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Mouse Stereotaxic Surgeries for Intracranial Viral Injection V.1

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We use this protocol and it's working

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Abstract

This procedure allows to inject a small volume of solution (in our case, either a suspension of genetically modified viruses, that will infect neurons and will induce the expression of desired proteins, often genetically-encoded probes; or a suspension of a chemical neuronal marker, “fluorogold”, that will be taken up that neurons with axonal projections in the area of injection) in a specific region of the brain.
An anesthetized mouse is placed on the stereotaxic apparatus, where its head is immobilized and positioned so that once the skull is exposed, specific anatomical landmarks (usually, the bone sutures) can be identified and used to calculate the relative position of different brain areas expressed as x/y/z coordinates. small hole can be drilled in correspondence of the desired x/y coordinates and the injection pipette can then be lowered to desired z coordinate, where the solution is slowly released.
After suturing the mouse and waiting an appropriate time for recovery and expression of the protein of interest, the mouse can be sacrificed and used for experiments.

Materials

-Anesthetic: isoflurane
-Anesthesia machine (Smiths Medical) with connector tubing, induction chamber and filter canisters for isoflurane waste
-Stereotaxic surgery frame and scope (David Kopf Instruments)
-Sterile surgery tools (forceps, fine scissors, needle holder as needed)
-Sterile drape
-Heating pad and temperature probe
-Non-steroidal analgesic (e.g. Metacam)
-Ophthalmic ointment
-Sterile 0.9% saline
-Antiseptics: povidone-iodine swabs and 70% ethanol swabs
-Hair clipper
-Drill with foot petal and sterilized drill bit
-Sterile cotton swabs
-Viral stock solution
-Suture material
-EMLA cream or bupivacaine line block
-Antibiotic ointment
-Glass micropipettes (Drummond Scientific) pulled with P-97 glass puller (Sutter Instruments). It is recommended to add some volumetric references on the pipettes based on their specifics.
-Post-surgery care: clean empty mouse cage on heating pad for recovery; clean mouse cage with extra gel food for post-surgery holding.

Troubleshooting

Safety warnings

Recommended PPE:
-Disposable gown
-Face mask
-Face shield/goggles
-Nitrile gloves
-Sterile gloves
-Depending on the biosafety level (BSL) recommended for type of virus injected, an appropriate biosafety cabinet might be required.

Surgical Set

5m

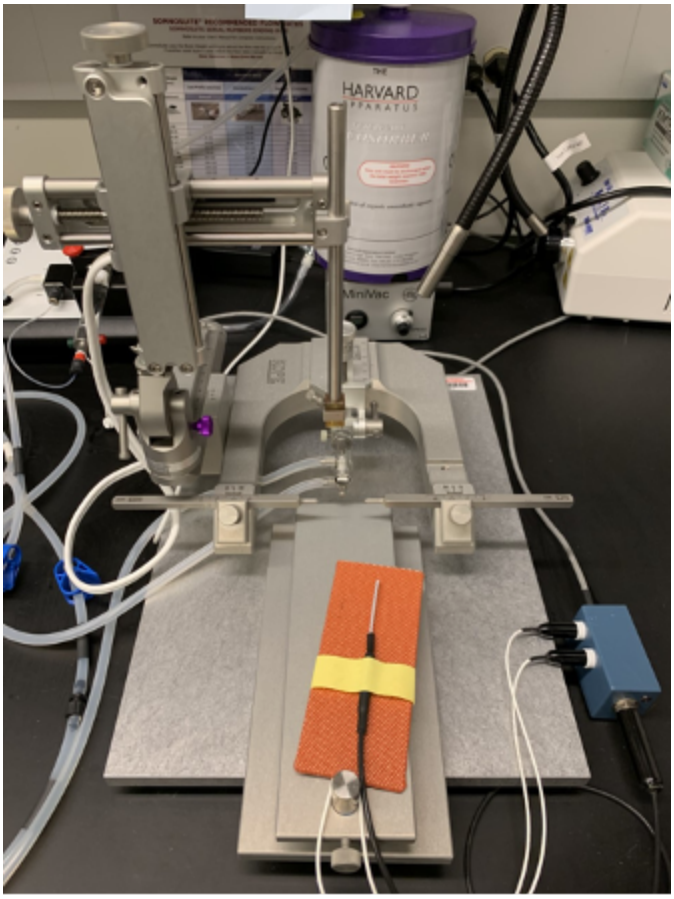
1 Prepare a clean empty mouse cage on a heating pad and a clean mouse cage with gel food for post-op care

2 Set up sterile working area including stereotaxic frame

3 Weigh mouse

4 Anesthetize mouse in induction chamber (recommended: 2.5% isoflurane, 200ml/min flow rate)

5 Hair over surgery area can be quickly clipped before transferring the mouse onto the stereotaxic frame



6 Once the mouse is deeply anesthetized (~ 00:05:00), stop anesthesia and move the mouse to the stereotaxic frame over the heating pad with the temperature probe and secure the mouse mouth on the nose cone. 5m

6.1 Restart anesthesia (directed towards the nose cone)

7 The heating pad settings should be adjusted so that the temperature probe placed under the mouse should read a body temperature between 33 °C - 37 °C

8 Apply ophthalmic ointment over eyes

9 Inject appropriate volume (based on mouse weight and desired dosage) of analgesic; an appropriate amount of saline can also be injected to prevent dehydration during the procedure

10 Carefully insert and secure the ear-bars. The position of the mouse head will be verified and adjusted once the skull is exposed, but it is recommended to make sure that the head is not visibly tilted

11 Clean the area of the incision with the povidone-iodine swab followed by the ethanol swab

11.1 Repeat Step 11, 3 times

12 It is preferred to apply line-block anesthetic (0.15% bupivacaine) under the skull skin before starting the procedure rather than applying EMLA cream on the sutured skin at the end of the surgery

Surgical Procedure

13 With the fine scissor, expose the skull by making an anterior-posterior incision

14 Visually identify bregma and lambda

15 Insert a glass pipette (a small volume of non-toxic food dye can be used to help marking the relevant spots) on the stereotaxic arm holder and lower it onto the skull

16 Mark bregma by gently touching the intersection of the coronal/sagittal sutures with the pipette tip, and zero the coordinates on the reader

17 Move to lambda (intersection of lambdoid and sagittal sutures) and measure its position relative to bregma

18 Minimize the deviation of dorso/ventral (D/V) and medio/lateral (M/L) distance between lambda and bregma by adjusting the position of the head

19 Re-zero the coordinates at bregma and repeat bregma/lambda measurements until satisfactory

20 Once the head is in the correct position, it is possible to identify the desired injection spot

21 It is recommended to use the measured anterior/posterior (A/P) distance between bregma and lambda to calculate an adjustment factor for the final coordinates: the measured B-L distance will be divided by the reference distance of 4.21. For an adult mouse, the obtained value (“adjustment ratio”) should be close to 1, and in this case no coordinates adjustment is required (but still optional). For smaller mice, the reference coordinates should be multiplied by the calculated adjustment ratio to obtain the final coordinates for the specific mouse

22 Move the pipette to the spot indicated by the adjusted A/P and M/L coordinates and mark it

23 Whether performing uni-lateral or bi-lateral injections, it is recommended to mark the spots on both sides of the skull, and to measure their relative dorso-ventral position. Their relative D/V deviation should be minimized by adjusting the position of the head

24 Once the desired spot has been marked, the marker pipette can be removed, and a hole is drilled in the skull at the indicated position

25 Blood and debris are cleaned with sterile saline and sterile cotton swabs

26 Insert micropipette with volumetric references in the holder and connect it to a syringe to apply positive/negative pressure

27 Draw up desired volume of viral solution in the syringe by applying negative pressure

28 Lower pipette loaded with the viral solution into the hole until the tip touches the dura. Zero the dorso-ventral coordinate

29 Gradually lower the pipette tip into the brain until the desired dorso-ventral coordinate is reached

30 Slowly inject the desired volume of viral solution (recommended: ~150nl/min) by gently and gradually applying positive pressure

31 Release pressure and leave the pipette in position for ~5-10 min so that the viral solution can spread and be absorbed by the tissue

32 Slowly retract viral injection pipette and discard it in an appropriate waste collection bin

33 Suture Skin

34 Optional: Repeat saline injection to prevent dehydration

Post-Surgery

1d 0h 25m

35 Remove animal from stereotaxic frame and place it in the clean, empty cage on heating pad until deambulatory (~ 00:10:00 - 00:15:00 25m

36 Once awake and deambulatory, mouse can be moved to the clean cage with gel food, also on heating pad

37 24:00:00 after surgery, a second dose of Metacam is administered and antibiotic ointment is applied on the sutured skin 1d

38 The health status of the mouse is monitored over the following days. If needed, additional doses of Metacam or saline can be administered

39 Mouse is normally kept in a cage on heating pad for at least 4 days and is then moved to standard housing

40 Mice are sacrificed for experiments at least 10 days after surgeries