Performing Rodent Survival Surgery

**Policy:** Survival surgical procedures in rodents requires aseptic techniques appropriate for the procedure being performed, including an appropriately prepared, dedicated surgical area, sterile instruments, and both animal and surgeon preparation. Anesthesia, analgesia, and peri-operative monitoring must be appropriate for the species and procedures performed, and must be recorded.

**Rationale:** According to the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* (“Guide”), a dedicated facility is not required for rodent survival surgery. However, a dedicated space appropriately managed to minimize contamination from other laboratory activities during surgery must be provided. Surgical procedures must be performed using aseptic techniques. Regulations further state that personnel conducting surgical procedures must be appropriately qualified and trained. In addition to outlining the facility, training, and procedural requirements, regulations/policies also provide definitions for major and minor surgical procedures. The *Guide* (1) defines major survival surgery as any surgical intervention that “penetrates or exposes a body cavity or produces substantial impairment of physical or physiological functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation).” The Animal Welfare Act similarly (2) defines a major operative procedure as “any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions.” Minor surgeries include procedures such a skin biopsies or vascular catheterization/cannulation that do not result in permanent impairment of physical or physiological functions. Laparoscopic procedures may be classified as major or minor depending on their impact on the animal (1).

**Procedures, Guidelines, and Exceptions:**

[Note: “Tables” refers to the document entitled *Recommended Surgical Disinfectants, Sterilants, and Suture Materials.*]

1. **Dedicated Surgical Area**
   A specific area within the laboratory must be identified as the site in which rodent survival surgery is performed; other laboratory functions may not be conducted in this area during the time of surgery. The surgical area must be impervious such that it can be easily cleaned and sanitized (see Table 1). The area should be returned to a level of hygiene acceptable for major survival surgery prior to a surgical session. The animal preparation site should be separate from the surgery area to avoid contamination of the surgical field with hair.

2. **Aseptic Techniques**
   a. **Animal Preparation**
      The surgical site and a wide surrounding area should be clipped (#40 blade or smaller for rodents) and prepared for aseptic surgery in standard fashion. To avoid contamination of the
surgical area, hair removal should be done at another location and the loose hair should be vacuumed or otherwise removed from the environment (tape is a practical and effective alternative for small amounts of hair). Depilatory creams are not generally recommended; there use should be described in the IACUC Proposal. If used, great care must be taken to ensure complete removal to avoid excessive skin irritation.

The surgical site should be prepared by swabbing in a circular fashion from the center (incision site) to periphery without returning the applicator to the center. Nolvasan® (Chlorhexidine) or Betadine® (Povidone iodine) scrub are recommended antimicrobial solutions for surgical skin preparation (see Table 2). The scrub solution should then be removed with sterile water, sterile saline, or alcohol. This should be repeated at least three times. While some recommend a final application of an antimicrobial (chlorhexidine or povidone iodine) solution, it is important to completely remove all scrub (which contains detergents) from the incision site. Use alcohol cautiously; excess volume can reduce body temperature and increases hazards associated with electrocautery.

A non-medicated ophthalmic ointment should be placed in the anesthetized animal’s eyes to prevent drying. In prolonged or very invasive surgeries, warm sterile saline or Lactated Ringers Solution (LRS) should be administered subcutaneously to prevent dehydration (1-2 ml in mice and 5-7 ml in rats).

b. Surgeon Preparation
A clean laboratory coat or scrubs, surgical mask, and hair bonnet/cap should be donned prior to scrubbing hands with an antimicrobial soap. If surgical procedures are performed within a HEPA-filtered cabinet (e.g., Biological Safety Cabinet), then caps and masks may be excepted. Sterile gloves must be worn during surgery; the use of non-sterile gloves may be acceptable if “tips only” techniques are employed.

Following preparation, the surgical site should be protected from contamination by the use of sterile surgical drapes with an opening over the proposed incision site. Anesthetized animals should be positioned for surgery such that the surgical site is readily accessible and yet position does not interfere with the animal’s ability to breathe.

c. Sterile Instruments and Surgical Packs
In general, surgical instruments must be clean and sterile (see Table 3). Steam sterilization of surgical packs, including instruments, drapes and gauze sponges is preferred (3). Sterilization indicators should be used to identify material that has undergone proper sterilization. Absorbent gauze sponges may be sterilized by steam or can be purchased from the Research Resources Center (RRC) in pre-sterilized packets. Instruments or equipment which cannot be treated by heat/steam or chemical agents may be sterilized by Ethylene Oxide (ETO). The RRC and Baxter II Vivarium (B2V) are equipped to perform ETO sterilization. Additional methods of sterilization include the use of dry heat or radiation.

Clean instruments may also be sterilized by a number of chemical agents (e.g., Clidox®, Sterad®), which may be available from the RRC. Sterilization by chemical means often takes several hours.
of exposure to the sterilant so care must be taken to read the label to assure proper use of the particular agent. When instruments sterilized by chemical agents are used to perform surgery, care must be taken to rinse instruments thoroughly with sterile saline or water to prevent chemical-tissue interaction, since many agents are toxic to animal tissues. Alcohol is not a sterilant or a disinfectant and may not be used to disinfect instruments or gloves without specific IACUC approval (4).

d. “Tips-Only” Techniques
Some surgical procedures require very small incisions and little tissue handling. In these cases, when only the tips of instruments will touch the surgical site, “tips only” technique may be appropriate. Surgical skin preparation and clear delineation of the sterile field, e.g., with sterile surgical drapes, is paramount to success using this technique. With attention to detail and handling of the instrument tips, the use of sterile gloves is unnecessary. It may also be acceptable to sterilize only the sections of the disinfected (see Table 4) surgical instruments that will touch the animal or animal’s tissue. In these cases, glass bead sterilizers are particularly useful. Glass bead sterilizers have been found to sterilize instruments in 10 seconds (5) and can also be used to help maintain instrument sterility when performing repetitive surgical procedures in rodents. Allow the instrument to cool after using a glass bead sterilizer.

When using “tips only” techniques, surgical instruments must be touched only on the handles. The sterile tips must not be allowed to touch anything that is not sterile, including non-sterile drapes, non-sterile gloves, or animal skin that is not surgically prepared. Conversely, the animal skin that has been surgically prepared must not be touched with non-sterile gloves or instrument sections. Similarly, sutures, catheters, and other sterilized materials must only be handled via the sterilized instrument tips.

e. Maintaining the Surgical Field
The surgical field, gloves and instruments must be protected from contamination during surgery. Any break in aseptic technique should be immediately rectified. Corrective measures include: 1) the exchange of soiled instruments, gloves, etc. for those which are sterile, 2) the flushing of contaminated tissue with sterile physiologic saline, and/or 3) the use of antibiotics or other prophylactic methods. In general, antibiotics should not be required for procedures of short duration with proper tissue handling and proper attention to aseptic technique. Should antibiotic use be indicated, consultation with RRF staff veterinarians is encouraged.

f. Repetitive Surgical Procedures
Performing the same procedure on a number of animals presents special challenges to maintaining sterile instruments and aseptic procedures. As mentioned above, glass bead sterilizers can be used to rapidly sterilize the ends of instruments between animals as long as all visible organic matter is removed prior to sterilization. As a general rule, glass bead sterilizers should not be used for more than 6-8 animals, depending on the procedure, and only once if intra-gastric or bowel procedures are performed. Segregating instruments during use can also help minimize infection. Instruments can be segregated based on use on a disinfected surface, such as the skin, or use in a sterile area, such as instruments used within internal body cavities...
(6). Performing serial procedures required greater attention to the sterile field. Sterilized instruments or gloves must only touch sterile drapes or incision sites prepared for surgery. Touching with non-sterile instruments or gloves renders an area or site non-sterile. When in doubt, re-sterilize.

g. Suture Materials/Methods
Sterile suture materials must be used for rodent survival surgery (see Table 5). Prepackaged sterile suture with needles attached are advisable and may be available from the RRC. Technical assistance with the selection of suture type, size and placement method is also available. Skin incisions should be closed with well-placed clips/staples or monofilament sutures in a simple interrupted pattern; suture glue may also be used. Incisions associated with the implantation of cranial electrodes should be additionally coated with an antibiotic ointment and may require daily debridement. Skin sutures or clips/staples must be removed when the wounds have healed. Usually this is between 7-10 days, although up to 14 days may be necessary for some procedures. The Project Director is responsible for removing skin suture/staples (i.e., not the RRF).

3. Anesthesia and Anesthetic Monitoring
Animals undergoing surgery must be maintained in a plane of anesthesia that renders the animal insensitive to pain throughout the procedure. Response to touch, tail flick, and toe/interdigital space pinch are tests which can be used to elicit responses in animals which are not fully anesthetized. Appropriate depth of anesthesia must be ascertained prior to skin incision and throughout the surgical procedure. Heart and respiration rates provide other indications of appropriate anesthetic effectiveness. Such reflexes should be closely monitored during surgery. An assessment of anesthetic depth and vital signs, such as respiration rate and depth, mucous membrane color, heart rate, should be conducted at least every 15 minutes. Records of this assessment should be maintained.

The use of the proper anesthetic agents for the species and the study is vital. A number of guides, texts, and references are available and should be used in selecting the appropriate products (see: Recommended Rodent Anesthetics and Analgesics). The RRF Veterinary Care staff should be consulted with additional questions regarding special formulations and use of anesthetic agents and reversal agents. Also please note that the IACUC maintains a policy regarding the use of pharmaceutical-grade medications and expired medical materials. If an animal responds to painful stimulus (withdrawal reflex or surgical manipulation) or if vital signs suggest that anesthetic depth is inadequate (e.g., increased respiratory or heart rate), additional anesthesia should be provided. For injectible agents, this is often accomplished with an additional dose of ¼ to ½ the initial dose.

Normal body temperature must be maintained during surgery. Due to a large surface area-to-body mass ratio, rodents are highly susceptible to hypothermia. A thermostatically-controlled pad employing circulating water or heated air is highly-recommended. Electric heating pads or lamps are not recommended; if used, special care must be taken to avoid overheating the anesthetized animal. This includes use of rectal probes as well as monitoring ambient air temperature at the level of the animal.
In addition to body temperature (which should not exceed 102°F in rodents), careful monitoring of depth of anesthesia and physiological functions and conditions, such as cardiac and respiratory rates and pattern, and/or blood pressure should be performed and documented (1). Intraoperative monitoring should be recorded (see: Rodent Survival Surgery Peri-operative Monitoring form).

4. Postoperative Treatment and Care
The Project Director or a trained member of his/her research staff has the primary responsibility for monitoring animals during the immediate post-operative period. Animals must be closely observed and recovery from anesthesia should occur in a warm cage. This is generally defined as the ability to ambulate and/or maintain normal posture. Following recovery animals may be returned to regular housing. A plan for post-operative treatment must be stated in the “Proposal to Use Laboratory Animals in Research and Teaching.” Post-operative analgesia, as well as any study-specific post-operative regimens, such as specific wound care and indwelling catheter maintenance, must also be described and include drug(s) to be used as well as dose and frequency of administration. The individual(s) responsible for the administration of post-operative therapy and recordkeeping must also be stated and provisions for the use of analgesics must be included in all treatment plans.

5. Peri-operative Recordkeeping
To assist in the oversight process, the date(s) of surgical procedures must be indicated on rodent cage cards. This will not only serve to remind research staff when suture/clip removal is necessary, but also allow monitoring and reporting by the RRF Animal Husbandry Staff. Cage card notation may simply be an inscription such as “Sx – XX/XX/XX,” or may be an indication of the procedure itself (e.g., “Appendectomy – XX/XX/XX”).

Survival surgery on all species requires that written documentation exist to verify that the procedures described in the animal use Proposal are in use, including anesthetic monitoring and analgesic administration. Dates and times (including AM/PM or military time) of all time-sensitive observations or treatments (post-operative evaluations, pain medication) must be recorded. Such documentation may be recorded in individual animal records or a laboratory notebook. If the notebook is not maintained with the animals, then it must be readily available for review by the IACUC, RRF Veterinary Care staff, and regulatory agencies. Records should include notation of all surgical procedures, medications administered, relevant observations, and other peri-operative care procedures. The IACUC has created a Rodent Survival Surgery Peri-Operative Monitoring form that can be used or modified as appropriate.

6. Analgesia and Recognition of Pain
Recognition of pain in animals is usually subjective. Knowledge of the signs of distress and pain in individual species is an important part of conducting research, particularly in studies requiring surgery. Surgical procedures that are known to be associated with pain in humans must also be assumed to cause pain in non-human animals. The proper selection and use of analgesics is an important part of postoperative care. Information on pain recognition and products which can be used to alleviate pain is available from the Research Resources Facilities.

Examples of signs of pain in the mouse are reflex withdrawal, biting response to handling,
piloerection, hunched back, sunken eyes and abdomen, dehydration and weight loss. Key signs of pain in the rat are vocalization, struggling, licking/guarding, weight loss, piloerection, hunched position, and hypothermia. Key signs of pain in the guinea pig are struggling, withdrawal, vocalization, easy restraint or capture, and unresponsiveness. *Note, however, that masking signs of pain, distress, and clinical illness is one of the most conserved instincts in these “prey species”; therefore, it is paramount to pro-actively treat post-operative pain* (see: *Recommended Rodent Anesthetics and Analgesics*).

**Special Notes:**

1) Injectables used during surgical procedures must not be contaminated. Maintaining needles, with or without syringes attached, in rubber stoppered vials is not acceptable. The use of sterile needles and syringes for the parenteral administration of drugs is mandatory. Needles should not be re-capped prior to disposal in an approved sharps container.

2) All drugs, fluids, suture material, etc. used or on-hand for use, must be within the use period allowed by the expiration date indicated on box or label. *Note: When combined into a single formulation, the commonly-employed anesthetic combination of Ketamine HC1 (37.5 mg/ml) and Xylazine (5 mg/ml) should be used within 30 days of preparation.*

3) Rodents do not normally actively regurgitate and hence preoperative fasting food is not mandatory. If, however, food is withheld, fasting should not exceed 12 hours. Water should not be withheld.

4) A strongly-preserved instinct in rodent species is the masking of clinical signs of pain and distress. Please note that IACUC Policy requires the use of post-operative analgesia for at least 48 hours following major survival surgery unless specifically exempted within an approved Proposal.

5) A *Rodent Survival Surgery Checklist* has been assembled to assist the laboratory in ensuring appropriate aseptic technique and peri-operative care.

**References:**


2) Animal Welfare Act; 9CFR, Subchapter A, Parts 1,2,3.


