Protocol

Mouse stereotaxic surgery for injection

1. Measure weight of mice with balance to decide amount of anesthetics.
2. Prepare Ketamine/Xylazine in 1 cc syringe (28G ½) based on mice’s weight.
	1. Ketamine / Xylazine Mixture (Ketamine - 10 mg/ml, Xylazine - 1 mg/ml) - Ketamine - 100 mg/Kg dose, 10 mg/Kg dose
3. Put mice in isoflurane for about 5-10 seconds and take out
4. IP injection of Ketamine and Xylazine, and wait 5-10 minutes until mice don’t have toe pinch. Write amount of anesthetics injected on the record.
5. Put antibiotic cream on eyes with autoclaved Q-tips. Inject small amount of 0.25% Bupivacaine between skull and head skin (make a small blob on head)
6. Mount mice on head stage using ear bars
	1. hold the animal toward to one side of bar and push another bar
	2. check head movement, should not move horizontally
	3. open up mouth, put mouth fixture in and tighten.
7. Scrub with Betadine using Q-tips on head, then scrub with EtOH.
8. place Hamilton syringe (Hamilton 80100, 1ul, 26G) in needle fixture
9. Make a incision with blade from posterior to anterior (start of skull to between eyes)
10. pull the incision using sterile Q-tips to expose the skull. Apply 30% hydrogen peroxide to dry the skull and further expose cranial cranial sutures.
11. Adjust syringe needle tip to Bregma, then record coordinates of Bregma from anteroposterior (AP), medial lateral (ML), and dorsal ventral (DV). Read coordinate from behind, back, and top, then write on Bregma-pre
12. Adjust needle tip to Lambda, and record the coordinates
13. Adjust mouth fixture to make DV coordinates to be equal between Bregma and Lambda, so mouse head is flat.
14. Reposition need tip to Bregma, and record coordinates on Bregma-post.
15. Calculate “1st site Mart” base on pre-Craniotomy coordinates, adjust needle tip to calculated position.
16. Mark the position to drill with high temperature cautery, check the mark with needle tip again.
17. Drill two skull screws on the contralateral side of the brain. Gently tap screws,pull up – the whole skull should come with the screw
18. Drill the hole with Foredom drill with foot switch
	1. Do not push drill tip hard on skull, which is thin and fragile
	2. Do not drill deep ( thickness of mouse skull is 300um)
	3. Drill a fairly wide hole (2 mm X 2 mm to accommodate both recording electrode AND optogenetic cannula
	4. Nick the dura with a bent 26 g needle
	5. Put saline on the brain once dura is pierced.
19. Viral injection –ChR2
	1. Check the needle with ddH2O to make sure needle is not cloaked. Fill Injection solution (6-OHDA (0.2ul) or virus (0.5ul)) in needle.
	2. Adjust needle to injection position, slowly lower the DV coordinates. move slightly deeper position (0.1-0.2 mm), then pull it back to the position. this make a room for drug injection
	3. Slowly inject the solution into brain over 2 minutes, and then wait 5-10 minutes to diffuse the drug after injection. (rate 0.1 ml/min)
	4. Slowly pull needle up
20. Electrode Implantation:
	1. Keep area wet with saline but clean of blood.
	2. Make sure head stage and implant can plug and unplug easily, prior to insertion.
	3. Place Microwire bundle in Stereotaxic arm. Remeasure Bregma and craniotomy location. Record DV position of brain surface, correlate with brain atlas. Keep track of pin #1’s location.
	4. Ground wire on array: wrap around a screw and feed into brain (separate hole)
	5. Ground stereostatic frame and skull screw to preamp.
	6. Attach preamp to head stage. Preamp should connect to oscilloscope
	7. Fire up preamp, scope, and speaker. Turn on rig
	8. Make sure everything is plugged into 1 wall outlet.
	9. Unplug everything non-essential, including lights.
	10. Move away antennas: such as microscope and hemostats and human bodies.
	11. Identify maximum depth. Begin to lower electrode slowly. Listen/watch for decrease in 60 Hz noise, for neurons. Check responses to stimuli: auditory, visual, and somatosensory. Determine best signals.
21. Optogenetic cannula
	1. At a steep angle in the AP direction, lower the cannula into place with a second stereotaxic arm
22. Closing
	1. Roll up a small kim-wipe and wick up any fluid inside the craniotomy hole, until no more is welling up.
	2. Put small amount of super glue down into skull hole around electrode.
	3. Place a small amount of Zip-Kicker in a syringe and put 1 drop into hole.
	4. Check again that skull is clean and dry, or else the head stage wont stay.
	5. Ventillate! Fan ± Window. Mask?
	6. Secure electrode with dental cement which you have mixed up. For 2 implants, use only a small amount on the 1st until you are ready to secure the 2nd. Put progressively goopier amounts.
	7. Let cement dry, at least 10 minutes.
	8. Close with surgical sutures/staples if needed.
	9. Place a small amount of antibacterial ointment on the surgical site.

