged infusion set **[2]**. Position the syringe on the infusion pump **[3]** and set the infusion pump to deliver 0.2 milliliters at a rate of 6 milliliters per hour, providing slow infusion of the microbubbles over the 2-minutes FUS exposure **[4]**.

3.8.1. Talent inverting the syringe. Videographer NOTE: 3.8.1 – 3.9.1 combined

3.8.2. Talent attaching the winged infusion set.

3.8.3. Talent positioning the syringe.

3.8.4. Talent setting the infusion pump to the appropriate rate.

3.9. Insert the winged needle into the catheter plug **[1]**. First, run the infusion pump **[2]**, wait 3 seconds, then start the FUS treatment by pressing the **on** button on the function generator. Repeat this twice per region with 5 minutes in between to allow the microbubbles to clear **[3]**.

3.9.1. Talent inserting the winged needle into the catheter plug.

3.9.2. Talent running the infusion pump. Videographer NOTE: 3.9.2 – 3.9.3 combined

3.9.3. Talent pressing the output enable button.

3.10. Press the **on** button again on the function generator to stop the FUS treatment when the infusion pump stops at 2 minutes **[1]**. Wait for 5 minutes for the microbubbles to clear, then start the infusion and the second FUS treatment **[2]**.

3.10.1. Talent pressing the on button on the function generator.

3.10.2. Talent starting the infusion and second FUS treatment.

3.11. Immediately after the second FUS treatment, inject gadobutrol contrast and the agent of interest. Total delivered volume of all agents should not exceed 5 milliliters per kilogram **[1]**.

3.11.1. Talent injecting gadobutrol and agent of interest.

3.12. To confirm BBB opening, place the animal back onto the MRI bed at the exact same location **[1]** and plug in the anesthesia line **[2]**. Collect MRI post scans with the same

imaging parameters as the prescan images to confirm gadobutrol MRI enhancement in the region of BBB opening **[3]**.

3.12.1. Talent placing the animal on the MRI bed. Videographer NOTE: 3.12.1 – 3.12.2 combined

3.12.2. Talent plugging in the anesthesia line.

3.12.3. Talent collecting MRI scans.

Results

4. Results: Focused Ultrasound BBB Opening

4.1. This protocol was used to induce localized blood brain barrier opening with both **[1]** the low-power immersion transducer **[2]** and the high power focused ultrasound transducer **[3]**.

4.1.1. LAB MEDIA: Figure 3 and Figure 4, just a, b, d, and e.

4.1.2. LAB MEDIA: Figure 3 and Figure 4, just a, b, d, and e. *Video Editor: Emphasize the images from Figure 3.*

4.1.3. LAB MEDIA: Figure 3 and Figure 4, just a, b, d, and e. *Video Editor: Emphasize the images from Figure 4.*

4.2. First, the low-power immersion transducer was targeted to either the anterior or medial brain hemisphere **[1-TXT]**, the animals were sacrificed with or without perfusion **[2-TXT]**, and the BBB opening was visualized via EBD autofluorescence **[3]**.

4.2.1. LAB MEDIA: Figure 3. *Video Editor: Label a "***medial***" and b "***anterior***".*

4.2.2. LAB MEDIA: Figure 3. Video Editor: *Label a "***+ perfusion***" and b "***- perfusion***".*

4.2.3. LAB MEDIA: Figure 3.

4.3. In later experiments, the FUS *(pronounce like 'fuss')* transducer was targeted to either the hippocampus **[1]** or the anterior cingulate cortex **[2]**. In addition to EBD, the MRI contrast agent gadobutrol was used to verify the targeted opening of the BBB in vivo **[3]**. After the animals were sacrificed, EBD autofluorescence confirmed the opening location **[4]**.

4.3.1. LAB MEDIA: Figure 4. *Video Editor: Emphasize a, b, and c.*

4.3.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize d, e, and f.*

4.3.3. LAB MEDIA: Figure 4. *Video Editor: Emphasize b and e.*

4.3.4. LAB MEDIA: Figure 4, just c and f. *Video Editor: Zoom in on c and f.*

4.4. To assess whether this technique could be used for targeted gene delivery, AAV9 expressing green fluorescent protein and gadobutrol contrast were injected immediately after blood-brain barrier opening in the hippocampus. The animal was imaged to verify the opening with gadobutrol contrast **[1]**.

4.4.1. LAB MEDIA: Figure 5 a and b.

4.5. Then, gene delivery was confirmed by GFP expression **[2]**.

4.5.1. LAB MEDIA: Figure 5 c. *Video Editor: Emphasize the green fluorescence in the image.*

Conclusion

5. Conclusion Interview Statements

5.1. **Megan Rich:** This protocol offers a benchtop approach to MRI-guided focused-ultrasound mediated blood brain barrier opening that can be easily adapted by other laboratories as a noninvasive, translational alternative to stereotaxic surgery.

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