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Standard Operating Procedure: Mouse Spinal Cord Injection Surgery

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We use this protocol and it's working

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Abstract

The intent of this Standard Operating Procedure (SOP) is to describe procedures for mouse spinal cord injection surgery.

Guidelines

RESPONSIBILITY

Principal investigator (PI), their staff, or any individual performing surgery on rodents or assisting in those procedures.

Materials

- Anesthetics (e.g. isoflurane) and induction chamber
 - o 2 charcoal filter canisters for isoflurane output
 - o Necessary tubing to connect equipment
- Stereotaxic surgery frame
- Drill with 0.5 mm drill bit, power supply, and foot pedal
- Warming pad, anal probe, and power supply
- Nanoliter injector (NANOLOTER-2020, WPI)
- Electric clipper
- Retractors
- Non-steroidal analgesic (e.g. carprofen)
- Sterile 0.9% saline
- Antiseptic solution for skin (e.g., povidone-iodine solution and 70% ethanol)
- Sterile ophthalmic ointment
- Sterile surgical instruments
 - o Scalpel
 - o Blunt Forceps
 - o Dumont forceps
 - o Fine Scissors
 - o Needle holder
- Sterile swabs and gelfoam spears
- Sterile drapes
- Glass pipettes for injection
- Suture material.



Surgical and Station Preparation:

- 1 Print and prepare spinal cord surgery forms from Chu Lab SharePoint.
- 2 Prepare solutions (e.g., PBS, AAV virus, and retrograde beads) needed for spinal cord surgery following Chu Lab inventory and SOPs.
- 3 Sterilize the necessary surgical tools and supplies, including scalpel, blunt forceps, dumont forceps, fine scissors, and retractor hooks in an autoclave.
- 4 Disinfection surgery station and place down a bench pad.
- 5 Setup necessary equipment including:
 - a. Stereotaxic frame
 - b. WPI glass pipette injector and controller
 - c. Warming pad
 - d. Drill
 - e. Induction Chamber
- 6 Check the isoflurane levels, make sure the isoflurane is enough to complete surgery.
- 7 Put a metal plate under the surgery frame to allow the magnetic retractors stand.
- 8 Disinfect an area on the working bench for the sterile surgical tools and supplies.

Pre-operative Procedures:

9

Note

Perform pre-operative procedures at a safe distance from the surgical environment in order to prevent contamination with hair.



Weigh the mouse and then put it into an induction chamber for anesthesia.

9.1 Open valves to oxygen tank and adjust to a flow of 1 liter per minute . Adjust the level of vaporizer to 3 percent isoflurane in oxygen and wait for the cessation of movement.

10 Shave the neck and the upper back of mouse for cervical injection.

11 Place mouse in the stereotaxic frame. Once nose cone is in place, close valve to chamber and open valve to nose cone and reduce vaporizer to 1.5-2 percent isoflurane in oxygen.

12 Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.

13 Place anal probe underneath body to regulate temperature. Heating system prove setpoint is  36.9 °C .



Surgical Procedures:

1m

14 Administer carprofen ( 5 µL , s.c.).

15 Using 70% ethanol on a sterile cotton swab in a circular motion, sterilize surgery site from the center of the surgical site to the exterior. Then apply povidone-iodine solution using cotton swabs in a circular motion from the center of the surgical site to the exterior. Repeat this process for a second time.

16 Tape the limbs and tail of mouse to the surgery frame, make sure its body is steady during surgery.

17 Place a suitable wedge under the lower neck/upper back of mouse to lift the cervical spinal cord.

18 Apply ophthalmic ointment to the eyes to protect them during the surgery.

19 Make a 2 cm long incision using a scalpel starting at the base of the mouse head and extending through the middle of the back.

20 Use three retractors to retract the skin and create a triangular surgical window.



- 21 Using micro scissors to cut the fascia that might hold the trapezius muscle and adipose tissue. Adjust the three retractors to tuck away the musculature and adipose tissue.
- 22 Locate the second thoracic vertebra (T2) and snip the muscles that are attached to T2.
- 23 Use another two retractors to retract the midline muscles to fully expose the T2.
- 24 Use T2 as a landmark to locate the appropriate cervical segments for injection.
- 25 Gently clean the exposed spinal segments using swabs and gelfoam spears to obtain a clear view of the segments.
- 26 Carefully peel the meninges to make sure that glass pipette can puncture into the spinal cord.
- 27 Fill the glass pipette with miner oil and mount it to the WPI injector.
- 28 Draw up the necessary volume of solution (e.g., retrograde beads, AAV virus, and PBS) into the glass pipette and WPI injector.
- 29 Move the glass pipette tip to the surface of target site using micro-manipulator. The tip should be  500 μL away from the midline.
- 30 Gradually lower the glass pipette tip to target D-V using the micro-manipulator and wait  00:01:00 .
- 31 Set the volume and rate of release of solution on the WPI injector controller. Typically,  300 μL of the AAVs solution is injected at a rate of 50 nl/min.
- 32 Once injection is done, wait  00:03:00 -  00:05:00 before retracting glass pipette to avoid backflow.
- 33 Gradually withdraw glass pipette until completely removed from the spinal cord.

1m

8m





- 34 Repeat the procedure if more than one injection is required.
- 35 Suture muscles and skin with sterile suture (polyamide-nylon, PDS, Vicryl; size: 3-0 or 5-0) and sterilize skin with betadine.

Post-Operative Procedures:

- 36 Remove animal from stereotaxic frame and place animal into a cage on a warming pad. Observe until animal is awaking and regained righting reflexes.
- 37 Document surgery on a surgical procedure cage card and return cage to appropriate room in the vivarium.
- 38 Administer Carprofen ( 5 μ L , s.c.) the next day.
- 39 Continue daily observations for 3 days post-operation and record on the back of the surgical procedure cage card. Extend observation period as necessary to ensure a healthy recovery.