# Aseptic mouse surgery protocol for Ketamine induced Gamma recordings and MEP stimulation

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**Surgery Table Preparation:**

1. Ensure all tools that will be in contact with mouse tissue are autoclaved. <https://www.gimaitaly.com/DocumentiGIMA/Manuali/EN/M35709EN.pdf>
2. Wipe down everything on surgical table with ethanol spray.
3. Put tooth picks and sugi’s in sterilization bag and autoclave as well.
4. Drill bits, head bar, electrode implant, other attachments not suitable for the autoclave in ethanol.
5. Lift plastic cover and turn power board on.
6. Turn on the Thermacage, it should be set to 35 degrees: <https://www.scanbur.com/products/housing/warming-cabinets/warming-cabinet-mini-thermacage>
7. Stick a plastic bag bin on the side of the table with tape.
8. Check isoflurane levels and refill if needed.
9. Turn on heat mat.
10. Turn on gas canister, but do not turn on the motor yet. Note down the initial weight of the gas canister. The canister will beep when it reaches 1010g indicating a canister change is required.
11. Turn on microscope light.
12. Place Large size(Jean) surgical gloves out on the surgical table.
13. Ensure sterillium is on the table to clean gloves between touching stereotax.
14. Turn on stereotaxic positioner.
15. Put down 3 drapes, clean sterile, dirty, and mouse head shave area.
16. Get the timer out and place on the surgical table.
17. Put out, Betadine, Saline, Ethanol.
18. Put low grade cleaning saline on the mouse head cleaning area in a petri dish.
19. Place surgical tools in order of usage, sugu's, cotton buds, pipette puffers out.
20. Get the butane out to blow away bone fragments.
21. Put drill bit in drill. Check drill bit alignment.
22. Check glue and dental cement area to ensure there is enough for the experiment.
23. Place the silicon into the dirty area ready to cover the exposed skull.
24. Prep a large syringe tip for positioning alignment.
25. Put petri dishes out for waste.
26. Put a tissue in mouse chamber.
27. Place the blue paper towel with depilatory cream out ready for mouse shaving.
28. Place the blue paper towel on dirty area to put glue, wipe things etc
29. Place the paper tape on the table to stick the thermoprobe to the heat mat.
30. Place blue paper tape on dirty side, ready to stick down thermoprobe/mouse tail together.
31. Weigh the mouse and record its details
    1. Surgery card: name of the mouse, date, procedure, initial weight, analgesics: dex and carprofen, my initials
32. Prepare drugs based on mouse weight.
    1. Saline - place two 0.5ml syringes on ephys exp heat mat.
    2. Carprofen - reduce pain and inflammation.
    3. Dexamathasone - anti inflammatory
    4. Vetergesic - reduce pain

**Surgery:**

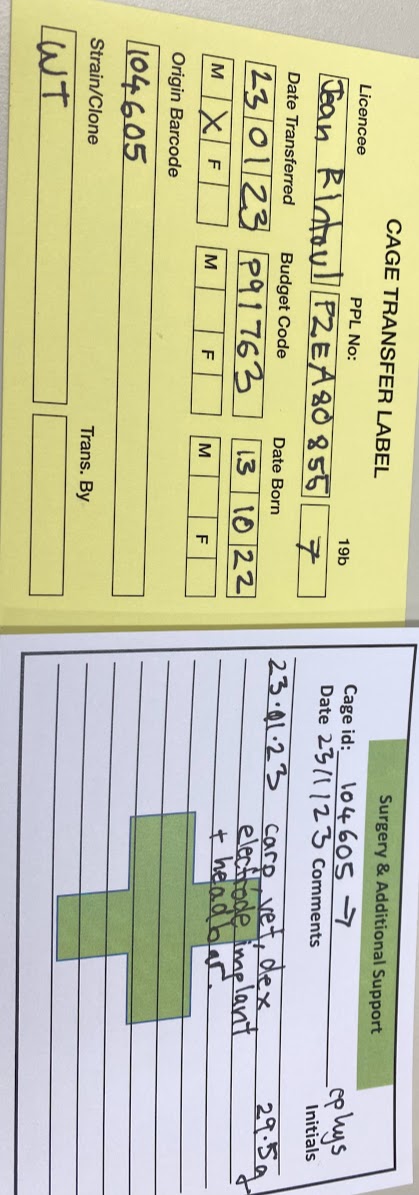
1. Turn on the oxygen machine to 4.5
2. Turn up the oxygen on the gas inhalation bar to 2.0
3. 3-way valve, check it's going into **CHAMBER**. **Note:** The 3-way valve has a hole through it in each of the prongs, so to direct it through put the blocking side on the direction you no longer want gas to flow.
4. Move mouse into chamber, gently in tube.
5. Close chamber and turn isoflurane to 3. Start timer for 2 minutes.
6. Note mouse breathing rate slow down.
7. 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.
8. Put Optixcare/Vaseline/Lacrilube on eyes with a toothpick to keep them moist.
9. Commence hair removal with depilation cream. Once hair comes loose, clean the depilation cream away with saline to prevent skin irritation.
10. Once hair is removed (2-3 mins), switch to mask on anaesthetic system in preparation to move it quickly to the surgical area.
11. Check the paw reflex and if not present, fix the mouse in the stereotax frame using ear pieces. Use a tooth pick to open it’s mouth to hook its teeth onto the nose cone.
    1. Put the mouse teeth in the mouthpiece hole and tighten the nose cone for extra stability
    2. Observe the breathing when tightening the ear pieces and adjust fixation if needed. Breathing rate should be one breath per second.
    3. Ensure the mouse head is straight (midline) while fixed
    4. Make sure the head is stable enough for drilling
12. Maintain anaesthesia at level 2.
13. Insert the anal temperature probe and cover the mouse with a sterilised piece of tin foil to keep it warm
14. Toe pinch.
15. 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.
16. Toe pinch.
17. Swab head 3 times, moving inside to out alternately with betadine and ethanol and saline to remove extra hair. This should remove and clear up hair, whilst also sterilizing the surgical area.
18. Toe pinch before commencing surgery.
19. Trim with small scissors between ears. Vertically cut up one side, then a diagonal cut near front of head. Repeat on other side, removing skin into petri dish on dirty side.
20. Remove conjunctive tissue left on the skull with dry and wet (saline) sugus
21. Trim the muscle at the back of the skull on the skull near the ears, pushing it back slightly.
22. **Use a scalpel to roughen the skull particularly around the areas where the head bar will make contact. This will help adhesion as it creates a larger surface area to adhere to, when the dental cement is applied.**
23. Use gel-like glue (loctite super glue power flex) to glue cut muscles to the bone all around the exposed area, ensuring all skin is glued to bone.
    1. Glue cut back muscle to the one underneath
    2. Then glue skin to where the two muscles are glued
    3. Glue the front and sides, covering the muscles
    4. Fill in the resulting holes on the back sides with glue
    5. Make sure all tissue at the edges covered with enough glue to prevent conjunctive tissue growing back
24. Put the large syringe tip in the stereotaxic positioner and locate bregma. Now, move to lambda and note Z offset until it is within 0.05mm.
25. Mark bregma with ink, for later ease of location.
26. **Mark 2 0.5mm drill holes at: (VEP and MEP location this time)**

* **PFC Location: AP 2.*4, ML 0.4mm. Right of bregma.***
* **VEP Location: AP -3.*5, ML 2.25mm. Right of bregma for left side visual stimulation.***
* **MEP Forelimb Location: AP +0.0 mm, ML 2.0mm left of bregma.**
* Brain Atlas Reference: <http://labs.gaidi.ca/mouse-brain-atlas/?ml=2.5&ap=-.4&dv=3.2>

1. Cement round 1: Mix cement and place cement over the edges where bone and skin/glue meet. Place cement along the back in particular, filling in an holes. Allow to dry for 15 minutes. This gives a clean and dry platform for the head bar to be attached. Note: Ensure the cement doesn’t go too far to the side, as this will make it hard to fit into the Neurotar.
2. Cement 2: Put the head bar over the exposed area. Do the cement positioning quickly once it starts to assure a firm hold.
   1. Mix dental cement with methyl methacrylate in a well until it has a thick texture and fill a syringe puller with it, then attach the large syringe needle ready to deposit it.
   2. Start with running a layer of dental cement around carefully make a layer on the area of skin/glue/bone where the head bar will attach. Apply dental cement wall around on the head, especially on the sides, before putting head bar on so that there are no holes left.
   3. Dispense some more cement and prep the syringe. Put it on the back of the head bar, moving around the perimeter twice, filling in the holes and leaving enough space so it will still fit on the neurotar. Pick it up with forceps and quickly place over the top of the other dental cement on mouse head.
   4. Place the prepared head bar on the head.
   5. Make sure midline parallel to head bar side and both hemispheres at a similar level.
3. Wait 15 minutes for cement to dry. During this time, adjust the end electrodes on the implant so that they align over the marked drill holes.
4. Drill a hole over marked drill hole sites. Blow away bone fragments with butane.
5. Cement 3: Place a small amount of cement over the back of the head bar then place the implant on top, angled so that the electrodes are over the top of the drill holes and embedded in the stim gel.
6. **Press down slightly (implants that have no ball on the end) such that the wire goes slight through the skull. Try to do this once only as minimal trauma to tissue is better.**
7. Wait a few minutes.
8. Mix the silicon and apply a thin layer around the electrode location as a sealant and stabilizer.
9. Wait for it to dry for 5 minutes.
10. Using multimeter, test the conductivity from the end of the screw to the end of the wire.
11. Move stereo tax away.
12. Toe pinch.
13. Decrease the isoflurane so that the mouse can breathe oxygen for a short time, then transfer it to the heated recovery chamber.
14. Mouse will awake a little off balance.
15. Wait 30 minutes to an hour while monitoring the mouse recovery within the heated chamber.
16. Return mouse to pre-prepared home cage.

**Straight after the surgery**

1. Green cross cage card with the type of procedure written on it, with the analgesia plan on it.



1. Prepare the home cage:
   1. If moving the animal into separate cage: fill in the transfer label
   2. Remove hoppers, tubes and metal grid
   3. Put wooden stick, and cardboard nesting material in cage to make it comfortable.
   4. Place a food pellet in petri dish with water so that it softens.
   5. Put carprofen analgesic in the water. 6-7 ticks(1 tick = 0.01ml/one mark on the syringes used) per 150ml based on weight of mouse.
2. Return the animal into the home cage when it recovers, likely between 30 minutes and 1 hour.
   1. Mouse should be moving around, cleaning itself and eating
   2. Use cupped hands to move animal back into cage.

**Post op**

1. Observe the animal over the next few days and keep warm in recovery chamber if needed. Daily weighing of animal (inclusive of head device) can help check if animal is eating. Check for signs of ill health, hair piloerection or slowed movement.
2. Record weight over next 3 days on surgery card – the mouse should recover initial weight by then.
3. Put carprofen in water over next 2 days + saline if needed to keep hydrated.

**Aseptic surgery - important points**

1. All surgical tools and single-use items (sugis, cotton buds, cocktail sticks) should be autoclaved prior to the surgery. Drill bits and head bar should be washed and disinfected by leaving them in ethanol.
2. Disinfect hands with sterillium whenever non-aseptic area touched with hands (e.g. mouse body post shaving, changing the sterotax settings and frames, checking animal reflex)
3. Clean and dirty areas should be kept separate throughout the surgery
4. Single-use cleaning items such as suggies and cotton buds should be dipped only once in a liquid of choice - avoid contamination
5. Do not touch the surface of single-use cleaning items with your hands, only with sterilised surgical tools
6. Regularly check the withdrawal reflex by pinching animal back paws with a dedicated forceps
   1. Isoflurane: every 5 mins
   2. If hand used to check the reflex, disinfect your hands
7. Measure time under anaesthesia: surgery should be around 1h 30min - 2h long

**Surgical prep - clean area**

* No sugis in a separate petri dish present here
* Electrode and drill bit should be out clean, prepared with the surgical tools
* Important to keep tools and head bar on two separate pieces of green drapes

A group of items on a table

Description automatically generated

**Surgical prep - dirty area**

A close up of a machine

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