# ECoG mouse surgery protocol:

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Summary order of operations:

1. Mark craniotomy and reference location using stereotax.
2. Drill hole and insert reference screw.
3. Stick head bar on with cement.
4. Drill craniotomy.
5. Attach connection PCB with ECoG to the back of the head bar, and cover with PDMS using the gel glue on outer edge of PDMS to hold in place over the craniotomy. Ensure no glue touches craniotomy itself.

**Ephys Area Experiment Preparation:**

1. Turn on the oxygen tank for the ephys experiment area to 15psi. Open copper side valve and main blue knob. Note the pressure left in the tank.
2. Check the height of the neurotar clamp, turn on the heat mat, turn on the miocroscope light.
3. Check isoflurane level and fill if needed.
4. Turn on the gas evacuation apparatus and write down the beginning weight on the canister. Do not yet turn on the motor. The canister will beep when it reaches 1010g.

**Surgery Table Preparation:**

1. Lift plastic cover and turn power board on.
2. Check isoflurane levels and refill if necessary.
3. Turn on heat mat.
4. Turn on gas canister, but not yet the motor or power up. Note down the initial weight.
5. Turn on microscope light.
6. Turn on stereotax positioner.
7. Put down 3 drapes, clean sterile, dirty, and mouse head shave area.
8. Get the timers out and place them on the surgical table.
9. Put out, Betadine, Saline, Ethanol.
10. Place head plate, stim electrodes on clean area.
11. Place surgical tools in order of usage, sugu's, cotton buds, two sized pipette puffers out.
12. Get the butane out to blow away bone fragments.
13. Put drill bits in the hand drill. Check drill bit alignment.
14. Check glue and dental cement area to ensure there is enough for the experiment.
15. Prep a large syringe tip for positioning alignment, and another for glue application, then one small syringe for skull flap lift,
16. Put petri dishes out for waste, and ethanol and betadine and sterile water/saline.
17. Put a tissue in mouse chamber.
18. Place the blue paper towel with depilatory cream out ready for mouse shaving.
19. Place the blue paper towel on dirty area to put glue, wipe things etc
20. Obtain the mouse. Weigh the mouse and return it to the cage.

Weight: 24g

1. Prepare drugs based on mouse weight.
   1. Saline - place on ephys exp heat mat. (one or two 0.5ml syringes?)
   2. Carprofen - reduce pain and inflammation.
   3. Dexamathasone - anti inflammatory
   4. Vet - reduce pain.

**Surgery:**

1. Turn on the oxygen machine to 4.5
2. Turn up the oxygen on the anesthetic system to 1.5
3. 3 way valve, check it's going into chamber.

**Note:** The 3 way valve has a hole through it in each of the prongs, so to direct it through put the blocking side on the direction you no longer want gas to flow.

1. Move mouse into chamber, gently in tube.
2. Close chamber and turn isoflurane to 3. Start timer for 2 minutes.
3. Note mouses breathing rate slow down.
4. At 2 minutes, switch over the 3 way valve to the nosecone position, remove the mouse and place in the nosecone. Put isoflurane down to 2.
5. Put vaseline on eyes with a toothpick to keep them moist.
6. Commence hair removal with depilitation cream.
7. Once hair is removed(2-3 mins), switch to mask on anesthetic system.
8. Quickly move mouse to stereotax, hooking teeth in and screwing nosecone over mouse to maintain anaesthesia at level 2.
9. Place thermoprobe in mouse butt.
10. Secure mouse with earbars. Note breathing rate – it should be one breath per second.
11. Toe pinch. Cover with warming cover.
12. Administer drugs and saline. Inject saline at the rate of 10ml/kg/hour subcutaneously to prevent mouse from anti-dehydration. I.e. 0.25 ml for mouse of 25 g per hour.
13. Toe pinch.
14. Swab head 3 times, moving inside to out alternately with betadine and ethanol. This should remove and clear up hair, whilst also sterilizing the surgical area.
15. Toe pinch before commencing surgery.
16. Trim with small scissors between ears. Vertically cut up one side, then a diagonal cut near front of head. Repeat on previous side, removing skin into petri dish on dirty side.
17. Use a sugu dipped in saline to remove the tissue over the top of the skull.
18. Trim the muscle at the back of the skull on the skull, pushing it back slightly.
19. Put the large syringe tip in the stereotax positioner and locate bregma. Now, move to lambda and note Z offset until it is within 0.05mm.
20. **Mark craniotomy and ground reference position with black ink**, making craniotomy about 2.0mm in diameter.

* ML:1.25 -> 3.5 (2.25mm in diameter)
* AP: -2.5 -> 4 (1.5 mm in height)
* Mark reference screw rostral of Lambda. Brain Atlas Reference: <http://labs.gaidi.ca/mouse-brain-atlas/?ml=2.5&ap=-.4&dv=3.2>



1. Move stereotax away.
2. Toe pinch.
3. Place head bar over mouse head, and trace location. Ensure skin is cut away and skull dried in area where dental cement will be laid.
4. Drill a hole over the reference mark and insert a screw which will be the ground reference as it touches the CSF in another area of the brain, add silver paint to ensure a conductive connection. The screw should have a 36AWG thickness silver wire twisted around it which will serve as the GND reference. The silver wire should have been stripped at both ends before experiment begins.
5. Prepare cement and fix the head bar.

Cement Instructions:

* Dispense cement(2 part) onto the special reflective paper and mix.
* Apply within 1 minute of dispensing.
* It works better when the surfaces to be connected(i.e. skull and headplate) are dry.
* Fix the head bar using the syringe to apply. Do not get any cement near craniotomy area.
* It will take 5 minutes to fully dry. Wait this long before proceeding with the craniotomy.

1. Using the hand-held smaller drill, drill around outside of craniotomy until skull becomes thin looking.
2. Drill the craniotomy until the skull cracks. Alternately blow bone fragment with butane, and add saline with the small pipette? to gauge remaining thickness and clean area. When area is thin enough bone flap should lift up using the end of a small syringe tip. Use tweezers to press on bone flap to see if it is movable.
3. Clean area by rinsing with saline until any bleeding stops. **Be careful** to use sugu only over skull edge and not directly over craniotomy.
4. Hydrate craniotomy with sterile saline/water.
5. Gently place the ECoG array over the craniotomy.
6. Prepare more cement. Place a small amount of cement around the GND screw. Place a small amount of cement at the back of the ECog Array to secure it in place.
7. Repeat saline hydration injection in mouse (0.25ml per 25g per hour)

Note: If I have a skin/muscle bleed, stop it with this glue.

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1. Use the more viscous gel glue to cover craniotomy with a PDMS coverslip over the ECOG and craniotomy to help keep it hydrated and it’s position stable.
2. Administer ketamine/xylazine, as per table below. Turn of the isoflurane and start a timer for the ket/xyl re-administration.

**Ketamine/Xylazine specific instructions:**

Table

Description automatically generated**Note: A tick is 0.01ml, or one mark on the insulin syringe we use.**

On the top you can see dosage and concentrations of drugs we use. The units in the table are in terms of 'ticks' in our insulin syringes so e.g. 2 ticks = 0.02 ml. In practice it is hard to dose anything finer than 0.5 tick. If the mouse is v light (~20g), we’d give it 2 ket + 1 xyl ticks. If heavier (~25-30g), it can get 2.5 ket + 1.5 xyl. I’d err on the side of caution because it is easy to overdose them and then the experiment is over. The mouse will be anaesthetized very fast if it is straight after isoflurane induction, or may take several minutes if done from awake. Expect the mouse to wake up after an hour after first dose. Check for responses every 10 minutes. If you get a response, readminister 1 tick of ket and see if this is sufficient.

1. Prepare E-Phys area as per experiment document, and move mouse there.