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**Title: Targeted Intracranial Delivery of Secreted Immune Molecules to Investigate Neuroimmune Interactions**

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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**

If **Yes**, can you record movies/images using your own microscope camera?

**No**

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

**Binocular stereomicroscope AmScope SE508**

**SCOPE shots: 3.1.2, 4.2.2**

***Videographer: Please film the above-mentioned shots using the scope kit***

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

***Videographer: Please record the instrument's display for the shots labeled as SCREEN. The screen should be in focus and the fonts should be clearly visible***

**3. Filming location:** Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? **0.2 miles**

**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: 26

Number of Shots: 50 (7 SC, 2 Scope)

# Interviews

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

**Authors:** Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

## INTRODUCTION:

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

- 1.1. **Nicole Scott-Hewitt:** This protocol provides a customizable method for administering secreted immune proteins into the brain for characterization of neuroimmune interactions.
  - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Julia Dziabis:** Microglia are difficult to manipulate directly; targeted delivery of their signaling molecules provides experimental control over their effects across distinct contexts.
  - 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. **Nicole Scott-Hewitt:** This protocol allows for downstream investigation of the functional impacts of an immune protein or molecule of interest.
  - 1.3.1. 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?~~

- 1.4. **Julia Dziabis:** Beyond immunohistochemistry, additional assays can be performed, such as qPCR, Western blotting, ELISA, and electrophysiological recordings from targeted brain regions.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Nicole Scott-Hewitt**: Future studies could co-administer multiple factors, including interacting immune factors or inhibitors, or examine tissue responses across different time points.
- 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 at Duke University

# Protocol

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

## 2. Animal Preparation

**Demonstrators:** Nicole Scott-Hewitt, Julia Dziabis

**Authors:** Let us skip the step of placing the animal in the chamber as per our veterinary review guidelines

- 2.1. To begin, place an anesthetized 4-month-old C57-Black-6-J mouse on a clean surface [1] and confirm the depth of anesthesia with a toe-pinch test [1-TXT]. **NOTE:** Authors used female mouse for filming, but the results in the manuscript state male mice were used, hence deleting the gender from the narration to avoid confusion. Informed the authors as well
- 2.1.1. WIDE: Talent placing the anesthetized animal on a clear surface.  
**TXT: Anesthesia: Isoflurane**  
**Induction: 3.5 - 4%**  
**Maintenance: 2% at 1 L/min**
- 2.1.2. Close-up of Talent pinching the toe of the mouse to confirm the loss of the reflex.
- 2.2. Move the animal to the warmed heating pad on the stereotax and position its snout in the nose cone [1]. Apply a generous coating of eye lubricant gently to each eye [2].
  - 2.2.1. Talent moving the mouse onto the warmed heating pad and fitting the nose cone.
  - 2.2.2. Close-up of Talent applying eye lubricant to each eye of the mouse.
- 2.3. Using a sterile cotton applicator, apply hair removal cream to the top of the head, and massage it into the fur [1]. Remove the cream and the fur using applicators dipped in 0.9 percent saline. Repeat as needed until the area is free of fur [2].
  - 2.3.1. Close-up of Talent applying hair removal cream to the top of the head with a sterile cotton applicator.
  - 2.3.2. Close-up of Talent removing the cream and the fur with an applicator dipped in 0.9% saline.

- 2.4. At the start of the surgery, administer meloxicam subcutaneously at 4 milligrams per kilogram of body weight [1].
  - 2.4.1. Close-up of Talent injecting 4 mg/kg of meloxicam subcutaneously into the mouse.
- 2.5. Secure the animal's head between the earbars, keeping the skull flat and level. Gently press down to confirm stability [1]. If movement occurs, carefully reposition the earbars [2].
  - 2.5.1. Close-up of Talent securing the head of the mouse between the earbars on the stereotax.
  - 2.5.2. Close-up of Talent pressing gently down on the head and resecuring the earbars.
- 2.6. Apply betadine to the head with a sterile cotton applicator, in an inside-to-outside circular motion [1]. Then, wipe the area with 70 percent ethanol and repeat this sequence 2 more times [2].
  - 2.6.1. Close-up of Talent applying betadine to the head in an inside-to-outside circular motion. **Author's NOTE: 2.6.1 and 2.6.2 were combined**
  - 2.6.2. Close-up of Talent applying 70% ethanol to the head with a sterile cotton applicator.

### **3. Skull Exposure and Craniotomy Preparation**

- 3.1. Using a scalpel, make a vertical incision in one fluid motion from just above the eyes to midway between the ears [1]. Gently separate the skin with a sterile cotton-tip applicator to expose the skull. Confirm that the bregma and the lambda are visible [2].
  - 3.1.1. Close-up of Talent making the vertical incision from just above the eyes to midway between the ears with the scalpel.
  - 3.1.2. SCOPE: separating the skin with a sterile cotton-tip applicator to expose bregma and lambda.
- 3.2. Using ridged forceps, attach the sterile 1.35-millimeter bit to the drill [1]. Secure the drill to the stereotax arm and place the bit over the center of the bregma, just touching the skull [2]. Then, zero the Z coordinate [3].

- 3.2.1. Close-up of Talent attaching the 1.35 mm drill bit to the drill with ridged forceps. **Authors:** Please confirm that the drill bit is 1.35 mm as given in the section 2 of the manuscript protocol. **Yes**
- 3.2.2. Talent securing the drill into the stereotax arm and positioning it above the center of bregma. **Author's NOTE:** This one might have been split into 2 shots – 3.2.2 was securing the drill, 3.2.3 was positioning above bregma and zeroing the display
- 3.2.3. SCREEN: The Z position being zeroed at bregma on the digital stereotax display.  
*Videographer: Please record the instrument's display for the shots labeled as SCREEN. The screen should be in focus and the fonts should be clearly visible*

**Response from authors: It is on a small instrument display, not a screen.**

**Authors:** Our videographer will film the instrument display for the shots labeled as SCREEN

- 3.3. As a quality-control check for head placement, move the drill bit to lambda [1] and lower it until it just touches the skull. Record the Z coordinate [2].
- 3.3.1. Shot of moving the drill bit to lambda.
- 3.3.2. SCREEN: The Z position at lambda being recorded on the digital stereotax display as bit is lowered.
- 3.4. Raise the drill bit and move it to minus 2.1 millimeters in the Y position, which is about midway between the bregma and the lambda [1]. Move the X position to minus 1.5 millimeters, then to +1.5 millimeters. Record the Z position at both locations when the bit just touches the skull [2]. Review the recorded positions and adjust the head placement if needed [3].
- 3.4.1. Close-up of Talent moving the drill bit to Y = -2.1 mm on the stereotax arm.
- 3.4.2. SCREEN: The Z positions at X = -1.5 mm and X = +1.5 mm being recorded on the digital stereotax display.
- 3.4.3. Talent adjusting the head placement.

**Authors:** I have modified the narration in step 3.4 above. Please check if it is correct.

#### **4. Craniotomy**

- 4.1. Zero the coordinates again at the bregma and move the stereotax arm to the desired X and Y coordinates [1].
- 4.1.1. SCREEN: The stereotax arm being moved to the desired X and Y coordinates on the digital display.

- 4.2. Turn on the drill at approximately 1,000 rpm and slowly lower the arm in the Z dimension to drill through the skull. Do not contact the brain tissue underneath [1]. A shiny, moist brain surface indicates successful penetration [2].
  - 4.2.1. Close-up of Talent lowering the running drill at 1000 rpm to drill through the skull.
  - 4.2.2. SCOPE: Shot of the surface of the brain appearing shiny and moist.
- 4.3. Remove the drill from the stereotax arm [1]. If performing multiple surgeries, detach the bit with sterile ridged forceps and place it in the bead sterilizer for 10 seconds [2]. Then, return the bit to the sterile field on a surgical towel [3].
  - 4.3.1. Talent removing the drill from the stereotax arm.
  - 4.3.2. WIDE: Talent placing the drill bit into the bead sterilizer for 10 s with ridged forceps.
  - 4.3.3. Talent placing the drill bit on the surgical towel.
- 4.4. Moisten a sterile cotton-tipped applicator with 0.9% saline and wipe away drilling debris from the exposed skull [1]. Check the eye lubricant and reapply as needed [2].
  - 4.4.1. Close-up of Talent wiping the drilling dust from the exposed skull with an applicator dipped in 0.9% saline.
  - 4.4.2. Close-up of Talent reapplying eye lubricant to the eyes of the mouse.

## 5. Protein Injection

- 5.1. Secure the injector to the stereotax arm [1] and load the protein of interest into the injector. The labeled C1q protein was used in this case [2-TXT]. Re-zero at the bregma and adjust to the desired X and Y coordinates [3]. Then, lower the injector tip through the drilled hole it reaches the brain surface, and zero the Z plane [4].
  - 5.1.2., Talent securing the loaded injector into the stereotax arm. **Author's NOTE: 5.1.2 was performed and recorded before 5.1.1, VO adjusted**
  - 5.1.1. Talent loading the protein of interest into the injector. **TXT: See text protocol for preparation of labeled proteins**
  - 5.1.3., SCREEN: The injector being re-zeroed at bregma and moved to the desired X and Y coordinates on the digital stereotax display.
  - 5.1.4., Close-up of Talent lowering the injector tip through the drilled hole to the surface of the brain.

- 5.2. Slowly advance the injector tip approximately 0.2 millimeters beyond the target Z coordinate, then retract it to the target depth [1].
  - 5.2.1. Close-up of Talent inserting the injector tip ~0.2 mm past the desired Z coordinate and returning it to the coordinate.
- 5.3. Next, inject the protein at a rate less than 500 nanoliters per minute, and record the time of injection [1].
  - 5.3.1. SCREEN: The injection being delivered at a rate no faster than 500 nL/min on the microinjector display.
- 5.4. To prevent backflow, keep the injector in place for 5 to 10 additional minutes after the delivery is complete [1].
  - 5.4.1. Shot of holding the injector in place for 5 - 10 min after the injection.
- 5.5. Then, slowly withdraw the injector from the brain over 1 to 2 minutes [1]. Then, remove the injector from the stereotax [2].
  - 5.5.1. Close-up of Talent slowly withdrawing the injector from the brain over 1 - 2 min.
  - 5.5.2. Talent removing the injector from the stereotax arm.

## **6. Wound Closure and Animal Recovery**

- 6.1. Loosen the earbars and rehydrate the incision with a sterile cotton applicator dipped in saline [1]. Apply 1 to 2 drops of 0.25% bupivacaine topically to the skin [2].
  - 6.1.1. Close-up of Talent loosening the ear bars and rehydrating the incision site with a saline-dipped sterile cotton applicator. **Author's NOTE: Action added to the narration and the shot**
  - 6.1.2. Close-up of Talent applying bupivacaine topically to the skin around the incision.

**Authors:** Please provide the concentration and the volume of bupivacaine applied. **0.25% Bupivacaine, 1-2 drops**

- 6.2. Use sterile forceps to close the incision site [1], and apply a tissue adhesive to seal the wound [2].

6.2.1. Close-up of Talent closing the incision site with sterile forceps.

6.2.2. Close-up of Talent applying tissue adhesive along the closed incision. **Author's**

**NOTE:** 6.2.1 and 6.2.2 were performed together in one shot

**Authors:** Sutures or wound staples are given as alternatives to tissue adhesive. Please specify which of the 2 shots described here would you prefer. **Tissue adhesive**

6.3. After releasing the nose cone [1], place the animal in a warm, clean cage containing hydrogel and rehydrated milled food to recover. Record the time when isoflurane anesthesia is discontinued [2].

6.3.1. Talent loosening the earbars and nosecone. **Author's NOTE:** Earbars were loosened prior at step 6.1 instead and here the nosecone was loosened

6.3.2. Talent placing the mouse into a warmed, clean recovery cage.

6.4. Monitor the animal and record when it first stands [1].

6.4.1. Talent observing the mouse in the recovery cage and recording the time it first stands.

6.5. ~~Once the animal is fully ambulatory for at least 30 minutes, provide hydrogel and rehydrated milled food [1].~~ If maintained beyond 24 hours, monitor the animal's body weight daily [1]. Administer postoperative meloxicam and follow the study-specific humane endpoint criteria [3].

**Authors:** These are only representative shots to smoothly finish the protocol and bridge the results

6.5.1. ~~Talent returning the mouse to a clean home cage containing hydrogel and rehydrated milled food.~~ **Author's NOTE:** We used the same cage as the recovery cage as it had the hydrogel and rehydrated food

6.5.2. Talent weighing the animal.

6.5.3. *Video editor: Reuse 2.4.1 if the animal in 2.4.1 look similar* Talent injecting meloxicam SQ. **TXT: See text protocol for post-operative care, tissue harvest and immunohistochemistry**

**Author's NOTE:** We did not have a previously surgerized animal to inject after 24 hours and did not want to give 2 doses of meloxicam to the same animal, so we did not record a video of this step

# Results

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## 7. Results

- 7.1. One hour after a unilateral intracerebroventricular C1q injection into a C1q (C-1-q) knockout mouse, immunohistochemistry revealed abundant C1q in the periventricular parenchyma, with colocalization in neuronal somas in the hippocampal CA3 (C-A-3) region [1]. No C1q signal was seen around the contralateral ventricle [2].
  - 7.1.1. LAB MEDIA: Figure 3A. *Video editor: Please emphasize the red signal in the left panel, and then the CA3 inset on the right*
  - 7.1.2. LAB MEDIA: Figure 3B.
- 7.2. In a wild-type animal, the tagged C1q showed the same peri-ventricular distribution without antibody amplification [1].
  - 7.2.1. LAB MEDIA: Figure 3C. *Video editor: Please emphasize the magenta peri-ventricular signal in the left panel*
- 7.3. Heat treatment of the tagged C1q produced visible aggregates under brightfield microscopy [1].
  - 7.3.1. LAB MEDIA: Figure 4A and Figure 4B. *Video editor: Please show the 2 panels side by side and emphasize the aggregates in B*
- 7.4. Robust C1q labeling was observed in the hemisphere injected with native tagged protein [1]. In contrast, no C1q labeling was detected in CA3 following injection of heat-inactivated protein [2], although the protein accumulated around the ventricular edges [3].
  - 7.4.1. LAB MEDIA: Figure 5A and Figure 5B. *Video editor: Please emphasize the red middle panel of Figure 5A*
  - 7.4.2. LAB MEDIA: Figure 5A and Figure 5B. *Video editor: Please emphasize empty middle panel of Figure 5B*
  - 7.4.3. LAB MEDIA: Figure 4C. *Video editor: Please emphasize the red signal*
- 7.5. The percentage of C1q-positive neurons was significantly reduced in the hemispheres injected with heat-inactivated C1q [1], while the total number of neurons in the CA3 region was unchanged [2].

7.5.1. LAB MEDIA: Figure 5C. *Video editor: Please emphasize the lower HI C1q-594 data points*

7.5.2. LAB MEDIA: Figure 5D.

#### 1. Isoflurane

Pronunciation link: No confirmed link found

IPA: /ˌaɪ.səˈflʊr.eɪn/

Phonetic Spelling: eye·suh·FLUR·ayn

#### 2. Anesthesia

Pronunciation link: [<https://www.merriam-webster.com/dictionary/anesthesia>](<https://www.merriam-webster.com/dictionary/anesthesia>)

IPA: /ˌæn.əsˈθiː.ʒə/

Phonetic Spelling: an·uhs·THEE·zhuh

#### 3. Meloxicam

Pronunciation link: [<https://www.merriam-webster.com/dictionary/meloxicam>](<https://www.merriam-webster.com/dictionary/meloxicam>)

IPA: /məˈlɔːk.sə.kæm/

Phonetic Spelling: muh·LOK·suh·kam

#### 4. Subcutaneously

Pronunciation link: [<https://www.merriam-webster.com/dictionary/subcutaneously>](<https://www.merriam-webster.com/dictionary/subcutaneously>)

IPA: /ˌsʌb.kjuːˈteɪ.ni.əs.li/

Phonetic Spelling: sub·kyoo·TAY·nee·uhs·lee

#### 5. Craniotomy

Pronunciation link: [<https://www.merriam-webster.com/dictionary/craniotomy>](<https://www.merriam-webster.com/dictionary/craniotomy>)

IPA: /ˌkreɪ.niˈɑː.tə.mi/

Phonetic Spelling: kray·nee-AA·tuh·mee

#### 6. Bregma

Pronunciation link: No confirmed link found

IPA: /ˈbreg.mə/

Phonetic Spelling: BREG·muh

#### 7. Bupivacaine

Pronunciation link: [<https://www.merriam-webster.com/dictionary/bupivacaine>](<https://www.merriam-webster.com/dictionary/bupivacaine>)

IPA: /bjuˈpɪv.əˌkeɪn/

Phonetic Spelling: byoo·PIV·uh·kayn

#### 8. Intracerebroventricular

Pronunciation link: No confirmed link found

IPA: /ˌɪn.trəˌsɛr.ə.brʊʊ.vənˈtrɪk.jə.lə/

Phonetic Spelling: in·truh·ser·uh·broh·ven·TRIK·yuh·ler

#### 9. Immunohistochemistry

Pronunciation link: No confirmed link found

IPA: /ˌɪm.jə.noʊˌhɪs.toʊˈkɛm.ə.stri/

Phonetic Spelling: im·yuh·noh·his·toh·KEM·uh·stree

#### 10. Periventricular

Pronunciation link: No confirmed link found

IPA: /ˌpɛr.i.vənˈtrɪk.jə.lə/

Phonetic Spelling: peh·ree·ven·TRIK·yuh·ler

#### 11. Parenchyma

Pronunciation link: [<https://www.merriam-webster.com/dictionary/parenchyma>](<https://www.merriam-webster.com/dictionary/parenchyma>)

IPA: /pə'reŋ.kə.mə/

Phonetic Spelling: puh·RENG·kuh·muh

#### 12. Colocalization

Pronunciation link: No confirmed link found

IPA: /ˌkoʊ.loʊ.kə.lə'zeɪ.ʃən/

Phonetic Spelling: koh·loh·kuh·luh·ZAY·shuhn

#### 13. Neuronal

Pronunciation link: [<https://www.merriam-webster.com/dictionary/neuronal>](<https://www.merriam-webster.com/dictionary/neuronal>)

IPA: /nɜ'roʊ.nəl/

Phonetic Spelling: noo·ROH·nuhl

#### 14. Somas

Pronunciation link: [<https://www.merriam-webster.com/dictionary/soma>](<https://www.merriam-webster.com/dictionary/soma>)

IPA: /'soʊ.məz/

Phonetic Spelling: SOH·muhz

#### 15. Hippocampal

Pronunciation link: [<https://www.merriam-webster.com/dictionary/hippocampal>](<https://www.merriam-webster.com/dictionary/hippocampal>)

IPA: /ˌhɪp.ə'kæm.pəl/

Phonetic Spelling: hip·uh·KAM·puhl

#### 16. Contralateral

Pronunciation link: [<https://www.merriam-webster.com/dictionary/contralateral>](<https://www.merriam-webster.com/dictionary/contralateral>)

IPA: /ˌkɒːn.trəˈlæt̬.ə.əl/

Phonetic Spelling: kon·truh·LAT·er·uhl

#### 17. Peri-ventricular

Pronunciation link: No confirmed link found

IPA: /ˌpɛr.i.vɛnˈtrɪk.jə.lə/

Phonetic Spelling: peh·ree·ven·TRIK·yuh·ler

#### 18. Aggregates

Pronunciation link: [<https://www.merriam-webster.com/dictionary/aggregate>](<https://www.merriam-webster.com/dictionary/aggregate>)

IPA: /ˈæɡ.rɪ.gəts/

Phonetic Spelling: AG·rih·guhts

#### 19. Hemisphere

Pronunciation link: [<https://www.merriam-webster.com/dictionary/hemisphere>](<https://www.merriam-webster.com/dictionary/hemisphere>)

IPA: /ˈhɛm.əˌsfɪr/

Phonetic Spelling: HEM·uh·sfeer

#### 20. Ventricular

Pronunciation link: [<https://www.merriam-webster.com/dictionary/ventricular>](<https://www.merriam-webster.com/dictionary/ventricular>)

IPA: /vɛnˈtrɪk.jə.lə/

Phonetic Spelling: ven·TRIK·yuh·ler