**STANDARD OPERATING PROCEDURE SOP NG0003**

**Mouse recovery stereotaxic surgery for the skull clearance and implantation of electrodes**

**Version:** 1.2 **Date:** 22/11/2022 **Author(s):** Patrycja Dzialecka

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**Objectives**

This Standard Operating Procedure (SOP) describes the guidelines to perform recovery stereotaxic surgery aiming to implant skull stimulating electrodes in mice. This SOP also includes guidelines for the post-surgery care of mice.

**Scope**

This SOP applies to any individual carrying out the implantation of skull stimulating electrodes & skull clearance for calcium imaging through stereotaxic surgery in 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 |
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**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 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

**Surgery**

1. Weigh the animal and record its details
   1. Surgery card: name of the mouse, date, procedure, initial weight, analgesics: dex, vet and carprofen, my initials
2. Anaesthetise the mouse
   1. Isoflurane: 3% induction for 2-3 minutes and 1.5-2% continously OR
   2. With ketamine + xylazine IP injection (2 ket + 1 xyl / 2.5 ket + 1.5 xyl, depending on weight)
3. Administer a dose of carprofen (3 ticks of 1:10 dilution), a dose of vet (1 tick + 9 saline), 1 tick of dex and .5 ml of saline subcutaneously to the mouse
4. Shave the skull and cheeks with the shaving creams
   1. Cover the eyes with vaseline and clean any cream if it gets in
   2. Expose back and all sides for the wide headbar
5. Move the mouse to the stereotaxic frame
6. Check the paw reflex and if not present, fix the mouse in the stereotax frame using ear pieces
   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
   3. Ensure the mouse head is straight (midline) while fixed
   4. Make sure the head is stable enough for drilling
7. Insert the anal temperature probe and cover the mouse with a sterilised piece of tin foil to keep it warm
8. Clear the head with cotton buds dipped in iodine and ethanol
   1. At least three times, clean all skin area in circular motion starting from the middle
   2. Remove any leftover hair with extra ethanol
9. Remove the skin on top of the skull
   1. Check pedal reflex before starting the cut
10. Remove conjunctive tissue left on the skull with dry and wet (saline) suggies
11. Scrape the whole skull with a scraper (most scraping)
    1. Scrape diagonally, evenly in both directions
    2. Use butane or air to blow away scraped bits of the skull (air puffs)
    3. If bleeding, clean with saline
    4. Essential to keep the skull clean and dry
12. Cut the muscles around the headbar placement
    1. Cutting the back muscle (1st only) and the side one (ipsilateral to the side where most headbar/electrode is) to ensure they are no longer attached to the bone
    2. If bleeding a lot, use cold saline to flush blood out
13. Scrape the surface again, removing any remaining muscle fragments
    1. Clean with saline to remove any bone fragments and dry afterwards
14. Use gel-like glue (loctite super glue power flex) to glue cut muscles to the bone all around the exposed area
    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
15. Let the exposed, scraped skull to dry for a few mins
    1. Can apply some air puffs during that time
16. Carefully clean the skull with acetone-dipped suggies
17. 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
18. Give the skull 5-10 mins to dry
19. Apply a thin layer of nail polish
20. Wait 5-10 minutes for the polish to dry
21. 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
22. Wait 10 minutes after the headbar is put on
23. While waiting: (optional) Put some glue at the back of the headbar to insulate it from electrodes (or dental cement)
24. While waiting: Find and mark your coordinates using a stiff wire dipped in ink
    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: AP –3.5, ML 1.25 & 3.25 (target 2.25). V1 coords: AP -3 to -5, ML 0.75 to 3.5
25. Drill vertical holes through the points down to dura
    1. If head not stable enough, tighten the ear bars (not ideal but acceptable)
    2. Clean holes ideal, always return to the same spot with the drill
    3. Drill to the dura, not completely through
    4. Avoid damaging dura and bleeding!
    5. Important to remember: skull thinner at the front of the skull (and more lateral?)
26. Fill the drilled holes with conductive gel before placing electrodes in
    1. Better contact (for now) and less chance of glue entering inside
27. Place your electrode pins in the holes and glue them to the skull
    1. Position your electrodes in the holes, holding them with a forceps
       1. A cotton bud stick can be used to support the socket
    2. Ensure the electrodes are stable
       1. Putting cement on the headbar back before electrode placement works well
       2. Alternatively, a cottom bud stick can be used to support the socket
    3. Glue the electrodes with tiny bit amount of glue on the skull, all around both of them. Use a super thin brush to go all around the holes
    4. Positioning a small drop of glue and sliding it down to the skull can ensure clean prep
28. Fix the back socket of the electrodes with extra dental cement
29. Apply a few drops of nail polish to fill the well and fix the electrodes better
    1. Avoid air exposure resulting in bubbles. If any bubbles visible under the scope, remove with a 27G thin needle. Play close attention to area around the electrodes
    2. Fill in any gaps with the cocktail stick
30. Wait a few minutes with the mouse in the frame for the glue to dry
31. Cover the electrodes and skull using a piece of tape when everything dry enough, ensuring the tape won’t touch the skull surface
32. Administer mouse with extra .5 ml of saline subcutaneously
33. Check again that the mouse fits into neurotar form when outside of the stereotax
34. Put the mouse into recovery chamber until it wakes up

**Straight after the surgery**

1. Prepare the cage
   1. If moving the animal into separate cage: fill in the transfer label
   2. Remove hoppers, tubes and metal grid
   3. Put small fluffly tissue and remove the old normal one
2. Return the animal into the cage when it recovers
   1. Mouse should be moving around, cleaning itself and eating

**Post op**

1. Observe the animal over the next few days and keep warm in recovery chamber if needed
2. Record weight over next 3 day – the mouse should recover most of initial weight by then
3. Inject or 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 (suggies, cotton buds, cocktail sticks) should be autoclaved prior to the surgery. Drill bits and headbar should be washed and disinfected by leaving them for a few seconds in the germinator. Electrodes can be disinfected in the germinator and/or ethanol
2. Disinfect hands with alcohol 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. Ketamine-xylasine: every 10-15 mins
   2. Isofluorane: every 5 mins
   3. If hand used to check the reflex, disinfect your hands
7. Measure time under anaesthesia: surgery should be around 1h 30min - 2h long
8. If pedal reflex observed, administer additional dose of ketamine
   1. Adjust as needed e.g. half a dose of ketamine (1 tick) if more time needed or 1 in 10 (1 tick ket + 9 tick saline) if surgery almost finished

**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).