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Mouse Stereotaxic Surgeries

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ABSTRACT

This protocol allows for stereotaxic surgeries to administer unilateral or bilateral injections of small volumes of viral vectors or other suspensions into the mouse parenchyma. After using an anesthesia chamber to put the mouse to sleep, it is then placed and stabilized on the stereotaxic frame and is prepared for surgery. The mouse's bregma coordinates is used to calculate the appropriate coordinates needed to inject into specific brain regions. This protocol is optimized for different brain regions based on the coordinates listed in the guidelines.

MANUSCRIPT CITATION:
Williams GP, Schonhoff AM, Jurkuvenaite A, Gallups NJ, Standaert DG, Harms AS. CD4 T cells mediate brain inflammation and neurodegeneration in a mouse model of Parkinson's disease. Brain. 2021 Aug 17;144(7):2047-2059. doi: 10.1093/brain/awab103. PMID: 33704423; PMCID: PMC8370411.

Schonhoff, A.M., Figge, D.A., Williams, G.P. *et al.* Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. *Nat Commun* **14**, 3754 (2023). https://doi.org/10.1038/s41467-023-39060-w

Protocol status: Working
We use this protocol and it's working

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GUIDELINES

SNpC	Striatum	Intraventrical	Entorhinal Cortex
M/L: 1.2	M/L: 2.0	M/L: 1.0	M/L: -3.9
A/P: -3.2	A/P: 0.5	A/P: 0.3	A/P: -3.52
D/V: -4.6	D/V: -3.2	D/V: -2.7	D/V: -4.2

For pain management we use Slow Release (SR) Buprenorphine. Firstly, and very importantly, buprenorphine SR CANNOT be diluted. The drug is specifically formulated so it can have “sustained release” action and dilution will cause the drug to be essentially non-efficacious. Here are some tips for handling the relatively viscous drug:

- Recommend warming the Buprenorphine SR-LAB vial in a incubator to around 1-2 degrees below animal body temperature. Warming it before drawing it into a syringe greatly reduces the polymer matrix viscosity
- Use a larger needle (e.g. 17g) to insert to the vial with drugs to avoid pressure resistance
- Use a 1 cc syringes featuring a low dead-space plunger with precise 0.01 ml graduation markings

In terms of concentration, the 0.5 mg/mL concentration as recommended by ARP is the best for calculating the appropriate doses. For ~25g mouse, we inject 50uL of the drug.

If using anal heating probe and pad, set probe to mouse internal temperature of 35deg C.

MATERIALS

- Sterotaxic frame
- Benchmark digital coordinate box
- Light source
- Distilled water (sterile)
- Betadine and Isopropyl alcohol
- Sterile cotton swabs (autoclaved)
- 10 uL Hamilton Syringe (Cat# 80030)
- Isofluorane and Oxygen
- Drill and bits
- Sterile ocular lubricant (petroleum ophthalmic ointment)
- Heating pad
- New clean cage
- Hair trimmer
- Timer
- Wound clips
- Gloves
- Surgical utensils: Scalpel, tweezers (sterile)
- Glass bead sterilizer
- Anesthesia box
- Filter for isofluorane collection
- Anesthesia chamber
- Automatic injector
- Disinfectant spray

SAFETY WARNINGS

Be sure to wear appropriate PPE when performing stereotaxic surgeries.

SETUP

1 Sterilize all surfaces with disinfectant prior to setting up and lay out materials and utensils needed for surgery.

2 Make sure stereotaxic frame is ready for surgeries on level table and all cords are fully attached. Switch on the injector, light source, and digital coordinate box.

3 Sterilize all utensils (including drill bit) in the glass bead sterilizer for 30 seconds. Clean the Hamilton syringe by rinsing with distilled water 10 times. Place all sterilized utensils on the bench protector near working area.

4 Connect the oxygen/isoflurane line to the anesthesia chamber and the filter to collect excess. Set oxygen flow to 0.5-0.8 (silver ball) at all times.

5 Knock down the mouse using the anesthesia chamber:

Set isoflurane to 3% to knock the mouse down…watch the breathing carefully through the whole procedure. Proceed only if pain reflex is not there by pinching the back limb of the animal.

6 Take the mouse out of the anesthesia chamber, and trim its hair in the place where you will be making an incision on the head. While placing the mouse in ear bars monitor the mouse breathing, and change isoflurane to 2.5% if needed.

7 Load the Hamilton syringe with virus or injecting liquid. Always use an excess of liquid and check for bubbles. Place the syringe into the injector. *Alternate…use the injector to load the syringe by using the “fast reverse” function*.

8 Position the mouse into the stereotaxic frame and tightening the ear bars, the mouse’s head should be secure and flat. Always monitor breathing and pain reflexes accordingly!!!!

9 Sterilize the skin by swabbing with isopropanol, betadine, followed by isopropyl alcohol again, 3 times making sure to avoid contact with the eyes.

PROCEDURE

10 Use a sterile scalpel to make a straight incision from behind the eyes to the base of the skull. Use sterile utensils to expose the bregma (perpendicular area where the fissures meet on top of the skull).

11 Set the injection rate and volume on the injector. (2 ul at 0.25 ul per minute if injection will be unilateral, and 0.5ul/min if injecting bilaterally)

12 Line up the needle with the mouse's bregma. Check alignment from all visual angles.

13 Reset the digital coordinate's box to "0" for anterior/posterior, medial/lateral.

14 Raise the needle to avoid scraping the skull and mouse's skin. Use the digital box to move the needle to the appropriate anterior/posterior and medial/lateral coordinates.

a. Positive anterior/posterior coordinates should move the needle towards the nose while negative anterior/posterior coordinates should move the needle towards the cerebellum.

b. Positive medial/lateral coordinates should move the needle to the right side of the animal while negative medial/lateral coordinates should move the needle to the left side of the animal.

15 Common coordinates used for our injections can be found in the guidelines section.

16 Drill at the appropriate anterior/posterior and medial/lateral coordinates using the drill. Do not drill all the way through the skull, you could damage the brain! Look for a small color change in the skull (i.e. white to a dark pink/red color). This should indicate that the skull is very thin and the needle should be able to puncture the skull.

17 Lower the needle to check if the drilled location matches the anterior/posterior and medial/lateral coordinates. The needle should be able to puncture the skull releasing a very small amount of blood and/or CSF. ALWAYS WATCH THE NEEDLE WHEN LOWERING!! If any resistance is met, the needle will bend and be damaged! If the needle does not fit into the drilled location, pull it up and drill appropriately. DO NOT CHANGE THE ANTERIOR/POSTERIOR OR MEDIAL/LATERAL COORDINATES OR BUMP THE INJECTOR! If the injector is bumped, return to bregma and begin all over again.

18 Bevel the needle (liquid should be visible at the tip of the needle). Lower the needle to where the bevel of the needle contacts the dura. Reset the dorsal/ventral coordinates to "0" on the benchmark digital box.

19 Lower the needle to the appropriate dorsal/ventral coordinates.
Nb. Negative dorsal/ventral coordinates should push the needle down into the brain.

20 Push “run” on the injector and watch carefully as the virus is injected into the brain. ALWAYS MONITOR THE INJECTION TO ENSURE THE PLUNGER ON THE SYRINGE DOES NOT BEND! This can damage your syringe and cause an unknown amount of virus to be injected into the brain.

21 It should take about 8 minutes for the 2 uL injection to finish. Wait 2 minutes before slowly retracting the needle.

22 5. Slowly retract the needle (dorsal/ventral coordinates should move towards the positive). Too fast of a retraction creates a vacuum and will pull out all injected liquid.

23 After the injection for one animal is done, make sure needle is not in your way, and clean off any blood or dried CSF for next injection.

24 Clip the incision site closed with wound clips and remove the mouse from the stereotaxic frame.

25 Return the mouse to its cage halfway on a heating pad and carefully monitor its recovery. Administer buprenorphine pain relief.

26 A mouse has fully recovered when it is walking around the cage. It may be necessary to place wet food on the floor of the cage for the following day. Monitor carefully for several days following the procedure and look for signs of pain/distress.

27 Clip the incision site closed with wound clips.