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Title: Widespread Transduction of Mouse Neocortical Neurons by Subarachnoid Injection of AAV2

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **yes** , all done

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **04/30/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 21
Number of Shots: 43

Introduction

1.1. **Alexey Malyshev**: Our research is focused on developing a method that allows viral transduction of a large volume of neural tissue in the mouse neocortex.

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

What are the current experimental challenges?

1.2. **Alexey Malyshev**: Typically, a suspension of viral particles is injected directly into the brain parenchyma. Here, the area of infection is determined by the diffusion of the viral particles in the brain tissue, which has a limited range.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.15*

What advantage does your protocol offer compared to other techniques?

1.3. **Maria Smirnova**: We inject AAV2 viral vector viruses into the subarachnoid space via the brain surface. We found that subarachnoid administration of the virus led to an almost four-fold increase in the infection area, compared with intraparenchymal injection.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.19.1*

How will your findings advance research in your field?

1.4. **Maria Smirnova:** The new technique provides widespread transduction of neocortical neurons in adult mice and helps to preserve brain tissue for subsequent optical or electrophysiological recordings of neuronal activity.

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

What research questions will your laboratory focus on in the future?

1.5. **Ivan Smirnov**: Our injection method allows selective expression of the transgene in a large population of layer five pyramidal neurons even when using a strong non-selective promoter. However, the mechanism of such selective infection is not fully understood.

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

Ethics Title Card

This research has been approved by the Ethics Committee of the IHNA RAS

Protocol

2. Stereotaxic Viral Delivery into the Mouse Brain Using Microsyringe Injection

Demonstrator: Maria Smirnova

2.1. To begin, secure the front teeth of an anesthetized mouse in the tooth bar and mount the animal mask **[1]**.
- 2.1.1. Talent placing the front teeth of the anesthetized mouse in the tooth bar and mounting the anesthesia mask. **TXT: Anesthesia: 5% Isoflurane inhalation**

2.2. Carefully position the mouse on the ear bars **[1]**. Shave the head from the eyes to behind the ears **[2]**. Then swab the shaved surface with 70% ethanol followed by a 5% alcohol solution of iodine **[3]**.
- 2.2.1. Talent adjusting and fixing the ear bars.
- 2.2.2. Talent shaving the mouse's head with clippers.
- 2.2.3. Talent cleaning the shaved scalp using ethanol and iodine.

2.3. Apply 4 % lidocaine solution topically **[1]** and inject 0.02 milliliters of dexamethasone subcutaneously to reduce pain and inflammation **[2]**.
- 2.3.1. Talent applying lidocaine to the scalp.
- 2.3.2. Talent administering dexamethasone injection subcutaneously.

2.4. With a sterile scalpel and scissors, make a 4 to 5-millimeter midline incision starting between the ears to open the scalp **[1]**. Expand the incision using scissors to avoid damage to the skull **[2]**.
- 2.4.1. Talent making a small midline incision with a sterile scalpel.
- 2.4.2. Talent carefully extending the incision with scissors.

2.5. Swab the skull surface with 3% hydrogen peroxide to visualize bregma and lambda **[1]**. Immediately stop the reaction with 0.9% sodium chloride saline **[2]**. Then use a bone scraper to remove tissue from the skull surface **[3]**.
- 2.5.1. Talent applying hydrogen peroxide on the skull.
- 2.5.2. Talent stopping reaction with saline.
- 2.5.3. Shot of the skull surface being scraped.

2.6. Mount the pre-prepared motorized injector with a Hamilton syringe on the stereotaxic arm **[1]**. Illuminate the exposed skull using a surgical light source and focus the microscope onto the bregma **[2]**.
- 2.6.1. Talent attaching Hamilton syringe to stereotaxic arm.

2.6.2. Talent adjusting microscope and lighting onto the skull.

2.7. Looking through the microscope, manipulate the stereotactic arm to align the needle tip directly over bregma **[1]**. Align bregma and lambda horizontally using the needle tip, return to bregma, and record the coordinates **[2-TXT]**.

2.7.1. SCOPE: 2.7.1.mp4 00:00-00:17
2.7.2. SCOPE: 2.7.2.mp4 00:13-00:33
TXT: Use atlas co-ordinates to calculate the relative co-ordinates of the target area

2.8. Move the needle to the calculated coordinates. Lower it to the new position and mark it **[1]**. Select the site for viral injection near the target while considering viral spread **[2]**.
2.8.1. SCOPE: 2.8.1.mp4. 00:20-00:39
2.8.2. Talent identifying final injection site considering spread.

2.9. Now, use a sterile dental burr of 0.5 to 0.8 millimeters diameter to create a small craniotomy while viewing through the microscope **[1-TXT]**. ~~Move the stereotaxic arm away to protect the microsyringe during drilling **[2]**.~~
2.9.1. SCOPE: 2.9.1.mp4 00:05-00:15,00:21-00:35
TXT: Avoid applying excessive pressure
2.9.2. ~~Talent repositioning stereotaxic arm away.~~
AUTHOR'S NOTE: Skip 2.9.2

2.10. ~~Apply 0.9% sodium chloride saline during pauses to prevent heating **[1]**.~~ Use pressurized air to blow away bone dust **[2]**.
2.10.1. ~~Talent pausing to apply saline.~~
AUTHOR'S NOTE: Skip 2.10.1
2.10.2. Talent using air to blow away dust.

2.11. ~~When the thinned bone is soft and transparent, stop drilling **[1]**. When a sufficiently large indentation forms in the bone, stop the rotation and monitor the thickness of the bone **[2-TXT]**.~~
AUTHOR'S NOTE: Skip 2.11.1-2.11.2
~~2.11.1. SCOPE: The drilling is being stopped.~~
2.11.2. ~~SCOPE: The indentation on the bone is being seen. **TXT: Appearance of bone cracks indicated sufficient thinning**~~

2.12. Bathe the hole with sterile saline and remove the excess with a cotton swab **[1]**. Then use a 27-gauge needle with a hook-shaped tip or fine tweezers to remove remaining bone, taking care not to damage the dura **[2]**.

2.12.1. Talent bathing the hole with sterile saline and removing the excess with a cotton swab.
2.12.2. SCOPE: 2.12.2.mp4 00:01-00:09,00:11-00:25

2.13. Cover the skull with a sterile, saline-moistened paper towel. Then place a clean transparent film on top of the skull surface **[1]**.
2.13.1. Talent placing moistened paper towel on skull then layering transparent film over the paper.

2.14. Move the stereotaxic arm back and align the microsyringe needle above the transparent film **[1]**. Then dispense excess oil until 2 microliters remain **[2]**.
2.14.1. Talent moving the stereotaxic arm and shot of the microsyringe needle being aligned above the transparent film.
2.14.2. Shot of the excess oil being dispensed over the film.

2.15. Pipette the viral injection volume and another 2 microliters of virus onto the transparent film **[1-TXT]**. Looking through the microscope, lower the needle tip into the center of the virus drop **[2]**.
2.15.1. SCOPE: 2.15.1.mp4 00:00-00:13
TXT: Fast green dye (0.1%) is added in the injected solution to visualize its spread within the subarachnoid space
2.15.2. SCOPE: 2.15.2.mp4 00:00-00:11

2.16. Now load the virus into the microsyringe with a motorized injector **[1-TXT]**.
2.16.1. Talent loading virus into the microsyringe. **TXT: Discard used tip and film into container with 2% bleach**

2.17. Remove the moistened paper from the skull and dry the area with a cotton swab **[1]**. Reposition the stereotaxic arm and needle above the insertion site **[2]**.
2.17.1. Talent removing moist paper and drying skull with a cotton swab.
2.17.2. SCOPE: 2.17.2.mp4 00:01-00:15

2.18. Next, dispense a drop of virus to check for needle clogging **[1]**. Make a small slit in the dura using a 30-gauge needle with a hook-shaped tip **[2]**.
2.18.1. SCOPE: 2.18.1.mp4 00:00-00:07
2.18.2. SCOPE: 2.18.2.mp4. 00:02-00:20

2.19. Lower the syringe needle to the dura and calculate insertion depth based on the surface contact point **[1]**. Insert the needle slowly into the cortex to a depth of 300 micrometers **[2]**, wait for 2 to 3 minutes, retract to 200 micrometers and wait another 2 to 3 minutes **[3]**.
2.19.1. SCOPE: 2.19.1.mp4. 00:00-00:22
2.19.2. SCOPE: 2.19.2.mp4. 00:00-00:15

2.19.3. SCOPE: 2.19.3.mp4 00:03-00:22

2.20. Begin viral injection at 0.06 microliters per minute for a total of 1 microliter **[1]**. After infusion, keep the needle at the target site for 10 minutes to allow virus dispersion **[2]**. Then retract the needle slowly to avoid backflow **[3]**.

2.20.1. SCOPE: 2.20.1.mp4. 00:00-00:15

2.20.2. SCOPE: 2.20.2.mp4 00:00-00:10

2.20.3. SCOPE: 2.20.3.mp4. 00:10-00:21

2.21. Dispense a drop of virus to confirm needle is not clogged **[1]**. Close the scalp incision using 5-0 *(Five-zero)* absorbable or non-absorbable sutures **[2]**.

2.21.1. SCOPE: 2.21.2.mp4 00:03-00:16

2.21.2. Talent suturing the incision on the scalp.

Results

3. Representative Results

3.1. Subarachnoid administration of AAV2 *(A-A-V-Two)* led to a significantly larger area of neuronal infection compared to traditional intracortical injection, with nearly fourfold expansion in both mediolateral and rostrocaudal directions **[1-TXT].**

3.1.1. LAB MEDIA: Figure 3A. **TXT: AAV2: Adeno-Associated Viruses** *Video editor: Highlight the taller gray bars labeled "s" in both "m/l" and "r/c" groups*

3.2. Subarachnoid virus injection resulted in strong green fluorescent labeling of neurons in cortical layers 2/3 *(Two-Three)* and 5 **[1]**, while layers 4 and 6 showed no labeled cell bodies but displayed visible dendrites and axons respectively **[2]**.

3.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the bright green cell bodies located in the L2/3 and L5 layers.*

3.2.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the green strands running through layer L6*

3.3. Fluorescent particles that were added to the injected virus suspension during subarachnoid injection were found along the brain surface **[1]**, indicating virus spread via cerebrospinal fluid, with broader surface coverage than neuronal labeling **[2]**.

3.3.1. LAB MEDIA: Figure 2A and B *Video editor: Highlight the bright orange-red spread labeled "AAV2 injection" and outline the full glowing area.*

3.3.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the green fluorescent area marked "AAV2 injection" and show how far it spreads compared to the center point.*

3.4. Large surface distribution of green fluorescence in sagittal sections confirmed widespread cortical infection following subarachnoid injection **[1]**, in contrast to the small, localized infection seen with standard injection **[2]**.

3.4.1. LAB MEDIA: Figure 1A.

3.4.2. LAB MEDIA: Figure 1B.

3.5. In supragranular layers, a substantial fraction of transduced neurons co-expressed parvalbumin or calbindin **[1]**, while no co-labeled interneurons were detected in layer 5 **[2]**.

3.5.1. LAB MEDIA: Figure 4A and 4C. *Video editor: Highlight the yellow-orange cells in layers L2/3*

3.5.2. LAB MEDIA: Figure 4B and 4D. *Video editor: Please highlight only the green cells with pyramidal shape*

PRONUNCIATION GUIDE:

1. Subarachnoid

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/subarachnoid
- **IPA:** /ˌsʌb.əˈræk.nɔɪd/
- **Phonetic Spelling:** sub-uh-RAK-noidmerriam-webster.com+1merriam-webster.com+1

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2. Parvalbumin

- **Pronunciation Link:** https://www.merriam-webster.com/medical/parvalbumin
- **IPA:** /ˌpɑːr.vælˈbjuː.mɪn/
- **Phonetic Spelling:** par-val-BYOO-minmerriam-webster.com+6merriam-webster.com+6merriam-webster.com+6

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3. Dexamethasone

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/dexamethasone
- **IPA:** /ˌdɛk.səˈmɛθ.əˌsoʊn/
- **Phonetic Spelling:** dek-suh-METH-uh-sohnmerriam-webster.com+1merriam-webster.com+1

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4. Lidocaine

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/lidocaine
- **IPA:** /ˈlaɪ.dəˌkeɪn/
- **Phonetic Spelling:** LYE-duh-kaynmerriam-webster.com

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5. Bregma

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/bregma
- **IPA:** /ˈbrɛg.mə/
- **Phonetic Spelling:** BREG-muhmerriam-webster.com+11merriam-webster.com+11merriam-webster.com+11merriam-webster.com+4merriam-webster.com+4merriam-webster.com+4

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6. Lambda

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/lambda
- **IPA:** /ˈlæm.də/
- **Phonetic Spelling:** LAM-duhmerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3

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7. Craniotomy

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/craniotomy
- **IPA:** /ˌkreɪ.niˈɑː.tə.mi/
- **Phonetic Spelling:** KRAY-nee-AH-tuh-mee

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8. Dura

- **Pronunciation Link:** https://www.merriam-webster.com/medical/dura
- **IPA:** /ˈdʊr.ə/
- **Phonetic Spelling:** DOOR-uhmerriam-webster.com+5merriam-webster.com+5merriam-webster.com+5

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9. Parenchyma

- **Pronunciation Link:** https://www.merriam-webster.com/dictionary/parenchyma
- **IPA:** /pəˈrɛŋ.kɪ.mə/
- **Phonetic Spelling:** puh-REN-ki-muhmerriam-webster.com+13merriam-webster.com+13merriam-webster.com+13merriam-webster.com