

Nov 14, 2024

Stereotaxic intracranial virus injection in mice

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5r514g1b/v1>

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Protocol Citation: Fuu-Jiun Hwang, Richard Roth, Jun B. Ding, Yue Sun 2024. Stereotaxic intracranial virus injection in mice. protocols.io <https://dx.doi.org/10.17504/protocols.io.8epv5r514g1b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: August 28, 2024



Last Modified: November 14, 2024

Protocol Integer ID: 106629

Keywords: ASAPCRN, mouse, Stereotaxic, stereotaxic intracranial virus injection, stereotaxic brain injections of virus, conducting stereotaxic brain injection, virus, mice

Funders Acknowledgements:

ASAP-020551

Grant ID: ASAP-020551

Abstract

This protocol outlines steps for conducting stereotaxic brain injections of virus in mice.

Attachments



Ding Lab Stereotaxic...

19KB

Guidelines

Anesthesia

- **Isoflurane:** Administer 1.5-2.0% isoflurane with a steady oxygen flow rate of 1 L/min via a standard isoflurane vaporizer.
- **Ketamine/Xylazine:** Use as alternative anesthetics if Isoflurane is unavailable. Prepare a ketamine/xylazine cocktail and administer it IP at a dose of 80-100 mg/kg for ketamine and 5-10 mg/kg for xylazine before the surgery. Administer 1 mg/kg Atipamezole IP to reverse the effects of xylazine post-surgery.
- Check for the absence of a withdrawal reflex by performing a tail or toe pinch. Once the animal is confirmed to be unconscious, transfer it to a stereotaxic frame and fit a small nose cone snugly over its snout. The nose cone should deliver a constant, non-rebreathing stream of isoflurane/oxygen and be connected to an activated charcoal scavenging unit.
- Adjust the dosage of isoflurane (approx. 1.5-2.0%) to eliminate blink and pedal reflexes without halting spontaneous respiration.
- Apply ointment to the eyes to prevent drying.
- Administer Buprenorphine SR or Ethiq-XR to reduce postoperative pain.



Materials

Plunger (for micro-injection)
1 mL syringes
27G or 30G 1/2 needles
Iodine swabs
Q-tips
Saline
Eye ointment
Scissors
Clamps
Forceps
Scalpel
Petri dishes
70% ethanol
Mineral oil
Glass capillary (Drummond Scientific)
Micropipette puller (Sutter)
Isoflurane or Ketamine/Xylazine
Buprenorphine SR or Ethiq-a-XR
Stereotaxic frame with dissecting scope
Electric razor
Micro Drill
Heating pad
Desired virus

Safety warnings

 Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval (e.g. IACUC) should be obtained before performing these experiments. Approval was obtained by the Stanford University IACUC before any procedures were performed.

Before start

Pre-Surgery Preparation

- Autoclave surgical instrument sets. Use hot bead sterilizer (250°C for 60 seconds) between animals if reusing instruments. Allow instruments to cool completely before use.
- Provide heat support at all points during the procedure (preparation, surgery, and recovery).
- Scrub area with 10% Clorox, followed by 70% ethanol.
- Gather necessary tools: plunger, 1 mL syringes, 27G or 30G 1/2 needles, iodine swabs, Q-tips, saline, eye ointment, scissors, clamps, forceps, Petri dishes, 70% ethanol.

Injection setup

- Prepare the injection needle by pulling a glass capillary (Drummond Scientific) using a micropipette puller (Sutter). Trim the tip to the desired length.
- Fill the needle with mineral oil and expel all bubbles. Assemble the plunger, needle holder, and pump. Insert the needle through the holder, ensuring the plunger passes through, and secure the needle holder.
- Lower the plunger to expel most of the oil. Place the virus solution under the needle. Aspirate the virus solution at ~ 100 nl/s to avoid air bubbles.

Surgery

- 1 Once the animal is anesthetized, secure it in a stereotaxic frame using a blunted ear bar (or jaw bar for young animals). Ensure the head is level.
- 2 Shave the fur on scalp with an electric razor and clean the skin with a betadine scrub, followed by 70% alcohol solution.
- 3 Make a small incision (~1 cm) in the skin using a scalpel.
- 4 Remove connective tissues and clean with a q-tip/swab to expose the bregma and lambda.
- 5 Under the dissecting scope, position the needle to touch the bregma and lambda, ensuring they are at the same Z position (within ± 0.02 cm difference). Adjust the ear bar or nose bar as needed.
- 6 Repeat this step to level the left and right side of the skull by touching the skull 2 mm left and 2 mm right of the midline, ensuring they are at the same Z position (within ± 0.02 cm difference).
- 7 Move the needle to the bregma and set the X,Y and Z coordinates to zero. Then, move the needle to the injection site based on the coordinates. Mark the injection site on the skull.
- 8 Create a small hole in the skull at injection site with a micro drill. Break the dura with a 27G or 30G needle.
- 9 Lower the needle to the injection site and insert slowly to the desired depth.
Allow the needle to sit for 5~10 minutes if performing shallow cortical injections.
- 10 Inject desired volume (typically 10-500 nL) of virus at a speed of 50-100 nL/min.
- 11 After injection, leave the needle in the brain for 5-10 minutes to ensure proper distribution. Withdraw the needle slowly.



Safety information

Use caution when handling and disposing of needles.

- 12 Bring the skin edges together with blunt forceps and suture the skin with 3-5 stitches.
- 13 Place the mouse in a recovery cage on a heating pad and allow it to fully recover from anesthesia (approx. 5-10 minutes).