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Mouse Stereotaxic Surgery

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ABSTRACT

This protocol describes the steps for performing stereotaxic surgery in mice. It is applicable to intracranial injections (e.g. virus, drug) and placement of implants (e.g. optical fibers, electrode arrays) into targeted regions of mouse brains.

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1 Prepare drugs and viruses

1.1 Draw up in syringes all drugs that you will need for the surgery (typically anesthetics, analgesics; also sterile normal saline and desipramine in the case of 6-OHDA injection).

1.2 If using 6-OHDA, retrieve an aliquot from the -20 deg C freezer and dilute with normal saline to the desired concentration (5 ug/uL for MFB injections, 2.5 ug/uL for intrastriatal injections), wrap in aluminum foil and place in an ice bucket.

1.3 If using viruses, obtain aliquots from the -80 deg C freezer and dilute with normal saline to the desired titer and place in an ice bucket.

2 Surgery room setup

2.1 Turn on the bead sterilizer and light.

2.2 Turn on the injection device you will be using (Hamilton syringe or Micro4).

2.3 Set a clean, empty cage halfway onto the heating pad on the prep area, and turn on the heating pad to medium.

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2.4 Place your sterile surgical tools (forceps, scalpel, small scissors, hemostat, and surgical clips) on one side of the stereotax.

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

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

2.7 Place a packet of sterile swabs nearby.

2.8 Make sure the small heating pad on the stereotax is on, and cover with folded napkins and tape down.

3 Mouse preparation.

3.1 Inject mouse with ketamine/xylazine at 40/10 mg/kg (about 0.1-0.2 mL of our stock), then place it into the empty recovery cage.

> With multiple surgeries, use a separate recovery cage for each mouse.

3.2 If the mouse will be receiving 6-OHDA injection, also administer 1 mL IP saline and desipramine (0.3 mL of 2.5 mg/mL) before starting the procedure.

4 Anesthesia and incision.

4.1 Turn on the oxygen and adjust on the anesthesia machine to a flow rate of 0.4

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L/minute.

4.2 Once the animal has fallen asleep in the recovery cage, place it on the stereotaxic frame by gently opening its mouth and putting its teeth through the bite bar and slipping the nose cone over the snout. Then, turn the isoflurane flow rate to 2%.

4.3 Apply lubricant to the eyes bilaterally to prevent corneal damage.

4.4 When the animal does not respond to toe pinch, turn the isoflurane flow rate down to 1%. **For the remainder of the procedure, check the breathing rate (by eye) and responsiveness (by toe pinch) at least every 5 minutes, but preferably more often.** Adjust the anesthesia accordingly (typically between 0.6-1.0% for maintenance).

4.5 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.

4.6 Prep the scalp as follows:
- Use a swab to apply hair remover to a strip down the middle of the mouse's scalp, working it down to the scalp, and leave for 2-3 minutes.
- Wipe away hair remover and hair with a fresh swab. Once all hair remover has been wiped off, you should have a strip of bare scalp.
- To this area, apply alternating swabs of betadine and 80% ethanol x 3.

4.7 **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 Take the scalpel and use the rounded edge to scrape the surface of the skull, removing any muscle or other tissue.

If you will be placing an implant, this step is particularly important and you

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should make sure the area around the implant is completely free of tissue, and then take the pointed side of the scalpel and score the skull with a hashmark pattern (to create a rough surface for dental cement to adhere to).

5.2 Place the drill (with a fresh drill bit) onto the stereotaxic arm.

5.3 Balance the Bregma and Lambda:

- Using the dissecting microscope from this point forward, lower the drill bit until it just touches Bregma on the mouse's skull. Note the Z coordinate.
- Lift the drill bit and move it posterior and lower it to Lambda on the skull. Note the Z coordinate.
- If these are <0.05 mm different, you can proceed. If it is >0.05 mm different, adjust the mouse's placement in the ear bars or the height of the bite bar until the skull is flat in AP.

5.4 Balance left/right laterally from Bregma

- Return to Bregma with the drill bit. Move the drill bit 2 mm to the left of Bregma and lower to the skull surface. Note the Z coordinate.
- Lift the drill bit and move 2 mm to the right of Bregma and lower to the skull surface. Note the Z coordinate.
- This should be the same as on the left side. If not, again check the symmetry of the ear bars and repeat until the skull is flat.

5.5 Drill hole at desired coordinates:

- Put the drill bit at Bregma, lift up, and move in AP and ML to the desired coordinates using the stereotax.
- Using foot pedal and stereotax, drill through the skull at this location, preferably without piercing the dura or damaging the underlying brain.

Enlarge or drill any additional holes needed for injections or implants.

- For implantation of a 200 micron optical fiber (for optogenetics or photometry), the fiber can be inserted through a single drill hole.
- For larger optical fibers (eg 400 micron fibers for photometry) or DBS devices, a larger "cloverleaf" drill hole can be created by making a single hole at the desired coordinates, and then making 4 additional (overlapping) holes 0.2 mm in each of the cardinal directions from the original hole.
- For DBS or electrode array implants, drill an additional hole in the right frontal area for a skull screw.
- For electrode array implants, a rectangular craniectomy will be created using the drill as a machine tool around the perimeter of the desired area, and you will drill an additional hole in the right posterior area (over the cerebellum) for the ground wire.

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5.6 Remove the drill from the stereotax.

6 Injection.

6.1 Before injecting, make sure to intentionally puncture the dura at that site (or cut a dural flap in the case of an electrode array implant) by using a 32G needle whose tip has been bent to pierce the drill hole. A small bead of fluid (CSF or blood-tinged CSF) will typically appear in the hole.

6.2 If you will be injecting 6-OHDA, saline, or virus, attach the Micro4 injector to the stereotax.

6.3 Select the syringe that is labeled for use that substance or virus, and confirm it has a blunt injection needle which is tightly inserted in the tip.

6.4 Using the button on the back of the Micro4 injector to open the grips on the device, slide the syringe and plunger into the device, being careful not to hit the tip of the needle on anything.

6.5 Use the stereotax to position the needle about 1 cm above the skull.

6.6 Set the Micro4 controller to inject (using a fast setting) until the plunger is nearly (but not all the way) down.

6.7 Using a 10 uL pipettor, pipet some of the 6-OHDA, saline, or virus onto a small (1 cm square) piece of parafilm that you have placed on the mouse's skull. It will bead up on the surface.

6.8 Lower the tip of the needle into the bead of fluid until it is just about touching the surface of the parafilm.

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6.9 Set the Micro4 controller to withdraw (using a fast setting), and withdraw until the bead disappears.

6.10 Lift the needle again to about 1 cm above the skull and remove the parafilm from the area.

6.11 Set the Micro4 controller to inject (using a fast setting) and inject about 200 nL at a time until you can see a small bead appear at the tip of the needle. Wipe away this bead with the side of a swab.

6.12 Lower the needle down to and through the drill hole, until it is at the surface of the brain (this is sometimes hard to guess with a drill hole, but do your best).

6.13 Start the injection. For most injections < 1 uL in volume, we use 50-100 nL/minute as the injection rate. For injections of 1 uL or more in volume, we typically use 200 nL/minute for the injection rate.

6.14 After the injection has been completed, leave in place another 5 minutes, then withdraw the needle by 0.2 mm and leave another 5 minutes.

6.15 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 Implants

7.1 After all injections, but before implanting any devices, prepare Metabond (dental cement):
- Scoop a small amount of the powder into a small weigh boat.
- Add 3 drops of liquid + 1 drop of catalyst to the weigh boat
- Mix using a Metabond brush inserted in the brush handle.

7.2 Use the Metabond brush to paint the resulting white liquid over the exposed skull, trying to avoid all holes. If you cover a hole, use forceps or the end of a 32G needle to remove the dried Metabond.

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7.3 Apply a small amount of Metabond to the lower 3rd of any ferrule (for optical fiber ferrules) or electrode array connectors. **This will dry in 1-2 minutes.**

7.4 Implant optical fibers by first inserting the fiber-ferrule assembly in a set of several ceramic ferrules and sleeves stuck together (a ferrule "stick"), and placing the stick (fiber down) in one of the grooves on the end of the stereotaxic "wand", secured with a clamp.

- For optogenetics, fibers are inserted quickly to the desired DV depth (from brain).
- For photometry or DBS, fibers or DBS devices are inserted quickly 1 mm (for superficial targets) or 2 mm (for deep targets), then advanced 0.5 mm every 2 minutes until at the desired DV depth (from brain).
- For electrophysiology, arrays are inserted quickly 1 mm (for superficial targets) or 2 mm (for deep targets), then lowered 0.2 mm every 5 minutes until 100 microns above the desired DV depth (from brain).

7.5 Prepare Ortho-jet (dental acrylic)

- Scoop a small amount of Ortho-jet powder into a small silicone bowl
- Add a few drops of Orthojet liquid
- Mix to attain a syrupy texture

You will have two applications of dental acrylic.

7.6 For the first layer, using the back of a swab or another device, drip the dental acrylic over the base of your implant, completely covering the hole it was inserted in, the fiber or electrodes, and extending to the skull screw and/or ground wires.
You want a little of the acrylic to come up to the bottom of the ferrule or the base of the DBS connector/electrode array connector.
In the case of the electrode array implant, while the acrylic is still soft, advance the last 100 microns to your target DV.

Do not touch the mouse or implant until the first layer of acrylic has hardened.
You can test whether it is ready by checking the residual acrylic in your silicone bowl, and if this is hard, probing the edge of the acrylic with your forceps. If it has completely hardened, then you can start the second layer.

7.7 In the case of the electrode array implant, you will bend the loop ground wire between the ground wire hole and the implant in a C shape and tuck it against the right side of the implant.

7.8 Prepare another batch of dental acrylic and apply to make sure all exposed wire, skull screw is covered, and build a "helmet" of smooth dental cement around the rest of the implant, such that it is a smooth surface all around the

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implant.

- With optical fiber-ferrules, about ½ to 2/3 of the ferrule should remain exposed above the top of the acrylic, to provide access.
- With DBS implants, the female pins should remain exposed above the top of the acrylic, but the sides of the connector should be mostly covered.
- With electrode arrays, the resin should be completely covered and the plastic connector about ½ covered with acrylic.

Once the implant is secured with solid acrylic, the stick and wand on which it was inserted can be gently removed.

8 Closing/Suturing

After completion of all injections and implants, the scalp can be sutured.

- For non-implant surgery, this typically involves 3-4 sutures along the length of the incision.
- For implant surgery, this sometimes involves 1 suture (most often posterior), but sometimes no sutures are required as the scalp fits just around the implant. **You do not want the scalp to be too tight around the implant.**

8.1 Open a packet of sterile suture.

8.2 Use a hemostat to grab the end of the curved needle that is closest to the thread, and pull the suture out of the stiff backing.

8.3 Your first incision should be placed in the middle of the area you desire to close.

- Using the hemostat in your right hand (if you are right handed) and the forceps in your left hand to pick up the edge of the scalp, drive the tip of the needle down through the scalp on the right side of the incision, near the edge.
- Then use the forceps to grab the scalp on the left side of the incision, and drive the tip of the needle up through the scalp.
- Overall the needle should make a right to left “U” trajectory.
- Now let go of the needle with the hemostat, and grab the end that has come through the left side, and pull the needle until just 2 cm of thread remain on the right side.
- Using your forceps in your left hand, and hemostat in the right, grab the thread on the left side, loop it twice around the end of the hemostat, and then use the tip of the hemostat to grab the bit of thread on the right side and pull it through the loops.
- This can be gently tightened, and is your first knot.
- Now use the same technique, but with only one loop, to tie additional knots over the first one.
- After you have a total of 3-4 knots, you can trim the ends to about 3 mm in

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length.
Repeat this process in front of and behind the first stitch, to close the remainder of the incision.

8.4 Remove mouse from stereotax as follow:
- Turn the isoflurane to off
- Remove the ear bars
- Loosen the nose cone
- Gently pull the mouse from the stereotax
- Place the mouse in the recovery cage

8.5 Inject mouse with analgesics:
- Buprenorphine (0.05 mg/kg IP)
- Ketoprofen (5 mg/kg SQ).

9 Monitoring and cleanup

9.1 As the mouse is recovering, and before starting the next surgery, clean and sterilize your instruments:
- Wipe tips of all instruments clean with a kimwipe and ethanol
- Place instrument tips briefly in the bead sterilizer
- Return instruments to your surgical area
- Clean the surgical area and replace the napkins.

9.2 Wait until mouse has recovered from anesthesia, responds to tail pinch and is ambulatory before returning to its home cage
Affix a surgery tag to the cage card and place the cage on the rack in the mouse room.

9.3 At the end of the day:
- Clean and sterilize all instruments and return to the instrument box.
- Remove all consumables from the surgical area, then spray and wipe down with dilute bleach.
- Clean the injection needles by repeatedly flushing with water and replacing in their boxes.
- Put sharps (suturing needle, drug injection syringes) and biohazards (any remaining virus) into the red sharps container.
- Make sure the isoflurane and oxygen flow rates are set to zero on the anesthesia machine and turn off the oxygen.
- Turn off the heating pad and bead sterilizer.

10 Postoperative Care

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10.1 One day post surgery, check on mice and administer additional ketofen and buprenorphine.

10.2 Continue checking daily until there are no signs of pain, then at least weekly thereafter.

10.3 In 6-OHDA treated mice, monitor daily for 7 days, then at least weekly thereafter.

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