1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe procedures for rodent stereotaxic surgery.

2. RESPONSIBILITY

Principal investigators (PI) and their staff, veterinary care staff or any individual performing stereotaxic surgery on rodents, or assisting in those procedures.

3. MATERIALS

3.1. Anesthetics
3.2. Non-steroidal analgesic (such as carprofen or meloxicam; see Rodent Analgesia SOP)
3.3. Lidocaine-bupivacaine, 1:1 mixture (local analgesic)
3.4. Sterile 0.9% saline
3.5. Sterile ophthalmic ointment
3.6. Electric razor
3.7. Gauze
3.8. Antiseptic detergent (e.g., chlorhexidine 2% solution or povidone-iodine solution)
3.9. 70% alcohol
3.10. Heating disc, warming pad or warm-water circulating pad (do not use electric heating pads), insulating material (thermal drapes, bubble wrap)
3.11. Sterile surgical instruments
3.12. Sterile gauze and swabs
3.13. Drill, sterile stainless steel screws, sterile cannulae
3.14. Suture material or wound clips (Autoclips)
3.15. Dry bead sterilizer or cold sterilization agents (e.g. glutaraldehyde) and 70% alcohol (as a rinsing agent)

4. PROCEDURES

4.1. Perform pre-operative procedures at a safe distance from the surgical environment in order to prevent contamination with hair.

4.2. Pre-operative Care:

4.2.1. Anesthetize the animal according to Rodent Anesthesia SOP. (Can be done after step 4.2.4)
4.2.2. Apply ophthalmic ointment in both eyes to prevent corneal desiccation. Reapply as needed.
4.2.3. Administer a non-steroidal anti-inflammatory analgesic (such as carprofen or meloxicam as per rodent analgesia SOP)
4.2.4. Administer from 0.2 to 0.5mL/10g body weight of 0.9% sterile saline subcutaneously.
4.2.5. Shave top of the head and remove hair.
4.2.6. Secure animal in the stereotaxic frame.
4.2.7. Place a heat source under the animal or wrap the animal with insulating material (ex: thermal drapes, bubble wrap).
4.2.8. Wash the surgical site with 2% chlorhexidine solution or povidone-iodine solution. Be careful not to wet the animal.
4.2.9. Apply 70% alcohol with gauze or swabs to the head. Be careful not to wet a large area on the animal as the evaporation of alcohol will lead to heat loss.
4.2.10. Apply 2% chlorhexidine solution or povidone-iodine solution with gauze or swabs to the head.

4.2.11. Repeat steps 4.2.9 and 4.2.10 twice.

4.2.12. Surgeon's preparation:
   4.2.12.1. Wash hands.
   4.2.12.2. Wear a surgical mask and a clean gown.
   4.2.12.3. Use aseptic technique.
   4.2.12.4. Wear sterile or alcohol disinfected gloves.
   4.2.12.5. The surgeon must avoid touching non-sterile surfaces.

4.3. Stereotaxic surgery:
   4.3.1. Ensure that all the available materials are at hand.
   4.3.2. Begin surgery with clean and sterile surgical instruments, handle them aseptically.
   4.3.3. Designate a sterile area on the working surface for the sterile material (instruments, suture material, drapes, gauze, etc).
   4.3.4. Prior to surgery, verify depth of anesthesia by loss of animal's pedal withdrawal (toe pinch) reflex.
   4.3.5. Expose the cranium by making a surgical anterior-posterior incision with a scalpel blade (for lesions) or by cutting a circular fold of skin with scissors (for cannula placement).
   4.3.6. Avoid contact of tissues with fingers by using the tip of instruments.
   4.3.7. Apply local anesthetics (mixture of bupivacaine and lidocaine) on the periosteum.
   4.3.8. Reflect the skin, scrape the skull and wipe the skull surface with sterile swabs to remove blood.
   4.3.9. Using a hand-held drill, drill burr holes (up to 2mm in diameter for rats and 1mm in diameter for mice) in to the skull for the insertion of stainless steel securing screws.
   4.3.10. Insert stainless steel screws.
   4.3.11. Make a craniotomy over the target area of the brain.
   4.3.12. Guide cannulae, temporarily fitted with internal cannulae, are inserted into the target area according to appropriate stereotaxic coordinates.
   4.3.13. Dental acrylic is applied around the cannulae, screws and any exposed cranium to secure the cannulae in place.
   4.3.14. When the cement hardens, the internal cannulae are replaced with “dummy” cannulae (obdurators) which are inserted in the guide cannulae to maintain patency.
   4.3.15. A plastic dust cap is placed to protect the cannulae assembly.
   4.3.16. Infiltrate the wound with a local anesthetic, e.g., mixture of lidocaine and bupivacaine, prior to closing the skin. Refer to Rodent Analgesia SOP.
   4.3.17. Skin is sutured with polyamide-nylon, PDS, Vicryl; size: 3-0 or 5-0 or incision can be closed using wound clips (Autoclips); 7mm or 9mm. Sutures or staples must be removed after 10 days.
   4.3.18. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood or debris (let cool completely) or in liquid sterilizing solution (e.g., glutaraldehyde or equivalent) for >5 minutes and then rinse with 70% alcohol. For liquid sterilization, it is recommended to use two alternating surgical kits in order to increase contact time with the solution.
   4.3.19. Dip suture material in 70% alcohol between each animal.

4.4. Surgical Monitoring and Supportive Care:
   4.4.1. Refer to Rodent Surgery SOP.

4.5. Post-operative Care:
   4.5.1. Refer to Rodent Surgery SOP.
   4.5.2. Repeat the administration of a non-steroidal anti-inflammatory analgesic (such as carprofen or meloxicam as per rodent analgesia SOP), every 24 hours for 1 to 2 days.
**Rodent Procedure Log**

**Investigator:**

**Procedure:** Stereotaxic Surgery

**Protocol:**

**Performed by:**

**Instructions:** complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (ex. surgeries, experimental infection). Keep the log in the housing room while active and in your files for 3 years for future review by the Quality Assistant and/or the FACC.

**ANALGESIA**
- **carprofen:** mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- **buprenorphine:** mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- **lidocaine/bupivacaine (local analgesic)**
- **other:**

**ANESTHESIA**
- **isoflurane** 2-2.5%
- **ketamine/xylazine/acepromazine**: mouse: 100 mg/kg (K) - 10 mg/kg (X) - 3 mg/kg (A) IP; rat: 50 mg/kg (K) - 5 mg/kg (X) - 1 mg/kg (A); IP or IM

**OTHER AGENTS ADMINISTERED**

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**Comments/footnotes:**
- *Dose can vary with the sex, the age, the strain, and the body condition of the animal.*

**Revised:** 2014-01-06
ANALGESIA

- Carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- Buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs

Other: ____________________________

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