University Animal Care Committee Standard Operating Procedure		
Document No: 10.11	**Subject:** Rodent Stereotaxic Surgery (Rat)	
Date Issued: March 23, 2011	**Revision:** 3	**Page No:** 1

Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe rodent stereotaxic surgery.

1. **Introduction and Definitions:** A stereotaxic instrument is a device which permits the precise location of a moveable object in space. It enables an investigator to place an electrode or cannula accurately in a small brain structure even though that structure is hidden from view deep inside the skull and brain. This makes it possible to stimulate or destroy such a structure without directly disturbing overlying brain tissue. The basis of stereotaxic surgical technique is that the various regions of the brain have a definite and predictable location with respect to the surrounding parts of the skull.

2. **Materials:**

- Isoflurane
- Xylocaine cream
- Weigh scale
- Analgesics (refer to SOP 10.1 Pain Management in Rats, or AUP)
- Sterile syringes (multiple sizes)
- Sterile needles (multiple sizes)
- PPE (cap, gloves, mask, clean lab coat or surgical gown)
- Standard sterile surgical gloves OR autoclaved nitrile exam gloves
- Clippers
- Polystyrene weigh boats
- 2% chlorhexidine surgical scrub
- Povidone-iodine solution
- 70 % isopropyl alcohol
- Heating pad
- Sterile surgical towels
- Sterile paper towels
- Sterile ophthalmic ointment
- Stereotaxic unit and ear bars
- Sterile drill bits, cannula guide, screwdriver and screws
- Sterile cannulas
- Sterile cannula dummies
- Sterile surgical kit
- Sterile gauze
- Sterile swab applicators
- Suture material

- 0.9% sodium chloride
- Lactated Ringers solution
- Dental acrylic
- Heat lamp
- Antibiotic ointment

3. **Procedures:**

- Use a decontamination spray to clean the drill.
- Prep surgical area according to SOP 10.3 “Aseptic Surgical Techniques (Rat)”.
- Open the surgical packs.
- Line the bottom of the induction chamber with clean paper towel.
- Weigh the rat and place in the induction chamber.
- Connect the isoflurane to the induction chamber and turn on the O_2 flow to a flow rate of 1-1.5L of oxygen/min.
- Turn the isoflurane vaporizer to 4%.
- Once anesthetized, remove from the induction chamber.
- Administer analgesics as per the approved Animal Use Protocol.
- Shave the top of the rats’ head; this should be performed away from the surgical area.
- Administer bupivacaine subcutaneously.
- If the animal is showing signs of regaining consciousness, place back in the induction chamber.
- Apply a small amount of xylocaine gel to the ear bars and position the animal on the ear bars. Alternatively, a small dollop of gel can be applied directly in the ears.
- Open the jaw and move the tongue to the side to avoid its teeth from getting caught on the bite bar. Place the bite bar in between the upper and lower jaws.
- Administer sterile ophthalmic ointment and reapply as required.
- Assess corneal/blinking reflex.
- Test for a pedal reflex to ensure the rat has reached a surgical plane of anaesthesia.
- Don standard sterile surgical gloves or autoclaved nitrile exam gloves using aseptic technique.
- Incise the skin over the skull using a scalpel blade and retract skin ensuring that it does not go beyond the start of the eyes.
- Gently remove the periosteum with the dental scraper.
- Retract the membrane in four locations.
- Wipe the top of the skull with a sterile cotton swab until bregma is visualized.
- Ensure that the left arm of the stereotax is at zero degrees.
- Insert the drill bit, ensuring it is tight and attach the drill to stereotax arm.
- Find the anterior-posterior line (vertical) line of best fit and take the anterior-posterior measurement (bottom, flat bar).
- Find the medial-lateral line (horizontal) of best fit and take the medial-lateral measurement (top, horizontal bar).
- Make your calculations. Place drill where required.
- Drill the cannula hole(s), and 4 screw holes. Gently assess depth with the dental scraper. Drill only once for the screw holes.

- Instill the screws. Hold them with tweezers or haemostats and use the screwdrivers. DO NOT screw them in fully, leave some head space.
- If filtered air is accessible, use an air hose to dry around the screws.
- Use a straight edge to ensure the cannula guides are straight and at 90 degrees.
- Remove the drill and replace with the cannula guide holder. Put the cannula guides on and recheck for straightness.
- Lower the cannula guides until they are in the center of the cannula hole. Place a drop of sterile saline in the cannula hole – slowly lower down to the skull to check placement of cannula guide (liquid should displace).
- Take dorsal-ventral (DV) measurements (the top, vertical bar).
- Calculate the cannula depth from the DV measurement and cannula depth.
- Lower the cannula to the calculated depth.
- Mix the dental acrylic in a weigh boat and transfer slowly to a disposable pipette. The solution should not be too runny as that will increase the exothermic reaction and may potentially cause tissue irritation.
- The scalp must be dry and free of any tissue for maximum adherence.
- Express the acrylic from the pipette and use the small sculptor to form it into a skullcap.
- Ensure all screws are completely covered, and that the cannulas are not protruding excessively. The cannula should have no more than a 1 mm projection.
- Prepare and add more acrylic cement, as necessary.
- Wait 3-5 minutes, or until the acrylic is firm, then withdraw the cannula guide holder.
- Put the pins in carefully.
- Administer analgesics as per approved protocol.
- Administer 5-10mL Lactated Ringers subcutaneously.
- Clean around the skull cap with a swab and sterile saline.
- Remove any acrylic cement from the surrounding skin.
- Apply a small amount of antibiotic ointment around the skull cap with a sterile swab.
- Remove the membrane clamps – cut off any dead skin if necessary.
- Remove the rat from the ear bars. Place in a clean cage lined with paper towel under a heat lamp and recover animals as per SOP 10.4 “Rodent Post-operative Care (Rat)”.
- Record the surgical procedure, injections, and rat identity number on the cage card. Appropriately identify the animal (ear punch, tail markings, etc.).
- All rats must be ambulatory with a strong righting reflex before being left alone in the recovery room.

References:

1) LeMoine, D., Bergdall, V., & Freed, C. (2015) Performance Analysis of Exam Gloves Used for Aseptic Rodent Surgery. *Journal of the American Association for Laboratory Animal Science,* Vol 54, 311–316.

Revised: November 26, 2015 / November 16, 2017 / October 22, 2020