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Virus Injections

Forked from Costa Lab Nanoject Virus Injections-CU

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Mafalda Vicente[1], Christine Weber-Schmidt[1], Ines Rodrigues-Vaz[2,3,1]

[1]Aligning Science Across Parkinson's (ASAP);
[2]Zuckerman Mind Brain Behavior Institute, Departments of Neuroscience and Neurology, Columbia University;
[3]Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown

Ines Rodrigues-Vaz

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We use this protocol and it's working

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Abstract

This is the standard stereotaxic/Nanoject injection mouse surgery protocol for Rodrigues-Vaz and Athalye et al, 2025.

Materials

Materials Needed

- 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
- Nanoject III
- Ensure to have enough isoflurane (fill to max line in holder on bottom right of regulator) for the duration of the surgery.
- Mineral oil
- Dental Drill
- Razor- ensure batteries are charged for hair removal

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

-Prepare capillary tubes for injection (Drummond 3.5″ capillary tubes #3-000-203-G/X) with Pipette puller; program-Heat 360; FIL 5; Vel 25; Del 10; Pul 50; (Sutter P-2000). Cut tip of capillary tubes leaving enough to go down 3mm – this should be straight and tip diameter should not be too small for virus flow.
-Turn on oxygen (~1.5 L/min) and extraction pump for 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 post shaving 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 Make incision (try to make only one cut) between the front of the ears and the beginning of the eyes (back to front) - hold skin taught with one hand while cutting. Insert clamp or hold skin open.

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

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+2.2mm and Bregma-2.2mm.

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

18 Calculate required coordinates and mark the position(s) on skull using ink on the end of the needle. For DLS we use the following coordinates: AP=+0.75 and ML=2.5 (left side).

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

Nanoject Set up & Injection(s)

20 Remove the 2 bottom black parts of nanoject – the chuck and the collet; slide them onto the capillary tube.

21 Place black ring and green seal ring onto the wire plunger of the Nanojet

22 Fill capillary tube with mineral oil, using the backfilling needle included in the Nanoject kit. Place syringe all the way into needle to avoid air bubbles.

23 Mount Nanojet to stereotax and slide the capillary tube onto the wire plunger; push collet, chuck and capillary towards the seal and screw the collet to the barrel of the Nanojet

24 Empty needle (press “EMPTY” in the “MANUAL” menu) until beep

25 Prep virus on cap of 0.2mL tubes (cut rim for better visualization) and use ticky-tack to place it in a surface to stabilize it; place tip of the capillary tube in contact with the viral solution and press “FILL” careful not to pick up air bubbles.

26 Lower tip of the capillary tube into final position for brain injection - for DLS: DV=2.4

27 Slowly inject solution = 4.6-20 nL/pulse.

28 Wait at least 5min prior to slowly removing capillary tube.

29 Seal skin together with sutures.

Completion of Surgery

30 When finished, switch off isoflurane and oxygen to the mouse.

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