INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE HOWARD UNIVERSITY
POLICIES AND PROCEDURES

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 Rattus 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: However, an area in the laboratory or part of a laboratory bench can be designated to be used only for rodent surgery.

2. The surgical area set aside in the laboratory must be a portion of the room that can be easily sanitized and located in an area with minimal traffic flow. As an enhancement (but not mandatory) a laminar flow hood may be used for survival surgery but a chemical fume hood is not suitable for survival surgery since it does not protect the sterile field. Terminal procedures such as perfusion can be carried out in a fume hood (stipulated safe for use with the perfusate to be employed)

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

4. Clean the surgery area with a suitable disinfectant: Always follow manufacturers' recommendations

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

5. Before commencing surgery set up the surgery area with all equipment and supplies needed before unwrapping instruments, donning sterile gloves, etc. Use a disposable clean towel or surgery tray to cover the work surface.

6. **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. Thermal support (37° C) must not overheat animals (i.e., > 40° C, with 42° C resulting in thermal injury). Heat lamps, contact devices, etc. are less desirable; while reflective foil may be equivalent to nothing at all and you cannot see the animal; hot pockets are variable in terms of heat uniformity and electrical pads can short out. Homeothermic body blankets with rectal thermometers are good; as well as warm air blower and pediatric incubators. Tecniplast produces a heat recovery rack unit that accommodates multiple cages at a time. The heat source should be placed under the work surface (again 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). Electric heating pads are not recommended due to the danger of burn injuries. 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. Steam (autoclave), ethylene oxide or chemical sterilization is required. 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% saline before implanting.

2. If performing surgery on more than one rodent, begin with at least 2 sets of sterile instruments. Open the instrument pack and the drape and sponge pack (if wrapped separately.) Use sterile suture, drapes and sponges prepared by autoclaving (or they can be purchased sterile). If the experimental design requires repetitive surgeries (i.e. performing the same surgical procedure on a number of rodents during the same surgical session or time frame) proceed as follows between animals: Clean all instruments thoroughly to remove all organic material followed by rinsing thoroughly and sterilize instruments for reuse. Alternatively, a hot 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. Active scavenging of waste anesthesia gases is required.

3. The f-air canister saturation must be monitored by tracking date and weight after use to determine when a new f-air canister should be placed. An "elephant trunk" anesthesia gas exhaust duct connected to the room exhaust air duct is strongly recommended.

D. Animal Preparation:

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

2. Evaluate prospective rodents to ensure that they are apparently in good health.

3. Do not withhold food in rodents before surgery unless specifically mandated by the protocol or surgical procedure. Water must also NOT be withheld unless it is scientifically justified. Withholding food for longer than six (6) hours in rats or mice must be discussed with a veterinarian and approved by the IACUC.

4. Animals should have a baseline weight taken prior to surgery. Animals should be prepared in an area away from the area designated for surgery (Note: animal preparation includes anesthetic induction, hair clipping and initial scrub).

5. Induce anesthesia and check anesthetic depth after the required induction time by verifying lack of withdrawal upon firm toe pinch. Performing a forelimb withdrawal reflex assessment preferable to a hind limb withdrawal reflex assessment.

6. After the animal is anesthetized, apply a bland sterile ophthalmic ointment (such as Paralube 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. Studies indicate that ketamine can stop tear production for up to 12 hours post operatively and has produced 42% keratitis in one case. Sterile ophthalmic lubricant should be used for all anesthetized rodents. These precautions also apply to nonsurgical procedures requiring anesthesia.

7. 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. After clipping the animal most use three wipes with Betadine and warm saline. The presenter preferred swabs to 4 x 4's (gauze) and 6 cycles of wipes with chlorhexidine. Alcohol is often avoided due to its chilling effect. Chlorhexidine swabs are commercially available.

8. 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 starting at the center and going toward the edge of the shaved area. Discard the chlorhexidine or iodine soaked gauze and use sterile warmed saline soaked gauze (rather than alcohol that may have a cooling effect on the Animal).starting from the center and working towards the edge of the shaved area.). **Avoid wetting large areas of fur with alcohol because of the potential to induce hypothermia.**

E. 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.

F. 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 C8).

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). **Glad Press and Seal Grad wrap is a recommended drape material.**

.

G. Rodent Anesthesia Monitoring

1. Perioperative assessment should include visual examination, body conditioning scoring (BCS), and assessment of the respiratory rate, etc. Good monitors include pulse oximeters, temperature monitors (esophageal probe instead of rectal probes is the trend), Doppler blood pressure measurement devices and use of multiparameter monitoring devices.

2. Anesthetic monitoring of small rodents includes testing of forelimb and hindlimb 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 be monitored electronically if possible during long or involved procedures. Monitor the rodent continually, documenting findings every 15 minutes and note the following:

    a. 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 may be more reliable than the forelimb but all may be pinched. 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 no longer sterile. Thus, you must change gloves and not use the hemostat within the sterile field.**

    b. 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.

    c. Evaluation of the color of the pinnae (ears), mucous membranes and toes. If these become bluish this is an emergency, indicating that the animal does not have enough oxygen. Pink is good and red 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 the animal is too warm. In such a case, the animal recovering from anesthesia must be protected removed from the heat source and the temperature lowered appropriately moved. The greatest danger facing rodents recovering from anesthesia is hypothermia.

    d. 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.

H. Surgical Procedure: 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 sterile saline-soaked gauze.

I. Closure of Incision(s):

1. Close the deeper tissue layers in one layer. 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.

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

3. 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:

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:

Animals must be monitored until they are ambulatory. Thereafter they should be observed daily for 3 to 5 days and the body weight checked. For surgical procedures lasting more than 10 minutes long SQ or IP fluids should be given that has been warmed to room temperature. Nutritional support should be sure to be received within 24 to 32 hours postoperatively, and when applicable, postprocedurally; with or without analgesia. If a novel nutritional supplement is to be given it should be presented to the animal preoperatively and can contain a preemptive analgesia

1. Recover each rodent in a separate cage with clean paper towel or other absorbent material inside the cage until it is in sternal recumbency. 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 cage placed over a safe heat source such as 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 can also be used. Avoid direct contact of the rodent with heat source. Use the lowest level of heat possible and the heat source can be positioned to emit heat to two-thirds or half of the cage bottom so that the recovering animal has the option to move away from excessive heat.

3. In instances of 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. For larger rodent species, an indwelling IV catheter can be used 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 given if required to compensate for excessive 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. During the next few days after surgery if the animal appears lethargic, or does not appear to be eating or drinking, or seems painful, reevaluate its condition. If indicated, repeat IP or SC fluid administration and analgesics and consult the veterinarian.

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

L. Records:

Keep appropriate and complete records of the surgical procedure, anesthesia and pre- and post-operative care including dose, route and date (+/- time) of administration of analgesics and antibiotics administered. All record notations must be signed/initialed and dated. Surgery performed 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).

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

REFERENCES:

1. PRIM & R Seminar March 2017, New Orleans, LA
2. National Academy of Sciences, 2011; The Guide for the Care and Use of Animals, Eighth Edition. 2011
3. 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
4. Forman, L.A., 2000; Rodent Surgery guidelines, Northwestern University, Chicago, IL.
5. 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.