

*University of Louisville*  
Institutional Animal Care and Use Committee  
***Policies and Procedures***

## **Performing Rodent Survival Surgery**

### **Federal Regulations and Policies**

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 including the use of sterile gloves, masks, sterile instruments. 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 as skin biopsies or vascular catheterization/cannulation.

### **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 surface of the surgical area must be impervious such that it can be easily cleaned and sanitized. The area should be cleaned and disinfected just before beginning a surgical session. The animal preparation site should be separate from the surgery area to avoid contamination of the surgical field with hair.

### **Sterile Instruments and Surgical Packs**

Surgical instruments must be clean and sterile. 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. Clean instruments may also be sterilized by a number of chemical agents (Cidex, Amersel, Alcide), which may be available from the Research Resources Center (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 prevent chemical-tissue interaction since many agents are toxic to animal tissues. To avoid adverse reactions, instruments should be rinsed with sterile water or saline before use. *Alcohol is not a sterilant or high level disinfectant and may not be used to disinfect instruments or gloves* (4).

Instruments or equipment which can not be treated by heat/steam or chemical agents may be sterilized by Ethylene Oxide (ETO). The Research Resources Center (RRC) and Baxter II Vivarium (B2V) are equipped to perform ETO sterilization. Absorbent gauze sponges may be sterilized by steam or can be purchased from the Research Resources Center in pre-sterilized packets.

Additional methods of sterilization include the use of dry heat or radiation. Glass bead sterilizers have been found to sterilize instruments in 10 seconds (5) and can be used to help maintain instrument sterility during performing repetitive surgical procedures in rodents.

Repetitive rodent surgeries present 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. 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). Gloves should also be changed or disinfected between cases.

## **Anesthesia**

Animals undergoing surgery must be maintained in a plane of anesthesia that renders the animal insensitive to pain throughout the procedure. Palpebral response to touch, tail flick, and toe or interdigital space pinch are tests which can be used to elicit responses in animals which are not fully anesthetized. Heart and respiration rates provide other indications of appropriate anesthetic effectiveness. Such reflexes should be closely monitored during surgery.

The use of the proper anesthetic agents for the species and the study is very important. A number of guides, texts, and references are available and should be used in selecting the appropriate products. The "University of Louisville Laboratory Animal Anesthesia and Euthanasia Manual for Rodents and Rabbits," 1997 edition, provides a brief overview of anesthetic agents and techniques. This guide and other references are available from the RRC. *Note:* The anesthetic combination of Ketamine HC1 (37.5 mg/ml) and Xylazine (5 mg/ml) should be used within 30 days of preparation. The RRF Veterinary Care staff should be consulted with additional questions regarding special formulations and anesthetic agents. Also please note that the IACUC maintains a policy regarding the use of Outdated Drugs/Supplies and Pharmaceutical-Grade Medications.

## **Animal Preparation**

The surgical site and a wide surrounding area should be clipped (#40 blade) and prepared for aseptic surgery in standard fashion. Preparation should include a pre-operative scrub with an antimicrobial agent followed by the application of an antimicrobial solution. The surgical site should be swabbed in a circular fashion from center to periphery without returning the applicator to the center. Nolvasan (Chlorhexidine) or Betadine (Povidone iodine) are recommended antimicrobial skin disinfectants. After an alcohol rinse, an antimicrobial solution should be applied to the area. Following preparation, the surgical site should be protected from contamination by the use of sterile surgical drapes. 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.

## **Surgeon Preparation**

A cap and mask must be donned by operator(s) prior to hand/arm scrub. Sterile gloves must be worn during surgery. A clean lab coat is recommended.

## **Intra-operative Procedures**

### ***Body Temperature***

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 lamp or heating pad may be used for this purpose, however care must be taken to avoid overheating the anesthetized animal. An electrical heating pad should be used with extreme caution to prevent burns. A thermostatically controlled circulating water pad is recommended. Careful monitoring of vital signs is required.

### ***Asepsis***

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. *Note: the use of peri-operative antibiotics is an inappropriate substitute for proper sterile technique.* In general, antibiotics should not be required for procedures of short duration with proper tissue handling and aseptic technique. Should antibiotic use be indicated, consultation with RRF staff veterinarians is encouraged.

### **Suture Materials/Methods**

Sterile suture must be used for rodent survival surgery; sterile wound clips/staples may be used in certain situations. Prepackaged sterile suture with needles attached are available from the Research Resources Center. Technical assistance with the selection of suture type, size and placement method is also available. All skin incisions must be closed with well placed clips/staples or non-absorbable, monofilament sutures in a simple interrupted pattern. Incisions associated with the implantation of cranial electrodes should be additionally coated with an antibiotic ointment and may require daily debridement. *Skin sutures, clips, staples must be removed when the wounds have healed.* Usually this is within 7-10 days; the project director is responsible for this procedure.

### **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 postoperative period. Animals must be closely observed and recovery from anesthesia should occur in a warm cage. Following recovery animals may be returned to regular housing. A plan for postoperative treatment must be stated in the "Proposal to Use Laboratory Animals in Research and Teaching." Such a statement may be as simple as "clinical signs of infection or illness will be treated appropriately after consultation with a RRF veterinarian." If a specific postoperative therapy regimen is desired or required, such as specific wound care and indwelling catheter maintenance, it must 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 postoperative therapy and recordkeeping must also be stated and provisions for the use of analgesics must be included in all treatment plans.

To further 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. 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.

### **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. In general, surgical procedures that are known to be associated with pain in humans should 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 in the Research Resources library.

Key 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.

### 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.
- 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.
- 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 never 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*.

### References

- 1) National Research Council, *Guide for the Care and Use of Laboratory Animals*, National Academy Press, Revised 1996.
- 2) Animal Welfare Act; 9CFR, Subchapter A, Parts 1,2,3.
- 3) Bloch, SS, *Disinfection, Sterilization and Preservation*, 3rd Ed. Lea and Febiger, Philadelphia 1983.
- 4) Rutala, W.A., APIC guidelines for selection and use of disinfectants, *Am. J. Inf. Control* **18**(2): 99-117, 1990.
- 5) Callahan, BM, *et al.* A comparison of four methods for sterilizing surgical instruments for rodent surgery, *Contemp. Top. Lab Anim. Sci.* **34**(2): 57-60, 1995.
- 6) Cunliffe-Beamer, TL, Applying Principles of Aseptic Surgery to Rodents, *Anim. Welfare Info. Ctr. Newsletter*, **4**(2): 3-6, 1993.