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GRIN lens implant (1 mm)

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We use this protocol and it's working

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Abstract

This procedure requires a viral injection ahead of time. This is the standard 1 mm GRIN lens implant protocol for Rodrigues-Vaz and Athalye et al, 2025.

Materials

- Sterile surgical tools
- Alcohol for cleaning
- Hydrogen Peroxide (for sterilizing skull).
- Sterile Saline (fill 1cc insulin syringe)
- Gauze pads & Betadine wipes
- Gel foam (to clean/stop bleeding)
- Paper towels for surgery and to elevate mouse to right height for ear bars
- Cotton tips/swabs
- KimWipes
- Ensure to have enough isoflurane (fill to max line in holder on bottom right of regulator) for the duration of the surgery.
- Dental Drill
- Razor- ensure batteries are charged for hair removal
- Vacuum system to hold lens / lens holder
- Cyanoacrylate glue
- Metabond
- Blunt needle
- GRIN lens
- Head bar
- Silicone sealant (Kwick-Sil or Kwick-Cast)

Safety warnings

-Wear appropriate PPE as required by your institution.

Ethics statement

This protocol was approved by Columbia University IACUC. Please do not perform any of these procedures unless there is prior approval from the institution's animal ethics committee.

Before start

-Collect mouse previously injected with desired virus (at least 5 days before this surgery)
-Collect lens to implant.
-Turn on oxygen (~1.5 L/min) and extraction pump for isoflurane machine.
-Turn stereotaxic heating pad on
-Administer analgesia on day of surgery subcutaneously: carprofen (5mg/kg body weight) repeated daily to ensure 72h of pain relief (by surgeon or approved lab personnel) or buprenorphine XR or SR (administered by CU animal care staff according to IACUC protocol and standard of care)

Placement of mouse in stereotaxic frame

1 Weigh mouse

2 Anesthetize mouse in isoflurane chamber (set initially to 3-5% for induction). Monitor until the mouse is under the anesthesia

3 Remove the mouse from chamber and shave head (can use isoflurane nose tube if working to maintain anesthesia)

4 Administer bupivacaine (2 mg/kg body weight) intradermal. Do this after shaving and before an incision is made.

5 Place upper front teeth of mouse into stereotaxic equipment and position tongue so that the mouse won't choke

6 Ensure nose is inside the anesthesia cone. Set isoflurane machine to 2% and decrease through surgery
(1-2% for surgery is typical). Confirm plane of anesthesia before starting and throughout surgery with toe pinch and breathing rate monitoring (by eye)

7 Holding the mouse by the ears, position the side holders of the frame and tighten over temporal lobes- if heads moves when pushing down, tighten further. There should be a straight line between the top of the frame and the corners of the ear and eye ("cheeks").

8 Looking from behind the mouse, check front-back alignment

9 Push down on the mouse's head to check everything is secure before beginning surgery

Cranial surgery

10 Add protective cream to the mouse's eyes (usually eye lubricant) using a sterile cotton swab

11 Clean surgery area with 3 alternating rubs of alcohol and then iodine/betadine swabs to sterilize

12 Prepare all sterile surgical tools and place them on one side of the stereotax

13 Change gloves to sterile gloves, cover mouse with sterile surgical drape

14 Expose skull - hold skin taught with forceps and cut skin with small scissors

15 Clean tissue from skull (periosteum) using hydrogen peroxide. The hydrogen peroxide will digest periosteum; clean well with cotton tip.

Note: due to previous surgery the periosteum and/or skull may be bloody; if needed use gel foam to stop bleeding.

16 Attach needle to stereotaxic frame holder and measure Bregma and Lambda dorsal-ventral (DV)
coordinates – they should be within 0.05 mm of each other. Adjust head until coordinates are within that range. Repeat the process with 2 different medial-lateral (ML) coordinates, comparing DV coordinates of Bregma+2.0 mm and Bregma-2.0 mm

17 After skull alignment, position the needle over Bregma. Make note of anterior-posterior (AP) and medial-lateral (ML) coordinates.

18 Calculate required coordinates and mark the position(s) on skull using ink on the end of the needle; mark edges of the lens: 0.5 mm from center mark. For lens implant over DLS we use the following coordinates: AP=+0.75 and ML=2.5.

19 Make hole using dentist drill. Rinse down the area with sterile saline if needed for a better view. Use absorbent padding (gel foam) if bleeding occurs.

20 Test that hole is big enough for the GRIN lens to fit.

Cortex removal and GRIN lens placement

21 Place lens in holder in vertical aligned position (vacuum holder).

22 Aspirate tissue above desired depth with blunt needle attached to vacuum system; wash tissue continuously to avoid blood drying inside

23 Align lens with hole and lower to desired depth – DV=2.0 for DLS; if there is resistance when lowering the lens, remove lens, align it and lower again making sure the lens is within hole.

Note: step 21 and 22 should be done within 5 minutes; crucial to assure that no blood dries inside hole for
the lens impairing future imaging. Wash blood often during these steps to avoid dried blood.

24 Use cyanoacrylate glue to hold lens in place; Zip-Kicker is used to speed up drying. Be careful that glue does not go on top of the lens, as that could impair imaging.

25 Remove vacuum holding system and use more glue to secure lens to the skull.

26 Using Metabond cement, build cap around lens securing it to the skull.

27 Place head bar and secure it, building crater around lens to protect it; tilt and turn mouse around as necessary while still under anesthesia to build cap.

28 Lens may be covered with a silicone sealant for protection(Kwick-Sil or Kwick-Cast).

Completion surgery

29 When finished, switch off isoflurane and oxygen and remove the mouse from stereotax.

30 Place mouse in the home cage, on a heating pad and keep under observation until they recover and are ambulatory.

31 Remove cage grid and place gel food on floor.