Regulations and Guidelines

The Animal Welfare Act states: All survival surgery will be performed using surgical gloves, masks, sterile instruments and aseptic techniques. Non-major surgery and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic technique, and defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions. The Guide for the Care and Use of Laboratory Animals states: The relative susceptibility of rodents to surgical infection has been debated; available data suggest that subclinical infections can cause adverse physiologic and behavioral responses (Bradfield et al 1992; Cunliffe-Beamer 1990) that can affect both surgical success and research results.

Justification for Applying Aseptic Technique in Rodent Surgery:

Importance of maintaining asepsis: Although mice and rats have been touted as being resistant to post-surgical infections, the literature contains numerous articles that documents how subclinical infections, such as Pseudomonas aeruginosa, Corynebacterium kutscheri, mouse hepatitis virus, or Spironucleus muris can become clinical diseases following stress or immune suppression (Foster, et al., 1982). Historically, researchers have performed surgery in rodents non-aseptically. However, the experimental evidence has been obtained to suggest that infections take a subclinical profile in rats and mice. Improvement is seen in post-op recovery by increasing food/water consumption due to implementing aseptic surgical technique. (Cunliffe-Beamer, T.L., 1972-73. Cunliffe-Beamer, T.L. Biomethodology, 1983) Experimental induced wound infections in rats were not associated with gross clinical or obvious behavioral signs. (Bradfield, Schachtman, McLaughlin, Steffen. Behavioral and Physiologic Effects of Inapparent Wound Infection in Rats, 1992, Lab. Animal Science, 42 (6), 572-578. Errata, Vol 43 (2), 20, 1993.) It is unsafe to assume there is anything special, in either way, about the resistance of rodents to infections. Rodent models have been used for antibacterial research in which rodents have been used to model human bacterial diseases, including surgery related conditions. This fact would suggest that there might be differences between rodents and other mammalian species, including humans, in the development of infections, including post-surgical infections (Morris T., Laboratory Animals 1995 vol. 29 page 26).

Definitions

Asepsis: A condition in which living pathogenic organisms are absent; a state of sterility.

Aseptic surgery: The performance of an operation with sterile gloves, instruments, etc., and utilizing precautions against the introduction of infectious microorganisms from without.

Special Considerations

Rats and mice have a high surface area to body volume ratios and rapid metabolism.

- With high metabolic rate and limited fat storage, energy depletion can be stressful.
- Pharmacological doses are higher than in larger species.
- Dehydrate faster per unit of time
- Rats and mice lose body heat rapidly through hairless areas. Hypothermia during surgery is a frequent cause of intra operative mortality.
- Higher cost in time- 1 hour surgery is equivalent to 4 hours in a cat.

Surgical Stress:

The major responses to surgery are characterized by an elevation in plasma concentrations of catecholamines, corticosterone, growth hormone, vasopressin, renin, aldosterone and prolactin, and by a reduction in plasma concentrations of FSH, LH and testosterone. Plasma insulin and glucagon concentrations fluctuate. These hormonal responses to tissue trauma produce an increase in glycogenolysis varies, but after
major surgery the response may persist for 4-6 hours. More prolonged changes in protein metabolism occur leading to negative nitrogen balance lasting for several days. Even minor surgical procedures can produce prolonged effects. Minimizing tissue trauma, preventing infections, controlling post-surgical pain and discomfort, and supporting the animal’s nutritional needs will reduce the magnitude of the metabolic response to surgery. The purpose of a survival surgical procedure is to produce an animal model that is defined and that has the smallest degree of non-treatment variability. An important objective is to return the animal to physiological normality, or to a defined state of abnormality, as rapidly as possible.

**Preoperative Preparation:**
- Assess health status. Recommendations: 1-2 weeks of acclimation to animal facility, to overcome the stress of transportation.
- Should be free of clinical signs of disease. The appearance should include normal posture and movement, glossy coat, bright eyes. Assess the character respiration (no sneezing, coughing or unusual respiratory sounds) and the cardiovascular status (bright pink coloration of ears and mucous membranes, albino animals). Normal intake of food and water should occur. For a review of transport stress, see van Ruiven R. Meijer GW, et al. Adaptation period of laboratory animals after transport: a review. Scand J Lab Anim Sci. 23(4) 1996 pp 185-190. Using various parameters, the minimum adaptation period of several species are listed, for example 12 hours to 3 weeks in rats.
- Fasting is generally unnecessary. Because rats and mice do not vomit, they do not have the risk of intra/post-op vomition as in other species. If you will perform surgery on the gastrointestinal tract, you can fast the animals, but briefly (a few hours). However, the reason for doing it should be considered carefully and weighted against the perturbation of normal metabolic processes needed for homeostasis. For example, starvation will not empty the stomach unless it is for more than 24hr, but it will seriously deplete glycogen reserves in the liver. (Behavioural and cardiac responses to sudden change in environmental stimuli: effect or forced shift in food intake, Steenbergen JM; Koolhaas JM; Strubbe JH; Bohus B. Physiology and Behaviour 45, 729-733. Also Vermeulen JK, Vries de A, Schlingmann F & Remie R, (1997) Food deprivation: common sense or nonsense, Animal Technology, Vol. 48, No 2, pg 45-54).
- Support normal body temperature during anesthesia. Rats and mice have high surface area and lose body heat rapidly by conduction. A major cause of surgical mortality is not the surgery or the anesthesia but hypothermia. Body temperature drops precipitously under sedation or anesthesia. Low body temperatures can cause irreversible shock and death. Place the animal on insulating materials (e.g. bubble wrap or folded drape). Provide heat source- but avoid overheating.
- Animal positioning: If limbs must be positioned for control of the surgical field, avoid placing excessive tension on the limbs, which may cause neural damage and shut off circulation. Use tape to hold down the limb(s) for positioning. Remember the animal indicates that it may be becoming light by limb movement. Also, avoid stretching the limbs into an unnatural position, which may traumatize joints as well as impair breathing. If limbs must be tied down, apply strips of white tape around the hind and forelimbs. You can also tie a length of tape over the back, from carpus to carpus, to stabilize the forelimbs and torso.
- Clip fur along the incision site. Clip a generous area to ensure fur does not contaminate the wound and a sufficient area that can be disinfected around the incision site. But avoid taking off too much fur, because this will reduce the animal’s ability to regulate his body temperature. Use the sticky side of tape to lift off the loose fur.
- Antiseptic preparation of surgical site: Alcohol is an ineffective antiseptic, use it to remove debris. Use tamed iodine or chlorhexidine solution. Apply antiseptics with a swab or sponge (moist, not dripping). Never use a squeeze bottle to apply disinfectant. A dripping sponge or a squeeze bottle can drench the whole body,
which lowers the animal’s body temperature.

- Draping is necessary when viscera or sterile instruments may come in contact with unprepared skin and fur. Options are: a) surgical paper drapes - cut fenestration for incision. They are inexpensive, autoclavable. b) Transparent, self-adhesive drapes, provided that the animal’s body is dry (use sterile gauze to pat dry prepared skin).
- Never use the anesthetized animal’s body as a table, do not rest your hands or instruments on the chest or abdomen. External pressure interferes with respiration and blood circulation.
- Endotracheal intubation: for rats use a 14-18 gauge venous catheter, trimmed to the length between the nose and thoracic inlet. See FLSC veterinary staff for guidance.

**General Preparation Surgery**

Some characteristics of common laboratory rodent surgery are smaller incision sites, fewer personnel in the surgical team, manipulation of multiple animals at one sitting, and briefer procedures as opposed to surgery in larger species. Modifications in standard aseptic suggestions for dealing with some of the unique challenges of rodent surgery have been published (Cunliffe-Beamer 1983-1993).

**Site**

The elaborate operating suites mandated by the NRC Guide for larger species are not necessary for rats and mice. What is necessary for survival surgery in these species is:

1) A clean, neat, disinfected area dedicated to rodent surgery for the duration of the procedure.
2) A separation of functions of animal prep, operating field and animal recovery. These may be adjoining areas on a long bench top. The rationale is to avoid contaminating the operating field with loose animal fur, splashes from incision site scrubbing, and bedding dust and fur from nearby cages.

**Instruments**

Surgical instruments must be autoclaved. If performing batch surgeries, i.e. using the same instruments on a series of animals, wipe them clean with 70% alcohol and re-sterilize the instrument tips between animals. You may need two sets of instruments to alternate use between animals. Bead sterilizers are preferred - allow instrument tips to cool before applying to animal’s tissues. Chemical sterilization such as glutaraldehyde must be followed by a saline rinse. If you are doing a full day of batch surgeries, then use a fresh set of autoclaved instruments for the morning and the afternoon series.

**Surgeon**

Use sterile surgical gloves, not exam gloves. If performing batch surgeries, rinse your gloved hand or fingers with chemical sterilant followed by saline rinse. Don’t forget the saline rinse for the gloves. Sterilant residues on gloves will be irritating to tissues and will increase the risk for local infection. Dry with a sterile towel. Put on new sterile gloves when these become uncomfortable, torn or punctured. An assistant should be available to anesthetize and prep the rodents for the surgeon to minimize contamination of the surgeon. That allows you to keep your gloves sterile between surgeries.

**Maintaining Asepsis (ungowned)**

1. Gloved hands should be held elevated above the waist and below the shoulders and should touch only the surgical incision and sterile objects, i.e. sterile instrument tray, sterile drape.
2. Once gloved, do not touch or lean over a non-sterile area. Do not drop your hands to your sides. Do not touch gloves to your skin or cloths.
3. Always lift an instrument from a sterile pouch or sterile surface. Do not drag instruments over the pack/drape edges because they can become contaminated.
4. Do not allow surgical instruments to fall below the edge of table. If an instrument does fall, the instrument is considered non-sterile and should not be picked up and reused until re-sterilized.
5. Sterile surfaces are to be kept dry. Moisture can lead to contamination of the surgical area.

**Intra-operative Analgesia:**

Anesthesia is a state where all perceived sensations are absent. Because drug effects can vary, you must
assess the depth of anesthesia prior to beginning a painful procedure such as surgery. The depth of anesthesia and the level of analgesia must be adequate to prevent the animal from feeling any pain in response to a surgical stimulus. Before making an incision, squeeze a paw firmly, but without injuring it, to test the animal’s perception or sensation of pain. If the animal withdraws its leg or if respiration rate increases, then the anesthesia is too light. Assess how much time elapsed from administering the anesthetic and compare that to the expected time of peak effect. You may have to wait longer for the anesthetic to take effect. Or, if surgery was delayed, the anesthetic may have worn off. If neither of these time factors may account for the inadequate anesthesia, it is possible that you may have to use a higher dose rate of your anesthetic.

**Special Issues:**

**Preemptive Analgesia:**

Preemptive analgesia is the prevention of pain before it occurs. As an adjunct to general anesthesia, a local anesthetic is used to desensitize a body area before making an incision. This reduces the pain of the surgical wound postoperatively and in healing. Much of the post surgical pain is the result of the sensations produced in the skin and body wall of the incision area before making an incision. This reduces the pain of the surgical wound postoperatively and in healing. When the skin and tissue are incised, local sensory nerves become excited and transmit impulses to the brain that are interpreted as pain. During general anesthesia, the animal is unconscious and is unable to perceive the neural stimulations from the incision site and so is unaware of painful sensations. However, when the anesthetic has worn off, the brain will process these neural excitatory impulses, which continue postoperatively for days until the incision is healed. The result is that the surgical wound is painful and sensitive to touch and movement. If a local anesthetic is infiltrated prior to the incision, it will block the sensory neuroexcitation caused by cutting the tissues. When the animal wakes up, he will have a reduction in sensory stimuli from the incision area, and pain of the surgical wound will be greatly decreased both initially and throughout the period of wound repair. Inject a local anesthetic subcutaneously to infiltrate it in the vicinity where the incision will be made. Allow a few moments for it to diffuse and take effect before beginning the surgery. Lidocaine can be used, but it is short acting and will provide no lasting effects. Inquire about local anesthetics and post-op analgesics from the FLSC Veterinary Staff.

**Intra-operative Care**

During anesthesia and surgery, assess and provide support for body temperature, respiration and cardiovascular function. In rodents, elaborate instrumentation is not necessary. For monitoring respiration and circulatory function, you can periodically observe the animal’s appearance for signs of problems. Respiratory Problems - the animal turns “blue” (hairless areas) if hypoxic. Evaluate the need for delivering oxygen - no special equipment is required. A tube delivering oxygen from a tank (turned to low flow) can be taped onto the table in the vicinity of the animal’s nose. Alternatively, a face mask may be made from a syringe case or syringe barrel. Maintain airway patency. Be careful in positioning the animal’s head and neck. Prevent blockage of the respiratory passages by blood, mucus, other material. If respiration rate falls progressively (40-90 breaths/ minutes acceptable): If surgery is in progress, assist ventilation by gentle compression of the chest at a rate of 1 breath/ second. If surgery is complete, administer an anesthetic antagonist (if appropriate) or a respiratory stimulant. Cardiovascular function - the animal’s hairless areas, normally pink if an albino, turn “white” if tissue perfusion is poor. Assess the cause of cardiac impairment. Anesthetic overdose - if appropriate, use an antagonist or an anticholinergic. Hypothermia is the greatest cause of rodent surgical mortality. Hemorrhage of 3-4 ml in a rat will cause irreversible shock. Good surgical technique will minimize blood loss. Blood transfusion is ideal for inbred strains; no cross-matching necessary (keep a donor handy if the risk of hemorrhage is high). Outbred strains generally have no problems when transfused 1X. Blood volume is approximately 70 ml/kg. Hemorrhage and loss of 10 % volume is tolerable, but 20-25% loss will cause shock.
Consider using fluid therapy- to support cardiovascular function or to prevent dehydration. Animals will have reduced food and water intake for 1-2 days after surgery. Providing sterile, warmed, physiological fluids (SQ, IP or IV) can be used to compensate for hemorrhage and reduction in water intake postoperatively.

- 0.7 cc/100 g Lactated Ringer’s Solution (LRS) warmed to body temp may be injected SQ before the procedure if a prolonged recovery is expected or extensive hemorrhage may be likely. This rate would provide a volume of 1.4 cc for a 200g rat and 0.14 cc for a 20g mouse.
- Or infuse IV at a rate of 2 ml/100g/hr. A tail vein catheter may be placed before the procedure to be available for IV infusions if necessary.

Consider whether the animal will have a reduced water intake for 12-24 hours post-op. Provide replacement fluids. Fluid requirements are 40-80mg/kg/24 hr. This equates to about 12 cc for a 200g rat or 1.2cc for a 20 g mouse. Administer this dose divided in 2 doses daily SQ or IP. If unexpected mortality occurs during the procedure, monitor heart rate and blood pressure in succeeding animals.

**Notes on Surgical Technique**

Minimize contamination of the operative field during surgery by restricting the movement of gloved hands and sterile instruments. Plan the incisions to avoid large vessels in the skin or body wall. Handle tissues gently and avoid excessive force in tissue retraction, which can cause necrosis. Avoid or minimize hemorrhage. But if it occurs, blot away blood with gauze sponge to remove a medium for bacterial growth. Never use a wiping action with gauze sponges, which will traumatize tissues and may cause renewed bleeding. Use a blotting action instead. If a wound becomes contaminated, use warm, sterile Lactated Ringer’s Solution or saline to irrigate and cleanse the area.

**Needle Type:**

If suturing with a needle, use the right type of needle for the type of tissue.

- **Soft Tissues**- use a tapered (round-bodied) needle on internal tissues. This type of needle passesatraumatically through soft tissues and allows them to “seal” behind the needle. Don’t use a cutting edge needle in soft tissues because this type of needle would tear the tissue, undermining the suture line, and it is more likely to cut through blood vessels leading to more hemorrhage in vascular tissues (e.g. muscle).
- **Skin**- Use a cutting edge needle on the skin. The dermis has tough fibrous tissue. To pass a needle through it, cutting edges are needed to slide the needle through the skin. This minimizes trauma and irritation to the skin. As a result, the animal will be less likely to inflict self-trauma to the sutured incision, if a cutting edge needle were used. On the other hand, if a tapered needle were used, the needle would have to be tugged through. The tugging and stretching of the skin would increase soreness of the skin wound. Needles with swaged on suture impose less trauma on tissues than do threaded needles.

**Suture Material:** Use the right kind of suture material for the type of tissue.

- **Internal layers**- Use an absorbable material, unless permanent ligatures are needed. Example material: Vicryl, sizes 3-0 and 4-0 in a rat; 4-0 and 5-0 in a mouse.
- **Skin layer**- Use a non-absorbable mono-filament sutures in skin (Prolene, nylon).
  - Don’t use braided sutures, like silk because they tend to wick bacteria and cause irritation and infection. This raises the chances of animal self-trauma.
  - Same sizes as above.
  - Insert the needle about 5mm from the wound margin
  - Space interrupted sutures or autoclips about 5mm apart
  - Do not cut suture so short that they can unravel later.

**Suture Layers and Patterns:**

1. **Body wall (abdomen)** - The suture line should be in a simple, interrupted pattern, using absorbable suture
material. Do not use a continuous pattern. The body wall layer is an important one because it must take the tension in the body wall caused by animal movement. Animals do not restrict their mobility after surgery, and so this layer must hold fast against tension. If a continuous suture line were used, and if a knot slipped or the suture broke, then the entire incision would dehisce. But if simple interrupted sutures are used, then the incision line is better protected.

2. Subcutaneous tissue- The suture line should be in a single continuous pattern, using absorbable suture. This should be used in larger rats which have a sizeable amount of subcutaneous tissue; it is not used in mice. Closing this layer collapses the potential space between tissue layers, preventing a seroma and abscess from forming. The subcutaneous layer will not have the tension of the body wall, and so that suture strength is not needed. Therefore, the continuous pattern can be safely used for its advantage of speed in suturing.

3. Skin- The suture line should be in a simple, interrupted pattern, for the same reasons as for the body wall layer. Use a non-absorbable suture material or stainless steel autoclips.

Knot Tying
- Tie all sutures (any layer) with square knots with a third throw (3 half-hitches).
- Don’t cut knot strands to short. If cut too short, they will come undone later.
- If skin sutures are cut too long, the animal may chew on them and in so doing, remove the suture.
- When applying autoclips avoid excessive pressure that could cause tissue ischemia.

Dehiscence - suture lines coming undone.
- The animal will chew and remove sutures if they are irritating.
- Be aware whether the suture strand will poke a body part or fold of skin. In skin fold areas, suture strands may jab the skin and cause irritation. This may occur with mono-filament nylon, because the cut end is hard. Skin fold irritation may be avoided by altering the placement of the sutures, changing the length of suture strands or by softening the suture material with daily applications of petroleum jelly to the suture end only.
- Avoid drawing sutures too tight. Wound margins normally become moderately edematous. Tight sutures will strangulate tissue and be painful. Over tightening skin sutures is the most common reason animals remove their stitches. The same surgeon carrying out the same surgery but using clips would have far less suture removal, which suggests that it is over-tightening rather that the irritation of low grade infection that cause the dehiscence.
- Maintain good septic technique. Infection macerates wound margin and causes sutures to loosen and fall out.

Suture Removal:
Whether using suture or autoclips, you must remove these from the skin at 7-10 days after the surgery. The time will vary depending on the surgical site. If sutures or clips are not removed, they will become embedded in the skin and will cause irritation and possibly infection. At some point, the animal will chew and remove the sutures or clips because of the irritation.

Tissue Adhesives are acceptable for closing skin in rodents. Don’t bathe the skin wound because animals are likely to self-traumatize the area if there’s glue residue on the skin surface. Instead, use tissue adhesive sparingly. Carefully place a tiny drop via an applicator tube onto the subcutaneous tissues. Use a probe to push the opposing edges of skin together, margin to margin. Avoid getting adhesive on the fur, or the animal may later open up the wound in the process of removing the glue from its fur. Apply adhesive drops 3-5 mm apart.

Postoperative Care
If possible, use anesthetic/sedative antagonists to recover the animal more quickly from anesthesia. Yohimbine is used to reverse xylazine. Administer IP. To treat respiratory depression, doxapram at 5-10mg/kg IV or IP is used. Re-dose as necessary at 15 min intervals. Continue providing a source of heat until the animal is fully ambulatory. Provide clean bedding to avoid wound contamination. Assess food and water intake for several days. Animal may not drink for 1 day post-op and will therefore dehydrate. You can give fluid replacement by warmed LRS 40-80 ml/kg/24hr, PO, SQ, or IP. If animals are dehydrated, provide fluid therapy and consider doing so by IV infusion. Test for dehydration by pinching the skin into a tent and then releasing it (“tenting the skin”). If normal, the skin will snap back towards the body. If dehydrated, the skin will fall slowly into place.

Environment: Rats and mice prefer low lighting, quiet, and a place to hide. Provide some cover: enrichment
tubes, Enviro-dry™ once they recover from the anesthetic. Also, provide clean bedding, so that surgical wounds will be less likely to be contaminated by feces and urine. Observe the animals for signs of pain distress post-operatively. Remember that rats and mice are nocturnal and are less active during the day, making it difficult to assess their behavior at times of less than peak activity. Compare posture and activity with normal animals. Rats and mice are able to mask pain, which is an evolutionary adaptation important for prey animals. Therefore, pain may be evident in altered behavior or it may not. Most evident will be a reduction in food and water consumption. Therefore, if the procedure is likely to produce pain in humans, it should be assumed to be painful in animals and should be treated with analgesics. Abnormal signs—any may be seen: hunched posture, ruffled fur, red staining of eyes or nares, vocalization, greater or less tissue coloration, greater or less activity, resents being handled, reluctant to move, abnormal gait, aggressiveness, low water or food intake. The first 24 hours are critical for pain management.

If pain or distress is observed or anticipated:
1. Provide analgesia
2. Evaluate your surgical technique and the procedure for potential improvements, e.g. by decreasing tissue trauma, improving aseptic technique, method of suturing.
3. Upon euthanasia or death, submit animal for necropsy to determine if there are other problems including surgery-related infection.

Post-op analgesia: increasingly recommended are post-op analgesia and preemptive analgesics (which reduces pain postoperatively). Types of post-op analgesics: opioid and nonsteroidal anti-inflammatory (NSAID). Choice need not be limited to one or the other. Both can be given and are additive in effect. The drug and dosage will be determined by the attending veterinarian.

Topical Anesthetic—Preemptive Analgesia: Don’t forget preoperative (preferred) or intra-operative infiltration of the incision site. This may prevent the need for any follow-up analgesic treatment or at least delay it for up to 8 hours.

**Record-Keeping**

Record keeping for rodents is a regulatory requirement for AAALAC accreditation. Nonetheless it is a useful adjunct to research documentation for tracking changes in animal responses to anesthesia, surgical procedure and in recovery. Record data: animal ID, age, weight, sex, health status, anesthetic, duration of anesthesia, progress of surgery, perioperative care procedures, clinical observations of recovery, medications.

**References:**


Kohane DS: Sankar WN; Shubina M; Hu D; Rifai N; Berde CB. Sciatic nerve blockage in infant, adolescent, and adult rats: a comparison of ropivacaine with bupivacaine. Anesthesiology, 1998 Nov, 89(5); 1199-208; discussion 10A.


Steenbergen JM; Strubbe JH; Bohus B. Behavioral and cardiac responses to a sudden change in environmental stimuli: effect of forced shift in food intake. Physiology and Behaviour 45, 729-733.


Acknowledgments:
Contributions to this document were generously made by:
1) Prof. David B. Morton, Head Centre for Biomedical Ethics, Division of Primary Care, Public and Occupational Health, (Director Biomedical Services Unit), University of Birmingham, UK.

2) Pauline L. Wong, DVM, DACVA, Veterinary Anesthesia Consulting Services and Anesthesia/CPC, Veterinary Medical Teaching Hospital, University of California, Davis, CA.

This is a handout has been modified from the handout used for a training session at Division of Comparative Medicine, Washington University. Materials were originally prepared by Nicole Duffee, DVM PhD.