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Title: Imaging Cerebrospinal Fluid Transport by Contrast-Enhanced Magnetic Resonance Imaging via Intraventricular Infusion in Rodents

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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? **Yes**

Olympus SZ40

3.4.2-3.9.3

Videographer: Please film the SCOPE shots using the scope kit

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 51

Interviews

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

Shoot notes from the videographer:

This one went smooth overall, but definitely had a few rough patches, mainly because the two authors didn't speak English very well, but we made it work. The interview we tried a bunch of times but because they didn't speak good english they had to read directly off of monitor without looking away. Also, the microscope adapter i had did not work again, I think i'll likely need a better one, it's also cheap plastic and so since its been over a year now it has worn down. So, had to do scope shots my recording monitor from scope display, and unfortunately it was a bit shaky, but i think still usable.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Xin Yu:** This protocol describes an MRI-compatible intraventricular cannulation technique that allows in-scanner tracer infusion such that the entire dynamics of CSF tracer transport can be captured.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Junqing Zhu:** This MRI-compatible protocol can be applied to studies of CSF dynamics, waste clearance, and glymphatic transport in rodents.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Anbang Chen:** This protocol establishes an MRI-compatible intraventricular infusion technique that enables in-scanner tracer delivery and uninterrupted dynamic imaging of CSF transport.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Anbang Chen:** Key considerations for this protocol include accurate skull leveling and a clog-free, leak-free infusion line to ensure consistent cannula placement and tracer delivery.

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

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Anbang Chen:** This technique can be adapted for chronic implantation to allow repeated tracer delivery in longitudinal studies.

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

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Case Western Reserve University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shot list.*

2. Assembly of the Cannula–Tubing Infusion System

Demonstrators: Junqing Zhu & Anbang Chen

2.1. To begin, using serrated scissors, cut a 30-millimeter segment of a copper cannula [1] and a 2-meter length of polyethylene tubing [2].

2.1.1. WIDE: Talent using serrated scissors to cut a 30-millimeter segment of a copper cannula and a 2-meter length of polyethylene tubing.

2.1.2. Talent cutting polyethylene tubing.

2.2. Under a microscope, gently flare one end of the polyethylene tubing with a 30-gauge needle [1]. Insert the copper cannula into the expanded end [2] and secure the junction with a cyanoacrylate adhesive [2]. Then, connect the opposite end of the polyethylene tubing to a 30-gauge needle [3].

2.2.1. SCOPE: Talent flaring one end of the tube with a 30-gauge needle. **NOTE: Use take 3, author said this should not be a scope shot**

***Videographer:** Please film the SCOPE shots using the scope kit*

2.2.2. Talent inserting the copper cannula into the expanded end of the polyethylene tubing and securing the junction with a cyanoacrylate adhesive. **NOTE: False slate for Take 3, mislabeled as something else. Either way, author said only use take 2 for this shot.**

2.2.3. Talent connecting the opposite end of the polyethylene tubing to a 30 gauge needle.

2.3. Fill the cannula-tubing assembly with a degassed tracer solution [1] and connect it to a 50-microliter Hamilton syringe [2]. Wrap the syringe tip with polytetrafluoroethylene thread-seal tape to prevent leakage [3].

2.3.1. Talent filling the cannula-tubing assembly with a degassed tracer solution. **NOTE: 2.3.1, 2.3.2, and 2.3.3 were all combined into 2.3.1 - there was a lot of movement for this shot, unavoidable because of lab layout, but the various actions in the script should have still frames, movement should only be in between moments**

2.3.2. Talent connecting the assembly to a 50-microliter Hamilton syringe.

2.3.3. Talent wrapping the syringe tip with a polytetrafluoroethylene thread-seal tape.

2.4. Mount the Hamilton syringe onto the syringe pump [1] and withdraw an additional 30

microliters of the tracer solution into the syringe [2].

2.4.1. Talent mounting the Hamilton syringe onto the syringe pump.

2.4.2. Talent withdraws an additional 30 microliters of the tracer solution into the syringe. **NOTE: Do not use take 1**

3. Stereotaxic Cannula Implantation

3.1. After anesthetizing the rat, perform the toe pinch reflex to confirm an adequate anesthetic depth [1-TXT].

3.1.1. Talent performing the toe pinch reflex to confirm an adequate anesthetic depth.
TXT: Anesthesia: Ketamine (90 mg/kg) and Xylazine (10 mg/kg) NOTE: DO not use take 1

3.2. Shave the head and neck [1], remove the loose hair [2], and sterilize the exposed skin with 70 percent ethanol pads [3].

3.2.1. Talent shaving the head and neck. **NOTE: Shots 3.2.1, 3.2.2, and 3.2.3 were all combined into 3.2.1**

3.2.2. Talent removing the loose hair.

3.2.3. Talent sterilizing the exposed skin with 70 percent ethanol pads.

3.3. Next, secure the rat in a stereotaxic frame [1] and place a heating pad covered with absorbent paper beneath the animal [2]. Then, apply an ophthalmic ointment to both eyes to prevent corneal drying [3].

3.3.1. Talent securing the rat in a stereotaxic frame. **NOTE: Shots 3.3.1, 3.3.2, and 3.3.2 were all combined into 3.3.1. The moments described in the shotlist for 3.3.2 and 3.3.3 take place after the camera repositioning in the take**

3.3.2. Talent placing a heating pad covered with absorbent paper beneath the animal.

3.3.3. Talent applying an ophthalmic ointment to both eyes.

3.4. Adjust the nose clamp to level the skull [1]. Make a midline incision [2] and retract the skin with four hemostat clips to expose the bregma and lambda [3]. Then, apply 3% hydrogen peroxide to the skull to clear residual periosteum and enhance the visibility of the landmarks [4].

3.4.1. Talent adjusting the nose clamp to level the skull. **NOTE: Shot 3.4.1, do not use take 1**

3.4.2. SCOPE: Talent making a midline incision and retracting the skin with four hemostat clips to expose the bregma and lambda. **NOTE: Shots 3.4.2, 3.4.3, 3.5.1, 3.5.2 - the author said these should not be scope shots**

3.4.3. SCOPE: Talent applying 3 percent hydrogen peroxide to the skull.

3.5. Mount the electric drill with a fine drill bit onto the stereotaxic arm [1] and ensure the

stereotaxic arm is firmly locked [2].

3.5.1. SCOPE: Talent mounting the electric drill with a fine drill bit onto the stereotaxic arm.

3.5.2. SCOPE: Talent checks if the stereotaxic arm is locked.

3.6. Next, position the drill bit at the bregma [1], move 0.7 millimeters posterior and 1.4 millimeters lateral to the right [2]. Lower the drill bit until it contacts the skull surface [3] and record the dorsoventral coordinate [4].

3.6.1. SCOPE: Talent positioning the drill bit at the bregma. NOTE: Shots 3.6.1, 3.6.2, 3.6.3, and 3.6.4 were all combined into 3.6.1 AND the author said these should not be scope shots. Also the action in shotlist for 3.6.4 happens after the camera repositioning in the take

3.6.2. SCOPE: Talent moving the drill bit 0.7 millimeters posterior and 1.4 millimeters lateral to the right.

3.6.3. SCOPE: Talent lowering the drill bit to the skull surface.

3.6.4. SCOPE: Talent recording the dorsoventral coordinate.

Added new shot called 3.6.5 that shows the coordinates on the rat, if required use this as an inset or may be at the end of the VO

3.7. Under a microscope, advance the drill in 0.1-millimeter increments [1] and stop immediately when the skull is breached [2].

3.7.1. SCOPE: Talent advancing the drill under the microscope in 0.1 millimeter increments. NOTE: Shot 3.7.1 and 3.7.2 were combined into just 3.7.1 - AND only use Take 3 for this

3.7.2. SCOPE: Talent stops the drill immediately after breaching the skull.

3.8. Then, replace the drill with the pre-loaded cannula on the stereotaxic arm [1]. Advance the infusion pump briefly until the fluid just emerges from the cannula tip to confirm an unobstructed fluid path [2]. NOTE: The VO is edited for the deleted shot

3.8.1. SCOPE: Talent replacing the drill with the pre-loaded cannula on the stereotaxic arm.

~~3.8.2. SCOPE: Talent advancing the infusion pump until the fluid emerges from the cannula tip.~~ NOTE: Not filmed

3.9. Ensure the cannula is mounted perpendicular to the skull surface [1], then align it over the burr hole [2]. Advance the cannula at 0.1 millimeters per second to a final target depth of 3.5 millimeters below the dural surface [3].

3.9.1. SCOPE: Shot of the cannula mounted perpendicular to the skull surface.

3.9.2. SCOPE: Talent aligning the cannula over the burr hole.

3.9.3. SCOPE: Talent advancing the cannula to the target depth.

3.10. Apply a drop of tissue adhesive at the cannula-skull interface [1]. Once the adhesive has cured, close the scalp incision with sutures [2]. Gently bend the copper cannula parallel to the skull surface to avoid contact with the MRI coil [3].

3.10.1. Talent applying a drop of a tissue adhesive at the cannula-skull interface.

3.10.2. Talent closing the scalp incision with sutures. NOTE: Shots 3.10.2 and 3.10.3 were combined into just 3.10.2

3.10.3. Talent gently bending the copper cannula parallel to the skull surface.

4. Dynamic MRI During Tracer Infusion

4.1. Place the volume transmitter coil at the isocenter of the scanner [1].

4.1.1. Talent exhibiting the volume transmitter coil outside the scanner for demo.

4.2. Transfer the animal to an MRI-compatible cradle in the prone position [1]. Connect the physiological monitoring system, including the respiratory sensor and the rectal temperature probe [2]. Turn on the circulating warm water bath to maintain the body temperature at approximately 37 degrees Celsius [3].

4.2.1. Talent transferring the animal to a magnetic resonance imaging-compatible cradle in the prone position. NOTE: Shots 4.2.1 and 4.2.2 were combined into just 4.2.1 AND do not use take 1 for this shot

4.2.2. Talent connecting the respiratory sensor and the rectal temperature probe to the physiological monitoring system.

4.2.3. Talent turning on the circulating warm water bath.

4.3. Administer isoflurane via a nose cone during scanning session [1], adjusting the concentration between 0.25% and 1.0% to maintain a respiratory rate of 50 to 70 breaths per minute [2].

4.3.1. Talent positioning the nose cone to deliver supplemental isoflurane.

4.3.2. Talent adjusting the isoflurane concentration during the scanning session.

4.4. Place the infusion pump outside the magnet fringe field [1]. Set the infusion rate to 1 microliter per minute and the infusion duration to 20 minutes, corresponding to a total infusion volume of 20 microliters [2]. Carefully arrange and secure the infusion line along the cradle to prevent tension and displacement [3].

4.4.1. Talent placing the infusion pump outside the magnet fringe field.

4.4.2. Talent setting the infusion parameters. NOTE: Shots 4.4.2 and 4.5.1 were combined into just 4.4.2 AND do not use take 1 for this shot
Author asked that we cut shot 4.5.2 entirely, we did not film it

- 4.4.3. Talent arranging and securing the infusion line along the cradle.
- 4.5. Then, mount the surface array coil directly over the dorsal skull, centered on the brain [1]. ~~Place a small piece of absorbent paper between the coil and the incision site to absorb any wound seepage [2].~~ NOTE: The VO is edited for the deleted shot
- 4.5.1. Talent mounting the surface array coil directly over the dorsal skull.
- 4.5.2. ~~Talent placing a small piece of absorbent paper between the coil and the incision site.~~ NOTE: Not filmed
- 4.6. Advance the cradle into the magnet until the brain is at the isocenter [1]. Verify that the infusion line and the physiological monitoring leads remain unobstructed [2].
 - 4.6.1. Talent advancing the cradle into the magnet until the brain reaches the isocenter.
 - 4.6.2. Talent checking that the infusion line and the physiological monitoring leads remain unobstructed.
- 4.7. Perform the initial scanner setup [1] and acquire anatomical images to confirm successful cannula placement [2].
 - 4.7.1. SCREEN: 001_screenshot_InitialSetup.mp4: 00:00-00:10
 - 4.7.2. SCREEN: 002_screenshot_Anatomical Images.mp4: 00:08-00:14
- 4.8. After obtaining baseline images, begin the infusion pump and dynamic scan simultaneously, and continue image acquisition for 3 hours [1].
 - 4.8.1. Talent starts the infusion pump and the dynamic scan simultaneously

Results

5. Results

- 5.1. Successful intraventricular cannulation was confirmed by direct visualization of the cannula tip within the ventricular lumen in the sagittal view [1] and the axial view [2].
 - 5.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the red arrow*
 - 5.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the red arrow*
- 5.2. By the end of the 20-minute infusion, enhancement was apparent within the infused lateral ventricle, the third and fourth ventricles, the cisterna magna, and the parenchyma surrounding the ipsilateral lateral ventricle [1].
 - 5.2.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the bright signal in the 20-minute images.*
- 5.3. Across intrathecal compartments, T1 decreased rapidly during infusion, propagating from the ipsilateral lateral ventricle [1] to the third and fourth ventricles [2], with the cisterna magna showing a slight delay in the onset of T1 shortening [3].
 - 5.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the line labeled 'LV'.*
 - 5.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the lines labeled 'V3' and 'V4'.*
 - 5.3.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the line labeled 'CM'.*
- 5.4. Periventricular regions showed earlier and more pronounced T1 reduction [1], whereas more distal regions showed delayed and reduced T1 reduction [2].
 - 5.4.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the filled-marker line labeled 'V4 Proximal' and the filled-marker line labeled 'LV Proximal'.*
 - 5.4.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the open-marker line labeled 'V4 Distal' and the open-marker line labeled 'LV Distal'.*

1. Cannula

Pronunciation link: <https://www.merriam-webster.com/dictionary/cannula>

IPA: /'kænjələ/

Phonetic Spelling: KAN·yuh·luh

2. Polyethylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethylene>
IPA: /ˌpɑːliˈeθəˌliːn/
Phonetic Spelling: pol·ee·ETH·uh·leen
3. Cyanoacrylate
Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanoacrylate>
IPA: /ˌsaɪənəʊˈækɹəˌleɪt/
Phonetic Spelling: sy·uh·noh·AK·ruh·layt
4. Degassed
Pronunciation link: No confirmed link found
IPA: /ˌdiːˈgæst/
Phonetic Spelling: dee·GAST
5. Microliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪkroʊˌliːtər/
Phonetic Spelling: MY·kroh·lee·ter
6. Polytetrafluoroethylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polytetrafluoroethylene>
IPA: /ˌpɑːliˌtetrəˌflʊəroʊˈeθəˌliːn/
Phonetic Spelling: pol·ee·tet·ruh·floor·oh·ETH·uh·leen
7. Stereotaxic
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotaxic>
IPA: /ˌsteriəʊˈtæksɪk/
Phonetic Spelling: stair·ee·oh·TAK·sik
8. Anesthetizing
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnesθəˌtaɪzɪŋ/
Phonetic Spelling: uh·NES·thuh·ty·zing
9. Anesthetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetic>
IPA: /ˌænəsˈθetɪk/
Phonetic Spelling: an·uhs·THET·ik
10. Ketamine
Pronunciation link: <https://www.merriam-webster.com/dictionary/ketamine>
IPA: /ˈkiːtəˌmiːn/
Phonetic Spelling: KEE·tuh·meen
11. Xylazine
Pronunciation link: <https://www.merriam-webster.com/medical/xylazine>
IPA: /ˈzaɪləˌziːn/
Phonetic Spelling: ZY·luh·zeen
12. Ophthalmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
IPA: /ɑːfˈθælmɪk/
Phonetic Spelling: off·THAL·mik

13. Corneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/corneal>
IPA: /'kɔ:rneɪl/
Phonetic Spelling: KOR·nee·uhl
14. Hemostat
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostat>
IPA: /'hi:məˌstæt/
Phonetic Spelling: HEE·muh·stat
15. Bregma
Pronunciation link: <https://www.merriam-webster.com/dictionary/bregma>
IPA: /'bregmə/
Phonetic Spelling: BREG·muh
16. Lambda
Pronunciation link: <https://www.merriam-webster.com/dictionary/lambda>
IPA: /'læmdə/
Phonetic Spelling: LAM·duh
17. Periosteum
Pronunciation link: <https://www.merriam-webster.com/dictionary/periosteum>
IPA: /ˌperiˈɑ:stiəm/
Phonetic Spelling: pair·ee·OS·tee·uhm
18. Dorsoventral
Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsoventral>
IPA: /ˌdɔ:rsəʊˈventrəl/
Phonetic Spelling: dor·soh·VEN·truhl
19. Dural
Pronunciation link: <https://www.merriam-webster.com/dictionary/dural>
IPA: /'dʊrəl/
Phonetic Spelling: DUR·uhl
20. Isocenter
Pronunciation link: No confirmed link found
IPA: /'aɪsoʊˌsentər/
Phonetic Spelling: EYE·soh·sen·ter
21. Physiological
Pronunciation link: <https://www.merriam-webster.com/dictionary/physiological>
IPA: /ˌfɪziəˈlɑ:dʒɪkəl/
Phonetic Spelling: fiz·ee·uh·LOJ·ih·kuhl
22. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>
IPA: /ˌaɪsoʊˈflʊrˌeɪn/
Phonetic Spelling: eye·soh·FLOOR·ayn
23. Anatomical
Pronunciation link: <https://www.merriam-webster.com/dictionary/anatomical>
IPA: /ˌænəˈtɑ:mɪkəl/
Phonetic Spelling: an·uh·TOM·ih·kuhl

24. Intraventricular
 Pronunciation link: <https://www.merriam-webster.com/medical/intraventricular>
 IPA: /,ɪntrəˈvenˈtrɪkjələr/
 Phonetic Spelling: in·truh·ven·TRIK·yuh·ler
25. Cannulation
 Pronunciation link: <https://www.merriam-webster.com/medical/cannulation>
 IPA: /,kænjəˈleɪʃən/
 Phonetic Spelling: kan·yuh·LAY·shuhn
26. Ventricular
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ventricular>
 IPA: /venˈtrɪkjələr/
 Phonetic Spelling: ven·TRIK·yuh·ler
27. Lumen
 Pronunciation link: <https://www.merriam-webster.com/dictionary/lumen>
 IPA: /ˈluːmən/
 Phonetic Spelling: LOO·muhn
28. Sagittal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sagittal>
 IPA: /ˈsædʒɪtəl/
 Phonetic Spelling: SAJ·ih·tuhl
29. Axial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/axial>
 IPA: /ˈæksɪəl/
 Phonetic Spelling: AK·see·uhl
30. Cisterna magna
 Pronunciation link: <https://www.merriam-webster.com/medical/cisterna%20magna>
 IPA: /sɪˈstɜːnəˈmæɡnə/
 Phonetic Spelling: sih·STUR·nuh MAG·nuh
31. Parenchyma
 Pronunciation link: <https://www.merriam-webster.com/dictionary/parenchyma>
 IPA: /pəˈrɛŋkəmə/
 Phonetic Spelling: puh·RENG·kuh·muh
32. Ipsilateral
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ipsilateral>
 IPA: /,ɪpsəˈlætərəl/
 Phonetic Spelling: ip·suh·LAT·er·uhl
33. Intrathecal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/intrathecal>
 IPA: /,ɪntrəˈθiːkəl/
 Phonetic Spelling: in·truh·THEE·kuhl
34. Periventricular
 Pronunciation link: <https://www.merriam-webster.com/medical/periventricular>
 IPA: /,pərəˈvenˈtrɪkjələr/
 Phonetic Spelling: pair·uh·ven·TRIK·yuh·ler

