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Injection of AAV and Prism GRIN Lens Implantation – Combined Surgery Protocol

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We use this protocol and it's working

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Abstract

This protocol describes a combined surgical approach involving AAV injection and prism–GRIN lens implantation in mice.

Materials

Material checklist:

- Gelfoam spears
- Cotton tip applicators
- Eye ointment
- Betadine
- 70% EtOH
- Sterile water
- Injector + Hamilton syringe (10 µl, removable needle, 33G)
- Hand drill with 0.5 mm drill bit
- 25G or 27G sharp needle with bevel bent into a hook
- Scalpel
- Hydrogen peroxide
- Saline (on ice)
- Surgical instruments (blunt forceps, Dumont forceps, fine scissors, needle holder)
- Lens paper
- Fine-tip marker
- ProView integrated prism lens
- Stereotax rod with ProView holder and ProView screwdriver
- ProView integrated lens covers
- Dummy microscope
- Metabond (powder + scoop, base and accelerant)
- Metabond plate (on ice, in freezer overnight the day before)
- Kwik-Sil
- Analgesics/anti-inflammatory.

Station preparation

1 Autoclave surgery tools.

2 Disinfect bench and place a bench pad on the working surface.

3 Setup the necessary equipment including:

- Stereotaxic frame
- Injector
- Warming pad
- Drill
- Induction Chamber

4 Check the isoflurane levels. Levels visible on the front of the vaporizer.

5 Designate a sterile area on the working surface for the sterile material (instruments, suture material, gauze, etc.).

Prepare injector

6 Set the syringe type (1700), the injection volume and injection speed (100 µL /min).
Make sure injection mode is set to infusion.

7 Clean the Hamilton syringe with sterile water, then 70% ethanol then sterile water again. Make sure it is not clogged and that the plunger can move up and down smoothly.

8 Place the syringe in the injector. The needle of the syringe will be used for leveling the skull.

Prepare and level the skull

9 After anesthetizing the mouse, shave its scalp with a razor. Put it back into the anesthesia chamber if needed.

10 Fix the mouse to the stereotaxic frame, making sure it's stable in all planes.

11 Apply eye ointment.

12 Clean the scalp with betadine, then 70% ethanol then betadine again.

13 Cut scalp with a surgical blade. The midline incision goes from between the eyes to right before the ears, such as to expose the fronto-nasal suture, the bregma suture and the lambda suture.

14 Rinse skull with cold saline then clean it with a cotton tip soaked in hydrogen peroxide to get rid of all conjunctive tissues and dry the skull. This will allow the sutures to appear white and well defined.

15 Level skull using the injection needle

- In the AP plane: less than 50 µm DV difference between Bregma and Lambda,
- In the ML plane: less than 50 µm DV difference between point +2.0 and –2.0 mm ML to Bregma.

Injection

16 Move injection needle to target site according to the AP and ML stereotaxic coordinates of injection ROI.

Note

Injection site should be about 250 µm away from imaging plane.

17 Raise the needle and mark target site with a fine tip marker.

18 Drill a burr hole over the target site.

19 Clean the hole from bone powder and blood, rinsing with cold saline.

20 Remeasure the DV coordinates at dura level and calculate corresponding target DV coordinates.

21 Lower the injection needle to target DV, at 100 µm/s.

22 Wait for 00:01:00 . 1m

23 Inject 300 µL at 100 µL /min.

24 Wait for 00:05:00 . 5m

25 Raise the injection needle 500 µm at 100 µm/s and wait for 00:01:00 . 1m

26 Raise the injection needle completely out of the brain at the same speed.

Make a burr hole for the GRIN Lens

27 Move injection needle to target site according to AP and ML stereotaxic coordinates of implant ROI.

28 Raise the needle and mark target site with a fine tip marker.

29 Drill a 1 mm burr hole over the target site.

30 Clean the hole from bone powder and blood, rinsing with cold saline.

31 The hole should be just slightly larger than the lens so that the lens fits right within the hole. Adjust size if necessary.

32 Use needle hook or Dumont #5 forceps to remove bone fragments from the hole's edges.

33 Remove dura with a needle hook and irrigate with cold saline.

34 Wet little pieces of gel foam with cold saline and clean the exposed brain surface, very gently. Repeat until the cranial window is clean and clear from any debris or blood. If bleeding persist, place a piece of gel foam soaked in cold saline on it for 00:05:00 to stop the bleeding. Make sure the gel foam piece is very wet when removing it. 5m

Prepare and implant the prism lens

35 Screw the dummy microscope to the ProView Stereotaxic rod and attach the rod to the stereotaxic frame.

36 Gently attach the prism lens integrated to the baseplate to the dummy microscope. Do NOT tighten the screw on the baseplate, the magnets are enough for a stable attachment.

37 Soak a lens paper with 70% ethanol and clean all the surfaces of the lens.

38 Center the lens over the hole, zero at dura and calculate corresponding DV coordinates of ROI.

39 Lower the lens very slowly at 10 µm/5 s increments to reach DV target. The lens should have a 1 mm segment over the skull available for adhesive application.

Note

The prism lens is sharp enough to go through the brain tissue without the need to create a lens track.

Secure the lens to the skull

40 Remove any fluid that may have accumulated around the lens using a small gel foam piece.

> Note
>
> ***The skull and lens must be completely dry before applying any adhesive.***

41 If needed, apply Kwik-Sil using a 25G needle (or smaller) around the lens at the edge of the hole to protect any exposed brain tissue.

> Note
>
> ***Excessive use of Kwik-Sil might decrease stability.***

42 Mix metabond using 3 scoops of powder, 7 drops of base and 1 drop of catalyst. The mix will be fluid at first which will allow it to spread nicely on the skull and around the lens.

43 Apply around the lens and on the skull. As you apply it, it will harden progressively allowing you to build up several layers to reach the integrated baseplate.

44 Wait for cement to cure, about 00:15:00 . 15m

> Note
>
> ***Depending on room temperature, curing time might differ. Make sure the cement is very hard before proceeding.***

45 Grab the baseplate with a needle holder firmly, and slowly raise the stereotax rod to detach the dummy microscope from the baseplate.

46 Using forceps pick up the baseplate cover and place it on top of the baseplate with the notch aligned to the baseplate's screw.

47 Tighten the screw to secure the cover but be careful to not over tighten. When you feel a resistance, add a quarter turn.

Finishing up

48 Provide post-op analgesics and anti-inflammatory medicine.

49 Detach the mouse from the stereotax and put it back in its cage on the heating pad.

50 Wait for it to wake up and start moving.

Note

Mouse will have a little bit of trouble adjusting to the weight of the head stage. It should be able to move normally withing an hour post-anesthesia recovery.

51 Provide post-op care and close monitoring at least 5 days post-surgery.