Purpose of this document: This is a handout that accompanies a hands-on rodent surgery workshop in the Division of Animal Resources (DAR).

Regulations and Guidelines

- The Guide for the Care and Use of Laboratory Animals (National Research Council) can be downloaded from https://www.aaalac.org/the-guide/. The 2011 Guide states, “Inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses (Beamer 1972; Bradfield et al. 1992; Cunliffe-Beamer 1990; Waynforth 1980, 1987) affecting surgical success, animal well-being, and research results (Cooper et al. 2000). General principles of aseptic technique should be followed for all survival surgical procedures (ACLAM 2001).”
- According to the Guide, a dedicated surgical facility is not required for rodent surgery, but surgery must be performed using aseptic technique.

Justification for Applying Aseptic Technique in Rodent Surgery

Importance of maintaining asepsis (NRC Guide for the Care and Use of Laboratory Animals):

- Although mice and rats have been touted as being resistant to post-surgical infections, the literature contains numerous articles that document 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 on rodents in a non-aseptic manner. However, experimental evidence has been obtained to suggest that infections take a subclinical profile in rats and mice. Improvement in post-op recovery by increased food/water consumption due to implementing aseptic surgical technique has also been documented (Cunliffe-Beamer, T.L, 1972-73. Cunliffe-Beamer, T.L. Biomethodology, 1983). Subclinical infections can lead to behavioral and physiological changes (Behavioral and Physiologic Effects of Inapparent Wound Infection in Rats, Lab. Animal Science, 42 (6), 572-578, 1992. 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 no differences between rodents and other mammalian species, including humans, in the development of infections, including postsurgical 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 the outside environment.

Good Technique includes:

- Asepsis
- Gentle tissue handling
- Minimal dissection of tissue
- Appropriate use of instruments
- Effective hemostasis
- Correct use of suture materials and patterns
Special Considerations

- Rats and mice have a high surface area to body volume ratio and rapid metabolism. Accordingly, one must realize the following:
  - Rodents dehydrate faster per unit of time than larger animals.
  - Rodents lose body heat rapidly through hairless areas such that hypothermia during surgery is a frequent cause of intraoperative mortality if proper precautions are not utilized.

- 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 and lipolysis, and result in hyperglycemia. The duration of the hyperglycemia 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 infection, controlling postsurgical 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.

Hemostasis

- It is important to minimize bleeding during surgery because:
  - Blood is an ideal growth media for bacteria
  - Blood loss leads to poor recovery and increases the chance of death
  - Blood loss increases post-op recovery time
  - Blood loss may introduce research variables

- To minimize blood loss:
  - Dissect along tissue planes
  - Do not cut across muscle when possible
  - Identify, isolate, retract large vessels
  - Know the anatomy

Tissue Trauma

- Trauma and infection negatively impact the animal and also serve as a confounding variable for experimental data. Diminish tissue trauma and infection by adhering to the following four principles:
  - Surgery is gentle: Rough tissue handling results in increased pain.
  - Time is trauma: Organ exposure to room environment is potentially traumatic to tissues. The longer the exposure the greater the trauma. Find the right balance between speed and quality of work.
  - The solution for pollution is dilution: Infection (clinical and subclinical) occurs when the number of infectious particles overwhelm the animal’s immune system. Adhere as close as possible to the aseptic principles outlined in these notes to diminish the number of microorganisms in the surgical site. If contamination occurs, dilute the contaminant with use copious amounts of a warm physiological solution (sterile saline or lactated ringer’s solution).
  - Wet tissues are happy tissues: Avoid desiccation by maintaining tissues wet at all times with a warm physiological solution.
**Preoperative Preparation**

- Assess health status. Recommendations:
  - Before conducting surgery, one must provide a minimum of three days for the animal to acclimate to the animal facility subsequent to its arrival to allow the animal to overcome the stress of transportation and return to a normal physiological state.
  - The animal should be free of clinical signs of disease:
    - Appearance should include normal posture and movement, glossy coat, bright eyes.
    - Assess the character of respirations (no sneezing, coughing, or unusual respiratory sounds or discharges should be apparent) and the cardiovascular status (bright pink coloration of ears and mucous membranes is the norm).
    - Normal intake of food and water.

- Fasting rats, mice and hamsters is generally unnecessary.
  - Because rats, mice and hamsters do not vomit, they do not have the risk of intra/post-op vomiting as in other species. If you will perform a surgery on the gastrointestinal tract, then you can fast the animals but briefly (a few hours). However, the reason for doing it should be considered carefully and weighed 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 a sudden change in environmental stimuli: effect of 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, mice, and hamsters have a high surface area to body mass ration and lose body heat rapidly by conduction.
  - A major cause of surgical mortality is not necessarily the surgery itself or the anesthesia but hypothermia. Body temperature drops precipitously under sedation or anesthesia. Low body temperatures can cause irreversible shock and death.
  - Animals should be provided with a heat source during the pre-, intra- and post-operative periods.
  - Improper heating devices can also be very dangerous. Electric heating pads are not recommended for use with rodents as they have varying temperatures across the surface and thus may have “hot spots” which can cause burns on the animal’s skin.
  - The safest device is a warming device circulating warm water apparatus.

- 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 compromise circulation.
  - Only tie down the limb(s) that need to be positioned. 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, one can do so with tape.

- Never use the anesthetized animal’s body as a table. Do not rest your hands or your instruments on the chest or abdomen of small surgical patients such as rodents. External pressure interferes with respiration and blood circulation.
**General Preparations for Surgery**

The NRC *Guide for the Care and Use of Laboratory Animals* states: “Some characteristics of common laboratory-rodent surgery such as 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, can make modifications in standard aseptic techniques necessary or desirable (Brown 1994; Cunliffe-Beamer 1993). Useful suggestions for dealing with some of the unique challenges of rodent surgery have been published (Cunliffe-Beamer 1983, 1993).”

Please read and be familiar with the tenants of the GSU IACUC Policy titled “Aseptic Technique for Animal Surgery” found here: [https://ursa.research.gsu.edu/document/aseptic-technique-for-animal-surgery/](https://ursa.research.gsu.edu/document/aseptic-technique-for-animal-surgery/)

**Location**
- The elaborate operating suites mandated by the NRC Guide for larger species is not necessary for rodents.
- 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. Free of debris and equipment not related to surgery.
  3. 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 surgery area with loose animal fur, splashes from incision site scrubbing, and bedding dust and fur.
  4. Avoid locations that are beneath supply ducts to minimize contamination from dust.
  5. Avoid high traffic areas such as those near doorways to prevent unnecessary interruptions and creation of air turbulence.

**Sterilization of Instruments**
See Table 1 below for sterilization options for surgical instruments.

- Surgical instruments should be autoclaved. Be sure to use autoclave indicator tape to ensure the autoclave is functioning properly (stripes will appear on the autoclave indicator tape verifying that sterilization has occurred). Cloth (double wrapped) or paper (double wrapped) surgical packs are typically good for only a finite period after sterilizing (e.g. six months). Accordingly, record the sterilization date on the temperature tape on the outside of the pack. Sterilization indicators are recommended inside the pack to confirm sterilization conditions of time, temperature and penetration.
• If performing batch surgeries, i.e. using the same instruments on a series of animals, wipe them clean (i.e. with sterile saline) to remove gross contamination and re-sterilize the instrument tips (e.g. in a hot bead sterilizers – see next section below) between animals. You can also use multiple surgical packs or different surgical instruments between animals. When performing batch surgeries, one needs to begin with autoclaved instruments on a given day. One can then use the hot bead sterilizer to re-sterilize the instrument tips for up to five (5) animals on the given day after which time a new surgical pack is needed.

• **Hot bead sterilizer**
  - This method sterilizes only the tips of the instruments.
  - Beads must be pre-heated to the recommended temperature and the instruments exposed for the recommended time (generally tips of instruments are exposed for 15 sec).
  - Gross debris must be removed from the instrument prior to sterilization.
  - Instruments must be allowed to cool before touching tissues. Cooling can be facilitated by dipping them in sterile saline upon removal from the hot bead sterilizer.
  - Best used for sterilizing instruments between surgeries.
  - 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.

• **Liquid sterilants** (e.g. glutaraldehyde [Cidex])
  - If using cold sterilant solutions make sure instruments are exposed for the proper length of time and expiration dates of solutions are observed
  - Instruments must be removed from solution and rinsed with a sterile physiological solution
  - Rinsed instruments must be placed on a sterile field

• **Delicate instruments**
  - Delicate instruments, materials for implantation or items that otherwise may melt or become damaged when heated can be sterilized using ethylene oxide or a liquid sterilant.
  - The packs must be sufficiently aerated to prevent toxic side effects from residual ethylene oxide gas (this may require 24 to 72 hours)

• **Instrument packs**
  Once packs are opened, the contents must be maintained on a sterile field. These items must be opened in a way that prevents contamination of the sterile contents.

Table 1. Recommended instrument sterilants (instruments should be cleaned of all gross debris prior to sterilization. Follow manufacturer’s recommended concentrations and contact times for all sterilizing agents).

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist heat (steam)</td>
<td>Autoclave</td>
<td>Effectiveness depend upon temperature, pressure, and time (e.g., 121°C for 15 min vs. 131°C for 3 min)</td>
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<tr>
<td>Dry heat</td>
<td>Hot bead sterilizer, dry chamber</td>
<td>Fast. Instruments must be clean prior to use and must be cooled before contacting tissue. Note that the hot bead sterilizer acts only on the tips of instruments and should therefore be used to</td>
</tr>
<tr>
<td>Sterilization Method</td>
<td>Description</td>
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</tr>
<tr>
<td>Ionizing radiation</td>
<td>Gamma radiation Requires special equipment and training</td>
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<tr>
<td>Gas sterilization</td>
<td>Ethylene Oxide (ETO) plasma vapor ETO requires 30% or greater relative humidity for effectiveness against spores. ETO gas is irritating to tissue; all materials require safe aeration time</td>
<td></td>
</tr>
<tr>
<td>Hydrogen Peroxide (H2O2) &amp; Peracetic Acid</td>
<td>SporklenzR (full strength) Broad-spectrum. Sporicidal-fungicidal sterilants only with contact time of 11 hr or more. Instruments must be rinsed with sterile water or saline prior to tissue contact</td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>Chlorine dioxide (ClidoxR, AlcideR) A minimum of 6 hr is required for sterilization. Presence of organic matter reduces activity. Solutions must be freshly prepared (&lt;14 days). Instruments must be rinsed thoroughly with sterile water or saline prior to tissue contact</td>
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**Animal Prep**

- **Anesthesia**
  - Isoflurane gas anesthesia administration through a precision vaporizer is generally considered the preferred method of anesthesia in rodents. However, injectable anesthetics may also be used.
  - Gas anesthesia may be induced in an induction chamber or it may be preceded by an injectable anesthetic cocktail.
  - For maintenance of anesthesia, a gas mask or endotracheal tube may be used to deliver the anesthetic. Endotracheal intubation can be performed in a rat with a 14-18 gauge venous catheter and mice with a 20-22 gauge venous catheter, trimmed to the length between the nose and thoracic inlet. Specialized apparatuses and kits can be purchased to facilitate intubation of rodents.

- **Protect the eyes:** Anesthetized animals should have corneas protected with an ophthalmic ointment to prevent potential corneal ulcers. Avoid touching the eye with the tip of the ointment dispenser as it may scratch the cornea.

- **Hair Removal**
  - Remove fur along the incision site with small clippers. Clip a generous area to ensure fur does not contaminate the incision site, but avoid taking off too much fur because this will reduce the animal’s ability to regulate its body temperature. Use the sticky side of white tape to lift off the loose fur or use a mild vacuum device to do so.
• An easy alternative to clipping is hair plucking (mice only). Hair follicles in mice (not other rodent species) are usually in telogen or resting phase and hair can be removed without injury.

• Aseptic preparation of surgical site:
  o Standard surgical prep consists of three alternating scrubs of an iodophor (e.g. Betadine) or chlorhexidine (e.g. Nolvasan) scrub solution and 70% alcohol.
  o Using a gauze sponge (non-sterile is ok but sterile is preferred) or cotton tipped applicator (non-sterile is ok but sterile is preferred), cleansing should be done in a circular motion.
  o Begin at the center of the shaved area and work toward the periphery.
  o Never go back to the center with the same sponge.
  o Scrubs should be alternated between an iodophor or chlorhexidine scrub and alcohol, ending with an iodophor or chlorhexidine solution, NOT scrub. Scrub soaps are caustic to subcutaneous tissue. Allow 4-5 minutes of contact time before making the incision.

• Draping is necessary when viscera or sterile instruments may come in contact with unprepped skin and fur.
  o The most common drape is the paper drape.
  o It may be precut or one in which you must cut a hole.
  o Surgical paper drapes are inexpensive and autoclavable. A disadvantage to paper drapes is that they usually cover the animal making animal monitoring difficult.
  o Transparent, self-adhesive plastic drapes (i.e. Press’n Seal) offer the advantage of increased animal visibility. *Please see Appendix I for aseptic removal of Press’n Seal from packing.
  o Sterile gauze sponges can also be used as drapes.
  o A sterile stockinette can also be “unrolled” over the animal and used as a surgical drape.
  o During surgery
    • Be careful not to get paper or cloth drapes wet.
    • Wet material acts as a wick to pull bacteria through from the non-sterile surface below.
    • When this happens instruments should be considered contaminated.
Surgeon

- Use a minimum of sterile surgical gloves, a surgical mask, and a clean lab coat or a scrub shirt.

- Donning surgical gloves:
  - Open the package of gloves observing sterile technique
  - Remember, the inside of the package is STERILE – exam gloves are not the same as sterile surgical gloves

  - It is important to don the gloves in such a way that prevents contamination of the outer surface of the gloves.
  - One glove is lifted from the opened glove package by its turned down cuff.
  - The glove is pulled on the hand with a rotating motion.
  - Place the gloved fingers beneath the cuff of the other glove.
  - With the gloved fingers under the cuff, the glove is placed on the ungloved hand.
  - The folded cuff protects the gloved hand from contamination.
  - The glove is pulled over the sleeve of the lab coat following insertion of the hand.
  - The fingers are then slipped under the cuff of the first glove to pull it over the lab coat sleeve.

Maintaining Asepsis

- Gloved hands should be held elevated above the waist and should touch only the surgical incision and sterile objects, i.e. sterile instrument tray, sterile drape.

- 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 clothes.

- Do not allow surgical instruments to fall below the edge of the table. If an instrument does fall, the instrument is considered nonsterile and should not be picked up and reused until re-sterilized.

- Sterile surfaces are to be kept dry. Moisture can lead to contamination of the surgical area.
**Pain Control:**

*Anesthesia* is a state where all perceived sensations are absent. It is imperative that one assesses the depth of anesthesia prior to beginning a painful procedure such as surgery.

Before making an incision, squeeze each paw firmly but gently (toe pinch reflex) 3-4 times to test the animal’s sensation of pain. If the animal withdraws its leg or if the respiration rate increases, then the anesthesia is too light. If using an injectable anesthetic, 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.

- **Preemptive Analgesia:**

  Preemptive analgesia is the prevention of pain before it occurs. This involves the administration of a systemic and/or local analgesic before the pain insult occurs (e.g. before the surgical incision is made). The basic idea is that, by addressing pain control before the “insult” occurs, one does not have to “catch up” and try to relieve pain that already exists. This preemptive pain control tends to be more effective than “after the fact” pain control.

  When the skin and tissues 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 site is painful and sensitive to touch and movement.

  If a systemic analgesic is administered and/or a local anesthetic is infiltrated prior to making the incision, it will block or diminish the sensory neuroexcitation caused by incising the tissues. When the animal wakes up, it will have a reduction in sensory stimuli from the incision area, and pain of the surgical site will be greatly reduced both initially and throughout the period of surgical recovery.

  An effective and simple local preemptive analgesic protocol is as follows: prepare a 50/50 mix by volume of lidocaine 1-2% with 0.5% bupivacaine. Infiltrate the incision area subcutaneously prior to making the incision. Lidocaine provides almost immediate pain control with a duration of 20-40 minutes and bupivacaine provides longer pain control (lasting 4-6 hours). Since bupivacaine tends to sting upon injection, it should be injected after the animal is anesthetized. In general major surgery requires systemic analgesics as the lidocaine/bupivacaine infusion only provides pain management to the incision site. Lidocaine and bupivacaine doses should not exceed 10 and 6 mg/kg respectively.

**Intraoperative Care**

- **Monitoring:**
  - Anesthetized animals must be monitored during the procedure to assure they stay in the proper anesthetic plane.
  - The anesthetic plane can be assessed by pinching the all of the toes.
  - Typically, any reaction indicates the animal is too light (not in a surgical plane of anesthesia).
  - The color of the mucous membranes and exposed tissues such as the pink soles of the feet are easy to monitor. Bright pink and red as apposed to pale, dusky grey or blue indicates appropriate tissue perfusion and oxygenation.
  - Respirations should be steady. If an animal’s respiratory rate increases then the animal may be becoming too light.
  - Core body temperature can also be monitored and supported (e.g. circulating warm water pad).
  - Pulse oximetry can be used in larger rodents to monitor the pulse rate and oxygen saturation.
  - Electrocardiograms can also be used in larger rodents.
**Respiration** – animal turns “blue” (hairless areas) if hypoxic.
- Evaluate the need for delivering oxygen, assess the face mask to assure there are no blockages.
- 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/minute acceptable in rodents):
  - 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 (e.g. doxapram).

**Cardiovascular function** – the animal’s hairless areas (normally pink) turn “white” if tissue perfusion is poor.
- Assess the cause of cardiac impairment.
  - Anesthetic overdose – if appropriate, use an antagonist or an anticholinergics (e.g. atropine).
  - Hypothermia – greatest cause of rodent surgical mortality.
  - Hemorrhage - 3-4 ml of blood loss in a 200 g rat will cause irreversible shock.
    - Good and gentle surgical technique will minimize blood loss.
    - Blood transfusion – Ideal for inbred strains; no cross-matching necessary (keep a donor handy if the risk of hemorrhage is high).
    - **Outbred strains** - no problem likely when transfused once.
    - Blood volume is approximately 70 ml/kg. Hemorrhage and loss of 10% volume is tolerable, but 20-25% loss will cause shock.

<table>
<thead>
<tr>
<th>Blood Vol</th>
<th>10% loss</th>
<th>20% loss</th>
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</thead>
<tbody>
<tr>
<td>Mouse 20 g</td>
<td>1.5 ml</td>
<td>0.15 ml</td>
</tr>
<tr>
<td>Rat 200 g</td>
<td>15 ml</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

- 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.
    - Adult mouse: 2 – 3 ml SC or IP OR Adult rat: 5 – 10 ml SC or IP of sterile warmed LRS or physiological saline may be injected before the procedure if a prolonged recovery is expected or extensive hemorrhage may be likely.
    - Or, IV infusion of sterile LRS or physiological saline IV at a rate of 2 ml/100g/hr (maximum of 25ml/kg). A tail vein catheter may be placed before the procedure to be available for IV infusions if necessary.

- **Notes on Surgical Technique:**
  - Prevent 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.
  - Minimize hemorrhage. When hemorrhage occurs, wick away blood with a sterile gauze sponge, Q tips or gel foam spear. Never use a wiping action, which traumatizes tissues and may cause renewed bleeding. Use a wicking or blotting action instead.
  - If a wound becomes contaminated, use warm, sterile LRS or 0.9% saline to irrigate and cleanse the area (the solution for pollution is dilution).
**INSTRUMENT HANDLING:** Generally, scissors and hemostats are held with the thumb and the ring finger. This gives you the most controlled use of the instruments.

The following pictures illustrate proper instrument holding:

- **Safe surgical blade loading**
- **Safe surgical blade unloading**
- **Wrong instrument holding**
- **Pencil grip technique for holding thumb forceps**
- **Holding hemostats or scissors**
  - *Thumb & Ring finger*
- **Holding the needle driver**
  - *Thumb & Ring finger*
- **Palming the needle driver**
SKIN INCISION: Placing tension on the sides of the incision with the non-dominant index finger and thumb while holding the scalpel handle with the dominant hand improves accuracy.

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 (e.g. intestine, muscle, peritoneum). This type of needle passes atraumatically 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 can tear (cut) the tissue as it passes through and 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 (cutting or reverse cutting needle). The dermis has tough fibrous tissue. To pass a needle through it, cutting edges are needed to slide the needle through the skin. This is more of an issue in larger species (e.g. non-rodents). This minimizes trauma and irritation to the skin. As a result, the animal will be less likely to self-traumatize 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 incision site.

- Swaged-on needles (needles attached to suture material) impose less trauma to tissues than do non-swaged-on needles (suture material threaded through the eye of the needle).

ARMING THE NEEDLE:

- Load the needle in its middle third

- Hold the needle with the tip of the needle holder with the needle perpendicular to the jaws of the needle holder
Holding the needle too close to the site of suture attachment will result in needle bending and frustration.

**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, PDS, Dexon, Maxon, sizes 3-0 and 4-0 in a rat; 4-0 and 5-0 in a mouse. Silk is frequently used for cardiovascular procedures.

- Skin layer – Use a **nonabsorbable monofilament** sutures in skin (Prolene, nylon), wound clips, staples, stainless steel suture, and/or tissue glue.
  - *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.

**SUTURE LAYERS AND PATTERNS:**

1. Body wall (abdomen) – The suture line should be in a simple interrupted pattern, using absorbable suture material. A continuous pattern may also be used but it has some drawbacks. The body wall layer is an important one because it must be able to withstand the tension in the body wall caused by animal movement. Animals may 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 come apart (dehisce). However, if simple interrupted sutures are used, then the incision line is better protected as the incision site is closed with a multitude of suture knots.

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 from forming. The subcutaneous layer will not have the tension of the body wall such that the continuous pattern can be safely used for its advantage of speed in suturing.

3. Skin – Use sutures or wound clips. The suture line should be in a simple interrupted pattern, for the same reasons as for the body wall layer. Use a nonabsorbable/monofilament material.
   - Insert the needle about 5 mm from the wound margin.
   - Space interrupted sutures (or clips) about 5-8 mm apart.
   - Do not cut suture ends so short that they can unravel later.
   - Cyanoacrylate skin glue (e.g. Vetbond, Nexaband, Dermabond) can be used to appose skin edges for small incisions or to reinforce skin edges between sutures. Do not bathe the skin incision because animals are likely to self-traumatize the area if there’s glue residue on the skin surface. Carefully place a tiny drop via an applicator tube right over the skin. Use forceps to push the opposing edges of skin together, margin to margin. Avoid getting adhesive on the fur or else the animal may later open up the incision site in the process of removing the glue from its fur.
   - A lot of rodents gnaw at externalized sutures so a buried suture line is recommended in rodents. Alternatively, one can use wound clips, staples, or stainless steel sutures.
KNOT TYING

- Tie all sutures (any layer) with square knots. As relates to most suture material, 3-4 throws are appropriate. Prolene and nylon are slippery and may need 5 throws.
- Don’t cut knot strands too 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.

GRASPING TISSUES WITH THE THUMB FORCEPS

- Generally the body wall (muscle layer) is grasped with fine rat tooth forceps.
- Rat tooth forceps could be injurious to soft tissue.
- The skin and soft tissues are handle with non-rat tooth forceps (i.e. Brown Adson forceps).

IMPROVING SPEED AND ACCURACY WHILE DECREASING TISSUE TRAUMA

- Hold the long end of the suture with your non-dominant hand at a comfortable distance from the knot (not too close and not too far).
• Stabilize your hands on a towel or the table to minimize trembling.

• Suture with your dominant hand and use your non-dominant hand to stabilize the suturing hand to improve your moves.

• Use the middle finger of the suturing (dominant) hand to pull on the long strand

• Avoid excess trauma to the tissue, either with the suture or the forceps. Avoid excessive pulling on tissue while holding the suture. Make your moves smooth.

**EZ CLIP APPLIER (WOUND CLIPS)**

• EZ Clip Appliers provide an excellent, fast and easy to apply method of skin incision closure. It is particularly helpful on animals such as rodents with tendencies to chew out their skin sutures.
• Clips are easily removed with wound clip removing forceps.
• Place them approximately 5-8 mm apart

**DEHISCENCE** – suture lines coming undone.

• The animal will chew and remove sutures particularly if they are causing irritation.
• Be aware whether the suture strand will poke a body part or fold of skin. In skin fold areas, a suture strand may jab the skin and cause irritation. This may occur with monofilament 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 tying sutures too tight. Wound margins normally become moderately edematous. Tight sutures will strangulate tissue and be painful. Overtightening skin sutures is the most common reason for animals removing their stitches.
• Maintain good aseptic technique. Infection macerates the wound margin and causes sutures to loosen and fall out.
SUTURE REMOVAL

Sutures, staples, and wound clips must generally be removed from the skin at 10-14 days after the surgery. Otherwise, they have the potential to become embedded in the skin and cause irritation and possibly infection. At some point, the animal may chew and remove the sutures, staples, or wound clips because of the irritation. Remove sutures by lifting the knot and cutting the suture close to the skin. Staples and wound clips are removed with instruments designed for this purpose.

Antibiotics

- Prophylactic antibiotics are not a substitute for the practice of proper aseptic surgery.
- In some species (e.g. hamsters, guinea pigs, and rabbits), an inappropriate antibiotic can cause fatalities due to antibiotic toxicity.

Postoperative Care

- Consider the use of anesthetic/sedative antagonists, when available, to recover the animal more quickly from anesthesia (e.g. yohimbine or atipamezole can be used to reverse xylazine and medetomidine).
- Continue providing a source of heat until the animal has regained the righting reflex. The righting reflex is tested by placing the animal on its side or back. If the animal returns to its feet then the righting reflex has returned.
- Provide clean bedding to avoid wound contamination.
- Pay particular attention to food and water consumption for the first few days post-operatively.
  - One can administer fluid replacement by warmed LRS 40-80 ml/kg/24 hr, PO, SQ, IP.
  - Test for dehydration by pinching the skin into a tent and then releasing it (“tenting the skin”). If normally hydrated, the skin will readily snap back towards the body. If dehydrated, the skin will fall slowly into place.
  - Daily weighing is a sensitive method of monitoring the animal. While subtle changes in activity or appetite may not be observed, changes in weight will be quickly detected. Please be aware that some analgesics also depress appetite.
  - Supplying a softer, more palatable, easily accessible diet may encourage the animal to eat.
- Environment: Rodents prefer low lighting, quiet, and places to hide. Provide some cover: place a drape over the cage.
- Observe the animals for signs of pain or distress postoperatively.
  - Remember that rodents 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.
  - Rodents are able to mask pain. Potential signs of pain include a reduction in food and water consumption, altered behavior, abnormal posture (e.g. hunched posture), decreased grooming (unkempt hair coat), irritable, red staining around the eyes.
  - 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.
  - The first 24 hours are typically the most critical for pain management.

- **Neonates:**
  - Neonates or animals recovering from prolonged surgeries can suffer from hypoglycemia.
  - These animals can benefit from administration of oral glucose.
Glucose should never be given SQ or IP.

**Analgesia**

- Please note that it is best to administered analgesics preemptively (before surgery). In addition to preemptive analgesic administration, analgesics can be administered post-operatively if necessary.
- Types of analgesics: opioid and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used analgesics. See Table 2 below for analgesic dose recommendations.
- Choice need not be limited to one or the other. These analgesics work synergistically together, meaning when co-administered they increase the potency of effect with duration of action.

Table 2. Recommended doses for analgesics: opioids and NSAIDs. Some injectable agents have a wide dose range, for initial dosing, it is recommended to start with a median dose within the provided range. The response to a dose of an analgesic has been shown to vary considerably between animals of different strains, sexes and ages (Frommel & Joye 1964; Katz, 1980). So a selected regimen may overdose some animals while under-dosing others. Therefore, pain assessment on an individual basis is required.

<table>
<thead>
<tr>
<th>Analgesics</th>
<th>Mice (mg/kg, unless otherwise stated)</th>
<th>Rats (mg/kg, unless otherwise stated)</th>
<th>Syrian Hamsters (mg/kg, unless otherwise stated)</th>
<th>Siberian Hamsters (mg/kg, unless otherwise stated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>0.05-0.1 every 6-12 hr SC, IP</td>
<td>0.01-0.5 every 8-12 hr SC, IP</td>
<td>0.01-0.05 every 6-12 hr SC, IP</td>
<td>0.5 every 8 hr SC, IP</td>
</tr>
<tr>
<td>Buprenorphine SR1</td>
<td>0.5-1.0 every 72 hr SC</td>
<td>1.0-1.2 every 72 hr SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butorphanol</td>
<td>5 every 4-6 hr SC, IP</td>
<td>2 every 4-6 hr SC, IP</td>
<td>1-5 every 4-6 hr SC, IP</td>
<td></td>
</tr>
<tr>
<td>Carprofen (Rimadyl)</td>
<td>5 every 24 hr SC, IP</td>
<td>5 every 24 hr SC, IP</td>
<td>5 every 24 hr SC, IP</td>
<td></td>
</tr>
<tr>
<td>Flunixin meglumine (Banamine)</td>
<td>2.5 BID SC</td>
<td>2.5 BID SC</td>
<td>2.5 BID SC</td>
<td></td>
</tr>
<tr>
<td>Ketoprofen (Ketofen)</td>
<td></td>
<td></td>
<td>5 SID SC, IP</td>
<td>5 SID SC, IP</td>
</tr>
<tr>
<td>Meloxicam2</td>
<td>1-1.5 SID SC, IP, PO</td>
<td>1-2 SID SC, IP, PO</td>
<td>1-2 SID SC, IP, PO</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>2.5 every 2-4 hr SC, IP</td>
<td>2.5 every 2-4 hr SC, IP</td>
<td>2.5 every 2-4 hr SC, IP</td>
<td>80 every 2-4 hr SC, IP</td>
</tr>
<tr>
<td>Reverse Opioid with Naloxone3</td>
<td>0.05-0.1 IP, IV</td>
<td>0.01-0.1 IP, IV</td>
<td>0.01-0.1 IP, IV</td>
<td></td>
</tr>
</tbody>
</table>

1Buprenorphine SR has been reported to cause skin reactions at the injection site. Please consult DAR veterinarians for direction on administration prior to use.
2 In mice, it is recommended to dilute injectable meloxicam to a concentration of 0.5 mg/ml.
3 Naloxone is used as a reversal agent for opioids specifically morphine, buprenorphine, it should not be used to reverse butorphanol.

*SID = once a day. BID = twice a day.
*IP = intraperitoneal. IM = intramuscular. SC = subcutaneous. IV = intravenous.
### Signs of Pain and Distress

Table 3. Species-specific signs of pain and distress in laboratory rodents.

<table>
<thead>
<tr>
<th>Species</th>
<th>Signs of Pain</th>
<th>Severe or Chronic Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodents</td>
<td>Eyes partially closed; porphyria in rats (red stains around eyes, nose); rapid respiration; rough haircoat; increased vibrissal movement; apprehensive or aggressive; reduced exploratory behavior; scratching; biting; hunched posture; sudden running (escape); aggressive vocalization upon handling; guarding; twitching; trembling</td>
<td>Weight loss; dehydration; incontinence; soiled haircoat; sunken eyes; eyes closed; muscle wasting; sunken or distended abdomen; hunched posture; decreased vibrissal movements; unresponsive; separates from group; ataxia; circling; hypothermia; decreased vocalization</td>
</tr>
</tbody>
</table>

Adapted from information in ACLAM textbook series *Anesthesia and Analgesia in Laboratory Animals*. Edited by Kohn, Wixson, White and Benson.

Table 4. Physiological and biochemical changes associated with pain.

<table>
<thead>
<tr>
<th>Physiological changes</th>
<th>Biochemical changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increased plasma concs.</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Epinephrine, Cortisol, corticosterone, Glucose</td>
</tr>
<tr>
<td>Vasoconstriction (elevated blood pressure)</td>
<td>Glucagon</td>
</tr>
<tr>
<td>Increased heart rate</td>
<td>Sodium</td>
</tr>
<tr>
<td>Increased stroke volume</td>
<td>Endorphins, enkephalins</td>
</tr>
<tr>
<td>Increased cardiac output</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>Lipotropin</td>
</tr>
<tr>
<td>Rapid, shallow respiration</td>
<td>Substance P</td>
</tr>
<tr>
<td>Decreased tidal volume</td>
<td>Amino Acids</td>
</tr>
<tr>
<td>Hypoxemia</td>
<td>Lipids</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>Ketones</td>
</tr>
<tr>
<td>Peripheral blood count</td>
<td>Renin, Angiotensin 2, aldosterone, vasopressin,</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>ADH</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td></td>
</tr>
<tr>
<td>Neutrophilia</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from information in ACLAM textbook series *Anesthesia and Analgesia in Laboratory Animals*. Edited by Kohn, Wixson, White and Benson.
Appendices

Appendix I

Aseptic Removal of Press’n Seal from its Packaging
References:


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


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