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Intracranial injections of viral vectors in mouse striatum

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We use this protocol and it's working

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Abstract

The following protocol outlines the steps necessary for intracranial injections of viral vectors in dorsal or ventral striatum in mice. These steps are generalisable for different viruses and injection sites, however care must be taken to optimise injection coordinates and virus titre.

Materials

Reagents:

- Ethanol
- Sterile Saline
- Metacam
- Marcaine
- Eye Lubricant
- Chlorhexadine
- 3M™ Vetbond™ Tissue Adhesive, 3 mL

Equipment:

- Hamilton™ Small Hub Removable Needles
- Model UMP3T-1 UltraMicroPump 3 with SMARTouch™ Controller
- Small Animal Stereotaxic Instrument with Digital Display Console

Pre-Surgery Preparation

1 Check health of mice to undergo stereotaxic surgery: mice should look well-groomed and exhibit normal exploratory behaviour, have pink colouration to show good blood perfusion and oxygenation.

DO NOT operate on mice that do not meet these standards or that appear abnormal.

2 Collect virus from frozen storage (-80°C freezer) and keep on ice.

Note

Make sure that the virus is aliquoted into autoclaved PCR tubes and kept frozen at -80°C until use.

3 Prepare post-surgery recovery cages: include forage in the bottom of the cage, sizzle, cotton pads, wooden blocks, cylinder, pyramid house, long spout on drinking water bottles, wet mash food, metacam jelly.

4 Turn on the THERMACAGE recovery boxes; place some tissue in each chamber.

5 Spray designated surgical area, anaesthetic trolley and stereotaxic frame with disinfectant spray, wipe down, repeat leave for 5 min, and wipe down once again.

Note

Keep in mind that this does not sterilise the surfaces.

6 Ensure the surgical heating pad is on top of the stereotaxic frame.

Note

Height can be adjusted to get the mouse to the right level with bubble wrap.

Bubble wrap should be available to protect mouse-tail from the surgical heating pad.

7 Ensure syringes, needles, sterile swabs, sterile 0.9% saline, drill, hair clippers, are available and laid out for surgery. You will also need isopropanol swabs and a black marker. Ensure that all surfaces to be touched during the surgery outside the sterile field are appropriately covered with either clingfilm or barrier film.

7.1 Ensure that a sufficient amount of isoflurane anaesthetic has been added to the vaporizer reservoir for surgery.

7.2 You will need to prepare 4 syringes. These are the syringes for the Marcaine, Metacam, and two syringes for saline. The syringes should be filled slightly more than the amount needed for the average weight of the mouse (20 g) and placed briefly in the recovery chamber. One of the saline syringes should be filled all the way and placed on the sterile field to be used throughout the surgery.

Note

Make sure the syringes are not left too long to avoid heating the needle.

7.3 Check oxygen tanks are full (at least one full one in reserve on trolley – often use 1.5 small cylinders per day).

8 Place microinjector arm onto stereotaxic frame if using microinjector and Hamilton syringe for injections (1 turn = 50 nL)

8.1 Using the centrifuge tubes in the surgery area, make sure that the Hamilton syringe is functioning properly. This is done by taking up ethanol into the syringe and expelling it out. Repeat this three times. If working properly, the ethanol should be taken up and expelled quickly.

Note

Make sure the saline used is sterile.

8.2 Repeat the same process with the saline.

Note

Saline is more viscous than the ethanol, therefore it may be taken up slower but should still be smooth.

9 Load the virus into the Hamilton syringe.

9.1 Prepare the virus by spinning down the tube and cutting approximately halfway on the length of the tube.

9.2 Secure the open tube using the Blu-tack on the ear bar. Tighten the ear bar to prevent movement.

9.3 Move the Hamilton syringe to the centre of the tube and lower carefully till it is inside the tube. Now using the microscope, lower it further until it is 90% of the way in the tube.

9.4 Using a 200 nl/sec speed, load the appropriate amount of virus into the syringe. Withdraw the needle and move away from the surgical field.

9.5 Secure the remaining virus using masking tape over the opening of the tube and put it back on ice.

10 Place autoclaved surgical instruments onto sterile drapes on top of designated surgical area.

10.1 Prepare Marcaine (1:9 Marcaine to saline ratio) for local analgesia. Take care to use insulin syringes that are individually packed and sterile.

10.2 Prepare Metacam (1:4 Metacam to saline ratio) in a centrifuge tube for analgesia.

10.3 Check that the following instruments are available on the drape: scalpel, needle-holder, two kinds of scissors (one for cutting the sutures and the second for the incision) and two forceps.

10.4 Prepare some diluted hibiscrub (1:10) and some sterile saline (in falcon tubes) and place next to surgical instruments.

11 Turn on the surgical heating pad to allow it to heat up before the mouse is placed on it.

12 Place the probe under the drape to make sure the heating pad does not overheat the mouse when first put on the mat.

13 Fill the Hamilton syringe with the desired substance.

Anaesthetic Induction and Surgical Preparation

14 Collect mouse for surgery from holding room and record pre-operative weight and time.

15 Connect anaesthetic induction chamber to the vaporizer and ensure flow is directed for induction.

15.1 Place paper towels into the induction chamber.

16 Once 1 breath/s breathing rate reached and righting reflex absent, shave mouse head in induction box using clippers.

16.1 Once head is well shaven, quickly switch the anaesthetic flow from the induction chamber to the stereotaxic nose cone. Turn the isoflurane rate down to ~1.5-2 %.

17 Gently move the tongue of the mouse out of the way and place the mouse into the nose cone.

17.1 Place mouse in ear bars: left hand ear bar fixed, place this into left ear canal, pull gently back, then insert right hand ear bar.

17.2 Once in place tighten right ear bar and position nose cone close to mouse and tighten nose cone.

17.3 It is extremely important that the head it tightly locked in place: you can check by applying an appropriate amount of pressure with your index finger.

Note

If not properly secured, then your coordinates will be inaccurate.

18 Place the heating pad under the mouse with a paper towel between.

18.1 Use bubble wrap and 96 well plates to lift mouse to the appropriate height (in line with the nose cone).

18.2 Wrap the tail in bubble wrap for protection from the surgical heating pad.

18.3 Turn on the surgical heating pad (set to 37 degrees Celsius).

19 Cover mouse in surgical drape or cling film to maintain sterility.

19.1 Only place drape on once you are happy that the mouse is breathing at a steady level (no gasping), and no pinch reflex exists when the foot is pinched.

19.2 If mouse is gasping or not breathing turn rate down/off immediately: mouse should recover quickly (~10-20 s) when removed from isoflurane.

Note

May need to adjust nose cone too (if it is placed too tightly against the mouse nose it can restrict airflow).

20 Place eye lubricant onto eyes to prevent drying out.

Note

Take care to note the tip of the eye lubricant tube does not scratch the eye.

Only the lubricant should touch the surface of the eye.

21 Swab shaved head with ethanol swabs to get rid of any remanent fur.

21.1 Clean starting from the centre, making your way to the edge of the shaved area.

Note

Try not to use the same part of the swab repeatedly.

21.2 Once clean of hair, repeat the same process using diluted hibiscrub three times.

22 Gently lift the skin using sterile iris curved forceps.

22.1 Inject diluted Marcaine (1:9) under scalp for pre-incision analgesia.

Note

The amount will depend on the weight of the mouse.

A dome should be seen form under the skin when Marcaine is being injected. Allow 5min for
analgesia to take effect.

22.2 Inject appropriate dosage of Metacam subcutaneously according to mouse weight.

23 Inject saline subcutaneously.

24 Prepare yourself for aseptic procedure: wash hands with Hibi scrub, dry hands with autoclaved paper towels and put on sterile surgical gloves.

Surgery

25 **Throughout surgery regularly check under drapes that mouse breathing rate is slow and steady and that depth of anaesthesia is appropriate. Also, regularly check mouse temperature on heat controller unit.**

Make incision in-between ears towards back of skull (make just large enough so you can see both bregma and lambda and you are able to locate your drill site).

25.1 Keep the scalpel steady and make one uninterrupted incision.

Note

If needed, extend the incision using smooth cuts with the scissor.

Multiple attempts using the scalpel will result in jagged edges which will impede recovery and prevent correct tissue apposition.

Put any used tools on a different drape and not back on the sterile field.

25.2 Use sterile swab to clear, as gently as possible, subcutaneous tissue to see lambda and bregma.

Note

The lambda and bregma appears as a shiny substance at the cut site, you will know the tissue is clear when the shine has gone.

Put any used cotton swabs directly in the clinical waste bin and not back on the sterile field.

26 Check DV position at bregma and lambda to ensure skull is flat (using Hamilton syringe needle under the microscope).

26.1 Ensure there is no more than a 0.1 mm difference between bregma and lambda DV position.

26.2 Move nose bar up or down to level skull (if required; by loosening uppermost screw and moving nose bar along pivot).

27 Once bregma and lambda are levelled on the DV plane, set the ML (x-coordinate) and AP (y-coordinate) of bregma to 0: then move to the desired injection site using your pre-selected coordinates.

Note

The coordinates used by us for targeting the striatum were as follows:

Dorsal striatum (AP = +0.8 mm, ML = ± 1.80 mm, DV = -2.4 mm)
Ventral striatum (AP = +1.3 mm, ML = ± 1.20 mm , DV = -3.75 mm)

27.1 At the pre-selected coordinates, move the needle just above the skull. There should be enough room for the marker to make a dot under the needle.

27.2 Take note of where on this dot the pre-selected coordinates are by moving the needle closer to the skull.

28 Drill hole.

Note

Take care to try not to break dura, apply gentle pressure to drill (needs very little pressure).

28.1 Clear hole of bone debris with sterile saline and sterile swabs.

28.2 Pick through dura with sterile disposable needle tip.

28.3 Ensure that Hamilton syringe needle can access drill site, i.e. you drilled in the correct location. Keep the exposed brain tissue moist with saline.

29 Take the DV coordinates of the surface of the brain (you will see a slight change in the light refraction
when the needle makes contact with the brain surface). This point should be approximately halfway through the bevel of the needle.

29.1 Ensure Hamilton is dispensing correctly.

29.2 Lower the Hamilton syringe to the DV site slowly (about 1 mm per min).

29.3 Inject the substance slowly (no more than 200 nL/min) and wait 5 min to ensure appropriate diffusion. Retract syringe slowly (about 1 mm per min).

29.4 If injection site is deep, retract 0.5 mm and wait 5 more min, then retract fully.

30 Repeat **steps 27, 28, and 29** if doing bilateral injections.

31 Check no debris under scalp, clear with sterile saline if necessary.

32 Ensure that a sterile drape or clingfilm is covering the mouse and change into a new pair of sterile gloves.

33 Suture incision with non-absorbable, monofilament suture (4-6 stitches usually).

33.1 **Optional:** Add skin glue in between the individual sutures, no more than one drop should be needed for regular incisions. Use more if larger incisions were used.

Note

Take care that you do not put too much skin glue or the skin glue does not cover the sutures. This irritates the skin more and the mice are more likely to scratch and open the wound. This may also result in overgrooming so should be applied with care.

34 Inject 0.5 mL of 0.9% sterile saline subcutaneously (0.5 mL per site).

35 Remove mouse from ear bars.

36 Turn off anaesthetic and oxygen.

37 Weigh mouse and record post-op weight.

38 Record cage number mouse came from, date of birth of animal, genotype, tail markings, pre-op weight, post-op weight, time awake following surgery, all surgery co-ordinates, time injection took to complete,
volume of substance injected. Any other notable observations regarding health status of mouse during or after surgery or things which may eventually affect health of mouse should also be noted.

38.1 If doing multiple surgeries, start the second surgery with a fresh set of tools.

38.2 Clean the used set of tools with a tootbrush and hibiscrub.

38.3 Soak the tools in Reprozyme (enzymatic cleaner) and bead steriliser prior to using in a surgery.

Post-Operative Recovery

39 Allow mouse to recover in the THERMACAGE.

39.1 Monitor mouse until awake and exploring/feeding/grooming.

40 Move to surgical cage prepared earlier in Step 3.

40.1 Ensure metacam jelly is provided. Alternatively, metacam is available for subsequent daily administration (oral/s.c.), if not using the jelly method.

40.2 Place some old bedding into the new cage to make it familiar to mice and reduce the chance of fighting when rehoused.

41 Monitor animals for 7 days postoperatively, including mouse weight and behaviour as per post-operative monitoring form.

Note

Mice should be at a **steady weight no less than 5% of starting weight** for several days to halt daily postoperative monitoring.

41.1 Fresh metacam jelly or subcutaneous injections should be provided for the first three days postoperatively (at a minimum, can be continued if required according to postoperative monitoring scoring).

41.2 If mouse is losing weight and is below pre-surgery weight, place easily accessible forage in bottom of cage (banana chips etc.) and some jelly in a weigh boat in cage until mouse gains weight back.

41.3 If wound infected or animal lost more than 15% of its pre-surgery body weight for more than 48 hours, the animal should be culled.

42 Mice should be kept with littermates that have had surgery and not with those that have not.

Note

If only single mouse had surgery and other littermates have not then singly house surgery mouse to prevent others fighting with more vulnerable surgery littermate.

43 Mice should be monitored weekly, including weight and behaviour, until animal is culled for experiment.