

SOP #: 201.01

Page: 1 of 9

Rodent Surgery (Pre-, Peri-, and Post-Operative Procedures)

The intent of this Standard Operating Procedure (SOP) is to describe procedures for rodent surgery. This SOP is intended for use by qualified personnel on an IACUC approved protocol who will be performing surgery on rodents, or assisting in those procedures.

TABLE OF CONTENTS

1. Introduction
2. Materials
3. Procedures
4. Personnel Safety
5. Animal Related Contingencies
6. References
7. Appendix

1. INTRODUCTION

This SOP outlines the procedures performed when doing survival surgical procedures on rodents. Rodent includes rats, mice and guinea pigs. Section 3c, Surgical Monitoring and Supportive Care, is applicable to both survival and non-survival surgeries.

Definitions

a. Survival Surgery

Any surgical procedure from which an animal regains consciousness for any period of time.

i. Major Survival Surgery

Refers to any survival surgical procedure that:

1. Penetrates and exposes a body cavity;
2. Requires the use of more than a single application of a short-term anesthetic;
3. Or produces substantial impairment of physical or physiologic functions.

Examples of major surgery include laparotomy, castration, ovariectomy, thoracotomy, craniotomy, joint replacement, spinal transection, and limb amputation.

ii. Multiple Major Survival Surgeries

Multiple survival surgical procedures are not permitted on animals unless scientifically justified, and approved by NUS Institutional Animal Care & Use Committee (IACUC)

- b. Non-survival Surgery
Any surgical procedure from which an animal is euthanized before recovery from anesthesia.
- c. Aseptic Technique
Aseptic technique is used to reduce microbial contamination to the lowest possible practical level and includes:
 - i. Preparation of the animal, such as hair removal and disinfection of the operative site;
 - ii. Preparation of the surgeon, such as the provision of clean surgical attire, surgical scrub, and sterile surgical gloves;
 - iii. Sterilization of instruments, supplies, and implanted materials;
 - iv. The use of operative techniques to reduce the likelihood of infection.

2. MATERIALS

- a. Animal support:
 - i. Sterile isotonic solution for injection (e.g., saline 0.9%)
 - ii. Needles and syringes
 - iii. Analgesics
 - iv. Anesthetics (gas, injectables)
 - v. Electric razor or Hydroxide-based hair removal topical cream
 - vi. Supplemental heat source
- b. Animal preparation:
 - i. Antiseptic solution (e.g. povidone iodine)
 - ii. 70% Alcohol
- c. Surgical supplies:
 - i. Sterile surgical instruments
 - ii. Sterilization equipment (e.g., bead sterilizer)
 - iii. Sterile gauze, elastic wraps, drapes
 - iv. Sterile gloves
 - v. Suture material or skin staples

3. PROCEDURES

- a. Preoperative Care

Perform pre-operative evaluations to ensure that the animals are healthy, and disease-free. This should include visual inspection of the animal and assessment of the behavioral status of the animal. The animal should be alert and behaving normally, and should have a smooth coat and clear eyes. Physical or behavioral

abnormalities should be brought to the attention of the Comparative Medicine (CM) veterinary staff.

Withholding food or water is generally not necessary in rodents unless specifically mandated by the protocol or surgical procedure (e.g., gastrointestinal surgery).

In some cases, it may be preferable to initiate antibiotic or analgesic treatment prior to surgery. However, the routine use of antibiotics should never be considered a replacement for proper aseptic surgical techniques.

- i. Administer analgesics according to Rodent Analgesia SOP #104.
- ii. Anesthetize the animal according to Rodent Anesthesia SOP #101.
- iii. Apply bland ophthalmic ointment (Duratears®) to both eyes to prevent corneal drying.
- iv. Administer 0.2–0.5 mL/10g body weight of isotonic fluids subcutaneously for surgeries exceeding 30 minutes.
- v. Remove hair from the surgical area (e.g., with an electric razor, hair removing cream, or by plucking) with a generous border (at least 1 cm) to avoid contaminating the incision site, and remove loose hair and debris from the animal.
- vi. Place the animal in the surgical area.
- vii. Perform three surgical scrubs:
 1. Scrub surgical site with a povidone iodine soaked gauze/cotton.
 - a. Start at the center of the surgical site and move to the outside of the prepared area in a circular manner.
 - b. Do not overlap areas that have been previously scrubbed with the same piece of gauze.
 2. Discard the povidone iodine soaked gauze and use alcohol soaked gauze (70% alcohol) to rinse and remove excess iodine.
 - a. Do not excessively wet the animal.
 3. Repeat scrub and rinse process three times.
 - a. Discard each piece of gauze after each round with scrub or rinse.

Surgeon's preparation:

- i. Wear a surgical mask and a clean gown.
- ii. Wash hands thoroughly with soap and water.
- iii. Use aseptic technique.
 1. Wear sterile gloves.
 2. Avoid touching non-sterile surfaces.
 3. Once the gloves come into contact with a non-sterile surface, they are no longer sterile and the surgeon must re-glove.
- iv. Sterile surgical draping: Whenever possible, drape the animal with a sterile, impermeable covering to isolate the disinfected area.

1. Draping is performed by the gloved and gowned surgeon, in order to prevent contamination of the surgical field.
2. Due to the small size of rodents, there are limitations on the effectiveness and usability of draping during surgery. However draping should both isolate the surgical site while allowing sufficient monitoring of the anesthetized patient.
- v. For minor incisions, drape the surgical site when suturing the wound.

b. Surgical Principles

- i. Designate an area dedicated to rodent surgery. CM facilities have procedure rooms equipped with BSCs for this purpose. Please go to these links to book the procedure rooms:
CELS: <http://lsi.nus.edu.sg/cmpr/>
MD2: <http://lsi.nus.edu.sg/cmpr/md2/>

NOTE: An investigator's laboratory may be used as a survival surgery area provided such use is **scientifically justified by the investigator and the location is inspected and approved by the IACUC.**

- ii. Ensure that all required materials are ready and at hand prior to surgery.
- iii. Begin surgery with clean and sterile surgical instruments.
- iv. Clean and disinfect all surfaces in the surgical area.
- v. Designate a sterile area (typically a sterile drape) on the working surface for the sterile materials (instruments, suture material, drapes, gauze, etc.).
 1. Maintain aseptic conditions during all survival procedures.
 2. Once an instrument comes into contact with a non-sterile surface, it must be re-disinfected.
- vi. Verify depth of anesthesia by loss of animal's pedal withdrawal reflex prior to start of surgery.
- vii. Use efficient surgical planning to decrease surgical time, tissue contamination, and tissue damage.
 1. Handle tissues gently.
 2. Use a scalpel blade or scissors to make the smallest possible incisions.
 3. Use the tip of instruments to handle tissues. Avoid handling tissues with fingers.
- viii. Tissue closure
 1. Close tissue layers separately (i.e. peritoneum/abdominal muscles layer together, and then subcutaneous tissue and then skin).
 2. Subcutaneous tissues can be sutured independently from the skin in order to minimize dead space.
 3. Suture Materials: Refer to Table 1 and Table 2

Table 1. Acceptable Suture Materials

Suture	Characteristics and Frequent Uses
Vicryl®, Dexon®	Absorbable; 60-90 days. Suitable for internal wound closure. Recommended for skin closure if buried.
PDS®, Maxon®	Absorbable; 6 months. Suitable for internal wound closure where extended wound support is desirable.
Prolene®	Nonabsorbable. Suitable for skin closure.
Nylon	Nonabsorbable. Suitable for skin closure.
Stainless Steel Wound Clips, Staples	Nonabsorbable. Suitable for skin closure. Recommended for skin closure if exposed. Requires instrument for removal from skin.

Table 2. Recommended Suture Gauges /Sizes

Species	Location or Function
Mouse	Abdominal or peritoneal area: 4-0 to 5-0 Skin (subcuticular): 5-0 to 6-0 Skin (external): 4-0 to 6-0
Rat	Abdominal area: 4-0 to 5-0 Skin (subcuticular): 4-0 to 6-0 Skin (external): 3-0 to 5-0
Guinea Pig	Abdominal area: 4-0 to 5-0 Skin (subcuticular): 4-0 to 6-0 Skin (external): 3-0 to 5-0

NOTE: Remove skin suture within 10 days of surgery

- ix. Disinfect the instruments between each animal after removal of gross dried blood and debris.
 1. Place in a dry heat bead sterilizer for approximately 5–10 seconds, or spray it with 70% alcohol and let it stand for at least 15 min.
 2. Dip suture material in 70% alcohol between each animal and allow to sit for at least 15 minutes.

c. Surgical Monitoring and Supportive Care

i. Body Temperature Maintenance

1. Surgeries exceeding 15 minutes for mice or 30 minutes for rats require a contact heat source (e.g., warm circulating water blanket, hand warmer) to prevent hypothermia.

NOTE: Never allow the animal to come in direct contact with heat source. Place layer cloth, gauze, or paper towels etc. between heat source and animal.

2. Continually check the animal for signs of hyperthermia or hypothermia by placing hand on animal tissue in contact with or near heat source.
3. Hand warmers can become extremely hot and placing them near an animal or animal cage often provides adequate heat.

ii. Anesthesia Monitoring

Before incision is made, test of rear foot reflexes is made and observed continually the respiratory pattern, mucous membrane color and responsiveness to manipulations throughout the procedure.

Monitor the rodent continually and note the following:

1. Presence of reflexes.
 - a. Toe pinch method- giving the toe/foot a good squeeze. If there is no withdrawal reaction, the animal is anesthetized deep enough to commence surgery. Remember that after this has been done the gloves are not sterile anymore. A sterile gauze pad may be used to protect the sterile gloves.
 - b. Alternatively, a hemostat may be used to squeeze toe/foot. In this case, one must be careful not to squeeze too hard. Remember that after the hemostat has been used to squeeze toe, it is not sterile anymore and must not be used for surgery.
2. Respiratory rate and breathing pattern.
 - a. If respiratory rate becomes too slow or breathing pattern becomes too shallow, anesthesia needs to be lightened by dialing in a lower gas concentration or by reversal of a component of an injectable anesthetic.
 - b. If increased respiratory rate is observed, anesthesia supplement is to be considered.
3. Heart rate (when applicable) during long procedures.
4. Mucous membranes.
 - a. Mucous membranes (MM) are evaluated by the color of the ears and toes. If these become bluish this is an emergency, indicating that the animal does not have enough oxygen. Pink is good and red MM usually indicates that the animal is too warm. This is not likely to occur during surgery but may occur during recovery from anesthesia, especially if a heat lamp is used to keep the animal warm. In such a case, the animal recovering from anesthesia must be protected and the lamp moved.
5. Reaction to surgical manipulation
 - a. If the animal makes any kind of movement in response to incision or manipulation of organs, surgery must be temporarily stopped and anesthesia supplemented.

SOP #: 201. 01

Page: 7 of 9

iii. Anesthetic Complications- in the case of respiratory arrest, stop anesthesia, administer oxygen and gently compress the thorax rapidly. Contact a CM veterinarian or veterinary technician immediately.

d. Postoperative Care

NOTE: Postoperative care begins immediately following surgery and extends up to 10 days or until the incision has healed.

- i. Place the animal in a clean, quiet environment for anesthetic recovery.
- ii. Do not place anesthetized animals in a cage with fully conscious animals.
- iii. If recovering the animal in a cage, place the animal on a clean paper towel in order to prevent aspiration of bedding material.
- iv. Until the animal regains righting reflexes, observe and monitor respiratory rate, red coloration of the eyes (for albinos), mucous membrane color, and skin tent for hydration status.
- v. Keep the animal warm and dry in order to prevent hypothermia.
- vi. Repeat analgesics post-surgically and for the next 72 hours, as per Rodent Analgesia SOP #104.
- vii. For surgeries exceeding 60 minutes, administer 0.2–0.5 mL/10g body weight of isotonic fluids subcutaneously (e.g. Lactated Ringer's or 0.9% saline).
- viii. Examine the wound/incision daily (for at least 5 days)
- ix. Report sick animals as per Reporting Sick Animals SOP #610.

NOTE: For any complications or emergencies contact CM Veterinary staff.

e. Record Keeping

Maintain detailed records of all procedures, medications, complications, and post-operative monitoring. Use Post-Procedure Care cards (purple cards) refer to Appendix A. These cards are available in CM vivaria.

4. PERSONNEL SAFETY

- a. Medical emergencies: call NUH 6779 5555
- b. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonoses, and injury risks. Refer to the OSHE webpage for more information.

5. ANIMAL RELATED CONTINGENCIES

- a. Post contact information for emergency assistance in a conspicuous location within the animal facility.

b. Emergency veterinary care is available at all times including after working hours and on weekends and holidays: **90013073**

6. REFERENCES

- Rodent anesthesia SOP #101
- Rodent analgesia SOP #104
- Reporting Sick Animals SOP #610
- Cornell University
<http://www.research.cornell.edu/care/documents/ACUPs/ACUP201.pdf>
- Boston University <http://www.bu.edu/orccommittees/iacuc/policies-and-guidelines/rodent-surgery-guidelines/>
- NIH OACU http://oacu.od.nih.gov/ARAC/documents/Rodent_Surgery.pdf
- National Academy of Sciences, 2011; The Guide for the Care and Use of Animals, Eight Edition.
- NACLAR Guidelines http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/13557/Attach3_AnimalsforScientificPurposes.PDF (pp.37-39)
- NUS IACUC
<http://www.nus.edu.sg/iacuc/policies%20and%20guidelines/Guidelines%20for%20Rodent%20and%20Bird%20survival%20surgery>
- http://www.research.ucla.edu/rats_help/manual/PoliciesAndGuidelines/PoliciesGuidelines_RMBSurgery.asp
- Marcel I. Perret-Gentil, Principles of Veterinary Suturing The University of Texas at San Antonio , <http://research.utsa.edu/files/larc/PrinciplesVeterinarySuturing.pdf>

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.01	Anna Acuna	17 September 2012	201.01

7. APPENDIX

Appendix A- Purple Card

Date:
 Procedure:
 Analgesic(s)/dose:
 Antibiotic/dose:

Date	Time	√ as appropriate	Initial
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	
		<input type="checkbox"/> Analgesic <input type="checkbox"/> Antibiotic	

Suture removal date:
 Comments:

These cards should be placed by the Investigator staff to notify CM that animals have had a procedure (e.g. anesthetic episode, a surgery). The analgesic or antibiotic dosages should be expressed as mg/kg (w/w) and frequency of administration E.g. once a day (SID) or twice per day (BID). Tick (√) as appropriate and initial in the space provided after each treatment.