



STANDARD OPERATING PROCEDURE 309 RODENT STEREOTAXIC SURGERY

1. PURPOSE

This Standard Operating Procedure (SOP) intends to describe the procedure for rodent stereotaxic surgery.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. MATERIALS

- 3.1. Sterile isotonic solution for injection (e.g., 0.9% saline)
 - 3.2. Analgesics - Lidocaine-bupivacaine, 1:1 mixture (local analgesic), non-steroidal analgesic (such as carprofen or meloxicam; see Rodent Analgesia SOP)
 - 3.3. Anesthetics
 - 3.4. Sterile ophthalmic ointment
 - 3.5. Electric clipper or depilatory cream
 - 3.6. Antiseptic solution for skin (e.g., chlorhexidine 4% solution)
 - 3.7. 3% Hydrogen peroxide
 - 3.8. Heating pack, warming pad.
 - 3.9. Sterile surgical drapes
 - 3.10. Sterile surgical instruments
 - 3.11. Sterile gauze
 - 3.12. Suture material (4-0 monofilament absorbable) or wound clips (Autoclips)
 - 3.13. Hot bead sterilizer and 70% alcohol (as a rinsing agent)
 - 3.14. Drill, sterile stainless-steel screws, sterile cannula, sterile Hamilton (R) syringe.
 - 3.15. Stereotactic animal holder
 - 3.16. Stereotactic syringe holder for the accurate injection site.
 - 3.17. Syringe pump
 - 3.18. Rodent Procedure Log and Post-Procedure cage cards
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4. PROCEDURE

- 4.1. Document the details of the surgical procedure in the Rodent Procedure Log.
- 4.2. Perform pre-operative procedures at a safe distance from the surgical environment to prevent contamination with hair.
- 4.3. Pre-operative Care:
 - 4.3.1. Administer a non-steroidal anti-inflammatory analgesic, e.g., carprofen or meloxicam, or long acting or regular buprenorphine as per rodent analgesia SOP.
 - 4.3.2. According to Rodent Analgesia SOP (SOP 101).
 - 4.3.3. Anesthetize the animal according to Rodent Anesthesia SOP (SOP 201).
 - 4.3.4. Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.
 - 4.3.5. Administer subcutaneously from 0.2 to 0.5mL/10g body weight of isotonic fluids.
 - 4.3.6. Remove hair over the surgical area with a clipper, and depilatory cream, allowing a perimeter of at least 1cm or as large as possible around the surgical site. Remove loose hair with gauze.
 - 4.3.7. Wash the surgical site with 4% chlorhexidine or povidone-iodine solution. Be careful not to wet a large area of the animal.
 - 4.3.8. Secure the animal in the stereotaxic frame.
 - 4.3.9. Place a heat source under the animal or wrap the animal with insulating material, e.g., thermal drapes or bubble wrap.
 - 4.3.10. Preparation of the surgical site:
 - 4.3.10.1. Apply 70% alcohol with gauze or swabs in a circular motion from the center of the surgical site to the exterior. Be careful not to wet a large area on the animal, as the evaporation of alcohol will lead to heat loss.
 - 4.3.10.2. Apply 4% chlorhexidine or povidone-iodine solution with gauze or swabs in a circular motion from the center of the surgical site to the exterior.
 - 4.3.10.3. Repeat steps 4.3.8.2 and 4.3.8.3 two more times.
 - 4.3.11. Surgeon's preparation:
 - 4.3.11.1. Wash hands.
 - 4.3.11.2. Wear a surgical mask, bonnet, and clean gown.
 - 4.3.11.3. Use an aseptic technique.
 - 4.3.11.4. Wear sterile gloves.
 - 4.3.11.5. The surgeon must avoid touching non-sterile surfaces.
 - 4.3.12. Cover the animal with a sterile drape.
 - 4.3.12.1. The drape can be placed only when suturing the wound for minor surgical interventions to prevent sutures from meeting hair and skin around the surgical area.
 - 4.3.12.2. Surgical drapes must be sterile for the first animal and may be transferred to the following animal during serial surgeries. The top surface of the drape must never encounter non-aseptic areas and must not be soiled.
 - 4.3.12.3. Use the drape to shield the animal's eyes from surgical lights, as prolonged exposure to intense light may cause damage to the retina.
- 4.4. Surgical Principles/Aseptic technique:
 - 4.4.1. Ensure that all the available materials are handy.
 - 4.4.2. Begin surgery with clean and sterile surgical instruments, and handle instruments aseptically.

- 4.4.3. Designate a sterile area on the working surface for the sterile material (instruments, suture material, drapes, gauze, etc.).
- 4.4.4. Before surgery, verify the depth of anesthesia by loss of the animal's pedal withdrawal (toe pinch) reflex using smooth-tipped/non-toothed forceps.
- 4.4.5. Expose the cranium by making a surgical anterior-posterior incision with a scalpel blade (for lesions or injections) or by cutting a circular skin fold with scissors (for cannula placement).
- 4.4.6. Avoid contact of tissues with fingers by using the tip of instruments.
- 4.4.7. Reflect the skin.
- 4.4.8. Apply local anesthetics (mixture of bupivacaine and lidocaine) to the periosteum.
- 4.4.9. Scrape the skull to detach and push aside the periosteum. Wipe the skull surface with sterile swabs to remove blood.
- 4.4.10. Hydrogen peroxide solution can be applied to the skull to identify bregma; the sutures will appear white.
- 4.4.11. Intracranial injection:
 - 4.4.11.1. Using predefined stereotaxic coordinates, mark the intended injection site on the skull.
 - 4.4.11.2. Make a single burr-hole in the skull at the injection site using a hand-held drill.
 - 4.4.11.3. Position the syringe over the burr-hole.
 - 4.4.11.4. Lower the syringe until the needle touches the cortical surface and use this point as "zero" (Z zero). Lower the syringe needle to the desired depth.
 - 4.4.11.5. Inject slowly with the pump to avoid an acute increase of intracranial pressure and facilitate fluid diffusion. This step may take 10 minutes, depending on the total volume injected.
 - 4.4.11.6. After the injection, allow 2-5 additional minutes of rest before withdrawing the syringe.
 - 4.4.11.7. Withdraw the syringe slowly.
 - 4.4.11.8. It is not necessary to seal the burr-hole.
 - 4.4.11.9. Infiltrate the wound with a local anesthetic, e.g., lidocaine, before closing the skin. Refer to Rodent Analgesia SOP 101.
 - 4.4.11.10. skin is sutured with monofilament absorbable suture size: 4-0, or the incision can be closed using wound clips (Autoclips); 7mm or 9mm. Sutures or staples must be removed after 10 days.
- 4.4.12. Implantation of cannula
 - 4.4.12.1. Using a hand-held microdrill, drill burr holes (up to 2mm in diameter for rats and 1mm in diameter for mice) into the skull to insert stainless steel securing screws.
 - 4.4.12.2. Insert stainless steel screws.
 - 4.4.12.3. Make a craniotomy over the target area of the brain.
 - 4.4.12.4. Guide cannula, temporarily fitted with an internal cannula, is inserted into the target area according to appropriate stereotaxic coordinates.
 - 4.4.12.5. Dental acrylic is applied around the cannula, screws, and any exposed cranium to secure the cannula. The dental acrylic should create a strong head cap fixed to the skull, and the edges of the head cap should be smooth and cover all the bones.
 - 4.4.12.6. When the cement hardens, the internal cannula is replaced with a "dummy" cannula (obturator) inserted in the guide cannula to maintain patency.

- 4.4.12.7. A plastic dust cap is placed to protect the cannula assembly
- 4.4.13. Implantation of neural implants
 - 4.4.13.1. Using a hand-held drill, drill burr holes (up to 2mm in diameter for rats and 1mm in diameter for mice) into the skull to insert stainless steel securing screws.
 - 4.4.13.2. Insert stainless steel screws.
 - 4.4.13.3. Make a craniotomy over the target area of the brain according to the predetermined stereotaxic coordinates.
 - 4.4.13.4. Slowly and carefully insert implant(s) into the target area(s)
 - 4.4.13.5. Apply dental acrylic around the electrodes, screws, and any exposed cranium to secure it in place. The dental acrylic should create a strong head cap fixed to the skull. The edges of the head cap should be smooth and cover all the bones.
- 4.4.14. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood and debris (let cool completely) or in a liquid sterilizing solution (e.g., glutaraldehyde or equivalent) for a few minutes (>5 minutes) and then rinsed with 70% alcohol. For liquid sterilization, it is recommended to use two alternating surgical kits to increase contact time with the solution.
- 4.4.15. Dip suture material in 70% alcohol between each animal.
- 4.4.16. Recommended suture and wound closure.

4.5 Surgical Monitoring and Supportive Care:

- 4.5.1 Provide a contact heat source to prevent hypothermia.
- 4.5.2 Frequently monitor the presence of reflexes, the respiratory rate and breathing pattern, and heart rate when available.
- 4.5.3 Adjust the depth of anesthesia according to monitored parameters (presence of reflexes, respiratory rate, breathing pattern, heart rate).
- 4.5.4 In the case of respiratory arrest, stop anesthesia, administer oxygen, and compress the thorax rapidly between thumb and index at an 80-120/min frequency.

4.6. Post-operative Care:

- 4.6.1. Post-operative care begins immediately following surgery, lasts at least 3 days, and extends for up to 10 days.
- 4.6.2. Post-operative animals should be identified with a Post-Procedure cage card.
- 4.6.3. Do not return animals that have not recovered to an animal housing room.
- 4.6.4. Observe the animal until it regains righting reflexes; do not leave recovering animal unattended. Observe respiration and coloration of the eyes (for albinos), mucous membranes, and skin.
- 4.6.5. Prevent heat loss and maintain the animal in contact with a heat source or inside a heated cabinet until it regains righting reflexes.
- 4.6.6. Administer oxygen if necessary.
- 4.6.7. For surgeries exceeding 60 minutes, or if there is significant blood loss, administer an additional 0.2 to 0.5mL/10 g body weight of isotonic fluids subcutaneously.
- 4.6.8. Monitor animals daily for the first 3 days following the surgery and until they fully recover. Monitor and contact veterinary care staff if recovery is prolonged beyond 3 days. Record all supportive care provided.
 - 4.6.8.1. Repeat analgesics post-surgically according to Rodent Analgesia SOP 101.

- 4.6.8.2. Provide food at the bottom of the cage.
- 4.6.8.3. Administer isotonic fluids subcutaneously from 0.2 to 0.5mL/10g body weight.
- 4.6.8.4. Examine the wound daily for signs of inflammation or infection, such as redness, swelling, or purulent discharge.
- 4.6.8.5. Ensure adequate wound closure presence of sutures or wound clips.
- 4.6.8.6. Measure body weight.
- 4.6.9. Remove skin sutures or wound clips after 7 to10 days.

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Approved by the BGU Animal Policy and Welfare Oversight Committee