Procedures in Rodent Survival Surgery

Purpose: To ensure that rodent survival surgeries are completed using the basic rules of asepsis, gentle tissue handling, anesthetic maintenance, and proper post-operative care. To ensure that rodent survival surgeries are carried out in accordance with applicable governmental regulations, NIH policies and guidelines, university policies and *The Guide for the Care and Use of Laboratory Animals*[1]. Note that rodent species covered by USDA and the Animal Welfare Act[2] require more stringent documentation and record keeping than rats of the Genus Ratus and mice of the Genus Mus. However, it is incumbent upon the investigator to care for all rodents to minimize pain and distress and to optimize animal care. In addition, good animal care and use will optimize research results and minimize variability, thus making it possible to use fewer animals.

Introduction: Survival surgical procedures in all animals including rodents should adhere to Halsted's Principles of Surgery.[3,4] These include:

- Gentle tissue handling
- Accurate hemostasis
- Preservation of adequate blood supply
- Strict asepsis
- No tension on tissues
- Careful approximation of tissues
- Obliteration of dead space

Aseptic surgical procedures are designed to prevent post-surgical infection due to microbial contamination of the incision and exposed tissues. Aseptic technique results in decreased inflammation and gentle tissue handling results in decreased catabolism and enhances recovery and reduces postoperative complications, Infections in rodents can be sub-clinical, but still affect the behavior and/or physiology of the animal. Prevention of infection improves the welfare of the animal and eliminates a source of uncontrolled variation in the experimental results.

Procedures:

A. Surgical Area:

1. A dedicated surgical suite is not required for rodent survival surgery.
2. An area in the lab or part of a lab bench can be designated to be used only for surgery.
3. The surgical area must be a portion of the room that can be easily sanitized and located in an area with minimal traffic flow. A laminar flow hood may be used but a chemical fume hood is not allowed since it does not protect the sterile field. Prepare your surgery area,

arrange gas anesthesia mask, stereotaxic apparatus, etc. before unwrapping instruments and putting on gloves.

4. Prepare the surgical area by removing all extraneous equipment or other materials.

- Always follow manufacturers' recommendations; clean the area with a disinfectant
    - Alcohols (70% ethyl alcohol, 85% isopropyl alcohol) for 15 min in absence of organic materials or gross contamination.
    - Quaternary ammonium compounds (Roccal, Quatricide) are rapidly inactivated by organic materials and may support growth of gram negative bacteria.
    - Aldehydes e.g. glutaraldehyde (Cidex, Cide Wipes, Cetylcide-G) rapidly disinfects surfaces. Toxic. Follow OSHA exposure limits.
    - Phenolics (Lysol, TBQ) less affected by organic materials than other disinfectants.
    - Sodium hypochlorite (Clorox 10% solution) corrosive, activity reduced by organic matter
    - Chlorine dioxide (Clidox, Alcide) kills vegetative organisms within 3 min, corrosive, activity reduced by organic matter, must be made fresh.
    - Chlorhexidine (Novalsan, Hibiclens) rapidly bactericidal and persistent also effective against many viruses, active in the presence of blood.

5. Use a disposable clean towel or surgery tray to cover the work surface. A heating pad must be placed under the work surface (set at no greater than 40°C) for procedures lasting longer than 20 minutes or for procedures which open a body cavity (i.e., thoracotomy, laparotomy). **Loss of heat can significantly prolong the duration of anesthetic, which in turn increases the risk of complications**. The animal must not be placed in direct contact with the heating pad. A circulating warm water pad is best but other heat sources may be used. Electric heating pads are not recommended due to the danger of burn injuries.
6. Replace all disposable materials after each surgery session.

B. Instruments, Suture Materials, Towels, Gauze Pads and Drapes:

1. All instruments that come in direct contact with the surgical site must be sterile.
2. Steam (autoclave), ethylene oxide or chemical sterilization is required.
3. The instruments, sutures, etc. should be placed in a specially designed pack or wrapped in drapes or cloths, then steam autoclaved. Please label packs with date of sterilization. Any implants should be sterilized; fragile implants may be gas-sterilized or soaked in 2% glutaraldehyde (soak for 10 hours) or other chemical sterilant (not disinfectant) – rinse off copiously with sterile water or 0.9% NaCl before implanting.
4. If performing surgery on more than one rodent, begin with at least 2 sets of sterile instruments.
5. Open the instrument pack and the drape and sponge pack (if wrapped separately.)
6. Use sterile suture, drapes and sponges prepared by autoclaving (or they can be purchased sterile).

7. If the experimental design requires repetitive surgeries (i.e. performing the same surgical procedure on a number of rodents at the same time) proceed as follows between animals:
    - Clean all instruments thoroughly to remove all organic material rinse thoroughly and sterilize instruments. A bead sterilizer is recommended. (Note: alcohol is not a sterilant.) It is recommended that a new set of sterile, autoclaved instruments be used on every five animals.

C. Maintenance of Gas Anesthesia Apparatus

1. Regular or annual inspection and maintenance of the isoflurane vaporizer by a professional contractor is required.
2. The f-air canister saturation must be monitored by either tracking or documenting time used or by increased weight to determine when a new f-air canister should be placed.
3. Whenever possible active scavenging of waste anesthesia gases is recommended.
4. Environmental Health and Safety will determine personnel exposure to isoflurane annually and advise users.
5. An “elephant trunk” anesthesia gas exhaust duct connected to the room exhaust air duct is strongly recommended.

D. Animal Preparation:

Rodents scheduled for survival surgery must have completed the required acclimatization period or been released from quarantine by the Attending Veterinarian.

1. Evaluate prospective rodents to ensure that they are apparently health.
2. Do not withhold food in rodents before surgery unless specifically mandated by the protocol or surgical procedure. Water must NOT be withheld unless it is scientifically justified and approved procedure. Withholding food for longer than six (6) hours in rats or mice must be discussed with a veterinarian and approved by the IACUC.
3. Animal should be prepared in an area away from the surgical area (Note: animal preparation includes anesthetic induction, hair clipping and initial scrub).
4. Induce anesthesia and check anesthetic depth after the required induction time by verifying lack of withdrawal upon firm toe pinch (toe pinch method).
5. After the animal is anesthetized, apply a bland sterile ophthalmic ointment to the eyes to prevent drying, which could result in development of corneal ulcers. (Note: Animals do not close their eyes when anesthetized and they do not blink.)
6. Remove fur from the surgical site using electric clippers with#40 or #50 blade. Avoid the use of depilatory cream or use with caution if required and approved in the IACUC protocol The area to be shaved must be twice that expected for the surgical area or at least 2 – 3 cm of shaved skin on each side of the planned incision, in the event that a larger incision than planned may be required. Hair plucking, in mice, if often easier and more effective than using clippers.
7. Put on clean or sterile gloves and scrub the shaved skin with a chlorhexidine or povidone iodine soaked gauze/cotton. Start from the center of the shaved site (or start from where incision will be) and clean in concentric circles toward the edge of the shaved area.

Discard the chlorhexidine or iodine soaked gauze and use an alcohol soaked gauze (70% isopropyl alcohol) to remove excess chlorhexidine or iodine in a similar fashion as above (starting from the center working towards the edge). **Avoid wetting large areas of fur with alcohol because of the potential to induce hypothermia.**

E. Patient Surgical Scrub:

Move the animal to the surgical area.

1. Do not use the surgical area for any other purpose during the time of surgery.
2. Place animal on a clean absorbent pad, over a heating pad (if appropriate), or in appropriate stereotaxic apparatus.
3. Position with tape. Do not overstretch the legs or bind them in such a way as to restrict circulation.
4. Repeat chlorhexidine/alcohol or iodine/alcohol scrub two more times (as described above in C7).
5. If possible, cover the animal with a sterile (recommended) drape with a fenestration (opening) over the proposed incision site. The drape minimizes contamination of the surgical area and surgical instruments. (To perform sterile draping, the surgeon must already be aseptically prepared including use of sterile gloves).

F. Rodent Anesthesia Monitoring

Anesthetic monitoring of small rodents includes testing of rear foot reflexes before any incision is made, and continual observation of respiratory pattern, mucous membrane color and responsiveness to manipulations throughout the procedure. It is recommended that rectal temperature and heart rate are monitored electronically if possible during long or involved procedures. Monitor the rodent continually, documenting findings every 15 minutes and note the following:

1. Toe pinch method: The toe pinch method to evaluate depth of anesthesia is useful but not enough in itself. One must use two fingers and give the toe/foot a good squeeze. If there is no withdrawal reaction, the animal is judged deep enough to commence surgery. The hind leg is more reliable than the forelimb. A sterile gauze pad may be used to protect the sterile gloves. Alternatively, a hemostat may be used to squeeze toe/foot. In this case, one must be careful not to squeeze too hard. **Remember that after the hemostat or fingers have been used to squeeze toe, they are not sterile anymore. Thus you must change gloves and not use the hemostat within the sterile field.**

2. Respiratory pattern: Anesthesia will cause a distinct slowing of respiratory rate (RR). The surgeon must evaluate if RR becomes too slow and the anesthesia needs to be lightened

and if the depth of respiration becomes too shallow. Increasing RR indicates the need for supplemental anesthesia.

3. Mucous membranes (MM): MM is evaluated by the color of the pinnae (ears) and toes. If these become bluish this is an emergency, indicating that the animal does not have enough oxygen. Pink is good and red MM usually indicates that the animal is too warm. This is not likely to occur during surgery but may occur during recovery from anesthesia, especially if a heat lamp is used to keep the animal warm. In such a case, the animal recovering from anesthesia must be protected and the lamp moved.

4. Reaction to surgical manipulation: If the animal makes any kind of move in response to incision or manipulation of organs, surgery must be temporarily stopped and anesthesia supplemented

G. Surgeon:

1. Wear a clean lab coat or scrub top and remove all jewelry (rings, bracelets, watches) on the hands and wrists.

2. Don a face mask and hair bonnet or cap for all surgeries.

3. Wash and scrub hands with a disinfectant soap, or surgical scrub brush, and dry with clean towels.

4. Wear sterile gloves

5. Change gloves between animals or if they become contaminated.

6. Anything touching the drape or the sterile field must be sterile. If forceps are used to check the toe pinch response, the tips are considered contaminated.

7. Sterile gauze pads may be used to manipulate non-sterile objects.

H. Surgical Procedure: Please recall that only procedures approved in the IACUC protocol can be performed

1. Check level of anesthesia again using toe pinch method.

2. Make the incision using a sharp scalpel or scissors.

3. Check level of anesthesia again using toe pinch method.

4. Control any hemorrhage through direct digital pressure, electro-cautery, or with a hemostat and tying off vessels as appropriate.

5. Using a new scalpel or scissors, incise deeper layers of tissue, such as the abdominal wall. Take care to prevent damage to underlying structures.

6. Perform the intended surgical procedure. Work carefully. Avoid unnecessary crushing of tissues. If tissues are to be exposed for any length of time, they must be periodically lavaged with sterile saline, or covered with saline-soaked gauze.

I. Closure of Incision(s):

1. Close the deeper tissue layers in one layer.

2. Depending on the procedure, a simple, continuous suture pattern with a 3-0 or 4-0 (for rats) or 4-0 to 5-0 (for mice) synthetic absorbable suture may be used or a simple interrupted pattern using natural absorbable (chromic gut) may be used. Note that silk is not appropriate because of its wick function, predisposing to postoperative infections

3. Tighten all knots adequately. Only apply enough strength to the closure to appose tissue edges. Tissue should not be compressed.

4. Close the skin as a separate layer using simple interrupted suture pattern with monofilament non-absorbable suture such as nylon (silk is not appropriate due to wicking and poor tensile strength). Tissue adhesive or staples or wound clips may also be used.

J. For Multiple Surgeries:

1. After the first surgery, clean the instruments by rinsing in saline or distilled water and insert each instrument into a hot bead sterilizer for the recommended time. Be sure to allow time for cooling after immersion in the hot bead sterilizer. If gloves are soiled, change them; if not spray with disinfectant (chlorhexidine or 10% bleach). Follow all above procedures on the next animal. It is recommended that a new set of sterile, autoclaved instruments be used on every five animals. If known contamination has taken place, the instrument should not be reused before resterilization. **Investigators should work closely with the AV to assure that the challenges of consecutive surgeries within one work session are adequately addressed.**

K. Postsurgical Care:

1. Recover each rodent in a separate cage with clean bedding placing the rodent on a clean paper towel inside the cage until it is sternal This is to avoid aspiration of bedding while the animal is still anesthetized.

2. Recover the animal in a warm environment, for example in a clean bedded cage placed over a heating pad, a circulating warm water heater, or chemical pack such as “hot hands” covered with a clean towel. A warm water bottle or warmed saline bag covered with towel or a heat lamp can also be used. Avoid direct contact of rodent with heat source. Use the lowest level of heat possible.

3. In prolonged or very invasive surgeries, administer warmed balanced electrolyte solution such as Lactated Ringers Solution = LRS) given intraperitoneally (IP) or subcutaneously (SC). Administer 0.5 -1.0 ml SC or IP to mice and 3- 5 ml SC or IP to rats. Larger rodent species may be have an indwelling IV catheter placed and receive fluids (LRS) via IV drip during the procedure. Alternatively, SC fluids may be administered at a rate of 4 ml/kg for every hour of surgery. More may be needed if there was much bleeding during surgery. Additional fluids should be given if the animal is dehydrated or not drinking.

4. Monitor the color of pinnae (external ear) or footpad. If the color is too pink, this probably denotes overheating.

5. Check respiration rate and depth every 10 to 15 minutes, until they have recovered their balance and can right themselves. The animal must not be left unattended until it has recovered and is able to remain upright in a sternal position.

6. Report any complications VS. The veterinarian must be consulted if recurring problems are not resolved.

7. The animal must be monitored daily for a minimum of 72 hours and up to one week following surgery, assessing such parameters as appetite, and wound healing. Administer analgesics and other drugs as stipulated in the protocol or as recommended by the veterinarian using the rodent post procedure monitoring sheet to document care during recovery.

8. If during the next few days after surgery the animal(s) appear lethargic, or do not appear to be eating or drinking, or seem painful, reevaluate condition. If indicated repeat IP or SC fluid administration and analgesics and consult veterinary staff

7. Remove skin closure materials 7-10 days post-surgery.

L. Records:

1. Keep appropriate and complete records of the surgical procedure, anesthesia and pre- and post-operative care including dose, route and time of administration of analgesics and antibiotics administered. All record notations must be signed/initialed and dated. The IACUC recommends using the linked forms to be used for such records. All records must provide the same level of detail included in the linked forms and must be kept in one easily accessed centralized location that is contiguous to the animal housing location.

2. Surgery on USDA regulated rodents (i.e., gerbils, hamsters, guinea pigs, chinchillas) requires maintenance of more extensive records (please consult with VS for the appropriate form).

3. In addition, the cage of the animal(s) should be marked to indicate that a surgical procedure has been performed.

REFERENCES:

1. National Academy of Sciences, 2011; The Guide for the Care and Use of Animals, Eighth Edition. 2011
2. United States of America Code of Federal Regulations (7 USC 2131-2159), Animal Welfare Act(1970,1976,1985,1990,2002,2007). http://awic.nal.usda.gov/nal_display/index.php?info_center=3&tax_level=3&tax_subject=182&topic_id=1118&level3_id=6735&level4_id=0&level5_id=0&placement_default=0
3. Forman, L.A., 2000; Rodent Surgery guidelines, Northwestern University, Chicago, IL.
4. William Stewart Halsted, MD 1852 -1922, a very influential American surgeon who emphasized hygiene, was an early champion of newly discovered anesthetics, and introduced several new surgical procedures.