




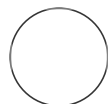
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Stereotaxic Surgery

 In 1 collection

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ABSTRACT

This protocol describes the steps for performing mouse stereotaxic surgery. It is applicable to intracranial injections of viruses and drugs.

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Before Surgery

- 1 Autoclave instruments (forceps, clamps, and scalpel holders; max 5 mice per tool pack) and cotton swabs.
- 2 Pull injector glass micropipettes.
- 3 Gather supplies needed for surgery (viruses, drugs, anesthetics, analgesics).

Surgery Setup

- 4 Disinfect surgical station.
- 5 Turn on injector (Nanoject III), glass beads sterilizer, digital readout system, lamp, and heating pad.
- 6 Load injector micropipettes with injectable (virus, drugs).
- 7 Set an area on one side of the stereotaxic frame to place sterile surgical tools, leveling tools and drill.
- 8 Set out cotton swabs, super glue, carprofen, bupivacaine, eye ointment, alcohol wipes and

betadine.

- 9 Place paper towels in the anesthesia chamber and on top of a heating pad in the stereotaxic frame.
- 10 Set a clean, empty cage for recovery onto a heating pad.
- 11 Start surgical log for the session.

Mouse surgical preparation


1m 30s

- 12 Turn on the oxygen (1 L/min) and adjust the anesthesia machine vaporizer to 5% isoflurane.
- 13 Weigh mouse.
- 14 Inject carprofen (5mg/kg) subcutaneously (s.c.).
- 15 Place mouse in anesthesia chamber until surgical plane of anesthesia is reached (check with toe pinch).
- 16 Shave scalp.

- 17 Place mouse on the stereotaxic frame: scruff mouse, gently pull tongue out and to the side with forceps, place front teeth onto the hole in the nose clip, pull cone over nose/mouth.
- 18 Switch isoflurane flow from anesthesia chamber to stereotaxic frame nose cone and lower it to 1-2%.
- 19 Mount the ear bars to held the mouse's head symmetrically held in the stereotaxic frame. Verify that the head is stable by lightly pushing the back.
- 20 Apply eye ointment bilaterally.
- 21 Prep incision site applying betadine with a cotton swab and ethanol sterile wipes.

Surgery

5m

- 22 For the remainder of the procedure, check the breathing rate and responsiveness for surgical plane of anesthesia with a toe pinch (this should be done every ~  00:05:00).
- 23 Use the scalpel to cut an incision down midline of scalp. Enlarge the surgical field using surgical clips to grab the skin on each side of the incision.

5m

- 24** Apply a splash of bupivacaine ([M] 0.6 mg/mL bupivacaine) under the skull skin.
- 25** Adjust lights and pre-level skull by eye.
- 26** Level the skull:
- 26.1** Using the microscope, lower the drill bit until it touches bregma. Lift the drill bit and move it posterior and lower it to lambda. Adjust the head's position so that bregma and lambda are aligned in the midline.
- 26.2** Position the drill in the middle between bregma and lambda and use the stereotaxic alignment indicator for leveling the head in X- and Y-directions.
- 27** Drill holes:
- 27.1** Re-zero drill tip at bregma.
- 27.2** Move to X and Y desired coordinates.
- 27.3** Drill through the skull without piercing the dura or damaging the brain.

27.4 Remove the drill from the stereotaxic frame.

28 Injection:

28.1 Puncture the exposed dura using a bent 32G needle. Use cotton swabs to clear any debris and fluids.

28.2 Load injector onto the stereotaxic frame and drive it to center of the drilled hole and zero Z-axis at the dura level or the surface of brain.

28.3 Select the appropriate program volume and rate (usually 2nL/s) for the virus/drug to inject and start the injection. Add saline to the skull with a syringe to prevent drying.

28.4 After the injection has been completed, leave in place another 5 minutes to allow diffusion.

28.5 Slowly withdraw the injector.

28.6 Repeat for other holes if needed.

29 Close incision:

29.1 Remove ear bars and pull skin back together using cotton swabs.

29.2 Apply a thin layer of glue along incision and pinch skin together using forceps.

29.3 Loosen the mouse cone and remove mouse from stereotaxic frame.

29.4 Place mouse gently in the recovery cage until ambulation; then return mouse to home cage.

29.5 Complete surgical log.

Between surgeries clean-up

30 If another surgery will be done, sterilize tips of instruments using the bead sterilizer. If surgical tools have been used 5 times, open a new pack of sterile tools and replace scalpel blade.

31 Disinfect stereotax with ethanol.

- 32** At end of day, turn off oxygen, anesthesia machine, injector, bead sterilizer, digital readout system, lamp, heating pads.

Post-operative care

- 33** Administer a second dose of carprofen (5 mg/kg, s.c.) 24 hours after surgery.