Guidelines for Survival Rodent Surgery

Scope: These guidelines apply to all surgical procedures performed on rodents at the NIH in which the animals are expected to recover from anesthesia Prior to performing any survival surgery techniques on rodents, an approved Animal Study Proposal must be in place with descriptions of the surgical procedures to be performed and personnel must be appropriately trained. Specific procedures to accomplish these guidelines can be obtained from your veterinarian.

General: It is important to note that rodents do not vomit, so it is not necessary to fast them prior to surgery (Horn et al. 2013). The following principles described in the *Guide for the Care and Use of Laboratory Animals* apply to rodent surgery.

- Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices are required.
- A designated animal procedure space for rodent surgeries is required; for example, a location within a procedure room or laboratory space free from clutter and easily disinfected prior to the surgical procedure may be used.
- When performing aseptic surgery and related activities, the area should be dedicated to rodent surgery such that cleanliness is ensured and contamination is minimized at the time of use.
- All survival surgery will be performed by using aseptic procedures, including masks, sterile gloves, sterile instruments, and aseptic techniques. Additionally, sterile gloves are preferred for 'tips only' technique.

The *Guide* states that it is important for research personnel to be appropriately qualified and trained in all procedures to ensure that good surgical technique is practiced. Good technique includes:

- Asepsis,
- Gentle tissue handling,
- Minimal dissection of tissue,
- Appropriate use of instruments,
- Effective hemostasis, and
- Use of suture materials and patterns or other wound closure techniques that minimize trauma and remain intact.

Analgesia, preservation of corneal integrity, nutritional support and maintenance of body temperature and hydration should be considered in the surgical plan. The surgical plan should also consider the availability of personnel to provide anesthetic induction, aseptic preparation of the surgical site, and post-operative care appropriate to the surgical procedure. Investigators must assure that the challenges of consecutive surgeries within one work session are adequately addressed.

Procedures:

Personal Protective Equipment:

- Clean jumpsuit or lab coat
- Mask
- Gloves
  - Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.
  - Using clean exam gloves and a "tips-only" technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field.
- Hair cover

Pre-Operative:

- Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery (see Table 1 below).
- After anesthetizing the animal, remove the hair from the surgical site by either clipping, plucking, or the use of depilatories. If a depilatory is used, thoroughly rinse the chemical from the rodent's skin or apply a neutralizing agent.
- Administer analgesics (preemptive analgesia) as appropriate and approved in your Animal Study Proposal.
- Protect the corneas from drying out by applying an ophthalmic ointment since anesthesia abolishes the blink reflex.
- Prepare the surgical site(s) with an appropriate skin disinfectant (see Table 2). If using a stereotaxic frame, the rodent should be placed in the frame *before* the skin disinfectant is applied.
- Surgeons should wash and dry their hands before aseptically donning sterile gloves.
- Nitrile examination gloves can be either autoclaved or gas sterilized as an economical alternative to pre-packaged sterile surgical gloves (LeMoine et al. 2015). Multiple pairs of gloves can be autoclaved in the same pack, but care must be used to avoid contamination of the gloves during donning.
- The same gloves can be worn between surgeries under the following circumstances:
    - The surgeon's gloves have not become contaminated during respective surgeries or
    - The "tips-only" technique is used. Examples of ways to prevent glove contamination are to have another person assist the surgeon by recovering and prepping subsequent animals for surgery, have the surgeon anesthetize and prep all animals having surgery before donning the gloves that s/he will wear during the procedure, etc.
- When feasible, the incision site should be draped aseptically with sterile material prior to making an incision to create a sterile surgical field. Draping is especially important when suture material will be used.
- Instruments, suture material, suture needle, etc. must never touch outside of the sterile surgical field.
- When working alone and manipulation of non-sterile objects (e.g. anesthesia machines, microscopes, lighting, etc.) is required, it may be helpful to use sterile aluminum foil or sterile plastic covers to manipulate the objects.
- Consult with your IC's Animal Program Director to ensure that your surgery practices meet the standards of aseptic surgery.

Operative:

- The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
    - If using the pedal withdrawal reflex to test depth of anesthesia, the rear paw has been shown to be more reliable than the forepaw.
    - If neuromuscular blocking agents (e.g. pancuronium, succinyl choline) are administered then alternative indicators of anesthetic depth must be monitored. Contact your veterinarian for equipment recommendations and information on how to interpret monitoring results. Animals on neuromuscular blockers must be mechanically ventilated.
- Provide an external heat source (preferably a feedback-controlled, infrared, warm water or air-circulating heating device) throughout anesthesia and surgery; contact your veterinarian for information about alternative thermal support devices. Electric heating pads and heat lamps are not recommended because of their potential to cause burns. Hypothermia is a common cause of mortality in rodents undergoing a surgical procedure due to their high surface area to body mass ratio.
- Begin surgery with sterile instruments and handle instruments aseptically (see Table 3).
- When using "tips-only" technique, the sterility of the instrument tips must be maintained throughout the procedure.
- Monitor and maintain the animal's vital signs and hydration.
- Close surgical wounds using appropriate techniques and materials (see Table 4).

Post-Operative:

- Move the animal to a warm, dry area and monitor during recovery. Return the animal to its routine housing only after it has recovered from anesthesia. (i.e. ambulating purposefully in the cage).
- Continue to provide analgesics as appropriate and approved in your Animal Study Proposal.
- If appropriate, consider giving warm fluids and/or nutritional support.
- Generally, remove skin closures 7 to 14 days post-operatively after verifying that the wound has healed.
- Maintain a surgical record with important operative and post-operative information (e.g., annotate cage card with procedure and date, body weight on the day of surgery, analgesic administration, wound closure removal, etc.).
- Continue frequent monitoring of the animal until it is stable (e.g., body weight, body condition, cage activity, etc.)

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APPENDIX

Definitions:

- MAJOR SURGERY: Major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transaction.
- MINOR SURGERY: Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, and most procedures routinely done on an "outpatient" basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of post-operative pain, have minimal complications, and return to normal function in a relatively short time.
- STERILIZATION: The process whereby all viable microorganisms are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if a growth supporting medium is supplied.
- DISINFECTION: The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

Table 1. Recommended Hard Surface Disinfectants (e.g., table tops, non-surgical equipment) Note: Always follow manufacturer's instructions for dilution and expiration periods.

AGENT	*EXAMPLES	COMMENTS
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive.
Quaternary Ammonium	Roccal®, Quatricide®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox ® 10% solution) Chlorine	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	Glutaraldehydes (Cidex® Cetylcide®, Cide Wipes®)	Rapidly disinfects surfaces.
Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants.
Chlorhexidine	Nolvasan® , Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.
Hydrogen peroxide Peracetic acid Acetic acid	Spor Klenz	Contact time 10 minutes.
*The use of common brand names as examples does not indicate a product endorsement.		

Table 2. Skin Disinfectants

Note: Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol can induce hypothermia in small animals.

AGENT	*EXAMPLES	COMMENTS
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7.
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for
*The use of common brand names as examples does not indicate a product endorsement.		

Table 3. Recommended Sterilants For Surgical Instruments & Equipment (i.e. implants and catheters) Note: Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

AGENT	*EXAMPLES	COMMENTS
Steam Sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time, e.g. 121°C for 15 min vs 131°C for 3 min
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast Instruments must be cooled before contacting tissue. *Only tips of instruments are sterilized with hot beads.*
Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time. Appropriate sterilization indicators should be used to ensure
Chlorine	Sterilant Levels of Chlorine dioxide (Clidox®, Alcide®) Sodium hypochlorite (Clorox® 10% solution)	Corrosive to instruments. Items must be clean and free of organic material. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylcide®, Metricide®)	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use. Product expiration dates must be adhered to as per manufacturer's instructions.
Hydrogen peroxide Acetic acid	Actril®, Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
*The use of common brand names as examples does not indicate a product endorsement.		

Table 4. Wound Closure Selection

MATERIAL*	CHARACTERISTICS AND FREQUENT USES
Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)	Multifilament, Absorbable in 60-90 days; 25-50% loss of tensile strength in 14-21 days. Ligate or suture subcutaneous tissues where an absorbable suture is desirable. Not routinely recommended for skin closure due to high capillarity.
Polydiaxanone (PDS®) or, Polyglyconate (Maxon®)	Monofilament, Absorbable in 6 months; 40% loss of tensile strength in 30-42 days. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Polypropylene (Prolene®)	Non-absorbable. Inert.
Nylon (Ethilon®)	Non-absorbable. Inert. General closure.
Silk	Non-absorbable. (Caution: Tissue reactive and may wick microorganisms into the wound, *so silk is not recommended for skin closure*). Excellent handling. Preferred for cardiovascular procedures.
Stainless Steel Suture/Wound Clips/Wound Staples	Non-absorbable. Requires instrument for removal.
Cyanoacrylate (Vetbond®, Nexaband®, Tissue Mend®)	Skin glue. For non-tension bearing wounds.
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Suture gauge selection: Use the smallest gauge suture material that will perform adequately.
Cutting and reverse cutting needles: Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.
Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.