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1. Introduction
Every research institution receiving funding from federal sources, or regulated by the US Department of Agriculture via the Animal Welfare Act, or accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) must establish and maintain acceptable standards for the care and use of research animals. Among the reference documents which set forth these standards are: the Animal Welfare Act, the Health Research Extension Act, the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, the Guide for the Care and Use of Laboratory Animals, the Institutional Animal Care and Use Committee Guidebook and related policy documents created by accrediting and regulatory agencies. The eligibility of an institution for federal funding, continued AAALAC accreditation, and good standing as a registered research institution with the US Department of Agriculture (USDA) are contingent upon its consistent compliance with the standards set forth in the above documents. Although there are slight differences in the requirements for rodent surgery from those of other species, and for human surgery, the standards that address animal welfare issues, including the appropriate use of anesthesia and analgesia are the same. All individuals performing rodent surgery are encouraged to read Guidelines for Rodent Survival Surgery by Bernal et al (see Suggested Reading section).
2. **IACUC Oversight**

The Institutional Animal Care and Use Committee (IACUC) is responsible for reviewing and approving all activities involving animal subjects, including the oversight of surgical activities. The Attending Veterinarian has specific responsibilities to provide veterinary medical care in these matters. Surgical plans must be clearly outlined in the written IACUC protocol and include post procedural plans for animal care. Significant changes must be approved by formal amendments to the approved protocol prior to their implementation. The IACUC may require demonstration of surgical competence and compliance with these guidelines by requiring that surgery be observed by the DLAR veterinarian or DLAR management. The IACUC will inspect all surgical sites within the research institution during the semi-annual program review and inspection process.

3. **General Considerations for Rodent Surgeries**

Due to their high surface area to body volume ratio, rodents become dehydrated and lose body heat quickly during anesthesia, more quickly than larger species. They have limited fat storage and energy reserves, which contribute to intra-operative hypothermia and post-operative weight loss. Pre-operative fasting of rodents is not necessary and not recommended. Excellent surgical technique and supportive care will help minimize the impact of anesthesia and surgery on the rodent’s overall health.

4. **Aseptic Technique**

Survival surgical procedures must be conducted using aseptic procedures. Aseptic technique is a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by pathogens. This includes proper preparation of the animal, surgeon, instruments, supplies and implanted materials, and proper procedures to maintain sterility and reduce the possibility of contamination. All individuals performing surgery must successfully complete the DLAR Surgery Training requirements.

5. **Surgical Facility/Area**

- In order to perform surgical procedures on rodents at sites outside of the DLAR facility, approval of each site must be obtained from the Attending Veterinarian and the IACUC. The surgery location must be designated in the approved IACUC protocol.
- Surgical standards for rodents in laboratories require a dedicated bench space which is free of other equipment and is sanitizable. It is disinfected prior to the beginning of the surgical session.
- The area is located away from air supply ducts or other drafts to minimize hypothermia of the animal and limit accumulation of dirt and dust on surfaces.
- The area is not used for any other activities during the surgical procedures.
- Access to this area by persons not directly involved in the activities should be limited during surgery.
- No food or drink is allowed in a laboratory.
- Drugs and supplies must be properly maintained and in date. Only pharmaceutical-grade anesthetics and analgesics may be used. (The only exception to this is the use of tribromoethanol (a.k.a. Avertin) for anesthesia in mice. IACUC approval is required.)
- Personnel must wear appropriate rodent surgery attire.
- The use of volatile anesthetics must be accompanied by appropriate fume scavenging devices in order to protect personnel.
- No animals are allowed to remain in a laboratory outside of the animal facility for more than 12 hours without written IACUC approval.
6. Pre-operative Preparation and Planning
   a. Surgical Records
      o Both the USDA and the NIH regulations require maintenance of proper medical records, including information about surgical procedures.
      o The information recorded in the surgical record should include:
         ✓ Procedure date
         ✓ IACUC protocol number
         ✓ Principal Investigator name
         ✓ Names of participants (surgeon, assistant)
         ✓ Animal ID
         ✓ Pre-Surgery information (weight, body temperature, health observations, drug doses, time of induction of anesthesia)
         ✓ Surgical procedure description
         ✓ Intraoperative observations (such as routine checks of anesthetic depth, respiratory rate and quality, body temperature, etc.)
         ✓ Post-operative monitoring observations (routine checks of respiratory rate and quality, rotation of animal position, etc.) until animal is ambulatory/fully recovered
         ✓ Time of recovery from anesthesia
         ✓ Post-Surgical observations (at least twice a day for first 3 days, once before 10 a.m. and then again after 2 p.m., then at least daily until suture or wound clip removal, analgesic doses)
         ✓ Date of suture or wound clip removal (7-14 days after surgery)
      o Records should be retained for 6 months for rodents or the life of the animal for USDA covered species. A copy of the records for USDA covered species must be supplied to DLAR as generated.

a. Anesthesia and Analgesia
   o Anesthesia:
      ✓ Many combinations of injectable and inhalation anesthetics are employed in rodent research. Recommendations are published in the DLAR Rodent Formulary.
      ✓ Follow the anesthetic regimen in the approved IACUC protocol.

   o Analgesia:
      ✓ U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training state: Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals. Surgical procedures require analgesia. (If the nature of the research precludes the use of analgesics, specific permission must be obtained from the IACUC for an exception to this requirement, i.e. “C” Pain Category protocol).

      ✓ Analgesics have optimal effects when they are administered prior to inducing any painful stimuli. With most rodents surgeries this involves administering analgesics after induction of anesthesia, before clipping the incision site and well before the skin incision.

      ✓ Follow the analgesic regimen in the approved IACUC protocol.
✓ Oral analgesics are available and are easy to administer, including self-administered flavored liquids or chewable tablets. It is recommended to acclimate the animals to the flavor of the medications several days prior to surgery to help prevent neophobia.

✓ Rodents exhibit an array of clinical signs as indicators of pain or discomfort. Among the most common ones exhibited are: hunched posture, self-mutilation, anorexia, withdrawn behavior, roughened hair coat, impaired/reduced locomotion, altered sleep/rest cycles, and uncharacteristic aggression.

b. Instruments and Materials
   o Acceptable methods of equipment sterilization:
     ✓ Steam autoclave
     ✓ Gas sterilization (ethylene oxide)

   o If surgeries are performed on multiple animals consecutively, the instruments must be sterilized between animals. After the initial sterilization using one of the above processes, a glass bead sterilizer may be used between each animal.
     ✓ Remove the gross debris from the used surgical instruments using povidoiqine- or chlorhexidine-soaked gauze and a brush as needed.
     ✓ Next submerge the instruments in alcohol.
     ✓ Then place the instruments into the glass beads so that the tips are fully emerged.
     ✓ Time: 15 seconds is required for sterilization. Do not leave the instruments in longer—they will become extremely hot and may injure your hand when removing them from the beads. Use a sterile gauze pad to remove instruments from the beads as a precaution.
     ✓ Place the re-sterilized instruments on the sterile field.
     ✓ Make sure the tips have cooled before using again on tissue as this can produce severe burns.

   o A new sterile instrument pack must be used after every 5 procedures.
   o Material may be supplied as sterile by the manufacturer, so long as it can be introduced to the surgical field in a sterile manner.

   o Chemical sterilants (e.g. "Cidex™", glutaraldehyde) are not recommended as they require extended contact time, must not be contaminated with organic debris, must be followed by rinsing in sterile water as they may cause tissue damage, and proper sterile handling and storage are required after removal from liquid. Manufacturer instructions must be followed to the letter. Alcohol alone is not an adequate liquid sterilant.

   o Plan ahead for replacing contaminated or misplaced instruments and supplies by having an adequate inventory of sterilized instruments on hand in the event of a problem.

c. Animal Preparation
   o Physical exam: Inspecting the condition of a rodent surgical patient prior to sedation helps to identify the presence of disease or other conditions which increase the risks of anesthesia and surgery. This starts as observation of activity and behavior cage-side followed by closer inspection of the animal.
Body weight: in order to calculate drug dosages accurately and to allow for post-operative health monitoring, body weight should be collected prior to anesthetic induction.

Hypothermia has a profound effect on rodents during anesthesia and recovery. All small patients should be supported by warming devices such as circulating water blankets, bubble wrap/other insulation around the patient, and increasing the operating room temperature. Electric heating pads are not recommended. Turn the warming device on several minutes prior to the induction of anesthesia.

An ophthalmic ointment should be used in the anesthetized animal’s eyes to prevent drying of the cornea. This should be done prior to hair removal to protect the animal’s eyes from the clipped hair.

Skin Preparation
✓ Unlike instruments and supplies, the animal’s skin at the incision site cannot be “sterilized”. Proper preparation can minimize contamination of the surgical field with skin microorganisms.
✓ Hair around the surgical site should be shaved with a surgical clipper using a #40 blade (clip “against the grain”). This procedure should be done in a location sufficiently separated from the surgical area to prevent hair contaminating the sterile operating area. Final hair removal may be aided by applying adhesive tape in a patting fashion to the clipped skin.
✓ Remove gross debris from the skin using alcohol before starting the scrub cycle.
✓ Scrub the surgical site with a surgical soap alternating with a rinse for three cycles. Scrub in a spiral pattern starting over the intended incision site and moving outward. A typical scrub would involve use of a povidone-iodine (e.g. Betadine®) or chlorhexidine (Nolvasan®) scrub, followed by a rinse with sterile water or alcohol. Contact time is an important factor in product efficacy with surgical prep liquids.
✓ Care should be taken not to excessively wet the surgical patient as this may promote hypothermia. Small sterile surgical sponges and cotton tipped swabs are useful for rodent surgical preps.

d. Surgeon preparation
✓ The surgeon must don a surgical cap, surgical mask, a sterile disposable gown, and sterile gloves. A new pair of sterile gloves is required for each animal.
✓ Contamination of sterile gloves during a procedure requires their replacement or re-sterilization.
✓ Surgeon and Assistant a.k.a. 2-person method is recommended. An assistant may perform anesthesia, animal prep, and post-op care, while the surgeon performs the procedure. The assistant must wear the appropriate PPE (cap, mask, gown, and exam gloves). Even with this technique, a surgeon will have to re-glove after contamination. It is always helpful to have an assistant, because it is difficult to anticipate everything you will need during a surgery session.

e. Supportive Care
✓ Rodents should receive judicious supportive care and monitoring during anesthesia and surgery. Supportive care includes monitoring both vital signs and analgesia during anesthetic and surgical procedures and throughout the recovery period. Plan in advance for providing appropriate supportive care based on the length and nature of a surgical procedure. The causes of perioperative morbidity
and mortality are usually multi-factorial. Adjusting the many, seemingly small factors associated with a procedure can have cumulative benefits on procedural outcomes.

- Maintain the animal’s physiological status as nearly normal as possible.
- Minimize animal pain and distress.
- Maintain body temperature via insulating materials and supplemental heat sources that do not pose a risk of thermal injury.
- Postoperatively, the animal may experience pain, discomfort, and distress unless treated with analgesics and appropriate supportive measures.
- Rodents should be provided with a source of warmth from the onset of anesthesia to the end of anesthetic recovery.
- Injecting subcutaneous sterile fluids, such as normal saline warmed to body temperature, help to compensate for blood loss during a procedure and depressed fluid intake post-procedure.

7. Surgical Procedure Requirements
   a. Monitoring
      - Monitoring During Anesthesia:
        - Temperature: a digital thermometer with rectal probe should be used for constant real-time body temperature monitoring.
        - Analgesia – toe / tail pinch, eye reflex
        - Respiration – gross changes in rate, character of breathing
        - Observe the color of mucous membrane and skin – blue (poor oxygenation), pale (poor blood perfusion)
        - Vital sign monitors are available for rodents, including pulse oximetry, ECG, blood pressure, and body-temperature-regulated surgical warming pads.
      - Monitoring Post-Procedural:
        - Rodents must be monitored until recovered from anesthesia as indicated by their ability to ambulate and resume normal activities such as grooming, eating, and drinking.
        - Assess appearance, activity, and behavior as indications of pain and discomfort.
        - Assess food and water intake.
        - Provide floor-level access to food and water (pellets and Hydrogel® on the cage floor) post-procedurally especially if stretching overhead for these items may be painful (e.g. neck incisions, laparotomy, etc.).
        - Supplemental moist food may be provided, such as Dietgel®. When applicable, it is recommended to initiate this several days prior to surgery to help prevent neophobia.
        - Assess incision and closure integrity. Measures should be taken to prevent the adherence of cage bedding to incision. The incision site is checked for integrity, discharge, inflammation, or self-trauma. These or other signs of complications will prompt a consultation with the DLAR veterinarian.

   b. Draping/Sterile Field
      - A surgical drape is strongly recommended. Paper drapes are convenient because you can customize the hole to fit the surgical site. Disposable surgical drape material is resistant to tearing when wet, and the blue/green color helps reduce glare from surgery lights.
      - Draping material consisting of povidone iodine-impregnated plastic film, with adhesive backing, can be used in rodent surgeries with good effect.
A common mistake is to make the drape too small. The larger the sterile field you create, the easier you will find it to avoid “breaks” in sterile technique.

A sterile surface for instruments to rest between use may include the drape if large enough, a tray or pan used to sterilize the instruments, the inside of the instrument pack wrapper, the inside of a glove wrapper, or a sterile towel or paper. It is insufficient to sterilize surgical instruments only to place them on a lab bench between uses.

c. **Tissue Handling**

Gentle tissue handling, minimal tissue dissection, and strict adherence to aseptic technique are important for timely wound healing with minimal pain and reduced risk of adverse effects.

d. **Incision Closure Material and Technique**

- **Suture:**
  - The selection of the proper surgical needle design (cutting vs. taper) and size promote competent technique.
  - Swaged on needles cause less tissue trauma than a manually threaded needles.
  - Proper selection of absorbable vs. non-absorbable suture material reduces wound healing complications, including infections.
  - Absorbable suture should be used when closing deep tissue layers (body wall fascia, muscle, subcutaneous tissue, etc.).
    - Vicryl® or Dexon® are recommended for ligation and suturing; absorbed in 60-90 days.
    - PDS® or Maxon® are recommended for ligation and suturing when extended wound support is necessary; absorbed in 6 months.
  - Non-absorbable suture should be used when closing skin.
    - Polypropylene or Prolene® is the suture of choice for skin incisions.

- **Wound clips:**
  - Stainless steel wound clips are available for rodent procedures.
  - 7mm is appropriate for mouse procedures.
  - 9mm is appropriate for rat procedures.

- **Tissue glue:**
  - Is only appropriate for small incisions on areas of the body that experience minimal skin movement (e.g. top of head).
  - Do not apply tissue glue over top of wound clips or sutures.
  - Care must be taken to apply the minimal amount needed to secure the incision closure. Excessive glue may cause tissue irritation and subsequent self-trauma.

- **Skin closure:** do not over-tighten skin sutures or wound clips. The tissue will naturally swell during the healing process. Too much tension in the closure material may cause animal discomfort which will lead to self-trauma and self-removal of sutures or clips.

- **Interrupted suture patterns should be used to prevent occurrence of wound dehiscence.**

- **Closing surgical sites in layers and obliterating surgical dead space help healing and minimizes infections.**

**8. Post-Operative Care**

Place recovering animals in a clean cage on a cage liner placed on top of the loose bedding and place the cage in a warm incubator or on a water circulating blanket.
Recovering animals should not be placed directly on loose bedding. Use caution with supplement heat as hyperthermia can be as detrimental as hypothermia.

- Non-ambulatory animals should not be placed in the same cage with ambulatory animals during recovery.
- Animals should not be returned to the housing room until they are fully recovered.
- Post-surgical analgesia must be provided as stated in the approved protocol.
- Avoid dehydration by administering appropriate fluid therapy before or after the procedure. This may be done by giving 1-2 ml per 100g body weight warm fluids (0.9% saline, LRS, or a 1:1 mixture of 4% dextrose and LRS) by subcutaneous injection. If there was significant blood loss or the animal is slow in recovering from anesthesia, additional fluids may be warranted.
- The research staff member responsible for post-surgical care must monitor the animal at least twice a day for first 3 days, once before 10 a.m. and then again after 2 p.m., then at least daily thereafter until wound clips or sutures are removed (7-14 days after surgery). Daily observations and treatments must be recorded in the animal’s post-surgical record. The incision site is checked for integrity, discharge, inflammation, or self-trauma. These or other signs of complications will prompt a consultation with the DLAR veterinarian.
- External wound clips, staples, and sutures need to be removed when the surgical site is healed, typically 7-14 days after surgery.
- The veterinary staff must be notified if post-surgical complications occur.

9. Quality Assurance/Compliance

- The Guide for the Care and Use of Laboratory Animals, p 105, requires the Attending Veterinarian to assure effective programs of anesthesia and analgesia, surgery, and postsurgical care. When conducted according to the guidelines in this document, rodent surgical procedures should result in a high success rate with few complications.
- Factors which might increase the incidence of problems include:
  - The implementation of new procedures
  - Training of new people
  - Technically-difficult surgical procedures
  - Utilizing rodent strains with compromised genetic backgrounds
  - Additional steps should be taken in preparation of the above circumstances to prevent complications.
- The IACUC and veterinary staff are obliged to assess the adequacy of current practices, and to implement and facilitate changes where necessary. A log (Monthly Survival Surgery Progress Report) must be maintained for surgical procedures, and must be submitted to DLAR by the 7th of each month. The lab should maintain a copy that is made available for inspection by IACUC members, veterinary staff or accreditation visitors on request. The log should include sufficient information to determine outcome of the procedure, including animal subject complications.
- Animals that die during a surgical procedure or post-operatively must be submitted to DLAR for a postmortem examination. This requires notifying the Attending Veterinarian’s office promptly so that the unfrozen carcass of the animal can be examined.
- IACUC Semi-annual Inspection:
- Whether or not surgery is taking place at the time of the inspection, site visitors can determine much about surgery practices by asking appropriate questions of faculty and their laboratory staff. Here are some things you can do to demonstrate compliance.
  - Perform only surgeries for which you have an active, approved animal use protocol.
✓ Train your staff in proper surgical technique. Familiarize them with these guidelines. Document this training, and have the training records available for the inspectors.
✓ Post these guidelines prominently in your lab.
✓ Keep a surgery log. Document surgical complications, or better, the lack of them. Have post-op records available for review.
✓ Convey to the inspector that you know what acceptable procedures are, that all surgeons are trained in acceptable procedures, and that you follow them.
✓ Emphasize those procedures you use to ensure sterility in difficult situations.
✓ Prepare for inspections with a mock inspection. A common question will be "Take me through a typical surgery."


1. Hypothermia
   a. Failure to conserve body heat with warming devices
   b. Too cool room temperature
   c. Using un-warmed fluids and irrigating solutions
   d. Excessive dampening during skin preparation
   e. Long procedure under anesthesia
   f. Open body cavities
   g. Inadequate heat support during recovery

2. Sepsis or wound infection
   a. Using non-sterile materials and instruments
   b. Contamination due to poor operative technique
   c. Rough or excessive tissue handling
   d. Length of procedures increase likelihood of sepsis
   e. Post-op contamination from self-mutilation (inadequate analgesia?)
   f. If post-operative infections become a problem, the first step should be to evaluate the aseptic technique of the operator. Clinical infections should always be reported to DLAR supervisory staff so that specific, individual guidance may be obtained

3. Dehydration and Hypovolemia
   a. Blood loss from excessive sample collections
   b. Surgical blood loss
   c. Evaporation during long surgical procedures
   d. Failure to replace fluid losses:
      e. Humidify oxygen gas with a vaporizer bottle during long procedures

4. Hypoventilation/Hypoxia
   a. Failure to provide supplementary O₂ when indicated

5. Lengthy Anesthesia or Surgery Duration
   a. Length of procedure often correlates with morbidity

6. Repeat dosing with injectable anesthetic agents

7. Surgical Procedural Error

8. Inadequate Analgesic Regimen

9. Inadequate Post-Procedural Monitoring/Care

Summary
  ○ Regulations require establishing and monitoring standards for rodent surgical procedures.
  ○ All surgical procedures must be conducted with the approval and oversight of the IACUC.
Supportive care involves managing pain, maintaining physiological status (conserving body temperature, supporting hydration) and providing wound management.

- Anesthetic agents should be selected and administered on a procedural basis and refined as necessary to assure appropriate doses.
- Rodent surgeries performed outside of the animal facility must be approved by the IACUC and have certain physical and procedural conditions.
- Prepping and draping the rodent surgical patient are important elements of aseptic technique.
- Surgical procedure techniques and outcomes are to be assessed by the Attending Veterinarian and the IACUC.
- Record keeping is required to facilitate surgical outcomes and adequacy of supportive care.
- Surgical deaths are to be reported to DLAR and enable post mortem examinations to be made in a timely manner.
- Multiple surgeries utilizing the same sterile equipment require special care to avoid cross contamination and breaks in aseptic techniques.

**Suggested Reading**


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