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Mouse Stereotaxic Surgery for Alpha Synuclein Pre-formed Fibrils (PFFs) Injection in the Dorsal Striatum V.2

Version 1 is forked from Mouse Stereotaxic Surgery

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We use this protocol and it's working

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Abstract

This protocol outlines the procedures for conducting stereotaxic surgery in mice for intracranial injections of PFFs into the dorsal striatum of the mouse brain.

Preparation of surgery apparatus

1 **Surgery room setup**

1.1 Turn on the bead sterilizer and light. Sterilize all the surgical instruments.

1.2
- Turn on the injection device. We use Nanoject III microinjector (Drummond Scientific) for minimal damage to the cortex and striatum.

1.3 Set a clean, empty cage halfway onto the heating pad in the prep area, and turn on the heating pad for mouse recovery after completion of surgery.

1.4 Place your sterile surgical tools (forceps, scalpel, small scissors, hemostat, and surgical clips) on one side of the stereotax (KOPF, Model 900 LS).

1.5 Dispense a small amount of Nair hair remover into a weigh boat and place to one side.

1.6 Dispense a small amount of Betadine into a weigh boat and place it to one side.

1.7 Place a packet of sterile swabs nearby.

1.8 Make sure the small heating pad on the stereotaxic is on, and cover it with sterile folded napkins.

Preparation of drugs for injection

2 **Prepare drugs and sonicated PFFs**

2.1 Draw up in syringes all drugs that you will need for the surgery (Lidocaine below 7 mg/kg, Bupivicaine below 8mg/kg, preoperative Ethiqa XR 3.25 mg/kg, Caprofen 5mg/kg SC, sterile normal saline,).

Sonicate PFFs and make them ready for injection.

Mouse preparation and anesthesia

3 **Mouse anaesthesia**

3.1 Transfer the mouse into an anesthesia induction chamber and induce anesthesia using 4% isoflurane with an oxygen flow of 1 L/min (Harvard Apparatus Mobile Anesthesia Systems, catalog # 750239). Maintain this setup until the mouse is completely anesthetized, which typically takes around 2.5 to 5 minutes (confirmed by lack of response to nociceptive stimuli such as pinching the toes and observing a normal breathing rate after full anesthesia is achieved).

Swiftly remove the mouse from the chamber and position it in a stereotaxic frame by gently opening its mouth and putting its teeth through the bite bar, and slipping the anesthesia nose cone over the snout.

Continue administering anesthesia using 1–1.5% isoflurane at an oxygen flow of 0.5–1 L/min. To sustain the mouse's body temperature around 37-38 degrees Celsius during the surgery, employ a warming pad equipped with temperature sensor probes (RightTemp® Jr, Kent) underneath the mouse.

Pre-operative treatment and PFFs injection

4 **Pre-operative treatment and surgery**

4.1
- Apply lubricant to the eyes bilaterally to prevent corneal damage and dryness.
- Provide Ethiqa XR injection subcutaneously.

4.2 For the remainder of the procedure, check the breathing rate and responsiveness (by toe pinch) at least every 15 minutes and maintain the record. Adjust the anesthesia accordingly (typically between 1.5-1.0% for maintenance).

4.3 Adjust the ear bars so that the animal’s head is symmetrically held in the stereotax. You can use the forceps to exert gentle downward pressure on the head and verify it does not move. If it moves, then adjust the ear bars.

4.4 Prep the scalp as follows:
- Use a swab to apply hair remover to a strip down the middle of the mouse’sscalp, working it down to the scalp, and leave for 2-3 minutes.
- Wipe away hair remover and hair with a fresh swab.
- Add alternating swabs of betadine and 80% ethanol x 3 to this area. Inject a mixture of lidocaine and bupivacaine at the scan as local anesthesia.

4.5 Confirm there is no toe pinch response.
Use the scalpel to cut a midline incision down the long axis of the scalp. Use the small scissors to enlarge the incision to the desired length. Use the surgical clips to grab the midpoint of the skin on each side of the incision to enlarge the surgical field.

5 Drilling and skull preparation.

5.1 Drill hole at desired coordinates:
- Place the Nanoject III microinjector (Drummond Scientific) fitted with a capillary needle at Bregma, lift it up, and move it in the AP and ML directions to position the desired coordinates for injection. For dorso-striatal injection of PFF, we are using +0.2 mm anterior, +/-2.0 mm lateral, and +2.6 mm ventral beneath the dura to Bregma coordinates.
- Using foot pedal, drill through the skull at this location, preferably without piercing the dura or damaging the underlying brain.

5.2 Remove the drill from the stereotax.

6
- Inject PFF using Nanoject III microinjector (Drummond Scientific) at the desired rate and desired volume. We inject 2 µL [M] 5 µg/µL at the rate of 2 µL per second in 100 cycles with a delay of 8 secound per pulse .

6.1 After the injection has been completed, wait for 8-10 minutes.

6.2 Slowly withdraw the needle while watching for fluid coming out of the hole upon withdrawal (which would indicate a failed injection, possibly due to a clog in the needle).

7 Close the opened skin using the appropriate suture. We are using PGA+ absorbable suture (Covetrus, SLU 057463).

Inject analgesic Carprofen (5.0 mg/kg IP, SC).

Transfer the mouse from the stereotaxic frame to another mouse cage provided with a heating pad for recovery. Once the mouse has recovered, please return it to its home cage.

Post-operative treatment and care

8 Provide Carprofen (5.0 mg/kg IP, SC) at an interval of 24 h for 2 days and post-operative care for 72 hours.