Survivable Stereotaxic Surgery in Mice


Materials:

Heating pad: CMA/microdialysis thermostat regulated heating pad: use the rectal thermometer (leave it under the mouse’s abdomen) for monitoring temperature.

Guide cannulae: Plastics One (5mm was used in test procedure)
Anesthesia:

1. Add isoflurane to the ISO machine.
2. Turn O₂ on by twisting main valve and make sure the flow rate is @ 1.
3. Turn chamber flow exit ON and turn ISO valve to 5%.
4. Place mouse in the pexiglass chamber and observe.
5. Once mouse is down, shave off fur with electric razor. Go from the ears to just in-between the eyes, move razor in different directions to effectively clean area of fur. **Careful not to shave wiskers.** Apply povidine/iodine to shaved area but protect the eyes from it.
6. Place mouse back into chamber for longer anesthesia.
7. Turn chamber flow OFF

**Procedure:**

1. Set up the stereotaxic instrument and all the materials needed. Make sure the area and instruments are cleaned and sterilized.
2. Turn the flow to the stereotaxic app. ON with ISO flow to 1.5%
3. Mount the animal onto the stereotaxic apparatus by placing the ear bars into the ear canal and tightening into place. First mount one ear bar in the ear canal, and then hold it in place and slide in the other ear bar. You know you are in the correct location when the head can no longer be moved side to side. Secure the mouth with the anterior mount of the stereotaxic and make sure that the head is level with a ruler. Put the ruler in a vertical position with respect to the stereotaxic instrument platform and check for a 90° angle between the ruler and the middle of the animal’s scalp).
4. Add petroleum jelly to the mouse’s eyes for protection.
5. Make an anterior/posterior incision on the scalp with a sterile scalpel extending from the lambda to just in-between the eyes of the animal. Use sterilized hemostats to pinch off the skin and keep the incision open. Using several sterile cotton swabs, dry off the exposed skull surface.
6. Put the guide cannula onto its mount, find bregma on the skull, and position the guide cannula right over this location. Write down the anterior/posterior and lateral coordinates. From bregma, find the correct coordinates needed for the placement of your probe with the aid of the stereotaxic atlas. Position the guide cannula to the correct coordinates by adding or subtracting from bregma. Bring your guide cannula down until it is touching the skull, and then record this ventral coordinate. Make a pencil mark with a sterile pencil at this location on the skull; this is where you will be drilling.

7. Remove the guide cannula and sterilize your drill bit. Carefully drill a hole at the pencil mark until you get through the width of the skull. Check with the guide cannula to see if it would clear the hole without touching the sides. Keep drilling and checking until the cannula can clear in a straight path. Once the hole is made, use a sterile needle to gently punch the meninges in order to allow unobstructed insertion of the cannula.
8. Next, using a hand drill, make three holes for skull screws. Sterilize three screws and place them onto the skull until they are tightly anchored on.

9. Clean the guide cannula with ethanol and saline, mount, and lower it slowly to the proper ventral coordinate. Make sure that the sides are not touching and that it is going in perfectly vertical.

10. Place the anchor screw medially and behind the posterior skull screws and hold it in place with tweezers. Cover the guide cannula, screws, and the rest of the skull with adhesive. After first batch of adhesive is dry, completely cover the area and the cannula and anchor screw enough to secure it.

11. As the cement becomes thicker and before it solidifies, separate the skin from the cement cup and mold the cement cup with the spatula to make sure the cement cap is smooth all around and does not irritate the skin later.
12. Allow the dental cement to completely dry before removing the animal from the apparatus. Remove the hemostats. Apply bacitracin all the way around the cement cap.

13. Place the animal in its own cage and monitor it until it becomes conscious before returning it to its room to recover.

Monitor animals until they recover from anesthesia on the day of surgery and daily post-op, until the end of the experiment, for signs of infection and evaluation of pain/distress. This includes weekends and holidays. Low spontaneous movement, distress vocalization upon handling, hunched posture, diarrhea, swelling and discharge in the area of the headmount, and lack of feeding/drinking are all signs of pain and distress. Buprenorphine (0.1-0.5mg/kg SC) is administered twice daily, and then, on an as-needed basis, if animal appears to be in pain. Local antibiotic treatment (bacitracin ointment) and systemic antibiotic treatment (penicillin 100,000 IU/kg IM every 12 hours for the first 48 hours post-op) are administered if post-operative infections occur. If any of these symptoms persist following administration of buprenorphine, supplemental fluid, and antibiotic treatment within 12 hours of surgery, the animal is euthanized.