UNIVERSITÉ LAVAL

Direction des services vétérinaires **Standard Operating Procedure**

Subject: Stereotaxic surgery in rodents	Number: C-3
Scope: A directive from the Direction des services vétérinaires to users and staff of Université Laval animal facilities (campus and affiliated research centres).	
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Purpose: Describe stereotaxic surgery procedures in rodents	Version 3

General considerations

- Several factors can cause the exact coordinates of the target area to vary slightly. If not accounted for, this can negatively impact the quality of the results and require the use of more animals. Before starting a protocol, it is best to include a few practice animals to ensure you have selected the correct area, especially when
  - new transgenic lines are used,
  - animals from different life stages are used, or
  - a new area of the brain is targeted with which the team has no experience.
- Perform surgery aseptically (see *SOP C-1 Aseptic surgery in rodents/ PNF C-1 Chirurgie aseptique chez les rongeurs*).
- Follow appropriate preoperative, surgical, postoperative, anesthesia, and analgesia procedures.
- Prepare a syringe of warmed fluid (saline or lactated Ringer's solution (LRS)) to irrigate the incised skin, control bleeding, and clean the surgical site. Frequent use of cold fluids can exacerbate hypothermia in animals.
- Depending on the animal's age and the targeted injection site, it is possible, with proper lighting, to identify anatomical landmarks in neonates and administer a direct injection without opening the skin. Perfecting this technique can eliminate wound dehiscence problems and limit animal losses.
- Before starting surgery and between each animal, disinfect the stereotaxic frame by spraying disinfectant on absorbent paper and wiping.

- Sterilize all materials (cotton swabs, gauze, fluids, etc.) and instruments in the autoclave.
- Before introducing them into the brain, electrodes, cannulas, and other implants that cannot be autoclaved must be sterilized with a proven method such as ethylene oxide gas or immersion in a solution designed for that purpose (e.g., glutaraldehyde or peracetic acid). Allow for adequate immersion sterilization time (usually more than 6 hours) and afterward rinse the implants with sterile water/saline and handle and store them aseptically.
- By following three conditions, you will ensure the long-term maintenance of implants:
    - utilize meticulous sterile techniques
    - adequately prepare the bone surface
    - securely anchor the cement to the bones and screws

Procedures

Animal preparation

- Weigh the animal.
- Administer a dose of systemic analgesic as per the protocol.
- Anesthetize the animal following the appropriate procedure in *SOP A-1 Analgesia and anesthesia in rodents (PNF A-1 Analgésie et anesthésie des rongeurs*).
- Shave the head and clean with antiseptic soap.
- Infiltrate the incision site and stereotaxic frame support points (i.e., the ear canals) with a local anesthetic (lidocaine and bupivacaine) as per *SOP A-1*. Wait three minutes before installing the ear bars.
- Before starting, check the depth of anesthesia by pinching the skin between the toes to verify the absence of the withdrawal reflex.

  Note: Given the pressure exerted on the skull by the fixation points, the anesthesia must be deep and consistent.
- Mount the animal in the stereotaxic frame. Insert the ear bars into the ear canals. For survival procedures, use blunt bars. For mice, zygomatic bars are recommended. Fine-tip bars are allowed for terminal surgery (the animal must be euthanized before recovery).
- Reapply ophthalmic ointment if it was removed when the animal was installed in the frame.

- Prepare the surgical site following *SOP C-1 Aseptic surgery in rodents (C-1 Chirurgie aseptique chez les rongeurs)*.
- Disinfect gloves with alcohol or put on a new pair of sterile gloves.

Surgery

- Cover the animal with a sterile surgical drape, leaving the head unobstructed. Respect asepsis as per *SOP C-1 Aseptic surgery in rodents*.
- Make an anterior/posterior incision through the skin and fascia covering the skull.

  Note: If scissors are used, straight ones are preferred. However, a scalpel is highly recommended for rats.
- Depending on the injection site, or if an implant must be attached to the skull, pull the fascia away from the bone using the scalpel and a cotton swab. Control bleeding by compression/friction with a cotton swab or by applying LRS.

  Note: For rats, it is often necessary to retract the skin to access the targeted area on the skull. Four small curved mosquito forceps can be placed at each corner of the wound, taking care to pinch the fascia and not the skin. A Colibri retractor can also be used. For mice, it is usually sufficient to push the skin laterally with a cotton swab. If necessary, a mouse eyelid retractor can be used.
- Keep the wound moist throughout the procedure by regularly sprinkling with saline or LRS.
- For very long surgeries, re-administer the lidocaine/bupivacaine mixture every 3 hours to the site and on the ear bars, being careful not to exceed the maximum toxic dose.

<u>Indirect injection</u>

- Use a needle holder to insert the sterile drill bit into the drill.
- Ensure the skull is level by comparing the bregma and lambda heights.
- Locate bregma.
- Perform the craniotomy at the appropriate stereotaxic coordinates, being careful not to puncture the dura mater accidentally.

  Note: We recommend you mark the craniotomy position with a sterile pencil.
- Use cotton swabs and LRS to stop the bleeding. If necessary, apply a hemostatic gel such as Gelfoam®.
- Remove bone debris from the craniotomy using forceps and/or cotton swabs soaked in saline or LRS. Use a 25G needle to pierce the dura mater without damaging the brain.
- Slowly lower the injection needle to the correct coordinates at the recommended speed of 1 mm/min.

- Administer the injection using a speed and volume appropriate to the species and target area.
- Wait 3 to 5 minutes to allow the product to diffuse and then slowly withdraw the needle.

  Note: We recommend you close the craniotomy with bone wax.
- Rinse the incision site with some saline or LRS.
- Close the surgical site aseptically by placing a sterile drape around it to avoid contaminating the sutures.

Preparing the skull to install an implant

- To maximize cement adhesion, ensure all fascia has been removed and all bleeding has stopped.

  Note: We recommend making grooves on the skull surface using a scalpel or needle tip, avoiding the sagittal and coronal sutures.
- Wash the bone surface with saline or LRS and dry it with a cotton swab.

Installing anchor screws

- Use a needle holder to insert the sterile drill bit into the drill.
- Ensure the skull is level by comparing the bregma and lambda heights.
- Drill the screw holes with a drill bit smaller in diameter than the screws and insert the screws carefully; do not go deeper than 1 mm. From 1 to 4 screws can be used to provide support for the dental cement. Avoid placing them too close to the site of interest so as not to interfere with the electrode recording or the insertion of the guide cannula. Avoid drilling into the skull at bone junctions since this will cause significant bleeding.

  Note: Screws should not penetrate the bone completely. Remove the screw and fill the hole with bone wax if this occurs.

Craniotomy

- If a craniotomy is needed, locate bregma.
- Make the craniotomy at the appropriate stereotaxic coordinates and avoid accidentally puncturing the dura mater. Use a drill bit size suitable for the cannula diameter.

  Note: We recommend you mark the position of the craniotomy with a sterile pencil.
- Remove bone debris from the craniotomy using forceps and/or cotton swabs soaked in saline or LRS and dry the skull with a cotton swab. Use a 25G needle to

pierce the dura mater without damaging the brain to facilitate the insertion of thin structures (e.g., micropipettes, cannulas, etc.).

Implanting a cannula

- Use the guide cannula to insert the cannula at the recommended speed of 1 mm/minute.
- With a 27G needle, apply a small drop of tissue glue to the skull at the base of the cannula. Be careful not to glue the guide cannula.
- Once the glue has dried, carefully remove the guide cannula without disturbing the cannula.

  Note: If the cannula moves on the skull, reapply a drop of glue.
- Apply a thin, fairly liquid layer of dental cement with a spatula or sterile cotton swab. Make sure it covers the entire area of exposed skull and the area all around the cannula and screws.
- Continue applying the cement to form the implant. Avoid leaving air bubbles that could weaken the structure.
- Block the cannula with a dummy cannula when it is not connected to an infusion system (e.g., osmotic pump).

  Note: Block double cannulas with a non-screwing double dummy covered with a plastic dust cap.

Implanting an electrode

- Insert the electrode with a device suitable for the electrode type at a recommended speed of 1 mm/minute.
- Apply a thin, fairly liquid layer of dental cement with a spatula or sterile cotton swab. Make sure it covers the entire area of exposed skull and the area around the electrode and screws.
- Continue applying the cement to form the implant. Avoid leaving air bubbles that could weaken the structure.

Implanting an optical fibre

- Insert the optical fibre with the appropriate device at the recommended speed of 1 mm/minute.
- Apply a thin, fairly liquid layer of dental cement with a spatula or sterile cotton swab. Make sure it covers the entire area of exposed skull and the area all around the optical fibre and screws.
- Continue applying the cement to form the implant, freeing up enough optical fibre to ensure a good connection. Avoid leaving air bubbles that could weaken the structure.

Installing a cranial window WITHOUT a craniotomy

Note: Rather than making a straight incision, use iris scissors to remove a circle of skin at the site where the window will be placed. Prepare the skull in the manner described above. Anchor screws are not needed for this procedure.

- Apply clear Metabond® to the skull to cover the entire exposed bone surface. Make sure there are no air bubbles.
- Place the window in the intended location before the cement hardens (<1 min), being careful not to create air bubbles that could affect imaging and the implant's strength.
- Apply dental cement around the window in several layers to form a small rim, avoiding air bubbles that could weaken the structure. Mould with a wooden applicator to smooth the outer edges that touch the skin.

Installing a cranial window WITH a craniotomy

Note: Rather than making a straight incision, use iris scissors to remove a circle of skin at the site where the window will be placed. Prepare the skull in the manner described above. Anchor screws are not needed for this procedure.

- Use a needle holder to insert the sterile drill bit into the drill.
- Demarcate the area around the craniotomy using the drill, applying only light pressure.
- Wash the bone surface with saline or LRS and wipe it with a cotton swab.
- Repeat the previous two steps until the bone fragment becomes mobile.
- Carefully remove the fragment with forceps, being careful not to puncture the dura mater accidentally.
- Use cotton swabs and LRS to stop the bleeding. If necessary, apply a hemostatic gel such as Gelfoam®.
- If necessary, remove bone debris from the craniotomy using forceps and/or cotton swabs soaked in saline or LRS and dry the skull with a cotton swab.
- Place the window on the skull to cover the opening.
- Apply tissue glue to the skull to cover the entire exposed bone surface and allow it to dry.
- Apply dental cement around the window in several layers to form a small rim, avoiding air bubbles that could weaken the structure. Mould with a wooden applicator to smooth the outer edges that touch the skin.

Installing a fixed anchor

- Be sure to follow the manufacturer's instructions, if applicable and install the anchor screws in the correct locations according to the implant design.

- Lower the fixation rod or plate at the desired angle onto the surface of the skull.
- Secure the plate or rod to the previously installed anchor screws and the skull by applying a thin layer of fairly liquid dental cement using a spatula or sterile cotton swab. Make sure it covers the entire area of the exposed skull and the area around the implant and screws.
- Continue applying the cement to form the implant. Avoid leaving air bubbles that could weaken the structure.

Cement moulding

- If needed, reapply ophthalmic ointment to protect the eyes.
- Apply dental cement in thin layers to prevent thermal damage to the brain, skull, and surrounding tissues (dental cement releases heat as it solidifies). Wait for the cement to dry completely before applying the next layer.

  Note: Generally, 3 layers of cement are required, the first very thin and liquid and the following increasingly thicker. If spacers are used, remove them immediately after applying the second layer (before the cement sets).

  Two moulding techniques for the final coat can be used, either an application over the skin or the creation of a cement cup. The site, implant type, and surgeon's experience may influence the choice of technique.
- Be very careful not to move the implanted structure or damage it, for example, by pouring cement into a cannula.
- If necessary, cut or polish the edges of the cement that touch the skin (the edges must be smooth).
- If the incision was too large, suture the anterior and/or posterior ends as needed, placing a sterile drape around the incision to avoid contaminating the sutures. During the next surgery, take care to make a smaller incision.

Postoperative period

- Before the animal recovers, trim the tips of its hind claws with small scissors or a claw cutter.
- If the surgery involved an implant, weigh the animal and record its weight on the DSV postoperative follow-up card in the space indicated (see Appendix I)
- Follow the procedure described in *SOP C-1*.
- Once the animal recovers, check its eyelid reflex (eyelid blinking when the corner of the eye is gently touched with a cotton swab). If there is no reflex response, apply eye ointment three times a day until the reflex returns.
- For animals with an implant, use a cage with a higher metal mesh to prevent injuries due to the implant.

- Continue postoperative analgesia as per the protocol.
- For animals with an implant or window, place a stereotaxic implant follow-up card on the cage and trim their claws once a week.

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SOP Revision History		
Version 2	August 31, 2015	Clarified soaking times for instruments and materials
Version 3	April 21, 2020	Added to General Considerations. Clarified immersion sterilization methods for implants. Added numerous clarifications to procedures. Added information on placements of electrodes, optical fibres, cranial windows, and fixed anchors. Clarified cement moulding techniques. Added postoperative weight measurement after implant placement. Added the stereotaxic implant follow-up card.