BACKGROUND

The University of Rhode Island’s Institutional Animal Care and Use Committee (IACUC) is charged with ensuring that all surgical facilities and procedures meet the criteria set by the federal regulations, including the Animal Welfare Act (AWA), the Animal Welfare Regulations (AWR), and the Public Health Service Policy (PHS). The PHS requires institutions to comply with the performance-based standards in the Guide for the Care and Use of Laboratory Animals (Guide).

AWR 2.31 (d)(ix) “Activities that involve surgery include appropriate provision for pre-operative and postoperative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques.”

The purpose of this policy is to clarify the requirements of the Principal Investigator (PI) and the Institution concerning surgical procedures performed on rodents. All investigators, laboratories, and facilities performing surgery (survival and terminal) on rodent species must adhere to the minimum standards addressed in this IACUC policy.

DEFINITIONS

The definition of “survival surgery” applies to any situation where surgical procedures are conducted and the animal recovers from anesthesia, regardless of the anesthetic time interval. The use of good surgical technique to improve experimental outcomes is well established in the reduction of post-surgical complications, including infections, improved survival rates, and a more rapid return to basal physiological state.

Major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, limb amputation).

Minor survival surgery does not expose a body cavity and causes little to no physical impairment (e.g., wound suturing, peripheral vessel cannulation, percutaneous biopsy).

Multiple survival surgeries: the situation in which a single animal undergoes more than one survival surgery (animal receives a surgical procedure, recovers, receives another surgical procedure, and again recovers).

Non-survival/Terminal surgery: any procedure in which the animal is euthanized prior to recovery from anesthesia. Consciousness is never regained after the animal is initially anesthetized.
POLICY AND PROCEDURE

REQUIREMENTS:

A. Planning Prior to Protocol Submission to the Institutional Animal Care and Use Committee (IACUC):
A veterinarian must be involved with the planning of all surgical procedures; this is most effectively done before the animal care protocol is submitted to the IACUC. It is suggested that the initial consultation be held while drafting the protocol. The purpose of this discussion will be to identify the roles of individual personnel and any training requirements; drugs, equipment and other supplies; the location for conducting the surgeries and what provisions may need to be made; pre-operative assessment and postoperative care; and the surgery schedule.

Plans for survival surgical procedures conducted in rodents should include a detailed description for each of the following:

a. Perioperative care and support including pre-operative medications, hypothermic prevention, ophthalmic protection (ointment).
b. Aseptic techniques including hair clipping and skin disinfection.
c. Anesthetics and tranquilizers.
d. Perioperative analgesics and/or anti-inflammatory agents (NSAIDS), as a method of preemptive pain management.
e. Nursing care and/or other treatments.
f. Location where the surgery will be conducted.
g. Qualifications and training of personnel who perform perioperative care and survival surgical procedures in rodents.

B. Responsibilities of Principal Investigators (PI):

a. Assure that all personnel are adequately trained in the anesthesia regimen and techniques, aseptic technique, good surgical technique, post-procedure support during the recovery period and record keeping.
b. Provide appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices. The veterinarian will provide guidance on acceptable practices and procedures.
c. Conduct all survival surgical procedures in a designated surgery area in the laboratory, which is uncluttered and not being used at the same time for other laboratory procedures.
d. Use aseptic procedures for all survival surgeries, regardless of the interval of survival.
e. Conduct a continuing and thorough assessment of the surgical outcomes to assure that the appropriate procedures are followed and potential complications are detected and addressed. In the event of unanticipated outcomes, consultation with the Attending Veterinarian (AV) is followed by taking appropriate corrective action including amending the approved IACUC protocol.

C. Expected Practice for Aseptic Surgery:

a. Attire:
   i. Surgical mask, clean attire and sterile gloves.
b. Instruments:
   i. Instruments must be sterilized (e.g., steam, ethylene oxide or other approved sterilant). If surgery will be performed on multiple animals then the sterilized
instruments must be maintained on an aseptic field and at a minimum “tips only” sterilized between animals.

c. Implants:
   i. All implanted devices, e.g., catheters, osmotic pumps, cannulae, and electrodes must be sterilized; the method of sterilization selected will depend on the composition of the implant.

d. Equipment:
   i. Equipment such as stereotaxic or restraint devices, monitoring equipment, etc. that are required in the surgical field must be disinfected prior to the initiation of surgery and between animals when multiple animal surgeries are conducted.

Any questions regarding aseptic procedures or permissible deviations from aseptic technique must be directed to the AV.

D. Training:

Professional and technical personnel and students who perform anesthesia, analgesia, surgery, and euthanasia must be trained to accomplish these tasks in a humane and scientifically acceptable manner before any surgery can take place. The AV is available to provide assistance with, or training in, aseptic and surgical techniques and the proper administration of anesthesia, analgesia and euthanasia.

_No surgical procedures may be performed on an animal until the IACUC protocol and the surgical location have been approved. All personnel must be properly trained and listed on the IACUC protocol_

E. Procedures:

   a. Pre-Operative:
      i. Conduct all survival surgical procedures in an approved surgery or designated area of the laboratory that is disinfected and uncluttered.
      ii. Attention should be given to maintain a clean operating theater at all times. Therefore, the preparation of the animal (e.g., clipping of fur, intubation, etc.) should not be done in the immediate operating area. There should be a separate but adjacent area where the animal will be physically prepared to undergo a surgical procedure. This area may double as a recovery location after conducting proper cleaning procedures.
      iii. Prepare the surgical site(s) with an appropriate skin disinfectant.
      iv. Surgeons and surgical assistants must wash their hands with an antibacterial soap prior to initiating the surgical procedure. To avoid contamination with aerosols released during scrubbing, the surgeon prep area should be separated from the operating area (Guide).

   b. Operative:
      i. Place an external heat source, such as recirculating water blanket, computer monitored and regulated heating pad or heat lamp or “Deltaphase® Isothermic Pads” (Braintree Scientific) specifically designed for rodent surgery and cover with a clean absorbent pad; to reduce the risk of burns, the heat source should never be in direct contact with the animal. The pad is then covered with a sterile
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surgical drape to define the sterile field; it is critical to maintain sterility of the surgical drape, particularly if you anticipate setting sterile instruments on it.

**Note:** non-regulated heat lamps and commercial household-type heating pads are not permitted during surgery to avoid tissue desiccation and injury. Hypothermia may not be a significant issue with short duration surgery and may not require a supportive heat source; consult a veterinarian.

ii. Anesthetize and maintain the animal in a surgical plane of anesthesia throughout the procedure. Apply a bland ophthalmic ointment (i.e. Puralube® sterile ocular ointment, or equivalent) to coat each cornea to protect from drying or abrasion.

**ANESTHESIA AND ANALGESIA** Contact the Attending Veterinarian for recommendations for appropriate anesthetics and/or analgesics. Anesthetized animals should NEVER be left alone during the procedure.

iii. The surgical site must be covered with a sterile drape after the surgeon has donned sterile gloves. Begin surgery with sterile instruments and handle instruments aseptically. When multiple survival surgeries are planned, it is recommended, when reasonable, to begin with at least (2) sets of sterilized instruments.

1. When using “tips-only” technique, maintain the sterility of the instrument tips throughout the procedure. Note: a pack of sterile instruments can be used on multiple animals, however, it is required that all instruments be sterile prior to the initiation of a surgical session and replaced at a prudent interval to minimize the potential for contamination.
2. When performing a series of similar surgeries keep instruments on an aseptic surface and replace surgical gloves at a prudent interval to minimize the potential for contamination.

iv. Monitor and/or maintain the animal’s vital signs throughout the procedure.

v. Close surgical wounds using appropriate techniques and materials. Proper wound closure is essential to avoid wound dehiscence. Wounds that enter a body cavity must be closed using a multi-layer closure in which the body wall is closed separately from the skin.

**RECOMMENDED GUIDELINES**

A. In the Operating Area

a. The animal must be properly secured to the operating table e.g. tying with gauze, umbilical tape, or other suitable restraint. “Pinning” (use of pins or hypodermic needles) of live animals for survival and non-survival surgeries is **PROHIBITED**.

b. Disinfect the area with appropriate surgical scrub. Iodophors (e.g., Betadine) or chlorohexidines (e.g., Nolvasan) should be used, and alternated with alcohol for three rounds of scrub. Alcohol alone is **NOT** an appropriate disinfectant.

c. Sterile drape should be placed over the animal. Transparent drapes are recommended to continuously monitor breathing. “Press ‘n’ Seal®” wrap may be used in place of a sterile drape. These have been shown to be nearly sterile when taken directly from the packaging, are clear to allow good monitoring and visualization, and help support thermoregulation by creating a sealed barrier around the animal. Using this method, the surgical incision can be made directly through the wrap and animal’s skin.
B. Surgeon and Surgical Assistant(s)
   All personnel taking part in the surgery must:
   - Wear clean lab coat, scrubs, or appropriate disposable gown.
   - Wear appropriate face mask.
   - Wash hands with antiseptic soap.
   - Wear sterile gloves.
   - Move carefully to avoid contamination of the surgical location.

   Gloves must be replaced if aseptic technique is disrupted, e.g., touching the isoflurane vaporizer with the sterile gloves, moving the animal with sterile gloves, etc. With proper planning, simple survival rodent surgeries may be performed by one person. If this cannot be accomplished because of the complexity of the procedure, then to consistently maintain aseptic technique there must be a surgical assistant trained to perform such tasks that would interfere with proper aseptic technique. If it is necessary for the surgeon to leave the surgical area during a procedure, then s/he must re-glove again before resuming surgery.

   a. Instruments and Surgical Materials
      It is extremely important to ensure that all instruments are appropriate for surgery
      i. All instruments must be cleaned and sterilized prior to the beginning of each surgical session. Alcohol alone is NOT a sterilant. Examples of methods of sterilization include steam autoclave, gas (e.g., ethylene oxide), and plasma sterilization.
      ii. Cold sterilization (e.g., Cidex) of surgical instruments must strictly follow manufacturer instructions. The FDA lists specific cold sterilants and the necessary conditions to be considered a sterilant or a disinfectant. Rinse with sterile water or sterile saline before using on an anesthetized animal. For mice and rats, if instruments are to be used for multiple surgeries in a single session, they must be sterilized between animals. Hot bead sterilizers are recommended in these cases.
      iii. For USDA rodents, just as in larger USDA species, a new pack of sterile instruments must be used for each animal. Hot bead sterilizers are not suitable, but consideration may be given to cold sterilization following the manufacturers' instructions. New autoclaved, gas sterilized, or plasma sterilized packs are should be used for each animal.
      iv. Do not use dull or rusted surgical instruments or those not manufactured for surgical use.
      v. The use of expired surgical materials for survival surgeries is inconsistent with acceptable veterinary practice or care and not permitted (NIH OLAW FAQ F.5).

   For any surgical procedure to be successful, steps must be taken to ensure that the animal is properly prepared for surgery, the surgeons are outfitted appropriately, and the surgical instruments are sterilized.

C. Post-Operative
   a. Move the animal to a warm, dry area and monitor and record observations at least every 15 minutes during recovery. Keep the animal warm until fully ambulatory: place the cage partially over a heating pad or alternately use an infrared heating lamp. To minimize the risk of overheating the animal, monitor the ambient temperature at the animal’s level and
maintain at 35 – 370 C or rectal temperature of 38 - 390 C. Return the animal to its routine housing only after it has fully recovered from anesthesia.

b. A sedated rodent should not be placed in a cage with other rodents until it is fairly ambulatory to avoid injury and even cannibalization of unresponsive cage-mates. This can even be an issue with a cage of animals that were anesthetized at the same time since individuals can recover at different rates. Animals can be housed together prior to recovery if they are under continual observation by laboratory personnel.

c. Provide analgesics as appropriate and approved in your IACUC protocol and monitor for indications of discomfort: abnormal posture or movement, inappetence, increased attention to surgical site. Report unusual findings to the AV.

d. Remove skin closures 10 to 14 days post-operatively. (The veterinarian may recommend a longer interval, depending on the nature of the surgery.)

D. Euthanasia
See URI Euthanasia of Research Animals Policy

E. Record Keeping Requirements
The USDA and PHS require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures.

Records must be readily available for review by the IACUC or their designee, the AV, and representative of the USDA regulatory agency. All rodent anesthesia and surgery records must be retained for a minimum of one (1) year.

Records documenting any survival surgery and any anesthetic procedure are to be retained by the lab. The following information must be included in the records:

a. PI name and protocol number
b. Title of procedure performed
c. Species and total number of animals
d. Name or Initials of Surgeon
e. Date of Surgery
f. Name and dose/dosage of all agents administered before, during, and after anesthesia and/or surgery. This includes anesthetics, analgesics, therapeutics, and any experimental agents delivered
g. Any complications (e.g., respiratory distress, bleeding, prolonged recovery or unanticipated mortality) that occurred during or after the procedure.

Records documenting post-operative monitoring and procedures are to be retained by the lab. The following information must be included in the records:

h. Any analgesic used and the dosage.
i. Observations of the post-surgical recovery of the animal; commonly used abbreviations – BAR (bright alert and responsive), are acceptable. The frequency of monitoring must adhere to what is described in your approved IACUC protocol. Typically this is a minimum of once daily monitoring for three consecutive days following the date of the surgery. Post-operative complications such as wound dehiscence or weight loss may require extended monitoring periods which should be documented.
j. Additional comments for any variations from the normal and expected events during the recovery period. This may include any notations on actions taken and the animal's response to these actions as well as any actions taken to alleviate pain and distress.
k. Weights of animals must be recorded if included in the IACUC protocol.

**Note:** This is done for a minimum of three (3) days for all rodent surgeries. If there is any indication that the animal is not doing well, the record must extend beyond this period.

**F. Exceptions**

All planned deviations from this policy must be presented to and approved by the IACUC prior to the performance of the surgical procedure. Emergency situations that involve deviations from IACUC-approved procedures must be reported to the AV and IACUC committee within one week of its occurrence.

**REFERENCES**

http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/iacucguideline-rodentsurgery.pdf

