# Aseptic mouse surgery protocol electrophysiological recordings and motor evoked potentials

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**Objectives**

This Standard Operating Procedure (SOP) describes the guidelines to perform recovery stereotaxic surgery aiming to implant electrodes in mice which can be used for electrophysiological recording as well as stimulation. This SOP also includes guidelines for the post-surgery care of mice.

**Document History**

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| --- | --- | --- | --- |
| **Version Number:** | **Edited by:** | **Effective Date:** | **Details:** |
| 1.0 | Patrycja Dzialecka | 23/03/2020 | Original protocol |
| 1.1 | Patrycja Dzialecka | 28/10/2020 | Update with next experiments |
| 1.2 | Patrycja Dzialecka | 22/11/2022 | Revision for lab upkeep 22 |
| 1.3 | Jean Rintoul | 27/11/2023 | Adaptation for electrophysiological recording and stimulation. |

**Materials**

* 0.5 ml insulin syringes
* Oxygen cylinder or oxygen generator
* Anaesthesia: isoflurane or ketamine-xylazine
* Analgesic drugs: carprofen (Ramadil), buprenorphine (Vetergesic) + dexamethasone to reduce brain swelling before drilling
* Isoflurane vaporiser
* Scavenger
* Scale
* Recovery box
* Timer
* Microscope
* Stereotaxic frame
* Heating pad
* Custom-made implants (made by Jon)
  + Platinum-iridium 0.25mm wire (Alfa Aesar; from VWR: 39383.BW)
  + Small
* Custom-made headbars (fitting neurotar frames)
* Drill bit (size 0.5)
* Drill
* 70% alcohol for sterilising gloves
* Sterile drapes
* Sterile transparent drape or tin foil to cover the mouse
* Sterile cotton buds
* Sterile absorption cotton triangles (suggies)
* Sterile toothpicks
* Cotton buds
* Veet shaving cream
* Toothpicks
* Betadine (mixed with sterile saline)
* Ethanol 70% for cleaning the mouse head
* Sterile surgery tools (small and larger scissors, very fine forceps x 2, scraper, 2 x normal forceps)
* Sterile saline
* Pen ink
* Loctite super glue (Gel for skin and Brush on for marking)
* Butane duster
* Small petri dishes for saline, betadine, and ethanol
* Large petri dish for waste, toothpicks, and triangular cottons
* X needle for skull marking
* Clear cement mixed with charcoal
* MMA (methyl methacrylate) to mix with cement
* Syringes
* X needles
* 27G needle to apply conductive gel into drilled holes
* Conductive gel
* Very thin brush (with a few leftover bristles)

**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. Place drill bits(0.5mm), 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 ready for trash.
8. Ensure the sharps container is on the surgery table at arm’s reach.
9. Check isoflurane levels and refill if needed.
10. Turn on heat mat.
11. 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.
12. Turn on microscope light.
13. Place surgical gloves out on the surgical table ready for use.
14. Ensure sterillium is on the table to clean gloves between touching the stereotax or dirty side of the surgery table.
15. Turn on stereotaxic positioner.
16. Put down 3 drapes, clean sterile, dirty, and mouse head shave area.
17. Get the timer out and place on the surgical table.
18. Put out, Betadine, Saline, Ethanol in petri dishes ready to clean the top of the mouse head.
19. Put low grade cleaning saline on the mouse head cleaning area in a petri dish.
20. Place surgical tools in order of usage, sugu's, cotton buds, pipette puffers out.
21. Get the butane out to blow away bone fragments.
22. Put drill bit in drill. Check drill bit alignment.
23. Check glue and dental cement area to ensure there is enough for the experiment.
24. Place the silicon into the dirty area ready to cover the exposed skull.
25. Prep a large syringe tip for positioning alignment.
26. Put a large petri dish out for waste.
27. Put a tissue in mouse chamber.
28. Place the blue paper towel with depilatory cream out ready for mouse shaving.
29. Place the blue paper towel on dirty area to put glue, wipe things etc
30. Place the paper tape on the table to stick the thermoprobe to the heat mat.
31. Place blue paper tape on dirty side, ready to stick down thermoprobe/mouse tail together.
32. Weigh the mouse and record its details
    1. Surgery card: name of the mouse, date, procedure, initial weight, analgesics: dex and carprofen, my initials
33. 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 anesthetized mouse and place in the nosecone. Put isoflurane down to 2.
8. Gently put Optixcare/Vaseline/Lacrilube on eyes 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 on the top of the head is removed (2-3 mins), switch to mask on anaesthetic system. Turn on the gas canister motor to filter any airborne isoflurane from mouse exhalation.
11. Move mouse from hair removal area to surgery stereotax, opening its mouth with a toothpick so the front teeth lodge into the hole in the mouth bar. Once the front teeth are in, move the nosecone up over the end of the nose. Tighten the nose cone.
12. Check the paw reflex and if not present, fix the mouse in the stereotax frame using ear pieces.
    1. Observe the breathing when tightening the ear pieces and adjust fixation if needed. Breathing rate should be one breath per second.
    2. Ensure the mouse head is straight (midline) while fixed
    3. Make sure the head is stable enough for drilling
13. Maintain anaesthesia at level 2.
14. Insert the anal temperature probe and cover the mouse with a sterilised piece of tin foil to keep it warm.
15. Toe pinch.
16. Administer pre-prepared 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.
17. Swab head 3 times, moving inside to out alternately with betadine, ethanol and saline to remove extra hair. This should remove and clear up hair, whilst also sterilizing the surgical area.
18. Toe pinch to check pedal reflex before commencing surgery.
19. Remove the skin on top of the skull.

* 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.
* Remove conjunctive tissue left on the skull with dry and wet (saline) sugus.
* Trim the muscle at the back of the skull on the skull near the ears, pushing it back slightly.
* **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 for the dental cement to adhere to. Scrape diagonally in both directions.**
* **Use butane to blow away scraped pieces of skull.**
* **Essential to keep the skull clean and dry.**

1. 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.
2. Let the exposed, scraped skull to dry for a few mins.
   1. Can apply some air puffs during that time.
3. Apply an even, thin layer of glue (Loctite Brush on, more runny than superglue) on top of the whole skull
   1. Don’t use air canister on it
   2. Best to use a glue model with a brush. Otherwise can use a cocktail stick, a spatula or a different brush with Loctite 401 glue
   3. Keep the surface shiny – if too much glue, it may go opaque and won’t be as transparent, and will also provide too much acoustic impedance when using ultrasound.
   4. Give the skull 5-10 mins to dry
4. Apply a thin layer of nail polish. Wait 5-10 minutes for the polish to dry.
5. Put the headbar over the exposed area.
   1. Mix dental cement with methyl methacrylate in a well until it has a thick texture and fill a syringe with it
   2. Start with running a layer of dental cement around on the headbar, filling in the holes
   3. Apply dental cement wall around on the head, especially on the sides, before putting headbar on so that there are no holes left
   4. Place the prepared headbar on the head
   5. Make sure midline parallel to headbar side and both hemispheres at a similar level
   6. Check that the mouse fits into printed neurotar model and adjust cement if needed
6. Wait 10 minutes after the headbar is put on
7. While waiting: (optional) Put some glue at the back of the headbar to insulate it from electrodes (or dental cement)

Positioning. Find and mark your coordinates using a tooth pick dipped in ink from a pen. Put the large syringe tip in the stereotaxic positioner and locate bregma. Mark 2 0.5mm drill holes at: (VEP and MEP location this time)**.** Brain Atlas Reference: <http://labs.gaidi.ca/mouse-brain-atlas/?ml=2.5&ap=-.4&dv=3.2>

* 1. Make sure bregma and lambda are on the same height – within 50-100 um. Adjust mouse in the frame if needed
  2. Make sure midline is straight
  3. Current coordinates used:
* **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.**

1. Wait 15 minutes for cement to dry. **During this time, adjust the end electrodes on the headbar implant so that they align over the marked drill holes.**

NOTE WELL: when positioning the head bar, ensure that it points straight back – so that it will fit into the neurotar with the extra plug attachment.

1. Drill vertical holes through the points down to dura.

* If head not stable enough, tighten the ear bars (not ideal but acceptable)
* Clean holes ideal, always return to the same spot with the drill
* Drill to the dura, not completely through
* Avoid damaging and bleeding!
* Important to remember: skull thinner at the front of the skull (and more lateral?)
* Blow away bone fragments with butane.

1. Placing the electrode implant.

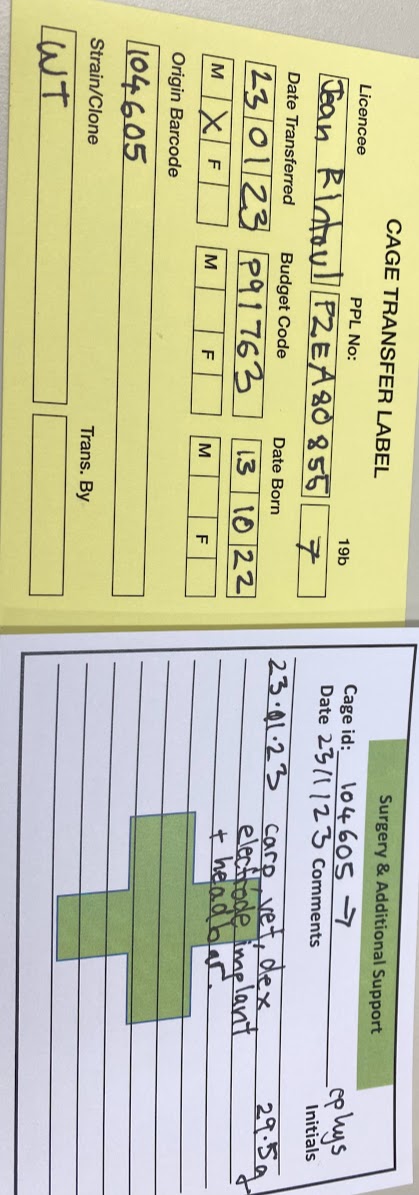
* Mix cement and apply a small amount to the back of the headbar and place the implant over the top, such that the two electrodes are located over the drilled holes.
* **Press down slightly (implants that have no ball on the end) such that the wire goes slight through the skull. Try to do this once only as minimal trauma to tissue is better.**

Wait a few minutes, then apply a bit more cement over the implant socket at the back of the head bar so it is stable and secure. **NOTE WELL: when positioning the head bar, ensure that it points straight back – so that it will fit into the neurotar with the extra plug attachment.**

1. Put clear near polish over the electrode sites such that they dry and provide a waterproof barrier.
2. Wait for it to dry for 5 minutes.
3. Test the impedance with a multimeter.
4. Administer mouse with extra .5 ml of saline subcutaneously.
5. Check again that the mouse fits into neurotar form when outside of the stereotaxic.
6. Move stereo tax away – unscrew the ear bars.
7. Decrease the isoflurane so that the mouse can breathe oxygen for a short time, then transfer it to the heated recovery chamber.
8. Put the mouse into recovery chamber until it wakes up.
9. Mouse will awake a little off balance.
10. Wait 30 minutes to an hour while monitoring the mouse recovery within the heated chamber.
11. 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 (sugus, 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 sugus 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.

**Surgery preparation: clean area**

* Absorption triangles should also be present in a separate petri dish
* Electrode and drill bit should be out clean, prepared with the surgical tools
* Cover the clean area with a green drape until the mouse is shaving in the prep area

A group of items on a table

Description automatically generated

**Surgery preparation: dirty area**

A close up of a machine

Description automatically generated

**Details on custom-made electrodes**

**Electrode implants**

The socket electrodes implanted during the surgery are built by soldering two short platinum-iridium wires (0.25 mm dia(meter), Alfa Aesar) to the socket (ref EX-MC2, Express Models). The electrode ends are smoothed to ensure minimal damage to tissue and bone during implantation. This is done by placing them in a hot flame produced by burning oxygen and acetylene for several seconds until a ball (~0.3-0.4 mm dia) is formed.

**Return electrodes**

The return electrodes are positioned on the shaved mouse cheeks prior to each stimulation session. These are created by placing either electrode gel (SignaGel, Parker; ~2-3 mm 11 dia) or a small piece of conductive copper tape (RS Components) on each cheek and connecting them to the stimulator with 26 AWG teflon-coated silver wires (WPI).