## Appendix C: Detailed surgical SOP for head fixation surgeries

This document gives an example SOP for the surgical implantation of a head fixation device. This should be used in addition to local guidance, local and national legislation, as well as other published guidelines such as those on aseptic surgical technique (1).

### Preparing for surgery

* Animals new to the facility should be given an acclimatisation period before surgery, typically five days. Habituation to the experimenter and rooms that the animal will be exposed to as part of surgery will also ensure lower stress levels.
* Introduce before surgery new items in the cage that will be important post-operatively. This includes extra bedding and jelly to deliver analgesia. At this point, provide non-medicated jelly to acclimatise mice to this novel foodstuff.
* Check the animal is in good health in the days preceding the surgery.
* Autoclave all instruments and materials to be used during the surgery. Autoclaved foil or similar can be used to cover the surfaces of instruments that cannot be autoclaved, for example anaesthetic vaporisers and surgical microscopes.
* Weigh the animal and adjust injection volumes of the drugs to be used to this weight.
* Induce anaesthesia and administer pre-operative analgesia. If the surgery is likely to take last longer than 30 minutes, 1ml isotonic saline should also be administered. Use the most refined methods for delivery.

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### Preparing the scalp

* After anaesthetic induction, hair can be optionally removed with an electric shaver, scissors, or depilatory cream. Electric shaving should not be done near the operating table to avoid contamination. Alternatively, if hair is left in place (for additional anchoring of the skin with the cyanoacrylate primer and dental cement), it must be thoroughly disinfected, for example with a dilute chlorhexidine solution.

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| **When shaving, take care not to damage the whiskers around the eyes and nose; whiskers and facial fur can be protected with petroleum jelly.** |

* Once the scalp has been cleaned, administer local anaesthetic to the centre of the area to be excised during surgery. Local anaesthetics take several minutes to act, so this should be done as soon as the scalp is prepared, before moving the animal to the operating table.
* Rodents’ eyelids stay open during anaesthesia leading to dryness and possible corneal damage. Eyes can be protected by applying sterile ophthalmic ointment or artificial eye drops. Alternatively, eyelids can be covered with petroleum jelly to keep the eyes closed.

### Transferring the animal to the operating table

* If using gaseous anaesthesia, quickly apply the anaesthetic mask with an oxygen flow of ~1L/minute and the isoflurane concentration to ~3% for rats and ~1.5% for mice. Throughout the surgery, monitor the depth of anaesthesia and reduce the level of isoflurane accordingly, this may be as low as 1% towards the end or surgery. Note that every animal responds differently and the precise isoflurane level should be adjusted according to the apparent depth of anaesthesia.
* Any increase in heart rate, or movement of the limbs or whiskers should be taken as a sign to stop all procedures and increase the level of anaesthesia until the animal no longer responds to a firm foot-pinch.

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| **It is important not to confound respiratory distress from applying too much anaesthesia for under-anaesthesia! In some animals, if the level of isoflurane is too high you may notice the animal breathing in gasps or developing a hunched posture. If unaccompanied by foot-pinch response, this is a clear sign that the isoflurane should be reduced immediately. Airways also need to stay clear to allow optimal breathing.** |

* After reaching a stable level of deep anaesthesia in the induction box, the animal can be mounted on the stereotactic frame.
* Local anaesthetics (lidocaine or bupivacaine) are applied under the scalp. Bear in mind that bupivacaine [has a slower onset than lidocaine (30 and 2 minutes, respectively) but longer duration of action](https://animalcare.ubc.ca/sites/default/files/documents/Guideline%20-%20Rodent%20Anesthesia%20Analgesia%20Formulary%20%282016%29.pdf) [(4-8 hours and <1 hour, respectively)](https://animalcare.ubc.ca/sites/default/files/documents/Guideline%20-%20Rodent%20Anesthesia%20Analgesia%20Formulary%20%282016%29.pdf) .
* Some stereotactic surgeries require head immobilisation with ear bars. The depth of anaesthesia needs to be carefully stabilised since the insertion of the ear-bars can be a strong irritant even for animals that are unresponsive to other stimuli. The use of non-puncture ear bars is recommended. Evaluate the absence of withdrawal (tail or toe-pinch) or blink (gentle corneal touch) reflexes before any painful manipulation and adjust anaesthesia and analgesia levels accordingly.
* If using closed-loop controlled heating-blankets systems, insert the lubricated rectal thermometer and turn on the heating pad, fixing the temperature probe to the mat with a small strip of paper tape and make sure temperature of the mouse is stabilised to 37oC.

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| **To prevent damage to the extremities, ensure that the temperature of the mat itself cannot reach excessively high temperatures, either by regulating the temperature of the mat (some systems may allow for this separately, or the probe would need to be positioned beneath the animal) rather than the core temperature of the mouse, or by using a system that restricts the maximum temperature of the heat source. Also ensure that the mouse is insulated by placing an absorbent pad between the animal and the heating mat and not in direct contact with the mat. The heating mat should also be insulated from the stereotactic frame.** |

* Cover the animal’s body from the neck down with a surgical drape (additional insulation can be provided by using veterinary bedding and bubble wrap). This will help to maintain a constant body temperature. A transparent drape will ensure visibility of the animal’s respiration at all times.

### Scalp excision

* To break up fat and oily deposits in the fur of the animal which could interfere with the binding of the headcap in the long-term, apply a 2% ethanol solution to the scalp before excision if not shaved.

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| **Some groups use a greater concentration such as 70% ethanol. 2% ethanol is sufficient to break the superficial tension of the fur without acting as an irritant or risking drying the skin underneath, which may compromise the welfare of the animal.** |

* Thoroughly disinfect the area using a topical disinfectant such as betadine or a chlorhexidine solution with a cotton swab. The scrubbed area should be larger than the skin section to be excised. Avoid disinfectant contact with the eyes and respiratory airways.
* Scalp excision should be as large as necessary and as small as possible: it should expose the skull area necessary for subsequent implant. Ideally, skin excision should not go over the skull muscles. Unless the skin opening is very small, avoid performing a slit and pulling the skin over since skin will tend to adopt its natural position, risking implant damage, itching, scratching and inflammation.
* Scalp excision should be performed with sharp surgical scissors and a minimal number of cuts to avoid skin nicks. To perform a single-cut scalp excision, pinch the skin at the centre of the area to be excised with small-toothed forceps and lift perpendicular to the operating table. Use large surgical scissors to cut the lifted skin by placing the open scissors parallel to the operating table. Before cutting adjust the vertical position of the pinched skin such that the blades of the scissors are at the level of the imaginary section of skin that will be excised. When cut, this should provide an oval shape excision with no nicks.
* Around the excised skin, protruding hairs can be trimmed with corneal scissors, using your fingers to keep the skin taut to avoid causing nicks. Wounded skin can be treated with topical anaesthetic ointments. At this stage, bleeding from the skin should be negligible.
* Keep in mind that the internal tissues of the animal are, in principle, free of pathogens. Therefore, it is not necessary to disinfect the tissues below the excision line. However, from this point on, any tool that contacts live tissue must be clean and sterile.

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### Preparing the skull

* After scalp excision, remove any loose hair with the help of sterile cotton swabs or small tweezers. Avoid exposing the skull to water peroxide, bleach solutions or other irritant products.
* The periosteum (connective tissue) is typically impregnated with local anaesthetic and thus becomes elastic and gelatinous. It must be removed to allow an adequate bonding of the skull with cementing materials. It can be cut out using corneal scissors and small forceps. When the periosteum becomes dry, it loses volume and becomes thin and fragile and can be easily removed by scraping.
* If using a stereotactic frame, roll, pitch and yaw of the skull should be adjusted at this point such that lambda and bregma are at the same height and their axis is perpendicular to the ear bars.
* Make any necessary landmark for future implants by using stereotactic tools, motorised systems or micro rulers. Long-term landmarks (e.g. bregma) can be made by carving the bone with a scalpel and filling the hollows with permanent markers (resistant to the solvents contained in the cementing materials to be used).
* Roughen the skull with the blade of a scalpel or with a dental drill at low speed in order to increase bone rugosity and cement adherence. Bleeding from the bone can occur if scraping is too deep.

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| **Some groups use a greater concentration such as 70% ethanol. 2% ethanol is sufficient to break the superficial tension of the fur without acting as an irritant or risking drying the skin underneath, which may compromise the welfare of the animal.** |

* Remove any tissue debris with the help of sterile cotton swabs or compressed sterile air cans. Use sterile saline or other physiological buffers to remove any blood clot on the bone. Make sure the skin and bone are dry before proceeding to the next step.

### Skin protection

* It is crucial to secure the skin to the skull before head-post implantation or craniotomies. This will keep the skin in place throughout the surgery, protecting it from mechanical damage and preventing the open wound from coming into contact with liquids (e.g. dental cement solvent which is highly irritant) and other debris during the rest of the surgery.
* Put a few drops of tissue adhesive (e.g. cyanoacrylate) on top of the dried skull. Spread the adhesive around the edges of the open skin with the help of a thin wooden stick (broken cotton swab). Ideally a thin band (1-2mm) of tissue adhesive should also cover the surrounding skin and hair. This can provide an impermeable protective barrier and act as an interface for further cementing materials.
* Depending on the specific characteristics of the implants, head-post and craniotomy, the order of the following steps should be performed according to the experimental limitations.

### Head-post implantation

* In mice, head-post implantation does not typically require the use of anchoring screws, but large implants and implants in larger animals like rats may be reinforced with small stainless steel anchoring screws.
* Anchoring screws should be inserted in bones that offer highest mechanical resistance. Screws should never traverse the skull and dura must remain intact. Suitable implantation points are the skull ridges due to their strength and thickness.
* Head-posts can be positioned on their definitive location with the help of stereotactic instruments. Alternatively, they can be transiently attached to the skull with cyanoacrylate glue. Once in place, the head-post can be covered with dental acrylic or dental cement. Wait enough time for the cement to cure.

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### Craniotomy

* Craniotomy is typically performed using dental drills. In cases where the skull is very thin (young mice or flattened skull), a scalpel can be used to cut the bone. Bone drilling should be ideally performed after delivering sterile saline or PBS solution at room temperature to the skull in order to soften the bone and reduce inflammation and bleeding. Thinned, soaked skulls become translucent (especially in mice) allowing visualisation of superficial blood vessels of the brain. Bone debris produced during the drilling process that occludes vision needs to be frequently removed with the help of a water suction system and sterile cotton swabs. Continuous buffer flow on top of the skull is most convenient. It can be implemented using water pumps or gravity systems to deliver the buffer into a silicone elastomer well on top of the skull. Overflowing is prevented by a suction pump.
* Always use sharp burrs. Keep them clean of bone residues and sterilise before use. Typical burrs used for craniotomy are FG ¼. For bone flattening it is preferable to use cylindrical burrs (typically 1 mm diameter and 3 mm long).
* Bleeding during craniotomy should be stopped rapidly. It can originate from blood vessels in the bone, sinuses, meninges or brain. Bleeding from the bone usually stops spontaneously if it originates in small vessels. Wetting the skull with saline usually helps clotting. Electric cauterisers can be used when bleeding fails to stop. Soaked gelfoam can be used to absorb blood and promote clotting. Bleeding from the sinuses is dangerous and usually leads to haemorrhagic death, thus drilling close over the sinuses should be performed with extreme care. Subdural bleeding can take place if drilling temperatures are too high and can damage brain cells. This can be prevented by keeping the bone moist with room temperature buffers.
* Dura mater is typically left intact in most chronic experiments to provide long-term protection of the brain. However, electrode insertion typically requires piercing the dura at electrode entry points or full dura removal. Micropipettes for viral delivery are typically bevelled to pierce the dura.

### End of surgery care

* Once surgery is complete, ensure the animal’s fur is clean of any eye ointment, cement or glue. Allow to come around from anaesthesia gradually.
* If the surgery has been over one hour, administer 1 ml isotonic saline. Move the animal to a heated recovery cage with access to sufficient water and food. Observe regularly until fully recovered from anaesthesia.
* If you are not using medicated jelly to deliver post-operative analgesia, injected analgesia may need to be delivered at this point or the day following surgery. Consult your local veterinary team for advice.
* Once fully recovered from anaesthesia, return to the homecage which should have additional bedding from the pre-operative period. If administering post-operative analgesia via jelly, remove the unmedicated jelly use dot habituate the animal and replace it with medicated jelly.

**Reference**

(1) Lilley E and Berdoy M (2017). *LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section.*