Rodent Surgery
Application of Aseptic Technique and Perioperative Care

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GENERAL CONSIDERATIONS OF RODENT SURGERY

Purpose of this document

This is a handout that accompanies a matching hands-on rodent surgery workshop. The principles and methods outlined in this handout are sound and up-to-date to the extent possible; however, your institutional policies and guidelines should be followed.

Regulations and Guidelines

- The **Guide for the Care and Use of Laboratory Animals** (National Research Council) can be downloaded from http://aaalac.org/resources/Guide_2011.pdf. 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 OLAW guidelines for rodents and the *Guide*, a dedicated facility is not required solely for rodent survival surgery, except for when the surgical procedure is conducted.
- The **UTSA IACUC Rodent Survival Surgery** policy can be found by going to http://research.utsa.edu/research-funding/institutional-animal-care-and-use-program_new/.

Justification for Applying Aseptic Technique in Rodent Surgery

The 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*, or mouse hepatitis virus can become clinical diseases following stress or immune suppression (Foster, *et al.,* 1982).
- Historically, researchers have performed surgery in rodents in a non-aseptic manner or using poor aseptic technique. However, experimental evidence has shown that infections take a subclinical (and clinical) 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). Experimentally induced wound infections in rats were not associated with gross clinical or obvious behavioral signs (Bradfield, Schachtman, McLaughlin, Steffen). 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.). Such changes negatively impact experimental results and question the accuracy of the resulting data.
- Rodent models have been used for antibacterial research 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).

The Surgical Environment

Definitions:

- ***Sterile***: Free from all living microorganisms and their spores.
- ***Asepsis***: Very insignificant numbers of microorganisms. 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.
- ***Contaminated/Colonized***: Bacteria/microorganisms present ($<10^6$/gram tissue on average animal). Clinical signs may or may not be present. Immune response may be able to ward off infection.
- ***Infected/Sepsis***: Bacteria/microorganisms present ($>10^6$/gram tissue on average animal). Clinical infection is evident and cultures are positive.

- ***Sterilization***: The process whereby all viable microorganisms are eliminated or destroyed. The criterion for adequate sterilization is the failure of organisms to grow if a growth-supporting medium is supplied.
- ***Disinfection***: The chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.
- ***Major Survival Surgery*** (Guide 2011 definition): As a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection.
- ***Minor Survival Surgery*** (Guide 2011 definition): Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an "outpatient" basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time.

Special Considerations

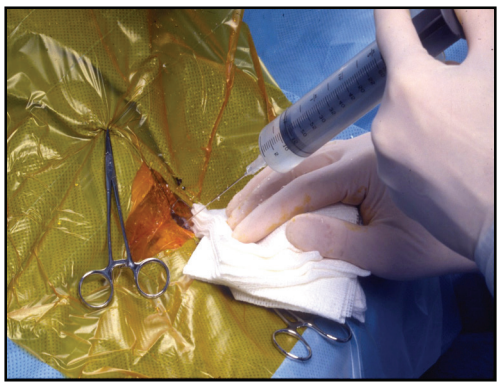
- Rats and mice have a high surface area to body volume ratio and rapid metabolism. Therefore,
  - rodents have a high metabolic rate and limited fat storage. Energy depletion can be stressful.
  - pharmacological doses tend to be higher in rodents than in larger species.
  - rodents dehydrate faster per unit of time.
  - rodents lose body heat rapidly. Hypothermia during surgery is a frequent cause of intraoperative mortality or prolonged and stressful recovery.
- 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 controllable variables. An important objective is to return the animal to physiological normality as rapidly as possible. Particular attention to excellent care during the first 4-6 hours post-op is key to the rodent's recovery.

Hemostasis

- It is important to minimize/control bleeding during surgery because blood loss:
  - Creates an ideal environment for bacterial growth.
  - Leads to poor recovery and stress.
  - Increases the chance of death.
  - Increases recovery time.
  - May introduce research variables.
- To minimize blood loss:
  - Dissect along tissue planes.
  - Do not cut across muscle when possible.
  - Identify, isolate and retract large vessels.
  - Know the anatomy.
  - Apply good hemostasis technique (direct pressure, cautery, hemostatic products, ligation, etc.).

Tissue Trauma & Contamination

- Trauma and infection negatively impact the animal and also serve as confounding variables for experimental data. Diminish tissue trauma and infection by adhering to the following **four principles**:



  1. ***Surgery is gentle***: Rough tissue handling results in increased pain, infection (clinical & subclinical) and recovery time.
  2. ***Time is trauma***: Organ exposure to room environment is toxic to tissues. The longer the exposure the greater the trauma. Find the right balance between speed and fine surgical technique. Incidence of infection increases three times when surgery is longer than 90 minutes.
  3. ***Wet tissues are happy tissues***: Avoid desiccation (drying) of exposed tissues by maintaining tissues moist at all times with warm saline or lactated ringer's solution (LRS).
  4. ***The solution for pollution is dilution***: Infection occurs when the number (generally ~10^6 infectious particles/gram [IP/gr] of tissue in immunocompetent animals) of infectious particles overwhelms 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 wound site. If contamination occurs during the surgical procedure, dilute the contaminant with use of copious amounts of warm rinse solution (sterile saline or lactated Ringer's solution).

Dealing with the Risk

- There is no such thing as a 100% guarantee of "sterility" or a risk-free environment, therefore bringing the number of infectious particles to low and practical risk levels should be the goal.
- The level of acceptable risk depends on:
  - Type and length of procedure.
  - Complexity of the procedure.
  - Species, physiological status and immune status.
  - Surgeon's training/skills/experience.
  - Animal preparation.
  - Surgical instrument/supplies preparation.
  - Aseptic technique.

Effects of Biomaterials

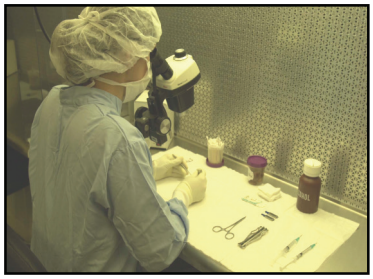
- Introduction of biomaterials into the body significantly decreases the number of infectious particles needed to result in infection.
- When biomaterials (e.g., catheters) are introduced into an animal, the following follows:
  - ~1000-10,000 bacteria/microorganisms present (Paston et al. J Clin Micro, 1993).
  - Animals may become clinically infected.
  - Cultures turn positive.

Preoperative Preparation of the Animal

- Assess health status. Recommendations:
  - Allow a minimum of a 3-day acclimation to the new environment to overcome the stress of transportation.
  - Should be free of clinical signs of disease:
    - Appearance should include normal posture and movement, glossy coat, bright eyes.
    - Assess the character of respiration (no unusual respiratory sounds or pattern) and the cardiovascular status (bright pink coloration of ears and mucous membranes).
    - Normal intake of food and water.
- Fasting rats and mice is generally unnecessary. Because rats and mice 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 disturbance of normal metabolic processes needed for homeostasis, in particular the resulting hypoglycemia. For example, starvation will not empty the stomach unless it is for more than 24 hours, 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, p 45-54).
- 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 and in some cases, respiratory compromise.
  - Secure limb(s) that need to be positioned.
  - Avoid stretching the limbs into an unnatural position, which may traumatize joints as well as impair breathing.
  - If limbs must be secured, apply strips of tape around the carpal area and forelimbs. You can also apply a length of tape over the back, from carpus to carpus, to stabilize the forelimbs and torso.
  - Never use the anesthetized animal's body as a table. Do not rest your hands or your instruments on the chest or abdomen. External pressure interferes with respiration and blood circulation.

General Preparations for Surgery

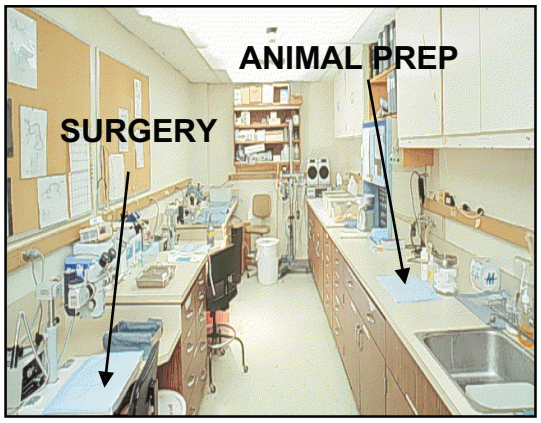
Useful suggestions for dealing with some of the unique challenges of rodent surgery have been published (Cunliffe-Beamer 1983, 1993)."



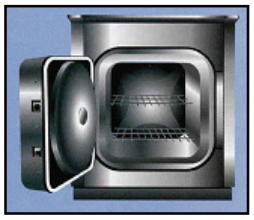
Location

- The elaborate operating suites mandated by the USDA Animal Welfare Act for larger species are not required for rats and mice.
- What is necessary and required for survival surgery in these species is:

1) A clean, neat (uncluttered), 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 or better yet, animal prep is best done when performed in a room separate from the room where surgery is to be performed. 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.

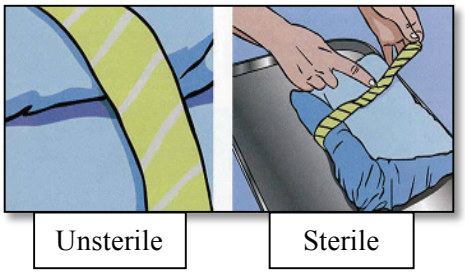


4) Avoid locations that are beneath supply ducts to minimize contamination from dust and air currents that may contribute to hypothermia in the animal.
5) Avoid high traffic areas such as those near doorways to prevent unnecessary interruptions and creation of air turbulence.



Instruments

- Surgical instruments should be autoclaved. Be sure to use an indicator to test that the instruments are sterile, e.g., the strip test or chemical color indicator shown in these pictures. Temp stripes turn black indicating proper sterilization has occurred and chemical color indicator turns brown.

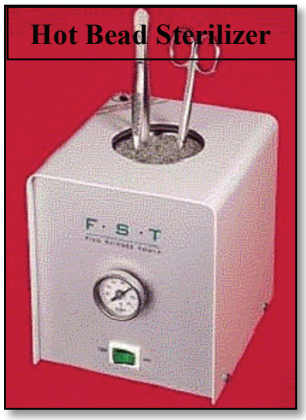


All Instruments must be double wrapped in linen or special commercially available autoclave bags. Expiration dates should be printed on all equipment packs. At UTSA packs are considered expired one year after sterilization, if a pack has ripped (exposing contents), or if it has become wet.

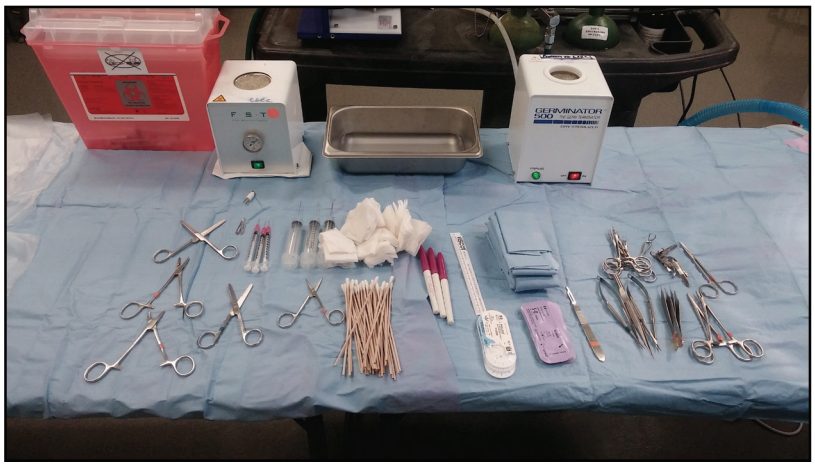
Autoclave settings should be as follows:

Autoclave Settings	Temp (F)	Pressure (PSI)	Time (min)
General Wrapped Items	250	20	30
Bottled Solutions	250	20	30
'Flashing'	270	30	4-7

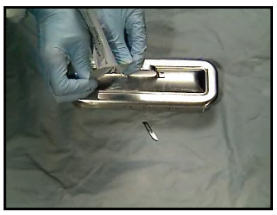
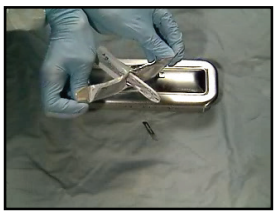
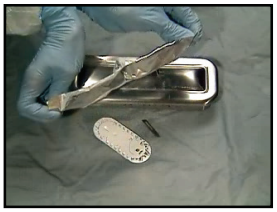
- 'Flashing' is when an instrument is autoclaved unwrapped for a shorter period of time. 'Flashing' is often used when a critical instrument is dropped.
- If performing batch surgeries, i.e., using the same instruments on a series of animals, wipe them clean with alcohol or sterile saline, brush debris in instrument grooves with a toothbrush and resterilize instrument tips (e.g. in a hot bead sterilizers – see next section below) between animals. You may need two sets of instruments to alternate use between animals.
- Hot bead sterilizer
  - Hot bead sterilizers are used for sterilizing instruments between surgeries. 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 60 sec or longer).
- Gross debris must be removed from the instrument prior to sterilization. A sterile hard-bristled brush is recommended to brush debris away from grooves.
- Allow instrument to cool before touching tissues.
- 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.
- No more than five rodent surgeries should be done using this sterilization method (depending on your institutional guidelines). A new set of autoclaved instruments must be used for the next group of animals. TIP: The use of two hot bead sterilizers will help distribute instruments between two systems, avoid overcrowding of instruments and reduce sterilization time.



- Liquid sterilants (e.g., glutaraldehyde [Cidex])
  - If using cold sterilant solutions make sure instruments are exposed for the proper length of time specified by the manufacturer. Adhere to expiration dates of solutions.
  - Instruments must be removed from solution and rinsed with sterile water, saline, or alcohol to remove sterilant chemical residue.
  - Place rinsed instruments 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.
  - The packs must be sufficiently aerated to prevent toxic side effects from residual gas.
  - This may require 24 to 72 hours.
- Instrument packs
  - Once packs are opened all other sterile equipment must be placed on the sterile field. These items must be opened in a way as to prevent contamination of the item or the surgical pack

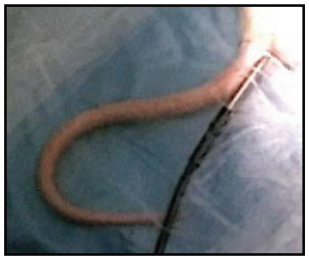
- Organize the instruments in your surgical pack
  - Point all tips in one direction.
  - It is helpful to place them in the order used.
  - Between surgeries cover the tips of the instruments with sterile material (e.g., gauze).
  - Note that the space between the pack and the draped animal is not sterile; do not lay instruments in this space.

Animal Preparation

Preventing hypothermia

- Support normal body temperature during anesthesia.
  - Rodents have a high surface area and lose body heat rapidly.

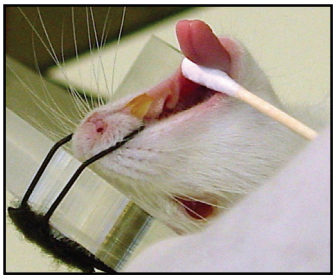
- A major cause of surgical mortality and physiological changes is not always the surgery or the effects of the anesthetic but ***hypothermia***. Body temperature drops precipitously under sedation or anesthesia. Low body temperatures can cause irreversible shock and death.
- Animals should be provided with a heat source during the pre-, intra- and post-operative periods.
- Homeothermic pads are superior to water circulating heating water blankets, which are in turn preferred over electric blankets. Electric heating pads are generally discouraged for use with rodents as they have varying temperatures across the surface.
- Homeothermic blankets are an ideal heat source as they monitor the animal's temperature and keep the animal within the set temperature.
- The tail is an important thermoregulatory organ in rodents. When heating the animal, when possible, place the tail over the heating pad and under covers as illustrated in the picture to the right.



- Place the animal on insulating materials (e.g. bubble wrap or folded drape).
- Place a heating lamp at a distance, which controls heating. However, a thermometer should always be placed adjacent to the animal to avoid burning the animal. Animals must be continuously monitored if using a heat lamp, especially during the post-op recovery period. You can test the environmental temperature by placing a thermometer in the vicinity of the animal for the approximate duration of a surgery (only 1-2° higher than body temp is necessary).
- It's easier to maintain normal body temperature than to reheat a chilled animal. If the animal is allowed to chill, there will be a reduction in circulation and organ function. Therefore, heating should be started immediately upon anesthesia induction.
- Supplemental heat is especially important when using chemical fume hoods or biosafety cabinets because the high currents generated in these hoods will tend to further cool the animal.
- Use warm fluids on tissue within an open cavity.
- Check body temperature throughout the procedure.
- Avoid keeping ambient temperature too cold if possible.
- Place animals in pre-warmed approved incubators during recovery if available.

Rat or Mouse Intubation

- Items required
  - Rat/mouse intubation kit
  - Rodent Work Stand
  - 2% lidocaine
  - Cut away speculum
  - Otoscope
  - Clear 14-18 ga catheter (intubation tube)

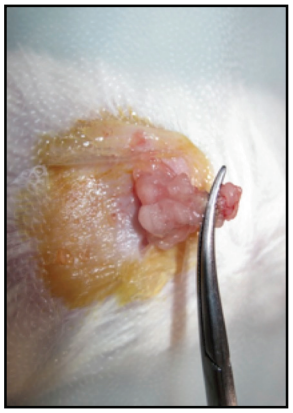


- Procedure
  1. Have everything ready before you start!
  2. Draw .3 mL of Lidocaine into a syringe, purge into applicator.
  3. Check to insure the intubation tube doesn't pass beyond the thoracic inlet, if necessary the tube should be shortened.
  4. Shortening the tube: Cut the tube at a 45º bevel with a sharp blade. A smooth rounded tip will pass easily through the cords. A sharp point will be traumatic and should not be used.
  5. Once the tube is ready, umbilical tape or suture (used to secure the tube) is tied to the catheter hub. A place is provided on the stand to hold the umbilical tape container.
  6. Place the tube over guide wire; making sure it is loosely fitted onto the hub so it can be easily advanced.
  7. Adjust the guide wire using the syringe plunger. It must be long enough to reach the midpoint of the trachea but should not reach the bifurcation of primary bronchi.
  8. Place speculum onto the otoscope. Keep the handle pointed up during intubation. The speculum is mounted so the cut away portion is facing the operator's dominant hand when the handle is held up. This angle allows for side access.

9. Configure work stand for rat; body positioners are rotated so the shallow stepped ends face away from the operator.
10. Once properly anesthetized, the rat is placed in a supine position on the stand. Fasten the body positioners to stabilize. Care is taken to insure the rat will not rotate to the right or left, this could compromise the technique.
11. Place selected intubation tube onto guide wire with tip on the inside of the guide wire. Note: If the tip is not appropriately placed it may pull away from the guide wire and cause difficulty or trauma as it passes the cords.
12. Apply the incisor loop and secure to the stand.
13. Tilt the stand to a 45º angle.
14. Place the small cotton swab under the tongue and rotate towards the operator to extend the tongue and lift the mandible.
15. Turn the speculum light on, rest elbow on bench/table, place hand on stand and insert the speculum gently and parallel to the hard palette. Carefully elevate until a clear view of the cords is obtained. The otoscope may be lowered or lifted to obtain a clear view but must remain parallel to the stand and should not be rocked or rotated down.
16. Once the cords are visualized insert the lidocaine applicator at side of mouth and advance toward cords. This helps eliminate laryngeal spasms and open up the larynx.
17. If the cords are not seen, apply gentle pressure to the soft palette.
18. Place 1 or 2 drops of lidocaine. Allow a few seconds for the lidocaine to take effect.
19. The guide wire is inserted at the corner of the mouth, passed through the cords into the trachea. Note: Do not insert the guide wire past the thoracic inlet.
20. Remove the speculum; advance the intubation tube over the guide wire and into the trachea. Advance until the ties are behind the incisors. Immediately remove the guide wire.
21. Lower the stand and check the tube for correct positioning.
    - Cooled mirror may show breath condensation.
    - Fogging seen at time of respiration indicates proper placement.
22. Do not attempt to intubate more than two times; trauma and swelling from repeat attempts can cause respiratory obstruction or death.
23. Carefully remove the incisor loop, loosen body positioners and gently turn the rat to the prone position.
24. The ties are secured over the whiskers and over the nose.
25. Recheck placement of intubation tube.
26. Considerable care must be taken when removing the rat from the stand and connecting to the breathing apparatus.

Note: Mouse intubation may be performed in a similar manner using a 20-21-ga IV catheter.

Animal Preparation



- Animals waiting for surgery should not be kept at a visual and olfactory distance from those animals undergoing surgery.
- Anesthesia
    - Isoflurane or sevoflurane 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 a pre-charged (with gas anesthetic) 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
    - We recommend the use of a calibrated precision vaporizer as the safest method of gas delivery. However, if gas is delivered without a precision vaporizer, a.k.a. the "Open-drop" method, the following guidelines may be used:

For induction, a concentration of 2-5% concentration of isoflurane gas is typically adequate. To use anesthetic gas at such specified concentrations, the volume of the induction chamber must be known precisely. After determining the chamber volume (it is recommended to record this permanently somewhere easily retrievable), add 0.05-0.2 ml of volatile anesthetic (in liquid form from the bottle) for each liter of chamber capacity. This can be done by applying the gas in liquid phase from its bottle to a cotton ball below the false floor of the container. For small containers, a piece of cotton can be enclosed in a histology tissue cassette and the agent may be poured or applied onto the cotton in the cassette. Use of 0.2 ml liquid agent per 1000 ml chamber volume will give about a 4% concentration of gas. In the experience of the veterinary staff at Emory University, using nine naïve ICR mice (5 males & 4 females; 2 months of age) introduced to the chamber sequentially after the introduction of isoflurane (0.2 ml/L chamber volume), recumbency was obtained in 57 +/- 21 seconds. However, for rapid and effective induction, the agent had to be replenished in the chamber approximately every 3 mice. Gas delivered by this method must be done under a chemical fume hood or type IIB Biosafety cabinets that are vented to the outside.

Volume of liquid agent/L chamber volume	Approximate concentration of isoflurane or halothane
0.05 ml	1%
0.1 ml	2%
0.2 ml	4%
0.3 ml	6%

This method of gas anesthesia delivery should only be used for very short procedures. Animals can very quickly become compromised using this technique, therefore monitoring of breathing pattern is crucial. Prolonged use (beyond a few minutes) will result in high mortality.

- Gas Anesthesia Scavenging
  - Waste anesthetic gas (WAG) from anesthetic gasses must be scavenged to minimize human exposure. Acceptable methods are:
    - Downdraft tables. These are usually only effective up to a height of 6-8 inches from the surface. Do not use induction chambers taller than this for induction of anesthesia.
    - Chemical fume hoods.
    - Type IIB biosafety cabinets that are vented to the outside.
    - Charcoal canisters. Charcoal canisters must be weighed before it is used for the first time, and after each use. Most canisters must be replaced after an increase in the recommended weight stated by the manufacturer. Depending on the size of the canister and the manufacturer's recommendations, the canister should also be weighed during especially long procedures to assure its continued effectiveness.
- Protect the eyes: Anesthetized animals should have their corneas protected with an ophthalmic ointment (not solution). Avoid touching the eye with the tip of the ointment dispenser as it may scratch the cornea. The use of petroleum-based products such as mineral oil and Vaseline are not acceptable.
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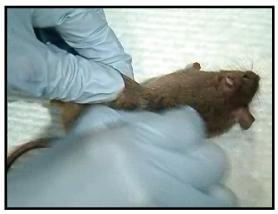
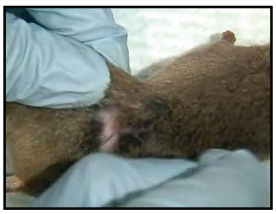
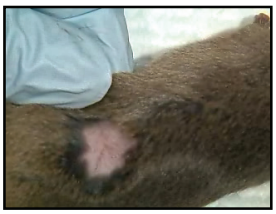
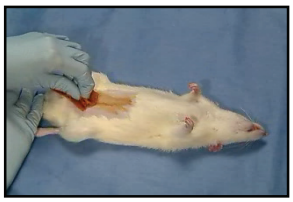
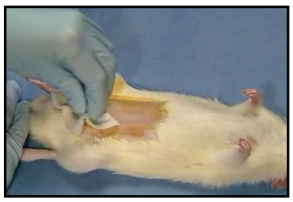
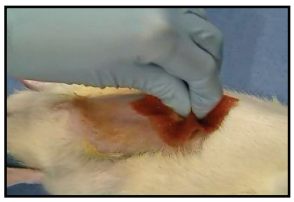
- Hair Removal
  - Remove fur along the incision site with small clippers. Clip a generous area to ensure fur does not contaminate the wound and a sufficient area that can be disinfected around the incision site, but avoid taking off too much fur, because this will reduce the animal's ability to regulate its body temperature. Use the sticky side of white tape to lift off the loose fur or a handheld vacuum cleaner.
  - An alternative to clipping is hair plucking. Hair follicles in mice (not other rodent species) are usually in telogen or resting phase and hair can be removed without injury.
  - Depilatory creams may also be used. Strict adherence to time of exposure (up to 45-60 seconds) is important as prolongued exposure of these creams on the skin may lead to chemical burns and localized inflammatory response.

- Antiseptic preparation of the surgical site:
  - The use of alcohol alone is generally not considered adequate.
  - Standard surgical prep consists of three alternating scrubs of a chlorhexidine scrub and 70% alcohol. *Although pictures in this presentation illustrate the use of iodophors (povidone iodine), chlorhexidine is my scrub and solution of choice.*
  - Using a gauze sponge or cotton tipped applicator, cleansing should be done in a circular motion.
  - Begin at the center of the hairless area and work toward the periphery.
  - Never go back to the center with the same sponge.
  - Scrubs should be alternated between a chlorhexidine scrub and alcohol, ending with an chlorhexidine solution, NOT scrub. Scrub soaps are irritating to tissue under the skin.
  - Be careful not to excessively wet the animal as this can exacerbate hypothermia.
  - The following step-by-step procedure is recommended and will serve as a guide for a proper surgical preparation:
    1. Remove hair.
    2. Apply 70% alcohol to degrease the area.
    3. Apply chlorhexidine **scrub**/soap (NOT solution) from center to periphery.
    4. Apply 70% alcohol from center to periphery.
    5. Repeat steps 3 & 4 two more times (or more).
    6. After last alcohol application, apply chlorhexidine **solution** (NOT scrub)
    7. Allow the solution to dry before making the incision – Combined chemical & desiccation actions result in the most lethal bacterial activity.

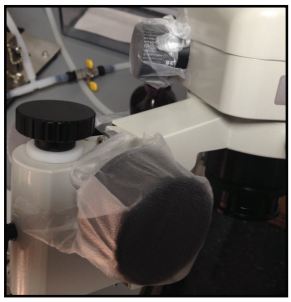
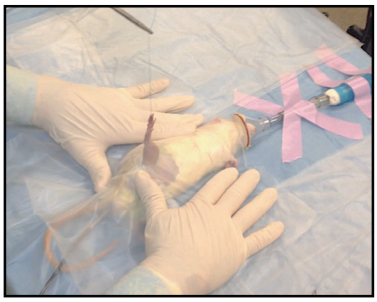
Chlorhexidine is recommended for skin prep

- Sterile draping is necessary to prevent viscera or sterile instruments from coming in contact with non-sterile areas like skin and fur. Types of drapes that may be used are:
  - Surgical impermeable paper drapes:
    - Inexpensive and autoclavable.
    - It may be precut or one in which you cut a hole.
    - A disadvantage to paper drapes is that they are not see-through and usually cover the animal making monitoring difficult.
  - Plastic drapes offer the advantage of more visibility.

- Transparent, self-adhesive drapes, provided that the animal's body is dry (use sterile gauze to daub dry prepped skin).

Glad's **Press'n Seal** provides a sterile, inexpensive and effective method to cover the surgical field. Although this is a food/grocery item, at UTSA we have tested it and results have been 100% negative for the presence of any microorganisms and organic material. The sticky part is placed on the animal, which allows easy monitoring due to the see-through nature of this material. Make sure the nose is not covered to avoid suffocation if a gas mask is not used. Press'n Seal may also be used to cover areas outside the surgical field that may need to be manipulated by the surgeon (e.g., gas anesthesia dials, knobs of the microscope or stereotaxic apparatus) and the surgical table.

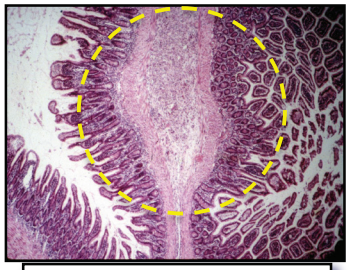



Press'n Seal – sticky side down

The sticky side of Press'n Seal should be down or it may stick to instruments and gloves. Permeable paper drapes are <u>not</u> recommended as they may allow wicking of bacteria into the wound.

<u>Surgeon</u>

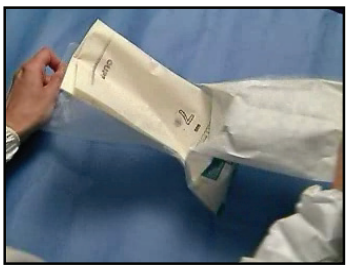
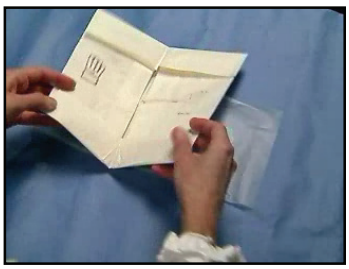
- Wash hands with an antiseptic soap.
- At minimum use sterile surgical gloves, facemask and a clean lab coat.
- When using powdered gloves remove all powder with sterile saline or alcohol. Powder from gloves is a foreign material that leads to foreign body reaction.
- If performing batch surgeries replace sterile gloves between animals.
- Sterile gowns are ideal but not required. If sterile gowns are not used, covering the arms with plastic sleeves and misting them with a disinfectant prior to donning sterile gloves will minimize contamination from the inadvertent touch of the gown sleeves with the sterile field.

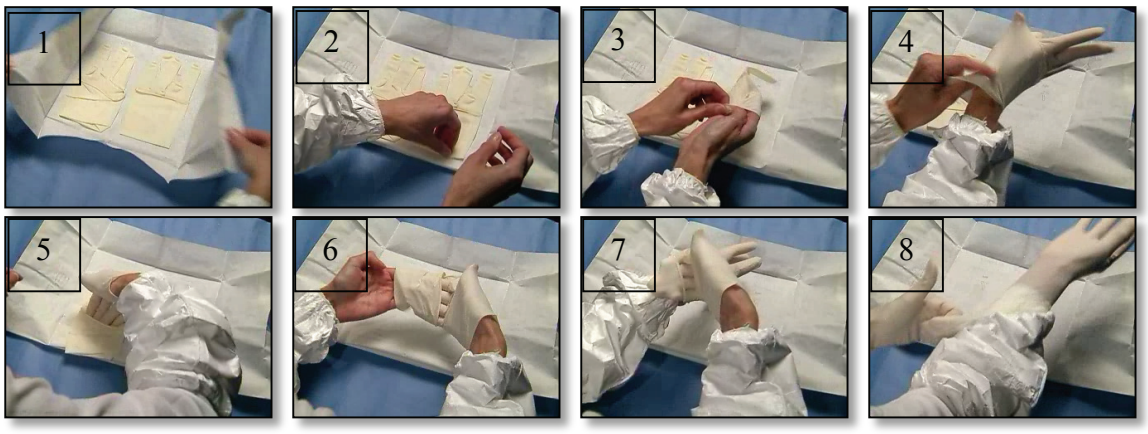


Granuloma formation from talcum powder on surgeon's gloves

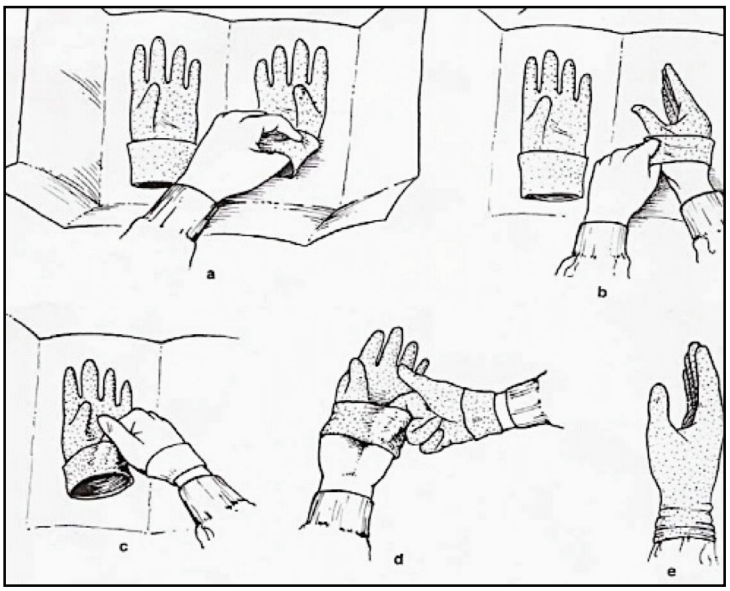
- Donning surgical gloves:
  - Open the package of gloves observing sterile technique.

Remember, the inside of the package is STERILE – exam gloves are not the same as sterile gloves.



- Donning sterile surgical gloves procedure:
  1. Don gloves in a way that prevents contamination of the outer surface of the gloves.
  2. One glove is lifted from the opened glove package by its turned down cuff.
  3. & 4. The glove is pulled on the hand with a rotating motion.
  5. Place the gloved fingers beneath the cuff of the other glove.
  6. With the gloved fingers under the cuff, the glove is placed on the ungloved hand. The folded cuff protects the gloved hand from contamination.
  7. Pull the cuff of the glove over the lab coat following insertion of the hand.
  8. The fingers are then slipped under the cuff of the first glove to pull it over the lab coat cuff.



Maintaining Asepsis

Once the surgeon, surgical areas, and animal are prepared, one must remain conscious throughout the procedure not to break the aseptic barrier that has been created.

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

- Do not allow surgical instruments to fall below the edge of the table. If an instrument does fall, the instrument is no longer considered sterile and should not be picked up and reused until resterilized.
- Replace sterile table coverings after each animal. The Use of Press'n Seal is a practical and inexpensive tool to accomplish this.

Press'n Seal table covering

ANESTHESIA AND ADMINISTRATION OF ANALGESICS

Anesthesia is a state where all perceived sensations are absent. Because drug effect 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, vigorously squeeze both rear toes firmly (toe pinch reflex) 3-4 times, to test the animal's perception of sensation and pain. If the animal withdraws its leg or if respiration rate increases, then the anesthesia is too light. The front toe pinch reflex may not be reliable as the pain perception may be present in the absence of a front toe pinch reflex.

Preemptive analgesia is the prevention of pain before it occurs or before the painful insult takes place. As an adjunct to general anesthesia, a preemptive local anesthetic(s) is used to desensitize a body area before making an incision. This reduces the pain of the surgical wound postoperatively. Preemptive analgesia is also accomplished by administering systemic analgesics before the pain insult occurs (e.g. before the surgical incision is made). In general, analgesics are more effective when administered prior to surgery and should be used in most cases unless there is a justifiable reason not to.

Much of the post-surgical pain is the result of sensations produced in the skin and body wall of the incision area. Anesthesia of the local nerves prior to incising these tissues will greatly reduce post-op pain and distress.

When skin and tissues are incised, local sensory nerves become excited and transmit impulses to the spinal cord/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 it 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 systemic analgesic is administered and/or a local anesthetic is infiltrated prior to the incision, such approach will block or diminish the sensory neuroexcitation caused by cutting the tissues even in the unconscious animal. When the animal wakes up, it 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.

An effective and simple analgesic consideration (that does not require the use of DEA controlled drugs) would be to prepare a 50/50 mix of lidocaine 1-2% with 0.5% bupivacaine. This is relatively inexpensive and easy to administer. The surgeon can infiltrate the incision area immediately after closure or better yet,

prior to making the incision while the animal is anesthetized. Lidocaine provides almost immediate pain control for 20-40 minutes and bupivacaine provides longer pain control for up to 4-6 hours. Lidocaine and bupivacaine doses should not exceed 10 and 6 mg/kg respectively. Higher doses may lead to heart arrhythmias.

METHODS OF DELIVERY OF INHALANT AGENTS TO RODENTS

The best method for the delivery of volatile agents to rodents involves the use of a precision vaporizer and an anesthesia chamber alone or in combination with a facemask appropriately sized for rodents. The **LARC has the equipment to safely and effectively administer inhalant anesthetics (isoflurane) to rodents using a precision vaporizer. Please contact LARC for details regarding use of this equipment.**

The rodent is placed within the chamber for induction at 4-5% isoflurane concentration. Pre-warmed induction chambers are recommended. Once anesthetized, the animal is removed from the chamber with anesthesia maintained by delivery through a facemask at 1-3% concentration. Both chamber and mask delivery incorporate the use of a precision vaporizer for precise control of the concentration of anesthetic gas delivered to the patient. Because oxygen flow is required to volatilize the liquid anesthetic placed within the vaporizer, oxygen is also delivered to the patient and helps to maintain the blood oxygen saturation. Oxygen should be delivered at 0.5 L/min in mice and rats. Adequate scavenging of waste anesthetic gases (WAG) is necessary to avoid exposure to personnel. In general, isoflurane or sevoflurane anesthesia is superior to injectable anesthesia. Animals are induced and recover more quickly and the depth of anesthesia is easily controlled with the vaporizer dial. This allows for greater control of the anesthetic depth and minimizes experimental variables.

Precision vaporizers must be recertified at the manufacturer's recommended interval. In the absence of a manufacturer's recommendation, certification is typically performed on an annual basis. Some IACUCs allow longer intervals depending on the degree of use of some systems.

If gas is delivered without a precision vaporizer, the guidelines given previously in these notes should be considered.

Calculating Vaporizer Oxygen Volumes:

Oxygen flow is calculated at 200-300 ml/kg/min but should never be kept at less than 500 ml/min, so for rats and mice maintain oxygen flows at 500 ml/min (0.5 L/min). 500 ml/min is an ideal oxygen flow rate.

ANESTHETIC MONITORING OF RODENTS

Parameters that can be used to assess the depth of anesthesia in rodents include:

- Recumbency and loss of purposeful movements.
- Muscle relaxation.
- Lack of vocalization and limb movement.
- Loss of response to aversive stimulation (e.g. pinching the rear toes or tail pinch).

Because the ratio of body surface area to body mass is greater in rodents than in larger species, thermal support is critical to the successful recovery of rodents from anesthesia. Body heat may be dissipated from the tail, soles of the feet and ears with a resultant profound decline in the core and surface body temperature. Hypothermia may, in turn, lead to a decline in both anesthetic metabolism and any urinary excretion of the anesthetic agent. When possible, ensure the tail (a major thermoregulatory organ in rodents) is heated during surgery.

In most instances, cardiovascular and respiratory assessments are limited to observations of chest wall movement to determine respiratory rate and palpation of the apical pulse through the chest wall. For complex and long procedures physiological monitoring is recommended. Physiological monitoring systems

like Kent Scientific's (www.kentscientific.com) Physiosuite or Somnosuite (the latter also delivers rodent precise isoflurane and sevoflurane anesthesia using the same system) provides simple monitoring resulting in improved post-op recovery.

Somnosuite

Physiosuite

SUPPORTIVE CARE OF ANESTHETIZED RODENTS

Methods to minimize heat loss to the environment during anesthesia of rodents include increasing the ambient temperature of the operating room; placement of a thermal blanket (e.g. homeothermic blanket or recirculating warm water blanket) or drape between the animal and the stainless steel operating table; use of heat lamps (carefully placed!); pre-heating of skin surgical prep solutions; minimization of organ exposure from body cavities during surgery; recovery of the animal on a warming blanket or within a temperature-supported cage; administration of warmed subcutaneous or intraperitoneal fluids before, during or after the anesthetic episode; housing on bedding during recovery to provide thermal insulation; and recovery with cage mates to permit animals to huddle together and thus provide thermoregulation. Do not place an unconscious rodent in a cage with an awake as the alert animal may mutilate the anesthetized rodent.

Fluid deficits can be corrected by subcutaneous or intraperitoneal injection of warmed saline, Lactated Ringers solution or replacement fluids (e.g., Normosol).

The pre-op administration of warm solutions can greatly decreased intra- and post-op mortality and it is highly recommended. In general, I make it a habit to administer fluids before surgery at a rate of 0.5-1.0 ml SC or IP in mice and 5-10 ml SC or IP in rats.

Ophthalmic ointment should be applied to the eyes as soon as the animal is anesthetized. The use of petroleum-based products such as mineral oil and Vaseline is not acceptable. Application of ophthalmic solutions will not provide the same level of eye protection that ophthalmic ointments do.

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 rear toes or tail for reflex response or by response to surgical painful stimuli.
  - Any reaction of the animal indicates the animal is too light and should be given more anesthetic.
  - 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 opposed to pale, dusky grey or blue indicates tissue perfusion and oxygenation.
  - Respiratory pattern and frequency will also give an indication of anesthetic depth.
  - Core body temperature can also be monitored in rats and mice.
  - Pulse oximetry can be used in to monitor pulse and oxygenation.
  - Electrocardiograms can also be used.
  - Respiration – animal turns "blue" (hairless areas) if hypoxic.
    - Evaluate the need for delivering oxygen… no special equipment is required. A tube delivering 100% oxygen from a tank (turned to low flow with a down regulator) can be taped onto the table in the vicinity of the animal's nose. Alternatively, a facemask may be made from a syringe case or syringe case.
    - Maintain airway patency.
      - Be careful in positioning the animal's head and neck.
      - Prevent blockage of the respiratory passages by blood, mucus or other material.
    - If respiration rate falls progressively or seems labored:
      - If surgery is in progress, assist ventilation by gentle compression of the chest.
      - If surgery is complete, administer an anesthetic antagonist (if appropriate) or a respiratory stimulant (e.g. doxapram 5-10 mg/kg IV or IP, repeated at 15 min intervals as needed). For anesthetic antagonist see Atipamezole under POST-OPERATIVE MONITORING section.
  - Cardiovascular function – the animal's hairless areas (normally pink) turn "pale" if tissue perfusion is poor.
    - Assess the cause of cardiac impairment:

- *Anesthetic overdose* – if appropriate, use an antagonist or an anticholinergic (e.g. atropine or glycopyrrolate).
- *Hypothermia* – perhaps the greatest cause of rodent surgical mortality.
- *Hemorrhage* of 3-4 ml loss in a 200 g rat will cause irreversible shock.
  - Surgical technique to 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 but reactions likely after more than one transfusion.
  - Blood volume is approximately 70 ml/kg. Hemorrhage and blood loss of 10% volume is tolerable, but 20-25% loss will cause shock.

	Blood Vol	**10% loss**	**20% loss (shock risk)**
Mouse 20 g	1.5 ml	0.15 ml	0.3 ml
Rat 200 g	15 ml	1.5 ml	3.0 ml

  - Consider the use of fluid therapy – to support cardiovascular function or prevent dehydration.
    - Animals may have reduced food and water intake for 1-2 days after surgery. Providing sterile, warmed, physiological fluids (SC or IP) can be used to compensate for hemorrhage and reduction in water intake postoperatively.
      Recommended fluid replacement
      - Mice: 17–33 ml/kg (~0.3-0.7 ml for a 20 g mouse) SC and 33 ml/kg (~0.7 ml for a 20 g mouse) IP.
      - Rats: 25 ml/kg (~5 ml for a 200 g rat) SC or IP.
      - 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.
      - Sterile LRS or physiological saline warmed to body temp may be injected before the procedure and it is highly recommended, especially if a prolonged recovery is expected or extensive hemorrhage may be likely. 0.5-1.0 ml SC or IP in mice and 5-10 ml SC or IP in rats
  - Consider whether the animal will have a reduced water intake for 12-24 hours post-op. Provide replacement fluids following guidelines in the previous section.
  - ECG – capability required for low amplitude and high rate monitoring.
  - Tail cuff for blood pressure.

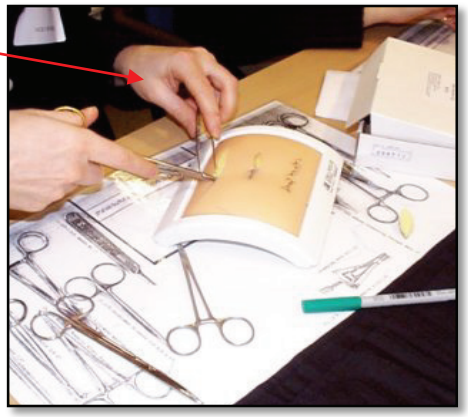
SURGICAL TECHNIQUE

Good Technique includes:

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

Skills are Practiced, Developed, and Refined
Practice, Practice, Practice

Wrong instrument holding – See following pages

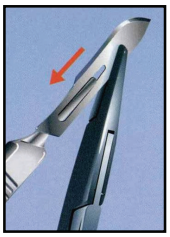


TIPS:

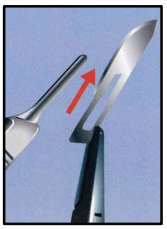
- Minimize contamination of the operative field during surgery by restricting the movement of gloved hands and sterile instruments.
- Plan the incisions to avoid large skin or body wall blood vessels.
- Handle tissues gently and avoid excessive force in tissue retraction, which can cause necrosis.
- Avoid or minimize hemorrhage, but if it occurs, wick away blood with a sterile gauze sponge, Q-tips or gel foam spears. Avoid using a wiping action, which traumatizes tissues and may cause renewed bleeding. Use a wicking or blotting action instead.
- If a wound becomes contaminated, use copious amounts of warm, sterile LRS or saline to irrigate and cleanse the area.

INSTRUMENT HANDLING: Generally scissors and hemostats are held with the thumb and the ring finger. Thumb forceps are held using a pencil grip technique.

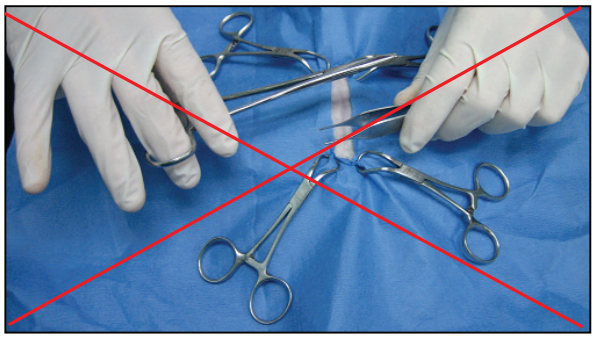
The following images illustrate proper (and improper) instrument handling:



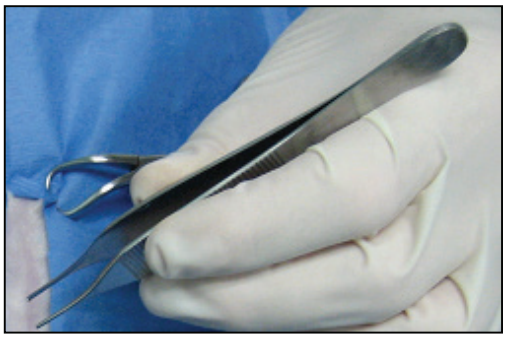
Safe surgical blade loading with the needle driver on to the blade handle



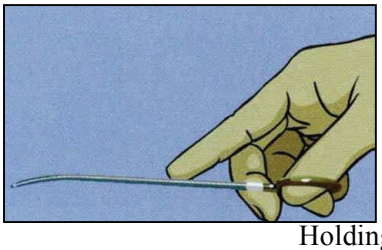
Safe surgical blade unloading

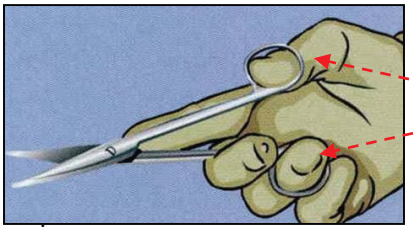


Wrong instrument holding

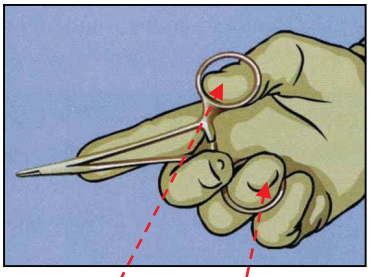


Correct holding: Pencil grip technique for holding thumb forceps

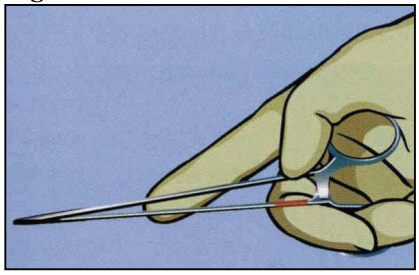




Holding hemostats or scissors
Thumb & Ring finger

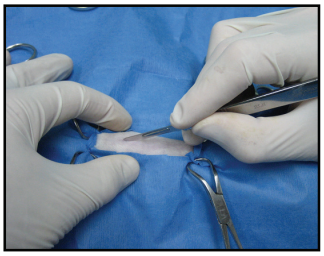


Holding the needle driver
Thumb & Ring finger



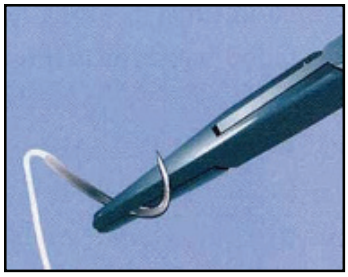
Palming the needle driver

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.

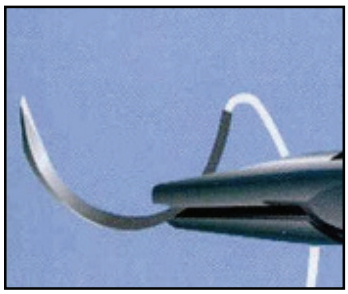


NEEDLE TYPE: If suturing with a needle, use the right type of needle for the type of tissue.

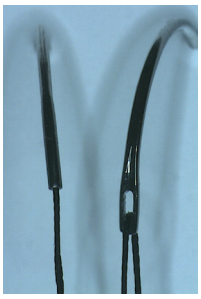
SOFT TISSUES – Use a **tapered** (round-bodied) needle on internal tissues (e.g. intestine, muscle, peritoneum). This type of needle passes atraumatically through soft tissues and allows them to "seal" behind the needle. Generally it is best not to use a cutting edge needle in soft tissues because this 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).



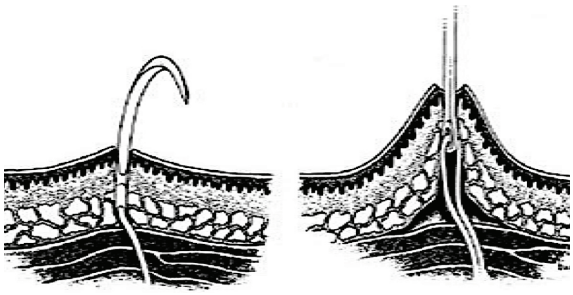
SKIN – Use a **cutting edge** needle on the skin (cutting or reverse cutting needle). The dermis has tough fibrous tissue. To pass a needle through it, cutting edges are needed to slide the needle through the skin. This minimizes trauma and irritation to the skin. As a result, the animal will be less likely to self-traumatize the sutured incision. 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.



Swaged needles impose less damage to tissues than do threaded (eye) needles and result in less tissue drag and trauma.



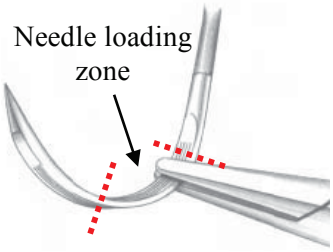
Swaged vs eye needle



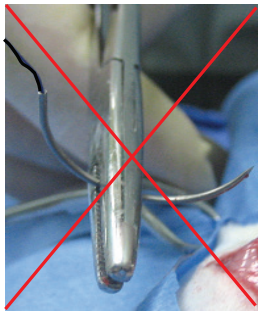
Tissue drag caused by swaged vs eye needle

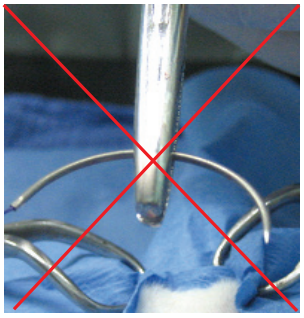
ARMING THE NEEDLE:

Load the needle in its middle third (the loading zone)

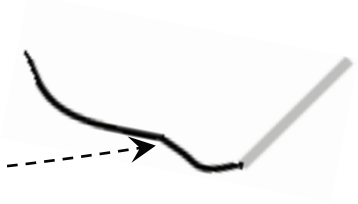


Hold the needle with the tip of the needle holder with the needle perpendicular to the jaws of the needle holder





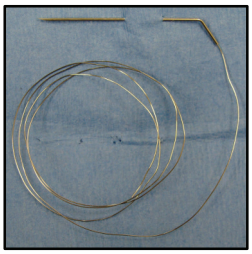
Holding the needle too close to the site of suture attachment will result in needle bending.



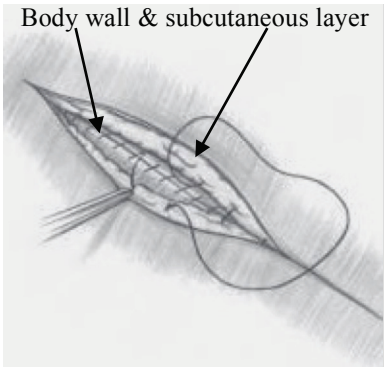
SUTURE MATERIAL: Use the right kind of suture material for the type of tissue.

- Internal layers – Use an **absorbable** material, unless permanent sutures are needed. Example material: Vicryl, PDS, Dexon, Maxon, in general with suture sizes of 4-0, 5-0 and 6-0 for rats; 5-0, 6-0 and 7-0 for mice. Silk is frequently used for cardiovascular procedures.

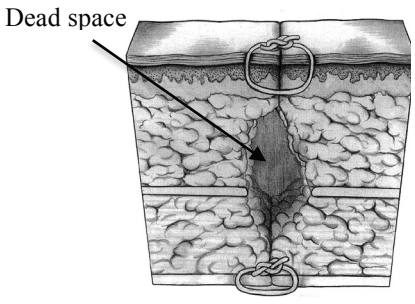
- Skin layer – Use a **nonabsorbable monofilament** sutures in skin (Prolene, nylon, stainless steel), wound clips, staples and/or tissue glue.
  - DO NOT use braided sutures, like "silk" because they can wick bacteria and lead to tissue reaction and infection. This raises the likelihood of self-trauma.
  - Sizes 4-0, 5-0 and 6-0 for rats; 5-0, 6-0 and 7-0 for mice.
  - Stainless steel (SS) to suture skin in rats may be assembled inexpensively by purchasing a spool of 30 ga. SS orthopedic wire.
    - A designated length of SS wire is threaded through a 22 ga. needle.
    - The needle is bent at ~120^0 to secure (kink) the wire in the needle.
    - The hub of the needle broken away and discarded.
    - The suture and needle must be autoclaved before using in survival procedures.
    - When closing skin, two throws are enough to secure the suture.



SUTURE LAYERS AND PATTERNS:

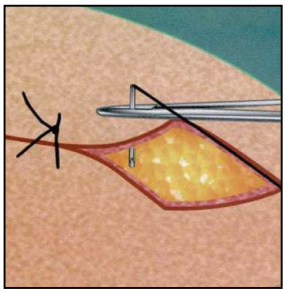
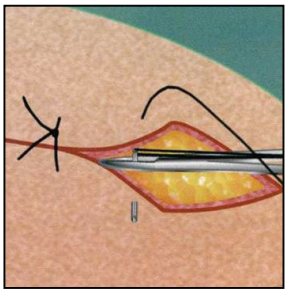
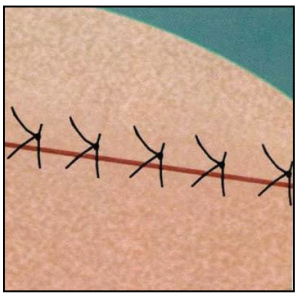


1. Body wall (abdominal) – The suture line should be a simple interrupted or simple continuous pattern, using absorbable suture material.

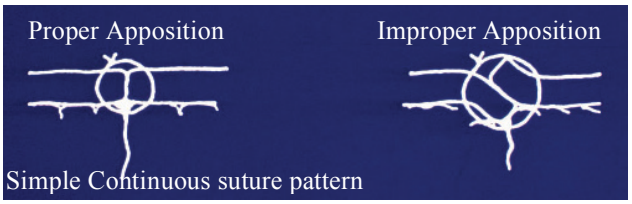


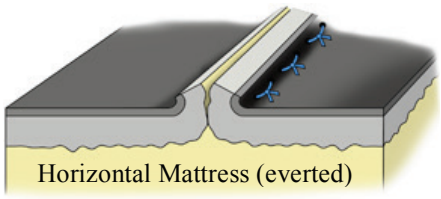
2. Subcutaneous tissue – The suture line should be in a single continuous pattern, using absorbable suture. This may be needed in some larger and obese rats with a sizeable amount of subcutaneous tissue. It is generally not used in mice. Closing this layer collapses the potential space between tissue layers also known as "dead space," preventing seroma and abscess formation.

3. Skin – The suture line should be closed with nonabsorbable/monofilament material in an interrupted pattern such as a simple interrupted or a horizontal mattress fashion.
   - Insert the needle about 4-5 mm from the wound margin.
   - Space interrupted sutures (or clips) about 5-8 mm apart.
   - Leave a longer tail on the suture to prevent unraveling and to make it easier to grasp when the skin suture has to be removed.

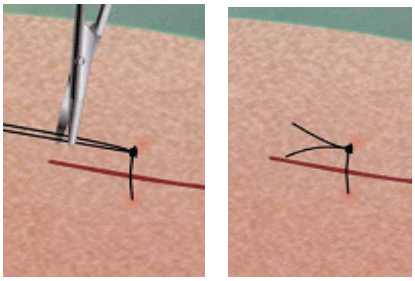
   - Some rodents gnaw at externalized sutures. In such cases a buried suture line (subcuticular pattern) or wound clips may be preferred.
   - Cyanoacrylate skin glue (e.g. Vetbond, Nexaband, Dermabond) can be used for non-tension bearing wounds to appose skin edges for small incisions or to reinforce skin edges between sutures. Don't "bathe" the skin wound because animals may be tempted to self-traumatize the area if there is excessive glue residue on the skin surface. Carefully place a tiny drop via an applicator tube onto the skin. Use forceps or fingers to push the apposing edges of skin together. Avoid getting adhesive on the fur, or else the animal may later open up the wound in the process of removing the glue from its fur.
4. Closure – Proper apposition:
   - Restore alignment of the tissues.
   - Balance adequate closure with too much suture. Suture is a foreign body and too much of it can affect healing.
   - Skin closure should be done by apposition of cut ends and not by overlapping of layers. An everting closure as in a horizontal mattress pattern is fine too.

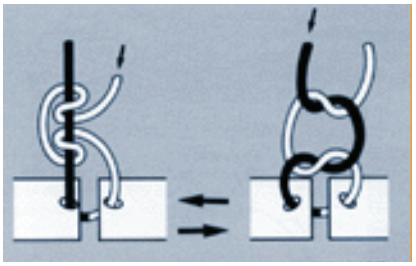




KNOT TYING/SECURITY

- Tie all sutures (any layer) with square knots (3-4 throws internal sutures, 5-6 throws skin). Square knots provide greater knot security against slippage when compared to slip knots.
- Don't cut knot strands too close to the knot. If cut too short, they may come undone later.
- If skin sutures are cut too long, the animal may chew on them and in so doing, remove the suture. Leave enough length to make it easier to remove at the time of tissue removal.

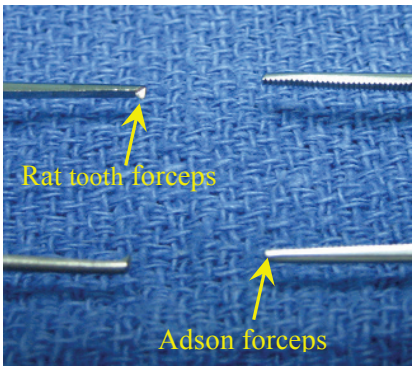




Slip Knot Square Knot

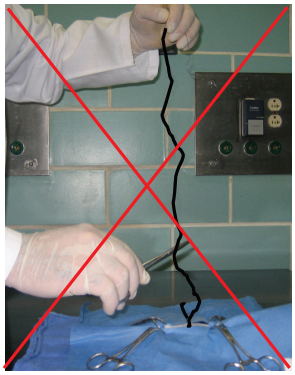
GRASPING TISSUES WITH THUMB FORCEPS

- Generally skin and body wall (linea alba) are grasped with fine rat tooth or Brown Adson forceps but not with Adson forceps. The force to grasp skin with Adson forceps will be greater than when using a rat tooth forceps leading to greater tissue damage.
- Rat tooth forceps could be injurious to soft tissue like gut, kidney, liver and other tissues.

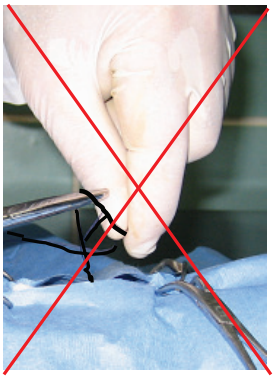


HOLDING THE SUTURE TOO FAR OR TOO CLOSE?

- When suturing, hold the long end of the suture with your non-dominant hand at a comfortable distance from the knot (not too far, not too close). Learn what your "sweet" long strand length should be, which is best learned through practice.



Suture held too far

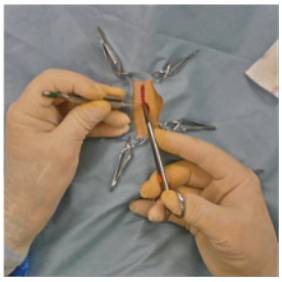


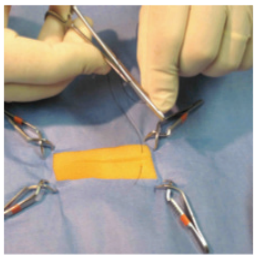
Suture held too close

IMPROVING SPEED AND ACCURACY WHILE DECREASING TISSUE TRAUMA
(Remember: *Surgery is Gentle*!!! *& Time is Trauma*!!!)

Stabilize your hands on a towel or table to minimize trembling

Note the comfortable, well-supported & rested position of the hands, while using the middle, ring & pinky fingers to stabilize the instruments & hands.



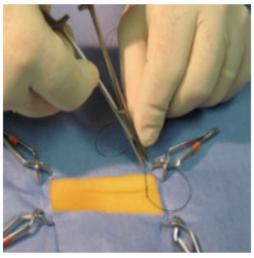


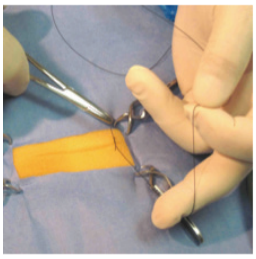
Hold the long suture strand with the non-dominant thumb and index finger. Rest the jaws of the needle driver on the non-dominant index finger to stabilize and improve your moves

Point the tip of the thumb and index towards the suture knot and incision

Synchronize the dominant and non-dominant hands to move the jaws of the needle driver towards the short tail of the suture

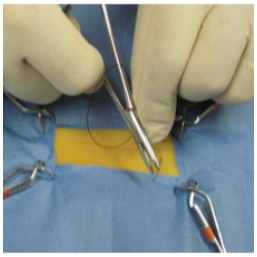
Grasp the short tail of the suture with the jaws of the needle driver

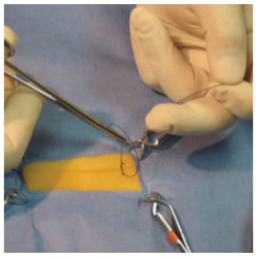




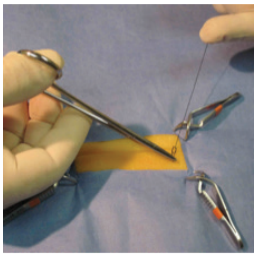
Use the dorsal surface of the non-dominant middle finger (distal phalanx) to pull on the long suture strand while pulling on the short strand with the needle drive

As above, grasp the short tail of the suture with the needle driver resting the jaws of needle driver on your non-dominant index finger to stabilize the needle driver and improve your moves





Use the palmar surface of the non-dominant middle finger (distal phalanx) to pull on the long suture strand while pulling on the short strand with the needle driver



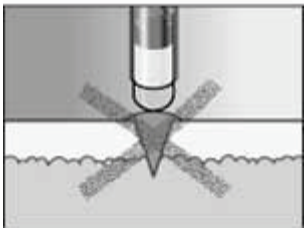
EZ CLIPS

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

TISSUE ADHESIVES

Wound closure should be simple, rapid and painless.
The ideal surgical adhesive would be safe for topical application, easy to apply, polymerize rapidly, support the approximated skin edges and maintain the skin edge securely for maximum wound healing and eliminate the need for suture removal. Tissue adhesives offer many of the advantages of the ideal wound closure devices. They are painless and have antimicrobial activity against gram-positive organisms. They have a low rate of dehiscence and a low infection rate, and provide excellent cosmetic results. The use of **Dermabond** and **Nexaband** Topical (2-Octyl Cyanoacrylate) as well as **3M Vetbond** (n-butyl cyanoacrylate), which polymerize in seconds after contact with tissue, significantly decrease the time for wound closure and eliminate the need for postoperative suture removal where appropriate. Additional benefits of these products include ease of use and formation of a protective barrier. Tissue adhesives are contraindicated when the wound is under tension.

A Guide for Using Tissue Adhesive



1. Use subcutaneous or subcuticular sutures as needed to eliminate wound tension. Tissue adhesive should not be used as a replacement for proper subcutaneous closure.
2. Be sure wound edges and surrounding skin are dry, to assure direct tissue contact and prevent premature polymerization of adhesive.
3. Manually approximate the wound edges with forceps or gloved fingers.
4. Use gentle brushing strokes to apply a thin film of liquid to the approximated wound edges, and maintain proper eversion of skin edges as you apply adhesive. Gradually build up 2-3 thin layers of adhesive. Ensure the adhesive is evenly distributed over the wound. The adhesive should extend at least ½ cm on each side of the apposed wound edges.
5. Avoid seepage into the wound as it may delay wound epithelialization and healing.
6. Avoid contact with eyes.

DEHISCENCE – Suture lines coming undone.

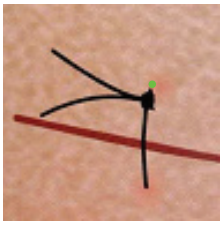
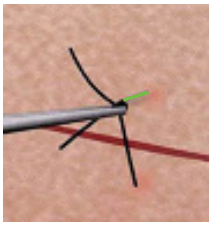
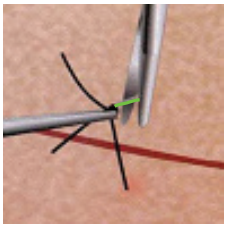
- The animal will chew and attempt to remove sutures if they are irritating.
- Be aware whether the suture strand will poke a body part or fold of skin. In skin fold areas, a suture strand may jab the skin and cause irritation. This may occur with monofilament nylon or polypropylene (Prolene), because the cut end is stiff. 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 for animals removing their stitches.

- Maintain good aseptic technique. Infection macerates the wound margin and causes sutures to loosen and fall out.

SUTURE REMOVAL

- Whether using sutures, clips, or staples, these must generally be removed from the skin at 10-14 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 may chew and remove the sutures or clips because of the irritation.

- Remove sutures by lifting the knot and cutting the suture portion that was under the skin prior to pulling, and then cut as close as possible to the skin as possible.

WHEN TO USE ANTIBIOTICS (or Not…)

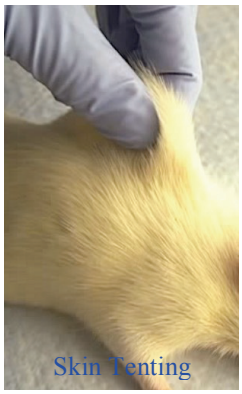
- Antibiotics are rarely needed when true aseptic principles are adhered to, but when needed, keep the following guiding principles in mind:
  - "Antibiotics may make a 3rd rate surgeon into a 2nd rate surgeon, but they will never make a 1st rate surgeon out of a 2nd rate surgeon" (Vince Mendenhall).
  - Antibiotics may be a source of variables and should only be used when there is justifiable necessity.
  - Pre-op antibiotics can reduce the risk of infection in complicated surgeries but should not be used as a substitute for proper asepsis (false sense of security…).
  - Consider antibiotic rotation to minimize development of resistant strains.
  - When appropriate, administer antibiotics before surgery so highest tissue levels are present at the time of surgery. In such cases, one pre-op dose is generally enough.
  - Use broad spectrum antibiotics.
- To evaluate if antibiotics are needed, keep the following principles in your decision-making process. In other words, antibiotics may be indicated under the following conditions:
  - In immune deficient strains (some strains are more compromised than others) or if the animal is severely stressed, ill or aged.
  - When entering certain hollow organs (e.g., bladder, respiratory track, gastrointestinal track).
  - When surgery involves extensive tissue dissection and/or blood loss.
  - If inadvertent contamination occurred during the surgical procedure.
  - When devices and biomaterials (e.g., catheters) are implanted.
  - When the procedure is being conducted by an inexperienced or poorly trained surgeon leading to excessive trauma. As experience increases, need for antibiotics decreases accordingly.
- In guinea pigs, rabbits and hamsters, inappropriate antibiotic use can cause fatalities due to their effect on their selected gut flora.

POSTOPERATIVE CARE

- If possible, use anesthetic/sedative antagonists to recover the animal more quickly from anesthesia.
  - **Atipamezole (Antisedan)**
    - 0.5-1 mg/kg SC, IM, IM, IV can be used to reverse ketamine/xylazine and ketamine/dexmedetomidine anesthetic cocktails.

- Administration of atipamezole is highly recommended after ketamine/xylazine or ketamine/dexmedetomidine anesthesia and can be administered immediately after completion of the surgical procedure to awaken the animals. However, atipamezole is an antagonist of xylazine and dexmedetomidine, not ketamine. Therefore early reversal (10–20 minutes after induction) is associated with undesirable behavioral disturbances due to the effects of ketamine.
- **Doxapram** 5-10 mg/kg IV or IP may be administered for respiratory depression. Redose as necessary at 15 min intervals.

- Continue providing an external source of heat until the animal is conscious enough and beyond if necessary. Animals are considered conscious enough when the righting reflex has returned. The righting reflex is tested by placing the animal on its side or back. If the animal places itself on its four feet, then the righting reflex has returned.
- Provide the post-surgical animal a new cage that contains clean bedding to minimize wound contamination.
- Assess food and water intake for several days.
  - Animals may not drink for one or more days post-op and may therefore dehydrate. Recommended fluid replacement for mice is 17–33 ml/kg SC and 33 ml/kg IP; and for rats is 25 ml/kg SC or IP.
  - If animals are dehydrated, provide further fluid therapy and consider doing so, ideally by IV infusion. However, since IV administration is difficult in rodents, SC or IP administration provide a good alternative. Oral dosing via gavage may also be done. Test for dehydration by pinching and pulling the skin just cranial to the shoulder blades into a tent and then releasing it ("tenting the skin"). If normally hydrated, the skin will snap back towards the body. If dehydrated, the skin will fall slowly into place.


Skin Tenting

  - 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. Some analgesics depress appetite and must be differentiated from that which occurs if an animal is not feeling well. Weighing animals may be stressful to rodents and in place of this, a Body Scoring System (BCS) can also be used to assess the animal's condition. BCS can be seen in the following pages.
  - Rodents have high-energy requirements due to their small size and high metabolic rate, yet they have minimal fat reserves, which can be mobilized to supply needed energy (more so in mice). Nutritional support is critical upon recovery to avoid hypoglycemia. Nutritional support can be provided by simply providing a high-quality pelleted rodent diet as soon as the animal has recovered sufficiently to ambulate and eat. Highly palatable and energy dense diet gels that contain moisture placed or products like Bacon Softies contained in containers at the bottom of the cage can be especially helpful for the recovery rodent and help reverse the post-op hypercatabolic state quickly. When considering other enticing post-op diets, keep in mind that rodents have a flavor preference for bacon, chocolate and berry.

DietGel
clearh2o.com


Bacon Softies
bio-serv.com

- Observe the animals for signs of pain or distress postoperatively.
  - 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.
  - Unless proven otherwise, if a procedure is likely to produce pain in humans, it should be assumed to be painful in animals and should be treated with analgesics.
  - Compare posture and activity with normal animals.
  - Rats and mice are able to mask pain. Therefore, pain may be evident in altered behavior or it may not. Most evident will be a reduction in food and water consumption. Because of their ability to mask pain, it is my opinion that when pain is evident, it is likely to be at the moderate to severe levels. Therefore, a good and timely post op analgesic regimen is paramount not only to protect against moderate/severe pain but for mild pain as well.
  - Abnormal signs of pain are hunched posture, ruffled fur, red staining of eyes and nares, vocalization, greater or less tissue coloration, greater or less activity, resenting being handled, reluctance to be moved, abnormal gait, abdominal presses, aggressiveness, low water or food intake, and subtle weight changes.
  - The first 24 hours are 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.
- **In general the following guidelines should be used during the immediate post-op period:**
  - Never leave unconscious animal unattended (monitor continuously until return of righting reflex, i.e. sternal).
  - Administer analgesics as stated in the protocol.
  - Provide supportive therapy as needed (e.g. supplemental heat, warmed SC or IP fluids).
  - Monitor/document animal and incision daily for at least five days.
  - Notify your veterinary group if any complications appear.
  - Document post-op findings and activities. The following is a suggested 3x5 card that may be used for post-op monitoring.

FRONT

Surgery Date_____

Sutures/Clips Removal (date)_____

RODENT POST-SURGICAL CARE CARD

Protocol # _____

Animal ID _____

PI _____

Contact Ph # _____

Surgery Site (e.g. abdomen, hind leg, head, etc.)

_____

Drugs, Doses and Routes (Anesthetics, Analgesics, Antibiotics, fluids, etc.)

_____

_____

_____

_____

_____

BACK

Post-operative monitoring/notes must be continued minimum of 5 days after surgery

Date/Time	Remarks (Incision, Pain, Rx, Behavior, etc)	Initials

Sample of 3x5 Post-op Rodent Record

BCS 1

Animal is emaciated

- **Skeletal structure extremely prominent; little or no flesh cover**
- **Vertebrae distinctly segmented**

BCS 2

Animal is under conditioned

- **Segmentation of vertebral column evident**
- **Dorsal pelvic bones are readily palpable**

BCS 3

Animal is well conditioned

- **Vertebrate and dorsal pelvis not prominent; palpable with slight pressure**

BCS 4

Animal is well over conditioned

- **Spine is a continuous column**
- **Vertebrae palpable only with firm pressure**

BCS 5

Animal is obese

- **Animal is smooth and bulky**
- **Bone structure disappears under flesh & SC fat**

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. …2+, 2, 2-)

Body Condition Scoring (BCS) Guide. Adapted from Ullman-Cullere, MH, and Foltz, CJ. 1999. *Lab Anim Sci* 49:319-323.

NON-PHARMACOLOGICAL METHODS OF PAIN CONTROL

Often ignored, non-pharmaceutical methods of pain control constitute an important adjunct to controlling post-operative pain. Anthropomorphizing this concept is perhaps the best way to understand the importance of this important method to address pain. For instance, imagine yourself having a splitting headache such as a migraine. What would you want your immediate surroundings be? You would want low or no lights, minimal noise, comfortable bed to lay on, and minimal disturbance. All these factors help reduce the stressors that worsen pain. These are examples of non-pharmaceutical methods of pain control. Just like humans, animals also benefit from this often-ignored method of pain control.

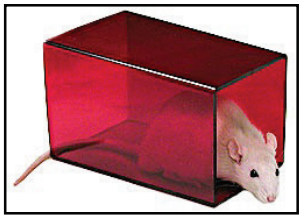
Examples of non-pharmacological methods of pain control in rodents are:

- Keeping **low ambient lights** in a quiet room.
- Maintaining the **animals normothermice**.
- Supporting **hydration** (supplemental fluids).
- Administration of **post-operative agents and analgesics as close as possible to their indicated therapeutic time**.
- Providing **energy dense, highly palatable, and easily accessible** (at cage bottom) **supplements** will help reverse the immediate post-op catabolic state and speed up recovery. Two examples of these products are illustrated below.

DietGel
clearh2o.com

Bacon Softies
bio-Serv.com

- **Social housing during the post-op recovery period**: Single housing has been used as the norm during the post-op period in rodents with the traditional belief that pair or group housing after surgery may result in animals disturbing each others' wounds. Evidence has shown that post-op social housing for mice and rats has definite benefits in terms of increased survival and faster recovery rates (Van Loo et al., 2007). Animal Care Committees should require justification when post-op single housing is requested in these species.
- **Enrichment**: Species-specific enrichment that promote species-specific behaviors such as hiding places, nesting and burrowing materials have been shown to decrease stress in the post-operative period and decrease blood pressure in hypertensive strains. Minimization of post-op stress is a critical non-pharmaceutical method of pain control (Disselhorst et al., 2010 and Baran et al., 2010).



ANALGESIA

Analgesics are often dismissed "in the name of science." The Argument for not using analgesics, "it may affect my research results!!!" is riddled with more myths than truths.

Using Post-op Analgesics - MYTH OR TRUTH???

Surgery is a form of stress and physiological stress upsets the body's homeostasis. The body responds to this by stimulating the nervous, endocrine, and immune systems leading to short and long-term physical effects on the body. In trying to justify withholding analgesics (because of the potential for "real" or "perceived" effects they may have on the animal's immune system), the researcher, Attending Veterinarian, and animal care committee should also consider the effects that unalleviated pain may have on the animal and the experimental results, which in many or perhaps most cases may affect data more profoundly than analgesics would. Withholding post-op analgesics may therefore introduce a powerful variable in your research.

Some of the effects of unalleviated pain may include:

1. Activation of the sympathetic nervous system.
2. Increased cardiac output.
3. Increased systemic vascular resistance.
4. Increased blood pressure.
5. Increased oxygen demand.
6. Vasoconstriction of coronary arteries.
7. Ischemia of the spleen.
8. Adverse renal effects.
9. Increased glucose, cytokines (IL6- C-protein), aldosterone, catecholamines, growth hormone, vasopressin, renin, prolactin, glycogenolysis and/or lipolysis, follicle stimulating hormone, luteinizing hormone and testosterone.
10. Fluctuating levels of insulin and glucagon.
11. Increased homeothermic responses.
12. Increased sodium and water retention.
13. **Release of endogenous glucocorticoids, which are immunosuppressive.**

Recommended Surgical Analgesic Protocols for Mice and Rats[1]

IMPORTANT CONSIDERATION: This is a guideline for classifying pain categories to common surgical procedures in mice and rats to aid the investigator, the Attending Veterinarian, and the animal care committee in deciding an appropriate peri-operative analgesic protocol. This guideline must be considered against other factors, such as length of procedure, extent of tissue dissection, degree of blood loss, materials implanted, unexpected surgical events, health status, age, strain, and surgeon's skills.

[1] Adapted from the Veterinary Bioscience Institute *Rodent Analgesia Webinar – Part 2*

Recommended peri-operative analgesic protocols for mice and rats

Mild Pain

Preemptive[2] (once)		Lidocaine/bupivacaine as local infiltration
Post-surgical	**Drug**	Buprenorphine, morphine or oxymorphone
	Frequency	Once

Mild to Moderate Pain – OPTION 1

Preemptive (once)		Lidocaine/bupivacaine as local infiltration
		AND
		Buprenorphine, morphine or oxymorphone
Post-surgical	**Drug**	Buprenorphine
	Duration	1-2 days

Mild to Moderate Pain – OPTION 2

Preemptive (once)		Lidocaine/bupivacaine as local infiltration
		AND
		Buprenorphine, morphine or oxymorphone
Post-surgical	**Drug**	Carprofen, ketoprofen or meloxicam
	Duration	1-2 days

[2] Administration of analgesics prior to induction of pain.

Moderate to Severe Pain

Preemptive (once)		Lidocaine/bupivacaine as local infiltration
		AND
		Buprenorphine, morphine or oxymorphone
Post-surgical	**Drug**	Buprenorphine
	Duration	2 days
		AND
	Drug	Meloxicam (use highest dose)
	Duration	2-3 days
		AND
	Drug	Morphine for severe pain
	Duration	As needed

Examples of mild, moderate, and severe post-surgical pain in mice and rats*

Mild:
- Subcutaneous pump or pellet implantation.
- Tail clipping.
- Intracerebral electrode implantation.
- Simple laparoscopic biopsies.

Moderate:
- Vascular catheterization.
- Embryo transfer.
- Ovariectomy.
- Orchiectomy.
- Craniotomy.

Severe:
- Orthopedic Procedures.
- Thoracotomy.
- Organ transplantation.
- Major laparotomy procedures.

General Dosages

	Mouse (mg/kg)	Rat (mg/kg)	*
Buprenorphine**	0.05-0.1 SC q8-12 h	0.01-0.05 SC q8-12h	O
Oxymorphone	0.2-0.5 SC q4h	0.2-0.5 SC q4h	O
Morphine	1-2.5 SC q2-6h	1-2.5 SC q2-6h	O
Ketoprofen	2-5 SC q24h	2-5 SC q24h	N
Carprofen	2-5 SC q12-24h	2-5 SC q12-24h	N
Meloxicam	1-2 SC, PO q12h	1-2 SC, PO q12h	N

*O = Opioid, N = NSAID (non-steroidal anti-inflammatory drug)
** Buprenorphine is the only opioid with long duration effect in rodents.

- Types of analgesics: Opioid and nonsteroidal anti-inflammatory drugs (NSAID).
- Choice need not be limited to one or the other as both can be given and are additive in effect.

NSAID dosing caution:
1. Ensure that animals are adequately hydrated (skin pinch test, or serum Total Protein test) before administering an NSAID to avoid renal damage.
2. NSAIDs must be used with caution beyond 3 days as they may have deleterious effects on the gastrointestinal mucosa. This may be especially true when using ketoprofen and flunixin.

Opioid dosing caution:
1. Opioid agents enhance sedative and respiratory depressive effects of anesthetics.
2. For rodents anesthetized without respiratory support (intubation, ventilation and oxygen supplementation), you may wish consider opioid administration until the end of surgery.
3. In this case, an NSAID may be the preemptive analgesic of choice.
4. If ventilatory support can be provided and an opioid is used as a preemptive analgesic agent, expect to reduce the dose of anesthetic agent (e.g. injectable and volatile anesthetics) by 30-50%.

Oral dosing caution:
1. Animals should be acclimated to oral medications before the surgery. When added to the drinking water, rodents may initially refuse to drink until they become adjusted to the flavor, which could be disastrous postoperatively. Rodents, particularly rats, can be neophobic.
2. When used in drinking water, analgesics should generally be administered 3-5 days prior to surgery.
3. Consideration to the use of analgesics in drinking water must take into account that, postoperatively, animals may decrease fluid intake and may therefore not receive the intended analgesic dose.

Lidocaine/bupivacaine intra-operative infiltration guidelines:
- Administer into the incision site and underlying tissues.
- Mix 1-2% lidocaine/0.25-0.5% bupivacaine (50/50) by volume.
- Consider dilution of the mix, esp. for mice (e.g. 1/10 dilution).
- Use of epinephrine prolongs action.

Local Anesthetic[3]	Onset	Duration	Do not exceed (toxic dose)
Lidocaine (xylocaine)	1-3 minutes	20-40 minutes	10 mg/kg
Bupivacaine	~20 minutes	4-6 hours	6 mg/kg

Adjuvants: Adding epinephrine (1:50:000 to 1:200,000) to plain solutions of local anesthetics just before administration shortens the onset time and prolongs the duration of action. A 1:200,000 dilution is obtained by adding 0.1 ml of 1:1000 epinephrine (with a tuberculin syringe) to 20 ml of local anesthetic.

[3] Should be used with great care on peripheral nerve blocks in areas with poor collateral circulation e.g., digits, tails. Use caution if patient has cardiac problems.

Injectable Mouse Anesthetics, Anesthetic Cocktails, Tranquilizers, and Sedatives (doses may vary according to strain, sex, and individual variations)

Drug	Dose		Route	Indications & Comments
Ketamine Xylazine	90-120 10	mg/kg	IP, IM	Surgical anesthesia
Ketamine Dexmedetomidine	50-75 1	mg/kg	IP	Moderate surgical anesthesia. Not for major surgery
Ketamine Xylazine Acetylpromazine	30.00 6.00 1.00	mg/kg	SC, IP	Surgical anesthesia
Tribromoethanol	125-250	mg/kg	IP	15-45 min surgical anesthesia, 60-120 min sleep time
Pentobarbital	40-85	mg/kg	IP	Surgical anesthesia
Atipamezole	0.5-1.0	mg/kg	IP, SC, IV, IM	Antagonist for reversal of ketamine / dexmedetomidine and ketamine / xylazine anesthesia
Acetylpromazine	2-5	mg/kg	IM, IP	Sedation
Diazepam	5	mg/kg	IP	Sedation
Xylazine	10	mg/kg	IP	Sedation

Injectable Rat Anesthetics, Anesthetic Cocktails, Tranquilizers, and Sedatives (doses may vary according to strain, sex, and individual variations)

Drug	Dose		Route	Indications & Comments
Ketamine Xylazine	80-90 8-10	mg/kg	IP, IM	Surgical anesthesia
Ketamine Dexmedetomidine	75 0.5	mg/kg mg/kg	IP	Moderate surgical anesthesia. Not for major surgery
Ketamine Xylazine Acetylpromazine	31.25 6.25 1.25	mg/kg mg/kg mg/kg	SC, IP	Simple to moderate surgical procedures, e.g. laparotomy, certain sterotaxic procedures
Ketamine Xylazine Acetylpromazine	37.5 7.5 1.5	mg/kg	SC, IP	Extensive surgical procedures
Pentobarbital	40-50	mg/kg	IP	Surgical anesthesia. For perfusion may use 80-120 mg/kg

Atipamezole	0.5-1.0	mg/kg	IP, SC, IV, IM	Antagonist for reversal of ketamine / dexmedetomidine and ketamine / xylazine anesthesia
Acetylpromazine	1-2	mg/kg	IM	Sedation
Diazepam	2-4	mg/kg	IM, IP	Sedation
Xylazine	1-3	mg/kg	IM	Sedation

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